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Molecular mimicry: a critical look at exemplary instances in human diseases

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Abstract. Molecular mimicry, the concept that antigenic determinants of microorganisms resemble antigenic determinants of the host, is frequently cited as a plausible mechanism to account for the association of infection and autoimmune disease. Based on analogous sequences of amino acids or on cross-reactions of monoclonal antibodies, numerous examples of such mimicry have been reported. There are, however, no clear examples of a human disease caused by molecular mimicry.

Key words. Autoimmunity; molecular mimicry; myocarditis; type 1 diabetes; Lyme disease; rheumatoid arthritis; ankylosing spondylitis; multiple sclerosis.

Introduction

The term molecular mimicry was coined by Damian [1], who was the first to suggest that antigenic determinants of microorganisms resemble antigenic determinants of their host. Such molecular mimicry, he opined, may obviate the development of an immune response to the microorganism, thereby protecting it from host defenses. It is ironic that the term has more recently assumed the opposite meaning, i.e., that antigenic determinants of a microorganism might elicit an autoimmune response causing harm to the host. Molecular mimicry has become a very popular explanation for the frequent association of infection with autoimmune disease, even though such an effect would seem to violate both evolutionary logic and experimental experience. There are, as yet, no firm instances of molecular mimicry by microorganisms serving as the initiating agents of human autoimmune disease, although a great deal of attention has been paid to this issue.

Here, we will examine several examples of human autoimmune diseases that are a sequela of an infectious process and review critically the evidence that molecular mimicry is the etiological mechanism. First, however, we will refer briefly to three recent examples testing experimentally whether microbial infections can induce a pathological autoimmune process.

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Test of the principle

To determine the capacity of microorganisms to influence the subsequent development of autoimmune disease through molecular mimicry, Barnett et al. [2] engineered recombinant vaccinia virus encoding the encephalitogenic peptide of myelin basic protein for PL/J mice. When PL/J mice were infected with the recombinant virus, they demonstrated no signs of the characteristic inflammation and demyelination of the T-cell-mediated autoimmune disease, experimental allergic encephalomyelitis (EAE). On the contrary, the mice were protected from the subsequent development of EAE after challenge with the encephalitogenic peptide or myelin basic protein given with complete Freund's adjuvant (CFA). The mice, however, were not protected when whole-mouse spinal cord homogenate was used for immunization, presumably because there are additional encephalitogenic determinants in the spinal cord preparation. Thus, protection was antigen specific. Sera of the infected mice contained antibodies

to the encephalitogenic peptide, indicating that the animals were primed by infection with the recombinant virus. Delayed-type hypersensitivity responses to myelin basic protein, however, were markedly reduced in the infected animals compared with controls. These experiments showed that an infectious agent that had been deliberately engineered to carry a molecular mimic generated the production of autoantibody but not autoaggression. In fact, the autoimmune response was deviated to favor the harmless antibody-mediated rather than the pathogenic cell-mediated response.

Another example of the effect of infection on subsequent autoimmune disease was described by Zinkernagel et al. [3]. They developed transgenic mice that expressed lymphocytic choriomeningitis virus glycoprotein in the pancreatic islet cells. The viral glycoprotein, therefore, was regarded by the host as self-antigen and no immune response ensued. Unprimed T cells administered by adoptive transfer from virus-infected donors were unable to cause autoimmune disease. Perhaps the virus-specific T cells were too rare, too low in affinity, or unable to localize in the target organ; or, the antigen was sequestered and the T cells remained ignorant. However, when the transgenic mice were infected with lymphocytic choriomeningitis virus, they developed insulitis and diabetes. Infection by the virus induced cytotoxic T cells that were specific for the viral glycoprotein expressed in the islets, and autoaggression resulted, because the activated T cells had gained access to the previously sequestered glycoprotein antigen.

A third instructive example was reported by Horwitz et al. [4]. Coxsackievirus B4 (CB4) infection has frequently been associated with insulitis and type 1 diabetes. The investigators infected diabetes-prone non-obese diabetic (NOD) mice with CB4 and found no significant acceleration of diabetes. In contrast, mice harboring a transgene encoding a diabetogenic T cell receptor specific for an islet cell antigen rapidly developed diabetes following CB4 infection. Since this T cell receptor did not cross-react with the virus, the results show that the diabetes induced by the viral infection was due to the bystander effect of local inflammation, tissue damage, and release of sequestered autoantigen, rather than to molecular mimicry.

These three experimental examples illustrate the interpretative difficulties in assigning molecular mimicry as the cause of a human autoimmune disease, even when the microbial infection is associated epidemiologically with the disease.

Rheumatic fever

Acute rheumatic fever occurring after infection with group A β-hemolytic streptococci represents the classic example of an epidemiologic association of an infection with an autoimmune disease. This systemic inflammatory sequela to streptococcal pharyngitis can lead to heart disease, affecting all layers of the heart. The incidence of acute rheumatic fever has decreased dramatically in North America and Europe, but it still ranks as a major cause of heart disease in other parts of the world. The reasons for the decrease are uncertain; they may relate to improved social conditions, prompt or preventive administration of antibiotics, or a change in the streptococcus itself. In the 1960s, Kaplan et al. [5] recognized that sera of patients with acute rheumatic fever contained heart-reactive autoantibodies and that myocardial tissue of rheumatic heart disease patients contained bound immunoglobulins reactive with streptococci. However Zabriskie et al. [6] could not identify the streptococcal antigens responsible for this cross-reaction. Other investigators in the 1960–1970 era reported a cross-reactive antigen shared by the streptococcal group-specific A carbohydrate and glycoproteins in heart valves. Goldstein et al. [7] and Van de Rijn et al. [8] isolated a low-molecular-weight streptococcal membrane antigen and showed that it could absorb heart-reactive autoantibody from patients with acute rheumatic fever. Manjula et al. [9] delineated the amino acid sequence of an α helix coiled-coil structure of streptococcal M6-type-specific protein, which exhibited strong structural homology to tropomyosin and myosin. Dale and Beachey [10] found that one prominent cross-reacting determinant of the organism prepared as a purified pepsin fragment was derived from the type 5 streptococcal M protein, and antibodies against purified M protein reacted with the heavy chain of myosin.

In further studies, Dale and Beachey [11] demonstrated that a purified pepsin fragment of the streptococcal M protein shared antigenic determinants with the sarcolemmal membrane protein of human heart. In further studies, the epitope of streptococcal M protein that cross-reacted with myosin was sequenced and mapped to a region within the N-terminal half of the M5 protein molecule. However, immunization of animals with any of the antigens described above failed to elicit cardiac lesions resembling rheumatic heart diseases. Cunningham and her colleagues [12] described a murine monoclonal antibody that was cross-reactive with streptococcal M protein, human cardiac myosin, and other coiled-coil molecules, and was also able to neutralize CB3. A 14- to 15-amino acid sequence was identified, which was largely shared by streptococcal M protein, the CB3 capsid protein, and human cardiac myosin. The same monoclonal antibody was cytotoxic to rat heart and fibroblast lines [12], and may provide important clues to the identity of the antigen responsible for molecular mimicry. More recent experiments by

Quinn et al. [13] have been directed towards reproducing the lesions of rheumatic fever by immunization with the peptides accounting for the streptococcal M protein-myosin cross-reactivity. Huber and Cunningham [14] showed that T lymphocytes obtained from CB3-infected mice demonstrated an immunodominant response to an 18-amino acid peptide derived from the M5 protein of *Streptococcus pyogenes*. The peptide bears both B and T cell epitopes of the M5 protein and cross-reacted with cardiac myosin. To determine whether the peptide is capable of inducing inflammatory heart disease, MRL/++ mice were immunized with the peptide in CFA. About 75% of the immunized animals developed myocarditis. Furthermore, when the peptide was conjugated to syngeneic spleen cells, it induced tolerance, so that virus-infected animals failed to develop the expected myocarditis. These experiments provide the best evidence yet that a peptide derived from streptococci is able to induce cardiac inflammation or tolerance, depending upon the method of administration.

The pathological changes produced in the heart by peptide immunization resembled the myocarditis seen following Coxsackievirus infection. In contrast, the typical pancarditis of rheumatic fever is a more extensive process, involving the pericardium, myocardium, and endocardium. Scarring of the endocardium leads to thickening and shortening of the cordi tendini and thickening of the valve leaflets. This produces severe cardiac insufficiency and stenosis of the cardiac valves, the most typical clinical finding of rheumatic heart disease. The classic myocardial lesion, the Aschoff body, begins as a loose focal mononuclear infiltrate around small arteries and evolves into a fibrous scar. Since it has not yet been possible to reproduce these characteristic histological changes or clinical findings by experimental immunization with streptococcal antigens, molecular mimicry does not provide a full explanation for the lesions of rheumatic heart disease.

Aside from carditis, streptoccal infection is implicated in autoimmune damage in the central nervous system, classically as Sydenham's chorea, although connections via molecular mimicry are unclear. Perhaps related, but in the realm of the exotica, is PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococci) [15].

Myocarditis

Patients with myocarditis usually present with signs of heart failure or arrhythmia. However, a certain diagnosis could not be made until the introduction of the endomyocardial biopsy in 1962. Histologically, myocarditis is characterized by the presence of mononuclear inflammation associated with adjacent myocyte damage. The inflammatory infiltrate is composed primarily of macrophages and lymphocytes, although in the more acute phases, polymorphonuclear cell infiltration is common. In the forms of myocarditis associated with hypersensitivity, eosinophilia is prominent, according to Rose and Baughman [16].

Myocarditis has both infectious and non-infectious etiologies. A number of drugs, especially adriamycin, have been implicated as toxic agents or as triggers of a hypersensitivity reaction. Most cases of acute myocarditis, however, are associated with infections by bacterial, rickettsial, viral, mycotic, protozoan, or helminthic agents. In Europe and North America, the most common agents are the Coxsackieviruses of group B3.

The mechanism of CB3-induced myocarditis is still unclear. Some strains of the virus have been shown to multiply in the heart and may be responsible directly for myocyte damage and the subsequent inflammatory reaction. Most myocarditis patients also produce autoantibodies reactive with cardiac tissue. A number of autoantibodies have been defined as specific for myosin, laminin, β-1-adrenergic receptors, adenine nucleotide translocator, and branched-chain ketodehydrogenase. The occurrence of these antibodies in patients with myocarditis as well as in the related disease, idiopathic dilated cardiomyopathy, has given rise to the view that these diseases may be the consequence of an autoimmune response generated in some way by the virus infection.

Because CB3 is the infectious agent most often implicated in human myocarditis, this organism has been widely used to investigate the pathogenetic mechanisms of the diseases in experimental animals. Following infection with a cardiotropic strain of CB3, all strains of mice develop an acute myocarditis. The disease is first manifested by cardiac inflammation and myocyte necrosis beginning on day 3 and increasing in severity until day 7. Some decrease in myocarditis is often discernible on day 9 and, by day 21, the inflammatory process has completely resolved. A few strains of mice, such as A/J and its congenics, however, proceed to an on-going form of myocarditis. Histologically, the disease process is characterized by diffuse rather than focal inflammation and consists mainly of a mononuclear, interstitial infiltrate. Little or no myocyte necrosis is evident in the latter phase of this disease. No infectious virus can be isolated from the heart after day 9, although viral RNA can be detected in a few myocardial cells. Heart-reactive autoantibodies are present in the animals that develop this second phase of myocarditis. This finding suggests that the second phase represents an autoimmune response provoked by the initial virus infection. The autoimmune response occurs in only a few strains of mice and is determined genetically by non-H-2 back-

ground genes, although H-2-encoded differences modify the severity of the disease.

The specificity of the autoantibodies present in the late phase of CB3-induced myocarditis provides an important clue to the antigen responsible for the autoimmune response. Alvarez et al. [17] and Neu et al. [18] have shown that cardiomyosin heavy chain is the major target antigen. Using purified mouse cardiac myosin incorporated in CFA, Neu et al. [19] induced cardiac lesions that resembled histologically the late-phase of CB3-induced myocarditis. Moreover, only those inbred strains that were genetically susceptible to the late phase CB3-induced myocarditis were susceptible to the myosin-induced disease (fig. 1). These results strongly support the view that the late phase of CB3-induced myocarditis, which occurs in genetically susceptible strains of mice, results from an autoimmune response to the cardiac-specific determinants on the myosin heavy chain.

The role of CB3 in the etiology of myocarditis is still unclear; Rose and Hill [20] detected no cross-reaction between the virus and myosin in assays using either T cells or B cells. Comparison of the amino acid sequence of the viral capsid polypeptides with the sequence of murine cardiac myosin failed to demonstrate candidate sequences of molecular mimicry. One myosin peptide (heavy-chain α residues 325–357) is highly myocarditogenic in A/J mice, but this sequence shows no homology with the structural polypeptides of CB3. Accordingly, rather than supporting a role for molecular mimicry, these results suggest that the virus acts by altering the local milieu, mobilizing endogenous myosin from its intracellular location within the myocytes and, attracting inflammatory cells and generating proinflammatory cytokines. In other words, the virus infection allows access of the immune system to a previously sequestered and presumably non-tolerated autoantigen.

A further perspective on the possible role of mimicry by viral antigens in the context of murine myocarditis is provided by Lawson [21] in this issue.

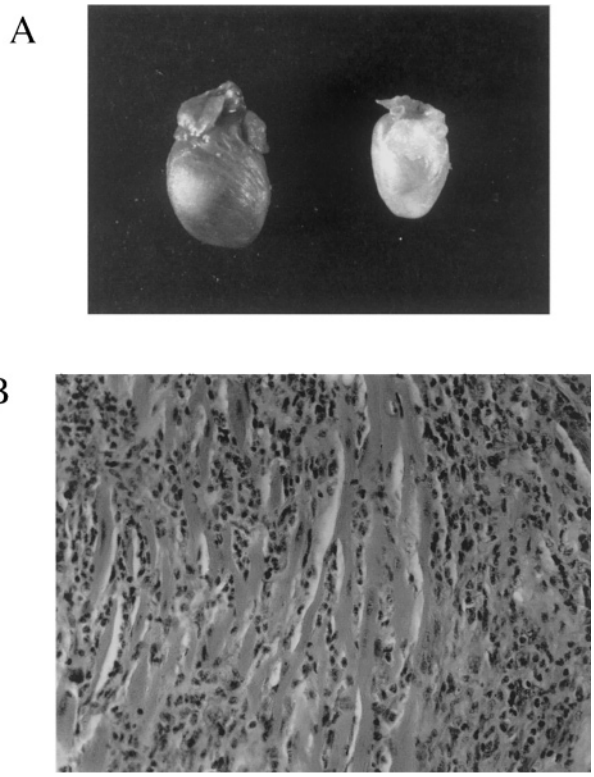

Figure 1. (*A*) Gross appearance of hearts from A/J mice. Left, mouse immunized with murine cardiac myosin plus complete Freund's adjuvant, showing large, pale heart. Right, mouse immunized with murine skeletal myosin plus complete Freund's adjuvant, showing normal appearance. (*B*) Microscopic view of heart from mouse immunized with murine cardiac myosin plus complete Freund's adjuvant.

Chagas' disease

Chagas' disease in its chronic evolution is a form of inflammatory cardiomyopathy that occurs in individuals infected with the protozoan, *Trypanosoma cruzi*. Current opinion, reviewed by Kalil and Cunha-Neto [22], is that the presence of a T-cell-rich infiltrate and the absence of a significant number of parasites in the heart signifies that the disease involves an anti-cardiac autoimmune response. Hence a great deal of attention has been directed to identifying an antigen of the parasite that could be shared with cardiac tissue, in order to explain the pathogenesis of the disease on the basis of molecular mimicry. Cossio et al. [23] found that antibodies to vascular interstitium were present in the sera of many patients with Chagas' disease and could be absorbed with *T. cruzi* epimastigotes. A 12-amino acid peptide has been described by Van Voorhis et al. [24] as the antigen of the parasite that is responsible for heart muscle disease, since antibodies to the peptide have demonstrated mimicry of *T. cruzi* epitopes with mammalian nerve. However, the human antibodies described to the vascular interstitium or the nerve antigen do not seem to correlate with chronic Chagas' cardiomyopathy. Bonfá et al. [25] and Kerner et al. [26] have described other cross-reactive molecules that include a highly conserved ribonucleoprotein and a microtubule-associated protein of the cytoskeleton. In another report, Ferrari et al. [27] described molecular mimicry at the epitope level between a ribosomal protein, PO, of *T. cruzi* and an epitope at the external domain of the cardiovascular β-1-adrenergic receptor; however, antibodies to this epitope did not appear to be more prevalent among Chagas' patients than among asymptomatic controls infected with *T. cruzi*.

Kalil and Cunha-Neto [22], in their own studies, identified a heart-specific epitope on cardiac myosin heavy chain that displayed molecular mimicry with an immunodominant *T. cruzi* antigen. Antibodies to cardiac

myosin were present in 100% of Chagas' sera tested, but in only 14% of sera from patients with asymptomatic *T. cruzi* infection. Such antibodies could not be found in the sera of patients with other cardiomyopathies, suggesting they were not formed simply as a consequence of heart damage itself. Furthermore, T cell clones isolated from the hearts of patients with Chagas' disease gave proliferative responses to cardiac myosin heavy chain and to *T. cruzi* peptide, and produced Th1-type cytokines. This evidence supports the view, but does not firmly establish, that molecular mimicry between constituents of *T. cruzi* and cardiac myosin is the pathogenetic basis of Chagas' heart disease.

Type 1 diabetes mellitus

Type 1 or insulin-dependent diabetes mellitus is most often seen in children and young adults, but this disease can develop at any age. Since the disease may take over 10 years after its initiation to become clinically apparent, exposure to an infectious agent may have occurred in the remote past. The fact that the sex ratio of incidence even in young adults is approximately 1:1 is an indication that the pathological process probably begins before puberty, since there is usually a strong female sex bias in autoimmune diseases that begin thereafter. Type 1 diabetes develops when there is complete or major loss of insulin production due to the immune-mediated destruction of β cells of the pancreatic islets. A number of autoantibodies are present in patients with type 1 diabetes including antibodies to GAD65, ICA512/IA-2 and insulin, and additional islet cell cytoplasmic antibodies are demonstrable by immunofluorescence; there are also islet cell surface antibodies which may contribute to pathogenesis through their location on the cell surface, according to Hagopian and Lernmark] [28]. GAD65 and its isoform GAD67 are enzymes that convert glutamate to the neuronal transmitter γ-aminobutyric acid (GABA) which is present in the brain as well as the pancreatic islets [29]. Antibodies to the autoantigen GAD may also be found in the neurological disease, stiff-man syndrome. ICA512/IA-2 is a protein tyrosine phosphatase-like molecule, one of a number of analogous transmembrane molecules [30, 31], but this member of the family actually lacks tyrosine phosphatase activity. The ICA512/IA-2 antigen is primarily associated with islet β cell secretory granules, in contrast to GAD65, which is associated with the small synaptic-like microvesicles on the islet β cell.

Numerous clinical and epidemiological investigations over many years have suggested an association between infection with CB4 and type 1 diabetes mellitus, reviewed by Szopa et al. [32] and Barrett-Connor [33]. This association is strengthened by the finding of analogous sequences between human GAD65 and a Coxsackievirus protein P2-C (Cox P2-C), an enzyme involved in the replication of Coxsackieviruses. The sequences involved are 257–267, KMFPEVKEKGM, in GAD65, and 35–46, KILPEVKEKHE, in the P2-C enzyme of the virus, with the homologous sequence PEVKEK as the critical element [34]. The homologous peptides from both Cox P2-C and GAD65 are both immunodominant and cross-reactive determinants, as judged by the induction of T cell reactivity measured by proliferation in response to the peptide after immunization with the full-length proteins [34]. However, serologic cross-reactivity between GAD65 and Cox P2-C has not been consistently demonstrated in human studies. Furthermore, at least 17 other viruses share some degree of homology with portions of GAD65, according to Jones and Armstrong [35]; this great degree of cross-homology would call for caution in drawing conclusions about the role of molecular mimicry between any particular Coxsackie peptide and GAD65 in the pathogenesis of type 1 diabetes. The PEVKEK connection was revisited by Myers et al. [36] who recently described a homology model of GAD65 in which the PEVKEK sequence was part of a surface-exposed loop. Of particular interest, mutagenic deletion of the 'PEVKEK loop' was prejudicial to the reactivity with GAD of the human GAD-reactive monoclonal antibody MICA 3.

The NOD mouse provides an excellent model of spontaneously occurring human type 1 diabetes mellitus and an opportunity to examine the mimicry idea. Deliberate infection of NOD mice with a strain of CB4 isolated from a patient with diabetes did not induce any significant diabetes in the short term although there was severe pancreas damage and pancreatitis resembling that seen after CB4 infection of other mouse strains [32, 37], and antibody responses to GAD65 were similar in infected and uninfected mice. This lack of response may indicate that the cross-reactive epitope shared by GAD65 and P2-C is a minor determinant relative to other Coxsackievirus constituents, and is incapable itself of generating a strong immune response. Therefore, molecular mimicry alone would not be sufficient to induce diabetes in the NOD mouse model. Other experiments have involved the infection of BDC2.5 mice, which bear a transgene encoding a diabetogenic T cell receptor, with CB4 [4]. By 2 weeks after infection, the blood glucose of the BDC2.5 mice had increased to diabetic levels in two-thirds of the mice compared with none of the uninfected transgenic litter mates. To demonstrate the immunological basis of the diabetes, splenocytes from the diabetic BDC2.5 mice were adoptively transferred to NOD/SCID recipients, in which they induced diabetes by 4 weeks. These results suggest

that diabetes induced by Coxsackievirus infection is the result of local tissue damage to islet β cells, and cellular infiltration in response to release of islet cell antigen, resulting in the stimulation of resting, autoreactive T cells. In other words, there is exposure of a sequestered intracellular autoantigen rather than a tolerance-breaking mimicking antigen. Another point against mimicry is that NOD mice reared under germ-free conditions are more susceptible to diabetes than NOD mice reared conventionally [38]. The reader is referred to the paper in this issue by Maclaren and Kukreja [39] for further discussion of epitope mimicry as a cause of diabetes.

Lyme disease

Following infection with *Borrelia burgdorfii*, about 10% of patients develop the late sequel of chronic arthritis. Since no spirochetal DNA is detectable in the joints and the condition is resistant to antibiotic therapy, this phase of the disease has been suggested to be autoimmune in origin. Gross et al. [40] have identified LFA-1 (CD11a/CD18, integrin $\alpha_L\beta_2$) as a candidate antigen for the induction of the antibiotic-resistant phase of Lyme arthritis. LFA-1 is a potential cross-reacting antigen by reason of sequence homology with an immunodominant epitope A on the outer surface proteins of *B. burgdorfii*, OSP-A. T cells derived from the synovial fluid of patients with Lyme arthritis proliferated in response to the homologous peptide component of LFA-1, as well as to intact LFA-1, and to OSP-A proteins. Patients who developed arthritis as a sequel of *B. burgdorfii* infection showed an increased frequency of HLA-DRB1*0401, and mice transgenic for DRB1*0401 demonstrated an increased level of T cell proliferation to the homologous peptide sequence of LFA-1; however, since the mice did not develop evidence of arthritis, the experimentally demonstrable cross-reactivity did not translate into a disease model of chronic Lyme disease.

Rheumatoid arthritis

Many pathways of investigation have suggested an association between rheumatoid arthritis (RA) and chronic infection. Microorganisms implicated in such a process have included *Proteus mirabilis* and *Mycobacterium tuberculosis* [41] and, more particularly, Epstein-Barr virus (EBV). Antibodies specific for Epstein-Barr nuclear antigen-1 (EBNA-1) have been detected in patients with RA and were shown by Fox et al. [42] to cross-react with a 62-kDa synovial membrane protein. Additional shared epitopes were described by Roudier et al. [43] between a T cell epitope common to the HLA-DRB1 β chain and EBV glycoprotein 110. In addition, Davies et al. [44] reported homology between type 2 collagen, a frequent reactant with early RA sera, and EBNA-1. Nevertheless, neither epidemiologic nor serologic investigation have provided really substantial support for the idea that a mimicking epitope of EBV initiates or sustains RA.

Heat shock proteins (hsps) are highly conserved across species and cross-reactivity between bacterial hsps and synovial antigens serve as a trigger for an immune-mediated inflammation of the joints. Patients with RA have been claimed to have elevated antibody levels to a 65-kDa hsp of *M. tuberculosis*, although the data are inconsistent [45], and Gaston et al. [46] reported that $\gamma\delta$ T cells from synovial fluid proliferated in response to this antigen. There is support for the role of mycobacterial hsp in RA from studies of adjuvant arthritis induced in rats by injection of CFA, containing killed *M. tuberculosis* in oil [47], and this disease was transferred by a T cell clone specific for mycobacterial hsp [48]. However, there is a high degree of antigenic cross-reactivity in mononuclear cells from sites of chronic inflammation [49] and adjuvant arthritis is not regarded as a close model of human RA. In terms of molecular mimicry in RA between hsp species and synovial antigens, a possible candidate is hsp60, the human homologue of hsp65, which shares sequence homologies with a number of autoantigens [50]. A further variant of the epitope mimicry concept in RA is the sharing of the disease susceptibility sequence QKRAA of the HLA-DR β chain with the gp110 protein of EBV, and the *Escherichia coli* chaperone protein DnaJ; J family chaperone proteins are well-expressed in synovial tissue [51]. More details on mimicry in RA are given by Davies [52] but, in our perception, the evidence is indirect, at best.

Ankylosing spondylitis

Ankylosing spondylitis and the following disease to be considered, multiple sclerosis, differ from those considered heretofore in that there is no serological expression of the presumed autoimmune process: disease expression is attributable entirely to T lymphocytes.

Ankylosing spondylitis is a rheumatic disease affecting the joints of the spine, the sacroiliac joints and, sometimes, the hip, shoulder or, smaller joints. An outstanding distinction of ankylosing spondylitis is its close association with an HLA class I molecule, HLA-B27. Notably, B27 is one of the relatively few class 1 MHC molecules with a close and non-linkage-related association with a human disease, perhaps pointing to cytotoxic (CD8) T cells in pathogenesis. Infection with enteric Gram-negative bacteria was suggested by Ebringer et al. [53] as an initiating event. Ankylosing spondylitis resembles the various forms of reactive

arthritis, in occurring shortly after infection with a variety of intracellular bacteria, including *Klebsiella pneumoniae* and *Yersinia enterocolitica*. As shown by Hermann et al. [54], isolates from the synovial fluids of patients with reactive arthritis and ankylosing spondylitis contain HLA-B27-restricted CD8 cytotoxic T cells that kill bacterially infected B27+ target cells. From findings of this type, the suggestion has arisen that bacterial proteins mimic human autoantigens and so induce a cell-mediated autoimmune response. However, bacterial antigens are not demonstrable in the joints in ankylosing spondylitis and the course of disease is progressive, with ultimate destruction of spinal and other joints.

A possible mechanism for induction of ankylosing spondylitis involves presentation of intracellular antigenic fragments derived from enteric bacteria by HLA-B27. Using HLA-B27 transgenic mice, David [55] has described the presence of MHC-class-II-restricted T cells, which may result from the appearance of free B27 heavy chains on the cell surface. The free B27 heavy chains would resemble MHC class II molecules by loading and presenting exogenous bacterial peptides to CD4 T cells. The actual target was postulated to be type 2 collagen, which is known to contain cross-reactive epitopes with enterobacteria. An alternative possibility is the MHC class II presentation of B27-derived peptides to CD4 T cells that have previously been activated by bacterial antigens, producing a form of allorecognition [56]. The process begins when infection by a Gram-negative organism activates CD4 T cells that recognize epitopes shared by bacterial proteins and B27 heavy chain. The inflammatory response will be restimulated periodically by infection with the provocative enteric bacteria.

HLA-B27, unlike any other B locus allele, shares a large number of hexapeptides and pentapeptides with Gram-negative bacterial proteins [57]. B27 binding of a peptide from its own sequence and of similar peptides derived from bacteria would provide a reasonable basis for cross-reactivity. The widespread distribution of these peptides, based on convergent evolution, makes it difficult to implicate particular microorganisms in the arthritogenic process. Finally, concepts of an infectious pathogenesis for ankylosing spondylitis need to be integrated (in ways that are as yet unclear) with data on particular structural and functional features of the B27 molecule itself [58].

Multiple sclerosis

Multiple sclerosis is a likely T-cell-mediated autoimmune disease in which infection is constantly being canvassed as a 'partner-in-crime.' Many different viruses have been implicated in the pathogenesis of multiple sclerosis, based on demonstrations of antibody in the serum or isolation from the cerebral spinal fluid. None of these reports, however, has withstood the critical test of widespread confirmation. Based on studies of the model EAE, the major encephalitogenic antigens are myelin basic protein, proteolipid protein, or myelin oligodendrocyte glycoprotein. Molecular mimicry has been suggested to explain a link between these infectious agents and the autoimmune pathogenesis of multiple sclerosis, which provides the opportunity for considering mimicry in terms of T cells. On that basis, Wucherpfennig and Strominger [59] studied the T cell reactivity of a number of viral proteins that can activate myelin-basic-protein-specific clones from multiple sclerosis patients. The findings were that several such peptides can be demonstrated, suggesting that a variety of pathogens can serve as potential triggers for the autoimmune response in multiple sclerosis. Observations following from the above study are based on T cell clones primarily reactive with peptides of myelin basic protein. These suggest that there is a high degree of degeneracy in recognition by the T cell receptor, and the autoantigen used to establish the T cell clone may represent a less efficient agonist than other self or microbial proteins [60, 61]. Such observations seem to favor the mimicry concept, and would also explain the difficulty in finding a single microorganism consistently associated with the disease. But there are inconsistencies. Noting the sequence homology between myelin basic protein and hepatitis B virus polymerase provided the rationale for immunizing rabbits with a shared peptide from these two proteins [62]; while immunization led to the production of lesions in the central nervous system reminiscent of EAE, hepatitis B virus has not been associated, clinically or epidemiologically, with multiple sclerosis.

Conclusions

Molecular mimicry remains a reasonable explanation for the often-described association of infection with autoimmune disease. However, in its simplest form, that of an epitope shared by a microorganism and a host macromolecule, there are as yet no firm examples of a human disease based on such a mechanism. The time has come, it seems, to reevaluate this mechanism in broader terms leading us to construct a 'balance sheet' (table 1).

The observation of epitope spread during the immune response may be one of the obstacles to identifying the initiating shared epitope if immunity to that epitope were to subsequently fade during evolution of the disease. This was commented on recently in the context of

Table 1. Infectious mimics and autoimmune diseases.

Strengths	Comments	Weaknesses	Comments
Temporal association between infection and an autoimmune disease.	See examples in text including streptococcal infection and carditis, Borrelia infection and Lyme disease, and the article on Campylobacter jejuni by Yuki [64] in this issue.	Good examples of mimicry and autoimmunity are found among the more rare rather than the more common autoimmune diseases.	Such 'good examples' include streptococcus-induced carditis and Chagas' disease (see text), C. jejuni and polyneuropathy [64], and herpes stromal keratitis [67].
Homologies between sequences of infectious organisms and identified autoantigens.	Sequence homologies are found in bacteria/viruses for many identified autoantigens, exemplified by PEVKEK in Coxsackie B4 and GAD65 (see text).	Spontaneous murine autoimmune models are not supportive of molecular mimicry.	Antigenic mimics are not required in spontaneous murine autoimmune diseases; in fact, infection protects NOD mice from diabetes (see text), and there is no role for infection in autoimmune disease of MRL-lpr mice [68].
Immunoreactivity with a mimicking sequence, demonstrable for T cells although doubtful for antibodies	See Vreugdenhil et al. [65].	Autoimmune B cell epitopes are usually highly conformational.	Mimicry would seem unlikely for conformational epitopes, but this limitation would not apply to T cell epitopes (see text).
The degeneracy of T cell receptors provides good opportunities in nature for cross-reactivity between microbial and self peptides.	See text, multiple sclerosis.	Some well-established autoantigens such as insulin have no known mimics	This indicates that mimicry is not universally applicable to the induction of autoimmunity.
Immunization with heterologous cytochrome c in mice breaks tolerance to homologous molecules.	Mamula et al. [66].	Failure to induce autoimmune disease by immunization with the mimicking epitope.	There are very few if any examples of experimental immunization with the mimic causing a replica of a human autoimmune disease.

EAE as representing the converse of 'original antigenic sin' [63], but that observation was made in relation to an antibody response to influenza virus rather than a T cell response, as pertains in EAE. Another difficulty are bystander effects, by which the infectious agent serves as an inflammatory agent as well as a local catalyst for the expression and presentation of host antigens, and which are considered by many as a likely alternative mechanism to account for the nexus between infection and autoimmunity. Finally, the evidence that a single T cell may recognize many different peptides, as discussed above in relation to multiple sclerosis, means that the molecular mimicry dogma of one T cell receptor to one shared epitope needs to be re-examined. Whatever the mechanisms, the connection between infection and autoimmune disease is of sufficient importance to justify the increased attention of the research community, because the convincing identification of an infectious organism as a triggering agent of autoimmunity would open promising opportunities for preventive and early interventions, as shown by the demise of rheumatic fever in the western world.

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