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INVITED REVIEW

Biotechnological agents for the immunotherapy of multiple sclerosis

Principles, problems and perspectives

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Summary

Based on exciting results in animal models, a number of novel immunotherapies employing biotechnological products, rather than conventional immunosuppressants, are being developed for the treatment of multiple sclerosis. The first part of this article is a review of some fundamental concepts of immunology and offers a hypothetical scenario for the immunopathogenesis of multiple sclerosis. The second part provides a critical overview of various immunotherapies relying on modern biotechnology. For each approach, the underlying immunological principles, experimental and clinical evidence, and foreseeable problems are separately addressed. Thus, it is hoped that this article serves a dual purpose, namely to provide an update on recent advances in immunology, and to serve as a useful source of reference to immunotherapies holding promise for future treatment of multiple sclerosis.

Keywords: multiple sclerosis; immunotherapy; autoimmunity; biotechnology; T lymphocytes

Abbreviations: APL = altered peptide ligand; CD = cluster of differentiation; cDNA = complementary DNA; CDR = complementarity determining region; EAE = experimental autoimmune encephalomyelitis; Fc = constant portion of immunoglobulin molecules; GM-CSF = granulocyte-macrophage colony-stimulating factor; HLA = human leucocyte antigen; ICAM = intercellular adhesion molecule; hsp = heat-shock proteins; Ig = immunoglobulin; IL = interleukin; IL-1ra = interleukin-1 receptor antagonist; IP-10 = interferon-γ-inducible protein-10; LFA = leucocyte function-associated antigen; MBP = myelin basic protein; MCP = monocyte chemoattractant protein; MHC = major histocompatibility complex; MIP = macrophage inflammatory protein; MMP = matrix metalloproteinase; MOG = myelin-oligodendrocyte glycoprotein; PCR = polymerase chain reaction; PLP = proteolipid protein; sFv = single chain variable portion of Ig molecules; TGF = transforming growth factor; TH = T helper (cell); TIMP = tissue inhibitor of MMP; TMC = trimolecular complex; TNF = tumour necrosis factor; TNF-R = TNF receptor; VCAM = vascular cell adhesion molecule; VLA = very late antigen

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Introduction

Much progress has been made recently in multiple sclerosis clinical trial methodology, largely based on advances in nuclear magnetic resonance imaging techniques. Complementary to these achievements, modern biotechnology is now offering a whole arsenal of potential treatments for multiple sclerosis, which rest on experimental results obtained during the past 15 years from systematic research into the immunopathogenesis of experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis. Some of the novel concepts are very promising, whilst others do not seem suitable for clinical application. In the first part of this article, the basic concepts of immunology and the pathogenesis of multiple sclerosis will be reviewed. In the second part, the different proposals for immunotherapy will be examined in the light of this knowledge.

Part 1: immunological principles

This section provides a brief overview of important immunological concepts. For more detailed reviews, the reader may wish to consult one of the major textbooks of immunology, e.g. the most recent edition of the textbooks by Janeway and Travers (*Immunobiology: The Immune System in Health and Disease*. Oxford: Blackwell, 1996) and Paul (*Fundamental Immunology.* New York: Raven, 1993). Additional sources of current information are the review journal *Immunology Today* and the review series *Annual Reviews of Immunology* and *Immunological Reviews*.

Cells and molecules of the immune system

The cells of the immune system reside in the central (primary) immune organs (bone marrow and thymus) or peripheral (secondary) lymphoid tissues (e.g. lymph nodes, spleen and gut-associated lymphoid tissue), or they circulate in the blood vessels and lymphatics. At any given time, there is an extensive traffic of immune cells migrating between these sites. However, under special circumstances, the cells of the immune system can reach essentially every site in the body.

The major types of immune cells include the B and T lymphocytes, which mediate antigen-specific immune responses, and the monocytes and polymorphonuclear leucocytes, which eliminate pathogens via antigen-independent mechanisms. B and T cells carry antigen-specific surface receptors, immunoglobulins (Igs) in the case of B cells and T-cell antigen receptors in the case of T cells. All these groups of immune cells are, of course, heterogeneous, as exemplified for T cells in Fig. 1.

The immune 'repertoire' of B and T cells is immense, comprising at least 10^8–10^{10} different specificities (i.e. different antigen-specific receptors). The different receptors are 'clonally distributed'. This means that with few exceptions, each B and T cell and its progeny (called a clone) carries only one type of antigen-specific receptor on its surface, although in thousands of copies.

The enormous diversity of antigen-specific receptors is generated in maturating B and T cells by a special process of somatic gene rearrangement, which is probably unique to the immune system. The genes for Igs and T-cell receptors are inherited as sets of gene segments, each of which encodes part of the antigen-specific receptor. Each set contains multiple segments. As B cells differentiate in the bone marrow, or T cells in the thymus, one segment of each set is randomly chosen and joined to form a contiguous stretch of DNA that codes for the entire receptor (Fig. 2A and B). Because different gene segments are joined in different cells, each cell forms a unique gene that codes for a unique receptor. This mechanism explains why a limited number of inherited genes can code for an almost unlimited number of proteins.

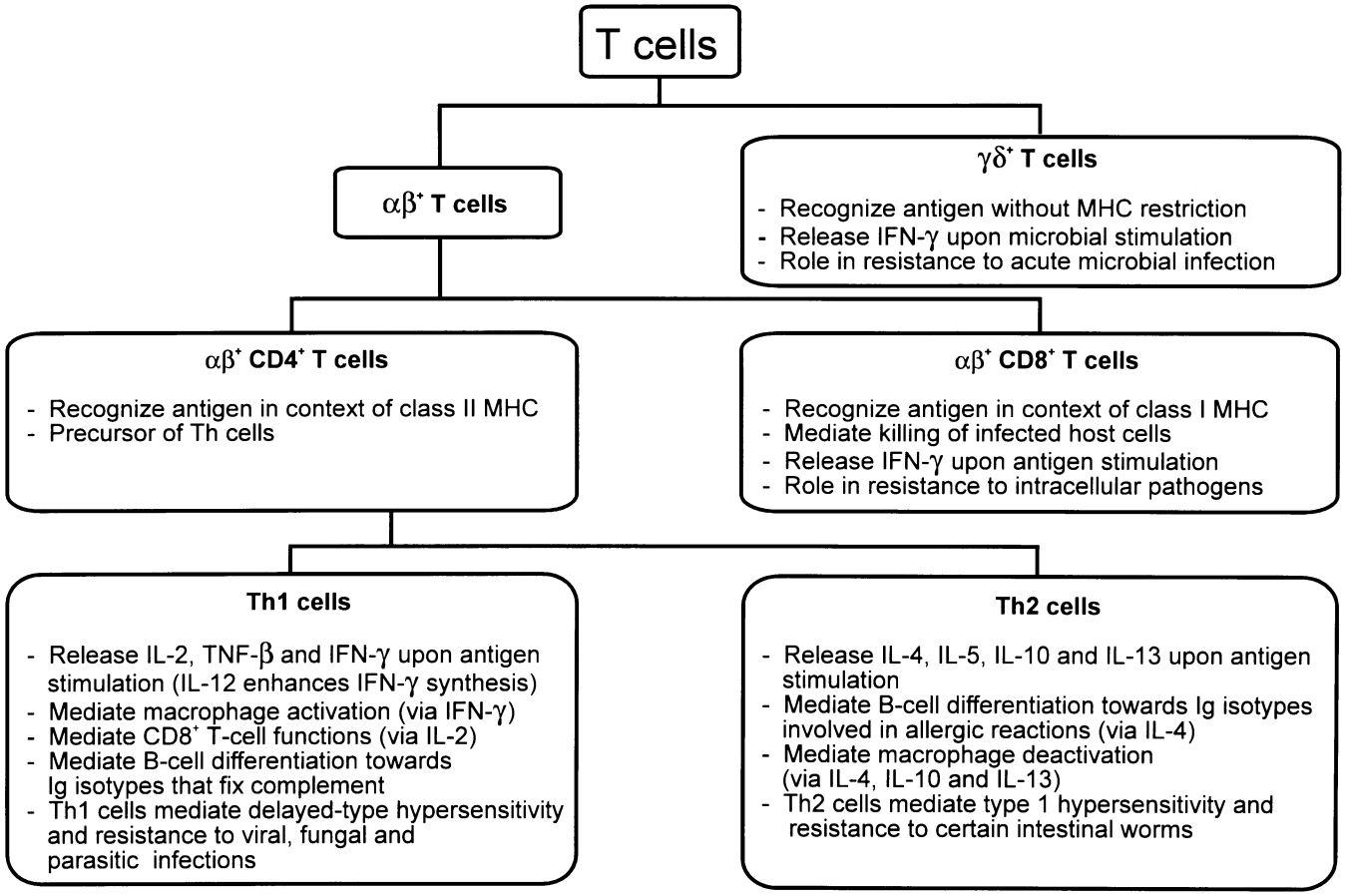

Fig. 1 Overview of the different types of T cells. T cells expressing the αβ T-cell receptor (αβ+ T cells) constitute >95% of the T cells in blood. The rare γδ+T cells are slightly enriched in certain multiple sclerosis lesions (e.g. Selmaj *et al*., 1991*a*). The αβ+ T cells can be subdivided into CD4+ and CD8+ cells, and the CD4+ T cells can be divided into TH1 and TH2 cells.

When the immune system is confronted with an invading pathogen, the clones of B cells and T cells that happen to carry receptors specifically recognizing that pathogen are stimulated and then proliferate. The clones that carry the best-fitting receptors are stimulated most strongly. This process of 'clonal selection' from a large pool of pre-existing immune cells explains the great adaptive potential of the system. Even the most exotic pathogen will, by pure chance, stimulate a few clones of B and T cells when it first invades an immunologically 'naive' organism. The *a priori* chances that the infection can be eventually controlled are good. Should a second encounter with the pathogen occur, long-lived 'memory' B and T cells will eliminate the pathogen more rapidly and efficiently, usually before it can cause symptoms. This is the basis of long-term immunity following infection or successful vaccination.

In most immune reactions, B and T cells closely cooperate. B cells can take up antigen via their specific Ig receptors, process it and re-express it on their surface in a form that can be recognized by the antigen-specific receptors of corresponding 'helper' T cells. Recognizing the antigen on the B cell, the helper T cell induces the B cells to grow and undergo further genetic modifications called 'isotype switching' and 'somatic hypermutation'. Igs are made in several distinct isotypes or classes (IgM, IgG, IgD, IgA and IgE), each of which has a distinct heavy-chain constant region encoded by a distinct constant-region gene (Fig. 2A). Isotype switching occurs by recombination events in the DNA of B cells. It does not significantly affect the specificity of antibodies, but significantly alters their functional properties, including serum half-life, complement activation and binding affinity for the different types of Fc (constant portion of Ig molecule) receptor.

Somatic hypermutation occurs in the rapidly dividing B cells in the germinal centres of peripheral lymphoid organs. It helps to increase the affinity of antibodies ('affinity maturation'). Like the somatic rearrangement of Ig genes (which occurs earlier in B-cell development), somatic hypermutation is a random process that allows a kind of 'Darwinian' selection of the best-fitting antibodies. In the variable-region genes of the dividing germinal centre B cells, somatic point mutations occur at an exceptionally high rate. Only B cells expressing receptors that can bind antigen tightly will survive and eventually leave the germinal centre to become antibody-secreting plasma cells or memory B cells. It is important to note that with few possible exceptions,

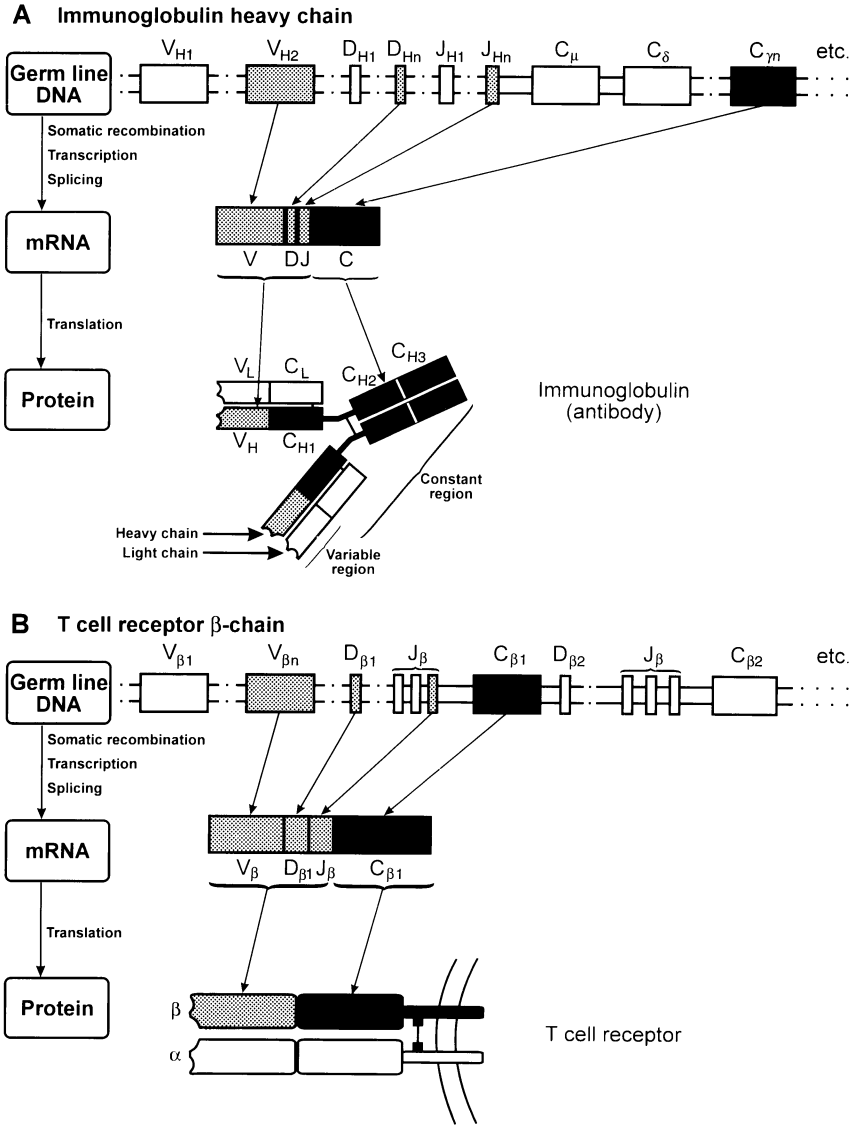

Fig. 2 Genetic organization and expression of Ig and T-cell receptor genes. (**A**) The germ line DNA encoding the variable region of the Ig heavy chain contains 51 variable (V_H), 27 diversity (D_H) and six joining (J_H) functional gene segments. The constant region genes code for the different Ig isotypes (e.g. C_μ for IgM, C_δ for IgD, etc.). A complete heavy-chain variable-region gene is assembled by somatic recombination events that first join the D and J segments, and then join the V gene segment to the combined DJ sequence. The heavy-chain constant-region sequences are spliced to the variable domain sequences during processing of the heavy-chain gene RNA transcript. The mRNA coding for the two types of Ig light chain (κ and λ) is constructed by similar mechanisms (not shown). (**B**) The germ line DNA encoding the T-cell receptor β chain genes contains 65 (46 functional) V_β, 13 J_β (not all are shown), two D_β, and two C_β segments (Rowen *et al*., 1996). Like the Ig heavy chain, the variable domain of the T-cell receptor β-chain is encoded by three gene segments, V_β, D_β, and J_β. Rearrangement of these gene segments generates a functional exon that is transcribed, spliced to join VDJ to C, and the resulting mRNA translated to yield the T-cell receptor β-chain protein. The mRNA coding for the T-cell receptor α-chain is constructed by similar mechanisms (not shown).

somatic hypermutation and affinity maturation are apparently restricted to B cells and do not occur in T cells.

The receptors of B and T cells recognize antigens in a fundamentally different way. B cells (and antibodies, the secreted form of the B-cell receptor) bind pathogens and their components in extracellular spaces. The antibodies neutralize the pathogen, make it digestible for phagocytes and activate the complement cascade. In contrast, T cells do not recognize soluble antigens, but only 'processed' antigens displayed on cells. More precisely, the T-cell system is specialized to recognize antigens as short peptides bound to proteins of the major histocompatibility complex (MHC) (Fig. 3).

The MHC is so called because it was originally discovered

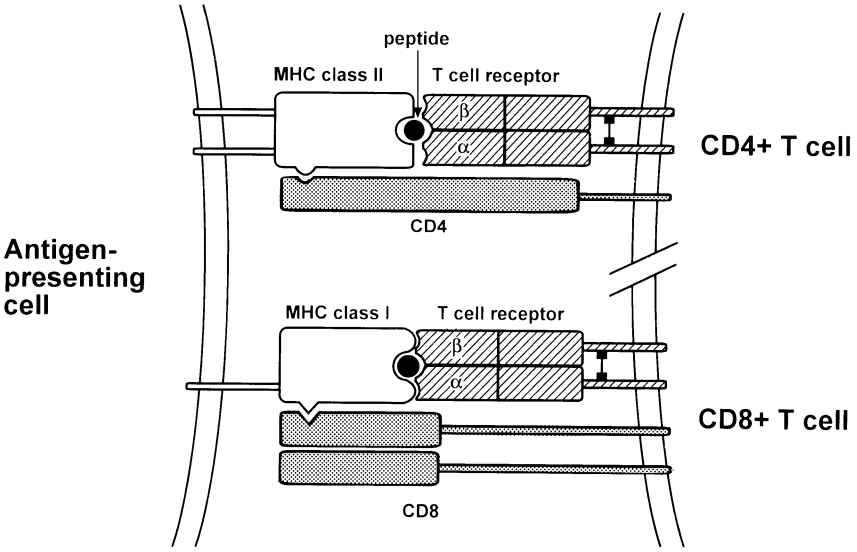

Fig. 3 Antigen recognition by CD4+ T cells (top) and CD8+ T cells (bottom). The T-cell receptor of CD4+ T cells recognizes an antigen peptide (indicated by black dot) bound to an MHC class II molecule (e.g. HLA-DR, -DP or -DQ) on the surface of an antigen-presenting cell. The T-cell receptor of CD8+ T cells recognizes an antigen peptide bound to an MHC class I molecule (e.g. HLA-A, -B or -C). CD4 and CD8 act as co-receptors.

through its effects on the rejection of transplanted tissues. The fundamental importance of the molecules encoded by the MHC only emerged much later (Zinkernagel and Doherty, 1997). In principle, there are two types of MHC molecules: class I, which includes human leucocyte antigens (HLA)-A, -B and -C, and class II, which includes HLA-DR, -DP and -DQ. The two classes of MHC molecule, which differ somewhat in structure and function, bind peptide antigens and present them to different types of T cells. The cluster of differentiation (CD)8+ cytotoxic T cells recognize peptides bound to MHC class I molecules, whereas CD4+ T cells recognize peptides bound to MHC class II molecules. The CD4 and CD8 molecules act as co-receptors. When the TCR recognizes a peptide–MHC complex on another cell, a co-receptor molecule also binds to the MHC portion of the complex, thus forming part of the recognition complex and helping to activate the T cell. CD4, which is found on helper and inflammatory T cells, binds only to MHC class II molecules; CD8, which is found on cytotoxic T cells, binds only to MHC class I molecules (Fig. 3).

The three-dimensional structure of the ternary complex between the TCR, the antigenic peptide and the MHC (class I) molecule has been solved by X-ray analysis of protein crystals (Garboczi *et al*., 1996; Garcia *et al*., 1996). When viewing such a static picture of a TCR 'caught' in the process of antigen recognition, one should consider that, in reality, antigen recognition is a highly dynamic process. It is thought that T cells 'count' the number of TCRs engaged by peptide–MHC complexes. The T cell becomes activated when that number reaches a certain threshold, i.e. several thousand TCR molecules (Viola and Lanzavecchia, 1996); *see* review by Rothenberg (1996). Each TCR can engage multiple peptide–MHC complexes serially, allowing each peptide–MHC complex to stimulate 100–200 TCRs sequentially. This helps to explain how T cells, each with ~10 000 antigen-specific TCRs on their surface, can recognize the corresponding peptide antigen, which is typically associated with as few as ~100 of the ~100 000 MHC molecules on a typical 'antigen-presenting cell' (*see* below). The 'counting' process seems to depend on a dose-related down-regulation of TCRs after antigen binding.

The peptides displayed by the class I and class II MHC molecules derive from different sources; MHC class I molecules bind peptides from pathogens that replicate in the cytosol of infected cells, typically viruses and some bacteria. These pathogens can only be destroyed by killing the cell containing them. Because MHC class I molecules are expressed on virtually all body cells, any cell that is infected can be destroyed in this way. Those MHC class I molecules that are expressed on noninfected cells (the vast majority under normal conditions) are associated with a 'self' peptide derived from some endogenous protein synthesized in the cell. These physiologically expressed 'self' peptides are normally ignored by the immune system for reasons explained below.

In contrast to MHC class I molecules, MHC class II molecules are expressed primarily on cells of the immune system (e.g. macrophages, B cells, and activated T cells in humans). The class II molecules bind peptides derived from proteins degraded in intracellular vesicles. Such peptides may come from an exogenous source or from intracellular bacteria and parasites. Exogenous antigens may enter the cell by phagocytosis/pinocytosis, or by receptor-mediated endocytosis. For example, external antigens bound by B-cell surface Ig are internalized and degraded in intracellular vesicles, generating peptides that are then carried to the B-

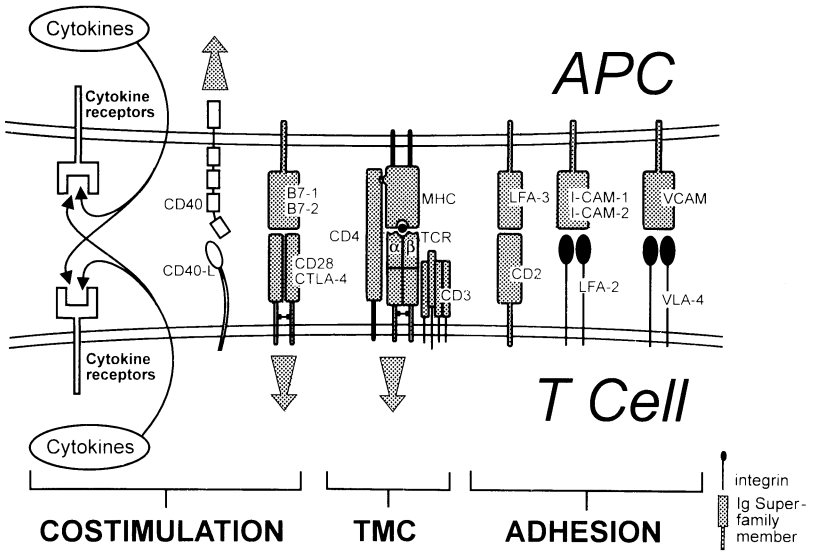

Fig. 4 Molecular interactions at the interface between a CD4+ T cell (bottom) and an antigen-presenting cell (APC) (top). Antigen-specificity is conferred by the clonotypic T-cell receptor (TCR) that recognizes an antigen peptide (black dot) bound to an MHC class II molecule (trimolecular complex, TMC) (centre). Signal transduction is mediated by the invariant proteins of the CD3 complex associated with the TCR. In addition to antigen-specific signalling via the TCR/CD3 complex, various costimulatory signals are transmitted by interactions between costimulatory molecules and their ligands (e.g. CD40 and CD40-L; CD28/CTLA-4 and B7–1/B7–2), or between cytokines and their receptors (left). Large arrows indicate direction of signalling. Interactions between various adhesion molecules strengthen the contact beween the T cell and the APC (right). Most adhesion and costimulatory molecules belong to either the integrin or Ig family.

cell surface by MHC class II molecules where they trigger CD4+ helper T cells.

Apart from the recognition of a foreign peptide bound to a self MHC molecule, the activation of T cells usually requires the simultaneous delivery of costimulatory signals by specialized antigen-presenting cells. Cells that act as specialized antigen-presenting cells include the dendritic cells, macrophages and B cells. Costimulatory signals are often mediated by the interaction of membrane-bound costimulatory molecules expressed on the T-cell surface (e.g. a molecule called CD28) with their ligands on the surface of the antigen-presenting cell (e.g. B7) (Fig. 4). T cells whose antigen-receptors are ligated in the absence of such costimulatory signals become inactivated (‘anergic’) rather than stimulated (Schwartz, 1996). The dual requirement for both receptor ligation and costimulation helps to prevent naive T cells from responding to antigens on self tissue cells, which lack costimulatory activity.

The intracellular signalling pathways (not shown in Fig. 4) are mediated by the TCR–CD3 complex or CD28 and CTLA-4 after binding of their respective ligands. TCR ligation is followed by phoshorylation of the CD3-ζ chain. CD3-ζ then binds and activates a cytosolic tyrosine kinase called ZAP-70 (zeta-associated protein-70). Subsequently, phospholipase C-γ is phosphorylated and activated, leading to the generation of second messengers that elevate intracellular Ca^{2+} (inositol trisphosphate) and protein kinase C (diacylglycerol), leading to a complex cascade of downstream events. The activation of protein kinase C and the elevation of calcium-ion concentrations within the cell are common features of the stimulation of cell proliferation in a number of cell types. The combination of these signals activates intracellular DNA-binding proteins and transcription factors that initiate a cascade of events resulting in the proliferation and activation of the T cell. Early events in CD28-mediated signal transduction may include binding and activation of phosphoinositide 3-kinase, and perhaps a protein tyrosine kinase. TCR and CD28 signals appear to be integrated at the level of the mitogen-activated protein kinase JNK. The JNK protein kinase phosphorylates the transcription factor c-Jun and thereby activates the AP-1 transcription factor, which is crucial for activation of the gene encoding interleukin-2 (IL-2).

These intracellular pathways of signal transduction, initiated by the binding of extracellular ligands to their specific receptors, represent an obvious target for pharmacological intervention. In many instances at least some of the intracellular signalling proteins are specifically linked to the ligand–receptor system so that relatively selective inhibition should be possible not only at the level of the receptor–ligand interaction but also at the level of intracellular signalling.

Self tolerance and autoimmunity

This leads to the question of how the immune system normally avoids reacting against self antigens, i.e. how it remains ‘tolerant’ to self antigens, one of the fundamental problems of immunology. It is becoming increasingly clear

that self-tolerance is a state that needs to be actively maintained by various mechanisms. It is also clear that an understanding of these mechanisms is essential for understanding the pathogenesis of autoimmune disorders, and for developing immunotherapies. Unfortunately, the present knowledge in this area is still far from complete. Some of the emerging concepts of tolerance and autoimmunity are briefly reviewed below.

Not surprisingly, the random processes that create the enormous diversity of TCRs by somatic gene rearrangements generate large numbers of cells that are either useless (because they do not recognize self MHC molecules), or dangerous (because they recognize self antigens on self MHC). The former cells are thought to be eliminated ('deleted') by positive selection, and the latter by negative selection. Both these processes occur in the thymus, an immune organ which can be considered as a kind of school in which T cells are educated. The T-cell precursors that enter the thymus must first learn to recognize self MHC molecules as the antigen-presenting facility. This is achieved by a selective process called 'positive selection'. Next, they must learn to tolerate the self antigens present on MHC molecules. This seems to be achieved, at least in part, by eliminating some of the T cells that recognize self peptides bound to thymic MHC molecules (negative selection).

It seems that in maturating thymocytes, the TCR sends graded signals, depending on the nature and affinity of the ligand it encounters. Weak signals are sufficient to select for survival, but inadequate to eliminate the developing (or to activate it once it matures). Such weak signals seem to lead to positive selection, while stronger ones cause deletion. Thymic selection is an extremely dynamic process, illustrated by the fact that ~98% of the thymocytes generated each day die in the thymus by programmed cell death (apoptosis). Only those few T cells that survive this extensive screening will eventually leave the thymus as mature T cells. In this way, a useful and non-damaging repertoire of TCRs is generated.

Thymic deletion of potentially autoreactive T cells has long been considered as the major mechanism for preventing autoimmunity. More recently, however, it has become clear that the normal immune system contains many T cells capable of recognizing autoantigens expressed in the thymus (for review, *see* Wekerle *et al*., 1996). Many proteins previously considered to be specific for 'peripheral tissues' and organs are in fact expressed in the thymus. Interestingly, these thymically expressed proteins include some encephalitogenic antigens of the nervous system, such as myelin basic protein (MBP) and S100β (Wekerle *et al*., 1996). T cells recognizing these antigens exist in the normal immune system. Therefore, the deletion of autoreactive T cells by thymically expressed autoantigens is, at best, incomplete. Immunologists are considering the possibility that thymic autoantigens positively 'shape' the immune repertoire rather than simply delete the autoreactive T cells (Cohen, 1992; Wekerle *et al*., 1996).

It is obviously very important to understand more about the mechanisms that prevent the numerous, potentially autoaggressive, T-cell clones from attacking the body's own tissues in the healthy organism. At present, these mechanisms are poorly understood. Autoreactive T cells that have not been deleted in the thymus are controlled by peripheral mechanisms of tolerance. As mentioned before, one such mechanism is 'clonal inactivation' that occurs when an autoreactive T cell happens to encounter 'its' autoantigen on a self-MHC molecule on a peripheral tissue, but fails to receive costimulatory signal(s) (Schwartz, 1996). For example, most tissue cells do not express B7, an important costimulatory molecule. Thus, it is possible that the peripheral tissues help to preserve tolerance by constantly inactivating those autoreactive T cells that passed the thymic control mechanisms. However, if an antigen-presenting cell that has costimulatory activity picks up a tissue-specific self-antigen and presents it to an autoreactive T cell, an autoimmune reaction can be induced. Clonal inactivation is not the only 'peripheral tolerance' mechanism. A different, relatively ill-understood and probably very complex mechanism is the active suppression of autoreactive T cells by 'suppressor T cells'. B-cell tolerance is achieved by similarly complex mechanisms, including clonal deletion and anergy (Hodgkin and Basten, 1995). Furthermore, some of the autoreactive B or T cells may not need to be strictly controlled, because their autoantigen is either inaccessible or expressed only in very low amounts (in which case self-tolerance is said to be maintained by 'clonal ignorance').

If the mechanisms of immunological tolerance fail, autoimmune diseases arise. For most autoimmune diseases, the precise triggering process(es) are not known. It is thought, however, that autoimmune diseases can be triggered by certain infections in genetically susceptible individuals. One possible mechanism for this loss of self-tolerance by infection is that the infectious agents induce a change in antigenicity of the infected tissues. For example, genes that are not normally transcribed might be 'turned on', or normal gene products might be altered at the post-transcriptional level so that they become autoantigenic. Furthermore, infectious agents might induce costimulatory activity on tissue cells, which express low levels of autoantigen but do not normally provide costimulatory signals. A second possibility is that antibodies or T cells generated in the response to an infectious agent cross-react with self-antigens (molecular mimicry). Until now, attempts to identify a particular infectious agent have failed for most autoimmune diseases. One of the reasons is that most autoimmune diseases develop gradually, making it difficult to determine the exact timepoint of disease onset. It is likely that the continued presence of the triggering infectious agent is not required for disease progression and that it has long disappeared when the first symptoms appear.

The immunopathogenesis of multiple sclerosis: a hypothetical scenario

Before discussing various immunotherapies, let us consider the current concepts of the immunopathogenesis of multiple

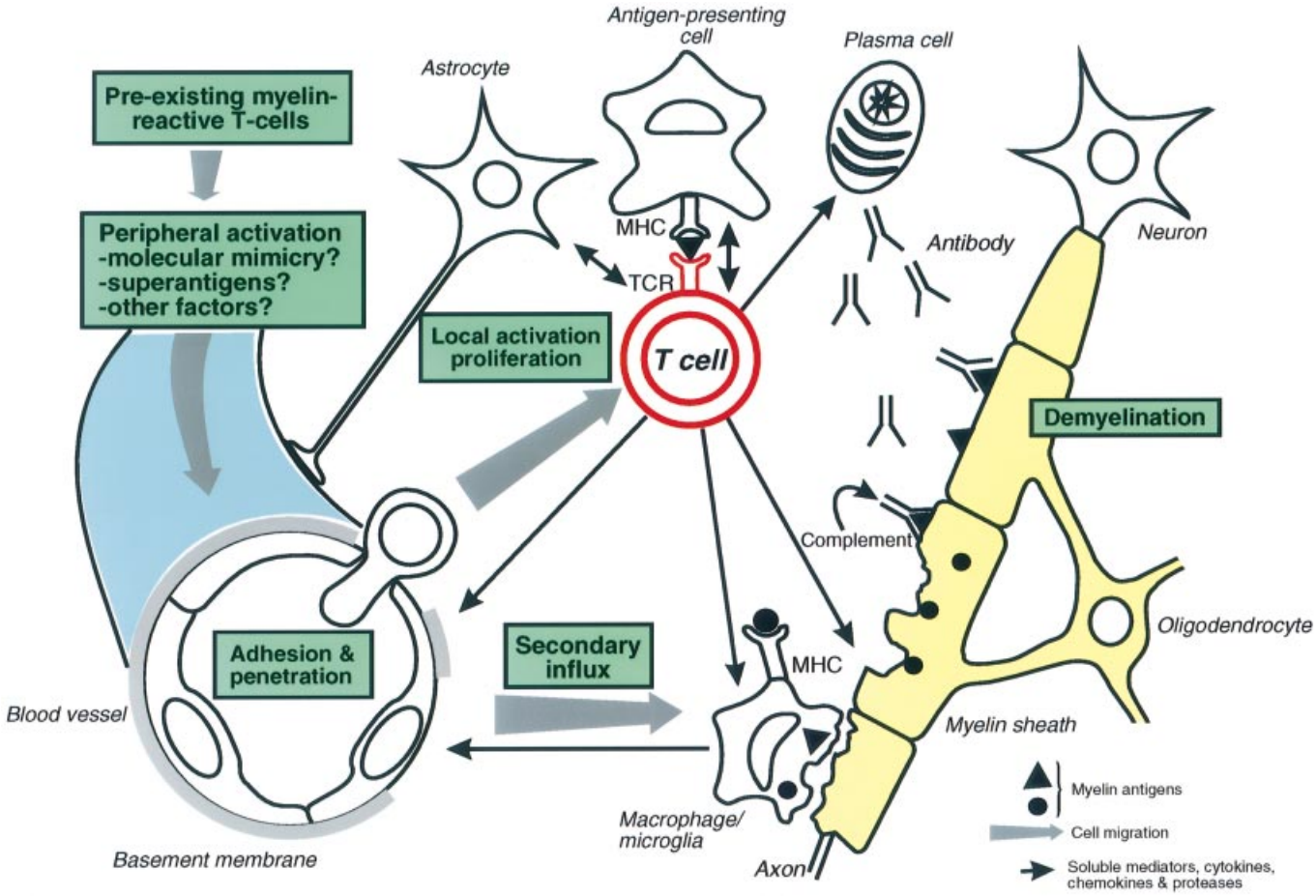

Fig. 5 Crucial steps in multiple sclerosis pathogenesis. Pre-existing autoreactive T cells are activated outside the CNS. The activated T cells traverse the blood-brain-barrier and are locally re-activated when they recognize 'their' antigen on the surface of local antigen-presenting cells. The activated T cells secrete cytokines that stimulate microglia cells and astrocytes, recruit additional inflammatory cells, and induce antibody production by plasma cells. Anti-myelin antibodies and activated macrophages/microglia cells are thought to cooperate in demyelination. *See* text for details. Modified from Gijbels (1995) with permission.

sclerosis. Based on animal experiments in EAE and observations in human multiple sclerosis, the following scenario has emerged (Fig. 5).

It is now accepted that potentially autoaggressive T lymphocytes specific for MBP or other autoantigens of the central nervous system pre-exist in the normal immune system of rodents (Schluesener and Wekerle, 1985) and primates (Burns *et al*., 1983; Genain *et al*., 1994). These autoreactive T cells must have escaped from the thymic control mechanism of clonal deletion. It is not known how these potentially autoaggressive T cells are controlled in normal individuals. Possible mechanisms include peripheral clonal inactivation ('anergy') and suppression (*see* previous section).

One of the first hypothetical events in multiple sclerosis pathogenesis is the activation of anergic, suppressed or ignorant T cells in the 'periphery' outside the CNS. How this initial activation occurs in multiple sclerosis patients is not known. Experimental models show that there are several possibilities that are not mutually exclusive and could be successively involved in different stages of the disease.

One possibility is that the initial activation of autoreactive T cells occurs via 'molecular mimicry' during bacterial or viral infection. Many bacterial and viral proteins share short sequence homologies with autoantigens. It is important to note that contiguous identity is not required; even seemingly unrelated amino acid sequences may, together with the 'presenting' MHC molecule, assume a structure that allows them to cross-stimulate autoantigen-specific T cells. MBP serves as a good example to illustrate this important point. One of the sequences preferentially recognized by human MBP-specific T cells is region $MBP_{85–99}$ of the MBP molecule (Wucherpfennig *et al*., 1991; Martin *et al*., 1992; Hafler and Weiner, 1995; Hohlfeld *et al*., 1995; Steinman *et al*., 1995; Hafler *et al*., 1996). Using previously established structural criteria for T-cell-stimulating epitopes, an extensive database search identified 129 viral and bacterial candidate peptides that matched the *structural* features (not necessarily the *sequence*) of the predicted molecular mimicry motif of this MBP region (Wucherpfennig and Strominger, 1995). Of these candidate peptides, seven viral and one bacterial peptide could indeed efficiently activate MBP-specific T-cell

clones *in vitro*, although their primary amino acid sequence was quite different from $MBP_{85–99}$ (Wucherpfennig and Strominger, 1995). These results exemplify that a single TCR can recognize distinct, but structurally related, peptides from multiple pathogens, allowing for extensive cross-reactivity with seemingly unrelated antigens.

A different form of cross-reactivity that could also lead to autoimmunity might occur at the level of the TCR. For a long time it was accepted dogma that each T cell (or T-cell clone) can express only one type of TCR, i.e. only one of the two alleles of the TCR Vα and Vβ chain is rearranged and functionally expressed (this phenomenon, which is also observed with Ig genes in B cells, is referred to as 'allelic exclusion'). There are, however, interesting exceptions. Allelic exclusion is not absolute for either the Vα or Vβ chain genes, and human T cells expressing two different TCR Vα (Padovan *et al*., 1993) or Vβ chains (Davodeau *et al*., 1995; Padovan *et al*., 1995) have been described. In such T cells, one Vβ (or one Vα) chain would be expressed along with two Vα (or two Vβ) chains so that two types of αβ TCR could form and appear on the cell surface. If one the two receptors was specific for say, a bacterial antigen and the other for an autoantigen, and the T cell became activated during a bacterial infection, then the activated anti-bacterial T cells would represent activated autoantigen-specific T cells that could trigger an autoimmune attack against the antigen recognized by their second (autoreactive) αβ TCR.

Furthermore, potentially autoaggressive T cells could be activated in the periphery by stimulation with a viral or bacterial 'superantigen'. Superantigens stimulate T cells by cross-linking their TCR β-chain with an HLA class II molecule expressed on another cell (Marrack and Kappler, 1990; Kotzin *et al*., 1993; Scherer *et al*., 1993; Fleischer, 1994). Because the superantigen-binding site of the TCR β-chain is shared between many different T-cell clones, superantigens can activate large numbers of T-cell clones specific for many different antigens, including autoantigens. Thus, superantigens could initially activate (or later re-activate) autoimmune T cells expressing a particular Vβ chain. This concept may be relevant to multiple sclerosis and has been indirectly supported by animal experiments in which relapses and exacerbations of EAE could be induced with staphylococcal superantigens (for review, *see* Brocke *et al*., 1994). It should be noted, however, that injection of the same superantigens into naive (nonprimed) mice did not induce any signs of EAE (Brocke *et al*., 1993). This would not support a role of superantigens as the *initial* trigger of multiple sclerosis, but would be consistent with their participation in later stages.

Apart from activation by molecular mimicry, dual TCR expression, or superantigens, autoreactive T cells could also be stimulated by completely nonspecific mechanisms, such as the exposure to high local concentrations of cytokines secreted in the course of unrelated inflammatory reactions. Furthermore, loss of self-tolerance could result from a change in autoantigen expression or breakage of an anatomical barrier. Experimental support has been provided for most of these mechanisms, and it is likely that different human autoimmune diseases are triggered by different mechanisms. In addition, different mechanisms may operate at different stages of the same disease.

Whatever the exact mechanism of the initial activation of autoreactive T cells and their subsequent reactivation during relapses, it is likely that this activation occurs outside the CNS. As discussed in detail below, the CNS microenvironment has a strong tendency to reduce and limit local immune reactions, and therefore is not a likely site for autosensitization. This notion, derived mainly from animal experiments, is indirectly supported by MRI findings in multiple sclerosis demonstrating that brain and spinal cord lesions often occur concurrently (Thorpe *et al*., 1996), strongly implicating a systemic trigger for disease activity.

Once the autoantigen-specific, pathogenic T cells have been activated, they must find their way into the CNS (Fig. 5). Although it is firmly established that the blood–brain barrier is impermeable for circulating large molecules and for most cells, there is compelling evidence that activated T cells can enter the CNS irrespective of their antigen specificity (Wekerle *et al*., 1986). Viewed teleologically, the selective properties of the barrier make sense. Consider the case of a systemic infection with a potentially neurotrophic virus, e.g. herpes simplex virus. T cells specific for viral determinants will be stimulated and activated in the periphery. This endows them with the capacity to traverse the blood–brain barrier so that they can scan the CNS for the presence of the virus. Thus, the CNS is by no means a 'blind spot' of the immune system but an area constantly surveilled by T cells engaged in an ongoing infection. If the T cells encounter 'their' foreign antigen in the CNS, they will eliminate it. Problems could arise only if the activated T cells (cross-)react against an autoantigen expressed in the CNS. In this case, the local recognition of autoantigen by T cells may initiate a vicious circle of immunopathological reactions (*see* below).

Upon activation, T cells increase their expression of 'adhesion molecules', and this allows them to attach to endothelial cells expressing the appropriate counter-receptors (Butcher and Picker, 1996). Endothelial cells can express tissue-specific ligands, thereby guiding T cells to specific sites, depending on the particular combination of adhesion receptors expressed on the T cells and endothelial cells, respectively (Butcher and Picker, 1996). The extravasation of leucocytes is thought to occur in four steps. The first of these is mediated by selectins, which recognize carbohydrate epitopes of glycoproteins. Selectin-mediated adhesion is weak, and allows leucocytes to roll along the vascular endothelial surface. The second step depends upon interactions between the leucocyte integrins, e.g. leucocyte function-associated antigen (LFA-1), with molecules on endothelium such as the Ig-related molecule, intercellular adhesion molecule (ICAM)-1. This binding arrests the rolling and allows the leucocyte to attach firmly to the endothelium.

In the third step the leucocyte squeezes through the endothelial wall (diapedesis). The fourth step is the migration of the leucocytes through the tissues along a concentration gradient of chemoattractant molecules (chemokines) secreted by cells at the site of inflammation. It is clear that the various adhesion molecules involved in the extravasation process are potential targets for immunotherapy.

Once having entered the brain, the activated autoreactive T cells must be confronted with CNS autoantigens within the CNS parenchyma. Animal experiments in which EAE can be transferred into previously healthy recipients by the injection of activated CD4+, MBP-specific, T cells (for review, *see* Wekerle *et al*., 1994) indicate that the first intruders are autoantigen-specific CD4+ T cells. CD4+ cells recognize their antigen as peptides embedded in MHC class II molecules located on the membranes of antigen-presenting cells (Fig. 3). Which of the MHC-expressing cells at the blood–brain barrier or in the adjacent CNS parenchyma could then act as antigen-presenting cells? Clearly, this question has not been definitely answered, but the most likely candidates include perivascular macrophages and microglia cells, at least during the initial stages of the autoimmune reaction (for reviews on microglia cells and their role in inflammatory CNS disease, *see* Fabry *et al*., 1994; Raine, 1994; Wekerle, 1994; Kreutzberg, 1996; Shrikant and Benveniste, 1996). One subpopulation of perivascular cells seems to express MHC class II antigens constitutively, that is even without requiring activation. Other brain cells require stimulation with proinflammatory cytokines to express MHC class II antigens along with various cellular adhesion molecules, including B7 (Wekerle *et al*., 1994; Williams *et al*., 1994). The cytokines could come initially from activated CD4+ autoreactive T cells entering the CNS and later also from other, secondary recruited inflammatory cells and from activated glia cells (Fabry *et al*., 1994). The proinflammatory cytokines that most efficiently induce and recruit additional inflammatory cells include interferon-γ, lymphotoxin and tumour necrosis factor-α (TNF-α).

Like all immunological reactions, the recruitment of local antigen-presenting cells must be considered as a dynamic process. A few strategically localized antigen-presenting cells, constitutively expressing MHC class II together with sufficient myelin peptides released from adjacent oligodendrocytes and myelin sheaths during physiological turnover, may be sufficient to stimulate some of the arriving CD4+ autoreactive T cells (Fig. 5). These T cells are re-activated, produce more proinflammatory mediators, and soon other local cells will be recruited to express MHC class II and costimulatory molecules (for review, *see* Shrikant and Benveniste, 1996). Thus, there is a hierarchy of inducibility of such 'facultative' antigen-presenting cells; perivascular monocytes and microglia cells are activated first and most easily (some express MHC class II molecules even constitutively), and activation of other microglia cells, pericytes and perhaps endothelial cells and astrocytes follows (Vass and Lassmann, 1990; Lassmann *et al*., 1991*a*, *b*, *c*; Fabry *et al*., 1994).

Once the inflammatory reaction has been fully ignited, the local brain microenvironment will change rapidly. The concentrations of inflammatory mediators rise sharply, leading to changes in the blood–brain barrier and allowing a secondary influx of monocytes and other inflammatory cells (Fig. 5). During the different stages of acute and chronic lesions, different cells may play different roles. This is exemplified by the complex role of astrocytes. In some situations, cytokine-stimulated astrocytes might act as fully competent (Fontana *et al*., 1984) or partially competent (Weber *et al*., 1994) facultative antigen-presenting cells during an early stage of lesion development. Under different conditions, however, the same astrocytes can produce local inhibitory signals that limit the activity of lesions during a later stage (Meinl *et al*., 1994; for review, *see* Shrikant and Benveniste, 1996).

An important lesson from EAE is that many proteins of the nervous system are potentially encephalitogenic (Wekerle *et al*., 1994; Lassmann and Vass, 1995). Apart from MBP, the autoantigen of classic EAE, other myelin antigens such as proteolipid protein (PLP) and myelin-oligodendrocyte glycoprotein (MOG) can all induce experimental encephalomyelitis under appropriate conditions. An experimental autoimmune encephalitis can even be induced with nonmyelin antigens such as S100β, which is abundantly expressed in astroglia but not oligodendrocytes (Kojima *et al*., 1994). Interestingly, any single autoantigen may produce different types of clinical course and pathology in different strains of the same animal species (note that each inbred strain corresponds to one individual of an outbred species!). Furthermore, the topography of lesions may be strictly dictated by the nature and origin of the autoantigen. Antigens present in the compact myelin, such as MBP and PLP, produce lesions located in areas with the thickest myelin sheaths (spinal cord, brainstem; Lassmann and Vass, 1995). In contrast, MOG, an antigen localized exclusively on the myelin surface, is present in high concentrations in areas with many thin myelin sheaths. The sites of inflammation observed after transfer of MOG-specific T cells include the periventricular and cerebellar white matter (Lassmann and Vass, 1995). The inflammation induced by S100β-specific T cells involves the cerebral cortex, retina and uvea in addition to the typical white matter lesions (Kojima *et al*., 1994). The cellular composition of the infiltrates also varies between the different EAE models. For example, the lesions are composed predominantly of T cells in S100β-induced disease (Kojima *et al*., 1994), whereas activated macrophages predominate in MBP-induced EAE lesions (Hickey *et al*., 1983; Matsumoto and Fujiwara, 1988).

Translated to multiple sclerosis pathogenesis, these observations could help to explain the heterogeneity of the disease (Lucchinetti *et al*., 1996). Like in EAE models, heterogeneity could reflect individual patterns in response to the same antigen, or a variable response to different antigens, or both. The situation is further complicated by the possibility

that different autoantigens might be involved during different stages of multiple sclerosis. Again, the basis for this concept of 'determinant spreading' lay in animal experiments. The concept maintains that in immunized animals an initial response, which may be restricted to only one dominant epitope of the autoantigen (perhaps mimicking a foreign antigen) may later spread to additional determinants (intramolecular spreading; for reviews, *see* Sercarz *et al*., 1993; Miller *et al*., 1995*a*). Even more complex, the encephalitogenic immune response may spread from one CNS autoantigen to another (intermolecular spreading). For example, in chronic-relapsing EAE induced by immunization with a peptide of PLP, the order in which new determinants are recognized during the course of disease follows a predictable sequential pattern, in that the immune response spreads to other determinants of PLP and to a different autoantigen, MBP (Yu *et al*., 1996*a*). The most popular explanation for determinant spreading is that the tissue damage that occurs during the early stage of the inflammatory response leads to the secondary sensitization of T cells against additional, different determinants (Sercarz *et al*., 1993). An additional mechanism could be 'clonal exhaustion' (depletion) of activated autoreactive T cells, e.g. by local apoptosis. At present, there is no solid evidence that determinant spreading occurs in human multiple sclerosis. This is not surprising, since it is not even known which of the various encephalitogenic autoantigens of the different EAE models are involved in multiple sclerosis.

Thus far, our discussion of the immunopathogenesis of multiple sclerosis has focused on autoreactive T cells. It is becoming increasingly clear, however, that B cells and their products, antibodies, are equally important, especially for demyelination. The lesions of classic MBP-induced EAE in Lewis rats, which are produced by the transfer of purified MBP-specific T cells alone, are mainly inflammatory, not demyelinating. If, however, a MOG-specific monoclonal antibody is co-injected with the T cells, large demyelinating lesions develop (Linington *et al*., 1988; Genain *et al*., 1995*a*). That the transfer of T cells is necessary, but by no means sufficient for demyelination, has not only been observed with MBP-specific T cells but also with T cells specific for other CNS autoantigens, such as MOG and S-100β. Also in those models, demyelinating lesions develop after co-injection of anti-MOG antibody (Linington *et al*., 1993; Kojima *et al*., 1994). These observations support the concept that T cells specific for various CNS autoantigens initiate inflammation and open the blood–brain barrier, whereas autoantibodies against surface antigens of myelin or oligodendrocytes are required to produce demyelination (for reviews, *see* Wekerle *et al*., 1994; Lassmann and Vass, 1995). Furthermore, autoreactive helper T cells might locally cooperate with autoreactive B cells in the production of anti-myelin autoantibodies, especially in chronic lesions (the ratio of T cells to B cells was ~190 : 1 in early and 6.6 : 1 in late lesions; *see* Ozawa *et al*., 1994). Locally produced Igs are presumably enriched in autoantibodies, because autoantigen-specific T cells, autoantigen-specific B cells, antigen-presenting macrophages and glia cells, and autoantigen are all concentrated in the same local microenvironment. In addition to locally produced Igs, systemically produced antibodies could gain access to the CNS when the blood–brain barrier becomes leaky as a consequence of local inflammation.

There is very little information on antibody-mediated demyelination. There is evidence that after binding to the myelin surface, demyelinating autoantibodies activate complement and attract macrophages/microglia (for reviews, *see* Compston *et al*., 1991; Hartung *et al*., 1995; Brosnan and Raine, 1996). The macrophages contribute to demyelination not only by physically 'stripping' the myelin but also by directed release of complement and inflammatory mediators, including reactive oxygen species and eicosanoids (*see* below). Furthermore, macrophages secrete TNF-α, also thought to participate in myelin injury (Brosnan *et al*., 1988; Selmaj and Raine, 1988; Selmaj *et al*., 1991*c*). In addition, some mediators and cytotoxic factors are presumably provided by activated glial cells, thus contributing to myelin injury (Selmaj *et al*., 1991*b*; Cannella and Raine, 1995). For example, reactive astrocytes in multiple sclerosis lesions contain NADH diaphorase activity, reflecting nitric oxide synthase catalytic activity (Bo *et al*., 1994). The synthesis of nitric oxide, a reactive nitrogen metabolite with broad antimicrobial and proinflammatory activity, is probably induced by proinflammatory cytokines such as TNF-α, IL-1 and interferon-γ (Lowenstein and Snyder, 1992; Choi, 1993; Liu *et al*. 1996*b*). In multiple sclerosis lesions, strong expression of inducible nitric oxide synthase was observed in macrophages in regions of active demyelination (De Groot *et al*., 1997).

A key assumption of the scheme shown in Fig. 5 is that only the initial steps of the immunopathological chain reaction involve specific antigen recognition by autoreactive T cells and autoantibodies. These initial events trigger a complicated inflammatory cascade. The most important cells of the later stages are the macrophages and glia cells, especially microglia (Perry *et al*., 1993). These cells produce a large number of biologically active substances and inflammatory mediators.

Macrophages phagocytose corpusculate tissue debris and then digest the ingested material. Soluble material, such as immune complexes (i.e. antigens bound to antibody) are internalized by macrophages following attachment to surface receptors (receptor-mediated pinocytosis). Macrophages express various receptors for Ig and two distinct receptors for the third component of complement. The various cytotoxic or proinflammatory substances produced by macrophages include protein-degrading enzymes (such as elastase, collagenase, lysosomal enzymes, etc.), cytokines and chemokines (especially IL-1, TNF-α, IL-6, IL-8, IL-12), complement components, active oxygen molecules (including free radicals) and a number of eicosanoids (including prostaglandin E_2, prostacyclin, thromboxane A_2, and leukotrienes B_4 and C_4). These factors stimulate other

inflammatory cells and contribute directly to local tissue injury, not only affecting myelin and oligodendrocytes but also the blood–brain barrier. The barrier becomes more leaky, allowing the influx of additional soluble factors from the blood, which further amplify the immune reaction (Fig. 5).

It seems that, in principle, macrophage- and microglia-induced demyelination may occur even in the absence of a myelin-specific antibody or T-cell response; transgenic mice in which the expression of a macrophage/microglia activating cytokine, IL-3, was targeted to astrocytes, developed multifocal, plaque-like demyelinating lesions containing large numbers of proliferating and activated foamy macrophage/microglia cells (Chiang *et al*., 1996). Thus, chronic CNS production alone of low levels of IL-3 promotes the recruitment, proliferation and activation of macrophage/microglia cells in CNS white matter with consequent primary demyelination.

Why does the clinical (and MRI) activity of individual multiple sclerosis lesions eventually resolve spontaneously without treatment? This question is as difficult to answer as is the question why and how lesions arise in the first place. At present, we assume that the primary systemic activation of autoreactive T cells is usually monophasic and abates when the precipating factors, e.g. infections are cleared. Furthermore, there is some evidence that during the recovery phase of clinical or MRI activity there is an increased systemic production of downregulatory cytokines such as transforming growth factor (TGF)-β and IL-10 (Rieckmann *et al*., 1994). This would indicate that systemic downregulatory factors at least contribute to the stabilization of lesions. In addition, local factors of lesion confinement are perhaps even more important during this phase. One such mechanism could be the local elimination of autoreactive T cells. There is indeed evidence that many T cells die via apoptosis ('programmed cell death') after local (re-) stimulation in the CNS (for review, *see* Bauer *et al*., 1995). Apoptosis occurs in activated T cells under certain conditions, e.g. ligation of the 'death receptor' Fas by its ligand (Fas-L). Both molecules are upregulated on activated T cells (Crispe, 1994; Lynch *et al*., 1995; Nagata and Golstein, 1995). It has been suggested that apoptosis, which would lead to the self-destruction of the autoantigen-specific T cells in the lesions, is particularly pronounced in the CNS, although it may occur in other tissues as well (Bauer *et al*., 1995). Local apoptosis of autoreactive T cells might indeed explain certain characteristics of CNS autoimmunity such as the monophasic course and resistance against re-induction of classical EAE, and the phenomenon of 'epitope spreading' discussed earlier in this section. Hypothetically, the population of autoreactive T cells that initiates a lesion could be partly or completely eradicated by this mechanism. Assuming that this is the case, the next wave of inflammatory activity would be mediated by T cells of slightly different specificity, explaining why the epitope recognized by the T cells has apparently 'spread' or shifted. It must be emphasized, however, that the concepts of epitope spreading and local apoptosis are based on EAE experiments. In multiple sclerosis, no autoantigen has been established and therefore, epitope spreading has not been demonstrated. Interestingly, however, apoptotic T cells have indeed been observed in multiple sclerosis lesions (Ozawa *et al*., 1994).

It is likely that other local mechanisms of lesion confinement exist, apart from the apoptosis of autoantigen-specific T cells. During lesion development, counter-regulatory or protective mechanisms seem to gain strength and eventually override the proinflammatory factors. In some instances, the same factor may play a dual role, proinflammatory or anti-inflammatory and protective, depending on the disease stage. For example, the same cells that initially produce proinflammatory signals, may later switch to the production of downregulatory factors. We found that human astrocytes produce soluble factors that inhibit T-cell proliferation, provided the astrocytes were first exposed to signals from activated T cells (Meinl *et al*., 1994). A dual role has also been proposed for the heat-shock proteins (hsp). High levels of hsp60 in structurally intact oligodendrocytes in acute multiple sclerosis lesions could indicate a protective role, whereas in chronic active lesions, hsp60 might act as an autoantigen recognized by T cells expressing the γδ receptor (for reviews, *see* Raine *et al*., 1996; Brosnan *et al*., 1996).

Not only do the functional properties of cells change during lesion evolution, the physical structure of the cellular microenvironment changes too. For example, myelin and oligodendrocytes are increasingly lost and partly replaced by reactive astroglia (Prineas, 1985; Raine, 1991; Lassmann and Vass, 1995). Because of the permanent structural changes, it is unlikely that existing lesions completely return to a normal immunological status. This may be one of the reasons why MRI activity (contrast enhancement) often recurrs in areas of pre-existing lesions (Barkhof *et al*., 1992; McDonald *et al*., 1992; Miller and Nauta, 1993).

The preceding discussion of the pathogenesis of multiple sclerosis could only provide a rough outline of the present concepts. Clearly, a thorough understanding of the pathophysiology of the disease is the basis for rational treatment and prevention (Hauser, 1994). The various strategies for the immunotherapy of multiple sclerosis will be the subject of the remaining part of this article.

Part 2: immunotherapeutic strategies

Introduction: advances in immunology and biotechnology

The novel immunotherapies discussed in the second part of this article can be considered as 'biotechnological therapies', because they attempt to manipulate immunological processes with biotechnological products designed to mimic, inhibit or otherwise interact with naturally occurring polypeptides or polynucleotides. The following aspects will be emphasized throughout the discussion of individual treatment strategies:

(i) immunological principles and rationale, (ii) experimental and clinical evidence and (iii) problems.

Therapies with conventional 'chemical' immunosuppressants will not be addressed (for critical reviews, *see* Ebers, 1994; Polman and Hartung, 1995; Thompson and Noseworthy, 1996). Likewise, strategies to foster myelin repair and remyelination, using neurotrophic factors or other agents, are fascinating but also beyond the scope of this article (for reviews on remyelination strategies, *see* Franklin, 1993; Compston, 1995; Duncan, 1996; McMorris and McKinnon, 1996; Miller *et al*., 1996*b*).

Biotechnological agents for immunotherapy are being developed not only for multiple sclerosis but also for other disorders and immunological conditions such as rheumatoid arthritis and organ transplantation (Lanzavecchia, 1993). A recurrent theme is that an optimal therapy should induce or reinduce a lasting state of tolerance that does not require long-term immunosuppression. As will be seen in the following, most of the novel immunotherapeutic agents do seem to require continuous long-term application, but strategies aiming at a lasting tolerance state are also in sight.

The therapies discussed here direct phases of the disease where inflammation and (auto)immune reactivity is prominent. Because immunological activity of the disease is likely to change over time, it cannot be expected that all therapies will be suitable for all phases and for all clinical types. Progress in clinical trial and MRI methodology should facilitate the rational selection, application and critical evaluation of immunotherapies and to determine their optimal time frame during the disease course (for reviews, *see* Whitaker *et al*., 1995; Miller *et al*., 1996*a*; Rudick *et al*., 1996*a*; Lublin *et al*., 1997).

The biological revolution

Heralded by the discovery of DNA as the primary genetic material and identification of its double-helical structure, the 'biological revolution' began in the fifties and has continued until today. Since every new discovery and technological breakthrough further speeds progress, the growth curve of knowledge is exponential rather than linear. Obviously, the advances of modern biology have a profound impact on medicine in general, and on immunology and immunopharmacology in particular. To mention only a few—perhaps arbitrary—landmark dates, the basic concepts of immunological tolerance were formed in the late fourties and early fifties; the immunological function of the thymus was first described in 1961; the structure of Igs was illucidated during the sixties; the generation of monoclonal antibodies was reported in 1975; the genetic basis of antibody diversity was described in 1976; the chemical cleavage and chain-termination methods for DNA sequencing were published in 1977. In the early to mid-eighties the T-cell receptor was identified as a protein and cloned as complementary DNA (cDNA). The polymerase chain reaction (PCR) was devised in the mid-eighties, and the structure of MHC molecules was solved in 1987. The techniques for the generation of transgenic mice and for gene targeting by homologous recombination were also developed during this time. Now, in the late nineties, the human genome project is progressing rapidly. Cytokines and neurotrophic factors are booming areas (Yuen and Mobley, 1996). Refined recombinant DNA techniques allow for genetic engineering and design of proteins almost 'at will'. Most of these discoveries have already had a profound impact on clinical medicine, especially in the pathophysiological understanding and diagnosis of genetic disorders. Therapeutic applications are growing rapidly. Novel techniques for drug design, such as combinatorial chemistry, rapid screening of compound 'libraries' and structure-based drug design are likely to revolutionize pharmaceutical chemistry (Petsko, 1996).

Protein engineering techniques

Novel proteins can be engineered by altering the DNA sequence of the cloned gene (site-directed mutagenesis). Large parts of a gene can be deleted by cutting out a segment with restriction endonucleases and ligating the remaining portions to form a smaller gene. Parts of two different genes can be ligated to create new combinations (the product of such a fused gene is called a 'fusion protein'). In fact, ingenious methods exist to bring about virtually any gene alteration. The techniques of protein and nucleic acid chemistry are highly synergistic for protein engineering, so that investigators can easily move back and forth between gene and protein.

Protein engineering techniques are, for example, widely used for the design of monoclonal antibody variants ('antibody engineering'). The major impediment to therapy with monoclonal antibodies in humans is that most monoclonal antibodies are made in mice, since it has been difficult to produce human monoclonal antibodies from hybridomas. Unfortunately, human patients rapidly develop an antibody response to these foreign mouse proteins. Once this has happened, all mouse monoclonal antibodies become useless in that patient.

One way to avoid this problem is to clone human V region genes into a 'phage display' library (Winter *et al*., 1994; Marks and Marks, 1996). The phage display technique allows the construction of antibodies of predetermined binding specificity by using libraries of associated heavy and light chain variable domains expressed on the surface of filamentous bacteriophages (so-called phage-antibodies). Rare phages are selected from the repertoire by antigen binding; soluble antibody fragments are expressed from infected bacteria; the affinity of binding of selected antibodies is then improved by mutagenesis techniques (Winter *et al*., 1994).

A second approach is to introduce transgenes for human Ig heavy and light chain loci into mice that lack endogenous Ig genes. B cells in these transgenic mice should have receptors encoded by human Ig genes but would not be

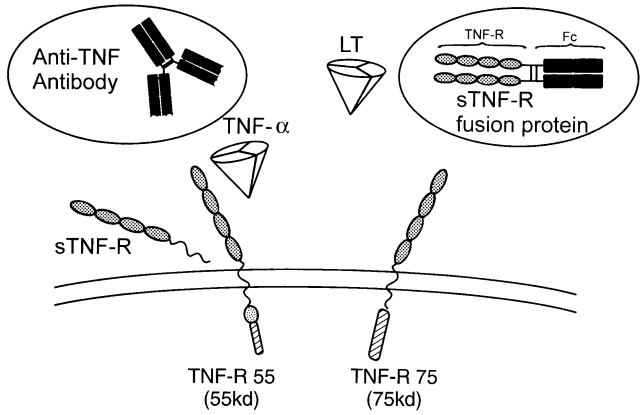

Fig. 6 Immunotherapies directed against tumour necrosis factor (TNF)-α and lymphotoxin (LT)-α. TNF-α and LT-α are homotrimeric cytokines that play a central role in the pathogenesis of multiple sclerosis. Both cytokines bind to two types of receptor, TNF-R55 and TNF-R75. Soluble forms of TNF-R (sTNF-R), which are shed from the cell surface, can bind and thus neutralize TNF-α and LT-α. For therapy, sTNF-R was fused to the Fc part of human Ig (upper right). Monoclonal anti-TNF antibodies could be used to neutralize TNF (upper left).

tolerant to most human proteins. It is therefore possible to produce monoclonal antibodies that are indistinguishable from human antibodies by immunizing such mice with human cells or proteins. A third approach is to replace large portions of mouse monoclonal antibodies with human sequences to generate 'chimeric' or 'humanized' monoclonal antibodies, which should be less immunogenic in humans than are unaltered mouse antibodies.

The single-chain variable portion of Ig molecules (sFv) represent another example of engineered antibody that consists of a single polypeptide chain in which the V_H and V_L domains are connected by a flexible peptide 'linker' (for review, *see* De Kruif *et al*., 1996). Diverse repertoires of sFv genes can be generated with the PCR either from V_H and V_L genes that had been rearranged *in vivo* or from V gene segments that are rearranged *in vitro*. The repertoire of sFv genes is cloned into a phage vector to generate 'phage antibodies' (phage display technology; *see* above). Inside each phage antibody is the vector DNA containing the gene for the sFv. Phage antibodies binding to a specific antigen can be separated from nonbinding phage antibodies by affinity chromatography on immobilized antigen. Repeated rounds of selection make it possible to isolate antigen-binding phage antibodies that were originally present at very low frequencies.

The sFv molecules fused or chemically linked to cytokines, toxins, enzymes or drugs can serve as carriers to target specific cells or sites. Antibodies coupled to toxins (called 'immunotoxins'; for review, *see* Thrush *et al*., 1996) could be used to kill activated T cells selectively. Bivalent antibodies, with both arms of the same specificity, can increase the affinity of antibody targeting. Bispecific antibodies, with two arms of different specificity, could be used to target a cell or ligand to another cell.

Other types of fusion proteins ('immunoadhesins') make use of those antibody properties that are associated with the Fc region. For example, a fusion protein of the extracellular domain of the TNF receptor (TNF-R) and the Fc portion of human IgG has the dual advantage that it is bivalent (i.e. contains two TNF-R moieties) and has a similar half-life and bioavailability to human IgG antibodies (Fig. 6). Many engineered proteins of this type are eventually recognized as foreign. Therefore, successful therapy with engineered proteins is often limited to a short period of time before neutralizing antibodies develop.

Immunological gene therapy and anti-sense oligonucleotide therapy

'In contrast to many previous medical revolutions such as anaesthesia, antibiotic therapy and organ transplantation, gene therapy has an upside-down history—one in which the conceptual advance has become widely accepted and firmly established as a medical principle before even a single instance of clinical efficacy has been demonstrated' (cited from Friedmann, 1996). This statement is all the more true for the application of gene therapy techniques to immunological diseases other than genetic immunodeficiency syndromes, although it is conceivable that gene therapy will eventually be applied to treatment of acquired autoimmune diseases such as multiple sclerosis. For example, recombinant vaccinia virus containing DNA of the antigen-specific receptor of autoaggressive T cells has been used for T-cell receptor vaccination to protect mice from EAE (Chunduru *et al*., 1996). Vaccination of H-2^u mice with naked DNA encoding T-cell receptor Vβ8.2 protected mice from EAE and shifted the autoimmune response from T helper cell (TH)1 to TH2 (Waisman *et al*., 1996).

Experimental arthritis could be suppressed by gene transfer of IL-1 receptor antagonist (IL-1ra) cDNA into joints, mediated by synoviocytes transduced with a retroviral vector carrying the IL-1ra cDNA (Makarov *et al*., 1996). Experimental autoimmune neuritis could be attenuated by injection of neuritogenic T-cell lines transduced with a recombinant retrovirus containing the mouse nerve growth factor gene (Kramer *et al*., 1995). This experiment suggests a more general strategy in which genetically engineered autoreactive T-cells are employed as vehicles to deliver therapeutically useful factors across the endothelial blood–nerve or blood–brain barrier (Kramer *et al*., 1995). Furthermore, T cells (or other cells) could be co-transfected with homing receptors that would determine the migration properties. In this way, it may eventually be possible to tailor-design cellular carriers for neurotrophic factors, cytokines or cytokine inhibitors, which can guide these therapeutic agents to any desired site.

Gene transfer of cytokines or cytokine inhibitors obviates

the need for continuous external application. For example, gene transfer of soluble TNF-R and IL-10 has been achieved in the mouse by intraperitoneal injection of cationic liposomes containing appropriate expression plasmid (Rogy *et al*., 1995). On the other hand, it would be an obvious disadvantage if the production of therapeutic factors could be turned off and it may indeed be possible in the future to control the activity of therapeutically introduced genes.

Rather than introducing functional genes, anti-sense oligonucleotide technology uses single-stranded oligonucleotides which hybridize to the complementary strand of messenger RNA and thereby prevent the mRNA from encoding a functional protein. Antisense methods are firmly established as research tools, e.g. for studies of gene expression, but their potential for clinical application is still at a relatively early stage (Stein and Cheng, 1993; Askari and McDonnell, 1996).

A different approach, to be mentioned here briefly, is to design short RNA molecules that can specifically bind and block proteins ('RNA decoys'). Using *in vitro* selection techniques, 'designer' decoy RNAs have been isolated from large pools of random RNA molecules that bind with high affinity to several proteins, including a few that do not naturally interact with nucleic acids. For example, a designer decoy RNA was identified that binds and blocks a monoclonal antibody recognizing the human insulin receptor; this nuclease-resistant variant decoy RNA was isolated from an RNA library containing 2′-amino pyrimidines (Lee and Sullenger, 1996). This RNA derivative is >10 000-fold more stable than unmodified RNA in serum and can inhibit anti-insulin autoantibodies from patients with insulin resistance, suggesting that decoy RNAs selected *in vitro* may be able to block oligoclonal autoimmune responses to self-antigens selectively in patients with autoimmune diseases (Lee and Sullenger, 1996).

Therapy with cytokines and anti-cytokines

Cytokines are soluble peptides that mediate intercellular communication. They act by binding to high-affinity receptors expressed on target cells and by inducing biochemical signals within those cells. Cytokines are not only released by cells of the immune system but also by many other cell types. Many effects of cytokines are redundant, i.e. different cytokines can induce similar or identical effects. Furthermore, most cytokines have a multitude of different biological effects, i.e. they are 'pleiotropic' (Table 1) (Paul, 1989; Paul and Seder, 1994). Neurotrophic factors, a special class of cytokines that act mainly on neuronal cells but may also affect immune cells (Torcia *et al*., 1996), are not included in Table 1.

The receptors for cytokines can be grouped into families (Taniguchi, 1995; Ihle, 1996). Signalling by these receptors depends on their association with a novel family of protein tyrosine kinases termed Janus kinases (Jaks), which couple ligand binding to tyrosine phosphorylation of intracellular signalling proteins recruited to the receptor complex. Among these are the signal transducers and activators of transcription (STATs), a family of transcription factors that contribute to the diversity of cytokine responses (Ihle, 1996). The functional redundancy of cytokines can be explained partly by the fact that the different members of a subfamily share a common signal-transducing receptor component.

The ligand cytokines can also be grouped into families, based on their structure, genetic organization, and cellular source (Paul and Seder, 1994). Cytokine families include the haematopoietins, interferons, Ig superfamily members, and the chemokines (Paul and Seder, 1994). It appears that cytokines and their receptors have all diversified during evolution. In higher animals, the 'cytokine network' has reached such an enormous complexity that it is impossible to conceptualize it in a simple scheme. The complexity of the cytokine network is further increased by the fact that the functional network involves not only soluble cytokines but also soluble cytokine receptors in addition to membrane-associated receptors and in some cases, membrane-associated cytokines ('tethered ligands'). A soluble form of receptor exists for many if not most cytokines and is usually generated by proteolytic cleavage of the receptor protein or by alternative splicing of its messenger RNA (Heaney and Golde, 1996).

The cytokine-based immunotherapies that can be envisaged for multiple sclerosis follow one of two basic strategies. The first strategy is to administer 'downregulatory' cytokines, such as interferon-β, IL-10, or TGF-β. It should be kept in mind, however, that cytokines with exclusively down-regulatory (or exclusively proinflammatory) properties do not exist. The second strategy is to administer inhibitors of proinflammatory cytokines. For example, one could use a genetically engineered ('humanized') monoclonal antibody for inhibition of TNF-α. Alternatively, one could design recombinant TNF receptor constructs for neutralization of circulating TNF. Finally, one could make therapeutic use of naturally occurring cytokine inhibitors or antagonists, such as the IL-1 receptor anatagonist, a naturally occurring competitive inhibitor of the proinflammatory cytokine IL-1 (Dinarello and Thompson, 1991; Arend, 1993).

One consequence of the complexity of the cytokine network is that it is virtually impossible to predict the overall effect that a given cytokine or cytokine inhibitor may have *in vivo*. Although it is obviously essential to test the safety and efficacy of cytokine-based therapies in animal models, totally unexpected, severe adverse effects may still occur in clinical trials in patients. This is emplified by a tragic episode that occurred in 1995 when genetically engineered IL-12 severely harmed several patients with renal cell carcinoma. It was particularly disturbing that the cytokine had been previously tested not only in animal experiments but also in patients in another pilot trial without adverse effects. It appears that a slight difference in mode of application was critical. This instance reminds us that any therapy is ultimately empirical, regardless of how solid its theoretical foundations appear to be.

Table 1 *Source and major effects of cytokines*

Cytokine	Sources	Effects on			
		B cells (B)	T cells (T)	Macrophages (M)	Other cells (OC)
IL-1	M, OC	+	+	+	+ (pleiotropic effects on many cells)
IL-2	TH1, OC	+	+	+	
IL-3	T, OC			+	+ (haematopoetic growth factor)
IL-4	TH2, OC	+	+ (TH2)		+ (pleiotropic effects on many cells)
IL-5	TH2, OC	+			+ (eosinophils)
IL-6	TH2, B, M, OC	+	+		+ (e.g. release of acute phase proteins; haematopoetic factor)
IL-7	Stromal cells	+ (pre-B cells)	+ (thymocytes)		
IL-8	M, T, OC		+ (attractant)		+ (e.g. neutrophil attractant; angiogenic factor)
IL-9	T	+	+		+ (erythroid precursors)
IL-10	T, B, M, OC	+	+ (TH2) − (TH1)	−	+ or − (OC)
IL-11	Stromal cells				+ (haematopoetic progenitors)
IL-12	M, B, OC		+ (TH1)		+ (NK, hematopoetic cells)
IL-13	TH2	+		+ or −	+ or − (OC)
IL-14	B, T, OC	+			
IL-15	M, many OC	+	+		
IL-16	T (CD8)		+ (chemo-attractant for CD4+ T)	++ (chemo-attractant for CD4+ M)	
IL-1 ra	M, OC	−	−	−	− (many OC)
IFN-α	B, T, M		Complex actions; *see* text		
IFN-β	Fibroblasts, many OC		Complex actions; *see* text		
IFN-γ	T, OC		Complex actions; *see* text		
IFN-ω	leucocytes, OC		limited information available		
TNF-α	M, T, OC	+	+	+	+ (pleiotropic effects on many cells)
LT (TNF-β)	T, OC		Similar to TNF-α		
GM-CSF	T, M, OC			+	+ (growth factor for progenitors and OC)
TGF-β	Many cells	−	−	+ or −	+ or − (highly pleiotropic; *see* text)
MCP-1	M, T, OC		+(attractant)	+ (attractant)	
MIP-1	M, B, T, OC		+ (attractant)	+ (attractant)	+ (e.g. attracts neutrophils, eosinophils)
IP-10	M, T, OC		+ (attractant)	+ (attractant)	+ (e.g. promotes adhesion to activated EC)
RANTES	T, M, many OC		+ (attractant)	+ (attractant)	+ (e.g. attracts eosinophils)

+ = stimulatory effect; − = inhibitory effect (blank = unknown, minor or absent effect); B = B-lymphocyte; EC = endothelial cell; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; IL-1 ra = IL-1 receptor antagonist; LT = lymphotoxin; M = monocyte/macrophage; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; IP-1 = interferon-γ-inducible protein; OC = other cells; RANTES = 'regulated on activation, normal T cell expressed and secreted'; T = T-lymphocyte; TGF = transforming growth factor; TH1 = T helper type 1 cell; TNF = tumour necrosis factor. Note that the Table can provide only a very rough overview. For many cytokines there is still only very limited information on their functional effects *in vitro* and complex role *in vivo*.

Interferons

Principles. Interferons represent a family of cytokines that interfere with viral replication. In addition, interferons have antiproliferative and various immunomodulatory effects (Trinchieri and Perussia, 1985; Paul, 1989; Paul and Seder, 1994; Farrar and Schreiber, 1993; Sen and Ransohoff, 1993; Arnason and Reder, 1994; Arnason, 1996). Interferon-α is part of a multigene family, whereas interferon-β and interferon-γ are encoded by single genes. Because interferon-β and interferon-α share components of the same receptor, they are referred to as type I interferons. Interferon-γ uses a separate receptor system and is referred to as type II interferon. Although the surface receptors are different, certain components of the intracellular signalling pathways are shared between the type I and type II interferons, so that the signalling pathways of the different interferons partially overlap (Darnell *et al*., 1994). Additional interferons have been discovered, but they are not characterized as well.

Interferon-γ is produced by activated T cells and natural killer cells (Trinchieri and Perussia, 1985; Paul, 1989; Farrar and Schreiber, 1993; Sen and Ransohoff, 1993; Paul and Seder, 1994). It is a potent activator of macrophages and monocytes, and induces an array of inflammatory mediators in these cells. An important function among many is to increase the expression of class I and class MHCII molecules on a variety of cell types, including both specialized and more adaptable antigen-presenting cells. This increase in

MHC expression facilitates antigen presentation, thereby augmenting and accelerating immune responses.

Type I interferons, interferon-α (actually a family of closely related proteins) and interferon-β, are produced by almost all mammalian cells upon stimulation. Attribution of interferon-α to leucocytes and interferon-β to fibroblasts is purely historical. One (but not the only) inducer of interferon synthesis is double-stranded RNA, which is part of the infectious cycle of viruses, but it is not found in mammalian cells. Interferon-β and interferon-γ trigger the synthesis of several host-cell proteins that contribute to the inhibition of viral replication. Like interferon-γ, type I interferons increase expression of MHC class I molecules and thereby enhance the ability of virus-infected cells to present viral peptides to CD8+ T cells. In contrast to interferon-γ, however, interferon-β and interferon-α do not induce the synthesis of MHC class II proteins; they suppress it (Ling *et al*., 1985; Inaba *et al*., 1986). This effect may be important for the immunomodulatory activity of type I interferons (Weinstock-Guttman *et al*., 1995).

The exact mechanism of interferon-β-mediated MHC class II inhibition is not completely understood. It appears that interferon-β somehow reduces the activity of the class II transactivator CIITA, a factor necessary for interferon-γ-induced MHC class II transcription (Lu *et al*., 1995). Apart from the inhibition of MHC class II expression, numerous other immunomodulatory effects of type I interferons have been described. However, the relative importance and mutual interdependence of these effects is not well understood (Weinstock-Guttman *et al*., 1995). Interferon-β has been shown to upregulate IL-10 expression and secretion by T cells and monocytes (Rep *et al*., 1996; Rudick *et al*., 1996*b*), indicating that part of the clinical effects of interferon-β are in fact mediated by IL-10 (*see* below for a discussion of the effects of IL-10). In addition, interferon-α and -β inhibit the production of IL-12 in mice *in vitro* and—during viral infection—*in vivo* (Cousens *et al*., 1997). Furthermore, interferon-β inhibited T-cell migration across basement membrane *in vitro*, presumably by decreasing the secretion of matrix-degrading enzymes (Leppert *et al*., 1996; Stuve *et al*., 1996). Treatment with interferon-β enhances serum corticosteroid levels in rats with EAE (Ruuls *et al*., 1996). However, it is doubtful that interferon-induced corticosteroid production is relevant to the therapeutic effects observed in multiple sclerosis, because corticosteroid levels were unchanged after s.c. application of interferon-β (Reder and Lowy, 1992).

Evidence. Both type I and type II interferons were initially tried in multiple sclerosis for their antiviral effect (Jacobs and Johnson, 1994). A pilot trial of systemic recombinant interferon-γ resulted in a sharp increase of exacerbations (Panitch *et al*., 1987). In contrast, type I interferons turned out to have a beneficial effect.

The effects of different recombinant interferon-β preparations on relapsing multiple sclerosis have been widely publicized, and the pros and cons of interferon-β are currently being debated among neurologists. Published results show a reduction of ~30% in the exacerbation rate [Sibley and IFNB (interferon beta) Multiple Sclerosis Study Group, 1993; IFNB Multiple Sclerosis Study Group, 1995; Jacobs *et al*., 1996], a significant reduction of MRI activity (Paty *et al*., 1993; IFNB Multiple Sclerosis Study Group, 1995; Stone *et al*., 1995; Jacobs *et al*., 1996) and a delay in time to sustained clinical progression (Jacobs *et al*., 1996). Recombinant interferon-α also seems to reduce exacerbation frequency and MRI activity (Durelli *et al*., 1994; Jacobs and Johnson, 1994). An important observation relevant also to interferon-β is that disease activity resumed after stopping treatment with recombinant interferon-α (Durelli *et al*., 1996).

The mechanism(s) of the complex *in vivo* effects of type I interferons are unknown and are obviously difficult to investigate *in vitro*. Antiviral and antiproliferative actions might contribute to the overall clinical effect in multiple sclerosis, although immunomodulatory effects such as reduction of transcription of MHC class II molecules are considered more important (Arnason and Reder, 1994; Weinstock-Guttman *et al*., 1995; Hall *et al*., 1997). Consistent with this notion, type I interferons are also effective in EAE (Abreu, 1985; Brod *et al*., 1995; Brod and Khan, 1996; Yu *et al*., 1996*b*).

Problems. The major short- and medium-term side effects observed thus far in multiple sclerosis trials of interferon-β include flu-like symptoms and (usually mild) laboratory abnormalities (IFNB Multiple Sclerosis Study Group, 1995; Jacobs *et al*., 1996). Skin necrosis at injection sites occurred in 1–3% of patients in a trial of interferon-β1b (IFNB Multiple Sclerosis Study Group, 1995). Whether or not the depressive symptoms and suicide attempts observed in this trial are causally related to the treatment is unknown (IFNB Multiple Sclerosis Study Group, 1995). It is also not clear whether the discontinuation of interferon treatment can have adverse or rebound effects. In rat EAE, discontinuation of treatment with recombinant rat interferon-β in the recovery phase resulted in a protracted and relapsing disease course with enhanced clinical severity (Ruuls *et al*., 1996).

A major unsolved problem of interferon therapy is the development of neutralizing antibodies (IFNB Multiple Sclerosis Study Group, 1995; Jacobs *et al*., 1996; IFNB Multiple Sclerosis Study Group, University of British Columbia MS/MRI Analysis Group, 1996). Antibodies of this type not only bind but also neutralize the biological effects of interferon-β. Neutralizing antibodies have been found in multiple sclerosis patients treated with interferon-β-1b and in patients treated with interferon-β-1a. Neutralizing antibodies have also been detected in patients treated with natural interferon-β (Fierlbeck *et al*., 1994), suggesting that the immunogenicity of interferons is not simply a consequence of structural differences between natural and recombinant interferons. It is interesting in this connection that naturally occurring autoantibodies against a number

of cytokines, especially IL-1α, IL-6, IL-10, interferon-α, interferon-β, and LIF (leukemia inhibitory factor), have been detected in serum of normal individuals and patients with various autoimmune diseases (for review, *see* Bendtzen *et al*., 1995). The functional importance of these autoantibodies is not clear, but they could be involved in the network of cytokine regulation.

In multiple sclerosis, neutralizing antibodies against interferon-β seem to attenuate or abolish the treatment effect (IFNB Multiple Sclerosis Study Group, 1995; IFNB Multiple Sclerosis Study, University of British Columbia MS/MRI Analysis Group, 1996). Neutralizing antibodies developed during the first year in 35% of patients (43 out of 124) treated with 8×10^6 units in the IFNB Multiple Sclerosis Study Group trial (IFNB Multiple Sclerosis Study Group and UBC MS/MRI Analysis Group, 1996). By the end of the third year, 38% of the remaining patients (34/89) were antibody-positive. A similar proportion of patients developed neutralizing antibodies in the low-dose treatment arm. In the future, it will be essential to observe the titres and effects of neutralizing antibodies in much greater detail, using reliable assays (Paty *et al*., 1996). In the worst possible scenario, neutralizing antibodies induced by therapy with recombinant interferon-β could crossreact against endogeneous interferon-β and ablate its physiological effects. Fortunately, there has been no evidence thus far for such a far-reaching effect of the therapeutically induced neutralizing antibodies, but the possibility must be kept in mind. It is likely that the extent of immunogenicity partially depends on the route of administration. The development of therapy-induced neutralizing antibodies is also a well-known phenomenon in therapies with interferon-α (Nolte *et al*., 1996). Whether truncated interferon derivatives or oral interferon can help to circumvent this problem is presently unknown (Rollwagen and Baqar, 1996).

Further open questions and unsolved problems relate to the optimal dosage, precise clinical indications, long-term effects and direct comparison of the different interferon preparations.

Tumour necrosis factor antagonists

Principles. TNF-α is a critical inflammatory mediator of EAE and multiple sclerosis, and may therefore be a target for immunotherapy. TNF-α is produced by macrophages and other cells and exerts diverse effects on different cells and tissues. As a mediator of inflammation, TNF-α stimulates the release of IL-1, many other cytokines and all metabolites of arachidonic acid (Beutler and Cerami, 1987; Beutler and Cerami, 1989; Jaattela, 1991; Vassalli, 1992; Tracey and Cerami, 1993; Paul and Seder, 1994). Through its capacity to influence leucocyte traffic by inducing or enhancing the expression of adhesion molecules on endothelial cells, TNF-α facilitates the local accumulation of macrophages, neutrophils and lymphocytes. In association with interferon-γ, TNF-α induces or enhances the production of reactive oxygen and NO (nitric oxide) derivatives, mediators that protect against microbial and parasitic invaders but may also damage the host. Since TNF-α can induce its own transcription, special, as yet incompletely understood, control mechanisms are required.

TNF is one of at least 10 (known) members of a family of ligands that activate a corresponding family of structurally related receptors (Bazzoni and Beutler, 1996). The receptors initiate signals for cell proliferation and apoptosis, which play a fundamental role in the normal development and function of the immune system. Mutations of the genes corresponding to the ligands or receptors can cause severe derangement of the immune response. All members of the TNF-ligand family are believed to consist of three polypeptide chains. All but lymphotoxin-β (which consists of a single lymphotoxin-α subunit and two lymphotoxin-β subunits) are made up of three identical subunits. All except lymphotoxin-α (which is entirely secreted) and TNF-α (which is predominantly secreted) are transmembrane proteins that act mainly through cell–cell contact.

There are two types of TNF receptors, TNF-R55 and TNF-R75. Like all other members of the TNF receptor family, they are believed to be transmembrane proteins that consist of two identical subunits. The two TNF receptors are recognized not only by TNF-α but also by the closely related cytokine, lymphotoxin-α (Jaattela, 1991; Heller and Kronke, 1994; Smith *et al*., 1994; Cleveland and Ihle, 1995). Lymphotoxin-β binds to a separate receptor (Bazzoni and Beutler, 1996).

Like the TNF-α ligand, the TNF receptors exist in a fixed membrane-bound and in a free soluble form (Fig. 6). Most, if not all cells express at least one type of TNF receptor. Binding of the homotrimeric ligand TNF-α activates the receptor in a way that is not fully understood (Bazzoni and Beutler, 1996). It is thought that ligand-induced changes bring the cytoplasmic regions of two receptors together, initiating a self-assembly process in which additional cytoplasmic proteins bind to form a complex with catalytic properties. There is evidence that the TNF receptors use different signalling pathways, including the sphingomyelin pathway, to mediate biological responses, but many aspects of TNF-mediated signal transduction are still unknown (Jaattela, 1991; Heller and Kronke, 1994; Smith *et al*., 1994; Cleveland and Ihle, 1995). Interestingly, corticosteroids also seem to exert at least part of their immunosuppressive actions by inhibition of TNF-α (Auphan *et al*., 1995; Scheinman *et al*., 1995). Corticosteroids stimulate the production of IκBα, a protein that holds the transcription factor NFκB in inactive form in the cytoplasm. NFκB, a major regulator of many cytokine and cell adhesion genes, is critically involved in the TNF signalling pathway (Heller and Kronke, 1994).

Transgenic mice expressing a mutant transmembrane TNF that is bioactive but not secreted develop chronic CNS inflammation, provided the mutant TNF is expressed in astrocytes but not neurons (Akassoglou *et al*., 1997).

TNF-α is expressed in multiple sclerosis lesions, where it is associated with CD3+ T lymphocytes, microglia cells and astrocytes (Selmaj *et al*., 1991*b*; Cannella and Raine, 1995). Furthermore, TNF-α and lymphotoxin are cytotoxic for oligodendrocytes *in vitro* (Brosnan *et al*., 1988; Selmaj and Raine, 1988; Selmaj *et al*., 1991*c*). In EAE, secretion of lymphotoxin and TNF-α by T cells specific for MBP correlates with the encephalitogenic potential of the T cells (Powell *et al*., 1990). Furthermore, several studies indicated that in multiple sclerosis patients there is a correlation between TNF levels in blood, serum or culture supernatant and the clinical course (Beck *et al*., 1988; Sharief and Hentges, 1991; Chofflon *et al*., 1992; Rudick and Ransohoff, 1992; Imamura *et al*., 1993; Rieckmann *et al*., 1994). Finally, the well-established genetic association of multiple sclerosis with HLA-DR2 can be at least partly explained by a propensity of HLA-DR2+ T cells to produce increased amounts of TNF-α and lymphotoxin (Zipp *et al*., 1995).

Evidence. The different strategies for TNF inhibition include pharmacological inhibitors of TNF synthesis and/or processing as well as biological inhibitors of TNF effects. The pharmacological inhibitors of TNF synthesis, thalidomide (Klausner *et al*., 1996) and pentoxifylline (Rieckmann *et al*., 1996; van Oosten *et al*., 1996*b*), were tested in pilot clinical trials in multiple sclerosis and other immunological diseases. The antidepressant rolipram, a selective inhibitor of phosphodiesterase type IV (Sinha *et al*., 1995), has shown promising effects on EAE in rats and common marmoset monkeys (Genain *et al*., 1995*b*; Sommer *et al*., 1995). However, this agent is currently unavailable for clinical trials in multiple sclerosis. Other selective inhibitors of type IV phospodiesterase are being developed for therapy. Also linomide (quinoline-3-carboxamide), a synthetic immunomodulator currently being evaluated for relapsing–remitting and progressive multiple sclerosis (Andersen *et al*., 1996; Karussis *et al*., 1996), exerts part of its effects by inhibition of TNF-α production (Gonzalo *et al*., 1993). Additional and possibly related effects of linomide include inhibition of apoptotic cell death triggered via the Fas pathway (Redondo *et al*., 1996) and reduction of induced nitric oxide synthase (Hortelano *et al*., 1997). It is not known what mechanisms are responsible for the immunomodulatory effects of linomide seen in multiple sclerosis and EAE.

A series of pyridinyl-imidazole compounds inhibit the production of IL-1 and TNF-α at the translational level (Lee *et al*., 1994). This class of cytokine inhibitors may provide new therapeutic candidates in the future (Lee *et al*., 1994). A different group of agents aims at the processing of the TNF-α precursor, a 233-amino acid membrane-bound precursor, into the mature, 157-amino acid cytokine. This process is dependent on matrix metalloproteinase enzymes (Chandler *et al*., 1997; Moss *et al*., 1997). Inhibitors of matrix metalloproteinases are very potent inhibitors of TNF processing (but not synthesis) *in vitro* (Gearing *et al*., 1994; McGeehan *et al*., 1994; Mohler *et al*., 1994).

Biological inhibitors of TNF include monoclonal antibodies against TNF-α and soluble TNF receptor constructs (Fig. 6). Several of these agents have shown positive effects in EAE (Ruddle *et al*., 1990; Baker *et al*., 1994; Selmaj *et al*., 1995; Klinkert *et al*., 1997) and rheumatoid arthritis (Elliott *et al*., 1994*a*, *b*). Anti-TNF-α agents being tested in rheumatoid arthritis include the human/murine IgG1κ chimeric neutralizing monoclonal antibody cA2 (Centocor); humanized anti-TNF-α IgG4 monoclonal antibody CDP571 (CellTech); soluble dimeric p-75 TNF receptor coupled to a human IgG1 Fc framework (Immunex); and soluble dimeric p-55 TNF receptor coupled to a human IgG1 Fc framework (Roche). In two multiple sclerosis patients treated with the monoclonal anti-TNF antibody cA2 in an open-label phase I trial, the number of gadolinium-enhancing lesions, CSF cell counts and IgG index increased after each treatment (van Oosten *et al*., 1996). A phase II trial of soluble dimeric TNF-R55 coupled to a human IgG1 Fc framework (Roche) has not been followed by a phase III trial.

In principle, the soluble receptor constructs generated by fusion of Ig Fc-region sequences to soluble TNF receptor have two major advantages over the soluble receptor, namely prolonged half-life and higher affinity (Heaney and Golde, 1996). A potential disadvantage of these constructs is their binding to Fc receptors of antigen-presenting cells, which could increase their immunogenicity. Conceivably, dimeric TNF-R could also be engineered by genetically linking two TNF-R domains via a linker peptide not related to Ig. Such agents would be expected to have a shorter half-life than the Ig-containing TNF-R constructs. One strategy would be to transfect autologous cells *in vitro* with the gene encoding the short-range soluble TNF-R construct and to use the transfected cells as vehicles. To this end, the TNF-R-transfected cells could be co-transfected with specific surface receptor(s) allowing them to home selectively to the target tissue where the action of the soluble TNF-R is needed.

Problems. Like other cytokine-based therapies, anti-TNF therapies are unselective. Therefore, unfavourable effects on physiological immune reactions, especially those which combat infections and tumours, cannot be excluded and must even be anticipated. Like other cytokines, TNF has pleiotropic effects *in vitro* and may have unpredicted effects *in vivo*. Demonstration of safety and/or efficacy in animal tests does not exclude unexpected side effects or other problems in patients. For example, dimeric p-75 TNF receptor coupled to a human IgG1 Fc framework (Immunex) prevents death in animal models of bacteremia and endotoxinemia, but fails to reduce (and may even increase) mortality in patients with septic shock (Fisher *et al*., 1996).

Like interferons, biological anti-TNF reagents would be expected to induce neutralizing antibodies. Fusion proteins containing constant region domains of Ig (like the soluble TNF receptor constructs) could be particularly immunogenic, because they would bind to the Fc receptors of antigen-presenting cells, thereby facilitating uptake and immunogenic

presentation. Furthermore, a general note of caution should be made regarding immunotherapies with soluble cytokine receptors. In some cytokine systems, soluble receptors or anti-cytokine antibodies do not neutralize the circulating cytokine but merely prolong its half-life, thereby potentiating its effects (Bendtzen *et al*., 1995). Indeed, low levels of soluble TNF receptor appear to enhance TNF signalling, perhaps by stabilizing the ligand, whereas higher concentrations inhibit TNF activity (Aderka *et al*., 1992).

Cytokine-induced 'immune deviation'

Principles. The 'immune deviation' approach attempts to 'deviate' or shift the spectrum of cytokines produced during an immune response by therapeutic application of regulatory cytokines. The approach is based on an important, relatively recent concept, postulating that there is a dichotomy in the population of CD4+ T cells. These cells, which were formerly simply referred to as 'helper' T cells, are now divided into at least two major subsets, TH1 and TH2 cells (Del Prete *et al*., 1994; Paul and Seder, 1994; Romagnani, 1994; Seder and Paul, 1994; Mosmann and Sad, 1996). In its present state, the concept of TH1 and TH2 cells is probably an oversimplification, attempting to put order into the bewildering complexity of cytokine effects. However, the concept is central to an understanding not only of cytokine-based but also of other immunotherapies.

According to the TH1/TH2 paradigm, TH1 cells act as 'inflammatory' T cells, which induce and directly participate in inflammatory ('delayed-type hypersensitivity') reactions and contribute to tissue injury. For example, the encephalitogenic T-cell clones that are used for transfer of EAE into normal recipient animals are always of the TH1 type. In contrast, TH2 cells stimulate antibody production by B cells and enhance eosinophil functions. In diseases like EAE and multiple sclerosis in which TH1 cells are thought to play a pivotal role, therapeutic 'immune deviation' would aim to convert the dominant TH1 response against CNS autoantigens into a TH2 response (Liblau *et al*., 1995).

In mice, TH1 and TH2 cells each produce a characteristic and distinct spectrum of cytokines. TH1 cells secrete IL-2, interferon-γ and lymphotoxin, whereas TH2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. Human TH2 cells produce similar patterns, although the synthesis of IL-2, IL-6, IL-10 and IL-13 is apparently not as strongly restricted to a single subset as in mouse T cells. Several other cytokines are secreted both by TH1 and TH2 cells, including IL-3, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF) and members of the chemokine families. T cells that produce both TH1 and TH2 cytokines are sometimes referred to as 'TH0' cells. Individual T-cell clones may show rather complex patterns of cytokine production, but it apppears that the majority can be broadly categorized as either TH1, TH2 or TH0. Differences between cells in cytokine expression may represent distinct stable phenotypes,

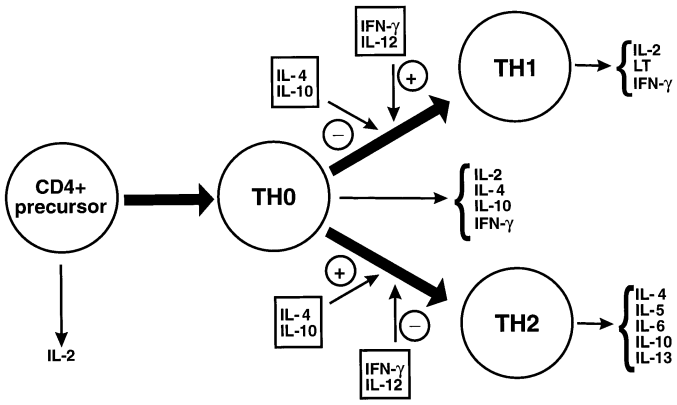

Fig. 7 Differentiation of TH1 and TH2 cells. CD4+ precursor cells maturate into TH0 cells. Under the positive or negative influence of various cytokines, TH0 cells differentiate into TH1 or TH2 cells. *See* Fig. 1 for overview of TH1 and TH2 cell functions. Note that the scheme is an oversimplification; in reality, the situation is much more complicated. Human TH1 and TH2 cells are generally less polarized in their cytokine spectrum than are murine TH cells. Fat arrows indicate differentiation pathways; thin arrows indicate positive or negative regulatory influences or cytokine secretion. IFN = interferon.

transient developmental stages or transient responses to stimulation conditions (Del Prete *et al*., 1994; Romagnani, 1994; Paul and Seder, 1994; Seder and Paul, 1994; Mosmann and Sad, 1996; Sornasse *et al*., 1996).

The characteristic cytokine products of TH1 and TH2 cells are mutually inhibitory for the differentiation and effector functions of the reciprocal phenotype (Fig. 7). For example, interferon-γ selectively inhibits the proliferation of TH2 cells, and IL-10 inhibits cytokine synthesis by TH1 cells. It is likely that *in vivo*, cytokine-induced changes in the overall pattern of cytokine secretion are brought about by changes both at the population level and single cell level (Murphy *et al*., 1996). For example, stimulation of human MBP-specific TH0-like cells with slightly modified antigenic peptides induced the production of TGF-β1 in these cells (Windhagen *et al*., 1995*b*). If a lasting change of the cytokine profile could be induced following optimally timed application of regulatory cytokines, this would open new possibilities for immunotherapy (Olsson, 1995; Rocken *et al*., 1996). Ideally, the modulating cytokine(s) would need to be applied only for a limited time, and would still induce a permanent change in the balance between TH1 and TH2 cells. Furthermore, if the therapeutic cytokines could be strategically administered during an active phase of the autoimmune reaction, immune deviation might selectively occur in the autoreactive T cells, but not in resting T cells of other specificity. Various approaches to achieve a generalized or antigen-specific immune deviation from TH1 to TH2 are listed in Table 2.

Evidence. Application of recombinant murine IL-4 ameliorated the clinical signs of EAE in SJL mice injected with MBP-specific T cells (Racke *et al*., 1994). The treatment

Table 2 *Strategies for 'immune deviation' (modified from Liblau* et al., *1995)*

(A)	Generalized immune deviation away from TH1 cells:
	Administration of IL-4, IL-10, or both
	Use of agents against interferon-γ or IL-12 (e.g. neutralizing anti-cytokine antibodies, anti-cytokine receptor antibodies, soluble cytokine receptors or synthetic cytokine antagonists)
	Use of agents that stimulate cAMP production or inhibit phosphodiesterase, e.g. pentoxifylline
	Use of other TH2-inducing compounds
(B)	Autoantigen-specific immune deviation away from TH1 cells:
	Immunization with autoantigens and concomitant stimulation of B7−2
	Immunization with autoantigens in the presence of IL-4
	Immunization with autoantigens and concomitant blockade of CD40L-CD40 interaction
	Targeting autoantigens to B cells for antigen presentation
	Oral administration (or inhalation) of autoantigens ('mucosal tolerance')
	Injection of autologous, activated, autoreactive TH2 cells
	High-dose systemic injection of soluble autoantigens
	Administration of peptide analogues of autoantigens ('altered peptide ligands')

did not prevent inflammatory infiltrates. It is assumed that IL-4 treatment induces a population of MBP-specific TH2 cells that do invade the CNS but do not induce tissue damage (Rocken *et al*., 1996). Preincubation of MBP-specific TH1 cells with IL-4 *in vitro* abolished the encephalitogenicity of the cells (Racke *et al*., 1994). A similar immune deviation could be achieved in PLP-induced EAE by immunization with a modified PLP peptide (Nicholson *et al*., 1995). Protection could be adoptively transferred with TH2 cells specific for the modified peptide ligand. In multiple sclerosis patients, treatment with the phosphodiesterase inhibitor pentoxyfylline reduced the expression of TH1-like cytokines in blood mononuclear cells, but increased the expression of the TH2-like cytokines IL-4 and IL-10 (Rieckmann *et al*., 1996).

Problems. The idea of permanently 'healing' an autoimmune disease by a short course of therapy is certainly appealing, and immune deviation is not the only approach aiming towards this goal, as will be discussed in later sections. Thus far, there is little evidence that a selective and lasting immune deviation can be achieved by a short course of therapeutic cytokines or other immunomodulators. *In vitro* experiments with human T cells indicate that the TH1 phenotype induced with IL-12 is more stable than the TH2 phenotype induced with IL-4 (Sornasse *et al*., 1996), indicating that it may be difficult to deviate from an established, ongoing autoimmune reaction, especially from TH1 to TH2. Furthermore, as indicated above, the TH1/TH2 paradigm is presumably a gross oversimplification. It would not be surprising if the 'therapeutic' application of TH2 cytokines had unexpected (and undesirable) consequences not accounted for by the simple TH1/TH2 scheme.

It is also important to note that the pathogenetic mechanisms of multiple sclerosis involve different types of T cells, as well as macrophages, B cells and antibodies. Thus, it would be much too simplistic to consider multiple sclerosis a disease exclusively mediated by TH1 cells. On the contrary, TH2 cells which support antibody production by B cells are likely to play an important role in certain forms and stages of multiple sclerosis. In those instances, it could be detrimental to stimulate TH2 cells and antibody production by immune deviation strategies, as was indeed observed in a MOG-induced marmoset EAE model (Genain *et al*., 1996; McFarland, 1996).

Transforming growth factor-β

Principles. TGF-β is contained in platelets and is produced by many nucleated cells and tumours. TGF-β is exceedingly pleiotropic and mediates far-ranging biological processes related not only to inflammation but also to development, tissue repair and tumourigenesis. TGF-β comes in three isoforms, TGF-β1, TGF-β2 and TGF-β3, in mammals (for review, *see* Stavnezer, 1995). The mature parts of the different isoforms have 70–80% sequence identity. In most *in vitro* assays, the biological activity of the isoforms is similar. However, there are differences in their potencies and some biological activities. Each isoform is secreted in a latent form that is activated extracellularly by proteolytic cleavage.

Most cells express three types of TGF-β receptors. One type of TGFβ receptor (type III) binds TGF-β but does not transmit signals. Signalling requires the formation of an oligomeric complex consisting of TGF-β receptors type I and II and TGF-β.

Evidence. A number of studies have demonstrated that TGF-β has beneficial effects in EAE (Schluesener and Lider, 1989; Johns *et al*., 1991; Kuruvilla *et al*., 1991; Racke *et al*., 1991; Stevens *et al*., 1994; Fabry *et al*., 1995). Conversely, neutralizing antibody to TGF-β1 enhances the severity of EAE (Johns and Sriram, 1993). Recombinant TGF-β2 was tested in a phase I dose-escalation trial in multiple sclerosis patients.

Problems. TGF-β is an exceedingly pleiotropic cytokine (Wahl, 1994). Its disease-limiting properties in autoimmune and chronic inflammatory diseases seem attractive, but the

very mechanisms it suppresses are also those necessary for defense against infectious pathogens. Furthermore, over-abundant systemic TGF-β could induce widespread tissue fibrosis and deposition of extracellular matrix (Wahl, 1994). TGF-β is nephrotoxic.

Interleukin-10

Principles. IL-10 is secreted by TH2 cells, macrophages and other immune cells (Moore *et al*., 1993). It has multiple *in vitro* activities on different cell types. For example, IL-10 suppresses the production of proinflammatory cytokines and mediators by macrophages and T cells, inhibits the expression of B7 and other costimulatory and adhesion molecules by antigen-presenting cells, and reduces T-cell proliferative responses. Furthermore, IL-10 induces a long-term, antigen-specific state of unresponsiveness ('anergy') in human CD4+ T cells (Groux *et al*., 1996).

Conversely, IL-10 stimulates B-cell growth and Ig production. IL-10 promotes TH2 cell responses and is a central regulator of the balance between TH1 and TH2 cells (*see* section on 'Cytokine-mediated immune deviation'). The IL-10 receptor, which is mostly expressed on immune cells, belongs to the same class as the interferon receptors.

Evidence. IL-10 prevents EAE in Lewis rats (Rott *et al*., 1994). In SJL mice, superantigen- and TNF-α-induced relapses of EAE could be prevented with IL-10 (Crisi *et al*., 1995). In another study, however, IL-10 had no effect on, or even caused worsening of, chronic relapsing EAE induced in SJL/J mice by injection of MBP-stimulated lymphocytes (Cannella *et al*., 1996). A phase I study of recombinant human IL-10 in healthy volunteers showed that a single i.v. bolus injection of 1, 10 or 25 μg/kg IL-10 induced a transient neutrophilia, monocytosis and lymphopenia (Chernoff *et al*., 1995). Mitogen-induced T-cell proliferation was transiently suppressed, as was the production of the proinflammatory cytokines TNF-α and IL-1β.

Problems. As with other cytokines, the *in vivo* effects of IL-10 in multiple sclerosis are unpredictable (Cannella *et al*., 1996) and need to be investigated in carefully designed clinical studies.

Interleukin-4 and -13

Principles. IL-4 and IL-13 are closely related (Paul and Seder, 1994; Zurawski and De Vries, 1994). They both inhibit the production of proinflammatory cytokines and chemokines by monocytes and promote human B-cell proliferation and activation. Like IL-10, these cytokines are produced by TH2 cells (*see* section on 'Cytokine-induced immune deviation'). There is evidence that IL-4 and IL-13 share a common receptor or receptor component. Human T cells apparently do not possess receptor for IL-13 (Sornasse *et al*., 1996).

Evidence. In SJL mice, the clinical signs of T-cell transfer EAE could be suppressed by injection of exogenous IL-4 together with the encephalitogenic MBP-specific T cells, although the number and distribution of infiltrating cells was similar to that in nontreated controls (Racke *et al*., 1994). It appears that IL-4 induced a population of MBP-specific protective TH2 cells (*see* section on 'Cytokine-induced immune deviation'). In Lewis rats, actively induced EAE could be suppressed by injection of hamster ovary cells transfected with human IL-13 (Cash *et al*., 1994).

Problems. These are discussed in the section on 'Cytokine-induced immune deviation'.

Interleukin-1 inhibitors

Principles. IL-1 is a major proinflammatory cytokine produced by monocytes/ macrophages (Dinarello and Thompson, 1991; Arend, 1993). Two types of IL-1 (IL-1α and IL-1β) have been described, which display similar activities. The production and action of IL-1 are regulated by multiple control pathways, some of which are unique to this cytokine ('IL-1 system'; for review, *see* Dinarello, 1996).

In addition to the two agonists, the IL-1 system consists of a specific activation system (IL-1 converting enzyme: ICE), a receptor antagonist (IL-1 receptor antagonist) produced in different isoforms, and two (type I and II) high affinity receptors. The biological activity of IL-1 is counter-balanced by three types of inhibitors, namely (i) the naturally occurring IL-1 receptor antagonist, which competitively binds the IL-1 receptor without inducing signal transduction; (ii) soluble IL-1 receptors, which bind IL-1 and diminish the free concentration of the soluble cytokine, thus hampering its binding to the cell surface receptor; and (iii) the membrane-associated 'decoy' (type II) receptor, a nonsignalling molecule whose function is to prevent the ligand from interacting with the signal-transducing (type I) receptor on the same cell. Recombinant IL-1 receptor antagonist and soluble IL-1 receptor constructs would be reasonable candidates for immunotherapy.

Evidence. IL-1 has been shown to enhance both actively induced and T-cell transfer EAE (Mannie *et al*., 1987; Jacobs *et al*., 1991). In contrast, soluble IL-1 receptor and IL-1 receptor antagonist suppress EAE (Jacobs *et al*., 1991; Martin and Near, 1995).

Problems. IL-1 provides an example for a complex cytokine circuit. Under normal conditions, the interactions between IL-1α and -1β, IL-1 receptor antagonist, and type I and II receptors must be delicately balanced. Therapeutic application of any one of the inhibitory components could be expected

to disturb this balance in a complicated way. For example, it has been shown that the inhibitory activity of the IL-1 receptor antagonist is enhanced *in vitro* by soluble type II receptor, but hindered by soluble type I receptor (Burger *et al*., 1995). This example shows that different types of cytokine inhibitors can abolish each other's effect.

Chemokines

Principles. Chemokines are chemoattractant cytokines that direct the migration of leucocytes towards sites of inflammation (Murphy, 1994; Bokoch, 1995; Furie and Randolph, 1995; Mackay, 1996). These molecules are potential targets for therapy. The receptors of chemokines are single-chain transmembrane molecules coupled to G proteins. Some chemokine receptors are promiscuous in ligand binding.

The chemokines are divided into three closely related polypeptide families, which display three highly conserved cysteine amino acid residues. The CXC chemokine family has the first two amino-terminal cysteines separated by one nonconserved residue; the CC chemokine family has the first two amino-terminal cysteines in juxtaposition, and the C-chemokine family has one lone amino-terminal cysteine. The CC chemokines include RANTES (regulation on activation, normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1, MCP-2 and MCP-3. The CXC chemokines include IL-8 and interferon-γ-inducible protein-10 (IP-10), and other members. An example of a C chemokine is lymphotactin. Most CXC chemokines attract neutrophils, whereas most CC chemokines attract monocytes and T cells. The CXC chemokines IL-8 and IP-10 also attract T cells. Furthermore, the CC chemokines MIP-1α and -1β, RANTES and MCP-1 can provide costimulatory signals to T cells (Taub *et al*., 1996).

Therapeutic strategies directed against chemokines include inhibition of chemokine production and blockade of the interaction between chemokines and their receptors. Compared with other cytokine-based therapies, anti-chemokine therapies are in a relatively early stage of development.

Evidence. The chemokines thought to be involved in the formation of inflammatory lesions in EAE and multiple sclerosis include IL-8, MCP-1, MIP, IP-10 and RANTES (Hulkower *et al*., 1993; Ransohoff *et al*., 1993; Godiska *et al*., 1995; Karpus *et al*., 1995). In actively induced Lewis rat EAE, MCP-1 expression was detected at the onset of inflammation in lymphocytes and endothelial cells in subarachnoid locations (Berman *et al*., 1996). After the onset of clinical signs, MCP-1 expression was widely distributed in the spinal cord. In chronic relapsing EAE, MCP-1, IP-10, RANTES and MIP-2 (KC) were upregulated in astrocytes during clinical relapses, whereas MIP-1α and RANTES were synthesized by infiltrating leucocytes (Glabinski *et al*., 1997).

After immunization of SJL mice with PLP peptide, secretion of MIP-1α (but not MIP-2 or MCP-1) related to the formation of inflammatory infiltrates (Karpus *et al*., 1995). Animals could be protected and treated with a monoclonal antibody against MIP-1. MIP-1 did not affect the activation of encephalitogenic T cells nor their transfer potential (Karpus *et al*., 1995).

Problems. These are the same as for other nonselective therapies. Theoretically, anti-chemokine therapies would seem more suitable for treatment of acute exacerbations than for long-term therapy.

Other cytokines

Principles. Table 1 indicates that many other cytokines and cytokine inhibitors may qualify as candidate targets or mediators of immunotherapy. Furthermore, it is likely that many more cytokines will be discovered in the future, many of which will be interesting for therapeutic applications.

Evidence. For example, adoptively transferred EAE could be inhibited with an antibody against IL-12, a potent inducer of interferon-γ and promoter of TH1 T-cell development (Fig. 7) (Leonard *et al*., 1995). Interleukin-15, a cytokine with IL-2-like activity, acts as a potent chemoattractant for T cells and appears to be a promising target for immunotherapy (McInnes *et al*., 1996).

Problems. These are the same as for other cytokine-based therapies.

Miscellaneous anti-inflammatory agents

The list of novel anti-inflammatory drugs is ever growing. It now includes such diverse agents as selective inhibitors of cyclooxygenase-2; inhibitors and antagonists of leukotrienes; selective inhibitors of inducible nitric oxide synthase; complement inhibitors such as soluble complement receptor (CR) 1 (Piddlesden *et al*., 1994) and anti-CR 3 monoclonal antibodies (Huitinga *et al*., 1993); inhibitors of matrix metalloproteinases; and many others. Therapy with high-dose i.v. polyclonal Igs (Tazekas *et al*., 1997) could also be added, although the rationale for their use in multiple sclerosis is based more on their potential effect on remyelination than their ill-defined immunomodulatory properties (Miller *et al*., 1996*b*; Rodriguez *et al*., 1996). Some, but not all, of these agents can be considered as biotechnological products. Here, only the matrix metalloproteinases and their inhibitors will be discussed in detail.

Inhibitors of matrix metalloproteinases

Principles. Matrix metalloproteinases (MMPs) are a family of zinc-containing endo-proteinases that digest specific components of the extracellular matrix, thus contributing to matrix equilibrium and structural integrity (Goetzl *et al*., 1996). The MMPs can be categorized into three major functional groups: (i) the interstitial collagenases (MMP-1 and -8 which preferentially cleave collagen types I, II, and III); (ii) the stromelysins (MMP-3, -10, and -11 which cleave laminin); and (iii) the gelatinases (MMP-2 and -9 which cleave type IV collagen).

Owing to their potentially hazardous effects, MMPs are strictly regulated at different levels. At the transcriptional level, MMP expression is controlled by various cytokines that act through positive or negative regulatory elements of the MMP genes. For example, the TH2 cytokines IL-4 and IL-10 inhibit the secretion of MMP by macrophages. Note that conversely, MMPs cleave the membrane-bound form of TNF-α, thus facilitating the secretion of this proinflammatory cytokine by macrophages and T cells (Goetzl *et al*., 1996; Chandler *et al*., 1997; Moss *et al*., 1997). MMP activity is further regulated at the post-transcriptional level by proteolytic activation of the latent proenzymes and by interaction with specific tissue inhibitors of MMPs (TIMPs), which bind and inactivate MMPs. Again, the expression and secretion of MMP-activating enzymes and TIMPs is influenced by different cytokines.

Dysregulation of MMP production and activation is thought to contribute to tissue injury in inflammatory and neoplastic diseases (Goetzl *et al*., 1996). Macrophages, T cells, eosinophils and neutrophils all produce MMPs with cell-specific patterns. The MMPs secreted by macrophages (e.g. MMP-1, -2, -3, -9, -12 and matrilysin) contribute substantially to the degradation, removal and remodelling of connective tissues. The production of these MMPs by macrophages is enhanced by surface determinants on activated T cells and suppressed by IL-4, IL-10, and in some instances by interferon-γ.

The gelatinase-type MMPs produced by T cells (MMP-2 and -9) serve principally to facilitate T-cell migration through basement membrane and other matrix structures. T-cell secretion of these MMPs is stimulated by cytokines and inflammatory mediators after adhesion of the T cells to endothelial cells via vascular cell adhesion molecule-1 (VCAM-1) or to basement membrane matrix via β1 integrins. In addition to MMPs, T cells and macrophages secrete heparanase, an enzyme which cleaves heparan sulfate moieties of the extracellular matrix and thus also facilitates cell movement and migration through extravascular tissues (Gilat *et al*., 1996). Furthermore, as mentionend in the section on TNF-α, MMPs facilitate the secretion of TNF-α in both T cells and macrophages by cleavage of the membrane-bound form of this proinflammatory cytokine (Chandler *et al*., 1997). In addition, MMPs facilitate shedding of L-selectin, an adhesion receptor, from the leucocyte surface. Release of L-selectin is thought to allow for a rapid detachment of leucocytes from the endothelial surface and to facilitate entry into subendothelial tissues (Bennett *et al*., 1996). From all these considerations it is clear that inhibition of MMPs or of the enzymes that activate MMP, e.g. tissue type plasminogen activator (Cuzner *et al*., 1996), could be advantageous in the treatment of autoimmune diseases and cancer.

Evidence. Immunocytochemical analysis shows that in the normal CNS, MMPs are mainly expressed in perivascular and parenchymal microglia (Maeda and Sobel, 1996; Cuzner *et al*., 1996). Most CNS microvessel endothelial cells express MMP-3 and -9 but not MMP-1 or -2. Large numbers of MMP-positive macrophages are present in acute multiple sclerosis lesions and in the active edges of chronic plaques. MMP-2-, -3- and -9-positive astrocytes can be detected in acute and chronic multiple sclerosis lesions (Cuzner *et al*., 1996; Maeda and Sobel, 1996). These data indicate that microglia-derived MMPs mediate turnover of the CNS extracellular matrix under normal conditions, whereas different cell populations express MMPs in sites of CNS injury.

In vitro, inhibition of MMP activity suppressed the migration of human T-cell lymphoblastoma cells through a model basement membrane (Xia *et al*., 1996). In different animal models of autoimmunity, MMP inhibitors significantly decrease oedema and inflammatory tissue damage, suggesting possible therapeutic benefits (for review, *see* Goetzl *et al*., 1996). A peptide (hydroxamate) MMP inhibitor prevented or reversed PLP-induced EAE in mice (Gijbels *et al*., 1994). Reversal of EAE appeared to be mediated mainly by an effect on the blood–brain barrier, since the degree of demyelination and inflammation was only slightly reduced in animals treated after the onset of clinical signs. Similar effects of treatment with a hydroxamate MMP inhibitor were observed in Lewis rat EAE (Hewson *et al*., 1995). Inhibition of MMP by oral treatment with D-penicillamine suppressed murine EAE (Norga *et al*., 1995). Inhibition of gelatinase secretion and migration of human T cells appears to one the many immunomodulatory effects of interferon-β (Leppert *et al*., 1996; Stuve *et al*., 1996).

Problems. Since MMP inhibitors are nonselective agents, all general precautions regarding nonselective treatments also apply here. The specific adverse effects of different MMP inhibitors will depend on their detailed chemical and pharmacological properties. For example, certain MMP inhibitors may block not only TNF-α secretion but also TNF-R cleavage (Williams *et al*., 1996). Conceivably, biological MMP inhibitors could be developed that selectively target those MMPs that are preferentially involved in a given immunopathological reaction. This is an active area of pharmacological research with possible applications not only in multiple sclerosis but also in rheumatoid arthritis and other diseases. It is presently unclear whether MMP inhibitors (or

other protease inhibitors not mentioned here) should be given as a long-term treatment, or only during acute episodes.

Therapies directed at adhesion, costimulation, or leucocyte-differentiation molecules

The physical interaction of a T cell with an antigen-presenting cell occurs at a specialized zone of transient intercellular membrane contact. In some respect, this type of cell–cell contact can be compared with the synaptic contact of neurons (Paul and Seder, 1994). At the 'immunological synapse', intercellular communication is mediated by both soluble factors (mostly cytokines) and by membrane-bound ligands that engage their complementary receptors (Fig. 4). The central element that confers specificity to the interaction is the trimolecular complex of an antigen-specific T-cell receptor and an antigenic peptide bound to an MHC molecule. This antigen-specific interaction is supported and strengthened by multiple interactions between various adhesion and costimulatory molecules and their counter-receptors. Depending on the strength of adhesion and on the combined effects of the different costimulatory signals, an integrated response is generated in the 'postsynaptic' T cell, and usually also in the 'presynaptic' antigen-presenting cell. Like a synapse of the nervous system, the immunological synapse can generate long-lasting ('memory') changes in antigen-specific T cells in addition to a short-term response.

The exact working of the immunological synapse is far from understood. Clearly, however, this structure has to be the central target for many selective and semiselective immunotherapies. As examples, immunotherapies targeted at cell adhesion molecules, costimulatory molecules or leucocyte differentiation antigens are considered in this section. Of these different approaches, therapies directed against T-cell costimulatory molecules appear to be the most attractive, because they would allow for relatively selective manipulation of the 'immunological synapse'. However, most of the adhesion, costimulatory and differentiation molecules discussed in this section have multiple functions so that the distinction becomes blurred.

Adhesion molecules

Principles. Adhesion molecules are cell surface proteins that participate in leucocyte circulation, homing to tissues and inflammatory sites, and in the transendothelial migration. Interaction of these molecules with their specific ligands mediates adherence of leucocytes to other cells, vascular endothelium and the extracellular matrix (for reviews, *see* Hemler, 1990; Raine *et al*., 1990; Ruoslahti, 1991; Springer, 1994; Hogg and Berlin, 1995; Imhof and Dunon, 1995; Schwartz *et al*., 1995; McMurray, 1996). In particular, adhesion molecules participate in essentially all cellular interactions between immune cells. Many of these molecules mediate not only adhesion but also transmission of costimulatory signals to lymphocytes. Therefore, the classes of 'adhesion molecules' and 'costimulatory molecules' overlap.

Based on their structure, adhesion molecules can be classified into several groups, including cadherins, selectins, integrins and Ig superfamily members (Table 3). Cadherins establish molecular links beween adjacent cells by forming zipper-like structures at adherens junctions. They are linked to the cytoskeleton through the catenins. These molecules, which play a key role in embryogenesis, will not be discussed further.

Selectins are expressed on leucocytes, platelets and endothelial cells. Their common structural component is an amino-terminal lectin-binding domain. Accordingly, selectins bind to glycosylated and sialylated ligands with a rapid association and dissociation constant. Selectins mediate leucocyte 'rolling' along the endothelial cell wall and are involved in the initial localization of leucocytes to inflammatory sites. There, the slowed, rolling leucocytes are exposed to chemoattractants and cytokines, leading to leucocyte activation, upregulation of additional adhesion molecules, adherence to the endothelial lining, and eventually diapedesis and chemotaxis (cf. Fig. 5).

Integrins are heterodimeric adhesion molecules composed of noncovalently bound α and β subunits. Different combinations of subunits form functionally different receptors. Integrins bind to a variety of extracellular matrix proteins (e.g. fibronectin, vitronectin, laminin, collagen) and to receptors of the Ig superfamily. A common ligand binding motif for several integrins is the amino acid sequence arginine–glycine–aspartate (abbreviated RGD), which is found, for example, in fibronectin. 'Peptidomimetic' drugs based on this motif are undergoing clinical trials in vascular thrombotic disease and could also be useful for inhibiting leucocyte binding in inflammatory diseases and metastatic cancer. Another amino acid motif, leucine–aspartate–valine (LDV), is especially important for the interaction of integrin with VCAM-1 and fibronectin in inflammatory reactions. Peptidomimetics of this motif are further candidate agents for the treatment of chronic inflammatory diseases.

Two prototypical integrin adhesion molecules are LFA-1 and the very late antigen (VLA)-4. LFA-1 is expressed on most circulating leucocytes, binds to ICAM-1 and ICAM-2, and acts as a cell adhesion and costimulatory molecule in T-cell activation (Fig. 4). VLA-4, also found on activated leucocytes, adheres to fibronectin VCAM-1, an adhesion receptor of the Ig family, is expressed on vascular endothelial cells. Integrins are known to exist in a low-affinity state and one or several high-affinity (activated) states. Migration of leucocytes across the vascular endothelium is probably mediated by fluctuation between these states.

The adhesion molecules of the Ig superfamily have Ig-like amino acid domains. They include LFA-2 (CD2), LFA-3, ICAM-1, ICAM-2, and VCAM-1. ICAM-1 and VCAM-1 are the principal counter-receptors of LFA-1 and VLA-4,

Table 3 *Cell adhesion molecules*

Name	Ligand	Expression (constitutive or induced)
Adhesion receptors of the selectin family		
L-selectin	Glycosylated mucinlike molecules	T, M, PMN
E-selectin	Sialylated mucinlike molecules	activated EC
P-selectin	P-selectin glycoprotein ligand 1	P, activated EC
Mucin-like vascular addressins		
CD34	L-selectin	EC
GlyCAM-1	L-selectin	High endothelial venules
MAdCAM-1	L-selectin, VLA-4	Mucosal lymphoid tissue venules
Adhesion receptors of the integrin family		
α4β1 (VLA-4)	VCAM, FN (CS-1), PP-HEV	T, B
α5β1 (VLA-5)	FN (RGD)	T, EC, epithelium, P
α6β1 (VLA-6)	LM	T, P
αLβ2 (LFA-1, CD11a/CD18)	ICAM-1, ICAM-2	Leucocytes
αMβ2 (MAC-1, CR3, CD11b/CD18)	ICAM-1, iC3b, FN, FX	M, PMN
αxβ2 (CR4, p150.95, CD11c/CD18)	FN, iC3b	M
α4β7	MAdCAM-1, VCAM	B, T
Adhesion receptors of the Ig superfamily		
ICAM-1 (CD54)	αLβ2, aMβ2	activated EC, Lymphocytes
ICAM-2 (CD102)	αLβ2	resting EC
ICAM-3 (CD50)	αLβ2	APC
VCAM (CD106)	α4β1	activated EC
LFA-2 (CD2)	LFA-3	T
LFA-3 (CD58)	CD2 (LFA-2)	Lymphocytes, APC

APC = antigen-presenting cell; CAM = cell adhesion molecule; CR = complement receptor; EC = endothelial cell; FN = fibronectin; HEV = high endothelial venule; ICAM = intercellular adhesion molecule; LFA = lymphocyte-function associated antigen; LM = laminin; M = monocyte/macrophage; P = platelet; PMN = polymorphonuclear leucocyte; PP = Peyer's plaque; VCAM = vascular cell adhesion molecule; VLA = very late antigen.

respectively (Table 3). ICAM-1 can be induced on a wide variety of cells. Cytokine stimulation of endothelial cells increases leucocyte adhesion primarily through increased ICAM-1 expression, which promotes adherence of LFA-1-positive leucocytes to inflammatory sites. VLA-4/VCAM-1 adhesion may be primarily responsible for prolonged cell adhesion to endothelium at inflammatory sites. An important feature of integrins is that they exist in different conformational states; an activated cell can transmit a signal from its cytoplasm that modifies the conformation of the extracellular domains of integrins on the cell surface, increasing the affinity of the integrins for their ligands ('inside out signalling').

In addition to avidity changes of existing adhesion molecules, adherence is regulated by modulation of expression of adhesion molecules. Differential expression and distribution of adhesion molecules determines homing of leucocytes to organs and sites of inflammation. Cytokines have a pivotal role in the regulation of adhesion molecule expression. For example, the proinflammatory cytokines TNF-α, interferon-γ, IL-1 and IL-6 increase the expression of many adhesion molecules. In addition to cytokines, many other soluble factors influence leucocyte chemotaxis, adhesion and accumulation at inflammatory sites. These factors include chemoattractants and leucocyte activators such as endotoxin, complement components, leukotriene B_4, histamine, thrombin, and chemokines. Adhesion interactions are potentially further regulated by soluble cell adhesion molecules, which could competitively bind to ligands or counter-receptors.

Clearly, an understanding of cell adhesion is important for clininal applications, and cell adhesion molecules are potential targets for immunotherapy. The principal validity of this approach has been demonstrated in experimental models of human disease and in pilot clinical trials. Most strategies of targeting adhesion molecules rely on monoclonal antibodies against adhesion molecules or recombinantly expressed soluble adhesion receptors. An additional approach is to design small peptide and nonpeptide inhibitors of adhesion molecules, such as, for example, RGD-peptide inhibitors of integrins (*see* above) or glycomimetic inhibitors of selectins.

Evidence. There is increased expression of various cell adhesion molecules in the CNS in EAE and multiple sclerosis, especially in and around microvessels, pointing to an important immunopathogenetic role of these molecules (for reviews, *see* Raine *et al*., 1990; Cross *et al*., 1991; Fabry *et al*., 1994; Raine, 1994). As expected, inflammatory cells in multiple sclerosis lesions express various adhesion molecules, consistent with their active role in the inflammatory process. Elevated levels of circulating adhesion molecules have also been observed, but their value as indicators of disease activity is limited (for review, *see* Hartung *et al*., 1995). Raised concentrations of soluble E-selectin were described in patients with primary progressive but not relapsing–remitting multiple sclerosis (Giovannoni *et al*., 1996).

In Lewis rats, T-cell transfer EAE could be prevented by a single intraperitoneal injection of a monoclonal antibody directed against α4β1 integrin (Yednock *et al*., 1992). It is thought that the antibody, which was administered 2 days after injection of the encephalitogenic T cells, acts mainly by blocking the entry of secondarily recruited host T cells and monocytes into the CNS (Yednock *et al*., 1992). Indeed, surface expression of α4 integrin by CD4+ T cells has been shown to be required for their entry into brain parenchyma (Baron *et al*., 1993). In guinea pigs, actively induced EAE was prevented and reversed with a monoclonal antibody against α4 integrin (Kent *et al*., 1995). An alternative strategy would be to use soluble adhesion receptors, for example soluble recombinant VCAM-Ig fusion protein, rather than antibodies.

Monoclonal antibody against ICAM-1 suppressed MBP-induced EAE in Lewis rats but had only a minor effect in the adoptive T-cell transfer model, indicating that the monoclonal antibody acted mainly on the induction phase of the immune response and to a lesser extent, on the transendothelial migration of T cells (Archelos *et al*., 1993). On the other hand, recombinant soluble ICAM-1 inhibited lymphocyte attachment to cultured cerebral endothelial cells, indicating that sICAM-1 might be a candidate agent for therapy (Rieckmann *et al*., 1995). Treatment with monoclonal antibodies against the counter-receptors of ICAM-1, the β2 integrins LFA-1 and the MAC-1 adhesion molecule (*see* Table 3), delayed the onset, and diminished the severity, of T-cell transfer EAE in mice (Gordon *et al*., 1995). In a different study, however, EAE was augmented by another anti-LFA monoclonal antibody (Welsh *et al*., 1993).

In patients with rheumatoid arthritis, a 5-day course of treatment with mouse anti-ICAM-1 monoclonal antibody reduced T-cell reactivity (as measured *in vitro*) for several months (Davis *et al*., 1995). This would indicate that treatment with anti-ICAM antibodies not only affects T-cell migration but also T-cell activation through the 'immunological synapse', leading to more lasting changes in T-cell reactivity.

Monoclonal antibody directed against CD2 suppressed or ameliorated both actively induced- and T-cell transfer-EAE in Lewis rats (Jung *et al*., 1995). Because antigen-induced activation of the T cells was not inhibited, the therapeutic effect was apparently brought about by inhibition of T-cell migration into the CNS (Jung *et al*., 1995).

Problems. By definition, anti-adhesion therapies are nonselective. Effective anti-adhesion agents are likely to affect leucocyte migration in general and could thus have undesired effects in situations where a strong inflammatory reaction is needed. Furthermore, therapeutic manipulation of adhesion molecules could affect important 'nonimmunological' tissue reactions such as wound repair, haemostasis and fertility. Indeed, much research activity in this area of pharmacology is related to angiogenesis, cancer, thromboembolic disorders and atherosclerosis. This is not surprising since many of the adhesion receptors listed in Table 3 are expressed on endothelial cells and platelets. At present, it appears that the widely distributed adhesion receptors are less attractive targets for immunotherapy than are the costimulatory molecules selectively expressed at the 'immunological synapse' (*see* next section).

Costimulatory molecules: the CD28/CTLA-4–B7-1/B7-2 and CD40–gp39 systems

Principles. In general, T and B cells require two distinct types of signal for effective activation. One signal originates from the ligation of the T-cell receptor complex and its coreceptors (CD4 and CD8) to an antigenic peptide bound to the presenting MHC molecule (trimolecular complex). The second signal is dependent on either soluble factors such as IL-2, or the ligation of cell surface molecules that provide essential costimulatory signals complementary to T-cell receptor engagement (Fig. 4). Two important costimulatory pathways, the CD28/CTLA-4 –B7-1/B7-2 and the CD40–gp39 system, are described in this section. Growing evidence indicates that interfering with these costimulatory signals causes negative signalling of the antigen-activated T cells and results in T-cell 'anergy' (antigen-specific nonresponsiveness; for review, *see* Schwartz, 1996).

B7–1 and B7–2 are both members of the Ig superfamily and act as counter-receptors for CD28 and CTLA-4 (Lenschow *et al*., 1996). B7–1 is expressed on activated B cells and T cells, and also on a variety of antigen-presenting cells including dendritic cells and activated monocytes. High levels of B7–2 are constitutively expressed on dendritic cells and monocytes, and are rapidly induced by activation on B cells and T cells (Lenschow *et al*., 1996). On B cells, B7–1 and B7-2-expression is regulated by a variety of cell surface glycoproteins, including signalling through CD40. Furthermore, B7–1 and B7–2 are regulated by cytokines. For example, IL-4 and interferon-γ increase the expression of B7. In contrast, IL-10 downregulates B7 expression, indicating that the immunosuppressive properties of IL-10 may, in part, be mediated by its regulation of the CD28/CTLA-4 ligands (Lenschow *et al*., 1996).

CD28 is constitutively expressed on the majority of human T cells. Expression is increased following T-cell activation. In contrast, CTLA-4 is not constitutively expressed, but induced after T-cell activation. Both CD28 and CTLA-4 exist mainly as disulphide-linked homodimeric glycoproteins. In addition, the two molecules are likely to exist in multiple isoforms (Lenschow *et al*., 1996). CD28 signalling facilitates the initiation of T-cell responses, prevents apoptosis and helps to sustain T-cell proliferation. Furthermore, B7/CD28 plays a fundamental role in the early development and differentiation of both TH1 and TH2 cells; in the absence of CD28 signalling, naive T cells are biased towards the TH1 type. There is evidence for a differential effect of B7–1 and B7–2 in that B7–1 and B7–2 seem to control TH1 and TH2 development, respectively (Lenschow *et al*., 1996).

The precise function of CTLA-4 is still controversial. CTLA-4 has a higher affinity for B7–1 and B7–2 than CD28. Early studies indicated that CTLA-4 plays a similar role as CD28 in the regulation of T-cell responses. However, more recent studies suggest that CTLA-4 acts as a downregulatory molecule in T-cell activation. Thus, under normal circumstances, the CD28/B7 system will serve to both upregulate and downregulate immune responses. Any disturbance in this delicate balance may dysregulate the immune response.

A second important costimulatory pathway is mediated by interaction of CD40 with its ligand gp39 (also referred to as CD40-L) (Fig. 4) (Foy *et al*., 1996). CD40 is a member of the TNF receptor family. Its distribution was originally thought to be restricted to B cells and dendritic cells, but more recently it has been shown to be expressed on other cells including macrophages, endothelial cells and thymic epithelial cells. The ligand of CD40, gp39, is structurally related to TNF-α and lymphotoxin. Like TNF, gp39 forms a trimer capable of binding, and thereby cross-linking, three CD40 molecules. It is expressed on several cell types, including activated T cells, basophils and eosinophils (Foy *et al*., 1996).

CD40–gp39 interactions play a pivotal role in expansion and differentiation of resting B cells. Cross-linking of surface CD40 is an extremely effective means to prevent apoptosis of B cells in germinal centres. Stimulation by gp39 is also critical for B cells to express high levels of costimulatory molecules and to mature to 'competent' antigen-presenting cells. Once matured, the B cells can reciprocally trigger T cells via costimulatory molecules to maximize the signalling of T-cell growth and lymphokine production. In addition to their prominent effects on B cells, CD40–gp39 interactions are thought to participate in negative thymic selection of T cells (Foy *et al*., 1996). Furthermore, CD40–gp 39 interactions seem to play a central role not only in host defense but also in the regulation of T-cell responses and in peripheral T-cell tolerance (Grewal and Flavell, 1996; Noelle, 1996). This has been suggested by experiments with a model of allograft rejection in which transplantation tolerance could be induced by the coadministration of allogeneic B cells and anti-gp39 antibody. These results indicate that antigens displayed on B cells are tolerogenic in the absence of a CD40-mediated costimulatory signal.

CD40L-deficient mice that carried a transgenic T-cell receptor specific for MBP failed to develop EAE after priming with antigen (Grewal *et al*., 1996). When these mice were provided with B7.1+ antigen-presenting cells, the transgenic T cells were stimulated to produce interferon-γ and induce EAE, demonstrating that CD40L is required to induce costimulatory activity on antigen-presenting cells for *in vivo* activation of CD4+ T cells.

Evidence. B7–2 (CD86) is constitutively expressed in cultured human microglia cells (but not astrocytes), and B-7–1 (CD80) can be induced in microglia (but not astrocytes) with interferon-γ (Satoh *et al*., 1995; Shrikant and Benveniste, 1996). In acute multiple sclerosis plaques, there is increased expression of B7–1 (CD80), predominantly in perivenular inflammatory lymphocytes, and of B7–2 (CD86), predominantly in microglia and macrophages, pointing to an important role of the B7–CD28 system in the local activation of T cells in CNS lesions (Williams *et al*., 1994; De Simone *et al*., 1995; Windhagen *et al*., 1995*a*).

Possibilities for therapeutic manipulation of the CD28–B7 pathway have received much attention. Targeting this pathway *in vivo* may represent a novel method of immunosuppression in which only the antigen-specific T cells are affected (made more tolerant), whilst the majority of T cells are not influenced. One of the most attractive features of this approach is that, at least theoretically, permanent antigen-specific tolerance could be achieved by a short course of treatment (Lenschow *et al*., 1996).

Most experimental therapies have relied either on anti-B7 monoclonal antibody or on a CTLA4-Ig fusion protein. Therapy with anti-B7–1 (but not anti-B7–2) monoclonal antibody blocked clinical relapses, ameliorated CNS pathology, and blocked epitope spreading in relapsing murine EAE induced with PLP peptide (Miller *et al*., 1995*b*). Treatment with anti-B7–1 predominantly induced TH2 T-cell clones, which prevented induction of EAE and abrogated established disease when transferred into PLP-immunized mice (Kuchroo *et al*., 1995). Administration of anti-IL-4 monoclonal antibody abolished the protective effect of anti-B7–1 antibody. Taken together, these results indicate that treatment with anti-B7 antibodies differentially influences the development of TH1 and TH2 cells, thereby altering the course of autoimmune disease.

Administering cytotoxic T-lymphocyte adhesion protein (CTLA)-4Ig to donor mice, or during *in vitro* activation of MBP-specific T cells, reduced the clinical severity of adoptively transferred chronic relapsing EAE (Perrin *et al*., 1995). In contrast, after the transfer of MBP-activated T cells, treatment of recipient animals with CTLA-4Ig had no effect. In actively induced EAE, systemic administration of CTLA-4Ig suppressed clinical disease (Cross *et al*., 1995; Khoury *et al*., 1995; Arima *et al*., 1996; Perrin *et al*., 1996). Immunohistological studies showed that CTLA-4Ig therapy reduced expression of TH1- (but not TH2)-type cytokines in the CNS, indicating that blocking the CD28–B7 pathway causes immune deviation from TH1 to TH2 (Khoury *et al*., 1995). In a rat model of cardiac allotransplantation, combined treatment with CTLA-4Ig and anti-CD4 antibody induced permanent tolerance, suggesting that an appropriate combination therapy targeted against different molecules of the immunological synapse can be very effective (Yin and Fathman, 1995). Similarly, combined treatment with CTLA-4Ig and anti-gp39 monoclonal antibody resulted in long-term acceptance of skin and cardiac allografts in mice (Larsen *et al*., 1996). Systemic injection of antigen-presenting cells treated *ex vivo* with an encephalitogenic MBP peptide and CTLA4-Ig (but not CTLA4-Ig alone) protected rats from

clinical EAE, indicating that *ex vivo* blockade of CD28-B7–1 leads to the generation of (presumably TH2) regulatory cells (Khoury *et al*., 1996).

Administering anti-B7 antibodies or CTLA-4 fusion protein are not the only ways to interfere with costimulatory signals in T cells. For example, application of antibody against EPR-1 (effector cell protease receptor-1), a cell-membrane receptor involved in signal transduction, inhibits protease-dependent mechanisms of lymphocyte costimulation (Duchosal *et al*., 1996). The role of EPR-1 in immunoregulation identifies this receptor molecule as a potential target for therapeutic immunosuppression.

Problems. The relative roles played by the different costimulatory molecules during an immune response (and hence the effects of treatment) are likely to depend on a variety of factors, including the nature of the antigen-presenting cell expressing the B7 molecules, the affinity of the B7 molecules for CD28 or CTLA-4, the cytokine milieu, and the levels of B7–1 and B7–2 expression. Furthermore, certain antibodies may have a stimulatory rather than 'blocking' effect on their target receptor. For example, both anti-B7–1 monoclonal antibody (Lenschow *et al*., 1996) and anti-B7–2 monoclonal antibody (Kuchroo *et al*., 1995) may exacerbate rather than suppress EAE under certain conditions. A further problem is that combined treatment with different antibodies may result in paradoxical exacerbation rather than improvement. For example, co-injection of anti-B7-1 and anti-B7–2 monoclonal antibody induced marked exacerbation of actively induced EAE in mice, although in this system, injection of either antibody alone had a beneficial (anti-B7–1) or neutral (anti-B7–2) effect (Perrin *et al*., 1996).

Another important factor is the timing of treatment. For example, B7–2 appears to play a critical role prior to antigen exposure (e.g. transplantation). In contrast, B7–1 plays an important role in the control of the immune response after antigen exposure (e.g. chronic autoimmune disease) (Lenschow *et al*., 1996). In EAE, a single injection of CTLA-4Ig suppressed disease, while multiple injections resulted in enhanced disease (Racke *et al*., 1995). This is consistent with an essential timing requirement for the coordinated interaction of B7 and CD28 family receptors.

Thus, the CD28/CTLA-4 –B7-1/B7-2 system of costimulatory molecules once again illustrates an important general principle, namely that the manipulation of any one component of a complex functional network may produce unpredicted overall effects, especially *in vivo*. CTLA-4 seems to be a major negative regulator of (auto)immune T-cell function. This explains, for example, why anti-CTLA-4 antibodies or F(ab) antibody fragments accelerate and exacerbate relapsing–remitting EAE mediated by T cells specific for peptide 139–151 of PLP in SJL/J mice (Karandikar *et al*., 1996; Hurwitz *et al*., 1997). The immunosuppressive effects of soluble CTLA-4Ig are presumably explained by inhibition of (stimulatory) interactions between B7 and CD28.

Therapies targeted against CD40 or CD40-L may theoretically inhibit both T and B-cell responses. Special caution is warranted since CD40 is expressed not only on antigen-presenting cells but also on other cells including endothelial cells. Therefore, anti-CD40 antibodies would be expected to bind to endothelial cells in many tissues, with unpredictable adverse consequences. For example, cytokine-stimulated human monocytes and endothelial cells were sensitized to the cytotoxic effects of an anti-CD40 immunotoxin (Francisco *et al*., 1996). Thus, anti-CD40-L antibodies or soluble CD40 ligand may be more promising candidates for therapy. These agents could be combined with CTLA-4Ig for more complete immunosuppression.

Leucocyte-differentiation molecules

Principles. The term 'differentiation' molecule is purely operational, indicating that the pattern of expression of these molecules can help to distinguish between the different maturational stages of a cell. Clearly, the various differentiation molecules have functions other than serving as labels for developmental stage. Most therapies targeted against these differentiation 'markers' aim at the depletion or functional inactivation of those cells that carry the respective marker.

Evidence. Monoclonal antibodies against T-cell differentiation molecules have been used for immunosuppressive treatment of transplantation rejection and various autoimmune diseases, including multiple sclerosis. Early phase I studies in multiple sclerosis showed that anti-T-cell monoclonal antibody infusions suppressed *in vitro* measures of the human immune response (Hafler *et al*., 1988). With repeated infusions, human anti-mouse antibodies were found in the circulation. Anti-CD3 infusions were associated with significant toxicity (Weinshenker *et al*., 1991). An open trial of a mouse-human chimeric anti-CD4 monoclonal antibody, cM-T412, induced a long-lasting reduction of circulating CD4+ T cells without major side effects (Lindsey *et al*., 1994). A controlled clinical trial of the same monoclonal antibody was essentially negative (van Oosten *et al*., 1996*a*). Patients treated with a single course of CAMPATH-1H, a 'humanized' monoclonal antibody against CD52, developed lymphopaenia that was sustained for over 1 year (CD52 is expressed on all lymphocytes and some monocytes) (Moreau *et al*., 1996). In this study, antibody infusion was often accompanied by significant clinical exacerbation, which correlated with increased levels of proinflammatory cytokines.

CD4 and CD52 are examples of widely expressed leucocyte differentiation antigens. Clearly, the more restricted the expression of a target molecule is, the greater the chances are that immunotherapy has a selective effect. For example, OX-40 is a surface molecule exclusively expressed on activated CD4+ T cells, usually $<1\%$ of peripheral CD4+ T cells. This protein is selectively upregulated, e.g. on MBP-specific T cells, at the site of inflammation during onset of

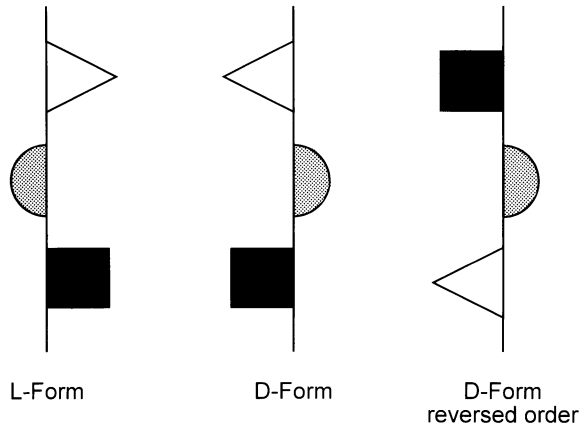

Fig. 8 Two-dimensional model of a peptide engineered by reverse synthesis and change in chirality. The aim of this approach (Jameson *et al*., 1994) is to generate a protease-resistant copy of the peptide consisting of three L-amino acids (*left*). The peptide consisting of the D forms of the same amino acids, arranged in the same sequence, represents a mirror image of original peptide (*middle*). However, the peptide consisting of the same D amino acids in reversed order (*right*) represents an exact copy of the original peptide. This can be demonstrated by rotating the peptide in the paper plane (*see* text for details).

EAE. An immunotoxin, constructed by coupling anti-OX-40 monoclonal antibody to ricin A, ameliorated adoptive transfer EAE in rats when treatment was administered on the first day of disease onset (Weinberg *et al*., 1996).

Monoclonal antibodies are not the only therapeutic agents for targeting leucocyte differentiation antigens. For example, EAE was inhibited with a small synthetic peptide analogue of CD4, which was designed by molecular modelling of the CD4 protein surface (Jameson *et al*., 1994; Marini *et al*., 1996). In contrast to naturally occurring proteins, the synthetic peptide consisted of D-amino acids. It was modelled by reverting the sequence of a critical 'hairpin loop' region of the CD4 molecule. The combined effect of reverse synthesis and change in chirality is that the synthetic peptide represents an identical copy of its naturally occurring counterpart (Fig. 8). Since virtually all proteases cleave peptide bonds between L-amino acids, the synthetic peptide is resistant to proteolysis. Further potential advantages of this approach are that the synthetic peptide showed little immunogenicity and that CD4+ T cells were inhibited but not depleted by the peptide treatment. Interestingly, the CD4 mimetic peptide apparently did not inhibit the CD4–MHC class II interaction, but acted at the level of the T cell, perhaps by uncoupling CD4 from the signal transduction machinery (Jameson *et al*., 1994).

Problems. Monoclonal antibodies against widely distributed leucocyte surface determinants are unlikely to find a major place in the immunotherapy of multiple sclerosis, owing to their side effects, immunogenicity and lack of selectivity. It is, however, conceivable that certain combinations of antibodies might induce a lasting state of self-tolerance in T cells, provided they are applied at an optimal time point. Whether this concept is valid and which antibodies should be combined needs to be investigated. Innovative strategies such as the reverse synthesis of D-amino acid peptides (Jameson *et al*., 1994) are likely to be strong competitors for antibody-based therapies.

Immunotherapies targeting the 'trimolecular complex'

It is the trimolecular complex (TMC) of antigen-specific T-cell receptor, antigenic peptide and MHC molecule that confers specificity to the interaction between the T cell and antigen-presenting cell at the immunological synapse (Fig. 4). Therefore, the TMC has long been considered as the ultimate target for highly selective, antigen-specific immunotherapy (Hohlfeld, 1989; Wraith *et al*., 1989). In principle, each component of the TMC can be targeted. The MHC molecule could be blocked by anti-MHC antibodies or 'blocking peptides', the antigen (or antigenic peptide) could be applied in such a way that the autoreactive T cells are inhibited rather than stimulated, and the T-cell receptor could be targeted with anti-T-cell receptor antibodies or by T-cell or T-cell receptor peptide vaccination.

Major histocompatibility complex inhibitors

Principles. Many autoimmune diseases are associated with certain MHC alleles. Therefore, attempts have been made to interfere with the onset of such diseases by inhibiting the T-cell populations that recognize antigens in the context of these particular MHC molecules. Both anti-MHC antibodies and MHC-binding 'competitor' peptides have been used for this purpose. It should be noted, however, that the results of some of the early experiments may have been misinterpreted, and that the concept of 'blocking peptides' is probably an oversimplification (Vaysburd *et al*., 1995).

Evidence. Experimental autoimmune diseases including EAE have been treated or prevented with antibodies directed against the MHC molecule presenting the autoantigen (Steinman *et al*., 1981) or at the complexes formed between class II molecules and autoantigenic peptides (Aharoni *et al*., 1991). Furthermore, MHC-binding peptides, which may or may not be related to the autoantigenic peptide (Lamont *et al*., 1990; Wauben *et al*., 1994; Gautam, 1995), have been used for blocking the binding site of those class II molecules that are associated with autoimmune diseases, thus reducing their capacity to bind autoantigen (for review, *see* Adorini *et al*., 1993). Vaccination with peptides from MHC class II beta chain hypervariable region causes allele-specific suppression of EAE, possibly due to the generation of auto-anti-MHC class II antibodies that interfere with T-cell sensitization (Bright *et al*., 1996).

Problems. Since the MHC molecule is the least variable component of the TMC, MHC-blocking strategies based on anti-MHC antibodies or 'promiscuous' MHC-binding peptides are comparatively nonspecific, even though they are targeting the TMC. In addition, anti-MHC antibody therapies are facing the same problems as other antibody-based therapies. Regarding MHC-blocking peptides, many practical issues remain unresolved. For example, appropriate delivery systems are required to achieve the sustained plasma levels of soluble MHC antagonists necessary for effective inhibition of T-cell activation. Further problems, which are shared with other peptide therapies, are the poor oral bioavailability and very short plasma half-life after parenteral administration.

Altered peptide ligands

Principles. For many years, antigen-induced stimulation of T cells was considered strictly as an 'all-or-nothing' phenomenon; if the T-cell receptor bound tightly to the MHC–peptide complex, the T cell was fully activated and would differentiate, produce cytokines, help B cells or kill target cells, and proliferate; in other words, antigen stimulation would always generate a full immune response. This simple view has dramatically changed in recent years, and a whole new field of T-cell biology has opened. This is centred around the concept of 'partial agonism' and 'antagonism' of peptide ligands (for review, *see* Sloan-Lancaster and Allen, 1996). 'Altered peptide ligands' (APL), which differ by as few as one or two amino acids from the peptide generating the full immune response, can bind to the T-cell receptor without triggering the full programme of T-cell activation. For example, an APL may partially activate a helper T cell to produce IL-4 and help B cells, but fail to induce proliferation. Although APLs only need to be different from the antigenic (unaltered) ligand in a subtle manner, how these subtle differences are translated into the observed differences in biological response is not clear. Current theories can be grouped into the kinetic and conformational models. In the kinetic model, the T-cell receptor has a reduced affinity for the APL as compared with the antigenic ligand. This results in a reduction in the time that the T-cell receptor remains engaged with its ligand. A short interaction with ligand then results in the transduction of a different signal through the T-cell receptor than that induced by the longer interaction with the unaltered ligand. In a conformational model, the transduction of signals through the T-cell receptor involves some kind of conformational change in the T-cell receptor upon recognition of ligand. This change is postulated to depend on the nature of the ligand (APL versus unaltered ligand). At present, the majority of experimental data tend to favour the kinetic model.

Although the exact mechanism of the 'partially agonistic' properties of APL is not completely understood, the field of 'APL engineering' has quickly gained a lot of attention, and APL are widely considered attractive candidates for antigen-selective immunotherapy. (A corollary to the novel concepts of T-cell stimulation is that a single T-cell receptor is not strictly monospecific for just one peptide/MHC combination; it may cross-react with a large number of different ligands; *see* Part 1.)

Peptide antigens need to make make contact with two much larger proteins, the MHC molecule (expressed on the antigen-presenting cell) and the T-cell receptor (expressed on the T cell; Figs 3 and 9). It is therefore possible to distinguish between residues of the antigenic peptide critical for MHC binding, T-cell receptor binding or both. Regarding MHC binding, three-dimensional atomic structures of several MHC class I and II molecules revealed that binding of the peptide 'main chain' is supplemented by the binding of peptide 'anchor side chains' into 'pockets' of the MHC molecule (Fig. 9) (Madden, 1995). Because the stereo-chemistry of these pockets varies between different MHC alleles, different MHC molecules tend to present a different spectrum of peptides.

Regarding T-cell receptor binding, peptides possess primary and secondary T-cell receptor contact sites (Sloan-Lancaster and Allen, 1996). APL are defined as analogues of immunogenic peptides in which these T-cell receptor contact sites have been manipulated, usually by substitution with another amino acid. While these peptides do not stimulate T-cell proliferation, they nevertheless have the capacity to activate some T-cell receptor-mediated functions. Antagonist peptides are defined as analogues that specifically down-modulate the agonist-induced response. These operationally defined functions are not mutually exclusive and often depend on the experimental system.

The properties of individual APL depend on their structural homology with the agonist ligand; peptides that contain very conservative substitutions at defined secondary T-cell receptor interaction sites act both as partial agonists and efficient antagonists. More deviant peptides retain the antagonist function, but loose their partial agonist property. Extensively substituted peptides loose both functions and become neutral. Of note, amino acid substitutions at the primary T-cell receptor contact site completely abolish all detectable activity (Sloan-Lancaster and Allen, 1996).

Taken together, these observations suggest a 'threshold-of-activation' model for T-cell signalling. According to this model, successful stimulation of individual T-cell functions depends on a certain critical level of activation, with each function requiring a particular threshold (Sloan-Lancaster and Allen, 1996). How the binding of an APL is transmitted via the T-cell receptor across the T-cell membrane to modify intracellular events is not completely clear. It appears, however, that peptide affinity is a critical factor. Agonist ligands have higher T-cell receptor affinity than antagonist ligands, and antagonist ligands have higher affinity than neutral ('null') ligands. Apparently, the rate of dissociation of ligand from T-cell receptor is a major determinant of the agonist and antagonist properties of a ligand (Rabinowitz *et al*., 1996). It is thought that T-cell activation involves a

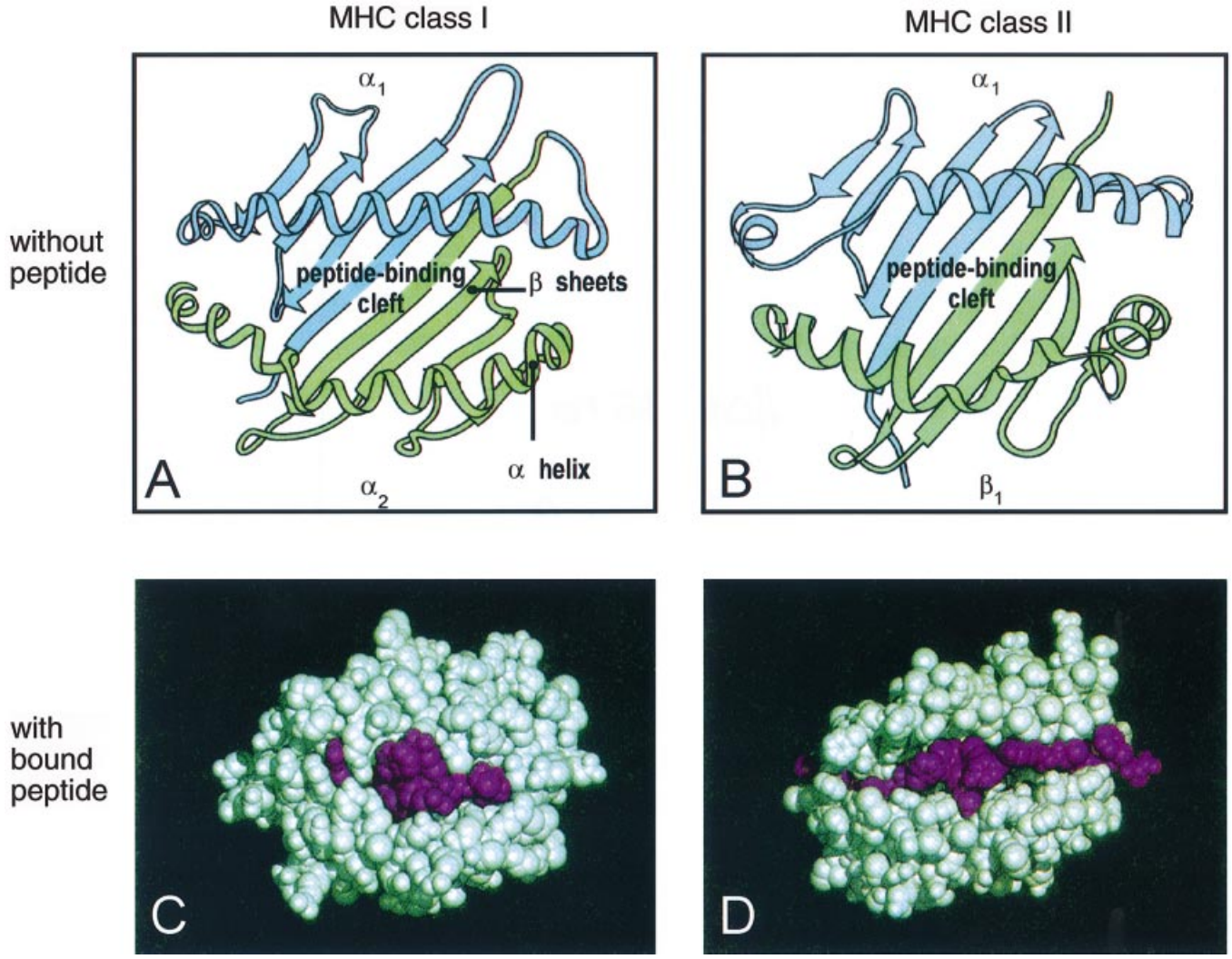

Fig. 9 Model of the antigen-binding cleft of MHC class I and II molecules as it would be 'seen' by the T-cell receptor. In MHC class I molecules (**A**, **C**) the peptide is bound in an elongated conformation with both ends tightly bound at either end of the cleft. In MHC class II molecules (**B** and **D**) the peptide is also bound in an elongated conformation but the ends of the peptide are not tightly bound and the peptide extends beyond the cleft. The 'ribbon diagrams' **A** and **B** illustrate that the sides of the cleft are formed by two α helices, whilst the floor consists of the β-pleated sheet formed by the pairing of the α_1 and α_2 domains of the MHC class I α chain (**A**), or the pairing of the α_1 and β_1 domains of the MHC class II α and β chains (**B**). **C** and **D** show computer graphics representations of the antigenic surface formed by the top part of the MHC molecule (light green) and bound peptide (purple). Subtle modifications of the 'upper' (T-cell receptor-facing) part of the peptide can convert an antigenic (encephalogenic) peptide into an 'altered peptide ligand'. *See* section on altered peptide ligands for details.

number of distinct signalling 'modules' that can be selectively triggered after ligand binding. Differential activation of these modules seems to depend on the way the CD3 proteins, which are always associated with the T-cell receptor, are initially phosphorylated during the cascade of signalling events (Sloan-Lancaster and Allen, 1996).

In principle, stimulation of T cells by APL could have several different consequences which could be useful for immunotherapy. Most desirable for therapeutic purposes would be a long-lasting change of the properties of autoreactive T cells. Such effects have indeed been observed in some experimental systems. For example, certain APL can modulate the cytokine pattern of T cells (Windhagen *et al*., 1995*b*). Furthermore, APL could induce a form of 'anergy' in T cells [anergic T cells are subsequently unable to respond to stimulatory ligands (Sloan-Lancaster and Allen, 1996)]. A less attractive, presumably only transient effect of APL would be simple competition with the unaltered ligand for T-cell receptor binding ('passive antagonism').

Evidence. APL are highly effective inhibitors of disease in different EAE models (Smilek *et al*., 1991; Franco *et al*., 1994). One possible mechanism is downregulation of the production of TH1 cytokines such as interferon-γ and TNF-α in encephalitogenic T cells (Karin *et al*., 1994). This would imply that therapy with a single APL peptide modulates not only T cells expressing the appropriate T-cell receptor but also 'bystander' T cells expressing different T-cell receptors

(Kuchroo *et al*., 1994). It seems indeed that treatment with APL may selectively silence the pathogenic T cells and, in addition, affect the secondarily recruited bystander T cells via production of TH2 cytokines (e.g. IL-4) and reduction of TNF-α in the lesion (Brocke *et al*., 1996). Genetically engineered variants of APL might be used for more efficient presentation, for example by targeting fusion proteins of APL and antibody to surface receptor expressed on antigen-presenting cells, e.g. the type I Fc receptor for IgG (Liu *et al*., 1996*a*; Legge *et al*., 1997).

Problems. Therapy with APL peptides is hampered by the same general problems as other peptide-mediated therapies, especially problems relating to peptide delivery and bio-availability. Furthermore, if APL therapy affected only T cells capable of reacting with the APL, then this strategy would be likely to fail in human disease. The reason for this pessimistic view is that the human autoimmune T-cell response is much more complex than the strikingly limited T-cell response observed in some EAE models (*see* section on T-cell vaccination). It must be anticipated that APL therapy, like other selective immunotherapies, will only work if the therapy can be 'individualized' by developing specific APL 'cocktails' for individual patients or groups of patients with similar immunological features.

On the other hand, if the encouraging data suggesting a more widespread effect of APL treatment on bystander T cells ('bystander suppression' is the term for this favourable phenomenon) can be corroborated, then this treatment could hold substantial promise (for review, *see* Steinman, 1996). But even then, there must be concern that the autoimmune reaction finds a way to evade the effects of APL therapy, for example, by epitope spreading. It is therefore important to perform more detailed studies on the long-term effects of APL treatment in different experimental systems, especially chronic EAE models. Whether the effects of APL observed in experimental systems can be achieved *in vivo* in the human immune system and if so, whether they can be employed for the treatment of multiple sclerosis patients, is presently open.

Oral tolerance

Principles. The term 'oral tolerance' refers to the observation that proteins passing through the gastrointestinal tract generate antigen-specific hyporesponsiveness (for review, *see* Weiner *et al*., 1994). Because other mucosal surfaces, e.g. in the respiratory tract, have similar properties, the term 'mucosal tolerance' is often used interchangeably.

It is thought that oral tolerance evolved to allow the gut-associated immune system to be exposed to external proteins without becoming sensitized. If animals are fed with proteins such as ovalbumin or MBP and are then immunized, the immune response against the fed antigen (but not against control antigen) is subsequently reduced. There is evidence that depending on the amount of antigen fed, orally administered antigens either induce activity in regulatory cells that 'suppress' the antigen-specific response (low doses), or inhibit antigen-specific T cells by induction of clonal anergy (high doses). (The doses being used in clinical trials are designed to induce regulatory T cells rather than anergy).

Antigens that pass through the gut preferentially induce TH2-type T cells that secrete IL-4, IL-10 and TGF-β. Such cells leave the gut and migrate to the organs that contain the fed antigen. The TH2 cells then are locally stimulated to release their anti-inflammatory cytokines. In this way, oral tolerance could be viewed as a natural drug delivery system in which anti-inflammatory cytokines are delivered to selected sites (Weiner *et al*., 1994). Because the regulatory cells activated during oral development of tolerance are triggered in an antigen-specific fashion, but suppress in an antigen-nonspecific fashion, they mediate 'bystander suppression' when they encounter the fed autoantigen at the target organ. Therefore, proponents of oral tolerance often stress that this therapy has the advantage that extracts of target organ (i.e. brain in the case of multiple sclerosis) can be administered without the need to know what the autoantigen is. In terms of cost, safety and simplicity, oral administration is clearly an optimal route for drug delivery.

Evidence. Orally administered autoantigens suppress several experimental autoimmune models in a disease- and antigen-specific fashion; the diseases include experimental autoimmune encephalomyelitis, uveitis and myasthenia gravis, collagen- and adjuvant-induced arthritis, and diabetes in the NOD (non-obese diabetic) mouse (Weiner *et al*., 1994). In some EAE models, intranasal application of encephalitogenic peptide seems to be more effective than oral administration (Metzler and Wraith, 1993). Whether this and other variant protocols involving development of mucosal tolerance (e.g. feeding MBP-toxin conjugates; *see* Sun *et al*., 1996) have any advantages has not been established.

Evidence for 'bystander suppression' comes from experiments suggesting that MBP- or PLP-induced EAE in the mouse can be suppressed by feeding MBP (Al-Sabbagh *et al*., 1994), and PLP-induced disease can be suppressed by MBP-specific regulatory T-cell clones (Chen *et al*., 1994). Oral tolerance is being tested clinically in a number of human diseases, including multiple sclerosis, rheumatoid arthritis, uveitis and juvenile diabetes. A pilot trial in relapsing–remitting multiple sclerosis was too small to draw conclusions and lacked MRI evidence (Weiner *et al*., 1993).

Problems. Whether oral or mucosal tolerance will have a place in the treatment of multiple sclerosis depends largely on the results of ongoing clinical trials. For how long mucosal antigens need to be applied for treatment of chronic autoimmune diseases is not known. Safety concerns regarding oral preparations of myelin or other CNS antigens are clearly important but do not *per se* invalidate the concept. It should be noted that oral tolerance may not work for all autoantigens, as indicated by experiments in a highly artificial model

system (Blanas *et al*., 1996). In these experiments, transgenic mice expressing ovalbumin under the control of the rat insulin promoter were lethally irradiated and then reconstituted with bone marrow from another transgenic mouse line that contained ovalbumin-specific CD8+ T cells. The transgenic mice became diabetic after feeding with ovalbumin. The authors conclude that 'caution should be applied when attempting oral administration of autoantigen to treat human diseases that may be affected by cytotoxic T cells (Blanas *et al*., 1996).

Other strategies of tolerance induction by modification of antigen presentation

Principles. It has long been known that the immune response to a protein antigen depends on the dose, form and route of administration. For example, antigens injected subcutaneously usually elicit a strong response, while antigens injected directly into blood tend to induce nonresponsiveness or tolerance. The exact mechanisms by which the route of antigen administration controls the nature and intensity of the immune response are not known, but it can be assumed that the types of antigen-presenting cells and special characteristics of the local environment play an important role (for review, *see* Wraith, 1995).

Stimulation with high doses of antigen may induce programmed cell death (apoptosis). Furthermore, it has been shown that antigen recognition, in the absence of the costimulatory signals that are normally provided by specialized antigen-presenting cells, induces a state of 'anergy' in T cells (T and B cells are said to be anergic when they cannot respond to their specific antigen despite optimal conditions for stimulation); for review, *see* Schwartz (1996). These observations have inspired experimental strategies in which antigens are presented to T cells in 'unusual ways', for example in the form of soluble complexes of MHC molecules with bound peptide antigen.

Evidence. Tolerance to MBP was induced by intraperitoneal injection of synthetic peptides of immunodominant determinants of MBP (Gaur *et al*., 1992). If they were administered before immunization with MBP, EAE was prevented, and if they were administered after immunization, EAE was ameliorated. Peptide-induced tolerance apparently resulted from the induction of anergy in antigen-specific T cells. Therapy with modified MBP peptide analogues that form long-lived peptide–MHC complexes *in vivo* was more effective than therapy with the unmodified MBP peptide (Samson and Smilek, 1995). Administration of an acetylated synthetic peptide of MBP (called PAL 68–86) suppressed EAE in Lewis rats (St Louis *et al*., 1997).

Therapy with high doses of MBP abrogated EAE in mice (Critchfield *et al*., 1994; Racke *et al*., 1996). The therapeutic effect was brought about by deletion of encephalitogenic CD4+ MBP-specific T cells through apoptosis. In principle, protein engineering techniques could be used to 'tailor-design' tolerogenic molecules (Zambidis and Scott, 1996). For example, i.v. treatment of SJL/J mice with a chimeric fusion protein of MBP and PLP (MP4) prevented EAE induced either by active immunization or by adoptive transfer of activated T cells (Elliott *et al*., 1996). Mice treated with MP4 were resistant to disease when rechallenged with an encephalitogenic PLP peptide.

In Lewis rats, EAE could be prevented by injection of spleen cells coupled to myelin antigens (Vandenbark *et al*., 1995). It is thought that this treatment induces selective tolerance in encephalitogenic T cells through a partially reversible form of anergy.

Soluble complexes of antigenic peptide bound to MHC class II molecules induced antigen-specific apoptosis of MBP-specific, virus-transformed CD4+ T cells *in vitro* (Arimilli *et al*., 1996). Induction of apoptosis by soluble MHC class II-peptide complexes is considered a possible therapeutic strategy to delete autoreactive T cells *in vivo*.

Problems. The strategies based on modified antigen presentation can only be applied if the autoantigen is known. In the case of soluble MHC–peptide complexes, it is also necessary to know the immunodominant encephalitogenic epitope (if there is any). As discussed in the immunopathogenesis section, these conditions are not presently met in multiple sclerosis.

Copolymer-1

Principles. Copolymer-1 is a synthetic basic random copolymer of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in a molar residue ratio of 6.1 : 1.9 : 4.7 : 1.0. It was originally studied along with other basic copolymers in an attempt to simulate the activity of MBP in inducing EAE, but was then found to suppress EAE in various species including guinea pig, rabbit, mouse, rhesus monkey and baboon. A detailed personal account of the discovery and development of copolymer-1 may be found in Arnon (1996). Note that different encephalitogenic determinants of MBP are involved in the different species, suggesting a broad effect of copolymer-1 on MBP responses. Furthermore, it appears that the suppressive effect of copolymer-1 is not restricted to MBP, but extends to EAE induced in mice with encephalitogenic PLP (Teitelbaum *et al*., 1996) or MOG (Ben-Nun *et al*., 1996) peptides. On the other hand, copolymer-1 had no effect on experimental myasthenia gravis and experimental thyroiditis, indicating some specificity for myelin-induced autoimmunity (Arnon, 1996).

It is difficult to envisage a plausible mechanism that could explain all these observations. It has been proposed that copolymer-1 competes with MBP, PLP and MOG for binding to MHC class II molecules expressed on antigen-presenting cells (Arnon, 1996). This would place copolymer-1 into the

category of MHC inhibitors/competitors. It seems that the binding to MHC class II molecules, which is 'promiscuous' (i.e. independent of the MHC II allotype), does not require internalization or processing of copolymer-1 by antigen-presenting cells. Since D-copolymer-1, a stereoisomeric form of copolymer-1, binds as efficiently to MHC class II molecules as copolymer-1 but does not inhibit EAE, one could speculate that MHC binding is a necessary but insufficient step that must be followed by a more specific step such as induction of regulatory ('suppressor') cells or T-cell receptor antagonism (Arnon, 1996). Still, the alleged selectivity of copolymer-1 for myelin autoantigens (MBP, PLP, MOG) and concomitant lack of suppressive activity in reactions against other autoantigens presently remains unexplained.

Evidence. Possible therapeutic effects of copolymer-1 were investigated in a number of clinical trials in relapsing–remitting (Bornstein *et al*., 1987; Johnson *et al*., 1995) and chronic progressive (Bornstein *et al*., 1991) multiple sclerosis. A phase III multicentre, double-blind, placebo-controlled trial in patients with relapsing–remitting multiple sclerosis showed a 29% reduction in relapse rate (Johnson *et al*., 1995), so that copolymer-1 has been suggested as a reasonable alternative to interferon-β for treatment of early relapsing–remitting multiple sclerosis (Wolinsky, 1995). Further studies are planned to obtain more detailed information on the effects of copolymer-1 on clinical progression and on MRI activity in different clinical types of multiple sclerosis. Whether combination therapy with copolymer-1 and interferon-β will show additive clinical effects, also awaits further study.

Problems. As long as the exact mechanisms of action are unknown, it is difficult to judge how 'specific' copolymer-1 therapy really is. Copolymer-1 was generally well tolerated in the published trials. The most common adverse experience was an injection-site reaction. A transient systemic reaction occurred at least once in 15% of patients treated with copolymer-1 (Johnson *et al*., 1995). This sporadic and unpredictable reaction occurred within minutes after injection, and was characterized by flushing or chest tightness with palpitations, anxiety, or dyspnoea and usually lasted between 30 s and 30 min.

Copolymer-1-reactive antibodies were found in the serum of treated patients, with maximum levels attained after a few months of treatment. The antibodies, which do not cross-react with MBP, apparently do not affect the biological activity of copolymer-1.

Vaccination with T cells or T-cell receptor peptides

Principles. The term T-cell vaccination was coined by I. R. Cohen in 1981 (Ben-Nun *et al*., 1981). The underlying concept considered organ-specific, autoaggressive clones of T lymphocytes as the pathogenic agents of autoimmune disease, analogous to pathogenic microbial agents. Cohen suggested that, as in microbiology, autoaggressive T cells can be attenuated to eliminate their pathogenic potential, while conserving their capacity to stimulate counter-regulatory cells. Obviously, this concept rests on two pillars. First, the healthy immune repertoire must contain potentially self-reactive T-cell clones, and these clones must be normally suppressed by counter-regulatory mechanisms. Secondly, in autoimmune disease, the equilibrium between autoaggressive T cells and the suppressive mechanisms is lost, but can be re-established by appropriate stimulation of the counter-regulatory mechanisms.

The original concept of T-cell vaccination relies on the injection of autoantigen-specific T-cell clones, which must be isolated from the prospective recipient, cultured and expanded, attenuated or inactivated *in vitro*, and then re-injected as a vaccine. More recently, peptides of the antigen-specific T-cell receptor of the autoreactive T cells rather than whole T cells have been used for 'T-cell receptor peptide vaccination' (for review, *see* Vandenbark *et al*., 1996*b*). The rationale for this therapy is the following. Each T-cell receptor contains an immunogobulin constant-like domain, which is shared between different T cells, and an Ig variable-like domain, parts of which are either unique to one T-cell clone or shared among only a few T-cell clones (Fig. 2B). As with Igs, the most variable region of the T-cell receptor is the complementarity determining region (CDR)3. Other relatively variable parts of the T-cell receptor are the CDR2 and CDR1 regions. Instead of using whole T cells for vaccination, T-cell receptor peptide vaccination uses short synthetic peptides of the T-cell receptor CDR regions of autoaggressive T cells. It is thought that vaccination with such CDR region T-cell receptor peptides stimulates counter-regulatory T cells, which recognize the immunizing T-cell receptor peptides with their T-cell receptors. It is further assumed that the autoreactive T cells express not only intact T-cell receptor on their surface but also MHC-bound peptides derived from their own T-cell receptor during normal protein turnover. (In principle, these endogenous T-cell receptor peptides could be associated with either MHC class I or MHC class II molecules). Thus, it would be expected that regulatory CD4+ or CD8+ T cells stimulated with the immunizing T-cell receptor peptides cross-react with the endogenous T-cell receptor peptides expressed on the autoaggressive T cells, allowing for selective inhibition or killing of the autoaggressive T cells.

Evidence. Successful T-cell vaccination against autoimmune disease was achieved in several variations. Originally, an encephalitogenic T-cell line was shown to lose its pathogenic potential after irradiation. Transfer of these attenuated T cells rendered the recipients resistant to subsequent active autosensitization with MBP (Ben-Nun and Cohen, 1981; Ben-Nun *et al*., 1981). Other procedures for attenuation include exposure to hyperbaric pressure and

fixation by cross-linking agents such as glutaraldehyde. More recent vaccination protocols relied on the transfer of sublethal doses of untreated or attenuated activated T-line cells. Such transfers lead to the expansion of recipient T cells specifically reacting against the originally transferred T line. Most, but not all, of the induced T cells express the CD8 membrane phenotype and recognize their target epitope in the context of MHC class I products (Sun *et al*., 1988*a*, *b*). These CD8+ regulatory T cells are specifically cytotoxic against the relevant autoaggressive CD4+ T-cell line, but fail to respond to other syngeneic T lines with different or even similar specificities.

In human pilot trials of T-cell vaccination, multiple sclerosis patients were vaccinated with MBP-reactive T cells cloned from their blood (Medaer *et al*., 1995; Zhang *et al*., 1995). Selected MBP-reactive T-cell clones were activated *in vitro* and irradiated to render them incapable of proliferation. Patients received three subcutaneous injections of two to four vaccine clones (15×10^6 cells for each clone). The treatment was well tolerated, but the number of patients was too small to allow conclusions about clinical efficacy (Medaer *et al*., 1995). Frequency estimations of MBP-specific T cells indicated that in the majority of recipients, MBP-reactive T cells remained undetectable in blood over a period of 1–3 years, but reappeared in some patients during clinical exacerbations (Zhang *et al*., 1995). The reappearing MBP-reactive T cells were different from the MBP-specific T cells present before vaccination, suggesting a shift of the T-cell repertoire to other determinants of MBP. The immunization induced predominantly CD8+ regulatory T cells capable of lysing the immunizing clones in a clonotype-specific manner. The T-cell responses induced by immunization were restricted to the immunizing clones and did not affect the MBP-reactive clones not used for immunization (Zhang *et al*., 1995).

In several rodent models of autoimmunity, the pathogenic, autoantigen-specific T lymphocytes use a strikingly limited number of available variable-region elements for their antigen receptor. Classic examples are the PL/J mouse (Acha-Orbea *et al*., 1988; Urban *et al*., 1988) and the Lewis rat (Burns *et al*., 1989; Chluba *et al*., 1989). In these animal strains, essentially all encephalitogenic T-cell clones use the T-cell receptor Vβ8.2 element. These observations led to two partly interdependent therapeutic strategies. First, it was shown that monoclonal antibodies against T-cell receptor V-region determinants can profoundly interfere with inducibility and even progress of organ-specific autoimmune disease (Zamvil *et al*., 1988). This strategy is not entirely specific, as it presumably affects any T-cell response using the V element recognized by the monoclonal antibody. Secondly, immunization of rats against synthetic peptides representing either the CDR3 region (Howell *et al*., 1989) or CDR2 region (Vandenbark *et al*., 1989) of the T-cell receptor of autoaggressive MBP-specific T cells was reported to prevent actively induced EAE, and to shorten ongoing disease (for review, *see* Vandenbark *et al*., 1996*b*). It appears that immunization with T-cell receptor peptides stimulates regulatory anti-Vβ8.2-specific T cells that inhibit (but do not delete) the encephalitogenic target T cells. Inhibition seems to be mediated, at least partially, by soluble factors, raising the possibility that the presence of regulatory T-cell receptor-specific T cells in the CNS might inhibit not only the stimulating Vβ8.2+ T cells but also 'bystander T cells' expressing different V genes (Vandenbark *et al*., 1996*b*).

In a human pilot trial of T-cell receptor peptide vaccination, multiple sclerosis patients were immunized with synthetic peptides encompassing the CDR2 regions of Vβ5.2 and Vβ6.1 (Bourdette *et al*., 1994; Chou *et al*., 1994). The rationale for choosing these particular V elements was based on previous results from the same group of investigators indicating that Vβ5.2 and to a lesser extent Vβ6.1 are preferentially expressed by MBP-specific T cells from multiple sclerosis patients as compared with normal controls (Kotzin *et al*., 1991). After immunization with T-cell receptor peptides, delayed type hypersensitivity skin reactions and antibody responses to the immunizing peptides were observed in some patients. T-cell receptor peptide-specific T cells isolated from the blood of immunized patients were predominantly CD4+ and expressed mRNA for interferon-γ, GM-CSF, IL-4, IL-5, and to a lesser extent, IL-2 (Chou *et al*., 1994). The number of patients enrolled in this pilot trial was too small to allow conclusions about the clinical efficacy of T-cell receptor peptide vaccination. In a double-blind placebo-controlled pilot trial conducted by the same group of investigators, 23 HLA-DR2 positive patients with primary or secondary chronic progressive multiple sclerosis were vaccinated with one of two T-cell receptor Vβ5.2 CDR region synthetic peptides or placebo (Vandenbark *et al*., 1996*a*; accompanying editorial by Antel *et al*., 1996). Peptides were injected weekly for the first 4 weeks, and then monthly for a total of 1 year. The trial design did not allow for demonstration of statistically significant differences in clinical outcome of peptide-vaccinated patients as compared with placebo-treated patients (Vandenbark *et al*., 1996*a*). The immunological data obtained in this trial indicate that vaccination with T-cell receptor peptide increases the frequency of circulating T-cell receptor-peptide-specific (regulatory?) T cells. T-cell receptor-peptide-specific T cells isolated from vaccinated patients inhibited the proliferation of autologous MBP-specific T cells *in vitro*. This inhibition was apparently not dependent on direct recognition of endogenously processed T-cell receptor on the MBP-specific T cells, and was mediated by secretion of soluble factors such as IL-10 by the T-cell receptor peptide-specific T cells. The authors speculate that, *in vivo*, T-cell receptor peptide-specific regulatory cells might suppress all TH1 cells in their immediate vicinity, including cells bearing Vβ elements other than Vβ5.2 ('bystander suppression').

Vaccination with whole T cells or T-cell receptor peptides is likely to be improved by novel DNA-based vaccination techniques, which introduce the immunizing antigen in the form of DNA. The DNA encoding the relevant antigen is incorporated into appropriate vectors or applied as 'naked

DNA vaccine' (Pardoll and Beckerleg, 1995). For example, immunization of mice with recombinant vaccinia virus expressing a Vβ8.2 gene significantly reduced the induction of EAE and the proliferative response of MBP-reactive T cells (Chunduru *et al*., 1996). Suppressive vaccination also seems to work with naked DNA encoding Vβ8.2 (Waisman *et al*., 1996). In the vaccinated mice, there was an elevation in the production of the TH2 cytokine IL-4, and a reduction in the TH1 cytokines IL-2 and interferon-γ.

Problems. Although it could be (and indeed is) argued that T cell or T-cell receptor vaccination might affect not only the T cells or T-cell receptor used for immunization but also 'bystander' T cells, it is clear that the chances of success depend critically on the extent of diversity of the human T-cell response to MBP and other encephalitogens. There is now growing consensus that the human T-cell response against the most widely studied potential encephalitogen, MBP, is extremely complex (for reviews, *see* Martin *et al*., 1992; Hohlfeld *et al*., 1995; Hafler *et al*., 1996). Many different antigenic determinants are recognized by T-cell lines selected for reactivity against whole MBP, although certain MBP sequences, e.g. 80–105, are recognized more frequently than others. Furthermore, the T-cell receptor used by MBP-specific T cells, even of T cells specific for $MBP_{80–105}$, are also remarkably heterogenous, both intra- and interindividually (Hafler *et al*., 1996). In a large collection of T-cell receptor sequences derived from MBP-specific human T-cell clones, there was no evidence for preferential usage of certain T-cell receptor Vβ elements (Hafler *et al*., 1996). In our own studies, only one of 215 long-term T-cell clones expressed Vβ5.2 (Meinl *et al*., 1993), peptides of which have been used for T-cell receptor peptide vaccination trials (Vandenbark *et al*., 1996*a*). Differences in T-cell receptor expression could relate to differences in patient characteristics. Therefore, it is important that patients are characterized in detail for T-cell receptor expression characteristics before T-cell receptor vaccination can be expected to produce a meaningful result. It must be anticipated that T-cell receptor vaccination works only if patients are treated with individualized vaccines that are tailor-designed according to the results of pre-treatment immunological characterization. Obviously, this requirement will substantially influence the cost and feasibility of T-cell receptor vaccination therapy.

A further level of complexity is introduced by the possibility that the human T-cell clones characterized *in vitro* differ in their functional *in vivo* properties. Experimental evidence directly supporting this possibility comes from experiments with MBP-specific T-cell clones isolated from the blood of normal rhesus monkeys, using exactly the same 'split well' cloning technique that is used for the generation of human antigen-specific T-cell clones. After re-injection into the autologous monkey, only one of three MBP-specific T-cell clones was encephalitogenic *in vivo* (Meinl *et al*., 1997). Obviously, the encephalitogenic potential of human MBP-specific T-cell clones cannot be tested by autologous transfer, but it is likely that human T-cell clones also differ in their pathogenic potential. If it was possible to assess the pathogenic potential of human T cells, T-cell-directed immunotherapy would become more realistic.

Interestingly, there is a minority of multiple sclerosis patients who exhibit a strikingly restricted T-cell response to MBP. In one-third of the multiple sclerosis patients in our own study, ~80% of the T-cell lines established by stimulation with whole MBP responded to a single, patient-specific cluster of immunodominant epitopes noted in MBP regions 80–105, 108–131 and 131–153 (Meinl *et al*., 1993). In one of these patients, we have compelling evidence that some MBP-specific T-cell clones persist for many years in the peripheral blood (Meinl *et al*., 1993; Hohlfeld *et al*., 1995). Patients with this kind of restricted, persistent autoimmune response would seem the most logical and obvious candidates for T-cell or T-cell receptor vaccination therapy, although it must be emphasized again that even in these patients, multiple T-cell receptors are used for recognition of the immunodominant epitope cluster (Meinl *et al*., 1993; Hohlfeld *et al*., 1995), and that the encephalitogenic potential of these T-cell clones may vary (Meinl *et al*., 1997).

In conclusion, T-cell and T-cell receptor vaccination in human multiple sclerosis is complicated by the remarkable complexity and diversity of the human autoimmune T-cell response. A further problem is that the role of MBP and other potential autoantigens is still unknown in multiple sclerosis. Therefore, it should be anticipated that further progress in this area of selective immunotherapy depends much more on the identification of the pathogenic autoantigen(s) and T-cell receptors than on an increase in the number of patients treated according to existing protocols.

Conclusions and outlook

It should have become clear from the discussion of the individual treatments that some of these proposals have a realistic chance for clinical application, whereas others have not. It is difficult to make a firm prediction, but 'semiselective' therapies based on cytokines or anticytokines are promising candidates. The popular concept of 'immune deviation' away from TH1 cells forms the basis for several other interesting strategies (Table 2). However, it will be important that our understanding of the pathogenesis of multiple sclerosis develops along with our treatment strategies. Note for example, that there is growing evidence for a remarkable heterogeneity of pathogenetic mechanisms which involve different types of T cells and macrophages, as well as B cells and antibodies. Thus, it would be too simplistic to consider multiple sclerosis a disease exclusively mediated by TH1 cells.

Among the many problems to be tackled in the future, the pathogenesis and treatment of (primary) progressive multiple sclerosis requires special attention (Thompson *et al*., 1997). Ideally, it would be possible to identify subtypes of multiple sclerosis and thus be able to tailor treatment according to

the different pathogenetic mechanisms. Immunotherapies requiring only a short course of treatment are especially attractive, since genetically engineered proteins are usually still immunogenic and will eventually induce neutralizing antibodies. Highly selective therapies such as APLs and T-cell (or T-cell receptor-peptide) vaccination, and DNA-based therapies such as anti-sense oligonucleotides and immunological gene therapies are still at an early stage of development, and it is not yet possible to assess their future potential.

It will be interesting to look back in 5 or 10 years to see which of the different experimental strategies discussed here turn out to be successful. Only time will tell, but it is clear that the prospects for treating multiple sclerosis are better today than one would have anticipated only a few years ago.

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References

Abreu SL. Interferon in experimental autoimmune encephalomyelitis (EAE): effects of exogeneous interferon on the antigen-enhanced adoptive transfer of EAE. Int Arch Allergy Appl Immunol 1985; 76: 302–7.

Acha-Orbea H, Mitchell DJ, Timmermann L, Wraith DC, Tausch GS, Waldor MK, et al. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. Cell 1988; 54: 263–73.

Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med 1992; 175: 323–9.

Adorini L, Guéry J-C, Rodriguez-Tarduchy G, Trembleau S. Selective immunosuppression. [Review]. Immunol Today 1993; 14: 285–9.

Aharoni R, Teitelbaum D, Arnon R, Puri J. Immunomodulation of experimental allergic encephalomyelitis by antibodies to the antigen-Ia complex. Nature 1991; 351: 147–50.

Akassoglou K, Probert L, Kontogeorgos G, Kollias G. Astrocyte-specific but not neuron-specific transmembrane TNF triggers inflammation and degeneration in the central nervous system of transgenic mice. J Immunol 1997; 158: 438–45.

Al-Sabbagh A, Miller A, Santos LMB, Weiner HL. Antigen-driven tissue-specific suppression following oral tolerance: orally administered myelin basic protein suppresses proteolipid protein-induced experimental autoimmune encephalomyelitis in the SJL mouse. Eur J Immunol 1994; 24: 2104–9.

Andersen O, Lycke J, Tollesson PO, Svenningsson A, Runmarker B, Linde AS, et al. Linomide reduces the rate of active lesions in relapsing-remitting multiple sclerosis. Neurology 1996; 47: 895–900.

Antel JP, Becher B, Owens T. Immunotherapy for multiple sclerosis: from theory to practice. [Review]. Nature Med 1996; 2: 1074–5.

Archelos JJ, Jung S, Maurer M, Schmied M, Lassmann H, Tamatani T, et al. Inhibition of experimental autoimmune encephalomyelitis by an antibody to the intercellular adhesion molecule ICAM-1. Ann Neurol 1993; 34: 145–54.

Arend WP. Interleukin-1 receptor antagonist. [Review]. Adv Immunol 1993; 54: 167–227.

Arima T, Rehman A, Hickey WF, Flye MW. Inhibition by CTLA4Ig of experimental allergic encephalomyelitis. J Immunol 1996; 156: 4916–24.

Arimilli S, Mumm JB, Nag B. Antigen-specific apoptosis in immortalized T cells by soluble MHC class II-peptide complexes. Immunol Cell Biol 1996; 74: 96–104.

Arnason BGW. Interferon beta in multiple sclerosis. [Review]. Clin Immunol Immunopathol 1996; 81: 1–11.

Arnason BGW, Reder AT. Interferons and multiple sclerosis. [Review]. Clin Neuropharmacol 1994; 17: 495–547.

Arnon R. The development of Cop-1 (Copaxone), an innovative drug for the treatment of multiple sclerosis. [Review]. Immunol Lett 1996; 50: 1–15.

Askari FK, McDonnell WM. Molecular medicine: antisense-oligonucleotide therapy. [Review]. N Engl J Med 1996; 334: 316–8.

Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis [see comments]. Science 1995; 270: 286–90. Comment in: Science 1995; 270: 232–3.

Baker D, Butler D, Scallon BJ, O'Neill JK, Turk JL, Feldmann M. Control of established experimental allergic encephalomyelitis by inhibition of tumor necrosis factor (TNF) activity within the central nervous system using monoclonal antibodies and TNF receptor-immunoglobulin fusion proteins. Eur J Immunol 1994; 24: 2040–8.

Barkhof F, Scheltens P, Frequin STFM, Nauta JJP, Tas MW, Valk J, et al. Relapsing-remitting multiple sclerosis: sequential enhanced MR imaging vs clinical findings in determining disease activity. AJR Am J Radiol 1992; 159: 1041–7.

Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA Jr. Surface expression of α4 integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med 1993; 177: 57–68.

Bauer J, Wekerle H, Lassmann H. Apoptosis in brain-specific autoimmune disease. [Review]. Curr Opin Immunol 1995; 7: 839–43.

Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. [Review]. N Engl J Med 1996; 334: 1717–25.

Beck J, Rondot P, Catinot L, Falcoff E, Kirchner H, Wietzerbin J. Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? Acta Neurol Scand 1988; 78: 318–23.

Ben-Nun A, Cohen IR. Vaccination against autoimmune

encephalomyelitis (EAE): attenuated autoimmune T lymphocytes confer resistance to induction of active EAE but not to EAE mediated by the intact T lymphocyte line. Eur J Immunol 1981; 11: 949–52.

Ben-Nun A, Wekerle H, Cohen IR. Vaccination against autoimmune with T-lymphocyte line cells reactive against myelin basic protein. Nature 1981; 292: 60–1.

Ben-Nun A, Mendel I, Bakimer R, Fridkis-Hareli M, Teitelbaum D, Arnon R, et al. The autoimmune reactivity to myelin oligodendrocyte glycoprotein (MOG) in multiple sclerosis is potentially pathogenic: effect of copolymer 1 on MOG-induced disease. J Neurol 1996; 243 Suppl 1: S14–S22.

Bendtzen K, Hansen MB, Ross C, Poulsen LK, Svenson M. Cytokines and autoantibodies to cytokines. [Review]. Stem Cells (Dayt) 1995; 13: 206–22.

Bennett TA, Lynam EB, Sklar LA, Rogelj S. Hydroxamate-based metalloprotease inhibitor blocks shedding of L-selectin adhesion molecule from leukocytes. Functional consequences for neutrophil aggregation. J Immunol 1996; 156: 3093–7.

Berman JW, Guida MP, Warren J, Amat J, Brosnan CF. Localization of monocyte chemoattractant peptide-1 expression in the central nervous system in experimental autoimmune encephalomyelitis and trauma in the rat. J Immunol 1996; 156: 3017–23.

Beutler B, Cerami A. Cachectin: more than a tumor necrosis factor. [Review]. N Engl J Med 1987; 316: 379–385.

Beutler B, Cerami A. The biology of cachectin/TNF–a primary mediator of the host response. [Review]. Annu Rev Immunol 1989; 7: 625–55.

Blanas E, Carbone FR, Allison J, Miller JFAP, Heath WR. Induction of autoimmune diabetes by oral administration of autoantigen. Science 1996; 274: 1707–9.

Bö L, Dawson TM, Wesselingh S, Mörk S, Choi S, Kong PA, et al. Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. Ann Neurol 1994; 36: 778–86.

Bokoch GM. Chemoattractant signaling and leukocyte activation. [Review]. Blood 1995; 86: 1649–60.

Bornstein MB, Miller A, Slagle S, Weitzman M, Crystal H, Drexler E, et al. A pilot trial of Cop-1 in exacerbating-remitting multiple sclerosis. N Engl J Med 1987; 317: 408–14.

Bornstein MB, Miller A, Slagle S, Weitzman M, Drexler E, Keilson M, et al. A placebo-controlled, double-blind, randomized, two-center, pilot trial of Cop-1 in chronic progressive multiple sclerosis. Neurology 1991; 41: 533–9.

Bourdette DN, Whitham RH, Chou YK, Morrison WJ, Atherton J, Kenny C, et al. Immunity to TCR peptides in multiple sclerosis. I. Successful immunization of patients with synthetic Vβ5.2 and Vβ6.1 CDR2 peptides [published erratum apperars in J Immunol 1994; 153: 910]. J Immunol 1994; 152: 2510–9.

Bright JJ, Topham DJ, Nag B, Lodge PA, Sriram S. Vaccination with peptides from MHC class II beta chain hypervariable region causes allele-specific suppression of EAE. J Neuroimmunol 1996; 67: 119–24.

Brocke S, Gaur A, Piercy C, Gautam A, Gijbels K, Fathman CG, et al. Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. Nature 1993; 365: 642–4.

Brocke S, Veromaa T, Weissman IL, Gijbels K, Steinman L. Infection and multiple sclerosis: a possible role for superantigens? [Review]. Trends Microbiol 1994; 2: 250–4.

Brocke S, Gijbels K, Allegretta M, Ferber I, Piercy C, Blankenstein T, et al. Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein. Nature 1996; 379: 343–6.

Brod SA, Khan M. Oral administration of interferon-α is superior to subcutaneous administration of interferon-α in the suppression of chronic relapsing experimental autoimmune encephalomyelitis. J Autoimmun 1996; 9: 11–20.

Brod SA, Scott M, Burns DK, Phillips JT. Modification of acute experimental autoimmune encephalomyelitis in the Lewis rat by oral administration of type 1 interferons. J Interferon Cytokine Res 1995; 15: 115–22.

Brosnan CF, Raine CS. Mechanisms of immune injury in multiple sclerosis. [Review]. Brain Pathol 1996; 6: 243–57.

Brosnan CF, Selmaj K, Raine CS. Hypothesis: a role for tumor necrosis factor in immune-mediated demyelination and its relevance to multiple sclerosis. J Neuroimmunol 1988; 18: 87–94.

Brosnan CF, Battistini L, Gao YL, Raine CS, Aquino DA. Heat shock proteins and multiple sclerosis: a review. [Review]. J Neuropathol Exp Neurol 1996; 55: 389–402.

Burger D, Chicheportiche R, Giri JG, Dayer J-M. The inhibitory activity of human IL-1 receptor antagonist is enhanced by type II interleukin-1 soluble receptor and hindered by type I interleukin-1 soluble receptor. J Clin Invest 1995; 96: 38–41.

Burns J, Rosenzweig A, Zweiman B, Lisak RP. Isolation of myelin basic protein-reactive T-cell lines from normal human blood. Cell Immunol 1983; 81: 435–40.

Burns FR, Li XB, Shen N, Offner H, Chou YK, Vandenbark AA, et al. Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar Vα and Vβ chain genes even though the major histocompatibility complex and encephalitogenic determinants being recognized are different. J Exp Med 1989; 169: 27–39.

Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. [Review]. Science 1996; 272: 60–6.

Cannella B, Raine CS. The adhesion molecule and cytokine profile of multiple sclerosis lesions [see comments]. Ann Neurol 1995; 37: 424–35. Comment in: Ann Neurol 1995; 37: 419–21.

Cannella B, Gao Y-L, Brosnan C, Raine CS. IL-10 fails to abrogate experimental autoimmune encephalomyelitis. J Neurosci Res 1996; 45: 735–46.

Cash E, Minty A, Ferrara P, Caput D, Fradelizi D, Rott O. Macrophage-inactivating IL-13 suppresses experimental autoimmune encephalomyelitis in rats. J Immunol 1994; 153: 4258–67.

Chandler S, Miller KM, Clements JM, Lury J, Corkill D, Anthony DCC, et al. Matrix metalloproteinases, tumor necrosis factor and

multiple sclerosis: an overview. [Review]. J Neuroimmunol 1997; 72: 155–61.

Chen Y, Kuchroo VK, Inobe J-I, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science 1994; 265: 1237–40.

Chernoff AE, Granowitz EV, Shapiro L, Vannier E, Lonnemann G, Angel JB, et al. A randomized controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. J Immunol 1995; 154: 5492–9.

Chiang C-S, Powell HC, Gold LH, Samimi A, Campbell IL. Macrophage/microglial-mediated primary demyelination and motor disease induced by the central nervous system production of interleukin-3 in transgenic mice. J Clin Invest 1996; 97: 1512–24.

Chluba J, Steeg C, Becker A, Wekerle H, Epplen JT. T cell receptor β chain usage in myelin basic protein-specific rat T lymphocytes. Eur J Immunol 1989; 19: 279–84.

Chofflon M, Juillard C, Juillard P, Gauthier G, Grau GE. Tumor necrosis factor α production as a possible predictor of relapse in patients with multiple sclerosis. Eur Cytokine Netw 1992; 3: 523–31.

Choi DW. Nitric oxide: foe or friend to the injured brain? [Comment]. [Review]. Proc Natl Acad Sci USA 1993; 90: 9741–3. Comment on: Proc Natl Acad Sci USA 1993; 90: 9808–12.

Chou YK, Morrison WJ, Weinberg AD, Dedrick R, Whitham R, Bourdette DN, et al. Immunity to TCR peptides in multiple sclerosis. II. T cell recognition of Vβ5.2 and Vβ6.1 CDR2 peptides [published erratum appears in J Immunol 1994; 153: 910]. J Immunol 1994; 152: 2520–9.

Chunduru SK, Sutherland RM, Stewart GA, Doms RW, Paterson Y. Exploitation of the Vβ8.2 T cell receptor in protection against experimental autoimmune encephalomyelitis using a live vaccinia virus vector. J Immunol 1996; 156: 4940–5.

Cleveland JL, Ihle JN. Contenders in FasL/TNF death signaling. [Review]. Cell 1995; 81: 479–82.

Cohen IR. The cognitive paradigm and the immunological homunculus. [Review]. Immunol Today 1992; 13: 490–4.

Compston A. Brain repair. [Review]. J Intern Med 1995; 237: 127–34.

Compston A, Scolding N, Wren D, Noble M. The pathogenesis of demyelinating disease: insights from cell biology. [Review]. Trends Neurosci 1991; 14: 175–82.

Cousens LP, Orange JS, Su HC, Biron CA. Interferon-α/β inhibition of interleukin 12 and interferon-γ production in vitro and endogenously during viral infection. Proc Natl Acad Sci USA 1997; 94: 634–9.

Crisi GM, Santambrogio L, Hochwald GM, Smith SR, Carlino JA, Thorbecke GJ. Staphylococcal enterotoxin B and tumor-necrosis factor-α induced relapses of experimental allergic encephalomyelitis: Protection by transforming growth factor-β and interleukin-10. Eur J Immunol 1995; 25: 3035–40.

Crispe IN. Fatal interactions: Fas-induced apoptosis of mature T cells. [Review]. Immunity 1994; 1: 347–9.

Critchfield JM, Racke MK, Zuñiga-Pflücker JC, Cannella B, Raine CS, Goverman J, et al. T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. Science 1994; 263: 1139–43.

Cross AH, Cannella B, Brosnan CF, Raine CS. Hypothesis: antigen-specific T cells prime central nervous system endothelium for recruitment of nonspecific inflammatory cells to effect autoimmune demyelination. [Review]. J Neuroimmunol 1991; 33: 237–44.

Cross AH, Girard TJ, Giacoletto KS, Evans RJ, Keeling RM, Lin RF, et al. Long-term inhibition of murine experimental autoimmune encephalomyelitis using CTLA-4-Fc supports a key role for CD28 costimulation [see comments]. J Clin Invest 1995; 95: 2783–9. Comment in: J Clin Invest 1995; 95: 2429–30.

Cuzner ML, Gveric D, Strand C, Loughlin AJ, Paemen L, Opdenakker G, et al. The expression of tissue-type plasminogen-activator, matrix metalloproteases and endogenous inhibitors in the central nervous system in multiple sclerosis: comparison of stages in lesion evolution. J Neuropathol Exp Neurol 1996; 55: 1194–204.

Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. [Review]. Science 1994; 264: 1415–21.

Davis LS, Kavanaugh AF, Nichols LA, Lipsky PE. Induction of persistent T cell hyporesponsiveness in vivo by monoclonal antibody to ICAM-1 in patients with rheumatoid arthritis. J Immunol 1995; 154: 3525–37.

Davodeau F, Peyrat M-A, Romagné F, Necker A, Hallet M-M, Vié H, et al. Dual T cell receptor β chain expression on human T lymphocytes. J Exp Med 1995; 181: 1391–8.

De Groot CJA, Ruuls SR, Theeuwes JWM, Dijkstra CD, Van der Valk P. Immunocytochemical characterization of the expression of inducible and constitutive isoforms of nitric oxide synthase in demyelinating multiple sclerosis lesions. J Neuropathol Exp Neurol 1997; 56: 10–20.

De Kruif J, Van de Vuurst de Vries A-R, Cilenti L, Boel E, van Ewijk W, Logtenberg T. New perspectives on recombinant human antibodies. [Review]. Immunol Today 1996; 17: 453–5.

De Simone R, Giampaolo A, Giometto B, Gallo P, Levi G, Peschle C, et al. The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. J Neuropathol Exp Neurol 1995; 54: 175–87.

Del Prete G, Maggi E, Romagnani S. Human Th1 and Th2 cells: functional properties, mechanisms of regulation and role in disease. [Review]. Lab Invest 1994; 70: 299–306.

Dinarello CA. Biologic basis for interleukin-1 in disease. [Review]. Blood 1996; 87: 2095–147.

Dinarello CA, Thompson RC. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. [Review]. Immunol Today 1991; 12: 404–10.

Duchosal MA, Rothermel AL, McConahey PJ, Dixon FJ, Altieri DC. In vivo immunosuppression by targeting a novel protease receptor. Nature 1996; 380: 352–6.

Duncan ID. Glial cell transplantation and remyelination of the central nervous system. [Review]. Neuropathol Appl Neurobiol 1996; 22: 87–100.

Durelli L, Bongioanni MR, Cavallo R, Ferrero B, Ferri R, Ferrio

MF, et al. Chronic systemic high-dose recombinant interferon alfa-2a reduces exacerbation rate, MRI signs of disease activity, and lymphocyte interferon gamma production in relapsing-remitting multiple sclerosis. Neurology 1994; 44: 406–13.

Durelli L, Bongioanni MR, Ferrero B, Ferri R, Imperiale D, Bradac GB, et al. Interferon alpha-2a treatment of relapsing-remitting multiple sclerosis: disease activity resumes after stopping treatment. Neurology 1996; 47: 123–9.

Ebers GC. Treatment of multiple sclerosis. [Review]. Lancet 1994; 343: 275–80.

Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, et al. Randomized double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. Lancet 1994a; 344: 1105–10.

Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Bijl H, et al. Repeated therapy with monoclonal antibody to tumour necrosis factor α (cA2) in patients with rheumatoid arthritis. Lancet 1994b; 344: 1125–7.

Elliott EA, McFarland HI, Nye SH, Cofiell R, Wilson TM, Wilkins JA, et al. Treatment of experimental encephalomyelitis with a novel chimeric fusion protein of myelin basic protein and proteolipid protein. J Clin Invest 1996; 98: 1602–12.

Fabry Z, Raine CS, Hart MN. Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. [Review]. Immunol Today 1994; 15: 218–24.

Fabry Z, Topham DJ, Fee D, Herlein J, Carlino JA, Hart MN, et al. TGF-β2 decreases migration of lymphocytes in vitro and homing of cells into the central nervous system in vivo. J Immunol 1995; 155: 325–32.

Farrar MA, Schreiber RD. The molecular cell biology of interferon-γ and its receptor. [Review]. Annu Rev Immunol 1993; 11: 571–611.

Fazekas F, Deisenhammer F, Strasser-Fuchs S, Nahler G, Mamoli B, for the Austrian Immunoglobulin in Multiple Sclerosis Study Group. Randomised placebo-controlled trial of monthly intravenous immunoglobulin therapy in relapsing-remitting multiple sclerosis. Lancet 1997; 349: 589–93.

Fierlbeck G, Schreiner T, Schaber B, Walser A, Rassner G. Neutralizing interferon β antibodies in melanoma patients treated with recombinant and natural interferon β. Cancer Immunol Immunother 1994; 39: 263–8.

Fisher CJ Jr, Agosti JM, Opal SM, Lowry SF, Balk RA, Sadoff JC, et al. Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. N Engl J Med 1996; 334: 1697–702.

Fleischer B. Superantigens. [Review]. APMIS 1994; 102: 3–12.

Fontana A, Fierz W, Wekerle H. Astrocytes present myelin basic protein to encephalitogenic T cell lines. Nature 1984; 307: 273–6.

Foy TM, Aruffo A, Bajorath J, Buhlmann JE, Noelle RJ. Immune regulation by CD40 and its ligand gp39. [Review]. Annu Rev Immunol 1996; 14: 591–617.

Francisco JA, Kiener PA, Moran-Davis P, Ledbetter JA, Siegall CP. Cytokine activation sensitizes human monocytic and endothelial cells to the cytotoxic effects of an anti-CD40 immunotoxin. J Immunol 1996; 157: 1652–8.

Franco A, Southwood S, Arrhenius T, Kuchroo VK, Grey HM, Sette A, et al. T cell receptor antagonist peptides are highly effective inhibitors of experimental allergic encephalomyelitis. Eur J Immunol 1994; 24: 940–6.

Franklin RJM. Reconstructing myelin-deficient environments in the CNS by glial cell transplantation. [Review]. Semin Neurosci 1993; 5: 443–51.

Friedmann T. Human gene therapy–an immature genie, but certainly out of the bottle. [Review]. Nat Med 1996; 2: 144–7.

Furie MB, Randolph GJ. Chemokines and tissue injury. [Review]. Am J Pathol 1995; 146: 1287–301.

Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature 1996; 384: 134–41.

Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, et al. The αβ T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. Science 1996; 274: 209–19.

Gaur A, Wiers B, Liu A, Rothbard J, Fathman CG. Amelioration of autoimmune encephalomyelitis by myelin basic protein synthetic peptide-induced anergy. Science 1992; 258: 1491–4.

Gautam AM. Self and non-self peptides treat autoimmune encephalomyelitis: T cell anergy or competition for major histocompatibility complex class II binding? Eur J Immunol 1995; 25: 2059–63.

Gearing AJN, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, et al. Processing of tumour necrosis factor-α precursor by metalloproteinases. Nature 1994; 370: 555–7.

Genain CP, Lee-Parritz D, Nguyen M-H, Massacesi L, Joshi N, Ferrante R, et al. In healthy primates, circulating autoreactive T cells mediate autoimmune disease [see comments]. J Clin Invest 1994; 94: 1339–45. Comment in: J Clin Invest 1994; 94: 921–2.

Genain CP, Nguyen M-H, Letvin NL, Pearl R, Davis RL, Adelman M, et al. Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. J Clin Invest 1995a; 96: 2966–74.

Genain CP, Roberts T, Davis RL, Nguyen M-H, Uccelli A, Faulds D, et al. Prevention of autoimmune demyelination in non-human primates by a cAMP-specific phosphodiesterase inhibitor. Proc Natl Acad Sci USA 1995b; 92: 3601–05.

Genain CP, Abel K, Belmar N, Villinger F, Rosenberg DP, Linington C, et al. Late complications of immune deviation therapy in a nonhuman primate. Science 1996; 274: 2054–7.

Gijbels K. A study on the role of interleukin-6 and matrix metalloproteinases in inflammatory demyelinating disorders in the central nervous system [PhD thesis]. Louvain: University of Louvain, 1995.

Gijbels K, Galardy RE, Steinman L. Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. J Clin Invest 1994; 94: 2177–82.

Gilat D, Cahalon L, Hershkoviz R, Lider O. interplay of T cells and cytokines in the context of enzymatically modified extracellular matrix. [Review]. Immunol Today 1996; 17: 16–20.

Giovannoni G, Thorpe JW, Kidd D, Kendall BE, Moseley IF,

Thompson AJ, et al. Soluble E-selectin in multiple sclerosis: Raised concentrations in patients with primary progressive disease. J Neurol Neurosurg Psychiatry 1996; 60: 20–6.

Glabinski AR, Tani M, Stricter RM, Tuohy VK, Ransohoff RM. Synchronous synthesis of α- and β-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. Am J Pathol 1997; 150: 617–30.

Godiska R, Chantry D, Dietsch GN, Gray PW. Chemokine expression in murine experimental allergic encephalomyelitis. J Neuroimmunol 1995; 58: 167–76.

Goetzl EJ, Banda MJ, Leppert D. Matrix metalloproteinases in immunity. [Review]. J Immunol 1996; 156: 1–4.

Gonzalo JA, Gonzalez-Garcia A, Kalland T, Hedlund G, Martinez C, Kroemer G. Linomide, a novel immunomodulator that prevents death in four models of septic shock. Eur J Immunol 1993; 23: 2372–4.

Gordon EJ, Myers KJ, Dougherty JP, Rosen H, Ron Y. Both anti-CD11a (LFA-1) and anti-CD11b (MAC-1) therapy delay the onset and diminish the severity of experimental autoimmune encephalomyelitis. J Neuroimmunol 1995; 62: 153–60.

Grewal IS, Flavell RA. A central role of CD40 ligand in the regulation of $CD4^+$ T-cell responses. [Review]. Immunol Today 1996; 17: 410–4.

Grewal IS, Foellmer HG, Grewal KD, Xu J, Hardardottir F, Baron JL, et al. Requirement for CD40 ligand in costimulation induction, T cell activation, and experimental allergic encephalomyelitis. Science 1996; 273: 1864–7.

Groux H, Bigler M, de Vries JE, Roncarolo M-G. Interleukin-10 induces a long-term antigen-specific anergic state in human $CD4^+$ T cells [see comments]. J Exp Med 1996; 184: 19–29. Comment in: J Exp Med 1996; 184: 1–8.

Hafler DA, Weiner HL. Immunologic mechanisms and therapy in multiple sclerosis. [Review]. Immunol Rev 1995; 144: 75–07.

Hafler DA, Ritz J, Schlossman SF, Weiner HL. Anti-CD4 and anti-CD2 monoclonal antibody infusions in subjects with multiple sclerosis. Immunosuppressive effects and human anti-mouse responses. J Immunol 1988; 141: 131–8.

Hafler DA, Saadeh MG, Kuchroo VK, Milford E, Steinman L. TCR usage in human and experimental demyelinating disease. [Review]. Immunol Today 1996; 17: 152–9.

Hall GL, Compston A, Scolding NJ. Beta-interferon and multiple sclerosis. [Review]. Trends Neurosci 1997; 20: 63–7.

Hartung H-P, Archelos JJ, Zielasek J, Gold R, Koltzenburg M, Reiners K-H, et al. Circulating adhesion molecules and inflammatory mediators in demyelination: a review. [Review]. Neurology 1995; 45 (6 Suppl 6): S22–32.

Hauser SL. Prevention strategies for multiple sclerosis. [Review]. Ann Neurol 1994; 36 Suppl: S157–62.

Heaney ML, Golde DW. Soluble cytokine receptors. [Review]. Blood 1996; 87: 847–57.

Heller RA, Kronke M. Tumor necrosis factor receptor-mediated signaling pathways. [Review]. J Cell Biol 1994; 126: 5–9.

Hemler ME. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. [Review]. Annu Rev Immunol 1990; 8: 365–400.

Hewson AK, Smith T, Leonard JP, Cuzner ML. Suppression of experimental allergic encephalomyelitis in the Lewis rat by the matrix metalloproteinase inhibitor Ro31–9790. Inflamm Res 1995; 44: 345–9.

Hickey WF, Gonatas NK, Kimura H, Wilson DB. Identification and quantitation of T lymphocyte subsets found in the spinal cord of the Lewis rat during acute experimental allergic encephalomyelitis. J Immunol 1983; 131: 2805–9.

Hodgkin PD, Basten A. B cell activation, tolerance and antigen-presenting function. [Review]. Curr Opin Immunol 1995; 7: 121–9.

Hogg N, Berlin C. Structure and function of adhesion receptors in leukocyte trafficking. [Review]. Immunol Today 1995; 16: 327–30.

Hohlfeld R. Neurological autoimmune disease and the trimolecular complex of T-lymphocytes. [Review]. Ann Neurol 1989; 25: 531–8.

Hohlfeld R, Meinl E, Weber F, Zipp F, Schmidt S, Sotgiu S, et al. The role of autoimmune T lymphocytes in the pathogenesis of multiple sclerosis. [Review]. Neurology 1995; 45 (6 Suppl 6): S33–8.

Hortelano S, Diaz-Guerra MJM, Gonzalez-Garcia A, Leonardo E, Gamallo C, Bosca L, et al. Linomide administration to mice attenuates the induction of nitric oxide synthase elicited by lipopolysaccharide-activated macrophages and prevents nephritis in MRL/Mp-lpr/lpr mice. J Immunol 1997; 158: 1402–8.

Howell MD, Winters ST, Olee T, Powell HC, Carlo DJ, Brostoff SW. Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides [published erratum appears in Science 1990; 247: 1167]. Science 1989; 246: 668–70.

Huitinga I, Damoiseaux JGMC, Dopp EA, Dijkstra CD. Treatment with anti-CR3 antibodies ED7 and ED8 suppresses xperimental allergic encephalomyelitis in Lewis rats. Eur J Immunol 1993; 23: 709–15.

Hulkower K, Brosnan CF, Aquino DA, Cammer W, Kulshrestha S, Guida MP, et al. Expression of CSF-1, *c-fms*, and MCP-1 in the central nervous system of rats with experimental allergic encephalomyelitis. J Immunol 1993; 150: 2525–33.

Hurwitz AA, Sullivan TJ, Krummel MF, Sobel RA, Allison JP. Specific-blockade of CTLA-4/B7 interactions results in exacerbated clinical and histologic disease in an actively-induced model of experimental allergic encephalomyelitis. J Neuroimmunol 1997; 73: 57–62.

IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial [see comments]. Neurology 1993; 43: 655–61. Comment in: Neurology 1993; 43: 641–3.

IFNB Multiple Sclerosis Study Group. Interferon beta-1b in the treatment of multiple sclerosis: Final outcome of the randomized controlled trial. Neurology 1995; 45: 1277–85.

IFNB Multiple Sclerosis Study Group, University of British Columbia MS/MRI Analysis Group. Neutralizing antibodies during treatment of multiple sclerosis with interferon beta-1b: experience during the first three years. Neurology 1996; 47: 889–94.

Ihle JN. STATs: signal transducers and activators of transcription. [Review]. Cell 1996; 84: 331–4.

Imamura K, Suzumura A, Hayashi F, Marunouchi T. Cytokine production by peripheral blood monocytes/macrophages in multiple sclerosis patients. Acta Neurol Scand 1993; 87: 281–5.

Imhof BA, Dunon D. Leukocyte migration and adhesion. [Review]. Adv Immunol 1995; 58: 345–416.

Inaba K, Kitaura M, Kato T, Watanabe Y, Kawade Y, Muramatsu S. Contrasting effect of α/β and γ interferons on expression of macrophage Ia antigens. J Exp Med 1986; 163: 1030–5.

Jaattela M. Biologic activities and mechanisms of action of tumor necrosis factor-α/cachectin. [Review]. Lab Invest 1991; 64: 724–742.

Jacobs L, Johnson KP. A brief history of the use of interferons as treatment of multiple sclerosis. [Review]. Arch Neurol 1994; 51: 1245–52.

Jacobs CA, Baker PE, Roux ER, Picha KS, Toivola B, Waugh S, et al. Experimental autoimmune encephalomyelitis is exacerbated by IL-1α and suppressed by soluble IL-1 receptor. J Immunol 1991; 146: 2983–9.

Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. Ann Neurol 1996; 39: 285–94.

Jameson BA, McDonnell JM, Marini JC, Korngold R. A rationally designed CD4 analogue inhibits experimental allergic encephalomyelitis [see comments]. Nature 1994; 368: 744–6. Comment in: Nature 1994; 368: 692–3, Comment in: Nature 1994; 370: 334.

Johns LD, Sriram S. Experimental allergic encephalomyelitis: Neutralizing antibody to TGF-β1 enhances the clinical severity of the disease. J Neuroimmunol 1993; 47: 1–7.

Johns LD, Flanders KC, Ranges GE, Sriram S. Successful treatment of experimental allergic encephalomyelitis with transforming growth factor-β1. J Immunol 1991; 147: 1792–6.

Johnson KP, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis. Results of a phase III multicenter, double-blind, placebo-controlled trial [see comments]. Neurology 1995; 45: 1268–76. Comment in:]. Neurology 1995; 45: 1245–7.

Jung S, Toyka K, Hartung H-P. Suppression of experimental autoimmune encephalomyelitis in Lewis rats by antibodies against CD2. Eur J Immunol 1995; 25: 1391–8.

Karandikar NJ, Vanderlugt CL, Walunas TL, Miller SD, Bluestone JA. CTLA-4: a negative regulator of autoimmune disease. J Exp Med 1996; 184: 783–8.

Karin N, Mitchell DJ, Brocke S, Ling N, Steinman L. Reversal of experimental autoimmune encephalomyelitis by a soluble peptide variant of a myelin basic protein epitope: T cell receptor antagonism and reduction of interferon-γ and tumor necrosis factor α production. J Exp Med 1994; 180: 2227–37.

Karpus WJ, Lukacs NW, McRae BL, Strieter RM, Kunkel SL, Miller SD. An important role for the chemokine macrophage inflammatory protein-1α in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. J Immunol 1995; 155: 5003–10.

Karussis DM, Meiner Z, Lehmann D, Gomori J-M, Schwarz A, Linde A, et al. Treatment of secondary progressive multiple sclerosis with the immunomodulator linomide. Neurology 1996; 47: 341–6.

Kent SJ, Karlik SJ, Cannon C, Hines DK, Yednock TA, Fritz LC, et al. A monoclonal antibody to α4 integrin suppresses and reverses active experimental allergic encephalomyelitis. J Neuroimmunol 1995; 58: 1–10.

Khoury SJ, Akalin E, Chandraker A, Turka LA, Linsley PS, Sayegh MH, et al. CD28-B7 costimulatory blockade by CTLA4Ig prevents actively induced experimental autoimmune encephalomyelitis and inhibits Th1 but spares Th2 cytokines in the central nervous system. J Immunol 1995; 155: 4521–4.

Khoury SJ, Gallon L, Verburg RR, Chandraker A, Peach R, Linsley PS, et al. Ex vivo treatment of antigen-presenting cells with CTLA4Ig and encephalitogenic peptide prevents experimental autoimmune encephalomyelitis in the Lewis rat. J Immunol 1996; 157: 3700–5.

Klausner JD, Freedman VH, Kaplan G. Thalidomide as an anti-TNF-α inhibitor: implications for clinical use. [Review]. Clin Immunol Immunopathol 1996; 81: 219–23.

Klinkert WEF, Kojima K, Lesslauer W, Rinner W, Lassmann H, Wekerle H. TNF-α receptor fusion protein prevents experimental auto-immune encephalomyelitis and demyelination in Lewis rats: an overview. J Neuroimmunol 1997; 72: 163–8.

Kojima K, Berger T, Lassmann H, Hinze-Selch D, Zhang Y, Gehrmann J, et al. Experimental autoimmune panencephalitis and uveoretinitis transferred to the Lewis rat by T lymphocytes specific for the S100β molecule, a calcium binding protein of astroglia. J Exp Med 1994; 180: 817–29.

Kotzin BL, Karuturi S, Chou YK, Lafferty J, Forrester JM, Better M, et al. Preferential T-cell receptor β-chain variable gene use in myelin basic protein-reactive T-cell clones from patients with multiple sclerosis. Proc Natl Acad Sci USA 1991; 88: 9161–5.

Kotzin BL, Leung DYM, Kappler J, Marrack P. Superantigens and their potential role in human disease. [Review]. Adv Immunol 1993; 54: 99–166.

Kramer R, Zhang Y, Gehrmann J, Gold R, Thoenen H, Wekerle H. Gene transfer through the blood-nerve barrier: nerve growth factor engineered neuritogenic T lymphocytes attenuate experimental autoimmune neuritis. Nat Med 1995; 1: 1162–6.

Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. [Review]. Trends Neurosci 1996; 19: 312–8.

Kuchroo VK, Greer JM, Kaul D, Ishioka G, Franco A, Sette A, et al. A single TCR antagonist peptide inhibits experimental allergic encephalomyelitis mediated by a diverse T cell repertoire. J Immunol 1994; 153: 3326–36.

Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, et al. B7–1 and B7–2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. Cell 1995; 80: 707–18.

Kuruvilla AP, Shah R, Hochwald GM, Liggitt HD, Palladino MA,

Thorbecke GJ. Protective effect of transforming growth factor β1 on experimental autoimmune diseases in mice. Proc Natl Acad Sci USA 1991; 88: 2918–21.

Lamont AG, Sette A, Fujinami R, Colon SM, Miles C, Grey HM. Inhibition of experimental autoimmune encephalomyelitis induction in SJL/J mice by using a peptide with high affinity for IA^s molecules. J Immunol 1990; 145: 1687–3.

Lanzavecchia A. Identifying strategies for immune intervention. [Review]. Science 1993; 260: 937–44.

Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C, et al. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 1996; 381: 434–8.

Lassmann H, Vass K. Are current immunological concepts of multiple sclerosis reflected by the immunopathology of its lesions? [Review]. Springer Semin Immunopathol 1995; 17: 77–87.

Lassmann H, Rossler K, Zimprich F, Vass K. Expression of adhesion molecules and histocompatibility antigens at the blood-brain barrier. [Review]. Brain Pathol 1991a; 1: 115–23.

Lassmann H, Zimprich F, Rossler K, Vass K. Inflammation in the nervous system. Basic mechanisms and immunological concepts. [Review]. Rev Neurol (Paris) 1991b; 147: 763–81.

Lassmann H, Zimprich F, Vass K, Hickey WF. Microglial cells are a component of the perivascular glia limitans. J Neurosci Res 1991c; 28: 236–43.

Lee S-W, Sullenger BA. Isolation of a nuclease-resistant decoy RNA that selectively blocks autoantibody binding to insulin receptors on human lymphocytes. J Exp Med 1996; 184: 315–24.

Lee JC, Laydon JT, McDonnell PCM, Gallagher TF, Kumar S, Green D, et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 1994; 372: 739–46.

Legge KL, Min B, Potter NT, Zaghouani H. Presentation of a T cell receptor antagonist peptide by immunoglobulins ablates activation of T cells by a synthetic peptide or proteins requiring endocytic processing. J Exp Med 1997; 185: 1043–53.

Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. [Review]. Annu Rev Immunol 1996; 14: 233–58.

Leonard JP, Waldburger KE, Goldman SJ. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J Exp Med 1995; 181: 381–6.

Leppert D, Waubant E, Burk MR, Oksenberg J, Hauser SL. IFN-β1b inhibits gelatinase secretion and in vitro migration of T cells: a possible mechanism for treatment efficacy in multiple sclerosis. Ann Neurol 1996; 40: 845–52.

Liblau RS, Singer SM, McDevitt HO. Th1 and Th2 $CD4^+$ T cells in the pathogenesis of organ-specific autoimmune diseases [see comments]. [Review]. Immunol Today 1995; 16: 34–8. Comment in: Immunol Today 1995; 16: 457–8.

Lindsey JW, Hodgkinson S, Mehta R, Mitchell D, Enzmann D, Steinman L. Repeated treatment with chimeric anti-CD4 antibody in multiple sclerosis. Ann Neurol 1994; 36: 183–9.

Ling PD, Warren MK, Vogel SN. Antagonistic effect of interferon-β on the interferon-γ-induced expression of Ia antigen in murine macrophages. J Immunol 1985; 135: 1857–63.

Linington C, Bradl M, Lassmann H, Brunner C, Vass K. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. Am J Pathol 1988; 130: 443–54.

Linington C, Berger T, Perry L, Weerth S, Hinze-Selch D, Zhang Y, et al. T cells specific for the myelin oligodendrocyte glycoprotein mediate an unusual autoimmune inflammatory response in the central nervous system. Eur J Immunol 1993; 23: 1364–72.

Liu C, Goldstein J, Graziano RF, He J, O′Shea JK, Deo Y, et al. FcγRI-targeted fusion proteins result in efficient presentation by human monocytes of antigenic and antagonist T cell epitopes. J Clin Invest 1996a; 98: 2001–7.

Liu J, Zhao M-L, Brosnan CF, Lee SC. Expression of type II nitric oxide synthase in primary human astrocytes and microglia: role of IL-1β and IL-1 receptor antagonist. J Immunol 1996b; 157: 3569–76.

Lowenstein CJ, Snyder SH. Nitric oxide, a novel biologic messenger. [Review]. Cell 1992; 70: 705–7.

Lu H-T, Riley JL, Babcock GT, Huston M, Stark GR, Boss JM, et al. Interferon (IFN) β acts downstream of IFN-γ-induced class II transactivator messenger RNA accumulation to block major histocompatibility complex class II gene expression and requires the 48-kD DNA-binding protein, ISGF3-γ. J Exp Med 1995; 182: 1517–25.

Lublin FD, Reingold SC, and the National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Guidelines for clinical trials of new therapeutic agents in multiple sclerosis: relations between study investigators, advisors, and sponsors. [Review]. Neurology 1997; 48: 572–4.

Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity in pathogenesis. [Review]. Brain Pathol 1996; 6: 259–74.

Lynch DH, Ramsdell F, Alderson MR. Fas and FasL in the homeostatic regulation of immune responses. [Review]. Immunol Today 1995; 16: 569–74.

Mackay CR. Chemokine receptors and T cell chemotaxis. [Review]. J Exp Med 1996; 184: 799–802.

Madden DR. The three-dimensional structure of peptide-MHC complexes. [Review]. Annu Rev Immunol 1995; 13: 587–622.

Maeda A, Sobel RA. Matrix metalloproteinases in the normal human central nervous system, microglial nodules and multiple sclerosis lesions. J Neuropathol Exp Neurol 1996; 55: 300–9.

Makarov SS, Olsen JC, Johnston WN, Anderle SK, Brown RR, Baldwin AS, et al. Suppression of experimental arthritis by gene transfer of interleukin 1 receptor antagonist cDNA. Proc Natl Acad Sci USA 1996; 93: 402–6.

Mannie MD, Dinarello CA, Paterson PY. Interleukin 1 and myelin basic protein synergistically augment adoptive transfer activity of lymphocytes mediating experimental autoimmune encephalomyelitis in Lewis rats. J Immunol 1987; 138: 4229–35.

Marini JC, Jameson BA, Lublin FD, Korngold R. A CD4-CDR3 peptide analog inhibits both primary and secondary autoreactive $CD4^+$ T cell responses in experimental allergic encephalomyelitis. J Immunol 1996; 157: 3706–15.

Marks C, Marks JD. Phage libraries: a new route to clinically useful antibodies. N Engl J Med 1996; 335: 730–3.

Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives [published erratum appears in Science 1990; 248: 1066] [see comments]. [Review]. Science 1990; 248: 705–11. Comment in: Science 1990; 248: 685–6.

Martin D, Near SL. Protective effect of the interleukin-1 receptor antagonist (IL-1ra) on experimental allergic encephalomyelitis in rats. J Neuroimmunol 1995; 61: 241–5.

Martin R, McFarland HF, McFarlin DE. Immunological aspects of demyelinating diseases. [Review]. Annu Rev Immunol 1992; 10: 153–87.

Matsumoto Y, Fujiwara M. Adoptively transferred experimental allergic encephalomyelitis in chimeric rats: identification of transferred cells in the lesions of the central nervous system. Immunology 1988; 65: 23–9.

McDonald WI, Miller DH, Barnes D. The pathological evolution of multiple sclerosis. [Review]. Neuropathol Appl Neurobiol 1992; 18: 319–34.

McFarland HF. Complexities in the treatment of autoimmune disease [comment]. Science 1996; 274: 2037–8. Comment on: Science 1996; 274: 2054–7, Comment on: Science 1996; 274: 1707–9.

McGeehan GM, Becherer JD, Bast RC Jr, Boyer CM, Champion B, Connolly KM, et al. Regulation of tumour necrosis factor-α processing by a metalloproteinase inhibitor. Nature 1994; 370: 558–61.

McInnes IB, al-Mughales J, Field M, Leung BP, Huang F-P, Dixon R, et al. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. Nat Med 1996; 2: 175–82.

McMorris FA, McKinnon RD. Regulation of oligodendrocyte development and CNS myelination by growth factors: prospects for therapy of demyelinating disease. [Review]. Brain Pathol 1996; 6: 313–29.

McMurray RW. Adhesion molecules in autoimmune diseases. [Review]. Seminars in Arthritis and Rheumatism. Arthritis Rheum 1996; 25: 215–33.

Medaer R, Stinissen P, Truyen L, Raus J, Zhang J. Depletion of myelin-basic protein autoreactive T cells by T-cell vaccination: pilot trial in multiple sclerosis. Lancet 1995; 346: 807–8.

Meinl E, Weber F, Drexler K, Morelle C, Ott M, Saruhan-Direskeneli G, et al. Myelin basic protein-specific T lymphocyte repertoire in multiple sclerosis. Complexity of the response and dominance of nested epitopes due to recruitment of multiple T cell clones. J Clin Invest 1993; 92: 2633–43.

Meinl E, Aloisi F, Ertl B, Weber F, de Waal Malefyt R, Wekerle H, et al. Multiple sclerosis: Immunomodulatory effects of human astrocytes on T cells. Brain 1994; 117: 1323–32.

Meinl E, Hoch RM, Dornmair K, de Waal Malefyt R, Bontrop RE, Jonker M, et al. Encephalitogenic potential of myelin-basic-protein-specific T cells isolated from normal rhesus macaques. Am J Pathol 1997. In press.

Metzler B, Wraith DC. Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: influence of MHC binding affinity. Int Immunol 1993; 5: 1159–65.

Miller DH, Barkhof F, Nauta JJP. Gadolinium enhancement increases the sensitivity of MRI in detecting disease acitivity in multiple sclerosis. Brain 1993; 116: 1077–94.

Miller SD, McRae BL, Vanderlugt CL, Nikcevich KM, Pope JG, Pope L, et al. Evolution of the T-cell repertoire during the course of experimental immune-mediated demyelinating diseases. [Review]. Immunol Rev 1995a; 144: 225–44.

Miller SD, Vanderlugt C, Lenschow DJ, Pope JG, Karandikar NJ, Dal Canto MC, et al. Blockade of CD28/B7–1 interaction prevents epitope spreading and clinical relapses of murine EAE. Immunity 1995b; 3: 739–45.

Miller DH, Albert PS, Barkhof F, Francis G, Frank JA, Hodgkinson S, et al. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. Ann Neurol 1996a; 39: 6–16.

Miller DJ, Asakura K, Rodriguez M. Central nervous system remyelination. Clinical application of basic neuroscience principles. [Review]. Brain Pathol 1996b; 6: 331–44.

Mohler KM, Sleath PR, Fitzner JN, Cerretti DP, Alderson M, Kerwar SS, et al. Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. Nature 1994; 370: 218–20.

Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. [Review]. Annu Rev Immunol 1993; 11: 165–90.

Moreau T, Coles A, Wing M, Isaacs J, Hale G, Waldmann H, et al. Transient increase in symptoms associated with cytokine release in patients with multiple sclerosis. Brain 1996; 119: 225–37.

Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. [Review]. Immunol Today 1996; 17: 138–45.

Moss ML, Jin SLC, Milla ME, Burkhart W, Carter HL, Chen W-J, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-α. Nature 1997; 385: 733–6.

Murphy PM. The molecular biology of leukocyte chemoattractant receptors. [Review]. Annu Rev Immunol 1994; 12: 593–633.

Murphy E, Shibuya K, Hosken N, Openshaw P, Maino V, Davis K, et al. Reversibility of T helper 1 and 2 populations is lost after long-term stimulation. J Exp Med 1996; 183: 901–13.

Nagata S, Golstein P. The Fas death factor. [Review]. Science 1995; 267: 1449–56.

Nicholson LB, Greer JM, Sobel RA, Lees MB, Kuchroo VK. An altered peptide ligand mediates immune deviation and prevents autoimmune encephalomyelitis. Immunity 1995; 3: 397–405.

Noelle RJ. CD40 and its ligand in host defense. [Review]. Immunity 1996; 4: 415–9.

Nolte K-U, Günther G, von Wussow P. Epitopes recognized by neutralizing therapy-induced human anti-interferon-α antibodies are

localized within the *N*-terminal functional domain of recombinant interferon-α2. Eur J Immunol 1996; 26: 2155–9.

Norga K, Paemen L, Masure S, Dillen C, Heremans H, Billiau A, et al. Prevention of acute autoimmune encephalomyelitis and abrogation of relapses in murine models of multiple sclerosis by the protease inhibitor D-penicillamine. Inflamm Res 1995; 44: 529–34.

Olsson T. Critical influences of the cytokine orchestration on the outcome of myelin antigen-specific T-cell autoimmunity in experimental autoimmune encephalomyelitis and multiple sclerosis. [Review]. Immunol Rev 1995; 144: 245–68.

Ozawa K, Suchanek G, Breitschopf H, Bruck W, Budka H, Jellinger K, et al. Patterns of oligodendroglia pathology in multiple sclerosis. Brain 1994; 117: 1311–22.

Padovan E, Casorati G, Dellabona P, Meyer S, Brockhaus M, Lanzavecchia A. Expression of two T cell receptor α chains: dual receptor T cells. Science 1993; 262: 422–4.

Padovan E, Giachino C, Cella M, Valitutti S, Acuto O, Lanzavecchia A. Normal T lymphocytes can express two different T cell receptor β chains: implications for the mechanism of allelic exclusion. J Exp Med 1995; 181: 1587–91.

Panitch HS, Hirsch RL, Schindler J, Johnson KP. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. Neurology 1987; 37: 1097–102.

Pardoll DM, Beckerleg AM. Exposing the immunology of naked DNA vaccines. [Review]. Immunity 1995; 3: 165–9.

Paty DW, Li DKB, UBC MS/MRI Study Group, IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial [see comments]. Neurology 1993; 43: 662–7. Comment in: Neurology 1993; 43: 641–3.

Paty DW, Goodkin D, Thompson A, Rice G. Guidelines for physicians with patients on IFNβ-1b: The use of an assay for neutralizing antibodies (NAB). Neurology 1996; 47: 865–6.

Paul WE. Pleiotropy and redundancy: T cell-derived lymphokines in the immune response. [Review]. Cell 1989; 57: 521–4.

Paul WE, Seder RA. Lymphocyte responses and cytokines. [Review]. Cell 1994; 76: 241–51.

Perrin PJ, Scott D, Quigley L, Albert PS, Feder O, Gray GS, et al. Role of B7:CD28/CTLA-4 in the induction of chronic relapsing experimental allergic encephalomyelitis. J Immunol 1995; 154: 1481–90.

Perrin PJ, Scott D, Davis TA, Gray GS, Doggett MJ, Abe R, et al. Opposing effects of CTLA4-Ig and anti-CD80 (B7–1) plus anti-CD86 (B7–2) on experimental allergic encephalomyelitis. J Neuroimmunol 1996; 65: 31–9.

Perry VH, Andersson P-B, Gordon S. Macrophages and inflammation in the central nervous system. [Review]. Trends Neurosci 1993; 16: 268–73.

Petsko GA. For medicinal purposes [comment]. Nature 1996; 384 (Suppl): 7–9.

Piddlesden SJ, Storch MK, Hibbs M, Freeman AM, Lassmann H, Morgan BP. Soluble recombinant complement receptor 1 inhibits inflammation and demyelination in antibody-mediated demyelinating experimental allergic encephalomyelitis. J Immunol 1994; 152: 5477–84.

Polman CH, Hartung H-P. The treatment of multiple sclerosis: current and future. [Review]. Curr Opin Neurol 1995; 8: 200–9.

Powell MB, Mitchell D, Lederman J, Buckmeier J, Zamvil SS, Graham M, et al. Lymphotoxin and tumor necrosis factor-alpha production by myelin basic protein-specific T cell clones correlates with encephalitogenicity. Int Immunol 1990; 2: 539–44.

Prineas JW. The neuropathology of multiple sclerosis. [Review]. In: Vinken PJ, Bruyn GW, Klawans HL, editors. Handbook of clinical neurology, Vol. 47. Amsterdam: Elsevier, 1985: 213-57.

Rabinowitz JD, Beeson C, Lyons DS, Davis MM, McConnell HM. Kinetic discrimination in T-cell activation. Proc Natl Acad Sci USA 1996; 93: 1401–5.

Racke MK, Dhib-Jalbut S, Cannella B, Albert PS, Raine CS, McFarlin DE. Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor-β1. J Immunol 1991; 146: 3012–7.

Racke MK, Bonomo A, Scott DE, Cannella B, Levine A, Raine CS, et al. Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. J Exp Med 1994; 180: 1961–6.

Racke MK, Scott DE, Quigley L, Gray GS, Abe R, June CH, et al. Distinct roles for B7–1 (CD-80) and B7–2 (CD-86) in the initiation of experimental allergic encephalomyelitis. J Clin Invest 1995; 96: 2195–203.

Racke MK, Critchfield JM, Quigley L, Cannella B, Raine CS, McFarland HF, et al. Intravenous antigen administration as a therapy for autoimmune demyelinating disease. Ann Neurol 1996; 39: 46–56.

Raine CS. Demyelinating diseases. [Review]. In: Davis RL, Robertson DM, editors. 2nd ed. Textbook of Neuropathology. Baltimore: Williams & Wilkins, 1991: 535–620.

Raine CS. Multiple sclerosis: immune system molecule expression in the central nervous system. [Review]. J Neuropathol Exp Neurol 1994; 53: 328–37.

Raine CS, Lee SC, Scheinberg LC, Duijvestin AM, Cross AH. Adhesion molecules on endothelial cells in the central nervous system: an emerging area in the neuroimmunology of multiple sclerosis. [Review]. Clin Immunol Immunopathol 1990; 57: 173–87.

Raine CS, Wu E, Ivanyi J, Katz D, Brosnan CF. Multiple sclerosis: a protective or a pathogenic role for heat shock protein 60 in the central nervous system? Lab Invest 1996; 75: 109–23.

Ransohoff RM, Hamilton TA, Tani M, Stoler MH, Shick HE, Major JA, et al. Astrocyte expression of mRNA encoding cytokines IP-10 and JE/MCP-1 in experimental autoimmune encephalomyelitis. FASEB J 1993; 7: 592–600.

Reder AT, Lowy MT. Interferon-β treatment does not elevate cortisol in multiple sclerosis. J Interferon Res 1992; 12: 195–8.

Redondo C, Flores I, Gonzales A, Nagata S, Carrera AC, Merida I, et al. Linomide prevents the lethal effect of anti-Fas antibody and

reduces Fas-mediated ceramide production in mouse hepatocytes. J Clin Invest 1996; 98: 1245–52.

Rep MHG, Hintzen RQ, Polman CH, van Lier RAW. Recombinant interferon-β blocks proliferation but enhances interleukin-10 secretion by activated human T-cells. J Neuroimmunol 1996; 67: 111–8.

Rieckmann P, Albrecht M, Kitze B, Weber T, Tumani H, Broocks A, et al. Cytokine mRNA levels in mononuclear blood cells from patients with multiple sclerosis. Neurology 1994; 44: 1523–6.

Rieckmann P, Michel U, Albrecht M, Brück W, Wöckel L, Felgenhauer K. Soluble forms of intercellular adhesion molecule-1 (ICAM-1) block lymphocyte attachment to cerebral endothelial cells. J Neuroimmunol 1995; 60: 9–15.

Rieckmann P, Weber F, Günther A, Martin S, Bitsch A, Broocks A, et al. Pentoxifylline, a phosphodiesterase inhibitor, induces immune deviation in patients with multiple sclerosis. J Neuroimmunol 1996; 64: 193–200.

Röcken M, Racke M, Shevach EM. IL-4-induced immune deviation as antigen-specific therapy for inflammatory autoimmune disease. [Review]. Immunol Today 1996; 17: 225–31.

Rodriguez M, Miller DJ, Lennon VA. Immunoglobulins reactive with myelin basic protein promote CNS remyelination. Neurology 1996; 46: 538–45.

Rogy MA, Auffenberg T, Espat NJ, Philip R, Remick D, Wollenberg GK, et al. Human tumor necrosis factor receptor (p55) and interleukin 10 gene transfer in the mouse reduces mortality to lethal endotoxemia and also attenuates local inflammatory responses. J Exp Med 1995; 181: 2289–93.

Rollwagen FM, Baqar S. Oral cytokine administration. [Review]. Immunol Today 1996; 17: 548–50.

Romagnani S. Lymphokine production by human T cells in disease states. [Review]. Annu Rev Immunol 1994; 12: 227–57.

Rothenberg EV. How T cells count. [comment]. Science 1996; 273: 78–4. Comment on: Science 1996; 273: 104–6

Rott O, Fleischer B, Cash E. Interleukin-10 prevents experimental allergic encephalomyelitis in rats. Eur J Immunol 1994; 24: 1434–40.

Rowen L, Koop BF, Hood L. The complete 685-kilobase DNA sequence of the human β T cell receptor locus. Science 1996; 272: 1755–62.

Ruddle NH, Bergman CM, McGrath KM, Lingenheld EG, Grunnet ML, Padula SJ, et al. An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis. J Exp Med 1990; 172: 1193–200.

Rudick RA, Ransohoff RM. Cytokine secretion by multiple sclerosis monocytes. Relationship to disease activity. Arch Neurol 1992; 49: 265–70.

Rudick R, Antel J, Confavreux C, Cutter G, Ellison G, Fischer J, et al. Clinical outcomes assessment in multiple sclerosis. [Review]. Ann Neurol 1996a; 40: 469–79.

Rudick RA, Ransohoff RM, Peppler R, VanderBrug Medendorp S, Lehmann P, Alam J. Interferon beta induces interleukin-10 expression: Relevance to multiple sclerosis. Ann Neurol 1996b; 40: 618–27.

Ruoslahti E. Integrins. [Review]. J Clin Invest 1991; 87: 1–5.

Ruuls SR, de Labic MCDC, Weber KS, Botman CAD, Groenestein RJ, Dijkstra CD, et al. The length of treatment determines whether IFN-β prevents or aggravates experimental autoimmune encephalomyelitis in Lewis rats. J Immunol 1996; 157: 5721–31.

Samson MF, Smilek DE. Reversal of acute experimental autoimmune encephalomyelitis and prevention of relapses by treatment with a myelin basic protein peptide analogue modified to form long-lived peptide-MHC complexes. J Immunol 1995; 155: 2737–46.

Satoh J-I, Lee YB, Kim SU. T-cell costimulatory molecules B7–1 (CD80) and B7–2 (CD86) are expressed in human microglia but not in astrocytes in culture. Brain Res 1995; 704: 92–6.

Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr. Role of transcriptional activation of IkBα in mediation of immunosuppression by glucocorticoids [see comments]. Science 1995; 270: 283–6. Comment in: Science 1995; 270: 232–3.

Scherer MT, Ignatowicz L, Winslow GM, Kappler JW, Marrack P. Superantigens: bacterial and viral proteins that manipulate the immune system. [Review]. Annu Rev Cell Biol 1993; 9: 101–28.

Schluesener HJ, Lider O. Transforming growth factors β1 and β2: cytokines with identical immunosuppressive effects and a potential role in the regulation of autoimmune T cell function. J Neuroimmunol 1989; 24: 249–58.

Schluesener HJ, Wekerle H. Autoaggressive T lymphocyte lines recognizing the encephalitogenic region of myelin basic protein: in vitro selection from unprimed rat T lymphocyte populations. J Immunol 1985; 135: 3128–33.

Schwartz MA, Schaller MD, Ginsberg MH. Integrins: emerging paradigms of signal transduction. [Review]. Annu Rev Cell Biol 1995; 11: 549–99.

Schwartz RH. Models of T cell anergy: Is there a common molecular mechanism? [comment]. [Review]. J Exp Med 1996; 184: 1–8. Comment on: J Exp Med 1996; 184: 19–29.

Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by $CD4^+$ T cells. [Review]. Annu Rev Immunol 1994; 12: 635–73.

Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 1988; 23: 339–46.

Selmaj K, Brosnan CF, Raine CS. Colocalization of lymphocytes bearing γδ T-cell receptor and heat shock protein $hsp65^+$ oligodendrocytes in multiple sclerosis. Proc Natl Acad Sci USA 1991a; 88: 6452–6.

Selmaj K, Raine CS, Cannella B, Brosnan CF. Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. J Clin Invest 1991b; 87: 949–54.

Selmaj K, Raine CS, Farooq M, Norton WT, Brosnan CF. Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. J Immunol 1991c; 147: 1522–9.

Selmaj K, Papierz W, Glabinski A, Kohno T. Prevention of chronic relapsing experimental autoimmune encephalomyelitis by soluble tumor necrosis factor receptor I. J Neuroimmunol 1995; 56: 135–41.

Sen GC, Ransohoff RM. Interferon-induced antiviral actions and their regulation. [Review]. Adv Virus Res 1993; 42: 57–102.

Sercarz EE, Lehmann PV, Ametani A, Benichou G, Miller A, Moudgil K. Dominance and crypticity of T cell antigenic determinants. [Review]. Annu Rev Immunol 1993; 11: 729–66.

Sharief MK, Hentges R. Association between tumor necrosis factor-α and disease progression in patients with multiple sclerosis [see comments]. N Engl J Med 1991; 325: 467–72. Comment in: N Engl J Med 1992; 326: 272–3.

Shrikant P, Benveniste EN. The central nervous system as an immunocompetent organ. Role of glial cells in antigen presentation. [Review]. J Immunol 1996; 157: 1819–22.

Sinha B, Semmler J, Eisenhut T, Eigler A, Endres S. Enhanced tumor necrosis factor suppression and cyclic adenosine monophosphate accumulation by combination of phosphodiesterase inhibitors and prostanoids. Eur J Immunol 1995; 25: 147–53.

Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. [Review]. Annu Rev Immunol 1996; 14: 1–27.

Smilek DE, Wraith DC, Hodgkinson S, Dwivedy S, Steinman L, McDevitt HO. A single amino acid change in a myelin basic protein peptide confers the capacity to prevent rather than induce experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA 1991; 88: 9633–7.

Smith CA, Farrah T, Goodwin RG. The TNF receptor superfamily of cellular and viral proteins: Activation, costimulation, and death. [Review]. Cell 1994; 76: 959–62.

Sommer N, Loschmann P-A, Northoff GH, Weller M, Steinbrecher A, Steinbach JP, et al. The antidepressant rolipram suppresses cytokine production and prevents autoimmune encephalomyelitis [see comments]. Nat Med 1995; 1: 244–8. Comment in: Nat Med 1995; 1: 211–4.

Sornasse T, Larenas PV, Davis KA, De Vries JE, Yssel H. Differentiation and stability of T helper 1 and 2 cells derived from naive human neonatal $CD4^+$ T cells, analyzed at the single-cell level. J Exp Med 1996; 184: 473–83.

Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. [Review]. Cell 1994; 76: 301–14.

Stavnezer J. Regulation of antibody production and class switching by TGF-β. [Review]. J Immunol 1995; 155: 1647–51.

Stein CA, Cheng Y-C. Antisense oligonucleotides as therapeutic agents–is the bullet really magical? [Review]. Science 1993; 261: 1004–12.

Steinman L. A few autoreactive cells in an autoimmune infiltrate control a vast population of nonspecific cells: a tale of smart bombs and the infantry. [Review]. Proc Natl Acad Sci USA 1996; 93: 2253–6.

Steinman L, Rosenbaum JT, Sriram S, McDevitt HO. In vivo effects of antibodies to immune response gene products: Prevention of experimental allergic encephalitis. Proc Natl Acad Sci USA 1981; 78: 7111–4.

Steinman L, Waisman A, Altmann D. Major T-cell responses in multiple sclerosis. [Review]. Mol Med Today 1995; 1: 79–83.

Stevens DB, Gould KE, Swanborg RH. Transforming growth factor-β1 inhibits tumor necrosis factor-α/lymphotoxin production and adoptive transfer of disease by effector cells of autoimmune encephalomyelitis. J Neuroimmunol 1994; 51: 77–83.

St Louis J, Chan EL, Singh B, Strejan GH. Suppression of experimental allergic encephalomyelitis in the Lewis rat, by administration of an acetylated synthetic peptide of myelin basic protein. J Neuroimmunol 1997; 73: 90–100.

Stone LA, Frank JA, Albert PS, Bash C, Smith ME, Maloni H, et al. The effect of interferon-β on blood-brain barrier disruptions demonstrated by contrast-enhanced magnetic resonance imaging in relapsing-remitting multiple sclerosis. Ann Neurol 1995; 37: 611–9.

Stuve O, Dooley NP, Uhm JH, Antel JP, Francis GS, Williams G, et al. Interferon beta 1-b decreases the migration of T lymphocytes in vitro: effects on matrix metalloproteinase-9. Ann Neurol 1996; 40: 853–63.

Sun D, Ben-Nun A, Wekerle H. Regulatory circuits in autoimmunity: recruitment of counter-regulatory $CD8^+$ T cells by encephalitogenic $CD4^+$ T line cells. Eur J Immunol 1988a; 18: 1993–9.

Sun D, Qin Y, Chluba J, Epplen JT, Wekerle H. Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions. Nature 1988b; 332: 843–5.

Sun JB, Rask C, Olsson T, Holmgren J, Czerkinsky C. Treatment of experimental autoimmune encephalomyelitis by feeding myelin basic protein conjugated to cholera toxin B subunit. Proc Natl Acad Sci USA 1996; 93: 7196–201.

Taniguchi T. Cytokine signaling through nonreceptor protein tyrosine kinases. [Review]. Science 1995; 268: 251–5.

Taub DD, Turcovski-Corrales SM, Key ML, Longo DL, Murphy WJ. Chemokines and T lymphocyte activation: I. β chemokines costimulate human T lymphocyte activation in vitro. J Immunol 1996; 156: 2095–103.

Teitelbaum D, Fridkis-Hareli M, Arnon R, Sela M. Copolymer 1 inhibits chronic relapsing experimental allergic encephalomyelitis induced by proteolipid protein (PLP) peptides in mice and interferes with PLP-specific T cell responses. J Neuroimmunol 1996; 64: 209–17.

Thompson AJ, Noseworthy JH. New treatments for multiple sclerosis: a clinical perspective. [Review]. Curr Opin Neurol 1996; 9: 187–98.

Thompson AJ, Polman CH, Miller DH, Brochet B, Filippi M, Montalban K, et al. Primary progressive multiple sclerosis: a review. [Review]. Brain 1997. In press.

Thorpe JW, Kidd D, Moseley IF, Kenndall BE, Thompson AJ, MacManus DG, et al. Serial gadolinium-enhanced MRI of the brain and spinal cord in early relapsing-remitting multiple sclerosis. Neurology 1996; 46: 373–8.

Thrush GR, Lark LR, Clinchy BC, Vitetta ES. Immunotoxins: an update. [Review]. Annu Rev Immunol 1996; 14: 49–71.

Torcia M, Bracci-Laudiero L, Lucibello M, Nencioni L, Labardi D, Rubartelli A, et al. Nerve growth factor is an autocrine survival factor for memory B lymphocytes. Cell 1996; 85: 345–56.

Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. [Review]. Annu Rev Cell Biol 1993; 9: 317–43.

Trinchieri G, Perussia B. Immune interferon: a pleiotropic lymphokine with multiple effects. [Review]. Immunol Today 1985; 6: 131–6.

Urban JL, Kumar V, Kono DH, Gomez C, Horvath SJ, Clayton J, et al. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. Cell 1988; 54: 577–92.

Vandenbark AA, Hashim G, Offner H. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 1989; 341: 541–4.

Vandenbark AA, Celnik B, Vainiene M, Miller SD, Offner H. Myelin antigen-coupled splenocytes suppress experimental autoimmune encephalomyelitis in Lewis rats through a partially reversible anergy mechanism. J Immunol 1995; 155: 5861–7.

Vandenbark AA, Chou YK, Whitham R, Mass M, Buenafe A, Liefeld D, et al. Treatment of multiple sclerosis with T-cell receptor peptides: results of a double-blind pilot trial. Nat Med 1996a; 2: 1109–15.

Vandenbark AA, Hashim GA, Offner H. T cell receptor peptides in treatment of autoimmune disease: rationale and potential. [Review]. J Neurosci Res 1996b; 43: 391–402.

van Oosten BW, Lai M, Hodgkinson S, Barkhof F, Miller DH, Thompson AJ, et al. Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody Cm-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. Neurology 1996a; 47: 1531–4.

van Oosten BW, Rep MHG, van Lier RAW, Scholten PET, von Blomberg BME, Pflughaupt KW, et al. A pilot study investigating the effects of orally administered pentoxifylline on selected immune variables in patients with multiple sclerosis. J Neuroimmunol 1996b; 66: 49–55.

Vass K, Lassmann H. Intrathecal application of interferon gamma. Progressive appearance of MHC antigens within the rat nervous system. Am J Pathol 1990; 137: 789–800.

Vassalli P. The pathophysiology of tumor necrosis factors. [Review]. Annu Rev Immunol 1992; 10: 411–52.

Vaysburd M, Lock C, McDevitt H. Prevention of insulin-dependent diabetes mellitus in nonobese diabetic mice by immunogenic but not by tolerated peptides. J Exp Med 1995; 182: 897–902.

Viola A, Lanzavecchia A. T cell activation determined by T cell receptor number and tunable thresholds [see comments]. Science 1996; 273: 104–6. Comment in: Science 1996; 273: 78–9.

Wahl SM. Transforming growth factor β: the good, the bad, and the ugly. [Review]. J Exp Med 1994; 180: 1587–90.

Waisman A, Ruiz PJ, Hirschberg DL, Gelman A, Oksenberg JR, Brocke S, et al. Suppressive vaccination with DNA encoding a variable region gene of the T-cell receptor prevents autoimmune encephalomyelitis and activates Th2 immunity [see comments]. Nat Med 1996; 2: 899–905. Comment in:]. Nat Med 1996; 2: 857–9.

Wauben MHM, Joosten I, Schlief A, van der Zee R, Boog CJP, van Eden W. Inhibition of experimental autoimmune encephalomyelitis by MHC class II binding competitor peptides depends on the relative MHC binding affinity of the disease-inducing peptide. J Immunol 1994; 152: 4211–20.

Weber F, Meinl E, Aloisi F, Nevinny-Stickel C, Albert E, Wekerle H, et al. Human astrocytes are only partially competent antigen presenting cells: possible implications for lesion development in multiple sclerosis. Brain 1994; 117: 59–69.

Weinberg AD, Bourdette DN, Sullivan TJ, Lemon M, Wallin JJ, Maziarz R, et al. Selective depletion of myelin-reactive T cells with the anti-OX-40 antibody ameliorates autoimmune encephalomyelitis. Nat Med 1996; 2: 183–9.

Weiner HL, Mackin GA, Matsui M, Orav EJ, Khoury SJ, Dawson DM, et al. Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis [see comments]. Science 1993; 259: 1321–4. Comment in: Science 1993; 259: 1263.

Weiner HL, Friedman A, Miller A, Khoury SJ, al-Sabbagh A, Santos L, et al. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. [Review]. Annu Rev Immunol 1994; 12: 809–37.

Weinshenker BG, Bass B, Karlik S, Ebers GC, Rice GPA. An open trial of OKT3 in patients with multiple sclerosis. Neurology 1991; 41: 1047–52.

Weinstock-Guttman B, Ransohoff RM, Kinkel RP, Rudick RA. The interferons: biological effects, mechanisms of action, and use in multiple sclerosis. [Review]. Ann Neurol 1995; 37: 7–15.

Wekerle H. Antigen presentation by central nervous system glia. [Review]. In: Kettenmann H, Ransom B, editors. Neuroglial Cells. New York: Oxford University Press, 1995: 685–99.

Wekerle H, Linington C, Lassmann H, Meyermann R. Cellular immune reactivity within the CNS. [Review]. Trends Neurosci 1986; 9: 271–7.

Wekerle H, Kojima K, Lannes-Vieira J, Lassmann H, Linington C. Animal models. [Review]. Ann Neurol 1994; 36 Suppl: S47–53.

Wekerle H, Bradl M, Linington C, Kaab G, Kojima K. The shaping of the brain-specific T lymphocyte repertoire in the thymus. [Review]. Immunol Rev 1996; 149: 231–43.

Welsh CT, Rose JW, Hill KE, Townsend JJ. Augmentation of adoptively transferred experimental allergic encephalomyelitis by administration of a monoclonal antibody specific for LFA-1α. J Neuroimmunol 1993; 43: 161–7.

Whitaker JN, McFarland HF, Rudge R, Reingold SC. Outcomes assessment in multiple sclerosis clinical trials: a critical analysis. [Review]. Multiple Sclerosis 1995; 11: 37–47.

Williams K, Ulvestad E, Antel JP. B7/BB-1 antigen expression on adult human microglia studied in vitro and in situ. Eur J Immunol 1994; 24: 3031–7.

Williams LM, Gibbons DL, Gearing A, Maini RN, Feldmann M, Brennan FM. Paradoxical effects of a synthetic metalloproteinase inhibitor that blocks both p55 and p75 TNF receptor shedding and TNF-α processing in RA synovial membrane cell cultures. J Clin Invest 1996; 97: 2833–41.

Windhagen A, Newcombe J, Dangond F, Strand C, Woodroofe MN, Cuzner ML, et al. Expression of costimulatory molecules B7–1 (CD80), B7–2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. J Exp Med 1995a; 182: 1985–96.

Windhagen A, Scholz C, Hollsberg P, Fukaura H, Sette A, Hafler DA. Modulation of cytokine patterns of human autoreactive T cell clones by a single amino acid substitution of their peptide ligand. Immunity 1995b; 2: 373–80.

Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR. Making antibodies by phage display technology. [Review]. Annu Rev Immunol 1994; 12: 433–55.

Wolinsky JS. Copolymer 1: A most reasonable alternative therapy for early relapsing-remitting multiple sclerosis with mild disability [editorial comment] [see comments]. [Review]. Neurology 1995; 45: 1245–7. Comment on: Neurology 1995; 45: 1268–76, Comment in: Neurology 1996; 46: 883.

Wraith DC. Induction of antigen-specific unresponsiveness with synthetic peptides: specific immunotherapy for treatment of allergic and autoimmune conditions. [Review]. Int Arch Allergy Immunol 1995; 108: 355–9.

Wraith DC, McDevitt HO, Steinman L, Acha-Orbea H. T cell recognition as the target for immune intervention in autoimmune disease. [Review]. Cell 1989; 57: 709–15.

Wucherpfennig KW, Weiner HL, Hafler DA [see comments]. T-cell recognition of myelin basic protein [see comments]. [Review]. Immunol Today 1991; 12: 277–82. Comment in: Immunol Today 1991; 12: 464–5.

Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995; 80: 695–705.

Xia M, Leppert D, Hauser SL, Sreedharan SP, Nelson PJ, Krensky AM, et al. Stimulus specificity of matrix metalloproteinase dependence of human T cell migration through a model basement membrane. J Immunol 1996; 156: 160–7.

Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against α4β1 integrin. Nature 1992; 356: 63–6.

Yin D, Fathman CG. Induction of tolerance to heart allografts in high responder rats by combining anti-CD4 with CTLA4Ig. J Immunol 1995; 155: 1655–9.

Yu M, Johnson JM, Tuohy VK. A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease. J Exp Med 1996a; 183: 1777–88.

Yu M, Nishiyama A, Trapp BD, Tuohy VK. Interferon-β inhibits progression of relapsing-remitting experimental autoimmune encephalomyelitis. J Neuroimmunol 1996b; 64: 91–100.

Yuen EC, Mobley WC. Therapeutic potential of neurotrophic factors for neurological disorders. [Review]. Ann Neurol 1996; 40: 346–54.

Zambidis ET, Scott DW. Epitope-specific tolerance induction with an engineered immunoglobulin. Proc Natl Acad Sci USA 1996; 93: 5019–24.

Zamvil SS, Mitchell DJ, Lee NE, Moore AC, Waldor MK, Sakai K, et al. Predominant expression of a T cell receptor Vβ gene subfamily in autoimmune encephalomyelitis [published erratum appears in J Exp Med 1988; 168: 455]. J Exp Med 1988; 167: 1586–96.

Zhang J, Vandevyver C, Stinissen P, Raus J. In vivo clonotypic regulation in human myelin basic protein-reactive T cells by T cell vaccination. J Immunol 1995; 155: 5868–77.

Zinkernagel RM, Doherty PC. The discovery of NHC restriction. [Review]. Immunol Today 1997; 18: 14–7.

Zipp F, Weber F, Huber S, Sotgiu S, Czlonkowska A, Holler E, et al. Genetic control of multiple sclerosis: increased production of lymphotoxin and tumor necrosis factor-alpha by HLA-DR2+ T cells [see comments]. Ann Neurol 1995; 38: 723–30. Comment in: Ann Neurol 1995; 38: 702–4.

Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. [Review]. Immunol Today 1994; 15: 19–26.

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