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Mimicry by Virus of Host Molecules:  
Implications for Autoimmune Disease

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Molecular mimicry defines the shared identity of molecules from disparate genes or proteins. Thus, although their origins are as separate as a virus and the self-determinant of a human or lower animal, two molecules’ linear amino acid sequences or their conformational fits may be shared. Such molecular homologies between proteins occur frequently and likely play roles in the processing of viral proteins inside cells. The homologies shared between viruses and host cytoskeletal proteins likely indicate that shared determinants on cell linker proteins guided viral proteins along highways and stop points inside cells. Most importantly, these unexpected cross-reactivities have broad and major implications for understanding autoimmune disease. Molecular mimicry is detected either by using humoral or cellular immune components, that cross-react with two presumably unrelated protein structures, or by computer searches to match descriptions of proteins in storage banks. The use of both these approaches to define molecular mimicry and establish its potential role in autoimmune disease is the topic of this chapter.

I. Introduction

By molecular mimicry we mean the shared identity of molecules from disparate genes or proteins (Oldstone and Notkins, 1986). Thus, although their origins are as separate as a virus and the self-determinant of a human or lower animal, two molecules’ linear amino acid sequences or their conformational fits may be shared. For a variety of reasons including false signals from enriched guanine–cytosine sequences or as introns designed to be spliced away may provide false hybridization signals, we have focused on molecular mimicry at the protein level. Such molecular homologies between proteins occur frequently and have broad and major implications for understanding autoimmune disease. Further, such homologies likely play roles in the processing of viral proteins inside cells. This realization came from repeated observations of homologies shared between viruses and host cytoskeletal proteins. This phenomenon led Dales et al. (1983) to hypothesize that shared determinants on cell linker proteins guided viral pro-
teins along highways and stop points inside cells. Additionally, these unexpected cross-reactivities attendant to mimicry warrant cautious use of reagents in diagnostic virology, microbiology laboratories, and synthetic vaccines, even though these materials originated from hybridomas or from animals immunized with predetermined (peptide) amino acid sequences. Molecular mimicry is detected either by using humoral or cellular immune components that cross-react with two presumably unrelated protein structures, or by computer searches to match descriptions of proteins in storage banks. The use of both these approaches to define molecular mimicry and establish its potential role in autoimmune disease is presented in this chapter.

II. Molecular Mimicry between Host “Self” Proteins and Viruses

Several independent reports appeared in the early 1980s noting molecular mimicry between the viral antigen SV40-T and host cell proteins (Lane and Hoeffler, 1980), measles virus phosphoprotein and the cytoskeleton component keratin (Fujinami et al., 1983), and a herpes simplex glycoprotein of 140K and a separate epitope on keratin from that recognized by the measles virus phosphoprotein (Fujinami et al., 1983). To better determine the frequency of molecular mimicry, Srinivasappa and his colleagues at the NIH acquired from many laboratories over 600 monoclonal antibodies raised against viral polypeptides (Srinivasappa et al., 1986). These investigators then mapped the incidence of monoclonals cross-reactivity with host proteins expressed on a large panel of normal tissues (Table I). In the analysis were antibodies against 11 different viruses including DNA and RNA viruses known to cause human infection from the herpes virus group, vaccinia virus, myxoviruses, paramyxoviruses, arenaviruses, flaviviruses, alphaviruses, rhabdoviruses, and coronaviruses. The results indicated that over 3.5% of such monoclonals cross-reacted with host cell determinants expressed on uninfected tissues. These determinants occurred at a single site or in widely diverse places located in a wide panel of cells found in the nervous system, endocrine system, immune system, gut, heart, and muscle (Fig. 1). These and our other observations indicated that certain monoclonal antibodies stained restricted subsets of neurons in selected areas of the nervous system or unique subsets of lymphocytes within a defined functional lymphocyte class were stained.

III. Molecular Mimicry and Autoimmune Disease Suggested from Studies with Monoclonal Antibodies

The above data indicate common cross-reactivity at the monoclonal level between viral protein and host self-proteins. In this situation, antibodies to hor-
TABLE I

Molecular Mimicry: Viruses–Host Antigens

| Monoclonal antibodies | Reactive with virus | Number tested | Reactive with uninfected tissues |
|-----------------------|---------------------|---------------|---------------------------------|
| Coxsackie B4          | 66                  |               | 1                               |
| Japanese encephalitis | 34                  |               | 6                               |
| Lymphocytic choriomeningitis | 174     |               | 3                               |
| Measles               | 39                  |               | 5                               |
| Rabies                | 80                  |               | 2                               |
| Vesicular stomatitis  | 37                  |               | 2                               |
| Herpes simplex (I)    | 20                  |               | 1                               |
| Vaccinia              | 16                  |               | 1                               |
| Dengue                | 132                 |               | 0                               |
| Cytomegalovirus       |                     |               |                                 |
| Human                 | 24                  |               | 1                               |
| Mouse                 | 14                  |               | 0                               |
| Total                 | 636                 |               | 22 (3.5%)                       |

*aListed are the analyses of over 600 monoclonal antibodies done by Srinivasappa et al. (1986). Monoclonal antibodies against 11 different viruses including DNA and RNA viruses known to cause human infection from the herpes virus group, vaccinia virus, myxoviruses, paramyxoviruses, arenaviruses, flaviviruses, alphaviruses, rhabdovirus, and coronavirus cross-react with host cell determinants expressed on uninfected tissues. Twenty-two of such monoclonal antibodies, or 3.5% provide evidence of molecular mimicry.

...mones, lymphocyte subsets, or cells of the nervous system, etc., can develop as a consequence of virus infection, with all the inherent potential for participating in disease. The cross-reactivity between viruses and particular tissues offers some interesting insights into the association of virus infections with specific diseases. For example, coxsackie B virus has been found in individuals with myocarditis, an inflammatory disease of the heart muscle. One of the monoclonal antibodies described by Srinivasappa et al. (1986) was directed against the neutralizing domain of coxsackie virus but also interacted with heart muscle (Saegusa et al., 1986). Equally intriguing was the recent finding by Fujinami and Powell (1986) of a link between Theiler’s virus and the demyelinating disease it causes. In this instance, a monoclonal antibody directed against the major neutralizing domain of Theiler’s virus also reacted with galactocerebroside, the main component on the surface of oligodendrocytes. Because oligodendrocytes are cells that make the myelin lamella that wraps around axons, their destruction leads to demyelination. Interestingly, inoculation of this monoclonal antibody into the sciatic nerve results in several fingerprints of demyelination. These and...
Fig. 1. Determinants shared between viral and host self-proteins for 21 of 22 monoclonal antibodies showed to possess molecular mimicry (see Table I). Note that these monoclonal antiviral antibodies cross-react with one or more groups of uninfected cells representative of the nervous system, endocrine system, immune system, gut, heart, and muscle. See Srinivasappa et al. (1986) for experimental data.

other examples (reviewed in Oldstone and Notkins, 1986) suggest a mechanism whereby immune reactants directed against a viral or microbial component may cross-react with a host component and generate autoimmune disease.

A second immunopathologic sequella associated with molecular mimicry is the formation and trapping of immune complexes (Dales et al., 1983; Oldstone and Notkins, 1986). In this instance, antibodies induced against proteins of the infecting virus, but cross-reactive with host proteins such as cytoskeletal or other self-proteins released into fluids during normal cell turnover or enhanced turnover and lysis occurring during virus infection, form complexes with antigen in the circulation. These complexes may become trapped in vessels with fenestrated endothelial linings such as the renal glomeruli, small arteries and capillaries, and the choroid plexus. Here, they accumulate to set in motion the events of immune complex disease. Such events may well account for the high incidence of antisel (myosin, actin, smooth muscle, nucleic acids, etc.) antibodies producing during virus infection and the formation of immune complexes during such infections (reviewed in Oldstone and Notkins, 1986).
IV. Amino Acid Homologies and Immune Responses between Important Host Proteins and Viruses Serve as a Mechanism for Autoimmunity: Evidence

The analysis discussed above indicates that 3–4% of monoclonal antibodies generated against specific virus determinants also bind to host “self-determinants. Other experiments have established that a minimum of five to six peptides is required for the induction of monoclonal antibodies (Wilson et al., 1984). Since, on the basis of antibody cross-reactivity, many viruses share antigenic sites with normal host cell components, the next step was to look for cross-reactive capability in eliciting autoimmunity and related disease. This was approached by using a computer-assisted search of the Dayhoff data bank. After examining the 2511 amino acid sequences including 470,158 residues deposited in the protein data bank to look for overlapping peptides, homologies were noted among 2469 hexomers, 186 septomers, and 17 octomers; however these may not have been in tandem. Since the probability of the requisite 20 amino acids occurring in six identical sequences in a row between two proteins, if amino acids occur at a random frequency, is $20^6$ or one to 64 million, it is unlikely that such a homology would occur by chance.

To provide evidence that molecular mimicry could cause autoimmunity we chose to study the encephalitogenic site of myelin basic protein and the disease allergic encephalomyelitis (EAE). The entire amino acid sequence of myelin basic protein is known, and its encephalitogenic sites have been mapped in several animal species (Hashim, 1978). Computer-assisted analysis located several viral proteins in the Dayhoff files that have significant homology with the encephalitogenic site of myelin basic protein (Hashim, 1978); these are the nucleoprotein and hemagglutinin of influenza virus, coat protein of polyoma virus, core protein of adenovirus, polyprotein of poliomyelitis virus, EC-LF2 protein of Epstein–Barr virus, rabies virus glycoprotein, measles virus nucleoprotein, and hepatitis B virus polymerase. However, of the banked sequences, the myelin basic protein encephalitogenic site in the rabbit fit best with hepatitis B virus polymerase (HBVP):

\[
\begin{align*}
66 & \quad 75 \\
\text{Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys} & \quad \text{Encephalitogenic site, rabbit myelin basic protein}
\end{align*}
\]

\[
\begin{align*}
589 & \quad 598 \\
\text{Ile-Gly-Cys-Tyr-Gly-Ser-Leu-Pro-Gln-Glu} & \quad \text{HBVP}
\end{align*}
\]

As seen in Fig. 2, immune responses, both humoral and cellular, were generated in rabbits inoculated with the dexomer viral peptide reacted with 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 (Fig. 2).
Fig. 2. Documentation that molecular mimicry between virus and host self-protein can cause autoimmune disease. The sharing of 6 amino acids between hepatitis B virus and the rabbit encephalitogenic site of myelin basic protein (MBP) is shown. On inoculation of rabbits with the hepatitis B virus sequences 589–598, rabbits developed both humoral (upper left panel) and cellular (upper right panel) responses against whole native myelin basic protein. Further, of 11 such rabbits inoculated, 4 developed perivascular infiltration in the subventricular area and parenchyma of the mid-brain (lower right panel). See Fujinami and Oldstone (1985) for experimental details.

This outcome provided the first evidence for the potential of molecular mimicry to cause both autoimmune responses and autoimmune disease (Fujinami and Oldstone, 1985). Conceptually, molecular mimicry can produce autoimmunity when virus and host determinants are sufficiently similar to induce a cross-reactive response, yet different enough to break immunologic tolerance. Such principles for molecular mimicry undoubtedly follow those mapped for the induction and breaking of tolerance at both the B and T lymphocyte levels by heterologous serum protein (Weigle, 1980).

With current technology allowing cloning and rapid sequencing of genes, and utilizing nucleic acid sequences of the open reading frame to determine the protein sequences encoded by a gene, more data on viral polypeptides will soon be available. Such information with respect to homologies between microbial agents and the acetylcholine receptor, insulin receptor, and/or encephalitogenic receptor will likely show similarities and should improve understanding of the postinfectious encephalopathies and demyelination following virus infections, potential causes of myasthenia gravis and perhaps endocrine disorders such as diabetes. Important will be the recognition that unless homology and the subse-
sequent immunologic cross-reactivity involve a host protein that precipitates disease, e.g., the restricted encephalitogenic site of myelin rather than multiple other sites of myelin basic protein, disease will be unlikely to follow despite autoimmune response.

V. Future Directions and Mechanisms by Which Molecular Mimicry Occurs and Causes Disease

We have uncovered several other interesting examples of molecular mimicry, and these are currently under study in our laboratory. Included are mimicry between viruses and the α-chain of the human acetylcholine receptor (Fig. 3) and between a number of microbial agents and the human major histocompatibility (MHC) marker HLA B.27 (Fig. 4). In the first instance, 10% of sera samples tested from myasthenic patients bound to the selected amino acid sequences for the α-chain of the acetylcholine receptor depicted in Fig. 3. Affinity purification of such antibodies is underway, and their ability to cause depolarization of the receptor is being evaluated. Similarly, sera from HLA B.27 patients with and without ankylosing spondylitis are being studied for their cross-reactivity with amino acid sequences from several microbial agents, depicted in Fig. 4. Other interesting molecular mimicries under study are those between myelin and AIDS virus and between sequences representing other MHC and viruses.

The most likely mechanism by which molecular mimicry would cause disease is by eliciting an immune response against a determinant shared between the host and the virus to bring forth a tissue-specific immune response, presumably capable of destroying cells and eventually the tissue. The probable mechanism is the generation of cytotoxic cross-reactive effect of lymphocytes and/or antibodies that recognize “self-determinants” localized 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 immunogen—a hit and run event. By such a mechanism, the microbial infection that initiates the autoimmune phenomenon need not be present at the time overt disease develops. The likely picture would be that the virus responsible for inducing a cross-reactive immune response is cleared initially, but the components of that immunity continue to assault host elements. This cycle continues as the autoimmune response itself leads to tissue injury that, in turn, releases more self-antigen, thereby incuding more antibodies, and so on. Such a sequence of events would render the isolation or identification of an initiating infectious agent difficult or impossible. Indeed, such events likely occur with the encephalopathies that follow measles, mumps, vaccinia, or herpes zoster virus infections of humans. In such postinfectious diseases, the infected host develops encephalitis, frequently associated with such other symptoms of autoimmune disease as rash.
Fig. 3. Amino acid sequence homology between the immunodominant region of the α-chain of the human acetylcholine receptor and several viruses matched through the Dayhoff protein bank. When the viral amino acid sequences were inoculated into rabbits and then tested for their binding to the α-chain of the acetylcholine receptor note that herpes simplex virus (HSV) glycoprotein D residues 286 to 293 showed a higher degree of cross-reactivity than those of polyoma virus, which showed a greater degree of sequence homology. Specificity of binding was checked by quantitative blocking experiments. Analysis of sera from over 40 patients with myasthenia gravis indicated that their antibodies bound to the acetylcholine receptor sequence shown. See Dyrberg and Oldstone (1986) for details.

Fig. 4. Amino acid homologies between amino acid sequences from HLA B.27, Epstein–Barr virus, and Klebsiella pneumoniae. (Unpublished data of P. Schwimmbeck and M. B. A. Oldstone.)
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and pain in the joints and skeletal muscles. Recovery of the viral agent at this time is exceedingly rare, although the agent has been recovered easily several days earlier. This link between molecular mimicry and host proteins is further supported by studies showing that, after several types of acute virus infections, mononuclear cells from peripheral blood or cerebral spinal fluid proliferate in response to host antigens, one of which is myelin basic protein. Further, several clonal populations of lymphocytes have been harvested from the central nervous system fluid of patients with encephalitis, and these lymphocytes proliferate when incubated with the infecting virus, its antigens, or nervous system antigens. These and other issues are likely to be studied increasingly to provide insights as to both potential etiologic agents and pathogenic mechanisms responsible for a variety of autoimmune disorders of man.

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