

Th1/Th2 Balance: The Hypothesis, its Limitations, and Implications for Health and Disease

Parris Kidd, PhD

Abstract

One theory of immune regulation involves homeostasis between T-helper 1 (Th1) and T-helper 2 (Th2) activity. The Th1/Th2 hypothesis arose from 1986 research suggesting mouse T-helper cells expressed differing cytokine patterns. This hypothesis was adapted to human immunity, with Th1- and Th2-helper cells directing different immune response pathways. Th1 cells drive the type-1 pathway ("cellular immunity") to fight viruses and other intracellular pathogens, eliminate cancerous cells, and stimulate delayed-type hypersensitivity (DTH) skin reactions. Th2 cells drive the type-2 pathway ("humoral immunity") and up-regulate antibody production to fight extracellular organisms; type 2 dominance is credited with tolerance of xenografts and of the fetus during pregnancy. Overactivation of either pattern can cause disease, and either pathway can down-regulate the other. But the hypothesis has major inconsistencies; human cytokine activities rarely fall into exclusive pro-Th1 or -Th2 patterns. The non-helper regulatory T cells, or the antigen-presenting cells (APC), likely influence immunity in a manner comparable to Th1 and Th2 cells. Many diseases previously classified as Th1 or Th2 dominant fail to meet the set criteria. Experimentally, Th1 polarization is readily transformed to Th2 dominance through depletion of intracellular glutathione, and *vice versa*. Mercury depletes glutathione and polarizes toward Th2 dominance. Several nutrients and hormones measurably influence Th1/Th2 balance, including plant sterols/

sterolins, melatonin, probiotics, progesterone, and the minerals selenium and zinc. The long-chain omega-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) significantly benefit diverse inflammatory and autoimmune conditions without any specific Th1/Th2 effect. Th1/Th2-based immunotherapies, e.g., T-cell receptor (TCR) peptides and interleukin-4 (IL-4) injections, have produced mixed results to date.

(*Altern Med Rev* 2003;8(3):223-246)

Introduction

The Th1/Th2 balance hypothesis emerged in the late 1980s, stemming from observations in mice of two subtypes of T-helper cells differing in cytokine secretion patterns and other functions.¹ They suggested these "Th1" and "Th2" cells were "important regulators of the class of immune response." The concept subsequently was applied to human immunity,² and 10 years after the original discovery the effects of Th1 and Th2 in disease became a major research focus.^{3,4} Currently much of the literature elevates the Th1/Th2 balance concept to the level of paradigm. Although Th1 and Th2 cells are now virtually anointed with the responsibility for coordinating the immune system, critical investigators are finding discrepancies in the hypothesis.^{5,6}

Parris Kidd, PhD – University of California, Berkeley, PhD in cell biology. Contributing editor, *Alternative Medicine Review*. Health educator; biomedical consultant to the dietary supplement industry.
Correspondence address: 847 Elm Street, El Cerrito, CA 94530

This review critiques the substantial body of literature related to Th1/Th2 balance and its putative role in health and disease. The hypothesis is measured against the cumulative data and some limitations are defined. Emphasis is placed on what is really known from humans, rather than from animal models, which, by their very nature, have limited relevance to the human condition. Nutritional and nontoxic approaches to manipulating Th1/Th2 balance are discussed in the context of immune homeostasis (“immunostasis”) and the paramount need to first do no harm in the management of disease.

Background to the Hypothesis

Of all the body’s organ systems, the immune system may be the most challenging to coordinate. The system is a disparate, far-flung collection of individual immune cells, immune cell aggregates, immune tissues, and immune organs.⁷ This mode of structural organization renders the system able to respond promptly and effectively to anything within the body not perceived as self. Functionally, a great variety of diffusible substances are used to convey messages, give instructions, and generally enable the billions of immune cells to communicate with each other. The Th1/Th2 hypothesis rests largely on the cytokine patterns (chemicals released by these cells that promote cell-to-cell communication) of these two cell types.

The pivotal role played by T-helper cells in amplifying immune responsiveness is well established. Mossman and Coffman’s original proposal was that mouse T-helper cell populations could be subclassified based on the cytokines they secreted. After much probing and controversy, it now seems that Th1 cells and the pathway they dominate are heavily reliant on interferon-gamma (IFN-gamma), and to a lesser extent interleukin-2 (IL-2) and interleukin-12 (IL-12). Th2 cells are most heavily reliant on interleukin-4 (IL-4) and sometimes interleukin-5 (IL-5) as well. Th1 and Th2 cells also can differ in the arrays of receptors on their outer surface that they use to respond to cytokines and other messenger substances.

Perhaps coincidentally, but as happens often in science, the hypothesis emerged as a by-product of new cell-cloning techniques and new assays for cytokines.⁴ Two features further increased the appeal of the hypothesis.⁴ First, each cell subset produced cytokines that served as their own growth factors. A type of feed-forward loop promoted further differentiation of that subset (autocrine effects). Second, the two subsets produced cytokines that seemed to cross-regulate each other’s development and activity (Figure 1).

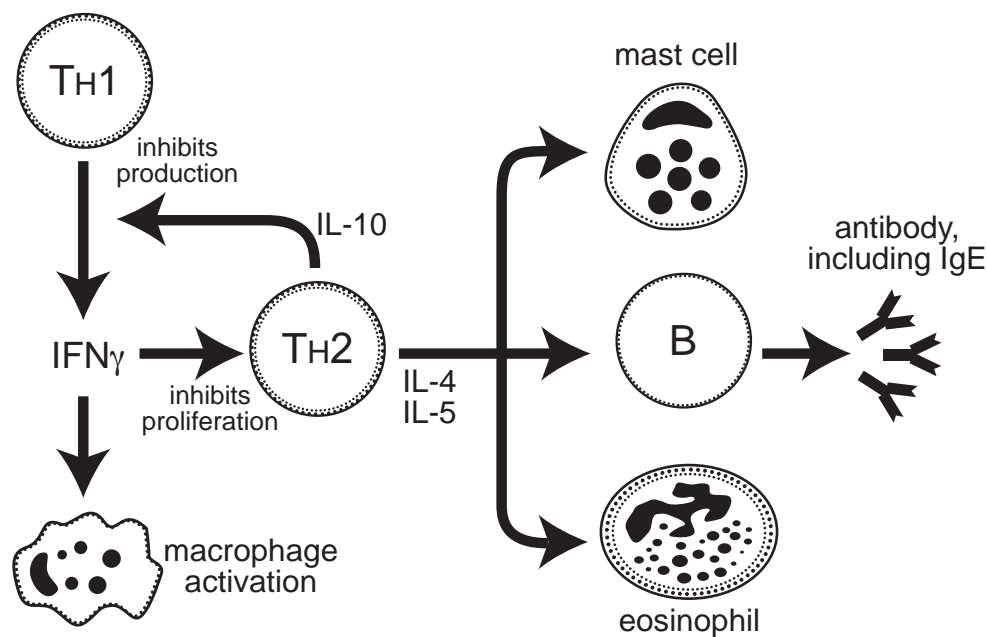
In the current research literature Th1 cells (now sometimes called “Type 1 immunity”) and Th2 cells (“Type 2 immunity”) are invoked to rationalize virtually all the known patterns of immune response. Th1 cells are hypothesized to lead the attack against intracellular pathogens such as viruses, raise the classic delayed-type hypersensitivity (DTH) skin response to viral and bacterial antigens, and fight cancer cells. Th2 cells are believed to emphasize protection against extracellular pathogens such as multicellular parasites. On the negative side, the Th1 pathway is often portrayed as being the more aggressive of the two, and apparently, when it is overreactive, can generate organ-specific autoimmune disease (e.g., arthritis, multiple sclerosis, type 1 diabetes).⁶ The Th2 pathway is seen as underlying allergy and related IgE-based disease, and predisposing to systemic autoimmune disease. But these stereotypes have proven to be oversimplistic, with the result that the hypothesis is increasingly criticized.^{5,8}

The Mechanics of the Hypothesis

A Central Role for T-Helper Cells in Immune Responses

There may be as many patterns of immune response as there are immune cells. The very power of this system is that it can follow a variety of different patterns depending on a multitude of factors, including the nature and concentration of the offending agent, the conditions that prevail in the immediate microenvironment of the responsive cells, and the host’s functional capacity to respond. In the face of these varying conditions,

Figure 1. Textbook Representation of the Th1/Th2 Hypothesis



Adapted from: Roitt I, Brostoff J, Male D. *Immunology (Fifth Edition)*. Philadelphia: Mosby; 1998.

Th1 cells secrete the cytokine interferon-gamma and activate inflammatory pathways mainly via macrophage activation. Th2 cells secrete cytokines interleukin-4 and -5 that upregulate antibody formation via B cells, mast cells, eosinophils, and other pathways. Th1 and Th2 cells can cross-inhibit each other.

the system must constantly be adaptive, mobilizing and functionally integrating its numerous cell types for rapid response. The Th1/Th2 hypothesis in its modern incarnation endows these two types of immune cells as the system supervisors.

From all that is known about immune system mechanics, Th1 and Th2 cells are definitely positioned in the functional web. But it is also established that the T-helper cells are downstream from the event that initiates the primary immune drive: perception of nonself or other antigens as potentially dangerous (Figure 2).⁹ Curiously, published studies on Th1/Th2 dominance often downplay the dendritic cells (DCs), monocyte-macrophages, and other antigen-presenting cells

(APCs). Judging from the current body of knowledge, these cells are just as strongly qualified to supervise immunity.^{7,10-13}

Differing Cytokine Patterns Define Th1/Th2 Dominance

Functional integration of the immune system is accomplished mainly by cell-to-cell communication that relies on small molecules. This is the language of immunity, a chemical kind of information transmission, simplistically “talk-crosstalk.” Every immune system cell is equipped to syn-

thesize and release a variety of small molecules that travel to other cells (both immune and nonimmune) and stimulate those cells to become either more active (up-regulated) or less active (down-regulated; modulated). These are the words of the immune vocabulary and chemically most of them are cytokines.^{14,15}

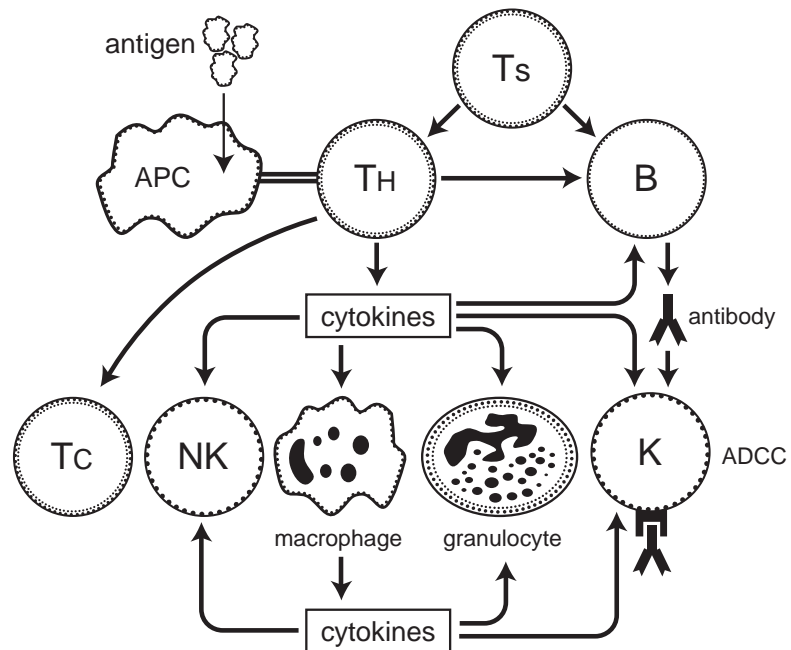
Cytokines are proteins or peptides, some of which have sugar molecules attached.⁷ They are a large group of molecules and include the interferons (IFNs), interleukins (ILs), and various colony-stimulating factors (CSFs). Also included are the tumor necrosis factors (TNFs) and transforming growth factors (TGFs), thought to be

particularly important in mediating inflammatory and cytotoxic reactions.⁷ The distinctive pattern of effect of each cytokine depends on concentration-dependent binding to specific receptors on the surface of the target cells and subsequent activation of cellular machinery at the cell membrane level or, in some cases, at the level of the nucleus and genetic machinery.^{11,15}

The Th1/Th2 concept rests largely on a dichotomy of cytokine profiles; however, as with other immune cells, the array of cytokines produced by the Th1 and Th2 cells varies greatly and is influenced by a large number of experimental variables, as well as the danger from artifacts. Some of the most important variables in cytokine biology include the species being researched (laboratory rodents differ substantially from humans). It is important to distinguish whether the study is done *in vivo* (most reliable), *ex vivo* (cells removed from the body and quickly assayed), or *in vitro* (in longer-term culture, least reliable because the cells typically undergo profound changes).^{15,16}

Most of the supposed differences in cytokine profiles between Th1 and Th2 responses were gleaned from earlier phases of research that relied heavily on mice and cultured cells. The more recent research involving human subjects has proved much of this earlier work to be highly simplistic or otherwise inaccurate.⁵ The most current literature (years 2002-2003) still carries much of this inaccurate information; currently the only defensible claims are that the Th1 cells produce far more IFN-gamma and IL-2 than do Th2 cells,

Figure 2. The Functional Flow of Immunity following Antigen Detection

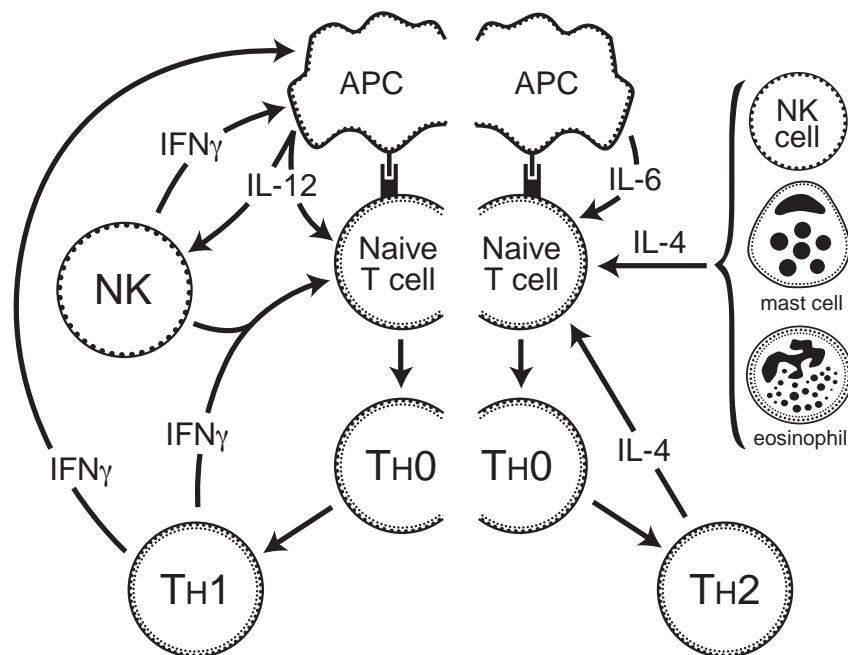


Adapted from: Roitt I, Brostoff J, Male D. *Immunology (Fifth Edition)*. Philadelphia: Mosby; 1998.

Antigen-presenting cells process antigens and present fragments to T-helper cells (Th). Subsequently these, with the help of cytokines, coordinate the activities of the various immune cell types to effectuate an immune response. Ts = T suppressor cell; Tc = cytotoxic T-lymphocyte; B = B cell; NK = natural killer cell; K = killer cell; ADCC = antibody-dependent cytotoxic cell.

while the Th2 cells produce far more IL-4 (and perhaps IL-5) than do the Th1 cells. IL-10, formerly assumed to be the major means by which Th2 cells down-regulate Th1 cells (Figure 1), is also produced in comparable amounts by other cell types.¹¹

Figure 3. A Rough Schematic of the Cytokine-directed Differentiation of Th1 and Th2 Cells from Naive T Cells



Adapted from: Lafaille JJ. The role of helper T cell subsets in autoimmune diseases. *Cytokine & Growth Factor Revs* 1998;9:139-151.

Antigen-presenting cells interact via antigenic sharing with the relatively undifferentiated naive T cells, secreting specific cytokines that urge them to differentiate ("polarize"), first into null T-helper cells (Th0), then into Th1 or Th2 cells. Natural killer cells likely assist in the polarization process.

Th1 and Th2 Polarization Built on Cytokine Patterns

Both the Th1 and Th2 subsets are produced from a non-committed population of precursor T cells – naive T cells. The differentiation proceeds within a few days of direct contact with naive cells by APCs.¹⁷ The process by which commitment develops is called polarization (Figure 3). The naive T cells may pass through a transient, pre-activation state (T0) on their way to becoming Th1 or Th2 cells. Both subsets contain effector cells that do the immediate work, and memory cells that retain the experience for future action as

necessary. Polarization of the T-helper cells could be indicative of a more profound polarization of the immune system as a whole. There is good evidence that a kind of type 1/type 2 polarization already begins with those cells having the primary contact with antigens, including the DCs, monocytes and macrophages, and other APCs. These APCs likely polarize into type 1 and type 2 cells in response to the type of antigen experience, then subsequently bias the polarization of the T-helper population functionally "downstream" from them.¹¹

The polarization process is driven mainly by cytokines. Experiments with "knockout" mice, substrains with specific genes subtracted from their genome, have made it easier to decipher how the many cytokines contribute to immune cell maturation.¹⁴ The cytokine micro-environment of a T cell, especially the various molecular types

and subtypes and concentration oscillations over time and distance, is a kind of dynamic signal matrix that interacts with receptors on that cell's surface to determine whether the cell matures into a Th1, Th2, or other differentiated T-cell type.^{14,15} Non-cytokine factors (e.g., chemokines, eicosanoids, oxygen free radicals, and various inflammatory mediators) all contribute to the signal matrix and influence the net outcome.

Chemokines are substances that attract cells to migrate in a particular direction (chemotaxis). Like cytokines, chemokines operate via binding to receptors on the outer surface of the

immune cell. The various chemokines also have overlapping effects on their target cells. Comparisons between Th1 and Th2 subsets suggest they differentially express surface chemokine receptors and that chemokines are involved in the initiation and amplification of type 1 and type 2 responses.¹⁸

Factors Complicating the Hypothesis

Despite the huge body of ongoing research focused on cytokines specific to a Th1 or Th2 response and on their potential applications as pharmacologic immune response modifiers, evidence indicates that many other dynamic factors influence Th1/Th2 maturation. Among these are: antigen dose, nature of the antigen, direct cell-to-cell interaction with APCs, the diversity and relative intensity of these interactions, and the cytokine receptors available on the naive cell. This “wholistic” scenario for Th1/Th2 maturation is more consistent with the great mass of existing evidence than are other scenarios that envision “master” cytokines controlling T-helper cell activity.

Th1/Th2 Differentiation Initiated by Antigen-Presenting Cells

Th1 or Th2 cell populations actually come into existence under the control of APCs. The most proximate step initiating the differentiation of a naive T cell involves intimate physical contact with one such cell from this population, distributed throughout the tissues as sentinels – early warning guards – for the immune system. They are mostly DCs, with long finger-like projections, extending great distances between the cells of non-immune tissues. Once a nonself antigen⁷ or other material judged dangerous⁹ above an exposure threshold is detected, the APCs spring into action. It likely becomes type-1 or type-2 biased, depending on the nature of its antigen exposure.

A DC or related APC exposed to an intracellular pathogen (or perhaps a cell wall antigen or other smaller fragment of the organism) will likely become type-1 biased. It promptly migrates to a nearby lymph node and begins to secrete

IL-12 (Figure 3). As this cytokine builds in concentration it begins to influence naive T cells to eventually become Th1 cells. Natural killer (NK) cells also respond to the IL-12 environment and proceed to release IFN-gamma, which reinforces the APC's production of IL-12 and also helps drive the naive T-cell commitment process. As they attain maturity, Th1 cells also produce IFN-gamma, which (together with the NK cells) stimulates the APC and naive T cells to polarize into more Th1 cells, in a self-reinforcing “autocrine” loop.

Like the Th1 cells, the emergence of Th2 cells is also dependent on their cytokine environment. Their maturation is likely initiated by the cytokine IL-6 from an APC, but also driving their maturation is IL-4 released by NK cells, mast cells, and eosinophils (Figure 3). As the Th2 cells mature they also produce IL-4, which together with the other participating cell types generates an autocrine loop to the naive T cells to make more Th2 cells. IL-6 also is produced by multiple cell types, including macrophages, endothelial cells, fibroblasts, and mast cells; the significance of this for Th2 determination is not yet apparent.

Commitment to Th1 or Th2 cells appears to be final, since efforts to reverse such differentiated cells have not been successful. If Th1/Th2 “reversal” ever does occur *in vivo*, as has been hypothesized, it must be on the basis of switched polarization from naive T cells.¹⁷

Regulatory T Cells Operate Outside of Th1/Th2

A key feature of the hypothesis is that Th1 and Th2 cells can antagonize each other's actions, either by blocking polarized maturation of the opposite cell type or by blocking its receptor functions.¹⁷ As examples, IFN-gamma secreted by Th1 cells can block the proliferation of Th2 cells, and high concentrations of IL-4 or IL-6 can block the generation of Th1 cells from naive T cells. However, other immune cell types can also intervene to block either Th1 or Th2 activity or both. These are the regulatory T cells.

Several subsets of regulatory T cells, the Tr cells, have been identified that appear to be distinct in function and phenotype (surface

characteristics) from the Th1 and Th2 populations.¹⁹ Type 1 Tr cells (Tr1) secrete high levels of IL-10 and low-to-moderate levels of TGF-beta. Type 3 (Tr3) cells primarily secrete TGF-beta and the CD4+CD25+ cells inhibit immune responses through cell-to-cell contact. The CD8+ Tr cells secrete either IL-10 or TGF-beta. Functional studies indicate Tr1 cells and other Tr populations may help terminate Th1-related inflammatory responses to pathogens, tumors, and alloantigens.²⁰

The Th3 subset of Tr cells was first identified in connection with studies of oral tolerance in multiple sclerosis. Treatment of MS patients by mouth with myelin basic protein (MBP) caused this cell population to increase in number and secrete more TGF-beta.²¹ Since this cytokine acts on multiple cell types, Th3 cells may be involved in many facets of immune regulation.²²

The CD4+CD25+ Tr cells comprise 5-10 percent of the total peripheral T-cell pool.¹⁹ Defined better by their surface markers than by cytokine profile, they carry the CD4 receptor but also the CD25, which is actually the IL-2 alpha receptor. These cells are potent immunosuppressants, probably by virtue of surface interactions with other cells than by cytokine secretion pattern.

Th1 Cells Boost Antiviral and Antibacterial Resistance

One aspect of the Th1/Th2 hypothesis is that the Th1 pathway primarily acts against intracellular pathogens, particularly viruses and bacteria. Findings regarding tuberculosis infection are consistent with this claim.

Th1 Cells Fight Mycobacterium tuberculosis

Infection with *Mycobacterium tuberculosis* (Mt) is a major health problem worldwide, with an estimated 7.5 million cases each year – more than 95 percent in developing countries.²³ The first line of host defense against Mt is provided by macrophages through nonspecific mechanisms of resistance, such as

phagocytosis. However, if a successful invasion takes hold the T cells become involved 2-3 weeks later. Studies of patients with familial susceptibility to Mt infection have confirmed that Th1 vigor is essential for protection.²³ Mutations affecting IFN-gamma receptors, IL-12 production, or IL-12 receptors (all Th1 mediated) also increase susceptibility to Mt and, in cases where IFN-gamma cannot be produced or responded to, the disease is severe and often fatal.^{23,24} There seems little doubt that successful immunity against Mt requires strong Th1 performance.

In 2002 a large team drawn from several African and European countries reported on an in-depth controlled study of Mt infection patterns and clinical courses in 414 infected patients, 414 paired community controls, and 414 household (healthy) controls in Gambia and Senegal.²³ Rather than assess Th1 and Th2 activity by serum levels of IFN-gamma and IL-4, which they rightfully deemed difficult to interpret, they measured Th1 activity by plasma soluble lymphocyte activating gene-3 (sLAG-3), and Th2 activity by plasma IgE, soluble CD30, and MDC/CCL-22 (a chemokine derived from macrophages). The patients were recruited at diagnosis and found to have significantly lower sLAG3 (Th1), whereas healthy household controls had higher sLAG-3 than the community controls. All the Th2 markers were consistently higher in the patients than in the community controls; household controls in contrast had lower Th2 indicators than the community controls. The implication from this data is that individuals with weaker Th1 and/or stronger Th2 activity were more at risk for infection than others in their household or community.

The infected patients were treated and then retested after 2-3 months of treatment or after 6-8 months when treatment ended.²³ After 2-3 months of treatment the sLAG-3 levels (Th1) were nearly quadrupled, and after 6-8 months they were nearly six times higher than at baseline – to a level higher than the community controls. Concomitantly, Th2 markers were reduced one-third after 2-3 months and by almost two-thirds by the end of treatment – to values lower than the community controls. In the household controls, Th1/Th2

values tended to become similar to those of the community controls, presumably because immediate exposure to Mt was removed during the treatment of their diseased contact.

From this large, well conducted, controlled study it is evident that clinical healing of tuberculosis is associated with a shift toward higher Th1 activity and lower Th2 activity.²³ The investigators noted that a full course of treatment was required to achieve this effect, and that patients who had poor clinical outcomes did not achieve this shift. They encourage further investigation into possible means to achieve early up-regulation of a Th1 response in TB patients to accelerate healing.

HIV-1 Infection and AIDS: Th1 only or Th1/Th2?

One of the most notable immunological defects in HIV-1 infected individuals is poor HIV-1-specific T-helper cell activity at all stages of the disease. Chronic HIV-1 infection also features depletion of T-helper cells that normally secrete IFN-gamma (a sign of Th1 activity).²⁵ Despite initial optimism, the experience with highly active antiretroviral therapy (HAART) is that it does not eradicate HIV-1 infection. Emphasis has been placed on finding mechanisms that promote immunological control of viral replication over the long term. One of these is to support, and wherever possible, to restore the Th1 pathway.

Vigorous activity of HIV-1-specific Th1 cells and cytotoxic T-lymphocytes (CTL) appears to play a critical role in containment of the viral infection, in part via lysis of virus-infected cells.²⁵ Research in animal models and in human cytomegalovirus infection also demonstrates a close link between T-helper cell vigor and CTL performance. A small subset of infected individuals have been able to survive with HIV-1 for over 20 years, clinically asymptomatic and with normal CD4+ counts in the absence of antiviral therapy. Many (although not all) of these individuals have vigorous virus-specific T-helper and CTL responses.²⁶

HIV-1 specific cellular immune responses (Th1) are inversely correlated with plasma viremia – the better the response the lower the virus

load. In repeated studies on chronically infected individuals untreated with antiretrovirals, the strongest T-cell proliferative responses against viral p24 antigen were associated with the lowest viral loads and vice versa.²⁵ These Th1 cells are the very ones that decline in numbers over time in untreated or HAART-unresponsive individuals. It is not understood how long-term nonprogressors manage to conserve these cell populations over decades without antiretroviral treatment.

Imami and collaborators found HIV-1 infected nonprogressor individuals also have fairly vigorous Th2 activity (measured as IL-4 secretion).²⁷ They also found the nonprogressors had a more restricted Th2 response to HIV antigens, and suggest this might reflect some kind of Th1/Th2 cross-modulation and/or cross-regulation that enables nonprogressors to keep the virus under control. Various researchers claimed to find a shift from Th1 dominance to Th2 dominance during HIV-disease progression, but others failed to confirm these reports.²⁸ Further work is needed to resolve this important discrepancy. Current understanding of HIV-1/AIDS strongly suggests a vigorous Th1 response in conjunction with a moderate but not overly vigorous Th2 response is important for long-term control of HIV-1.²⁹

Children infected with HIV-1 can be treated with HAART to lower viral load and allow their immune status to rebound. Children who achieve viral suppression after five months or more of HAART may not show increased percentages of CD4+ (total helper) cells, although their capacity to produce IFN-gamma (Th1) and IL-2 is partially restored.³⁰ The recovery is usually more robust in children under age three with good thymus mass. Furthermore, those children who respond favorably to HAART with virus suppression show higher production of IL-10, a cytokine doubtful to be simply Th2 dominant, but important for HIV-1 suppression. Therefore, it seems that in children with HIV-1, the Th1 pathway, with help from cells that produce IL-10 (whatever their identity), confers potential for long-term successful resistance to the virus.

Some “Th1 Overactivation Diseases” are Mixed Th1/Th2 Conditions

Rheumatoid arthritis (RA), type 1 diabetes, and multiple sclerosis (MS) are chronic inflammatory and autoimmune diseases. All three have been extensively researched and have “animal models” that supposedly mimic the human disease and allow for more in-depth mechanistic analysis. Many advocates of the Th1/Th2 hypothesis believe these diseases are examples of Th1 dominance, and a great deal of highly technical developmental research has been undertaken based on these assumptions. Indeed, if pathogenic Th1 dominance (or Th2, for that matter) were conclusively established for any major disease, the current intense focus on Th1 and Th2 interventions would be more justified. However, to date none of these diseases has been proven to be Th1 dominant.

Rheumatoid Arthritis

Although the pathogenesis of this disease is far from completely understood, T-helper cells do seem to play a central role in its autoimmune manifestations.³¹ Activated T-helper cells are found in the inflammatory filtrates, and T cell-directed therapies have provided some clinical benefit. Recent evidence suggests that their pathogenic role may extend beyond antigen recognition in the joint, to more systemic involvement.³¹

Gerli and collaborators isolated T-helper cells from the blood and synovial fluid of four patients with active RA: two untreated at onset of the disease, one in the early phase during treatment, and one in the chronic phase.³² The cells were cloned and assayed for cytokine activity. All four patients with active RA had strong predominance of Th1 clones in the blood and a slight prevalence of naive, undifferentiated T-cell clones in the joints. Those with early onset RA had primarily IL-4, indicative of Th2 activity. Those not yet being treated manifested IL-10 (perhaps from Th2 cells but also likely Tr cells). Gerli interpreted the data to mean RA is mainly a Th1-driven condition, although a Th2 response is evident early in the disease process.³²

Other indirect support for RA as Th1-driven is the clinical observation that pregnancy ameliorates the progression of RA.³³ Pregnancy improves the symptoms of RA in 75 percent of patients, leading to a significant resolution of inflammation and sufficient symptom relief to enable patients to taper off or even stop medications. In fact, the positive effect of pregnancy alone has been deemed greater than the benefit of some of the newer therapeutic agents.³¹ Exactly how pregnancy can have such marked benefit for RA is not known, but it seems clear that Th1 immunity becomes markedly diminished during pregnancy, in favor of Th2 dominance, which confers maternal tolerance of the developing fetus.

Diminished Th1 activity during pregnancy may help explain why pregnant women have a higher incidence of infections, especially with intracellular pathogens, than non-pregnant women.³¹ In addition, the DTH response indicative of Th1 activity is lowered during pregnancy. Interestingly, relapses of RA occur within six months postpartum in 90 percent of cases.³¹ At that time, pregnancy-associated alterations in Th1/Th2 balance can no longer be found, suggesting the Th2-dominant state during pregnancy has waned, allowing the pre-pregnancy Th1-dominated state to become reinstated.

Several studies have demonstrated decreased prevalence of allergic diseases in RA patients.³¹ The prevalence of hay fever is halved (4% in RA patients versus 8% in controls), and the symptoms are less severe. Atopy is 20-40 percent less prevalent in RA patients. As allergy and atopy are very likely Th2-dominant conditions, these findings are consistent with RA being driven by Th1 dominance and being down-regulated by the Th2 pathway when it becomes overactive.

Cytokines are increasingly being employed therapeutically to control malignancies and viral infections. Thus, IL-12 has been used to enhance cellular cytotoxicity. However, when IL-12 was applied to a woman with metastatic cervical cancer, her RA was severely exacerbated.³¹ IL-12 is a strong inducer of Th1 cell development with concomitant production of IFN-gamma, also a potent Th1 inducer (Figure 3).

IFN-gamma administration itself tends to induce autoimmune responses, and several clinicians have noticed first onset of RA or exacerbation of pre-existing RA following IFN-gamma administration.³⁴

The findings from various human and animal studies do not support such a clear-cut role for Th1 as the driving force in RA.³¹ For example, assessment of cytokine messenger ribonucleic acid (mRNA) isolated from blood of patients with RA yielded variable results. While 5/14 patients had elevated IFN-gamma (Th1), 3/14 had elevated IL-4 (Th2). From synovial tissue 8/10 samples had elevated IFN-gamma and 2/10 had elevated IL-4.³¹ In reactive arthritis, an RA-related disease triggered by persistent intracellular bacteria, 6/8 samples had IL-4.³⁵ The animal models of RA feature even more diverse and profound discrepancies from the expected.³¹

Schulze-Koops and Kalden rendered an articulate defense of the Th1 hypothesis for RA.³¹ They suggest the human findings may be obscured by heterogeneity of RA expression and small trial sample sizes, and that with the animal models biological differences from human RA may be involved. Certainly human subjects are highly variable in individual disease expression, and laboratory animal models of disease consistently differ from the human state. Schulze-Koop and Kalden also suggest that several of the current anti-RA drugs work by altering Th1/Th2 balance. But evidence for this is indirect and comes mostly from non-clinical settings. These Th1 advocates concede it may be overly simplistic to remold the RA data to make it fit the Th1/Th2 hypothesis. They admit it is possible Th1 is subject to simple guilt-by-association with RA, rather than being a major mechanism driving the disease.

To date little direct clinical effort has been made to modulate the Th1/Th2 balance in RA patients. IL-4 (that key Th2 cytokine) given IV had no apparent benefit and significant adverse effects.³¹

TCR (T-cell receptor) peptide therapy was pioneered by Vandenbark and a mixed academic-corporate group,³⁶ and involves vaccination with TCR-peptide fragments normally expressed on

Th1 cells. The rationale is that human autoimmune disease often involves Th1 cells reactive against self antigens. There is evidence all individuals carry such autoreactive Th1 cells but that in healthy people they are kept silent by natural regulatory mechanisms. Th1 cells have a penchant for displaying autoantigenic fragments of the normal TCR on their surface, which, according to the Th1/Th2 hypothesis, can be sensed by Th2 cells as signals to release Th2 cytokines (mostly IL-10) that down-regulate autoreactive Th1 cells. Vandenbark and colleagues made peptide portions of a series of TCR fragments into vaccines for subdermal or intramuscular injection. The vaccines were apparently safe and well tolerated, but not particularly beneficial. Of 484 RA patients vaccinated, one-third had measurable immunological response to vaccine injection. A minority experienced marginal clinical improvement and a handful showed excellent improvement.

Multiple Sclerosis

The data from MS patients falls short of definitive support for Th1 involvement. One study reported markedly elevated levels of the Th1 cytokine IL-12.³⁷ Another study found no difference in the cytokine pattern of myelin basic protein (MBP) reactive T-cells in patients with MS as compared with healthy individuals.³⁸ Yet another study found both IFN-gamma (Th1) and IL-4 (Th2) elevated in serum from MS patients in the acute stage, suggesting perhaps simultaneous overactivation of Th1 and Th2 subsets.³⁹ Experimental allergic encephalomyelitis (EAE), the animal model for MS and probably the most heavily studied of the animal models, likewise has yielded equivocal findings.

Singh and collaborators have reviewed the EAE findings,⁶ and found EAE can be passively transferred by injecting Th1 cells from an EAE animal into a non-EAE host. Th2 cells do not react this way, which gives strong support for Th1 dominance. Also, oral administration of MBP (one confirmed MS/EAE autoantigen) boosts Th2 cytokine production, which could be interpreted as down-regulation of Th1 activity. But when EAE

goes into spontaneous remission, a predictable occurrence, the Th2 cytokine IL-4 seemingly is not required; and MBP-primed Th2 cells can cause EAE in immunodeficient mice, rather than protect them as Th1 dominance would predict.

Re-examination of EAE in the light of recent findings leaves the door open for participation by the so-called regulator helper cells, whether Tr1, Th3, or CD4+CD25+. Thus, the cytokine IL-10 plays a critical role in down-regulating Th1 responses, but is more predominantly produced by regulatory (Tr) cells than by Th2 cells.⁴⁰ Under certain conditions IL-10 may actually worsen EAE.⁶ Therefore, the existing data from MS patients, especially when considered along with the EAE animal data, does not reasonably support Th1 dominance.

Vandenbark's group also tried TCR peptide therapy on MS patients. Of 171 patients studied to date, 50-90 percent supposedly showed immunological response to vaccination and as much as 35 percent had some degree of favorable clinical response.³⁶

Type 1 Diabetes

Type 1 diabetes in humans and in nonobese diabetic (NOD) mice is an autoimmune disease. One antigen involved is glutamic acid decarboxylase (GAD). Patients have benefited to some degree from adjuvant therapy in the form of vaccination with BCG, an early "immunotherapeutic" preparation from a *Bacillus* bacterium.⁶ BCG injections have also prevented the occurrence of diabetes in NOD mice. BCG vaccination boosts IL-4, a Th2 cytokine. However, administration of Th2 cells to NOD mice can worsen the disease, if the recipient mice are immunocompromised.⁴¹ This finding dictates caution in applying Th2-based therapy to human type 1 diabetes.

In summary, for three major autoimmune diseases – RA, MS, and type 1 diabetes – a Th1 dominance has not been well enough established to rationalize balancing intervention. On both pragmatic and theoretical grounds there is real possibility of making the patient sicker through efforts to intervene with Th2 cells or Th2 cytokines.

A Few Disorders Feature Th2 Overactivation

Allergen-reactive Th2 cells are thought to be primarily involved in the triggering of type 1 hypersensitivity disorders, including allergy, asthma, eczema, hay fever, and urticaria.⁷ In addition, Th2 cell-cytokine predominance has been hypothesized in chronic graft-versus-host disease, progressive systemic sclerosis, and systemic lupus erythematosus.

Allergy

Allergens entering the body by way of the respiratory or digestive tracts or through the skin are likely accosted by B cells or macrophages.⁶ The antigen is taken inside the cell, fragmented, then displayed on the cell surface to allergen-specific T cells, predominantly Th2 cells, since they secrete IL-4, IL-5, and IL-10 cytokines. IL-4, and probably other Th2 cytokines, help recruit B cells, mast cells, and eosinophils, all of which can produce IgE antibodies, which play a central role in induction of allergic symptomatology.⁴²

The IgE antibodies attach to basophils and mast cells by way of their surface IgE receptors. Subsequent allergen exposure leads to activation of such IgE-sensitized cells, which in turn release various mediators such as histamine, leukotrienes, and prostaglandins that affect the clinical manifestations of allergic reactions.^{6,7} Singh and collaborators suggest up-regulation of genes controlling IL-4 expression (Th2) and/or deficits in Th2 modulation may be responsible for the exaggerated Th2 response to environmental allergens in atopic individuals.⁶ IL-5 generated by allergen-reactive Th2 cells attracts and activates eosinophils, which are responsible for tissue destruction in allergic asthma.

Immunotherapy by injection of standardized, purified allergens has been used to successfully treat allergic disease.⁴³ Secrist and collaborators demonstrated that this procedure could reduce the production of IL-4 (Th2) in atopic individuals to levels observed in non-atopic individuals.⁴⁴ This could mean that immunotherapy can alter the reactivity of memory Th2 cells. Examination of skin biopsies from allergen-challenged

sites of patients who had undergone successful grass pollen immunotherapy showed increased mRNA for IL-12 (Th1); this was not seen in matched patients who had not undergone immunotherapy.⁴⁵ The current knowledge base is consistent with allergy being a Th2-dominant condition treatable by Th2-directed immunotherapy.

Asthma

Asthma is generally accepted to be an inflammatory condition and there is good evidence it is Th2 dominant.⁴⁶ Similar Th2-dominant patterns are evident both in the nonatopic form of asthma, for which self-antigens might drive the response; and in occupational asthma, induced by toluene diisocyanate, for example.

Acute severe asthma (status asthmaticus) also features a Th2 cytokine profile.⁴⁶ The Th2 nuclear transcription factor GATA3 is over-expressed, while its Th1 transcription counterpart Tbet is under-expressed. A working hypothesis has been that Th2 cytokines contribute to asthma pathology through their capacity to promote IgE synthesis, the maturation and activation of mast cells and basophils, and eosinophil infiltration leading to epithelial damage and airway hyper-responsiveness (AHR).⁴⁶ However, the mechanistic relationships between T-cell activation and the inflammatory pattern of asthma with its clinical features of AHR, airway narrowing, and cough are still not evident. Similarly, the links between Th2 dominance and IgE-mediated atopy, and day-to-day asthma of varying severity remain to be elucidated.

Immunotherapy in the form of whole-allergen injections has been used in the treatment of asthma for more than a century.⁴⁷ A meta-analysis indicates this approach is effective in selected patients with IgE-mediated disease.⁴⁸ Several studies have examined Th1/Th2 cytokines following stimulation with allergen: IFN-gamma (Th1) could be increased, decreased, or unchanged while IL-4 (Th2) could decrease or stay unchanged.⁴⁹ A mystery remains as to the mechanisms by which whole-allergen immunotherapy may affect clinical improvement.

In Italy, Majori and colleagues administered house dust mite (HDM) extract subcutaneously to patients experiencing year-round asthma and proven sensitivity to the mite *Dermatophagoides pteronyssinus*.⁴⁹ Blood was analyzed for cytokine status at baseline, at three months, and one year after starting the therapy. The ratio of IFN-gamma to IL-4 was significantly increased at three months and one year. This finding is in agreement with several other studies, all of which concluded down-regulation of Th2 had occurred. In this study, neither AHR nor bloodborne IgE improved, but skin-prick sensitivity to HDM was reduced. The investigators stated clinical symptoms improved, but did not offer supporting data. They conceded that the absence of IgE reduction could indicate that IL-4 was working through non-Th2 pathways. This ambiguous type of outcome occurs quite often in studies that attempt to correlate clinical improvement with Th1/Th2 cytokine pattern shifts.⁵

Another immunotherapy strategy of blocking individual cytokines or administering single cytokines as counterbalances has produced disappointing results in asthma, probably because of the plethora of cytokines known to be involved. Larche and colleagues have questioned whether treatments can be developed that target multiple cytokines yet remain more specific than corticosteroids.⁴⁶

One feature that complicates a strict Th2 interpretation of asthma is that the Th2-type cytokines from mast cells, basophils, and eosinophils can also have novel direct effects on airway smooth muscle contractility.⁵⁰ In addition, distinct from IL-4 and IL-5 which are identified directly with Th2 cells, novel or perhaps pseudo-Th2 cytokines, including IL-9, IL-11, IL-13, and IL-25 are likely important in the disease.⁴⁶

Asthma appears to be largely a Th2-driven disease, but much of this story seems incomplete. Also, it is clearly established that in atopic asthma and allergic rhinitis, APCs first acquire and process the allergen and then present it to the reactive T cells. Whether APCs could bypass Th1/Th2 and contribute more directly to asthma symptomatology is unknown. This issue profoundly relates to the validity of the entire Th1/Th2 hypothesis.

Atopic Dermatitis

In its acute state, atopic dermatitis can manifest increased expression of Th2 cytokines, while the chronic state shows elevated levels of the Th1-type cytokine IL-12.⁶ Further analysis reveals that Th2 pathways are likely operative during the initial phase of inflammation, whereas Th1 may become active later.⁵¹ Activation of both Th1 and Th2 cytokines has been observed in patients allergic to house-dust mite.⁵²

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by overproduction of a variety of anti-cell nuclear and other pathogenic autoantibodies. It is characterized by B-cell hyperactivity, polyclonal hypergammaglobulinemia, and immune complex deposition. T cells are known to contribute to disease development.⁵³ SLE can feature elevation of both Th1 cytokines (IL-2, IFN-gamma) and Th2 cytokines (IL-4).

In 2002 Chang and collaborators reported on a study of 20 previously untreated SLE patients analyzed for a variety of cytokines and compared with healthy controls.⁵³ They found no differences in IFN-gamma (Th1) or IL-4 (Th2); however, those patients who also had arthritis had higher IFN-gamma than did the other patients. Conversely, patients with serositis or CNS involvement had higher IL-4 than those who did not. A previous study found SLE patients with nephritis had higher Th1 cytokines than non-nephritis patients.⁵⁴

SLE is known to be a heterogeneous disease, and an association of genetic polymorphism with SLE susceptibility has been reported.⁵³ It may be that Th1 and Th2 cycle in dominance depending on disease stage and presentation.

Cancer: Dendritic Cells Confirmed Important

The Th1/Th2 hypothesis has become so entrenched in the immunology literature that Th1 and Th2 cells have been given the status of inducing and maintaining the “cellular” and “humoral”

immune responses, respectively. However, this dichotomy was not a sound theory to begin with, since all immune responses are ultimately initiated and controlled by cells. Dendritic and other antigen-presenting cells have been given short shrift, even though these cell populations are indispensable for sensing antigen intrusion and initiating a response, including priming and driving the polarization of naive T-helper cells into Th1 or Th2 cells. Nowhere is this more evident than in the case of cancerous tumors.

Dendritic cells and related cell types¹⁰ are dispersed throughout the tissues as sentinels – a sophisticated early-warning system for the presence of foreign antigen (non-self) or otherwise threatening material.^{7,9} DCs are probably the most important regulators of naive T cells, judging from their high capacities to make and release IL-12.⁵⁵ In the tumor microenvironment IL-12 production tends to be suppressed, often resulting in an underactivation of Th1 activity, which normally would mobilize against tumor antigens.

Functionally upstream from the polarization of naive T cells into Th1 and Th2 (and probably into other subsets including Th3 and other Tr populations), the DCs also become polarized. The polarized DC1s can then drive Th1 polarization, while the DC2s drive Th2.¹¹ In their polarization process the DCs are influenced, not just by cytokines or chemokines, but by eicosanoid prostaglandins such as PGE2, an inflammatory mediator produced by macrophages, fibroblasts, and epithelial and tumor cells. As reviewed in Shurin et al,⁵⁵ naive DCs exposed to high concentrations of IFN-gamma (as can be generated by activated NK and Th1 cells) can become DC1s, produce copious IL-12, and drive Th1 polarization. Those exposed to relatively high PGE2 likely are inhibited from elaborating IL-12 and polarize to DC2. Those exposed to high IL-10 as well as PGE2 likely become IL-12 inhibited and also unable to stimulate naive T cells; these may be effectively a DC3 subset that permits the emergence of Tr-type, regulatory-suppressor populations. The Tr cells tend to produce large quantities of IL-10, which can down-regulate both the Th1 and Th2 pathways.

Th1 pathways typically produce activation of cytotoxic T lymphocytes (Tc), NK cells, macrophages, and monocytes, all of which can attack cancer cells and generally defend against tumors.⁵⁵ IFN-gamma and other Th1 cytokines are typically lower in advanced cancer patients, while the Th2 marker IL-4 can be higher or unchanged.⁵⁶ Nodules of non-small cell lung cancer freshly removed from patients expressed a marked imbalance toward Th2, as did biopsy samples from basal cell carcinoma.⁵⁷ In prostate cancer patients IL-2 was low (Th1) and IL-10 high.⁵⁸ IL-10 is a confirmed Th1-suppressive cytokine, and heightened IL-10 is a common factor in cancer.⁵⁵

IL-10 has a variety of suppressive effects that include inhibiting Th1 cytokine production, down-regulating APC and NK cell function, and lowering overall T-cell proliferation.⁵⁷ Especially under the influence of IL-4 (Th2), tumor cells apparently up-regulate IL-10 that suppresses nearby killer cells. Tumor-derived IL-10 has been documented in lymphoma, ovarian carcinoma, melanoma, neuroblastoma, and renal cell and colon carcinoma.⁵⁷ IL-12 is another cytokine that can be up-regulated by Th1 activity and inhibited by Th2.⁵⁹ A low IL-12/IL-10 ratio was found in cervical cancer patients.⁵⁵ Recent clinical studies suggest elevated IL-10 is predictive of a poor prognosis.⁵⁷ With both IL-4 and IL-10 being proven inhibitors of Th1 and promoters of Th2 activity, the recognized capability of cancerous tissue to suppress immunity is readily rationalized.⁵⁷

Anti-cytokine approaches to cancer therapy are currently in progress, including possible insertion of pro-Th1 genes into tumors. However, compounding the topic of cytokine imbalance is the unknown but probably substantial contribution of non-cytokine substances produced by the tumor tissues to help suppress Th1 activity. These and other considerations make it highly unlikely that manipulation of one or a few cytokines can affect a therapeutic breakthrough in cancer. The Th1/Th2 hypothesis lacks definitive support from the cancer literature.

A Major Missing Link: Oxidative Stress

Oxidative stress plays a major role in inflammation. Macrophages are a type of APC that infiltrate inflamed tissues and release prodigious quantities of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS).⁷ These contribute to antioxidant depletion and the subsequent destruction of tissue cells, the beta cells during pancreatic inflammation, for example. In the process the macrophages themselves can be depleted of antioxidants, as reflected in their intracellular glutathione status.⁶⁰

Glutathione in its reduced form (GSH) is the single most important protective and regulatory antioxidant in cells.⁶¹ As it is oxidized it becomes oxidized glutathione (GSSG), and the intracellular ratio of GSH:GSSG is an excellent measure of the cell's overall antioxidant status. Beginning in the 1980s, Herzenberg's group in California and Roederer's group in Germany conducted groundbreaking research that showed T-helper cells infected with HIV-1 deteriorated in GSH status and progressively lost their functional capacity. By the late 1990s, Herzenberg and collaborators had applied this groundbreaking approach to macrophages and the Th1/Th2 concept.⁶²

Peterson, Herzenberg and collaborators worked with mice to show depletion of glutathione from APCs *in vivo* results in lowered Th1 activity and higher Th2 activity;⁶² GSH repletion had just the opposite effect. Later Murata's group in Japan demonstrated that macrophages with most of their glutathione in the reduced form (reduced macrophages, RM) are effectively type 1 cells capable of polarizing Th1.^{60,63,64} Macrophages with mostly oxidized glutathione (oxidized macrophages, OM) are effectively type 2 and could polarize Th2. Thus, it seems immune activity can have Th1 or Th2 character depending on the relative antioxidant status of the cells directing the process.

Murata's group also devised ways to raise or lower the macrophage intracellular GSH concentration at will. They found that high GSH inside the macrophage supports gene activity that leads to secretion of IL-12, the major Th1-

polarizing cytokine. Furthermore, exposure of the macrophage to IFN-gamma (also Th1) tends to raise its GSH, thereby reinforcing its orientation toward type 1. On the other hand, exposure to IL-4 (Th2) lowers its GSH and steers it toward type 2 activity. Thus, the macrophages are capable of accepting feedback from the T-helper cells, which affects their antioxidant status and influences their type 1 or type 2 tendencies. It can be concluded that antioxidant status at the level of the immune cell and its microenvironment can markedly affect the ultimate pattern of immune response. In addition, the immune system may be truly a cell collective with both the “upstream” DC/APC cells and the “downstream” cells (T cells, B cells, etc.) affecting the immune response.

Mercury Exposure Predisposes to Th2 Dominance and Autoimmunity

The heavy metal mercury is ubiquitous in the environment, has no low threshold for toxicity, and is implicated in diseases as diverse as multiple sclerosis, Parkinson’s disease, and autism. Wu and colleagues found that administering mercury (as mercuric chloride) to Brown Norway rats induced a systemic autoimmune syndrome.⁶⁵ This was characterized by autoantibodies, necrotizing vasculitis principally affecting the caecum, inflammatory polyarthritis, and marked increase of serum IgE. A similar syndrome can be produced in the mouse and is linked to elevated IL-4 (Th2). Wu’s group confirmed that mast cells were involved in this mercury toxicity syndrome.

Wu’s group also confirmed that mast cells can degranulate and up-regulate IL-4 production through depletion of their intracellular GSH.⁶⁵ They concluded, as have other researchers,^{60,62} that oxidative stress may favor a Th2-dominated immune state in the host. Interestingly, Peterson et al suggest certain mice strains characterized as “Th1” or “Th2” strains may be mere artifacts of differential susceptibility to GSH depletion. The C57BL/6 mouse, a typical Th1 strain, is substantially less sensitive to GSH depletion than the BALB/c mouse, a typical Th2 strain. Because parasite infection will often modestly deplete GSH in the host, the human Th2 orientation to parasites

could be an adaptation based on a glutathione depletion threshold effect.⁶²

Immunostimulation: Glucans and Th1 Up-regulation

In the course of their extensive investigations of the Th1 and Th2 phenomena, Murata’s group administered lentinan to mice.⁶⁴ Lentinan is a beta-1,3-D-glucan from the medicinal mushroom *Lentinus edodes*. This molecule closely resembles other glucans typically liberated from fungal cell walls during digestion or in the course of immune response. When they gave lentinan intraperitoneally (ip) to mice (lentinan is a large molecule, poorly bioavailable by mouth), the macrophage GSH status improved and their capability to produce IL-12 improved, thus orienting them toward type-1 immunity. This effect could be turned off by depleting GSH, then restored using alpha-lipoic acid, an antioxidant nutrient that replenishes GSH.⁶⁴

Murata and colleagues also collected T-helper cell populations from the mice following lentinan exposure, assessed their cytokine production capacity, and found they shifted toward Th1 production and away from Th2.⁶⁴ Knowing that IL-2 is very important for priming Th1 cells, they administered IL-2 (also ip) to the mice and got a result very similar to that with lentinan – increased macrophage intracellular GSH and skewing of the T-helper response toward Th1. They then took the next obvious step and gave lentinan and IL-2 together (in overlapping doses); again, the result was the up-regulation of macrophage GSH, as well as a synergistic effect on IL-12 production from the polarized Th1 population.

High-dose, systemic administration of IL-2 has been used for some time in cancer immunotherapy.⁶⁴ Clinical outcomes were not particularly successful because of limited antitumor efficacy and dose-related adverse effects. The use of IL-2 in combination with lentinan (or preferably, orally bioavailable mushroom extracts) may be one means to increase IL-2’s therapeutic efficacy and lower its adverse effects.⁶⁶ Addition of glutathione replenishers to this cocktail might further boost Th1 competence,

for example to combat recalcitrant viruses such as HIV-1 or hepatitis C, to restore lagging DTH competence in the elderly, or to correct Th1 immunocompromise that occurs in patients in recovery following surgery.^{67,68}

Nutrients and Other Orthomolecules That Modify Th1/Th2 in Humans

This section examines nutrient and other orthomolecules for which there is proof in humans of a Th1/Th2 specific effect. For purposes of this review, animal model findings were not considered sufficient for inclusion.

Melatonin

Melatonin provides a homeostatic link between the brain and the immune system. It has specific high-affinity binding sites on both Th1- and Th2-helper cells. The affinity of these binding sites suggests they could recognize physiological concentrations of melatonin in serum.⁶⁹ Also, being a potent antioxidant, melatonin may help ameliorate the effect of toxic cancer therapies. In a small controlled study conducted in Italy, melatonin was given by mouth to 14 consecutive patients with untreatable, metastatic solid tumors.⁷⁰ Patients were treated during two consecutive cycles with either melatonin (20 mg/day orally) and IL-2 (subcutaneous) or melatonin, IL-2, and naltrexone (oral). Blood lymphocyte counts significantly increased in both groups over baseline. Clinical outcomes were not disclosed. The investigators took this finding as encouragement to conduct further research into anticancer therapy with combination IL-2, melatonin, and naltrexone.

Melatonin does not have receptors on B cells and probably does not boost “humoral” (Th2-type) immunity.⁷¹ Some APCs (monocytes and macrophages) have melatonin receptors and respond readily to stimulation with enhanced APC and phagocytic capacity. Melatonin production is progressively reduced with advancing age, so its capacity to up-regulate APC and Th cells seems deserving of further exploration.

Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is produced by the adrenal glands and is found in the plasma mainly as its sulfated derivative, DHEAS. As one of the effectors of the hypothalamic-pituitary-adrenal (HPA) axis, DHEA supports the body’s adaptive stress responses. It may also be involved in immune regulation. One study examined plasma DHEAS in HIV+ men compared to healthy controls for autonomic function, Th1/Th2 cytokine levels, and helper-cell count (CD4+).⁷² The HIV+ group was found to have significantly lower autonomic function than did the controls, along with lower helper cell (CD4+) counts. Plasma DHEAS levels in the HIV+ men correlated positively with IFN-gamma (Th1), while IL-4 levels (Th2) correlated negatively with autonomic performance. These data, while circumstantial, are consistent with DHEAS supporting healthy Th1 resistance against HIV-1.

Another Th1/Th2 study examined serum DHEAS levels in male patients with atopic dermatitis, thought to be a Th2-dominant condition.⁷³ Serum DHEA was lower in the AD patients (ages 19-30 years) than in age-matched healthy controls. Patients with SLE, another likely Th2-biased disease, also have low serum DHEA, and this may limit IL-2 production from T cells (Th1).⁷⁴ These data are also circumstantial, but speculatively they could support DHEA’s involvement in immune homeostasis (anti-Th2/pro-Th1). By contrast, men and women with RA reportedly have low DHEA and DHEAS in their blood, synovial fluid, and saliva.⁷⁵ This would not be consistent with RA being a predominantly Th1 disease, but would support the emerging theory that RA is a mixed Th1/Th2 condition, as discussed in the previous section.

Progesterone

Progesterone very likely contributes to the natural suppression of cell-mediated immunity that accompanies pregnancy and allows for the fetus to be tolerated during gestation. Enhanced tolerance during pregnancy is a Th2-dominant effect, although many other factors could be involved besides Th1/Th2 balance. The improvement in RA

during pregnancy also is consistent with lowered Th1 and elevated Th2. Further evidence that progesterone may up-regulate Th2 comes from mice made arthritic by experimental inoculation with the agent of Lyme disease (*Borrelia burgdorferi*).⁷⁶ When the mice were impregnated the arthritis was ameliorated and IL-4 markedly increased (Th2). However, just giving progesterone resulted in the same effect. Recent studies report male RA patients respond positively to DHEA replacement therapy.⁷⁵

Minerals

Selenium

Selenium is an important antioxidant and has significant effect on the immune system. It is frequently deficient in HIV+ individuals with progressing disease, which parallels the diminished production of Th1 cytokines.⁷⁷ When selenium-deficient animals are supplemented with selenium, IL-2 action (Th1) is enhanced. This is likely to benefit the type-1 pathway and thereby improve antiviral, antibacterial, and antifungal resistance along with DTH.⁷⁸

Zinc

Zinc deficiency is a worldwide public health problem, with up to 2 billion persons deficient.⁷⁹ Growth retardation and susceptibility to infections are some of the consequences. Prasad and collaborators developed an experimental human model of zinc deficiency.⁷⁹ They assayed IL-2 status (Th1) in these subjects and found that IL-2 and IFN-gamma declined during the depletion phase and was corrected by repletion. IL-4 (Th2) did not change. Besides being linked to Th1 maturation, IL-2 is also essential to NK-cell activity. Zinc is also necessary for the activity of thymulin, a thymic hormone, and zinc deficiency also results in fewer T cells coming to maturity.⁸⁰

Probiotic Bacteria

Substantial evidence exists that gram-positive lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* can survive transiently in the human intestinal tract when taken

by mouth. As these probiotics make contact with the gut mucosa they can stimulate systemic, cell-mediated immunity that approximates type-1 activity.⁸¹ This effect has the potential to boost cancer resistance, antiviral activity, and even partially reverse immune senescence in the elderly.

Cross has reviewed in-depth the human and animal studies on probiotics for type-1 stimulation.⁸¹ Dietary supplementation using particular defined strains was found to increase IFN activity in the blood of human volunteers. Probiotics may also down-regulate conditions linked to Th2 overactivation. Scandinavian studies were cited that indicated children born to families who consume traditional *Lactobacillus*-rich fermented foods experience fewer allergies than those from families who consume more sterile foods.

Other epidemiological studies indicate lower incidence of atopic (IgE-mediated) skin and respiratory tract hypersensitivity complaints among children with stable gut populations of *Lactobacilli* and *Bifidobacteria* compared with those who had a paucity of gut bacteria. Clinical scores of eczema were reduced in breast-fed infants whose mothers consumed *L. rhamnosus* GG as a dietary supplement. A double-blind trial found that consumption of *L. rhamnosus* GG by at-risk babies born to atopic parents, or by their mothers before and after giving birth, cut the incidence of subsequent clinical atopy in these infants by half. Such children also can manifest elevation of blood IFN-gamma (Th1).

Probiotics may be uniquely useful for rebuilding declining immunity in the elderly.⁸¹ In this population cell-mediated immunity is most affected, including declines in T-helper response capacity and marked lowering of skin DTH. The benefits from probiotics were found to be greater the more advanced the age and/or the more impaired at baseline; both type-1 and type-2 cytokine patterns can be partially restored. A randomized trial determined that dietary supplementation with *Lactobacillus*-containing yogurt to ostensibly healthy seniors for 12 months reduced their allergic symptomatology (e.g., rhinitis, wheezing) compared to matched controls. Also improved was the activity of APC and NK cells, both prominent

in resistance to cancer. Controlled trials in Japan found *L. casei* (Strain Shirota) effective in lowering the recurrence of bladder cancer.⁸²

Phytochemicals: Phytosterols and Sterolins

A proprietary mixture of plant sterols, beta-sitosterol (BSS) in a proprietary 100:1 mixture with sterolin, its glucoside (beta-sitosterol glucoside, BSSG) has been investigated for its immune-modulating properties. After *in vitro* experiments suggested this combination enhanced Th1 function, clinical studies were undertaken with a proprietary mixture.^{83,84} A randomized, placebo-controlled trial established its clinical efficacy against pulmonary tuberculosis.⁸³ A long-term, open-label trial was conducted on more than 300 patients infected with HIV-1, one group receiving the treatment and the other no treatment.⁸⁴ Disease progression was slowed by the sterol mixture and CD4+ T-helper cell counts were conserved, with some of the treated subjects followed for more than five years. After six months the control group was given the option of taking the sterol mixture. These results suggested the sterol/sterolin mixture was safe and effective as a long-term immunotherapy for people living with the AIDS virus.

Other findings suggest HIV/AIDS might involve a pathogenic impairment of the Th1 pathway and up-regulation of Th2. Th1/Th2 cytokines were evaluated in a group of HIV patients on no therapy and a group of patients managed with the mixture of plant sterols/sterolins.⁸⁴ The technique of three-color flow cytometry was used to generate precise estimates of cytokine responses to cells collected from the blood, cultured and stimulated with a mitogen. IFN-gamma secretion (Th1) was found markedly elevated in cells from the HIV+ patients taking the sterol mixture, compared to healthy individuals and the untreated HIV+ patients, and significantly more cells stained for IFN-gamma.

IL-4 secretion (Th2) was higher in both the treated and untreated HIV+ groups compared to the healthy controls, with non-treated

individuals demonstrating the highest values.⁸⁴ When ratios of IFN-gamma to IL-4 were calculated (Th1:Th2 cytokines), the untreated HIV+ group had a much lower ratio (5.6/5.0, at 7 or 18 hours after stimulation) and was markedly different from the treated HIV+ group (30.7/24.6) or the healthy group (15.7/9.5). Both groups of HIV+ patients were drawn from an ongoing clinical trial in which those treated with the plant sterols were manifesting stable T-helper cell counts, whereas those not treated were showing the otherwise predictable decline in T-helper cell counts with the passing of time.⁸⁴

Anti-inflammatory Benefits of Fish Oil: Beyond Th1/Th2 Mechanisms

As discussed above, chronic diseases previously assumed to result from Th1 overactivity (RA, MS, and type 1 diabetes) are found, upon critical evaluation, to have substantially overlapping Th1 and Th2 activities. Such mixed Th1/Th2 involvement was allowed for in the original hypothesis.¹ However, the debate most often disregards the inflammatory process intrinsic to these diseases. Nonspecific by its very nature, inflammation may not respect Th1/Th2 dominance. Once set in motion, the inflammatory cascades release a broad array of pro-inflammatory cytokines, chemokines, eicosanoids, reactive oxygen species, and other mediators that may overwhelm Th1/Th2 balance in order to fully mobilize immunity against the perceived threat.

Meanwhile, the long-chain omega-3 fatty acid preparations (LCw3), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have proven anti-inflammatory effects. They have proven benefit against circulatory inflammatory states (heart attack, coronary artery disease, restenosis, hypertension); kidney nephropathy and transplant; inflammatory bowel diseases; and cancer cachexia (pancreas).⁸⁵ Repeatedly, studies have failed to demonstrate either pro-Th1 or pro-Th2 mechanisms. Yet, a substantial body of work indicates they substantially improve quality of life in diseases currently considered to be Th1 or Th2 oriented.

Calder and colleagues assessed 13 controlled studies with fish oils for RA, completed through year 2001.⁸⁶ All of these reported clinical improvement including reduced morning stiffness, reduced joint tenderness, reduced time to fatigue, and increased grip strength. Fish oil preparations benefit asthma and atopy,^{87,88} and there is preliminary evidence the LCw3 also benefit SLE.⁸⁹ In immunoglobulin A (IgA) nephropathy, the most common glomerular disease worldwide, initial trials with LCw3 have been sufficiently promising that further trials are underway comparing fish oil to prednisone, the only other encouraging treatment for the disease.⁹⁰

These striking findings with LCw3 suggest it is not essential to intervene specifically at the T-helper cell level in order to benefit the patient. Beyond their proven nutritional support for anti-inflammatory eicosanoid balance, EPA and DHA also operate through non-eicosanoid mechanisms that include down-regulation of pro-inflammatory cytokines.⁸⁵ Their proven benefits for subjects with inflammatory and autoimmune pathology argue against any central role for Th1/Th2 balance in ameliorating disease states.

Limitations of the Hypothesis

Th1/Th2 cytokine balancing is routinely touted in the immunological literature as a fundamental paradigm advance in the field. However, it remains an unproven hypothesis and many of its facets have become untenable. Dent commented, "Nothing in immunology is ever as simple as it first seems."⁵ Thus, helper cells are found not to be the only, or even necessarily the dominant, sources of type-1 and type-2 cytokines.⁵ The literature is surprisingly murky as to which cytokines are specifically type 1 and which are type 2. Furthermore, there is significant evidence to suggest dendritic cells and other antigen-presenting cells have central importance in immunity.^{7,10-13} Further usefulness of the Th1/Th2 hypothesis may well hinge on including APCs in the model.

But an expanded model may still have problems. For example, the relative ease with which macrophage APCs can see-saw between type-1 and type-2 polarization simply by a change

in their glutathione status (reduced/GSH becomes converted to oxidized/GSSG) could suggest that Th1/Th2 dominance is largely an artifact of experimental observation. Alternatively, such cells' sensitivity to their intracellular redox status could be directing their polarized functions. It is tempting to speculate that inflammation, which is typically an oxidizing phenomenon, ultimately depletes the Th1 cells' reserves of reduced glutathione, shifting them to Th2 activity. The resultant Th2 activation then triggers an end to the inflammatory process.

Th1/Th2 pathways are undoubtedly overlain by other inflammatory phenomena. All too often, in zealous efforts to squeeze findings from animal models and cell cultures into the Th1/Th2 paradigm, researchers lump broad-spectrum inflammatory cytokines (IL-6 or TNF-alpha, for example) together with the characteristic Th1 cytokines (IFN-gamma and IL-12). This results in confusion regarding the autoimmune diseases and their progressive inflammatory status. Furthermore, while Th1/Th2 proponents look mainly to IL-10 from Th2 cells to terminate autoimmunity, this cytokine may be the tool of another helper class: the regulators (Tr). The existence of helper regulatory Tr cells (CD4+) and non-helper Tr cells (CD8+) complicates the assumption that Th1 and Th2 cells can mutually inhibit each other (Figure 1).¹⁹

These limitations on the Th1/Th2 hypothesis are severe but do not necessarily rule it irrelevant. Dent sees virtue in the Th1/Th2 hypothesis as a means to understand tolerance, of the quasi-parasitic fetus developing in the uterus or of sperm in the reproductive tract, for example. The known Th2 dominance in these conditions seems appropriate for at least some stages of human life. Subversion of Th2 pathways are the likely means by which parasitic organisms survive in the host.⁵ Th1 dominance seems relevant to situations where the body requires a vigorous response against a viral presence. Viewed from the traditional perspective, the healthiest immune state could be one poised more or less equally between "cellular immunity" (approximating Th1) and "humoral immunity" (Th2). A more clinically

relevant distinction would be of innate immunity (regulated by APCs, including monocytes/macrophages, DCs, and other phagocytic cells), versus acquired or adaptive immunity regulated by Th1, Th2, and Tr cells.⁹¹

Much of the clinical work around Th1/Th2 dominance involves a drive to develop molecules tailored as immunotherapeutics. But the early euphoria that Th1/Th2 manipulation would catalyze major immunotherapeutic breakthroughs has yet to be fulfilled. Clinical interventions with cytokines, single or mixed, have had very limited success, which is not surprising given the plethora of known cytokines with characteristic overlap in specificity and the diverse arrays of surface receptors on each cell. However, human recombinant IL-4 was successfully given by injection to alleviate psoriasis in 20/20 patients.⁹²

The TCR-peptide therapy approach pioneered by Vandembark's group³⁶ has also had limited success. Perhaps one-third of MS patients experienced modest benefit; in RA, substantially fewer than one-third had any clinical improvement; and in psoriasis as few as seven percent of the patients may have experienced benefit. And, the approach does carry risks. An experimental Alzheimer's vaccine apparently triggered catastrophic brain degeneration in as many as 15 patients.⁹³ This vaccine may have exacerbated a tendency in older patients to be progressively more Th1-biased.⁹⁴ It would be reckless to proceed further with this approach until the differences between lab animal and human immune mechanisms are adequately understood.

Conclusion

Whether right or wrong, the Th1/Th2 hypothesis is a "top-down" model of cell interaction that assumes one class of immune cells is especially equipped to supervise the others. Many lines of evidence indicate this model is overly simplistic and requires major modification to satisfy the discordant data. Meanwhile, standing in contrast to this hierarchical model is the pre-existing model of immune cell integration (Figure 2), which fits the existing Th1/Th2 data just as well. The huge body of research available on immune mechanisms

remains consistent with all the categories of immune cells making collective effort to maintain the body's defenses, respond promptly to danger, and eliminate threatening agents.⁶⁻¹³ It is most likely that, rather than being dominated by Th1- or Th2-helper cells, the diverse cell categories (APC, monocytes/macrophages, granulocytes, Th1/Th2/Th3, Tr, killer and natural killer) are each called upon at times to make the most prominent functional contribution, deferring to others as circumstances dictate.

One advantage inherent in the more collective model for immune functioning is that keeping the immune system healthy and mobilizing it against disease would depend more on giving the body systemic support than on deploying tailored magic immunotherapeutics. Proven immunomodulators such as mushroom extracts take on significant relevance.⁶⁶ Fish oils, vitamin C, glutathione, and other antioxidants, as well as numerous plant extracts can further enhance immune cell functionality.

In managing immune hypofunction or other dysfunction, it is crucial to manage all forms of stress. Rooks reported diverse stressors, including sleep deprivation, calorie restriction, excessive exercise, examination stress, and cardiopulmonary bypass surgery, down-regulate Th1 and up-regulate Th2 activity.⁹⁵ These effects are mediated mainly by glucocorticoids, but also by the catecholamine hormones epinephrine and norepinephrine.⁹² As heroic efforts to tailor technological immune therapies go forward, the best immune intervention tools continue to be lifestyle modification, vitamins, minerals, orthomolecules, and selected nontoxic phytotherapies.

References

1. Mosmann TR, Cherwinski H, Bond MW, et al. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-2357.
2. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145-173.

3. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today* 1996;17:138-146.
4. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996;383:787-793.
5. Dent LA. For better or worse: common determinants influencing health and disease in parasitic infections, asthma and reproductive biology. *J Reprod Immunol* 2002;57:255-272.
6. Singh VK, Mehrotra S, Agarwal SS. The paradigm of Th1 and Th2 cytokines: its relevance to autoimmunity and allergy. *Immunol Res* 1999;20:147-161.
7. Roitt I, Brostoff J, Male D. *Immunology*. 5th ed. Philadelphia, PA: Mosby; 1998.
8. Zhai Y, Ghobrial RM, Busuttill RW, Kupiec-Weglinski JW. Th1 and Th2 cytokines in organ transplantation: paradigm lost? *Crit Rev Immunol* 1999;19:155-172.
9. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994;12:991-1045.
10. Lotze MT, Thomson AW. *Dendritic Cells*. San Diego, CA: Academic Press; 2001.
11. Moser M, Murphy KM. Dendritic cell regulation of TH1-TH2 development. *Nat Immunol* 2000;1:199-205.
12. Upham JW. The role of dendritic cells in immune regulation and allergic airway inflammation. *Respirology* 2003;8:140-148.
13. Moll H. Dendritic cells as a tool to combat infectious diseases. *Immunol Lett* 2003;85:153-157.
14. Aggarwal BB. *Human Cytokines*. London: Blackwell Science; 1998.
15. Balkwill F. *The Cytokine Network*. Oxford: Oxford University Press; 2000.
16. Romagnani G. Cytokines and the Th1/Th2 paradigm. In: Balkwill F, ed. *The Cytokine Network*. Oxford: Oxford University Press; 2000.
17. Lafaille JJ. The role of helper T cell subsets in autoimmune diseases. *Cytokine Growth Factor Rev* 1998;9:139-151.
18. Mantovani A, Sozzani S. Chemokines (Chapter 5). In: Balkwill F, ed. *The Cytokine Network*. Oxford: Oxford University Press; 2000.
19. McGuirk P, Mills KH. Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. *Trends Immunol* 2002;23:450-455.
20. Groux H, O'Garra A, Bigler M, et al. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;389:737-742.
21. Fukaura H, Kent SC, Pietruszewicz MJ, et al. Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. *J Clin Invest* 1996;98:70-77.
22. Gorelik L, Flavell RA. Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol* 2002;2:46-53.
23. Lienhardt C, Azzurri A, Amedei A, et al. Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity *in vivo*. *Eur J Immunol* 2002;32:1605-1613.
24. Newport MJ, Huxley CM, Huston S, et al. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. *N Engl J Med* 1996;335:1941-1949.
25. Norris PJ, Rosenberg ES. Cellular immune response to human immunodeficiency virus. *AIDS* 2001;15:S16-S21.
26. Harrer T, Harrer E, Kalams SA, et al. Cytotoxic T lymphocytes in asymptomatic long-term nonprogressing HIV-1 infection. Breadth and specificity of the response and relation to *in vivo* viral quasispecies in a person with prolonged infection and low viral load. *J Immunol* 1996;156:2616-2623.
27. Imami N, Pires A, Hardy G, et al. A balanced type 1/type 2 response is associated with long-term nonprogressive human immunodeficiency virus type 1 infection. *J Virol* 2002;76:9011-9023.
28. Tanaka M, Hirabayashi Y, Gatanaga H, et al. Reduction in interleukin-2-producing cells but not Th1 to Th2 shift in moderate and advanced stages of human immunodeficiency virus type-1-infection: direct analysis of intracellular cytokine concentrations in CD4⁺CD8⁺-T cells. *Scand J Immunol* 1999;50:550-554.
29. Keane NM, Price P, Lee S, et al. An evaluation of serum soluble CD30 levels and serum CD26 (DPPIV) enzyme activity as markers of type 2 and type 1 cytokines in HIV patients receiving highly active antiretroviral therapy. *Clin Exp Immunol* 2001;126:111-116.

30. Reuben JM, Lee BN, Paul M, et al. Magnitude of IFN-gamma production in HIV-1-infected children is associated with virus suppression. *J Allergy Clin Immunol* 2002;110:255-261.
31. Schulze-Koops H, Kalden JR. The balance of Th1/Th2 cytokines in rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2001;15:677-691.
32. Gerli R, Bistoni O, Russano A, et al. *In vivo* activated T cells in rheumatoid synovitis. Analysis of Th1- and Th2-type cytokine production at clonal level in different stages of disease. *Clin Exp Immunol* 2002;129:549-555.
33. Da Silva JA, Spector TD. The role of pregnancy in the course and aetiology of rheumatoid arthritis. *Clin Rheumatol* 1992;11:189-194.
34. Ioannou Y, Isenberg DA. Current evidence for the induction of autoimmune rheumatic manifestations by cytokine therapy. *Arthritis Rheum* 2000;43:1431-1442.
35. Simon AK, Seipelt E, Sieper J. Divergent T-cell cytokine patterns in inflammatory arthritis. *Proc Natl Acad Sci U S A* 1994;91:8562-8566.
36. Vandembark AA, Morgan E, Bartholomew R, et al. TCR peptide therapy in human autoimmune diseases. *Neurochem Res* 2001;26:713-730.
37. Tang L, Benjaponpitak S, Dekruyff RM, Umetsu DT. Reduced prevalence of allergic disease in patients with multiple sclerosis associated with enhanced IL-12 production. *J Allergy Clin Immunol* 1998;102:428-435.
38. Windhagen A, Anderson DE, Carrizosa A, et al. Cytokine secretion of myelin basic protein reactive T cells in patients with multiple sclerosis. *J Neuroimmunol* 1998;91:1-9.
39. Hohnoki K, Inoue A, Koh CS, et al. Elevated serum levels of IFN-gamma, IL-4 and TNF-alpha/unelevated serum levels of IL-10 in patients with demyelinating diseases during the acute stage. *J Neuroimmunol* 1998;87:27-32.
40. Bettelli E, Das MP, Howard ED, et al. IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J Immunol* 1998;161:3299-3306.
41. Pakala SV, Kurrer MO, Katz JD. T helper 2 (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. *J Exp Med* 1997;186:299-306.
42. Maggi E. The TH1/TH2 paradigm in allergy. *Immunotechnology* 1998;3:233-244.
43. Bousquet J, Becker WM, Hejjaoui A, et al. Differences in clinical and immunologic reactivity of patients allergic to grass pollens and to multiple-pollen species. II. Efficacy of a double-blind, placebo-controlled, specific immunotherapy with standardized extracts. *J Allergy Clin Immunol* 1991;88:43-53.
44. Secrist H, Chelen CJ, Wen Y, et al. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J Exp Med* 1993;178:2123-2130.
45. Hamid QA, Schotman E, Jacobson MR, et al. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254-260.
46. Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;111:450-463.
47. Freeman J. Vaccination against hay fever: report of results during the first three years. *Lancet* 1914; i:1178.
48. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med* 1995;151:969-974.
49. Majori M, Caminati A, Corradi M, et al. T-cell cytokine pattern at three time points during specific immunotherapy for mite-sensitive asthma. *Clin Exp Allergy* 2000;30:341-347.
50. Lacy P, Moqbel R. Immune effector functions of eosinophils in allergic airway inflammation. *Curr Opin Allergy Clin Immunol* 2001;1:79-84.
51. Bohm I, Bauer R. Th1 cells, Th2 cells and atopic dermatitis. *Hautartz* 1997;48:223-227.
52. Laan MP, Baert MR, Vrendendaal AE, Savelkoul HF. Differential mRNA expression and production of interleukin-4 and interferon-gamma in stimulated peripheral blood mononuclear cells of house-dust mite-allergic patients. *Eur Cytokine Netw* 1998;9:75-84.
53. Chang DM, Su WL, Chu SJ. The expression and significance of intracellular T helper cytokines in systemic lupus erythematosus. *Immunol Invest* 2002;31:1-12.
54. Akahoshi M, Nakashima H, Tanaka Y, et al. Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus. *Arthritis Rheum* 1999;42:1644-1648.

55. Shurin MR, Lu L, Kalinski P, et al. Th1/Th2 balance in cancer, transplantation and pregnancy. *Springer Semin Immunopathol* 1999;21:339-359.
56. Sato M, Goto S, Kaneko R, et al. Impaired production of Th1 cytokines and increased frequency of Th2 subsets in PBMC from advanced cancer patients. *Anticancer Res* 1998;18:3951-3955.
57. Huang M, Wang J, Lee P, et al. Human non-small cell lung cancer cells express a type 2 cytokine pattern. *Cancer Res* 1995;55:3847-3853.
58. Filella X, Alcover J, Zarco MA, et al. Analysis of type T1 and T2 cytokines in patients with prostate cancer. *Prostate* 2000;44:271-274.
59. Ria F, Penna G, Adorini L. Th1 cells induce and Th2 inhibit antigen-dependent IL-12 secretion by dendritic cells. *Eur J Immunol* 1998;28:2003-2016.
60. Murata Y, Amao M, Hamuro J. Sequential conversion of the redox status of macrophages dictates the pathological progression of autoimmune diabetes. *Eur J Immunol* 2003;33:1001-1011.
61. Kidd PM. Glutathione: systemic protectant against oxidative and free radical damage. *Altern Med Rev* 1997;2:155-176.
62. Peterson JD, Herzenberg LA, Vasquez K, Waltenbaugh C. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc Nat Acad Sci U S A* 1998;95:3071-3076.
63. Murata Y, Shimamura T, Hamuro J. The polarization of T(h)1/T(h)2 balance is dependent on the intracellular thiol redox status of macrophages due to the distinctive cytokine production. *Int Immunol* 2002;14:201-212.
64. Murata Y, Shimamura T, Tagami T, et al. The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. *Int Immunopharmacol* 2002;2:673-689.
65. Wu Z, Turner DR, Oliveira DB. IL-4 gene expression up-regulated by mercury in rat mast cells: a role of oxidant stress in IL-4 transcription. *Int Immunol* 2001;13:297-304.
66. Kidd PM. The use of mushroom glucans and proteoglycans in cancer treatment. *Altern Med Rev* 2000;5:4-27.
67. Decker D, Schondorf M, Bidlingmaier F, et al. Surgical stress induces a shift in the type-1/type-2 T-helper cell balance, suggesting down-regulation of cell-mediated and up-regulation of antibody-mediated immunity commensurate to the trauma. *Surgery* 1996;119:316-325.
68. Brune IB, Wilke W, Hensler T, et al. Downregulation of T helper type 1 immune response and altered pro-inflammatory and anti-inflammatory T cell cytokine balance following conventional but not laparoscopic surgery. *Am J Surg* 1999;177:55-60.
69. Gonzalez-Haba MG, Garcia-Maurino S, Calvo JR, et al. High-affinity binding of melatonin by human circulating T lymphocytes (CD4+). *FASEB J* 1995;9:1331-1335.
70. Lissoni P, Malugani F, Malysheva O, et al. Neuroimmunotherapy of untreatable metastatic solid tumors with subcutaneous low-dose, interleukin-2, melatonin and naltrexone: modulation of interleukin-2-induced antitumor immunity by blocking the opioid system. *Neuroendocrinol Lett* 2002;23:341-344.
71. Currier NL, Sun LZ, Miller SC. Exogenous melatonin: quantitative enhancement *in vivo* of cells mediating non-specific immunity. *J Neuroimmunol* 2000;104:101-108.
72. Schifitto G, McDermott MP, Evans T, et al. Autonomic performance and dehydroepiandrosterone sulfate levels in HIV-1-infected individuals: relationship to TH1 and TH2 cytokine profile. *Arch Neurol* 2000;57:1027-1032.
73. Tabata N, Tagami H, Terui T. Dehydroepiandrosterone may be one of the regulators of cytokine production in atopic dermatitis. *Arch Dermatol Res* 1997;289:410-414.
74. Suzuki T, Suzuki N, Engleman EG, et al. Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes in patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1995;99:251-255.
75. Cutolo M. Sex hormone adjuvant therapy in rheumatoid arthritis. *Rheum Dis Clin North Am* 2000;26:881-895.
76. Moro MH, Bjornsson J, Marietta EV, et al. Gestational attenuation of Lyme arthritis is mediated by progesterone and IL-4. *J Immunol* 2001;166:7404-7409.

77. Baum MK, Miguez-Burbano MJ, Campa A, Shor-Posner G. Selenium and interleukins in persons infected with human immunodeficiency virus type 1. *J Infect Dis* 2000;182:S69-S73.
78. Roy M, Kiremidjian-Schumacher L, Wishe HI, et al. Selenium supplementation enhances the expression of interleukin 2 receptor subunits and internalization of interleukin 2. *Proc Soc Exp Biol Med* 1993;202:295-301.
79. Prasad AS. Effects of zinc deficiency on Th1 and Th2 cytokine shifts. *J Infect Dis* 2000;182:S62-S68.
80. Dardenne M, Pleau JM, Nabarra B, et al. Contribution of zinc and other metals to the biological activity of the serum thymic factor. *Proc Natl Acad Sci U S A* 1982;79:5370-5373.
81. Cross ML. Immunoregulation by probiotic lactobacilli: pro-Th1 signals and their relevance to human health. *Clin Appl Immunol Rev* 2002;3:115-125.
82. Aso Y, Akaza H, Kotake T, et al. Preventive effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. The BLP Study Group. *Eur Urol* 1995;27:104-109.
83. Donald PR, Lamprecht JH, Freestone M, et al. A randomised placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1997;1:518-522.
84. Breytenbach U, Clark A, Lamprecht J, Bouic P. Flow cytometric analysis of the Th1-Th2 balance in healthy individuals and patients infected with the human immunodeficiency virus (HIV) receiving a plant sterol/sterolin mixture. *Cell Biol Int* 2001;25:43-49.
85. Alexander JW. Immunonutrition: the role of omega-3 fatty acids. *Nutrition* 1998;14:627-633.
86. Calder PC, Yaqoob P, Thies F, et al. Fatty acids and lymphocyte functions. *Br J Nutr* 2002;87:S31-S48.
87. Calder PC, Miles EA. Fatty acids and atopic disease. *Pediatr Allergy Immunol* 2000;13:29-36.
88. Broughton KS, Johnson CS, Pace BK, et al. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. *Am J Clin Nutr* 1997;65:1011-1017.
89. Das UN. Beneficial effect of eicosapentaenoic and docosahexaenoic acids in the management of systemic lupus erythematosus and its relationship to the cytokine network. *Prostaglandins Leukot Essent Fatty Acids* 1994;51:207-213.
90. Donadio JV Jr. Use of fish oil to treat patients with immunoglobulin A nephropathy. *Am J Clin Nutr* 2000;71:373S-375S.
91. Elenkov II, Chrousos GP. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Ann NY Acad Sci* 2002;966:290-303.
92. Ghoreschi K, Thomas P, Breit S, et al. Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. *Nat Med* 2003;9:40-46.
93. Munch G, Robinson SR. Alzheimer's vaccine: a cure as dangerous as the disease? *J Neural Transm* 2002;109:537-539.
94. Meydani SN, Endres S, Woods MM, et al. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J Nutr* 1991;121:547-555.
95. Rooks GA. Glucocorticoids and immune function. *Baill Clin Endocrinol Metab* 1999;13:567-581.