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Virus-induced Autoimmunity: Molecular Mimicry as a Route to Autoimmune Disease

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Introduction

Viruses are implicated in autoimmunity on the basis of three findings. First, autoimmune responses are made *de novo*, or those already present are enhanced concomitant with infection by a wide variety of human DNA and RNA viruses. This point is strengthend by the second finding that, in experimental animals, both acute and persistent virus infections can induce, accelerate, or enhance autoimmune responses and cause autoimmune disease. For example, we have shown that autoimmune manifestations normally present in NZB mice (anti-DNA antibodies, anti-red blood cell antibodies) or their $(NZB \times W)F_1$ relatives (anti-DNA antibodies) are enormously enhanced by persistent infection with DNA (polyoma) or RNA (lymphocytic choriomeningitis, LCMV) viruses; that is, antibodies form earlier and reach for higher titers in the infected mice than in their uninfected counterparts [1, 2]. Further, the NZW mice, which normally do not develop these autoimmune responses, do so upon polyma or LCMV infections. Indeed, the responses in NZB, $(NZB \times W)F_1$ or NZW mice are so marked that autoimmune diseases occur at a higher incidence with earlier time of death [NZB, $(NZB \times W)F_1$] or appear *de novo* (NZW) [2, 3]. A number of viruses, including retroviruses, are now known to perform similarly. Third, by evaluating molecular mimicry in human autoimmune disorders (Figure 1), we have uncovered a number of potential etiologic agents and mechanisms of autoimmune disease [4].

Viruses can induce autoimmune responses in many ways. For example, certain viruses have a mitogenic affect on unique blood lymphocyte subsets and hence can act as polyclonal activators. Viruses also direct the release of lymphokines and monokines. These molecules are important modulators of immune responses by acting directly as growth or differentiation factors or by regulating the expression

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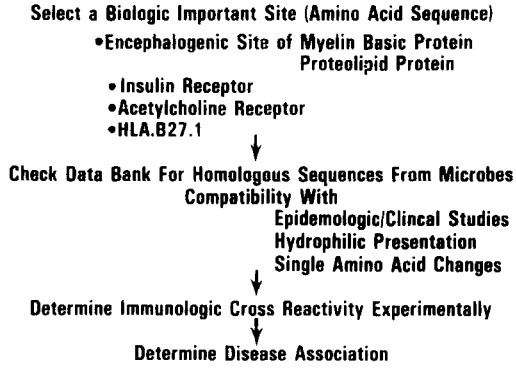

Figure 1. Cartoon of experimental approach utilized for clinical investigation of molecular mimicry as a potential cause of autoimmune disease.

of Class I and Class II major histocompatibility molecules (i.e. interferon effect). Viruses can also replicate selectively in particular lymphocyte subsets. By their presence, activation or replication they can cause immunosuppression or immunoenhancement (reviewed in [5, 6]). Moreover, some viruses and other microbes contain chemical structural components that mimic normal host 'self' proteins. An effector immune response, either B (humoral) or T (cytotoxic T cell), directed against the microbe might than also cross-react with 'self' protein, thereby evoking autoimmunity. The events of this scenario, termed molecular mimicry, comprise the body of this report.

We define molecular mimicry as similar structures shared by molecules from dissimilar genes or by their protein products. Either the molecules' linear amino acid sequences or their conformational fit may be shared, even though their origins are as separate as, for example, a virus and a normal host self determinant. Because guanine-cysteine (GC) sequences and introns designed to be spliced away may provide, respectively, false hybridization signals and nonsense homologies, molecular mimicry must be analyzed at the protein level. Such homologies between proteins have been detected either by use of immunologic reactants, humoral or cellular, that cross-react with two presumably unrelated protein structures, or by matching proteins described in computer storage banks. Regardless of the methods used for identification, it is now abundantly clear that molecular mimicry is common between proteins encoded by numerous DNA and RNA viruses and host 'self' proteins. Such events are relevant not only to autoimmunity but also as a likely mechanism by which viral proteins are processed inside cells [7].

Examples of molecular mimicry were first described as such in the early 1980s by investigators who found that monoclonal antibodies against SV40 T antigens cross-reacted with host cell proteins [8]. However, the importance of this observation became apparent when others realized that the monoclonal antibodies against a battery of viruses were cross-reacting with host determinants. For example, we showed cross-reactivity between measles virus phosphoprotein (72 K molecular weight) and the cytoskeleton component keratin (54 K molecular weight), and between a herpes simplex virus glycoprotein of 140 K and a separate epitope on keratin from that recognized by the measles virus phosphoprotein [9]. Further, we

Table 1. *Several anti-viral monoclonal antibodies that show cross-reactivity with interesting host antigens: potential implications for disaease*

Coxsackie B virus VP1 protein[1] / Myocardium	Viral myocarditis[2]
Measles virus hemagglutinin / Subset of human T lymphocytes	Viral immunosuppression
Theiler's virus VP1 protein / Galactocerebroside	Attack on oligodendrocytes, virus induced demyelination
HIV-1 gp41 / Astrocyte	Gliosis and CNS disorder in AIDS

[1]Immunochemical or immunofluorescent evidence of cross-reactivity.
[2]Speculation on disease on.

[7] found homology between vaccinia virus hemagglutinin and the cytoskeleton protein vimentin.

To determine the possible frequency of molecular mimicry, Srinivasappa and his colleagues [10] tested over 600 monoclonal antibodies obtained from several laboratories, all raised against viral polypeptides. These investigators then charted the incidence of the monoclonals' cross-reactivity with host proteins expressed in a large panel of normal tissues. In their analysis and our subsequent studies monoclonal antibodies were reactive with 14 different viruses, including such commonly found representatives DNA and RNA viruses as the herpesvirus group, vaccinia virus, myxoviruses, paramyxoviruses, arenaviruses, flaviviruses, alphaviruses, rhabdoviruses, coronaviruses, and human retroviruses. Most important, over 4% of such monoclonals cross-reacted with host-cell determinants expressed on uninfected tissues. Some of these monoclonal antiviral antibodies reacted with constituents of more than one organ. Hence, from these data, it is clear that molecular mimicry is common and not restricted to any specific class or group or virus. These and other reported cross-reactivities (Table 1) raise interesting questions about the etiology and pathogenesis of a number of autoimmune diseases.

Because the studies described above indicate that many viruses share antigenic determinants (linear or conformation) with normal host proteins, the next step was to determine experimentally whether molecular mimicry could elicit autoimmune diseases. Myelin basic protein was chosen as the host component to study because its entire amino acid sequence is known, and its encephalitogenic site of 8–10 amino acids has been mapped in several animal species. By computer-assisted analysis, several viral proteins listed in the Dayhoff files showed significant homology with the encephalitogenic site of myelin basic protein. Included were similarities and/or fits between myelin basic protein and the nucleoprotein and hemagglutinin of influenza virus, coat protein of polyoma virus, core protein of the adenovirus, polyprotein of poliomyelitis virus, EC-LF2 protein of Epstein-Barr virus (EBV), hepatitis B virus polymerase (HBVP), and others. However, the best fit occurred between the myelin basic protein encephalitogenic site in the rabbit and HBVP (Figure 2).

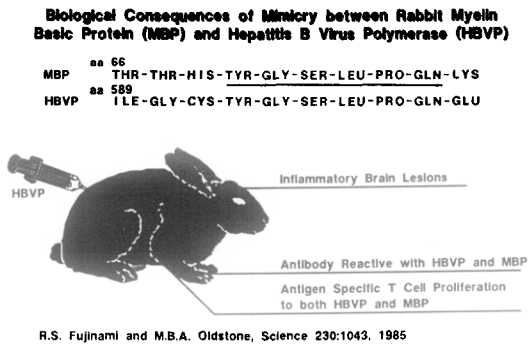

Figure 2. Evidence that molecular mimicry can cause autoimmune responses and autoimmune disease.

Interestingly, products of immune responses, both humoral and cellular, generated in rabbits inoculated with the octamer or decamer viral peptide reacted with whole myelin basic protein. Further, inoculation of the HBVP peptide into rabbits caused perivascular infiltration localized to the central nervous system, reminiscent of the disease induced by inoculation of either whole myelin basic protein or the encephalitogenic site of myelin basic protein (Figure 2) [11]. This experiment was seminal in that it conclusively showed molecular mimicry could cause both autoimmune responses and autoimmune disease. The most likely explanation for how molecular mimicry causes disease is that an immune response against the determinant shared by host and virus takes the form of a tissue-specific attack, presumably capable of destroying cells and eventually the tissue. The probable mechanism is generation by the pathogen of cytotoxic cross-reactive effector lymphocytes or antibodies that recognize specific determinants of 'self protein' located on target cells. Interestingly, the induction of cross-reactivity would not require a replicating agent, and the immunologically mediated injury could occur after removal of the pathogen—a hit-and-run event. Clearly, the virus infection that initiates an autoimmune phenomenon need not be present at the time overt disease develops. A likely scenario would be that the virus responsible for inducing a cross-reacting immune response is cleared initially, but the components of that immunity continue to assault host elements. The cycle continues as the autoimmune response itself leads to tissue injury that, in turn, releases more self antigen, thereby inducing more antibodies, and so on. Such a sequence might account for the viral encephalopathies occurring in humans after measles, mumps, vaccinia, or herpes-zoster virus infections; in these post-infectious diseases, recovery of the inducing agent has been rare.

This theory is reinforced by studies showing that, after any of several acute viral infections, mononuclear cells from the peripheral blood or cerebral spinal fluid proliferate in response to host antigens, one of which is myelin basic protein. Interestingly, several clonal populations of lymphocytes harvested from central nervous system fluids of humans with encephalitis proliferate in response to the infecting virus as well as to antigens of the nervous system.

Viruses or microbes also play by other game plans. For example, a virus with the capacity to persist in its host may continuously or cyclically express its antigens. Although expression of a viral genome may be restricted so that no infectious virus

Table 2. *Sequence similarities between microbial proteins and human host 'self' proteins*[1]

HLA-B27	70:	LYS ALA GLN THR ASP ARG GLU ASP LEU
KLEB PN	186:	SER ARG GLN THR ASP ARG GLU ASP GLU
HuAChR	160:	PRO GLU SER ASP GLN PRO ASP LEU
HSV GP D	286:	PRO ASN ALA THR GLN PRO GLU LEU
HLA DR	50:	VAL THR GLU LEU GLY ARG PRO ASP ALA GLU
HCMV IE-2	79:	PRO ASP PRO LEU GLY ARG PRO ASP GLU ASP
INSULIN r	66:	VAL TYR GLY LEU GLU SER LEU LYS ASP LEU
PAPILLOMA E2	76:	VAL LEU HIS LEU GLU SER LEU LYS ASP SER
COAGFACT XI	269:	ILE LYS LYS SER LYS ALA LEU
DENGUE	68:	ILE LYS LYS SER LYS ALA ILE
MYOSIN	138:	TYR GLU ALA PHE VAL LYS HIS ILE MET SER VAL
COX B3	2,152:	TYR GLU ALA PHE ILE ARG LYS ILE ARG SER VAL
BRAIN PROTEIN	156:	ASP SER THR LYS ASN ARG LYS THR ASP
HIV POL	222:	ASP SER THR LYS TRP ARG LYS VAL ASP

[1]Shared sequences currently under active evaluation for pathogenic role in selected diseases. Immunologic cross-reactivity has now been observed for the majority of these sequence pairs, and a disease association has been suggested for the first two pairs by investigation of specimens from patients with ankylosing spondylitis and myasthenia gravis, respectively.

replicates, production of a viral determinant in common with that of the host might continue. This would allow initiation of an immune response and/or autoimmunity, either one leading to cyclic, chronic, or progressive disease.

In any case, molecular mimicry would occur only when the virus and host determinants are sufficiently similar to induce a cross-reactive response yet different enough to break B or T-cell immunologic tolerance.

Lastly, evidence for the hypothesis that molecular mimicry causes autoimmune disease in humans emanates from studies on pathogenesis of the non-rheumatoid arthritides, ankylosing spondylitis (AS) and Reiter's syndrome (RS) [12–15], and on myasthenia gravis [15, 16]. Six consecutive amino acids (QTDRED) are identical between the hypervariable domain of HLA-B27 and *K. pneumoniae* nitrogenase (Table 2). Sera from a significant proportion of HLA-B27 individuals with RS (18 of 34) or AS (7 of 24), but not from appropriate controls, reacted with a synthetic peptide containing the homologous region of HLA-B27.1 and *K. pneumoniae* nitrogenase [12]. These results with American patients were confirmed by analysis of an additional 27 sera obtained from The Netherlands [Ivani and Oldstone, unpublished results, 1988]. As expected, sera from the latter AS and RS patients also reacted with a peptide derived from the *K. pneumoniae* nitrogenase (CNSR*QTDRED*ELI). Chen *et al.* [17] found that antibody to the hypervariable region of HLA-B27.1 is reactive with Yersinia (strain—pseudo tuberculosis), another microbe implicated in the AS/RS arthritides. These observations suggest that RS and AS are autoimmune diseases, with HLA-B27 serving as the autoantigen. Induction would be caused by a

microbe(s) encoding a protein with sequence homology to HLA-B27 (variable region), and the disease would presumably be related to an unusual concentration of HLA antigen in certain tissues-like joints. To address this latter issue, material was obtained from the joints of patients with AS and others with non-AS arthropathies. Accordingly, joint tissue from 11 of 12 AS patients studied heavily expressed HLA-B27 sequences, i.e. QTDRED occurred in synovial cells lining the joint space and in endothelial cells in synovial tissue [13]. In contrast, these sequences were not observed either in synovial tissue of individuals with non-arthropathies or in endothelial and other cells obtained by skin biopsy from non-AS but HLA-B27 positive individuals.

The large majority of patients with the autoimmune disease, myasthenia gravis, characteristically have detectable antibodies against the acetylcholine (AChR). The nicotinic AChR is composed of multiple subunits responsible for gating ion flow across membranes in response to binding of the neurotransmitter, acetylcholine. The actions of AChR antibodies, either experimentally induced or spontaneous, lead to the numerical reduction of available AChR and prevention of the neuromuscular junction's ability to transmit signals from nerve fibers to muscle fibers; medically, the outcome is myastheia gravis. For molecular dissection of the physiologically important binding sites, synthetic peptides representing unique regions of the α-chain of AChR have been used in association with α-bungarotoxin to compete with the binding of antibody specific for AChR. These interactions also map accessible sites on AChR that bind antibodies. Utilizing the strategy outlined in Figure 1, we found immunochemically significant cross-reactivity with the human AChR α-chain amino acid sequences 160–167 and herpes simplex virus glycoprotein D residues 286–293 (Table 2). For example, antibodies to the herpes simplex virus peptide reacted with both the corresponding AChR peptide and with the AChR native protein. Interestingly, a different herpes simplex virus glycoprotein sequence amino acid 381–389 also showed several similar amino acid sequences, but on immunochemical analysis failed to raise antibodies that cross-reacted with the AChR sequence [15, 16]. Affinity purification of antibodies from patients with myasthenia gravis, using the human AChR α-chain 157–170 peptide immobilized on thiopropyl-Sepharose, yielded IgG antibodies that bound to the native AChR and inhibited the binding of α-bungarotoxin to its specific binding site on the receptor. Thus, the human AChR α-chain 160–167 peptide specifically cross-reacts with a shared homologous domain on herpes simplex virus glycoprotein D, residues 286–293, by binding and inhibition studies, and elicits antibodies in myasthenic patients that bind to the native AChR protein; these antibodies are capable of causing a biologic effect. Immunologic cross-reactivity of this 'self' epitope with herpes simplex virus suggests that this virus may be associated with some cases of myasthenia.

The probability that a random six amino acid sequence will be identical in two dissimilar proteins is 1 in 20^6, assuming that all amino acids are represented equally. Nevertheless, the finding of sequence homology is, by itself, insufficient evidence of biologically meaningful mimicry. Two examples illustrate this point. First, like the *K. pneumoniae* nitrogenase, the EBV BBLF protein shares six continuous amino acids with HLA-B27, but the homology is in the conserved and not the variable domain of the HLA-B27.1 molecule. No immunochemically identifiable cross-reactivity occurs between HLA-B27-specific sequences and EBV peptides

containing the homologous sequence. Further, over 50 patients with AS or RS and of the HLA-B27 haplotype had no antibodies to the EBV peptides [12, 14]. Thus, whether or not the homology reflects the biologically meaningful domain is important.

Second, homology alone may not lead to a cross-reacting immune response, especially if the dissimilar amino acid(s) represents a radical substitution or interferes with binding. For example, despite a high degree of similarity between portions of the AChR α-chain (PESDQPDL) and polyoma virus middle T antigen (*PESDQ*DQ*L*), no cross-reacting antibodies form. Yet a more distant similarity between the AChR sequence and herpes simplex virus glycoprotein D (*P*NAT*Q*PE*L*) induces strong immunologic cross-reactivity [15].

Just as homologies and immunologic cross-reactivities have been found between the HLA-B27 variable domain and *K. pneumoniae* nitrogenase, between the human AChR and herpes simplex virus glycoprotein, and between other host and microbial proteins (Table 2), additional similarities will surely emerge as more genes and proteins are analyzed. Some of these examples may account for diseases in terms of an autoimmune response provoked by molecular mimicry. However, unless the homology and subsequent immunologic cross-reactivity involve a host protein that can precipitate disease (e.g. the restricted encephalitogenic site of myelin, and immunodominant domain of the insulin receptor or AChR), the autoimmune response is unlikely to lead to autoimmune disease.

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