Biology and Pathogenesis of Lymphocytic Choriomeningitis Virus Infection

M.B.A. Oldstone

1 Introduction

The strength of the lymphocytic choriomeningitis virus (LCMV) model rests on the following five foundations. First, the virus in vivo in its natural host, the mouse, or in vitro in cultured cells is non-cytolytic. This quality allows clear separation of effects caused by the virus from those caused by the host immune system. Consequently, the host cell control of viral infection as opposed to how virus interacts with cells to distort their functions without killing them can be decoded. Second, reactions to LCMV infection can encompass a widely diverse range of immune responses (Fig. 1). Usually when immunocompetent adult mice are injected with LCMV, they generate a marked immune response to eliminate the infectious agent. Although their innate responses include the production of interferon (IFN), macrophages and natural killer (NK) cells (Muller et al. 1994; see reviews Buchmeier et al. 1980; Borrow and Oldstone 1997; see chapter by Biron et al., this volume), it is the adoptive immune response – primarily the virus-specific $CD8^+$ CTL response – that is responsible for virus clearance. This protective response proceeds

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	Age of host	Route	Generation CTL	Phenotype	Immunologic reason
Virus	Adult	IP, SC, IV	↑CTL	Clear viral infection, immunity	Positively selected LCMV antigen specific CD8+ CTL in the periphery are clonally expanded and lyse virus infected cells. If virus infects leptomeningeal or choroid plexus cells in the brain CTL induces death.
		IC	↑CTL	Leptomeningitis, death	
Virus	Newborn	IP, IV, IC	↓CTL	Persistent infection	High affinity LCMV antigen specific T cells are negatively selected out by the thymus. Low affinity antigen specific cells reside in the periphery.
Immunosuppressive virus	Adult	IV	↓CTL	Persistent infection	Immunosuppressive viruses bind preferentially and at high affinity to α-DG, a cellular receptor for arenaviruses. Among immune cells, α-DG is preferentially expressed on interdigitating dendritic cells (DEC 205+, CD11c+).
Persistently infected adult mouse	+ Transfer immune memory T cells (CD8 + CD4)			Clear viral infection	Mice with low number and low affinity LCMV antigen specific CD8+ and CD4+ T cells are reconstituted with high affinity LCMV antigen specific CD8+ and CD4+ T cells. Virus cleared by CTL directed lysis and by action of IFN-γ and TNF-α cytokines.

Fig. 1. The several disparate scenarios following different routes of infection in different aged mice challenged with LCMV and the role of cytotoxic T lymphocytes (*CTL*)

mostly by lysing virally infected cells through a perforin-mediated pathway. The sensitivity and specificity of this viral antigen-specific $CD8^+$ and the viral antigen-specific $CD4^+$ T cell response have allowed experimentation that revealed how these cells function in acute infection and in the maintenance of immunologic memory or its loss during persistent infection. Quantitative analysis of such antigen-specific T cell responses during specific stages of expansion, burst size, retraction and maintenance was also achieved (see chapters by; McNally and Welsh and Homann, this volume). Third, LCMV easily establishes persistent infection in vivo via in utero or congenital vertical transmission, in newborn mice, or in immunocompetent adult mice inoculated with immunosuppressive viral variants. The basic mechanism(s) by which virus persists and the resultant immunosuppression that maintains this long-term infection are amenable to experimental analysis. For example, LCMV infection by all these modalities, except for immunosuppressive viral variants in adult mice, results in an aborted or inefficient anti-LCMV T cell response even though the same animals' immune responses to other viruses or humoral responses to particulate or soluble foreign antigens are not impaired (Tishon et al. 1993a). In contrast, a generalized suppression immune response occurs in normal adult mice following high-dose (2×10^6PFU or higher i.v.) LCMV inoculation (Tishon et al. 1993a). Although all these models accommodated persistent infection, the mechanisms by which it occurs differ. That induced in utero, congenitally or in newborn mice stems from the infection of thymic cells and the specific removal (negative selection) of lymphocytes with potential responsiveness to LCMV (reviewed in Borrow and Oldstone 1997). In contrast, the persistence and generalized immunosuppression following infection of adult mice with immunosuppressive variants is caused by selective LCMV infection of

DEC205$^+$ and CD11c$^+$ interdigitating dendritic cells (SEVILLA et al. 2000; SMELT et al. 2001), the major antigen-presenting cells of the host (Figs. 1, 2). Regardless of the mechanism of initiating infection, continuous expression of viral genes in differentiated cells like neurons, endocrine cells, or immune system cells, can alter homeostasis and cause disease (OLDSTONE 1989, 1993). This phenomenon can also be studied in cultures of specialized cells that make differentiation products such as GH, neurotransmitters, cytokines, or immunoglobulins (Ig). As with the in vivo scenario, infection in vitro affects more than 95% of cells within a few days, and the infection lasts during continuous passages. The fourth basic asset of LCMV is a simple one in that this virus contains four genes, two each placed on two distinct

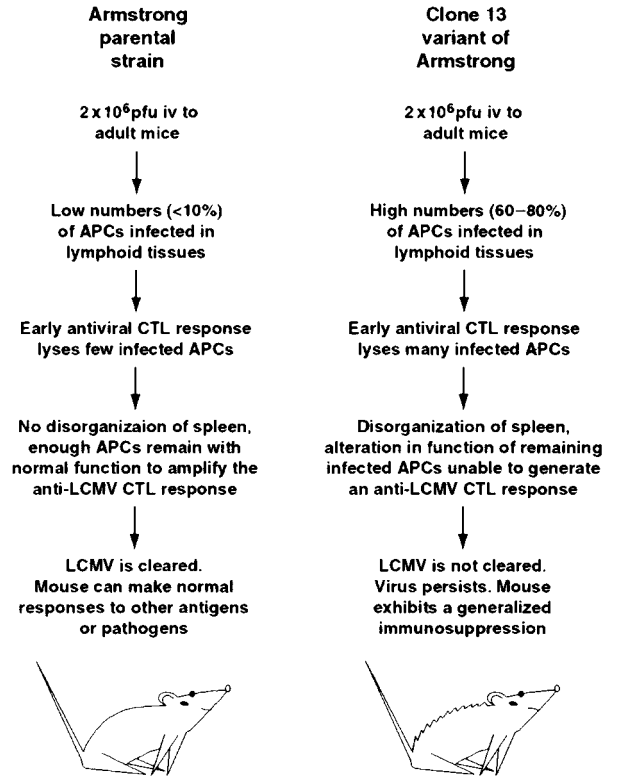

Fig. 2. Biologic differences between wild-type LCMV ARM, which generates a robust CTL response and clears the virus infection, and its variant Clone 13, which fails to generate a sufficient primary day 7 CTL response to clear the viral infection resulting in a persistent viral infection. The mechanism by which this occurs is shown in Fig. 5 (also see SEVILLA et al. 2000; SMELT et al. 2001)

pieces of genomic RNA. Thus, one can make genetic reassortants between LCMV strains or variants, each with differing phenotypes to map the viral gene(s) involved. Further, (see chapter by Lee and de la Torre, Vol. I) the ability to use reverse genetics to complement the impressive biologic observations from experiments with LCMV infection offers a unique opportunity to dissect and understand basic building blocks of viral pathogenesis. Fifth, and most germane, events initially uncovered by the study of LCMV in its natural rodent hosts have proved applicable to infections caused by a wide spectrum of DNA and RNA viruses and with cell-mediated immune control of bacteria, parasites and tumors in humans.

The foregoing precepts reflect the beauty of LCMV as a model of infection and its value to the field not only of viral but also of microbial pathogenesis. Table 1 in the Introduction to this volume and the contribution by Zinkernagel (this volume) list the remarkable number of key concepts in immunology and virology that were first defined in studies using this virus. These concepts include basic immunologic phenomena such as the major histocompatibility complex (MHC) restriction of T cell recognition (Zinkernagel and Doherty 1974), that tissue injury is mediated by antiviral immune responses associated with acute and persistent viral infections, and that the non-cytopathic LCMV can cause disease by interfering with the differentiated functions of infected cells.

2 Balance Between Acute and Persistent Infection

The understanding of virus-induced immune response disease and the related immunopathology has its roots in the studies reported by Rowe (1954). He showed that suppression of immune responses changed the ordinarily lethal, acute infection with LCMV to a persistent infection in susceptible mice. The work of Rowe and later of other investigators who used neonatal thymectomy, genetically athymic mice, irradiation, antilymphoid drugs, or antithymocyte sera, etc. (reviewed in Borrow and Oldstone 1997), extended the concept of immune response-mediated injury during viral infection. Thereafter, the role of lymphocytes in mediating virus-induced immunologic injury was delineated. First, experiments performed inde-

Table 1. Immune factors that control acute and persistent LCMV infection

	Control of:	
	Acute viral infection	Persistent viral infection
CD^8+ T cells	+ + +	+ + +
Perforin	+ + +	+ + +
CD^4+ T cells	No	+ + +
IFN-γ	No	+ + +
B cells	No	No
L-selectin	No	+ + +
CD40 ligand	No	+ + +

pendently by LUNDSTEDT (1969) and OLDSTONE et al. (1969) showed that lymphocytes obtained 6–9 days after an acute LCMV infection killed LCMV-infected targets in vitro. Next, such lymphocytes were found to be of thymic origin and to bear Thy1.2 markers on their surfaces (COLE et al. 1972; MARKER and VOLKERT 1973). GILDEN et al. (1972a,b) showed that mice surviving an ordinarily lethal dose of LCMV when immunosuppressed during acute LCMV disease died when reconstituted with syngeneic, immune T lymphocytes. ZINKERNAGEL and DOHERTY (1974) defined the need for two signals between the cytolytic T lymphocyte and its infected target, namely, virus specificity and H-2 restriction, so that the killing of LCMV-infected cells could proceed. Armed with these data, others (MIMS and BLANDEN 1972; ZINKERNAGEL and WELSH 1976) used virus-specific splenic lymphocytes to reconstitute immunosuppressed, infected H-2-matched mice and thereby lowered their viral titers.

In a series of mice whose genes encoding various T cell components, cytokine genes or effector molecules, like perforin, were knocked out or neutralized by adding antibodies to the products of these genes (YOUNG et al. 1989; WALSH et al. 1994; KAGI et al. 1995; TISHON et al. 1995; KLAVINSKIS et al. 1989a; LEIST and ZINKERNAGEL 1990), investigators showed that $CD8^+$ T cells and perforin were the cardinal players in the clearance of acute viral infection and that neither $CD4^+$ T cells, IFN-γ, nor tumor necrosis factor (TNF)-α were required (Table 1).

In perforin-deficient mice, $CD8^+$ T cells became activated with LCMV infection, but the virus was not eliminated. By contrast, mice carrying the spontaneous mutations *gld* (nonfunctional Fas ligand) or *lpr* (inactive Fas) controlled acute LCMV infection with normal kinetics, indicating that perforin-dependent cytotoxicity is crucially involved in the clearance of acute LCMV infection but that no measurable involvement of Fas-dependent pathways occurs (KAGI et al. 1995; WALSH et al. 1994). Further, the fact that perforin-deficient mice were unable to clear LCMV indicates that cytokine production alone by $CD8^+$ cytotoxic T lymphocytes (CTL) or $CD4^+$ T cells is likely to be insufficient to mediate the clearance of an acute viral infection. In agreement with these findings are results showing resolution of the acute LCMV infection in mice treated with antibodies to IFN-γ or TNF-α, and IFN-γ-deficient (knockout) mice are able to clear an acute infection caused by this virus (TISHON et al. 1995; KLAVINSKIS et al. 1989a; LEIST and ZINKERNAGEL 1990). However, antiviral cytokines can modulate the virus-immune response balance, and thus play a role in combating acute LCMV infection (GUIDOTTI et al. 1999). Recently, the use of tetramer- (MURALI-KRISHNA et al. 1998) and intracellular cytokine-staining techniques (BUTZ and BEVAN 1998; MURALI-KRISHNA et al. 1998) combined with the identification of MHC class I D^b-restricted peptides and the use of CTL clones (OLDSTONE et al. 1988; KLAVINSKIS et al. 1989a; YANAGI et al. 1990; GAIRIN et al. 1995; HUDRISIER et al. 1996; BAENZIGER et al. 1986; BYRNE et al. 1984; ANDERSON et al. 1985) and class II I^b-(VARGA and WELSH 1998; OXENIUS et al. 1998) has allowed fine mapping of the total anti-LCMV antigen-specific $CD8^+$ and $CD4^+$ T cell response (Fig. 3; BERGER et al. 2000; HOMANN et al. 2001).

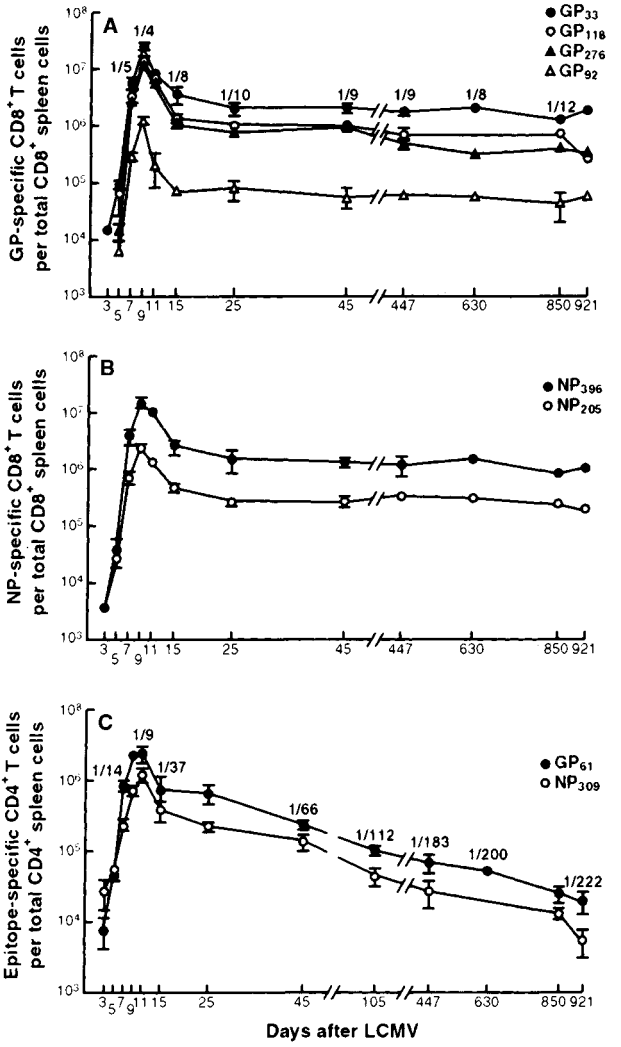

Fig. 3. Generation of LCMV-specific $CD8^+$ CTL and $CD4^+$ T helper cells during the course of an acute LCMV infection and the differences between maintenance of $CD8^+$ and $CD4^+$ antigen-specific memory (see the chapter by Homann, this volume; HOMANN et al. 2001)

Both class I MHC-restricted (largely $CD8^+$) and class II MHC-restricted (generally $CD4^+$) T cell responses are elicited after a virus infection (Fig. 3). "Professional" antigen-presenting cells, primarily dendritic cells (5–10,000× greater in activity than macrophages), and mainly also macrophages, process peptides made from viral proteins for presentation in association with class I and II molecules. MHC class II molecules induce $CD4^+$ T cell responses, whereas MHC class I induce primarily $CD8^+$ T cell responses. In most instances, peptides presented in association with class I are derived exclusively from proteins synthesized within the

cell. As viruses replicate intracellularly, peptide fragments of the viral proteins become associated and presented with class I MHC molecules. This sequence targets $CD8^+$ T cell activation and subsequent interaction with virus-infected cells. Identification of the epitopes of LCMV reactive with $CD8^+$ T cells has confirmed that non-structural internal virion proteins are frequently recognized in addition to virion surface proteins. Antiviral T cells enjoy several advantages from the recognition of non-structural, internal viral proteins: (1) the number of potential viral epitopes is increased; (2) these proteins tend to be more conserved among viruses than are virion surface proteins. Thus, T cell responses can cross-react between strains thereby providing protection against related viruses; and (3) targeting of T cells to the first non-structural viral proteins available in an infected cell promotes destruction of the infected cell before production of progeny virions is complete; consequently, the factory manufacturing infectious virus is removed.

The recent development of new techniques for quantitating antigen-specific T cells has allowed the reassessment of the magnitude and kinetics of both virus-specific $CD4^+$ and, in particular, $CD8^+$ T cell responses initially in LCMV infected mice (Murali-Krishna et al. 1998; Butz and Bevan 1998) and subsequently in virus-infected humans. Antigen-specific T cells can now be identified by staining with fluorescently labeled tetrameric complexes of MHC molecules folded around a specific peptide or, alternatively, on the basis of their ability to produce cytokines in response to antigen stimulation. These assays quantitated single cytokine-producing cells, either by ELISPOT or by intracellular staining of cells from which cytokine secretion was blocked using brefeldin A. The use of these techniques has shown that antigen-specific $CD8^+$ T cells can expand to far higher numbers than previously appreciated during the acute phase of a virus infection, e.g., more than 50% of the $CD8^+$ T cells in the spleen of mice and on occasion 80% were found to be virus-specific at the peak of the acute response to LCMV (Murali-Krishna et al. 1998; Butz and Bevan 1998; Homann et al. 2001).

LCMV can escape recognition by virus-specific $CD8^+$ T cells by acquiring mutations that prevent binding of viral peptide epitopes to MHC molecules or prevent recognition by the T cell receptor (TCR) (Aebischer et al. 1991; Lewicki et al. 1995a,b; Pircher et al. 1990). Several rules govern viral peptide-MHC interactions (reviewed by Whitton and Oldstone 2001; Bjorkman et al. 1987; Falk et al. 1991; Rotzschke et al. 1990; Garcia et al. 1996; Rammensee et al. 1995; Gairin et al. 1995; Young et al. 1994; Matsumura et al. 1992; Hudrisier et al. 1996). The bound peptide sequence is linear, resulting from proteolytic fragmentation of a viral protein synthesized within the cell. Studies of endogenously processed viral peptides indicate that they vary in length from 8–11 amino acids (aa) and display MHC allele-specific motifs. Mutational and crystallographic studies of MHC molecules complexed with viral peptide show that the molecule's flexible conformation allows these 8–11aa to bind within the MHC groove once their anchoring residues are fixed (Bjorkman et al. 1987; Rammensee et al. 1995; Falk et al. 1991; Matsumura et al. 1992). Analysis of residues flanking the anchoring residue(s) indicates the critical importance of minor pockets of MHC-binding clefts in the peptide selection process, leading to the concept that these structural factors

are likely to be responsible for the preferential choice of specific peptides observed in interactions between viral components and MHC molecules (HUDRISIER et al. 1996). Mutation of residues in a viral epitope that is important for MHC binding can ablate peptide MHC binding altogether, or reduce the affinity of a peptide-MHC interaction so that the peptide-MHC complex has an extremely short half-life and is unlikely to trigger T cell activation. Alternatively, mutations at residues involved in MHC binding can cause peptides to bind to MHC in a distorted conformation, so that the TCR contact surface is altered (BERTOLETTI et al. 1994a; reviewed in KOUP 1994 and OLDSTONE 1991, 1997). TCR recognition of peptide-MHC complexes can also be affected by changes in the TCR contact residues of an epitope. Altered peptide-MHC complexes may fail to interact with a particular TCR altogether, or they may be recognized but the T cell may receive a reduced or even a different type of signal when it recognizes this complex. The responding T cell may thus be only partly activated, e.g., to proliferate but not perform effector functions, or even anergized. Presentation of certain mutant peptides to T cells can thereby inhibit the response to the complex not just by competing with it for binding to MHC, but by negatively signaling the responding T cell population, a phenomenon known as T cell antagonism (BERTOLETTI et al. 1994b; KLENERMAN et al. 1994).

Studies with LCMV have shown that, under CTL selection pressure in vitro, it is possible to select viral variants bearing CTL escape-conferring mutations in all three of the most immunodominant epitopes recognized in H-2^b mice (LEWICKI et al. 1995a). However, as long as at least one of these three immunodominant epitopes has not mutated, the virus is cleared efficiently and effectively with the usual kinetics in vivo (LEWICKI et al. 1995b). Importantly, when all three immunodominant epitopes mutate in the same virus, the host can still mount lower affinity CTL responses against subdominant epitopes in the virus and control infection, although in this case more time is required to achieve viral clearance. Thus, under CTL pressure, escape variants can arise (PEWE et al. 1998; GOULDER et al. 1997; MOSKOPHIDIS and ZINKERNAGEL 1995; PHILLIPS et al. 1991), but the host has other options and can use epitopes lower in the hierarchy when immunodominant epitopes mutate. The inference drawn is that, unless a particular antiviral response is restricted to one epitope (e.g., in LCMV TCR transgenic mice when the majority of the host T cells are directed against a single viral epitope) (PIRCHER et al. 1990), mutations in dominant CTL epitopes are unlikely to make a major contribution to viral persistence in vivo. This view, derived from work with LCMV, is reinforced by observations of selected CTL escape variants in human immunodeficiency virus (HIV-1) infection (BORROW et al. 1997). Hence, results with LCMV suggest that during natural infections, the selection of CTL escape viral variants is not likely to constitute the sole mechanism for achieving viral persistence. Exceptions are the presence of a uniquely mono-restricted (focused) $CD8^+$ T cell response (PIRCHER et al. 1990) or a unique MHC/HLA haplotype (DE CAMPOS-LIMA et al. 1993). The MHC diversity and breadth of the TCR repertoire in most individuals allows for recognition of viruses even when one or more of the most dominant epitopes have mutated.

Naturally occurring persistent LCMV infection was first recorded by TRAUB (1936), who observed retention of infectious virus in tissues and circulation throughout the lifespan of mice. Two decades later HOTCHIN and CINTIS (1958) described a model whereby newborn mice, inoculated with LCMV within the first 24h of life, survived an ordinarily lethal intracerebral dose of virus (the same dose or one several logs less was lethal to adult mice) and showed continuous infection in sera and tissues over the natural course of life. This model provided a clinical and biological picture similar to the naturally occurring disease described by Traub. Several investigators have demonstrated that such persistently infected mice are relatively deficient in virus-specific immune lymphocytes, but are able to mount high titers of antiviral antibodies (reviewed in BUCHMEIER et al. 1980; BORROW and OLDSTONE 1997). Recently, use of a LCMV CTL vaccine showed that low affinity CTL can be generated in persistently infected mice (VON HERRATH et al. 2000).

Figure 1 shows elements that affect the balance between acute and persistent LCMV infections, and Table 1 lists immune factors that control the course of both acute and persistent infection. Inoculation of immunocompetent adult mice with LCMV by the intracerebral route usually leads to acute leptomeningitis, choroiditis, and inflammation of the ventricle with death occurring within 6–8 days. Cumulative evidence indicates that CTL are the major effector cells in this reaction (DOHERTY and ALLAN 1986; reviewed in BORROW and OLDSTONE 1997; JOLY et al. 1991; GAIRIN et al. 1991). When virus is introduced into adult immunocompetent animals by the peripheral route, animals may survive or die dependent on the balance struck favoring either immunity or immunopathologic injury. When newborn mice, less than 24h old, are inoculated with LCMV by any route, including intracerebral, virus persists in tissues and sera throughout the animals' lives. However, reconstitution of these newborns with LCMV-immune lymphocytes leads to clearance of infectious virus and viral nucleic acids (OLDSTONE et al. 1986; TISHON et al. 1993b; BERGER et al. 2000). When adult immunocompetent mice are either inoculated with an ordinarily lethal dose of virus coupled with an immunosuppressive agent, i.e., irradiation, thymectomy, antilymphocyte serum, cytoxan, or inoculated with an immunosuppressive virus variant (see Fig. 1 and 2), a persistent infection follows. In a persistent infection, established through inoculation of newborn animals, or when virus is transmitted vertically mother-to-fetus (Fig. 4), immunosuppression is restricted to the LCMV T cell response (OLDSTONE et al. 1973). That is, T cell responses are generated to other viruses as well as antibody responses to soluble and particulate antigens or viruses including LCMV. In contrast, when persistent infection is generated by inoculation of immunosuppressive variants (Fig. 2 and 4), a generalized suppression of the immune system occurs (TISHON and OLDSTONE 1991).

The requirements for clearance of a persistent LCMV infection differ from those of an acute infection (Table 1). Whereas virus-specific $CD8^+$ T cells alone control acute LCMV infections, long-term control of persisting virus requires $CD4^+$ T cell help to sustain the $CD8^+$ T cell response (reviewed in BORROW and OLDSTONE 1997; ALTHAGE et al. 1992; JAMIESON et al. 1991; MATLOUBIAN et al. 1994; BATTEGAY et al. 1994; AHMED et al. 1987; OLDSTONE et al. 1986; TISHON

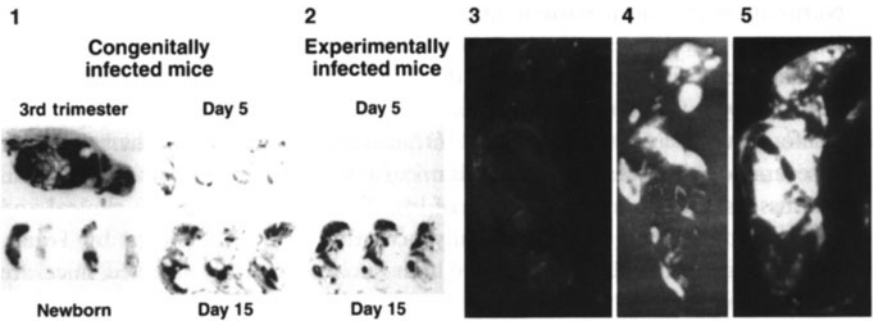

Fig. 4. Transmission of LCMV by the congenital route (*panel 1*) or after experimental inoculation of infectious virus (*panel 2* and *4*): inoculation of newborn mice; *panel 5*, inoculation of adult mice with immunosuppressive variant Clone 13) (*panel 3*, probing uninfected mouse) as observed in whole animal sections and with a ^{32}P riboprobe to LCMV NP. Note infected fetus in the mouse infected by vertical (congenital) transmission. For details of the technology for using whole mouse sections and probes to detect and follow the expression of LCMV or other viral sequences see BLOUNT et al. (1986) and LIPKIN et al. (1989)

et al. 1993b). The precise number of $CD8^+$ T cells and $CD4^+$ T cells required to clear a persistent LCMV infection was quantitated recently (BERGER et al. 2000). In adoptive transfers, the minimum number of cells needed to purge the virus and cure persistent infection is 3×10^6 LCMV-specific $CD8^+$ T cells and 0.7×10^6 LCMV-specific $CD4^+$ T helper cells, a ratio of 50 antigen-specific $CD8^+$ T cells to 1 antigen-specific $CD4^+$ T cell. In addition, although neither IFN-γ nor TNF-α is required for clearance of an acute LCMV infection, both are essential to eliminate persistent LCMV infection (Table 1) (TISHON et al. 1995; A. Tishon and M.B.A. Oldstone, unpublished data).

3 Persistent Infection

3.1 Establishment with LCMV: General

Persistent infection with LCMV can be established by negative selection when this virus invades the thymus. The result is the deletion of potential T lymphocytes of high to moderate affinity that would usually respond to LCMV. Low affinity LCMV-specific cells pass to the periphery and can be activated to respond to LCMV infection or vaccination (reviewed in BORROW and OLDSTONE 1997; VON HERRATH et al. 1994, 2000; BERGER et al. 2000). In addition to this well appreciated mechanism of central tolerance, LCMV can cause peripheral tolerance by infecting interdigitating $DEC205^+$ and $CD11c^+$ dendritic cells (SEVILLA et al. 2000; SMELT et al. 2001).

Initiation of immunosuppression in immunocompetent adult mice by LCMV variants was initially discovered over a decade ago (AHMED et al. 1984b; AHMED

and OLDSTONE 1988). Cloning and sequencing of the immunosuppressive variant LCMV Clone 13 revealed five nucleotide changes from the parental LCMV ARM which altered a single aa in the viral GP and single aa in the viral polymerase (SALVATO et al. 1988). Subsequent findings demonstrated the importance of the GP mutation for the selection of LCMV immunosuppressive variants in the spleen, its influence on the numbers of cells infected in lymphoid tissues and the fact that it has no or limited effect on infection of neurons (AHMED et al. 1991; BORROW et al. 1993; EVANS et al. 1994; MATLOUBIAN et al. 1990, 1994; VILLARETE et al. 1994; DOCKTER et al. 1996). Once inside the cell, a role for the viral polymerase in enhanced replication/transcription that affected viral yield per cell was reported (AHMED et al. 1988).

However, to enter the cell the virus first had to bind to its receptor. Alpha-dystroglycan (α-DG) is the receptor for several arenaviruses, including LCMV (CAO et al. 1998; see also Kunz et al., in Vol. I). DG plays a fundamental role in cell assembly and the organization of basement membranes (HENRY and CAMPBELL 1999). Several viral strains and variants, including LCMV Clone 13 (Figs. 2, 5), bind to α-DG at 2–3 logs higher affinity than other strains [including Armstrong (ARM); Figs. 2, 5] and variants of LCMV (SEVILLA et al. 2000; SMELT et al. 2001). This conclusion came from binding studies using α-DG immobilized on membranes or soluble α-DG to competitively block virus binding to cells expressing α-DG (Fig. 5). Further, viral reassortants made between Clone 13 and ARM and between Traub and ARM mapped attachment and infection of the virion to genes encoded in the S RNA (SMELT et al. 2001) (Fig. 5, panel 2). The S RNA contains the gene that encodes LCMV glycoprotein (GP) and nucleoprotein (NP). Since ARM and Clone 13 have the same NP aa sequence, but differ at a single aa position in their GP, i.e., ARM has phenylalanine (F) at aa 260, but Cl 13 has leucine (L) at that position, the GP1 mutation is implicated in the binding of LCMV to α-DG. Of over 35 cloned LCMV variants studied (SEVILLE et al. 2000), those having a small aliphatic aa at GP1 aa 260 [isoleucine (I) or L] or a serine (S) at GP1 153 bound at high affinity to α-DG. In contrast, viruses having bulky aliphatic aa F at GP1 aa 260 or aa 153 bound at low affinity to α-DG. Most important, strains that bound at high affinity to α-DG, when injected into adult immunocompetent mice, caused immunosuppression (failed to generate anti-LCMV CTL at day 7 post-inoculation) and were associated with persistent infection ($10^{3.5}$–10^5PFU LCMV in sera) over the next several weeks (SEVILLA et al. 2000). In contrast, viruses that bound at low affinity to α-DG were able to generate a robust CTL response by day 7 and cleared the viruses by day 15. Other studies (ODERMATT et al. 1991; BORROW et al. 1995) showed that the immunosuppressive LCMV strains disorganized splenic architecture and preferentially bound to cells in the white pulp (BORROW et al. 1995; SEVILLA et al. 2000; SMELT et al. 2001) (Fig. 5, panel 3), whereas the non-immunosuppressive strains bound to red pulp. Again, reassortants between immunosuppressive and non-immunosuppressive strains mapped this tropism of the virus for the spleen's white pulp to genes encoded in the S RNA (SMELT et al. 2001).

Analysis of cells of the immune system for expression of α-DG revealed its preferential expression by professional antigen-presenting interdigitating dendritic

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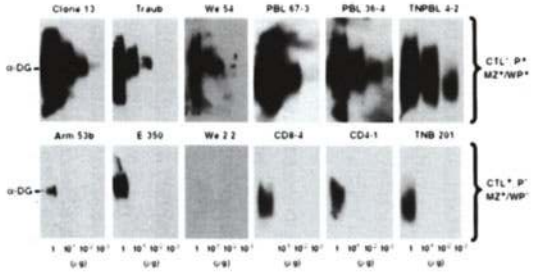

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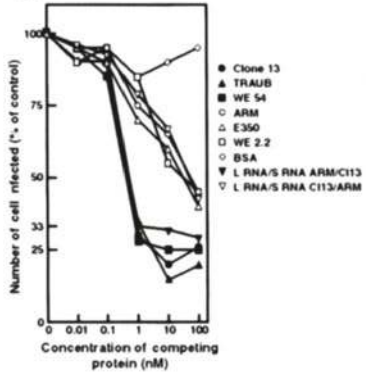

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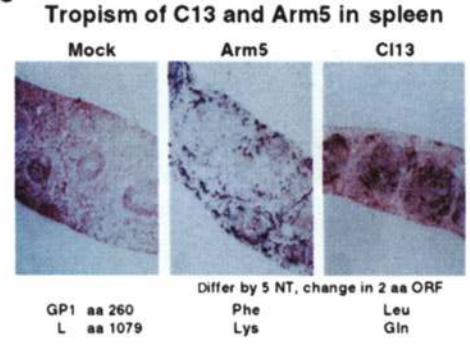

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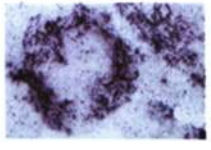
	Expression of α-DG
B cell	<1%
T cell	<1%
CD11b	<1%
CD11c	++++
DEC-205	++



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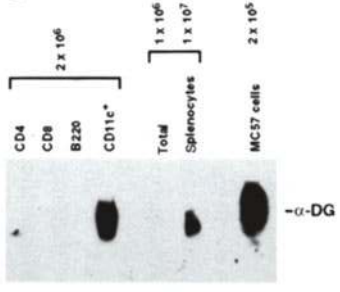

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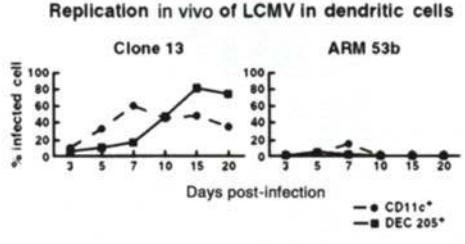

Fig. 5. Virus–dendritic cell interactions. Quasispecies selection of variants that bind at high affinity to the arenavirus cellular receptor α-DG leads to generalized immunosuppression (CAO et al. 1998; SEVILLA et al. 2000; SMELT et al. 2001). *Panel 1*, multiple strains of LCMV and variants generated during a persistent viral infection and isolated from a variety of immune cells bind at high affinity to α-DG immobilized on membranes (*top row*) segregating these strains from those (*bottom row*) that bind at low affinity to α-DG. *Panel 2*, division of viruses into two groups. Those that require 1–8nM of soluble α-DG to block virus attachment (high-affinity binders) and infection (*darkened symbols*) and those requiring >400nM (low-affinity binders) of soluble α-DG (*open circles*). *Panel 2* also shows that genes encoded by S RNA of LCMV (NP or GP) of a high affinity binder are responsible for binding to α-DG. Since the NP sequence of high affinity (Clone 13) and low affinity (ARM 53b) viruses is identical, binding to α-DG maps to the GP of these two strains, which differs by one aa. *Panel 3* shows that the high affinity Clone 13 α-DG binder preferentially localized to the white pulp of spleen, but the low affinity (ARM 53b) binder is tropic for red pulp. Note that the difference in GP aa residues is a single aa with Clone 13 having a leucine in position 260, as opposed to ARM 53b with a phenylalanine at that site. Study of over 50 virus strains and variants indicates the uniformity of the findings in *panels 1–3* and the binding of those viruses at high affinity to α-DG, which causes generalized immunosuppression and persistent infection. In contrast, the low α-DG binders generate a $CD8^+$ CTL response that clears the acute virus infection. *Panels 4–6* implicate infection of $CD11c^+$ and $DEC205^+$ dendritic cells in the binding to α-DG and generalized immunosuppression. The initial observation of organ-specific (spleen) selection of viral variants (AHMED and OLDSTONE 1988; AHMED et al. 1984b) preceded the discovery of α-DG as the receptor for LCMV (CAO et al. 1998) and the discovery of selected tropism for white and red pulp (BORROW et al. 1995; SEVILLA et al. 2000; SMELT et al. 2001). *Panel 4*, the use of selected monoclonal antibodies and a fluorescence activated cell sorter (FACS) shows that expression of α-DG is preferential on $CD11c^+$ and $DEC205^+$ dendritic cells but negligible on B and T cells and CD11b cells. *Panel 4* also displays virus nucleic acid sequences of a high affinity binding virus located to the white pulp of the spleen. *Panel 5*, quantitative studies of α-DG isolated from cells of the immune system again show preferential localization to dendritic cells ($CD11c^+$). *Panel 6*, replication of LCMV Clone 13 in $CD11c^+$ and $DEC205^+$ cells (up to 80% of such cells are infected) is shown. By comparison <12% of such cells are infected by LCMV ARM, a low α-DG binder

cells (IDC) (SEVILLE et al. 2000) (Fig. 5, panels 4–6). Study of the kinetics of IDC infection revealed that over 60% with as much as 80% of $CD11c^+$, $DEC205^+$ cells were infected with the immunosuppressive variant Clone 13, whereas less than 10% of these cells became infected with a non-immunosuppressive strain of the virus. Biochemical studies suggested the involvement of B7.1 coregulating factor in infected $DEC205^+$ and $CD11c^+$ cells in vivo (N. Sevilla et al., unpublished data), and in vitro analysis of a murine dendritic cell line infected with high affinity Clone 13 but not with low affinity LCMV ARM revealed participation of IL-12 transcription (A. Holz et al., unpublished data) as functional correlates for maintaining the generalized immunosuppression.

Thus, in conclusion, among cells of the immune system, $CD11c^+$ and $DEC205^+$ splenic dendritic cells primarily express the cellular receptor α-DG for LCMV. By selection, strains and variants of LCMV that bind α-DG with high affinity are associated with virus replication in the white pulp, show preferential replication in a majority of $CD11c^+$ and $DEC205^+$ cells, cause immunosuppression and establish persistent infection. Recent studies by Smelt et al. (unpublished data) show that LCMV strains entering $CD11c^+$ and $DEC205^+$ cells in the marginal zone are carried into the T cell-dependent area akin to the Trojan horse of Homer's *The Iliad*. In contrast, LCMV strains and variants that bind with low affinity to α-DG replicate mainly in the red pulp but only minimally in $CD11c^+$

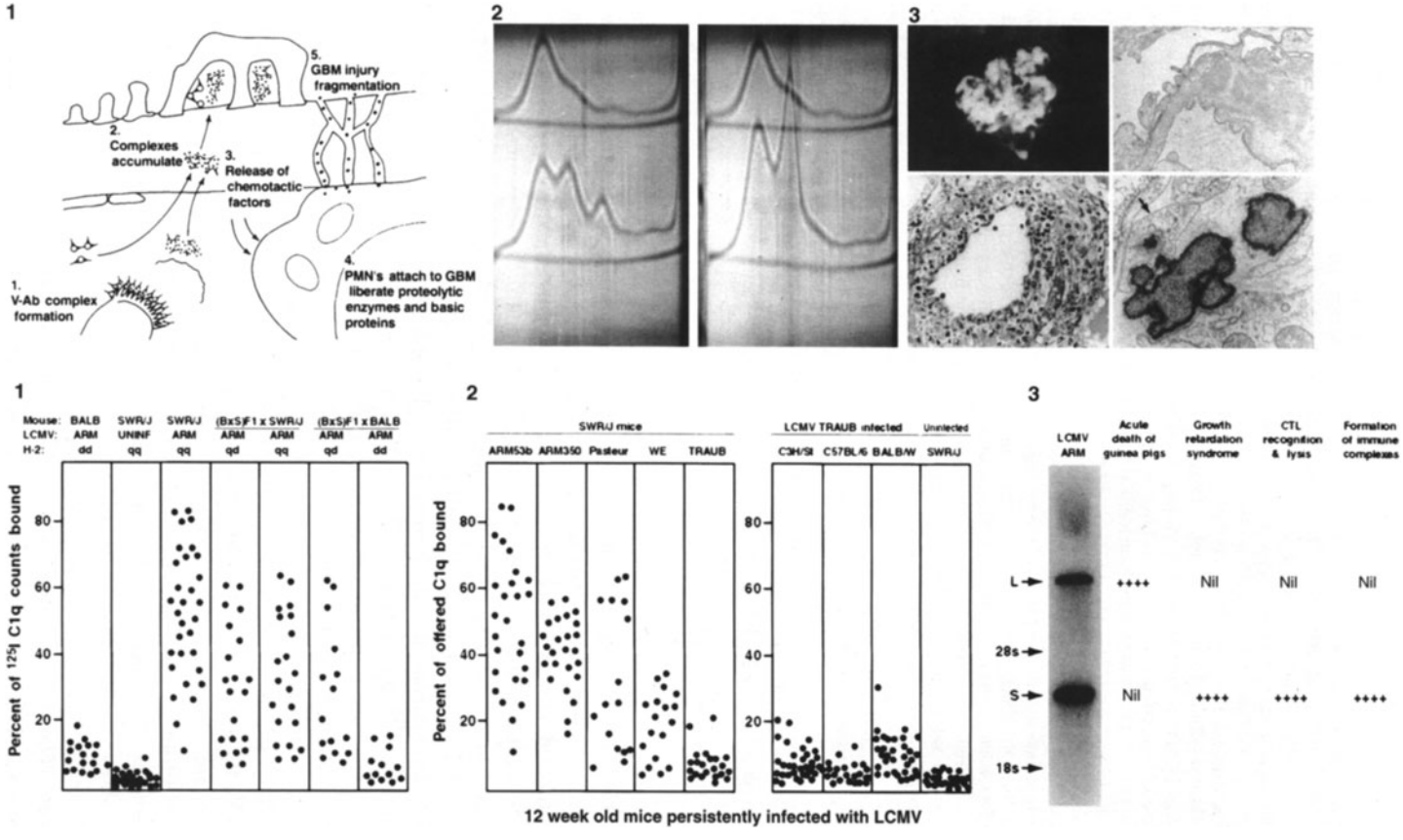
1
5. GBM injury fragmentation
2. Complexes accumulate
3. Release of chemotactic factors
4. PMN's attach to GBM liberate proteolytic enzymes and basic proteins
1. V-Ab complex formation
2
3
1
Mouse: BALB SWR/J SWR/J (BxS)F1 x SWR/J (BxS)F1 x BALB
LCMV: ARM UNINF ARM ARM ARM ARM ARM
H-2: dd qq qq qd qq qd dd
Percent of 125I C1q counts bound
2
SWR/J mice
ARM53b ARM350 Pasteur WE TRAUB
LCMV TRAUB infected
C3H/St C57BL/6 BALB/W
Uninfected
SWR/J
Percent of offered C1q bound
12 week old mice persistently infected with LCMV
3
LCMV ARM
Acute death of guinea pigs
Growth retardation syndrome
CTL recognition & lysis
Formation of immune complexes
L
28s
S
18s
++++
Nil
Nil
Nil
Nil
++++
++++
++++

Fig. 6. Virus-antiviral (*V-Ab*) immune complexes. Generation, disease consequences, host MHC gene control and role of viral genes. *Panel 1* (*top*), cartoon illustrating how V-Ab immune complex form and deposit in the renal glomeruli. *Panel 2* (*top*), *upper portion* shows ultracentrifugation profile of sera from normal control mice; the *lower part* shows sera from persistently infected mice containing circulating complexes. These V-Ab immune complexes are infectious as proven when removal of Ig by immunoprecipitation with an antibody to Ig markedly reduces (2 logs or more) the viral titer in the sera, yet similar precipitation with an antibody to albumin does not lower the viral titer. *Panel 3* (*top*; *clockwise* beginning at 12 o'clock) profiles tissue deposition of immune complexes in the renal glomerulus detected by immunofluorescence, detected by electron microscopy, immune complexes in the choroid plexus being engulfed by macrophages and immune complexes in an artery causing arteritis (OLDSTONE and DIXON 1969, 1972; LAMPERT and OLDSTONE 1974; OLDSTONE 1976). *Panel 1* and *panel 2* (*bottom*) show circulating immune complexes detected in sera using C1q. In *panel 1*, after persistent infection with LCMV ARM strain BALB mice (H-2^d) generate low to negligible amounts of C1q binding immune complexes, whereas SWR/J mice (H-2^q) generate high levels of C1q binding complexes. F1 hybrids between these two strains have high levels of complexes, but mice generated by crossing these F1 animals to each parent have high levels of C1q circulating complexes only when one H-2^q gene is present. Low to negligible amounts require both H-2^d alleles. Use of recombinant inbred mice maps the host genetic control to MHC I^A gene(s) (OLDSTONE et al. 1983). LCMV titers in sera of H-2^d and H-2^q and hybrid mice are equivalent. *Panel 2* (*bottom*) shows that SWR/J generate an abundance of V-Ab immune complexes when persistently infected with LCMV ARM 53b, E-350 or Pasteur strains; however infection with LCMV Traub or WE strains fails to generate significant levels of V-Ab immune complexes (TISHON et al. 1991). Reassortants between LCMV ARM 53b and LCMV Traub mapped the formation of V-Ab immune complexes to viral genes encoded on the S RNA (the GP and NP). *Panel 3* (*bottom*) shows other examples in which reassortants mapped LCMV pathogenic genes. The acute death of guinea pigs infected with LCMV WE mimicked aspects of Lassa fever viral infections and mapped to the L RNA that encodes the Z and polymerase (*L*) genes (RIVIERE et al. 1985b). Both the generation and function of CTL and inception of GH disease mapped to genes encoded on the S RNA of LCMV (RIVIERE et al. 1985a; AHMED et al. 1984a; OLDSTONE et al. 1985)

and DEC205$^+$ cells and generate a robust anti-LCMV CTL response that clears the virus infection. Differences in binding affinities can be mapped to mutations in the viral GP1 ligand that binds to α-DG and in several instances to a single aa change. Thus, receptor/virus interaction on dendritic cells in vivo can be an essential step in the initiation of virus-induced immunosuppression and viral persistence (Fig. 2).

3.2 Virus-Antibody Immune Complex Formation and Disease in Persistent Viral Infection

In most virus infections, antibody to virus interacts with virus or viral antigens in the circulation, resulting in the formation of V-Ab complexes. V-Ab complexes themselves are potent pathogenic agents and, once deposited in tissues, induce a phlogogenic response. V-Ab immune complex disease occurs frequently in chronic viral infections and is a common pathogenic mechanism of animal and human nephritides and arteritides. Persistent LCMV infection has been a paradigm in which the occurrence, basis and genetic control of immune complex disease have been dissected (reviewed in OLDSTONE 1975; OLDSTONE et al. 1983; TISHON and OLDSTONE 1991) (Fig. 6).

Evidence for V-Ab immune complex disease lies in (1) demonstrating circulating V-Ab complexes, and (2) showing localization of virus, host Ig and com-

plement at the sites of tissue injury. Several techniques used to show circulating V-Ab immune complexes (Fig. 6) are C1q precipitation, complement utilization, electron microscopy, analytical ultracentrifugation, monoclonal rheumatoid factor precipitation, platelet agglutination, precipitation of V-Ab complex with antibody directed toward the antibody or complement bound with the virus (anti-Ig or anti-C3 precipitation) and use of cultured cells that contain receptors for bound C3.

That virus travels in the circulation complexed with host Ig was demonstrated by precipitating Ig from the sera (using an anti-mouse IgG) of mice chronically infected with LCMV and showing significant reduction of the infectivity titer (reviewed in OLDSTONE 1976). These results with LCMV have been duplicated in mice chronically infected with lactic dehydrogenase virus and leukemia virus, mink infected with Aleutian disease virus, horses infected with equine infectious anemia virus, and humans infected with hepatitis B antigen (HB Ag), Epstein-Barr virus, cytomegalovirus (reviewed in OLDSTONE 1975) and HIV. In addition, in the sera of chronically infected mice, LCMV circulates complexed not only with Ig but also with C3, since immunologically specific precipitation of C3 as well as Ig removes two or more logs of infectivity.

Deposition of circulating V-Ab complexes in tissues has been confirmed by identification of viral antigens, host Ig and complement in a granular pattern along renal basement membranes. Immunofluorescence and electron microscopy of such tissues containing immune complexes yields the characteristic patterns pictured in Fig. 6. Identification and quantitation of the specific antiviral antibodies present in the deposited complex are accomplished by: (1) elution of the glomerular-bound Ig by either low ionic, high ionic or low pH buffers to dissociate V-Ab bonds; (2) recovery of the eluted Ig and its quantitation by immunoprecipitation; and (3) quantitation of the Ig after absorption with various virus, tissue and cellular antigens, again by any of several immunoprecipitation assays. Serum Ig is immunochemically isolated and assayed in a similar way. The ratio of antiviral antibody to total Ig in the tissue eluate over the antiviral antibody to total Ig in the serum depicts the concentration of antiviral antibody localized in the tissue. Although such elution studies have limitations – most notably loss of eluted antibody via recombination with eluted antigens, incomplete elution or denaturation of eluted antibody – they nevertheless provide the only direct quantitation of antibodies present in the injured tissues.

The major sites of V-Ab immune complex deposits are on the basement membranes of the renal glomerulus, endothelial walls, medium and small arteries and in the choroid plexus (Fig. 6) (OLDSTONE 1975). V-Ab immune complexes have also been found in other tissue, i.e., heart, lung, joints, skin.

Once complexes are formed and circulate, they are either phagocytosed and removed by cells of the reticuloendothelial system (RES) or deposited in tissues. Large complexes appear to be preferentially removed by the RES over small complexes. Also, some cells of the RES have receptors that recognize different classes of Ig and complement and show different binding affinities. Changes in structure or recognition units of Ig seem important in phagocytosis, since alteration by reduction and alkylation of antibody (before making the soluble complex)

decreases the complex's rate of removal from the circulation. RES activity undergoes depression after prolonged exposure to circulating immune complexes. The resulting decreased efficiency in RES function precedes the onset of immune complex deposition and subsequent development of proteinuria and nephritis. The phagocytic properties of mesangial cells of the kidney may then protect against such deposition in the glomerular basement membrane until mesangial cell overload eventually allows deposition on the glomerular basement membrane.

During the active process of immune complex deposition, increased vascular permeability occurs and is associated with the release of vasoactive agents. With this increase in vascular permeability, large complexes, usually 19s or larger, deposit along filtering membranes.

Evidence that immunopathologic consequences of LCMV-Ab immune complexes cause disease is supported by several observations. First, if mice infected at birth with LCMV are nursed by LCMV-immune foster mothers, they have a more rapid and severe onset of immune complex glomerulonephritis and arteritis as well as a shorter lifespan than conventionally reared carrier mice (Oldstone and Dixon 1972). Maternal antibody is found complexed to LCMV antigens in the glomeruli. Second, adoptive transfer of anti-LCMV antibody into persistently infected mice or the parabiosis of an immune syngeneic mouse to a persistently infected mouse results in enhancement and severe manifestations of chronic LCMV disease (Oldstone and Dixon 1969). Third, induction of LCMV infection-associated immune complex disease depends on both the mouse strain and infecting LCMV isolate, and disease severity correlates with the level of V-Ab complexes produced (Fig. 6).

Disease-susceptible inbred mouse strains are those that make high levels of antiviral antibody after neonatal infection with LCMV. For example, SWR/J mice persistently infected with LCMV ARM (high responders) make 50-fold more LCMV NP- and GP-specific antibody and have sevenfold higher levels of circulating complement-binding immune complexes than persistently infected BALB/WEHI mice (low responders) (Fig. 6). The SWR/J strain shows heavier deposits of virus-antibody complexes in their tissues, although both mouse strains carry the same load of infectious virus (Oldstone et al. 1983; Tishon et al. 1991). Breeding studies between high responder mice, low responder mice, their hybrid offspring, backcrosses of the hybrids to both high and low responder parents, and use of selected recombinant inbred mouse strains map the formation of C1q binding V-Ab complexes to genes located in the Ir region of MHC H-2 loci (Fig. 6). Thus, MHC class II genes controlling the strength of antibody responses played a prominent role in the V-Ab immune complex disease of mice despite similar levels of infectious virus in high and low responders.

Other studies showed the importance of LCMV strain in causing V-Ab immune complex formation and disease during persistent infection (Tishon et al. 1991) (Fig. 6). Although SWR/J mice persistently infected with LCMV ARM or E-350 contain very high levels of circulating and trapped immune complexes, and LCMV WE or Pasteur produce somewhat less but still substantial levels, LCMV Traub elicits a much lower antiviral antibody response. Thus, SWR/J mice

persistently infected with the Traub strain of LCMV have low to negligible levels of circulating immune complexes, with minimal immune complex deposition in tissues, even though their viral load is similar to that in animals infected with LCMV ARM (Fig. 6). Therefore, both host and viral determinants influence susceptibility to the development of immune complex disease, acting by modulating the level of antiviral antibody production, immune complex formation and resultant immunopathologic injury.

4 Persistent Virus Infection Alters the Function of Differentiated Cells Leading to Disease

4.1 General Concept and In Vitro Findings

Peter Medawar amply summarized viruses when he described them as "a piece of bad news wrapped in a protein coat" (MEDAWAR and MEDAWAR 1983). Viruses were first separated from other microbial agents by using the Pasteur-Chamberland filter. Viruses known or suspected to cause disease have been characterized, first, by the clinical picture and histological profile and, second, by virologic, immunologic, or molecular biologic assays. These first procedures, were initially applied by IVANOVSKI (1899) and BEIJERINCK (1899) to infection of tobacco plants with tobacco mosaic virus and by LOEFFLER and FROSCH (1898) to infection of cattle with foot and mouth virus. This and the second approach continue to be used today. Thus, the sine qua non for suspecting viral involvement has been a histopathologic picture of cellular necrosis, usually, but not always, including inflammatory infiltrates. Indeed, the histological picture frequently suggests the virus involved, e.g., "cytomegalo-like" cells during infection with cytomegalovirus, injury of anterior horn neurons in poliomyelitis, and the appearance of Negri or Lyssa bodies within certain neurons of the central nervous system in rabies. In other instances, such as encephalitis or myocarditis, the cellular necrosis and associated perivascular cuffing are not specific enough to suggest a single etiologic agent – any of several agents could be the cause. The decisive factors in the course of virally induced injury are initially the tropism of that virus for specific cells and then either the viral lytic ability per se or the interaction between the components of the host immune response and the infected cell or virion. In either case, viral infections are often classified in contemporary medical books according to the cells injured and the inflammation or transformation observed. As indicated, the type of disease is dependent on the specific cell infected (e.g., neuron, lymphocyte, or myocardial cell), and the severity reflects the number of host cells infected and the type injured. For example, the death of neurons is likely to be more devastating than death of fibroblasts.

A possibility of very recent vintage is that a virus might cause disease by altering the function of a cell without destroying that cell (reviewed in OLDSTONE

1984a,b, 1985, 1989, 1993). For example, while studying the effects of persistent LCMV infection on differentiated neuroblastoma cells, we noted abnormalities in the synthesis and degradation of acetylcholine caused by a decrease in the production of the appropriate acetylase or esterase (OLDSTONE et al. 1977) (Fig. 7, panels 1 and 2). Nevertheless, these cells were normal in morphology, growth rates, cloning efficiencies, and levels of total RNA, DNA, protein, and vital enzyme

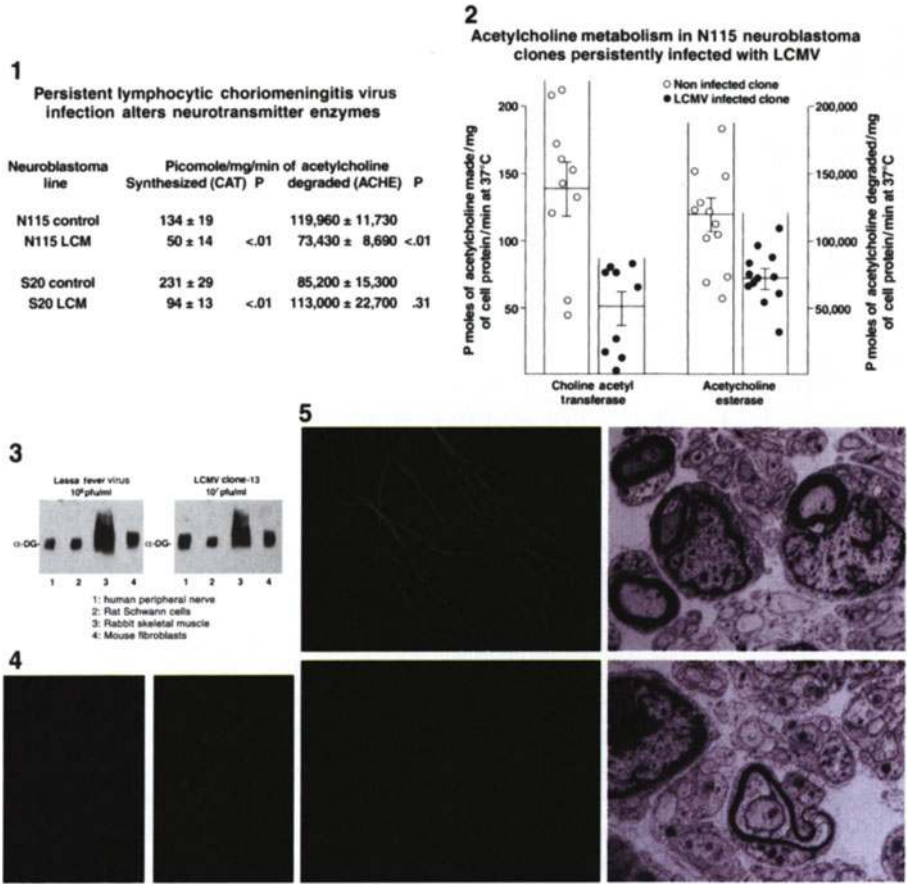

Fig. 7. Virus alters differentiated functions of neuronal cells in vitro (OLDSTONE et al. 1977; RAMBUKKANA and OLDSTONE 2001). *Panels 1* and *2* show diminished synthesis of the acetylase and the esterase of acetylcholine in neuroblastoma cells on the population (*panel 1*) and clonal cell level (*panel 2*). *Panel 3* shows several cell lines, including Schwann cells, expressing high concentrations of the LCMV receptor α-DG; Lassa fever virus and LCMV Clone 13 display similar high affinity binding to α-DG. Low affinity α-DG binding LCMV ARM cannot infect Schwann cell cultures, high affinity binding LCMV Clone 13 (*panel 4*) does infect (*green fluorescence* >95% of cells express LCMV NP) but not kill these cells [host strain (*blue*) no cell necrosis], instead causing a persistent infection. *Panel 5* (*upper panel*) control cells (dorsal root ganglia and neuronal cells) or LCMV ARM infected cells in culture allow normal axon formation with myelination as shown by the myelin basic protein (*green fluorescence*), and by electron microscopy. In contrast (*lower panel*), Clone 13 infection prevents myelin genesis yet does not lyse these cells and minimal myelin formation is detected by EM

synthesis. Similarly, Holtzer and his associates (HOLTZER et al. 1975, 1982) showed that Rous sarcoma virus (Prague strain ts mutant), grown at temperatures that were permissive for infection of differentiated chick chondroblasts, myotubes, or melanoblasts, altered the unique functions of these specialized cells. Thus, infected chondroblasts failed to make sulfated proteoglycans; myotubes failed to synthesize heavy and light chains of myosin, and melanoblasts did not produce melanin. At non-permissive temperatures, manufacture of these differentiated products was normal. By changing the temperature of these infected cells, Holtzer and colleagues observed either the selected deficiencies of differentiated products or a return to normal functions.

Subsequently, viruses have been shown to alter immunologic functions in a similar way. CASALI et al. (1984) showed that infection with measles virus or influenza virus of peripheral blood lymphocytes aborted their expected specialized functions, including the manufacture of Ig and the capacity to act as cytotoxic effectors. Similar results were shown by SCHRIER and OLDSTONE (1986) during human cytomegalovirus (HCMV) infection which rendered human T cells unable to lyse HCMV targets. Hence, these RNA (measles, influenza) and DNA (HCMV) viruses altered the differentiated functions of lymphocytes without lysing or destroying them.

Recently, with an in vitro system comprising dorsal root ganglia cultured with Schwann cells, LCMV infection aborted axon myelination (RAMBUKKANA et al. 2001) (Fig. 7, panels 3–5). These cultured neurons developed myelin sheaths as documented by the expression of myelin basic protein on axons and by electron microscopy of myelin formation. Because these cells have heavy concentrations of α-DG (RAMBUKKANA et al. 2001; S. Kunz and M.B.A. Oldstone, unpublished observations), their infection by either Clone 13, a high affinity LCMV binder to α-DG, or LCMV ARM, a low affinity binder, was studied. Clone 13 infected >95% of the cells, while LCMV ARM infected <10%. Despite universal LCMV Clone 13 infection, the cells showed no apoptosis or physical injury. Yet, as dramatically shown in Fig. 7, myelin failed to form in LCMV Clone 13-infected but not uninfected cultures or cultures infected with LCMV ARM. Clearly, the virus can alter the differentiated or luxury function of cells without disturbing their vital functions. These findings have been extended to other cells/organs infected with LCMV and with other RNA and DNA viruses both in vitro and in vivo (Table 2). The two best-studied in vivo systems, the growth hormone (GH) deficiency syndrome and alterations in behavior and learning, are presented below.

4.2 Growth Hormone Deficiency Syndrome

C3H/St mice infected neonatally with the ARM strain of LCMV exhibit a GH deficiency syndrome (Fig. 8) manifested as growth retardation and severe hypoglycemia (OLDSTONE et al. 1982, 1984). Decreases in body weight and length become apparent when the mice are 5–7 days old, and by 15 days the weight of infected animals is approximately 50% that of uninfected control animals. Such

Table 2. Viruses cause disease by altering a cell's differentiation function and unbalancing homeostasis

Pathology in the absence of cell lysis

Growth retardation and hypoglycemia
- 50% ↓ Growth hormone synthesis
- 5x ↓ Growth hormone mRNA (steady state)
- 16x ↓ Growth hormone mRNA (initiation of transcription)
- 5–10x ↓ Growth hormone transactivator GHF-1

Hypothyroidism
- 30% ↓ T3, T4
- 5x ↓ Thyroglobulin mRNA (steady state)

Chemical diabetes
- ↑ Blood glucose
- Normal or low pancreatic insulin

Neuronal dysfunction
- ↓ Gap 43
- ↓ Somatostatin mRNA
- ↓ GABA
- ↓ Cholinergic mRNA and protein
- Normal GAD (GABA) mRNA
- Normal MuBr 8 (SNAP mRNA)
- Normal amyloid B protein mRNA
- Normal actin mRNA
- ↓ Integrative function
- ↓ Ability to form myelin

Obesity
- ↓ Norepinephrine, ↓ Dopamine

Deformed whiskers

Cardiomyopathy

Faulty antigen presentation by dendritic cells
T lymphocyte suppression
- Failure to clear virus
- Viral persistence

persistently infected mice also become severely hypoglycemic, a result of GH deficiency, which is likely to be the reason that most of them die. The mechanisms underlying this growth retardation have been analyzed in great detail, and this is probably the best understood example of a disease resulting from direct viral interference with the differentiated function of cells in the absence of cytolysis or inflammation.

The retardation of growth and severe hypoglycemia are dependent on mouse strain and virus isolate (Oldstone et al. 1984; Bureau et al. 2001; Tishon and Oldstone 1990). Whereas C3H/St and certain other mouse strains, including CBA/N mice display the GH deficiency syndrome when infected neonatally with LCMV ARM, still other strains (e.g., BALB/WEHI and SWR/J mice) are disease resistant (Tishon and Oldstone 1990). Disease susceptibility is not MHC linked and involves multiple genes. Similarly, whereas the ARM and E-350 strains of LCMV

1

2

Parameters 15 day old mice	Virus infected across both	Uninfected control
Growth development	30% reduction in size	
Blood glucose (mg/dl)	41 (3-80)	135
Survival to 30th day of life	<5%	>95%
Growth hormone level in pituitary (µg hormone/mg pituitary)	16	36
Growth hormone messenger RNA integrity (bp)	800	800
Growth hormone messenger RNA (steady state)	5x reduction	
Growth hormone messenger RNA (initiation)	10x reduction	
TSH mRNA		
Actin mRNA		

3

L/S RNA A/A	W/W	W/A	A/W
PIT GH ↓-50%	Normal	↓-50%	Normal
WGT 3.9gm	6.8gm	2.6gm	5.9gm
B. GLU 56mg%	125mg%	37mg%	113mg%
Survival 5%	95%	5%	95%

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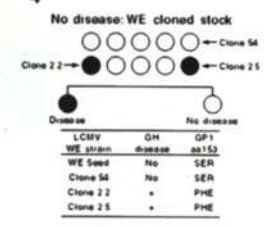

LCMV WE strain	GH disease	GP1 aa152
WE Seed	No	SER
Clone 54	No	SER
Clone 2 2	+	PHE
Clone 2 5	+	PHE

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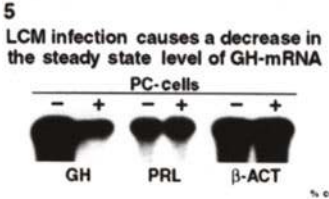

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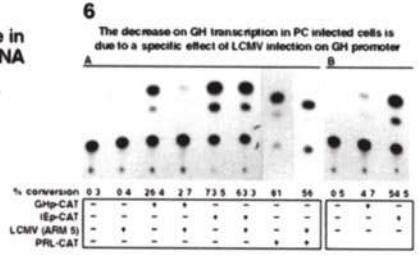

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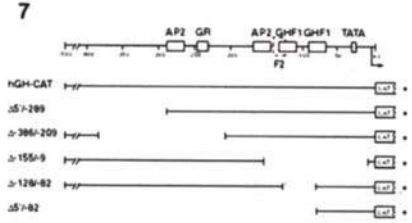

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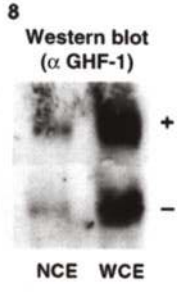

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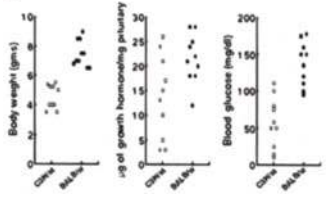

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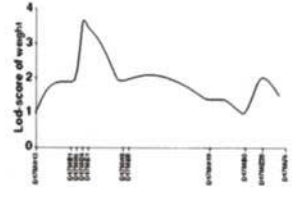

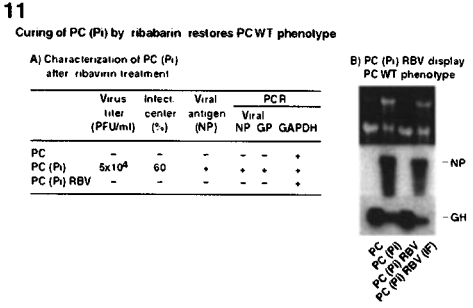

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- **Two viral genes are involved in GH disease**
  1. **GH Disease maps to S RNA of LCMV**
  2. **Viral GP: receptor binding/entry**
  3. **Viral NP: binding to GHFI transcription factor**
- **Host susceptibility gene(s) map to locus on chromosome 17 by site H-2D/D17 Mit 24/D17 Mit 51**

Fig. 8. Persistent virus infection alters the differentiation (luxury) function of a cell without lysing it, but disturbs homeostasis and causes systemic disease: mapping of the viral and host genes involved. *Panel 1* illustrates LCMV NP antigens in GH-producing cells of the anterior lobe of the pituitary in C3H/St mice during persistent infection with LCMV ARM. Mice were infected at birth and studied at day 12. *Panel 2* displays the phenotype of virus-infected and control mice (OLDSTONE et al. 1982, 1984a; VALSAMAKIS et al. 1987; KLAVINSKIS and OLDSTONE 1989). *Panel 3* shows the reassortants made from LCMV ARM (*A*), a strain that causes the GH disease, and LCMV WE (*W*), a strain that does not, to map the relevant viral genes. Reassortants between the long (*L*) and short (*S*) RNAs of these LCMV strains were used to infect C3H/St mice. *Upper panels* display LCMV infections in GH cells in the anterior lobe of the pituitary, and *lower panels* show the phenotypes. The results reveal that viral genes encoded on the S RNA, the GP or NP are responsible for the effect on growth and development (OLDSTONE et al. 1985). *Panel 4* provides evidence that the viral GP is involved by selecting variants of WE that have a single aa mutation in the GP but complete homology in the NP and cause or do not cause GH disease. Here, a single aa mutation in GP residue 153 was associated with GH disease; serine 153 with GH disease^{+} and phenylalanine 153 with GH disease^{nil} (BUESA-GOMEZ et al. 1996; TENG et al. 1996a,b). Presumably, the viral GP was required for binding to or entry into the GH-producing cell. In transgenic mice made so that LCMV NP under control of the GH promoter was placed in GH cells, GH disease developed, implicating the viral NP once it is inside the GH cells. In contrast, expressing the viral GP inside the GH cell failed to produce disease. *Panel 5* shows that LCMV infection of cells (PC) that produce both GH and prolactin (PRL) affects GH but not PRL transcription. In these PC cells, each cell transcribes both GH and PRL. *Panel 6* depicts the results from a CAT assay in which the CAT enzyme is under the control of the GH or PRL promoter. The viral effect maps to the GH promoter (DE LA TORRE and OLDSTONE 1992). *Panel 7* shows that, with the use of this GH promoter, deletion mutants map the effect of virus to a 61bp region in the GH promoter that encodes the transcription factor GHF-1 (PIT-1). *Panel 8* shows, at the protein level, the reduced GHF-1 from PC cells in the whole cellular extracted faction (WCF). Mapping of host genes involved in the virus-induced GH disease (TISHON and OLDSTONE 1990; BUREAU et al. 2001) appears in *panels 9* and *10*. On the basis of weight, GH levels and blood glucose measurements, C3H/St mice are susceptible but BALB/W mice are not (*panel 9*). F2 crosses between these two strains and the use of a microsatellite mapping technique to demonstrate host susceptibility gene(s) place the locus on chromosome 17 near the H-2D site between D17 MIT24 and D17 MIT51 (*panel 10*). *Panel 11* demonstrates the reversibility of the viral effect on differentiated function. LCMV infection of PC cells aborts GH transcription; however, use of the anti-arenavirus agent ribavirin (*RBV*) cures the infection and restores GH transcription. NP in the northern blot refers to expression of LCMV NP transcripts (DE LA TORRE and OLDSTONE 1992)

produce both growth retardation and hypoglycemia in C3H/St mice, and LCMV Pasteur does so to a lesser extent, LCMV WE and Traub have only a minimal effect on growth and do not cause hypoglycemia or the related death (OLDSTONE et al. 1985).

When growth is retarded in any murine virus combination, it always correlates with the level of infection in GH-producing cells in the anterior pituitary gland. Most (> 90%) GH-producing cells in the anterior pituitary of C3H/St mice infected with LCMV ARM or LCMV E350 become infected and contain viral antigen. With electron microscopy, mature virus particles can be detected budding from the surfaces of these cells (OLDSTONE et al. 1982). In contrast, Traub and WE strains of LCMV infect far fewer GH-producing cells (< 15%) in the same mouse strain. Studies with reassortant viruses generated between LCMV ARM and LCMV WE initially mapped the ability to infect GH-producing cells and cause growth retardation in C3H/St mice to the genes encoded on the viral S RNA segment (Fig. 8, panel 3) (OLDSTONE et al. 1985). More recently, LCMV clones have been isolated by selecting plaques from the LCMV WE population that, unlike sister clones or the parental population, retard growth in C3H/St mice (BUESA-GOMEZ et al. 1996; TENG et al. 1996a,b). Sequence comparison of the S RNA segment of WE clones that do and do not cause disease in C3H/St mice has revealed that a single aa change in the viral GP correlates with disease-causing potential (Fig. 8, panel 4). Although this aa difference affects the binding of some LCMV WE clones to their cellular receptor α-DG in vitro (SMELT et al. 2001), the α-DG gene is encoded on mouse chromosome 9, whereas susceptibility to GH disease maps to chromosome 17 (BUREAU et al. 2001). Further, Traub, WE and WE-54 strains, which bind at high affinity to α-DG, do not cause GH disease nor do they infect GH cells in the pituitary, although they replicate to high titers elsewhere. In contrast, LCMV strains like ARM, E-350 and WE2.2 and WE2.5 that bind at low affinity to α-DG infect GH cells in the pituitary. Hence, α-DG is not the relevant receptor on such cells. An alternative, yet to be discovered, receptor may be preferentially expressed on C3H/St and similar susceptible mice.

How does LCMV replication in GH-producing cells activate growth retardation? Oldstone and co-workers (OLDSTONE et al. 1982, 1984) initially reported that LCMV ARM infection of C3H/St mice resulted in a significant reduction (approximately 50% on day 16) in the level of GH in the pituitary, and Valsamakis and colleagues (1987) showed that this decrease correlated with a fivefold reduction in the steady-state level of GH mRNA. In turn, this event was related to a reduction in initiation of transcription of this gene, which appeared to be selective. Transcriptional initiation of another pituitary gene, the precursor of thyroid-stimulating hormone (TSH-β) or of the housekeeping genes, actin and proα2(1) collagen, were only minimally affected (VALSAMAKIS et al. 1987; KLAVINSKIS and OLDSTONE 1989).

A more in-depth characterization of the molecular mechanisms underlying LCMV-induced downregulation of GH mRNA synthesis involved a tissue culture model using a rat pituitary cell line (PC cells) that expresses both GH and prolactin (Fig. 8, panel 5). Persistent infection of these cells (each cell expresses both GH and

prolactin) by LCMV markedly downregulated GH mRNA transcription but caused comparatively minimal interference with prolactin transcription (DE LA TORRE and OLDSTONE 1992). Transfection experiments indicated that expression of the reporter gene, chloramphenicol acetyltransferase, in PC cells was significantly decreased by LCMV infection when the reporter gene was expressed under the control of the GH promoter (Fig. 8, panel 6) (DE LA TORRE and OLDSTONE 1992). By contrast, similar levels of chloramphenicol acetyltransferase activity were obtained in uninfected and LCMV-infected cells when chloramphenicol acetyltransferase expression was under control of either a cytomegalovirus immediate-early promoter or a simian virus (SV40) promoter. Next, the use of GH promoter deletion mutants (Fig. 8, panel 7) together with in vitro transcription assays using nuclear fractions from uninfected or LCMV-infected PC cells suggested that the viral effect on GH promoter activity is caused by interference with the GH transactivation factor, GHF1 (PitI). Finally, PitI protein levels were reduced in LCMV-infected PC cells (Fig. 8, panel 8).

Other studies of the molecular basis of the ability of LCMV to downregulate GH mRNA synthesis in PC cells have addressed which viral component(s) mediates the phenomenon. Infection of PC cells with a recombinant vaccinia virus expressing LCMV NP, but not with a control vaccinia virus recombinant or one expressing LCMV GP, significantly decreased the level of GH mRNA, indicating that the viral NP or its mRNA is a sufficient mediator. Confirmation came from results indicating that the interaction of LCMV NP or its mRNA with the PitI protein or its mRNA likely forms the molecular basis of this selective downregulation of GH mRNA transcription and, in turn, GH production, thereby retarding growth. Similarly, in transgenic mice, the GH promoter expressed LCMV NP and mimicked the GH deficiency syndrome caused by persistent LCMV infection.

Recently, the host genes involved in resistance/susceptibility to the virus-induced GH disease were better defined by microsatellite mapping (BUREAU et al. 2001) (Fig. 8, panels 9 and 10). Although C3H/St, BALB/WEHI or CDJ and SWR/J mice infected at birth with LCMV ARM harbored equivalent amounts of virus in their blood, brain, heart, liver, spleen and thymi through life, only C3H/St mice replicated high titers of virus in their anterior lobe of the pituitary gland infecting the majority (>90%) of GH-producing cells (Fig. 8, panels 1 and 3) (OLDSTONE et al. 1985; TISHON and OLDSTONE 1990). In contrast, less than 15% of GH-producing cells became infected in BALB and SWR/J mice. Half the F1 hybrid offspring produced by crossing the susceptible C3H/St GH-deficient strain with the BALB/WEHI GH-resistant mice then developed the disease, but the trait was not sex-linked (TISHON and OLDSTONE 1990). F1 hybrid backcrosses to the susceptible C3H/St parental strain or to the resistant BALB/WEHI parental strain indicated that more than two genes were involved. C3H/St mice have the H-2^k haplotype; even though some other H-2^k strains also developed GH disease after LCMV infection, other H-2^k mice did not (TISHON and OLDSTONE 1990). Further, C3H/Sw mice that have the H-2^b haplotype on the C3H background did develop this disease, further indicating that disease is not related to the H-2^k haplotype but to the C3H background genes. Collectively, these data suggest that the GH deficiency

disease induced by LCMV ARM in C3H/St mice is not linked to the mouse MHC haplotype or to sex and is not dependent on a dominant gene. Rather, multiple genes are involved, and these are related to the C3H background (Tishon and Oldstone 1990).

Microsatellite mapping across the mouse genome was utilized to identify areas of significant linkage between the clinical findings of growth deficiency induced by the viral infection and host genes, from over 100 individual susceptible C3H/St × resistant BALB/WEHI F1 mice crossed to similar F1 mice. Such studies revealed that the GH deficiency syndrome during persistent LCMV ARM infection maps to a region on chromosome 17 just outside the MHC H-2D site between D17 Mit24 and D17 Mit51. These data linked a region on chromosome 17 encompassing 2.5cM region to the pathogenesis of the GH disease induced by LCMV infection. Further, since murine α-DG, the known receptor for LCMV, residues on chromosome 9 (Yotsumoto et al. 1996) not chromosome 17, these findings indicate that an alternative receptor molecule or co-receptor restricted to C3H/St and other GH disease-susceptible mice plays a role in binding and/or entry of LCMV ARM into GH-producing cells.

4.3 Alterations in Behavior and Learning Associated with Persistent LCMV Infection

The link between persistent LCMV infection and clinical signs of severe disease can easily be overlooked. For example, when tested as adults, apparently "normal" mice persistently infected with LCMV can exhibit neurobehavioral abnormalities (Hotchin and Seegal 1977; de la Torre et al. 1996; Gold et al. 1994). These include an impaired spatial learning ability, as indicated by a deficit in the acquisition of discriminated avoidance performance and a reduced tendency to explore a novel environment (although their locomotor activity is not affected). During persistent infection of mice with LCMV, viral antigens and nucleic acids in the CNS are localized almost exclusively within neurons (Fig. 9, panels 2 and 3a) (Oldstone 1987, 1989). The highest levels of persisting virus are found in the hippocampus, neocortex, limbic system and certain regions of the hypothalamus, with lower levels in the brain stem, thalamus, and basal ganglia (Rodriguez et al. 1983). Virus persistence occurs in the absence of necrosis and inflammation in the CNS. Thus, it is likely that the neurobehavioral alterations seen in mice persistently infected with LCMV are a consequence of direct viral effects on the neuronal populations within which virus persists.

Details of the effects LCMV persistence has on neuronal functioning, and the contributions each of these virus-induced deficits in neuronal functions may make to the neurobehavioral phenotype exhibited by mice persistently infected with LCMV, are beginning to be understood. One series of studies has linked neurochemical abnormalities affecting neurotransmitters to LCMV-mediated neurologic deficits (Oldstone et al. 1977; Lipkin et al. 1988; de la Torre et al. 1996; Gold et al. 1994). Thus, pharmacologic analysis has shown that mice persistently infected with

LCMV are hypersensitive to the muscarinic cholinergic antagonist, scopolamine, as revealed during their performance in tasks involving learning and motor activity (GOLD et al. 1994). Moreover, in vitro studies (OLDSTONE et al. 1977) demonstrated that persistent infection of murine neuroblastoma cells with LCMV significantly lowered the intracellular levels of choline acetyltransferase and acetylesterase. A cholinergic dysfunction consequent to viral interference with neuronal production of key enzymes involved in neurotransmitter metabolism, therefore, may be one contributor to the learning deficits exhibited by mice persistently infected with LCMV.

The most compelling of these studies (DE LA TORRE et al. 1996) examined whether structural correlates existed for the CNS alterations described in mice persistently infected with LCMV. Specifically sought were alterations in synaptic density and neuronal plasticity, both of which can have profound effects on behavior. The investigators found that, although the overall synaptic density in the neocortex and limbic structures of LCMV-infected mice was preserved, the expression of growth-associated protein-43 (GAP-43), a protein proposed to play an important role in the neuronal plasticity processes accompanying learning and memory, was significantly decreased in the molecular layer of the hippocampus (Fig. 9, panels 3 and 4). In vitro analysis revealed that persistent infection with LCMV of PC12 cells, a cell line that undergoes differentiation from a chromaffin- to a neuron-like phenotype when grown in the presence of neurotrophic growth factor (NGF), prevented NGF induction of GAP-43 upregulation in these cells. NGF-mediated upregulation of amyloid precursor protein in these cells was not affected, indicating the selective viral affect on GAP-43 transcription (Fig. 9, panel 4). Just how LCMV infection affects NGF-mediated upregulation of GAP-43 expression in PC12 cells is not completely clear, but it may interfere with specific pathways of the NGF signal transduction mechanisms, including the protein kinase C-dependent pathway involved in the stabilization of GAP-43 mRNA (CAO et al. 1998). Similarly, LCMV persistence in neurons of mice may interfere with the regulation of GAP-43 expression in response to extracellular signals in the presynaptic terminals of the hippocampal circuitry. The resulting deficit in neuronal plasticity may contribute to the learning defects observed in these mice. Recent studies utilizing a RNA priming technique, hippocampal neurons from LCMV-infected and matched uninfected controls and novel gene array technology (SUTCLIFFE et al. 2000) have uncovered a series of four previously unknown and nine known genes whose transcription is either down- or unregulated with a twofold reproducibly comparing infected and non-infected neurons. Analysis of some of these genes bears the prospect of uncoding the molecular basis of neuronal dysfunction. The cartoon in Fig. 9, panel 1, illustrates the concept of viruses altering differentiated cell functions.

5 Conclusions

The foregoing discussion documents how valuable the study of LCMV infection in its natural rodent host has been to the general understanding of virus-immune

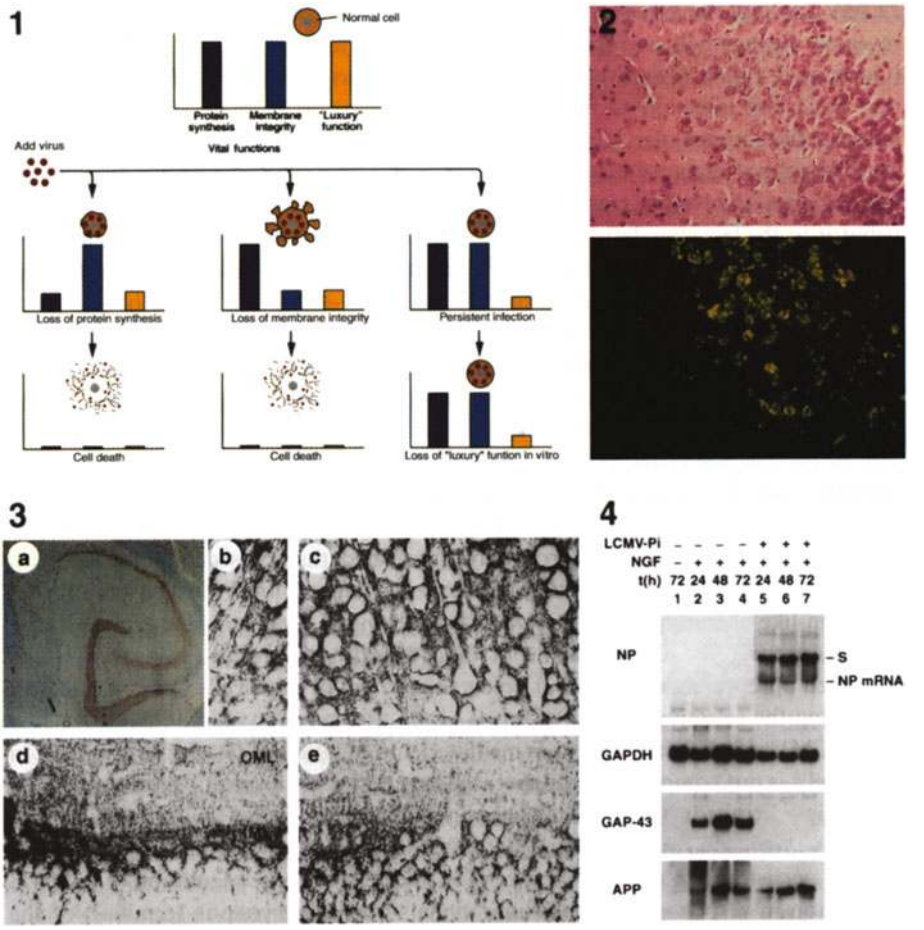

Fig. 9. Virus alters differentiated functions of neuronal cells in vivo and in vitro. *Panel 1* cartoon of virus altering the differentiated (luxury) function of a cell without lysing it. In contrast, viruses can lyse cells by cutting off their protein synthesis or destroying their membranes. *Panel 2* shows that mice persistently infected with LCMV contain virus in neurons of the cerebral cortex without structural destruction of those neurons or an inflammatory response in the area of infection. *Panel 3a* depicts the same phenomenon for hippocampal neurons. In vivo such infected hippocampal neurons undergo a decrease in GAP-43 protein (*panel 3e*) compared to age-matched controls (*panel 3d*). However, other neural markers like synaptophysin are not altered in infected (*panel 3c*) or uninfected neurons (*panel 3b*) (Oldstone 1987, 1993; Rodriguez et al. 1983; de la Torre et al. 1993, 1996). These persistently infected mice are defective in cognitive and memory performances (de la Torre et al. 1996; Gold et al. 1994). *Panel 4* recapitulates in vitro the effect of lowering the GAP-43 level during LCMV infection (LCMV: NP mRNA) of PC-12 neuron-like cells. However, transcription is selective, since amyloid precursor protein (APP), cFOS, etc., transcripts are not altered during the infection

system interactions and viral diseases. As described here and elsewhere in this volume, a remarkable number of key concepts in immunology and virology were first defined in studies of LCMV. Moreover, these conclusions have been extended to many DNA and RNA virus infections of humans. The complex spectrum of

variation that marks the outcomes of LCMV infection serves to illustrate the delicate balance governing virus-host interactions, and how seemingly small differences in either host or viral genes can profoundly influence the resolution of infection or the production of end-stage disease. One can safely anticipate that future studies of LCMV infection in mice will yield many more advances in the understanding of viral pathogenesis.

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