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18. The 924 $ry^{506}$ embryos with the M cytotype were injected (*4*) with p559CR1 (400 mg/ml) and pπ25.7wc (50 mg/ml) and gave rise to 144 fertile adults (G0). The 144 G0 adults were mated to homozygous $ry^{506}$ flies and the G1 flies were scored for $ry^+$ eye color. One or more progeny of 14 G0 individuals exhibited wild-type eye color and were, therefore, successful transformants. The strain $ry^{506}$ is homozygous for a deletion of a segment of the XDH-coding region and therefore cannot revert to $ry^+$ (provided by W. Bender, unpublished results). Balanced stocks of $ry^+$ P-element insertions were established. Six transformants with inserts mapping to the X (two transformants) or second chromosomes (four transformants) were crossed to a strain containing the third chromosome balancer TM3, $p^p$ $e^s$ $ry^{RK}$ $bx^{34e}$ *Sb* $trp^+$ (isolated by R. Karess, unpublished). The P[*ry*,559]; TM3, $ry^{RK}$*Sb* $trp^+/ry^{506}$ $trp^+$ flies were crossed to $ry^{506}trp^{CM}$ flies and their P[*ry*, 559]; TM3, $ry^{RK}$*Sb* $trp^+/ry^{506}trp^{CM}$ progeny were backcrossed again to the $ry^{506}trp^{CM}$ strain. Two $ry^+Sb^+$ flies (P[*ry*,559]; $ry^{506}trp^{CM}/ry^{506}trp^{CM}$) derived from each of the six transformants were tested for rescue of the *trp* phenotype. Two $ry^{506}Sb^+$ siblings ($ry^{506}trp^{CM}/ry^{506}trp^{CM}$) of each of the six transformants were also tested. These latter flies served as negative controls. All flies containing the P[*ry*,559] insertion were $trp^+$ in phenotype and all $ry^{506}Sb^+$ siblings tested that lacked the P-element insertion gave ERG recordings indistinguishable from the $ry^{506}trp^{CM}$ flies. The eight transformants with third chromosome inserts were not tested for rescue of the *trp* phenotype since the *trp* gene is also on the third chromosome. Consequently, these transformants could not be moved into a $trp^{CM}$ background as readily as the transformants with X and second chromosome inserts.
19. Starting from the λ559 clone, Wong *et al.* isolated a series of overlapping λ clones that span the breakpoints in 99C5-6 to which *trp* has been mapped (*11*). These workers probed RNA blots with bacteriophage DNA that had been labeled with $^{32}P$, and reported that the stretch of DNA represented by these clones encodes four RNA species in Oregon R, two of which were missing in $trp^{CM}$. We have prepared RNA from the same fly stocks (Oregon R and $trp^{CM}$), fractionated the poly(A)$^+$ RNA on a formaldehyde gel, blotted to nitrocellulose, and probed with *trp* genomic DNA (p559E1.7) that had been labeled with $^{32}P$. The results of this analysis showed that the size and concentration of *trp* RNA were similar in both fly stocks.
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# Amino Acid Homology Between the Encephalitogenic Site of Myelin Basic Protein and Virus: Mechanism for Autoimmunity


Abstract. *Amino acid sequence homology was found between viral and host encephalitogenic protein. Immune responses were then generated in rabbits by using the viral peptide that cross-reacts with the self protein. Mononuclear cell infiltration was observed in the central nervous systems of animals immunized with the viral peptide. Myelin basic protein (MBP) is a host protein whose encephalitogenic site of ten amino acids induces experimental allergic encephalomyelitis. By computer analysis, hepatitis B virus polymerase (HBVP) was found to share six consecutive amino acids with the encephalitogenic site of rabbit MBP. Rabbits given injections of a selected eight– or ten–amino acid peptide from HBVP made antibody that reacted with the predetermined sequences of HBVP and also with native MBP. Peripheral blood mononuclear cells from the immunized rabbits proliferated when incubated with either MBP or HBVP. Central nervous system tissue taken from these rabbits had a histologic picture reminiscent of experimental allergic encephalomyelitis. Thus, viral infection may trigger the production of antibodies and mononuclear cells that cross-react with self proteins by a mechanism termed molecular mimicry. Tissue injury from the resultant autoallergic event can take place in the absence of the infectious virus that initiated the immune response.*



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Many viruses share antigenic sites with normal host cell components (*1–3*), a phenomenon known as molecular mimicry. This commonality has been demonstrated by direct comparisons of amino acid sequences. Moreover, the finding of monoclonal antibodies that react with both host and virus constituents suggests that viruses have the potential to trigger autoimmune responses and resultant disease. Antibodies or cytotoxic lymphocytes generated against the virus might cross-react with self proteins, thus causing cellular injury leading to disease. Once the infectious agent initiates this process, it need not be present during the autoimmune destruction that follows.

Molecular mimicry occurs frequently and with various DNA and RNA viruses. Lane and Hoeffler (*3*), using a monoclonal antibody, showed that the large T antigen of simian virus 40 and normal proteins of host cells have common antigenic sites. Subsequently, we found that the measles virus phosphoprotein (P3), the 140-kilodalton (kD) protein of herpes simplex virus, and the hemagglutinin of vaccinia virus all reacted with distinct epitopes on intermediate filaments (*1*). In a study of more than 600 monoclonal antibodies to 11 different viruses, Srini-

vasappa *et al.* (*4*) found that over 3 percent of such antibodies also reacted with normal tissues. However, in most instances, the actual site or epitope of the virus or normal cell that reacts with a monoclonal antibody is not known.

The encephalitogenic site of myelin basic protein (MBP) is a host cell determinant capable of eliciting autoreactivity and autoimmune disease. We chose to study MBP because its entire amino acid sequence is known and its encephalitogenic site has been mapped in several animal species [reviewed in (*5*)]. Using computer-assisted analysis, we compared sequences of viral proteins in the Dayhoff computer bank with the encephalitogenic site of MBP and performed several immunologic studies to look for cross-reactivity and disease production (*6*).

Our analyses are based on the observed sequence homology between the MBP encephalitogenic site and hepatitis B virus polymerase (HBVP), the generation of antibodies and lymphocytes that cross-react with this virus peptide and MBP, and the induction of central nervous system (CNS) infiltrates in rabbits inoculated peripherally with selected viral peptide sequences.

Computer analysis showed amino acid homologies of several animal viruses and MBP. Included were similarities of MBP to the nucleoprotein and hemagglutinin of influenza virus, the coat protein of polyoma virus, the core protein of adenovirus, the polyprotein of Rous, avian sarcoma and Abelson leukemia viruses, the polyprotein of poliomyelitis virus, and the EC-LF2 protein of Epstein-Barr virus. Because the best fit occurred between the MBP encephalitogenic site and HBVP, these peptides were synthesized for use in the remaining studies.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 66 | | | | | | | | 75 |
| MBP | Thr | Thr | His | Tyr | Gly | Ser | Leu | Pro | Gln | Lys |
| | 589 | | | | | | | | 598 |
| HBVP | Ile | Gly | Cys | Tyr | Gly | Ser | Leu | Pro | Gln | Glu |

Of seven rabbits immunized with HBVP [eight amino acids (8AA)], five developed significant levels of antibody to MBP (Fig. 1). The titers ($\log_2$) of antibody to MBP ranged from 5.5 to 8.7. When such antibodies were tested against the HBVP peptide (immunogen), the titers ranged from 10.4 to 14.5. Competition blocking experiments with increasing amounts of HBVP (8AA) inhibited binding by HBVP antibodies to MBP in a dose-dependent manner. At 500 μg of MBP, 70 percent of the binding was inhibited. Mononuclear cells obtained at various times after immunization with 500 μg of HBVP (8AA) were cultured with 10, 50, or 100 μg/ml of HBVP or whole MBP. Twenty-one days after primary sensitization, mononuclear cells from all four test rabbits responded by proliferation after exposure to HBVP peptide [stimulation index (SI), 4.6 to 11.2]. Monuclear cells from two of these four rabbits also responded to MBP (SI, 2.8 and 2.9) (Fig. 2). Peripheral blood mononuclear cells obtained from two of four additional rabbits immunized with HBVP (10AA) also showed significant proliferation (SI > 2) when cultured with MBP.

Brains and cervical spinal cords of 11 rabbits were removed 28 to 32 days after immunization with HBVP (8AA and 10AA). Histologic evaluation indicated scattered lesions of perivascular mononuclear and meningeal infiltrates characteristic of experimental allergic encephalitis (EAE) in four of these rabbits (Fig. 3). In contrast, no such CNS lesions developed in either unimmunized rabbits (group of seven) or rabbits immunized with various viral peptides lacking sequence homology with the encephalitogenic site (group of ten) or in lung and kidney tissues from rabbits (group of four) immunized with HBVP. None of the rabbits immunized with HBVP developed clinical signs of EAE with the protocols used. By comparison, one of four animals injected with a similar dose of MBP encephalitogenic peptide (10AA) had clinical signs of CNS involvement, and three of the four had similar histologic lesions of perivascular infiltrates in brain and spinal cord. Four rabbits injected with MBP (8AA) had neither clinical nor histologic evidence of EAE.

We have shown that a viral peptide with amino acid sequences homologous to those of a host or self protein can induce cross-reactive antibodies and proliferating lymphoid cells in response to the self protein and thus generate an inflammatory response in vivo at the location of the self protein. We chose for study the self protein of the encephalitogenic site of MBP because it is the best studied material causing tissue-specific autoimmune disease and because it is thought to participate in the pathogenesis of several human disorders associated with demyelination. In our experiments, sharing of six amino acids was sufficient to produce immunologic cross-

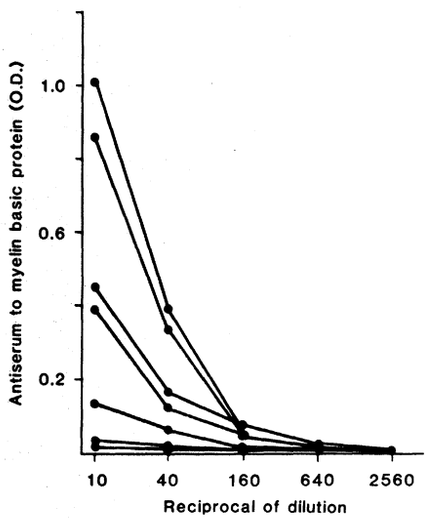


Fig. 1. Enzyme-linked immunosorbent assay for binding of HBVP antiserum to MBP-coated wells. In wells coated with 5 μg of bovine MBP, serum from five of seven rabbits immunized with HBVP bound at the levels shown. The second antibody used to perform this assay was horseradish peroxidase–conjugated, affinity-purified goat antibody to rabbit immunogloblin. Peptides were made in an Applied Biosystems 430A. Eight- and ten–amino acid peptides were synthesized from the encephalitogenic site for rabbit (positions 68 to 75 and 66 to 75). Materials generated were analyzed by reverse-phase high-performance liquid chromatography. New Zealand White rabbits, each weighing 2.5 to 3.0 kg were injected with 250 μg of peptide into each hind footpad (500 μg total in 200 μl). Peptides were mixed in complete Freund's adjuvant before being used for immunization. Inoculated rabbits and matched controls were examined daily for clinical signs of CNS dysfunction and were bled at days −7, 14, 21, and 28 after receiving peptides.

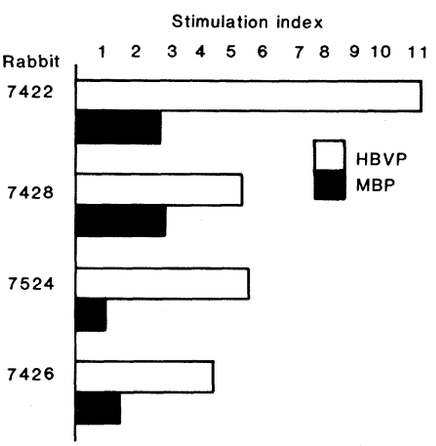


Fig. 2. Reactivity of HBVP-sensitized rabbit mononuclear cells to HBVP or MBP. Stimulation indices greater than 2 are considered positive. Positive responses to MBP were obtained for two of the four rabbits tested; all four rabbits showed positive responses for HBVP. Mononuclear cells were obtained from the peripheral blood of rabbits 21 days after immunization and processed by Ficoll-Hypaque density centrifugation. These mononuclear cells were pulsed with various peptides or whole MBP in concentrations ranging from 100, 50, and 10 μg per milliliter. After 4 days, cultures were pulsed with [$^3$H]thymidine, and trichloroacetic acid–precipitable counts were determined 24 hours later.

reactivity. Other investigators, using monoclonal antibodies with synthesized peptides, have also shown that antibodies recognize as few as six amino acids (*7*).

Our analysis provides clues for understanding autoimmune diseases. An immune response against a determinant shared by host and virus can bring forth a tissue-specific immune response that is presumably capable of eliciting cell and tissue destruction. The probable mechanism is generation of cytotoxic cross-reactive effector lymphocytes or antibodies that recognize specific determinants on target cells. Although viral glycopeptides may participate in such events, viral proteins thought to reside inside infected cells (not expressed on the outer plasma membrane) can induce the generation of cytotoxic T lymphocytes and may be among the major determinants recognized during both experimental and natural virus infections. Thus, in studies of influenza virus with both cloned and heterologous populations of effector T cells that are virus-specific and MHC-restricted (MHC, major histocompatibility complex), significant reactivities against the virus polymerase and ribonuclear protein were observed in mice and humans (*8*).

The induction of cross-reactivity does not require a replicating agent, and immune-mediated injury can occur after the immunogen has been removed: a hit-and-run event. Hence, virus infections that initiate the autoimmune phenomenon may not be present by the time overt disease develops.

During the cross-reacting immune response, virus may be cleared, but the components of the immune attack continue to assault self elements. The autoimmune response itself leads to tissue injury that, in turn, releases more self antigen, and the cycle continues. Therefore, although viruses may initiate disease, the likelihood of their recovery from tissue sites is small. Even in those postinfectious viral encephalopathies of humans for which the infectious agent that induced the immunologic event is known, the agent is seldom recovered (*9*). Furthermore, recent studies of patients with measles, a virus infection that can cause postinfectious viral encephalopathy, show that mononuclear cells from the peripheral blood and spinal fluid of such patients proliferate when cultured with MBP (*10*). This suggests the possibility of molecular mimicry as described here.

We speculate that autoimmunity occurs when viral determinants mirror host configurations that induce disease—for example, the encephalitogenic site of MBP—and an effective immune response initiated against the virus turns against the self site. Autoimmunity would not occur if the shared site did not participate in disease production. Autoimmune responses may result from the breaking of tolerance for bovine serum albumin or thyroglobulin at the B-cell level, or activation of competent T or B cells (or both) in EAE, as described by Weigle and associates (*11*). In this instance, the virus and host determinants must be sufficiently similar to induce a cross-reactive response but different enough to break tolerance. Similar events may play a role in autoimmune components associated with Guillain-Barré syndrome (peripheral myelin), myasthenia gravis (acetylcholine receptor), thyroiditis, diabetes, and multiple sclerosis. Data have recently accumulated showing cross-reactivity between determinants on viruses and lymphoid, CNS, and endocrine cells (*12*). A different sequence of events occurs in the autoimmune disorders that are chronic or relapsing and remitting. In this instance, viruses with the capacity to persist may continuously or cyclically express viral antigens. Although expression of the viral genome might be restricted so that no infectious virus replication occurs, production of the determinant or immunodominant region in common with that of the host could continue. The resulting antigen, properly presented, might then evoke immune responsiveness to the cross-reacting site leading to chronic and progressive disease.

Finally, molecular mimicry may also have a selective advantage for the virus. By mimicking sites or regions on host molecules that are suppressive or tolerance-inducing, a virus might be regarded as self and not be eliminated by an immune response. In addition, sites shared by viruses and intracellular proteins may aid viral maturation by signaling or directing the viral gene products to the appropriate cell compartment or structure.

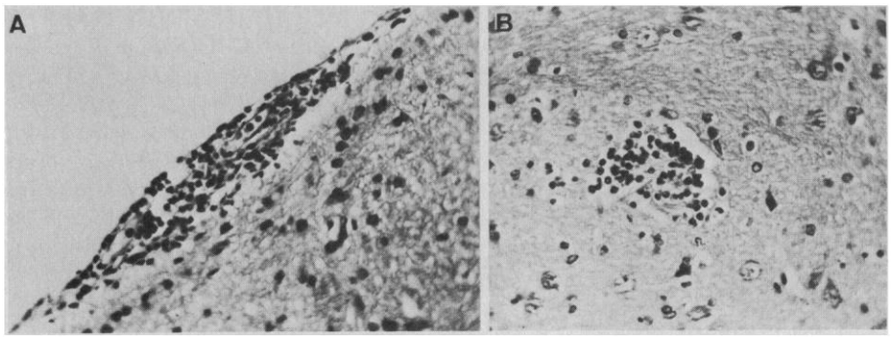


Fig. 3. Lesion from two individual rabbits immunized with HBVP. Section stained with hematoxylin and eosin (magnification ×400), showing infiltrating cells under the leptomeninges in the area of ventricle III and perivascular cell cuffing in the midbrain cerebral cortex.

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