

(and probably the SKR) was greatly facilitated by the fact that it acts via a second messenger system. Thus the presence of relatively few receptors leads to the production of many second messenger molecules and the opening of many ion channels. This amplification would not be present with receptors that directly gate ion channels. Nevertheless, these problems must be compared with those encountered in purification, reconstitution and sequencing. This classical approach has been applied with limited success to peptide receptors and ion channels in the nervous system. Thus it seems likely that receptor and ion channel cloners in the future may

choose to replace their radioactive ligands and affinity chromatography resins with a voltage clamp and frog oocytes.

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A supernova and the centennial celebration of the elegant Hotel del Coronado in San Diego set the stage for a recent interdisciplinary workshop on viral infections and neurological diseases\*. Topics discussed included viral neurotropism, inflammation and autoimmunity, and mechanisms for latency and persistence in viral infections of the nervous system.

The meeting was highlighted by a keynote address by the late George Khoury, concerning transactivating factors in control of viral replication and transcription. Cellular transactivating factors may interact with viral promoters in determining tropism. Virus-encoded transactivating factors can determine phenotypic manifestations of infection. Studies with the JC virus (JCV) system have shown that the early gene product, the T Ag, is critical to determining initiation of viral replication and transcription. In transgenic mice, JCV T Ag may lead to dysmyelination and neural tumors. In the HTVL-I system the *tat* protein has potential to serve as a transcriptional transactivator and is oncogenic in transgenic mice.

#### Viral neurotropism

The basis for neurotropism and the pathogenesis of neurological

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## Viruses, the immune system and central nervous system diseases

dysfunction in many viral infections of the central nervous system (CNS) remains unknown. Viral utilization of host cell neurotransmitter and complement receptors has been shown with reovirus and EBV infections, respectively. Infection of neural cells may be linked to abnormalities in neurotransmitter function<sup>1†</sup>.

The molecular basis of neurotropism is understood in greater detail in some viral systems. Recent work with poliovirus has shown the utility of crystallography and computer modeling in understanding structure–function relationships<sup>2</sup>. Adaptation of the Lansing type II strain of poliovirus in mice toward neurovirulence is due to changes in the capsid proteins<sup>3</sup>. In reovirus infections of mice, the route of viral dissemination, neural or hematogenous, is determined by the sigma genes<sup>4</sup>. Tropism in ecotropic murine leukemia virus infections of mice has been mapped to the envelope and long terminal repeat (LTR) genes. The envelope protein gp 70 determines CNS infection. Sites of infection within the CNS are encoded by the LTR. Wild type virus causes diffuse spongiform encephalopathy with hind limb paralysis. Chimeric virus constructed with an LTR derived from T-lymphocytes infects only striatum and cortex<sup>5</sup>.

#### Viral specificity and autoimmunization

Humoral and cellular immune responses directed against self antigens are recognized sequelae of exposure to some viruses and vaccines. Four percent of monoclonal antibodies from normal subjects that react with a variety of common viruses also react with antigens found in human tissues<sup>6</sup>. Proliferation of such clones could lead to autoimmune disease. T cell responses to MBP have been described in rats infected with mouse hepatitis virus JHM<sup>7,8</sup>. Lymphoproliferative cerebrospinal fluid (CSF) T cell responses to myelin basic protein (MBP) are common in patients with rubella<sup>9</sup> and postmeasles encephalomyelitis<sup>10</sup>.

The mechanisms responsible for the induction of these types of autoimmune responses remain obscure. In some viral infections, host proteins are incorporated into the viral envelope as the virus buds from infected cells. Host antigens presented to the immune system in this fashion could be seen as 'foreign'<sup>11</sup>. Infection with Semliki forest virus (SFV) may cause demyelination through this mechanism. Mice infected with SFV show T cell-mediated demyelination and have antibodies to glycolipids contained in the viral envelope. Superinfection with an

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unrelated enveloped virus enhances the antibody titer to glycolipid and aggravates the demyelination<sup>12</sup>.

Autosensitivity might also result from similarities between host and microbial antigens. In this model, termed 'molecular mimicry' (MM), infection with a microbial agent elicits a cellular or humoral response that recognizes self antigens homologous to microbial antigen(s). In theory, the virus, bacterium or parasite need not invade the CNS or be present after the initial phase of antigen presentation. MM has been invoked in several human or animal immune disorders including post-viral and postvaccination CNS demyelination, ankylosing spondylitis and Reiter's syndrome, myasthenia gravis, and renal transplant rejection. The search for MM begins with screening data banks for amino acid sequences shared by microbial agents and host proteins. The microbial agents selected should be associated with a known autoimmune disease. Similarly, the host protein examined should represent an epitope with biological significance. Subjects with the disease are screened for humoral cellular reactivity to the microbial agent and to synthetic peptides representing the region of homology between host and microbe. Finally, animals are immunized with these synthetic peptides in an effort to reproduce the disease<sup>13-15</sup>. MM may also be important in the pathogenesis of certain paraneoplastic syndromes, e.g. gammopathy-associated sensorimotor neuropathy (antibody-to-myelin associated glycoprotein or glycosphingolipid) or motor neuron disease (antibody-to-GMI and other gangliosides)<sup>11,16</sup>. The approach is limited to examination of linear epitopes present in protein data banks. It should become more powerful as we accumulate more information on the linear and three-dimensional structure of host and microbial antigens.

#### **Immune recognition of viral and tissue antigens**

The relationship between T cell structure and function is becoming better understood. T cells express an heterodimeric surface receptor complex (Ti) consisting of two disulfide-linked glycoproteins with V, D, J, and C elements, struc-

turally similar to immunoglobulins. T cell activation probably occurs through the interaction between antigen-MHC and the V regions of the Ti complex. This interaction can be mimicked with antibodies directed against the V region of Ti<sup>17</sup>.

Lymphocytes, monocytes and macrophages also have receptors for a variety of neurotransmitters. These receptors may be important to CNS modulation of immune function<sup>18</sup>. This close relationship between the CNS and the immune system has been underscored by recognition of the immunoglobulin gene superfamily, which includes adhesion molecules such as NCAM and MAG<sup>16</sup> immunocyte receptors, MHC antigens, and 1B236, a new CNS-specific molecule<sup>19,20</sup>.

Cytotoxic T-lymphocytes (CTL) are restricted in their recognition and lysis of virus-infected cells by class I-MHC gene products<sup>21</sup>. Lysis appears to be mediated through the release of cytotoxic granules. One of the most interesting constituents of CTL granules is the protein perforin 1 (cytolysin). Perforin 1 polymerizes into tubular aggregates that form transmembrane channels in target cells<sup>22</sup>. Interestingly, perforin 1 shows substantial amino acid homology to C9, which functions in an analogous fashion during antibody plus complement-mediated cell lysis<sup>23</sup>.

Anti-myelin basic protein (MBP) and anti-proteolipid protein T cell clones have been used to study the pathogenesis of experimental allergic encephalomyelitis (EAE), an established model for studying CNS demyelination<sup>24</sup>. Immunosuppressed mice reconstituted with anti-MBP T cell clones develop EAE, indicating that other immunocytes are not required for disease. In addition to macrophages, astrocytes probably also serve as antigen presenting cells. Astrocytes can be induced to express Ia though exposure to IFN- $\gamma$  or activated T cells<sup>24,25</sup>. In addition, astrocytes can mimic the effector functions of macrophages, such as phagocytosis of myelin debris, release of proteolytic enzymes and a toxic factor similar to TNF<sup>23</sup>. The capacity of endothelial cells to express MHC and to act as antigen presenting cells remains controversial and may vary according to species<sup>21</sup>.

Some viral infections, such as

adenovirus, can modulate the expression of MHC antigens on the surface of infected cells<sup>26,27</sup>. Downregulation of class I-MHC antigen expression on virus-infected cells, and the resulting inability of MHC-restricted CTL to kill these cells, could facilitate persistent viral infections. Other viral infections may lead to induction of MHC antigens. This may be particularly important in the pathogenesis of virus-induced inflammatory demyelinating diseases. The capacity of murine hepatitis virus JHM (MHV) to induce Ia expression on rat astrocytes *in vitro* and *in vivo* correlates with the severity of JHM-induced demyelination<sup>28</sup>. Viral induction of MHC in other glial cells may be due to release of an 80-100 kDa soluble factor that increases MHC mRNA<sup>29</sup>. Systemic infections may also induce MHC through release of circulating IFN- $\gamma$ .

#### **Macrophages, immunocytes and inflammation**

Macrophages are increasingly recognized as important to host defense against viral infection and neoplasia. The mechanisms by which macrophages are enlisted are incompletely understood. A non-glycosylated 10-25 kDa protein, factor-increasing monocytopoiesis (FIM), appears to be important. FIM is elaborated by macrophages in tissues and stimulates production and release of marrow monocytes<sup>30</sup>.

Reactive T cells produce a variety of soluble factors (lymphokines) responsible for mobilizing and activating macrophages as well as inflammatory cells: interleukin-2 (IL-2), interferon- $\gamma$  (IFN- $\gamma$ ); in addition, they produce IL-3 (polyclonal stimulator of hemopoiesis), IL-4 (B cell growth factor 1), and IL-5 (B cell growth factor 2 or IFN $\beta$ 2)<sup>25</sup>.

Activation of monocytes and macrophages by IFN- $\gamma$  or lipopolysaccharide is reflected in the expression of major histocompatibility (MHC) and other cell surface antigens<sup>31</sup>. One of these antigens, CR3, is important to migration of monocytes from the vascular to the tissue compartment<sup>32</sup>. Activated macrophages in turn release several soluble factors including interleukin-1 (IL-1), tumor necrosis factor (TNF) and nerve growth factor (NGF)<sup>25</sup>. IL-1 has

protean effects on the immune system. It activates T, B and NK cells and stimulates its own production at the level of transcription<sup>33</sup>. TNF has antiviral and antineoplastic properties. It has been purified and sequenced and its gene has been mapped to human chromosome 6 in proximity to the MHC locus<sup>34,35</sup>. The role of NGF is less clear. Macrophage-produced NGF may be important to in development and repair of the CNS<sup>32</sup>.

### Viral latency and persistence

The capacity to produce latent infections is characteristic of herpesviruses. Recently, the factors that result in a switch from latent to active Epstein Barr virus (EBV) infection have been investigated by introducing specific EBV DNA fragments into latently infected cells. A 2.7 kb DNA fragment containing one complete and several partial open reading frames (ORF) has been identified that drives the EBV infectious cycle from latency to activation. The product of the complete ORF is a polymorphic 16–41 kDa protein (p21 or zebra protein). This protein is expressed during activation of latent EBV infection by exogenous agents such as phorbol esters or butyrate<sup>36</sup>.

Another herpesvirus, murine CMV produces latent infection of B lymphocytes. During latent infection, only immediate early (IE) and early genes are transcribed. These gene products may be important to initiation and maintenance of latency<sup>37</sup>. Mice infected with HSV have also been used as a model to study viral latency in neurons. In HSV-1 infections of mice, the genome does not integrate with host DNA. After the acute phase of infection only IE genes are transcribed<sup>38</sup>. Stevens and colleagues have identified an antisense IE transcript in latently infected cells which could regulate IE gene expression<sup>39</sup>. Reduction in cell surface glycoproteins, products of late gene expression, might allow infected cells to escape host surveillance and establish latency or persistence. Similar mechanisms may be implicated in persistent infections with agents other than herpesviridae. Lymphocytic choriomeningitis virus (LCMV) is a segmented, single stranded ambisense RNA virus that causes persistent infection in mice.

Nucleoprotein mRNA is transcribed directly from genomic RNA; however, the virus must transcribe a genomic complementary intermediate as template for glycoprotein mRNA. The additional step required for glycoprotein synthesis may be important to restriction of cell surface expression of viral antigens and hence to viral persistence<sup>40</sup>. These lessons in viral pathogenesis have led to consideration of antisense oligonucleotide sequences as antiviral agents to control viral infections at the level of transcription<sup>41</sup>.

In contrast to HSV and LCMV, which infect neurons, the lentiviruses, visna and human immunodeficiency virus (HIV), which also cause persistent infection and neurological dysfunction, do not. The evidence that pathogenesis of neurological disease is indirect in lentivirus infections is two-fold. First, only a small number of cells ( $10^{-6}$ – $10^{-5}$ ) show viral transcripts<sup>42</sup>. Second, though visna infected animals may show viral nucleic acids in oligodendroglia and astrocytes, the majority of CNS cells with viral transcripts in visna infection and all of the CNS cells with viral transcripts in HIV infection are either monocytes, macrophages, or endothelial cells<sup>42–44</sup>. In HIV-infected brains, cells with HIV transcripts are frequently seen in proximity to blood vessels. Abnormalities in vascular permeability or release of soluble factors such as IL-1 and TNF may be pathogenic in HIV-dementia<sup>15,44</sup>. Viral transmission during HIV infection appears to be by cell–cell fusion. This makes it unlikely that antibodies play any role in control of viral spread<sup>45</sup>. In visna-infected animals, neutralizing antibody may actually enhance *in vivo* infectivity by promoting uptake of virus by macrophages<sup>46</sup>. The implications of these data for vaccine development are uncertain.

The striking difference between HIV-dementia, in which there is no inflammation or parenchymal damage, and visna in which there is intense meningeal and perivascular inflammation and demyelination, is reminiscent of the difference between lepromatous leprosy, a pure macrophage infection in the absence of T cell response, and tuberculoid leprosy, in which T cell-mediated inflammation is accompanied by extensive parenchymal

damage. Thus, mankind's newest and oldest plagues have much in common.

Numbers in the text refer to speakers as listed below: 1. Floyd E. Bloom – La Jolla, CA, USA; 2. James Hogle – La Jolla, CA, USA; 3. Vincent Racaniello – New York, NY, USA; 4. Kenneth Tyler – Boston, MA, USA; 5. Paul Jolicouer – Montreal, Canada; 6. Abner L. Notkins – Bethesda, MD, USA; 7. Helmut Wege – Würzburg, FRG; 8. Michael J. Buchmeier – La Jolla, CA, USA; 9. Peter Marquardt – Würzburg, FRG; 10. Richard T. Johnson – Baltimore, MD, USA; 11. Adreas J. Steck – Lausanne, Switzerland; 12. Hugh E. Webb – London, UK; 13. Robert S. Fujinami – La Jolla, CA, USA; 14. Thomas Dyrberg – Gentofte, Denmark; 15. Michael B.A. Oldstone – La Jolla, CA, USA; 16. Norman Latov – New York, NY, USA; 17. Ellis L. Reinherz – Boston, MA, USA; 18. Edward J. Goetzl – San Francisco, CA, USA; 19. Robert J. Milner – La Jolla, CA, USA; 20. Allan F. Williams – Oxford, UK; 21. Peter C. Doherty – Canberra, Australia; 22. Eckhart Podack – Valhalla, NY, USA; 23. Moon Lee Shin – Baltimore, MD, USA; 24. Hartmut Wekerle – Würzburg, FRG; 25. John Schrader – Vancouver, Canada; 26. Gilbert W. H. Jay – Bethesda, MD, USA; 27. Per A. Peterson – La Jolla, CA, USA; 28. Paul Massa – Würzburg, FRG; 29. Susan R. Weiss – Philadelphia, PA, USA; 30. Ralph van Furth – Leiden, The Netherlands; 31. Thomas Hamilton – Durham, NC, USA; 32. Siamon Gordon – Oxford, UK; 33. Charles A. Dinarello – Boston, MA, USA; 34. David V. Goeddel – South San Francisco, CA, USA; 35. Grace H. W. Wong – South San Francisco, CA, USA; 36. I. George Miller – New Haven, CT, USA; 37. Jay Nelson – La Jolla, CA, USA; 38. Nigel W. Fraser – Philadelphia, PA, USA; 39. Jack G. Stevens – Los Angeles, CA, USA; 40. Peter Southern – La Jolla, CA, USA; 41. David W. Kingsbury – Memphis, TN, USA; 42. Malcolm Martin – Bethesda, MD, USA; 43. Michel Brahic – Paris, France; 44. Clayton Wiley – La Jolla, CA, USA; 45. Jay Levy – San Francisco, CA, USA; 46. Opendra Narayan – Baltimore, MD, USA.

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