CHAPTER 23

Molecular Mimicry

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Prologue

Molecular mimicry defines 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–cytosine (GC) sequences and introns designed to be spliced away may provide, respectively, *false hybridization signals* and nonsense *homologies,* we focus here on molecular mimicry at the protein level. Such homologies between proteins have been detected either by use of immunologic reagents, humoral or cellular, that cross-react with two presumably unrelated protein structures, or by computer searches to match proteins described in storage banks. Regardless of the methods used for identification, it is now clear that molecular mimicry between proteins encoded by numerous DNA and RNA viruses and host "self" proteins is a relatively common event [1–3]. Among the broad implications of these data are leads for understanding virally induced autoimmunity and disease [2–8] as well as mechanisms by which viral proteins are processed inside cells [9]. Further, the unexpected cross-reactivities attendant to mimicry warrant cautious use or reagents in diagnostic virology and microbiology, even though these materials originated from hybridomas or from animals immunized with predetermined (peptide) amino acid sequences. This chapter is presented to define molecular mimicry and emphasize its effect on viral pathogenesis.

Molecular Mimicry Between Viruses and Host Cell Proteins

Examples of molecular mimicry were first described as such in the early 1980s by investigators who found that monoclonal antibodies against SV 40 T antigens cross-reacted with host cell proteins [10]. However, the importance of this observation became apparent only when others realized that the monoclonal antibodies against a battery of viruses were cross-reacting with host

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determinants [1,3,9,11]. For example, Fujinami et al. [3] showed cross-reactivity between measles virus phosphoprotein (72K molecular weight) and the cytoskeleton component keratin (54K molecular weight), and between a herpes simplex virus glycoprotein of 140K and a separate epitope on keratin from that recognized by the measles virus phosphoprotein. Dales et al. noted shared homology between vaccinia virus hemagglutinin and the cytoskeleton protein vimentin [9], whereas Sheshberadaran and Norrby found homolgy between the fusion protein of measles virus and a heat shock protein [11]. These and other observations of immunologic reactivity between viral proteins and cytoskeletal determinants suggested the hypothesis of shared determinants on cell linker proteins that might help to guide viral proteins along highways and stop points traveled inside cells (see Figure 7 of ref. 9). Others found that monoclonal antibody directed against a specific viral determinant to which it was raised, reacted with determinants of another totally unrelated virus [12].

Molecular Mimicry and Autoimmune Manifestations

The potential of molecular mimicry as an important event in pathogenesis leading to disease became evident from two groups of observations. The first was cross-reactivity at the monoclonal antibody level between viral protein and host self proteins. In such a system, antibodies to hormones, lymphocyte subsets, or cells of the nervous system developed as a consequence of virus infection, with all the inherent capacity to participate in disease. The cross-reactivity between viruses and particular tissues offers some insight into the association of viral infection and specific diseases. For example, coxsackievirusB4 has been found in individuals with myocarditis or inflammatory disease of the heart muscle. Of related interest, a monoclonal antibody directed against the neutralizing domain of coxsackievirus also interacted with the heart muscle (see figure 23.1). Equally intriguing was a link between Theiler's virus and the demyelinating disease it causes. 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 [13]. Because oligodendrocytes are cells that make the myelin lamellae wrapped around axons, their destruction leads to demyelination [14]. Interestingly, inoculation of this monoclonal antibody induced demyelination [13]. Figure 23.1 shows several examples of molecular mimicry detected by using normal host tissues to screen monoclonal antibodies against various viral proteins (see ref. 1 for details).

The second sequela associated with molecular mimicry is the formation and trapping of immune complexes [15]. In this instance, cytoskeletal or other self proteins of an infected host are released into fluids either as a result of normal cell turnover or enhanced turnover and lysis occurring during viral infection. Antibodies induced against proteins of the infecting virus,

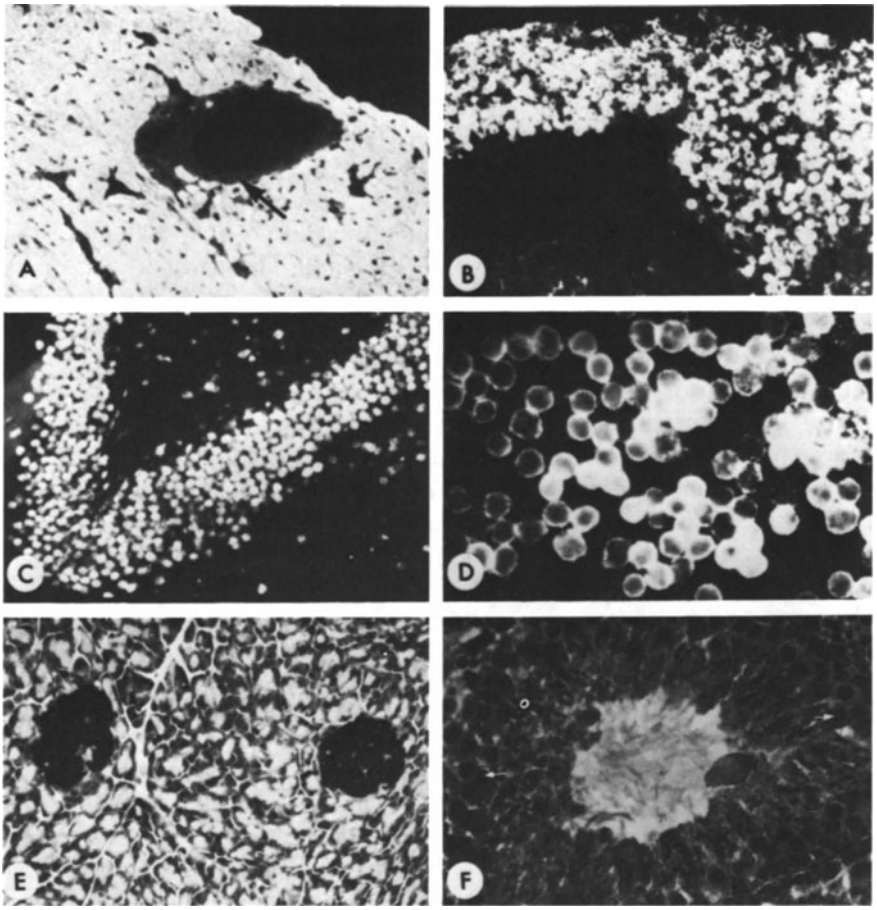

Figure 23.1. Reactivity of monoclonal antiviral antibodies with normal tissues detected by indirect immunofluorescence (**A,B,C,D**) or immunoperoxidase (**E,F**). Monoclonal antibody to coxsackievirus B_4 reacts with mouse myocardium; smooth muscle in coronary artery shows no reaction (arrow) (A). Monoclonal antibody to Japanese encephalitis virus reacts with mouse anterior pituitary cells (**B**). Two monoclonal antibodies to measles virus are shown, one reacting with nuclei of cells in mouse hippocampus (**C**), and the other with human T lymphocytes (**D**). A monoclonal antibody to herpes simplex virus that reacts with both hamster pancreatic B cells in the islets of Langerhans (**E**) and nuclei of spermatogonia (arrows) in the testis (**F**).

but cross-reactive with host proteins, can form antigen–antibody complexes in the circulation. These complexes may become trapped in vessels with fenestrated endothelial linings such as the renal glomeruli, small arteries, and capillaries in the choroid plexus. Here, they can accumulate to set in motion the events of immune complex disease. After viral infection of even antiviral immuniztion [9], the host antigen–antibody complexes may form

an important component of total immune-complex deposits. The principles of immune-complex disease have been detailed earlier in this series [15].

How Common Is Molecular Mimicry?

To 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. These investigators then charted the incidence of the monoclonals' cross reactivity with host proteins expressed in a large panel of normal tissues [1] (Figure 23.1). In the analysis were antibodies against 11 different viruses, including such commonly found representatives of DNA and RNA viruses as the herpesvirus group, vaccinia virus, myxoviruses, paramyxoviruses, arenaviruses, flaviviruses, alphaviruses, rhabdoviruses, and coronaviruses. The results were that approximately 4% of such monoclonals cross-reacted with host-cell determinants expressed on uninfected tissues. Moreover, some of these monoclonal antiviral antibodies reacted with antigens in more than on organ [1,16]. From these data, it is clear that molecular mimicry is common and not restricted to any specific class or group of virus.

Using Peptides to Induce Monoclonal Antibodies

We now know that a minimum of six to seven peptides are required for the induction of monoclonal antibodies [17]. Thus, the probability that the requisite 20 amino acids occur in six identical sequences between two proteins is 20^6 or 1 to 128,000,000, assuming all amino acids are represented equally and at random. After a search through the 2511 amino acid sequences in the Dayhoff protein data base (which includes 470,158 residues) to discover overlapping peptides, Wilson and colleagues [18] found 2469 hexamers, 186 septamers, and 17 octamers with homologies. This data provides a ballpark estimate of the expected frequency for mimicry by use of linear sequences.

Amino Acid Homologies and Immune Responses Between Important Host Protein and Virus as Mechanisms for Autoimmunity

Because, on the basis of antibody cross-reactivity, many viruses clearly share antigenic sites with normal host-cell components, the next step was to look for cross-reactivity capable of eliciting autoimmunity and related disease. 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. With the use of

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, hepatitis B virus polymerase, and others. However, the best fit occurred between the myelin basic protein encephalitogenic site in the rabbit and hepatitis B virus polymerase (HBVP):

66 75 THR-THR-HIS-TYR-GLY-SER-LEU-PRO-GLN-LYS,	Encephalitogenic site, rabbit myelin basic protein
589 598 ILE-GLY-CYS-TYR-GLY-SER-LEU-PRO-GLN-GLU,	HBVP

Interestingly, products of the immune responses, both humoral and cellular, generated in rabbits inoculated with the octomer or dexomer 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 [4]. This outcome clearly exemplifies the potential of molecular mimicry to cause both autoimmune responses and autoimmune disease.

Mechanisms by Which Molecular Mimicry Occurs and Causes Disease

The most likely explanation for how molecular mimicry causes disease is that an immune response against the determinant shared by host and virus can 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 effector lymphocytes or antibodies that recognize specific determinants of "self proteins" 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 immunogen—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 virus encephalopathies occurring in humans after measles, mumps, vaccinia, or herpes–zoster virus infectious;

in these postinfectious diseases, recovery of the inducing agent has been rare [19]. This theory is reinforced by studies showing that, after several types of acute viral infection, mononuclear cells from 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 have been harvested from central nervous system fluid of humans with encephalitis, that proliferate to the infecting virus as well as to nervous system antigens (B. Waksman, personal communication). Several relevant human diseases that may be associated with a molecular mimicry pathogenesis are recorded elsewhere [2,20,21]. Viruses 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 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 immunologic tolerance. Weigle and his colleagues [22] have mapped the induction and breaking of tolerance at both the B cell and T cell levels by using heterologous serum proteins, and the same principles undoubtedly govern microbially induced molecular mimicry.

With the revolution in current technology allowing cloning and sequencing of genes and their proteins, more data on viral polypeptides will soon be available. Then, just as homologies are being found between the acetylcholine receptor and/or the insulin receptor with several viral proteins [2] and between important proteins of both the central and peripheral nervous systems with several viruses [2,20], other similarities will surely emerge. However, unless such homology and the subsequent immunologic cross-reactivity involve a host protein that precipitates disease, e.g., the restricted encephalitogenic site of myelin rather than multiple sites on myelin basic protein, disease is unlikely to follow, despite an autoimmune response.

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