CURRENT TOPIC

Molecular mimicry in autoimmune disease

Susan Leech

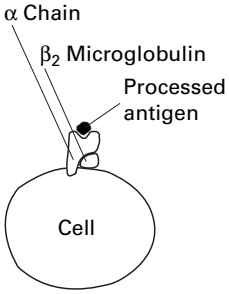

Figure 1 MHC class I: HLA A, HLA B, HLA C—for example, HLA B27. Expressed on the surface of virtually all cells except mature erythrocytes and trophoblast cells. Presents antigen (processed peptides) to CD8 T cells. Comprises an α polypeptide chain and β_2 microglobulin (invariant).

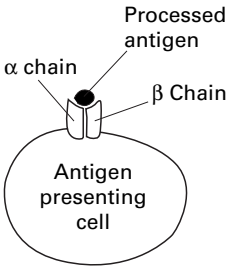

Figure 2 MHC class II: HLA DP, HLA DQ, HLA DR—for example, HLA DR3. Constitutively expressed on the surface of macrophages, monocytes, dendritic cells, and B lymphocytes. Presents antigen (processed peptides) to CD4 T cells. Comprises an α and β polypeptide chain.

Department of Child Health, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ, UK
S Leech

Correspondence to:
Dr Leech.

The origins of autoimmune disease are multifactorial. Environmental factors and a genetic predisposition result in tissue injury caused by autoreactive T cells or antibodies. Usually a single organ or individual cell type is affected in the absence of gross abnormalities of the immune system. Autoimmune diseases tend to have long, asymptomatic prodromal periods and the initiating events leading to loss of self tolerance occur long before the disease becomes clinically manifest. This makes the initiating factors harder to identify and they remain largely unknown in humans.

Several different pathological processes have the potential to break tolerance and cause autoimmune disease. Antigenic similarity between pathogenic organisms or foreign proteins and self proteins (molecular mimicry) is one of them.

The major histocompatibility complex (MHC) is a collection of genes on chromosome 6 that codes for the human leucocyte antigens (HLA). These are glycoproteins expressed on the surface of cells that bind short peptides, degraded or generated by the cell, and present them to T lymphocytes (figs 1 and 2). The term "molecular mimicry" was used in the 1970s to explain persistent viral infections. It was suggested that the MHC and viruses encoded similar peptide sequences, which allowed the host to regard an infecting virus as "self" and forego an immune response. More recently, it has been used as a hypothesis to explain autoimmune disease.[1] Several pathogens share antigenic determinants with host proteins. Often, these are used to gain entry into the cell; rhinovirus binds to an adhesion molecule, ICAM-1, on epithelial cells,[2] and human immunodeficiency virus (HIV) binds to CD4,[3] and enters the cell using a chemokine receptor. Most infections result in a specific immune response against the infecting organism. Cross reactivity may occur between the clones of T and B lymphocytes generated against an infecting agent and a host protein sequence (immunological crossreactivity). This "hit and run" mechanism could be induced to persist long after the pathogen has disappeared, by the presence of autoantigens driving the immune response.

How does molecular mimicry occur?

During T cell development, primitive cells leave the bone marrow and enter the thymus (fig 3). During the subsequent three weeks they develop a T cell receptor (containing CD3) on the cell surface along with an accessory molecule; either CD4 or CD8. The primitive T cells undergo both positive selection, which selects cells able to interact usefully with peptide presented by MHC, and negative selection, which deletes cells that are strongly reactive with self or are unable to interact with presented peptide antigens. Positive and negative selection occur using MHC present on cells within the thymus; it is postulated that much of the peptide presented by this MHC is derived from MHC itself.[4] The process of thymic selection eliminates 99% of precursor cells, which undergo apoptosis (programmed cell death), leaving only 1% to reach the periphery. The T cell receptor repertoire generated allows a certain degree of self reactivity in return for a reasonably comprehensive coverage of foreign antigens.

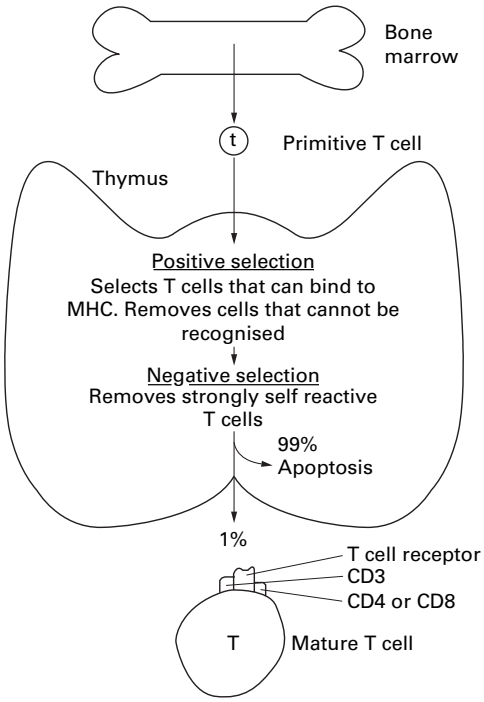

Figure 3 T cell development and thymic education.

If a foreign peptide (such as a virus) resembles these MHC derived peptides, it has the potential to activate such T cells. If a self antigen is also structurally similar, exposure to the foreign peptide results in these activated T cells becoming autoreactive.

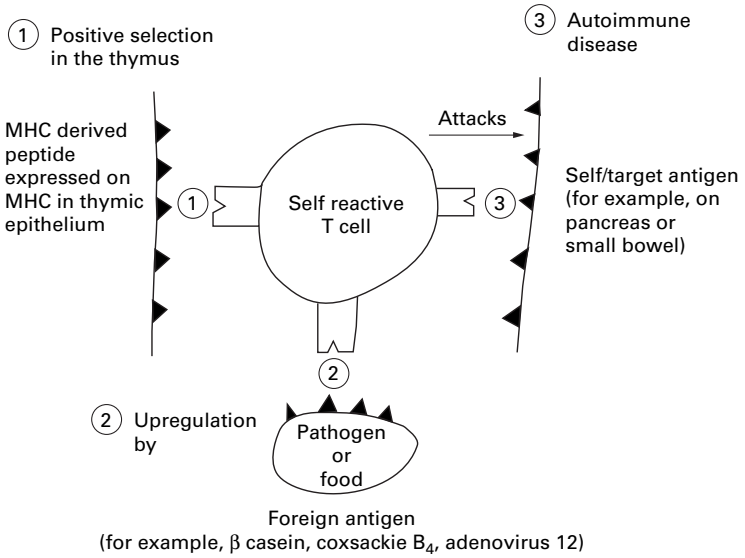

Figure 4 Three way molecular mimicry.

"Three way molecular mimicry" is thus said to exist between peptide sequences of three different origins:

(1) the MHC, which is critically involved in thymic T cell selection
(2) foreign antigens (such as bacteria, viruses, or food), which upregulate antigen presentation and provide co-stimulation
(3) self or target antigens, which are recognised by self reactive T cells (fig 4).

Identification of the mechanism of molecular mimicry fuses the influences of genetics and the environment into a single pathogenic process and is a powerful hypothesis in diseases where both can be shown, epidemiologically, to have an influence.

Ankylosing spondylitis

For example, in diseases with a genetic predisposition, the contribution of environmental factors to the pathogenesis can be estimated by the incidence of the disease in identical twins. The drop in concordance from 100% is an index of the size of the influence of environmental factors. Ninety five per cent of patients with ankylosing spondylitis carry the HLA B27 allele compared with 7% of the general population. Fifty per cent discordance in monozygotic twins implies a significant environmental factor. It was suggested that this might be a result of mimicry between HLA B27 and a microorganism. Several microbial agents (klebsiella, yersinia, and shigella) were epidemiologically associated with the disease. In 1979, cross reactivity between HLA B27 positive lymphocytes and a klebsiella antigen was reported.[5] The association has now been linked to the presence of an amino acid sequence, QTDRED (table 1), in the nitrogenase protein of klebsiella and in HLA B27 (amino acids 72–77).[6]

Table 1 Abbreviations used for amino acids

Q	Glutamine
T	Threonine
D	Aspartic acid
R	Arginine
E	Glutamic acid

Insulin dependent diabetes

In humans the HLA associations of insulin dependent diabetes mellitus (IDDM) are not absolute and environmental factors are important. Early work suggested an association of the MHC class I molecules, HLA B8 and HLA B15, with diabetes. Later, stronger associations were found with the class II molecules HLA DR3 and HLA DR4. The class II associations were assumed to be a result of the B8–DR3 and B15–DR4 linkage disequilibrium (that is, that these alleles appear together on the same chromosome more often than their single gene frequencies suggest). The DR3–DR4 heterozygote had a greater risk and HLA DR2 was protective. Identical twins have a relatively low (45%) concordance rate for IDDM. There is strong evidence for an environmental trigger such as coxsackie virus, rubella, and *Mycoplasma pneumoniae*. Diabetes occurs in up to 20% of patients following congenital rubella.[7] Three way molecular mimicry exists between a peptide of carboxypeptidase H (an enzyme expressed within islet cell granules and an autoantigen) and a peptide of the β chain of HLA DQ, which is processed and presented by HLA DR4,[8] and peptides of coxsackie virus coat protein. Another sequence in the β chain of HLA DQ resembles the islet cell autoantigen jun B (a nuclear transcription factor against which T cell autoreactivity has been demonstrated),[9] and the islet cell antigen, ICA 512,[10] has similarities with peptides in human herpesvirus 1 (herpes simplex virus 1) and 4 (Epstein-Barr virus).

COXSACKIE VIRUS

In the 1970s, epidemiological studies suggested an association between diabetes and viral infections. Several studies demonstrated raised titres of both IgM and IgG antibodies to coxsackie virus and later to coxsackie B4 in newly diagnosed type 1 diabetics. Initially, the association of coxsackie virus was thought to be a result of its tropism for β cells,[11] which might cause damage to these cells and initiate autoimmunity. More recently, autoimmune reactivity of T cells to the β cell enzyme glutamic acid decarboxylase (GAD; an enzyme that catalyses glutamic acid to γ aminobutyric acid (GABA)) has been shown to be important in the pathogenesis of diabetes.[12] The major peptide determinant of GAD recognised by patients with diabetes has a significant sequence similarity to a 15 amino acid sequence within the P2-C protein of coxsackie B virus. Antibodies to the P2-C protein cross react with GAD 65 and vice versa, suggesting that molecular mimicry might be responsible for the disease.[13]

Finland has one of the highest incidences of diabetes in the world (0.6%). In 1995, as part of Akerblom and Tuomilehto's childhood diabetes in Finland study group, a prospective study suggested antenatal exposure to coxsackie enterovirus increased the risk of diabetes in the child (particularly if onset was before 3 years of age).[14] Serologically verified enterovirus infections were twice as common in siblings who developed clinical diabetes compared with siblings who remained non-diabetic, several years before diagnosis. More recently, these antibodies have been shown to be generated in healthy children who then went on to develop

diabetes (although they were also present in non-diabetic controls),[15] and are higher in siblings of diabetic patients who then become islet cell antibody positive (particularly if they express the high risk HLA DQB1 genotype).[16]

β CASEIN

There is epidemiological evidence that a short breast feeding time and early ingestion of cows' milk is a risk factor for diabetes.[17] In cows' milk, the proportion of protein as casein is higher (85%) than in human milk (25%); a large proportion of this is β casein. Thirty seven per cent of patients with diabetes have autoantibodies to β casein at diagnosis. In about 50% of patients with recent onset diabetes, their lymphocytes show an enhanced proliferative response to β casein, which is virtually absent in normal controls and patients with autoimmune thyroid disease.[18] Sequence differences between human and bovine β casein could be responsible for the generation of an autoimmune response if milk proteins are introduced within the first weeks of life when foreign proteins can stimulate the immune system.[19] Enhanced cellular and humoral immune responses have been reported with other components of cows' milk, such as β lactoglobulin and bovine serum albumin. That multiple cows' milk proteins generate an immune response in patients with diabetes may suggest a disturbance in gut mucosal immunity in presentation of oral antigens.

Several sequence homologies between bovine β casein and peptides expressed by pancreatic β cells have been identified, including the islet cell antigen, ICAp69,[20] and carboxypeptidase H; both putative autoantigens in diabetes. The sequence in position 63–67 of β casein corresponds to a region of variation between the bovine and human forms, and the bovine form is identical to residues 415–419 of the β cell specific glucose transporter, GLUT-2. Autoantibodies to GLUT-2 have been described in 77% of patients with recent onset IDDM.[21]

Currently, the childhood diabetes in Finland study group is screening infants at birth and half of those with a high risk genotype (HLA DQ) are given a casein hydrolysate formula as supplementary milk and a diet free from dairy products and beef for the first 6–8 months of life. The other half will be given a normal diet. The children are to be followed up to 10 years of age and a reduction in the incidence of diabetes is expected to be seen in the dietary intervention group.

Adenovirus 12 in coeliac disease

Seventy per cent of monozygotic twins are concordant for coeliac disease, suggesting an environmental factor is also involved in the pathogenesis. In 1987, Kagnoff and colleagues[22] found that 89% of untreated coeliac patients had antibodies to adenovirus 12, and that these antibodies were also raised in treated children with coeliac disease (31%) compared with controls (0–13%). Antibodies to adenovirus 18 or echovirus 11 were not raised. A similar amino acid sequence between

Key messages

- Molecular mimicry is a mechanism by which immunological self tolerance can be broken, leading to autoimmune disease
- Three way molecular mimicry has been shown to exist between:
  (1) the MHC acting via T cell selection
  (2) foreign antigens that upregulate antigen presentation and provide co-stimulation
  (3) the host target antigen, recognised by self reactive T cells, driving autoimmune disease
- Molecular mimicry provides a way of intervening against and preventing autoimmune disease

A-gliadin and the E1b protein of adenovirus 12 was reported, and this sequence was found to be an antigenic determinant in active coeliac disease. This suggested that the viral protein might play a role in the pathogenesis of coeliac disease by virtue of immunological cross reactivity. In 1991, Mantzaris and Jewell[23] demonstrated that intraduodenal insertion of the peptide produced mononuclear cell infiltration and microscopic changes that were not seen in controls. However, later studies using monoclonal antibodies against the epitope sequence[24] and studies looking for persistent adenovirus DNA[25 26] in small intestinal biopsies have failed to show evidence of an association.

It is possible that infection with adenovirus sensitises the genetically predisposed host to A-gliadin by molecular mimicry, with resultant gluten sensitive enteropathy, using the hit and run mechanism.

Clinical developments

Molecular mimicry plays a major role in the initiation of common autoimmune diseases. Control of the initiating factor (for example, by vaccination against coxsackie virus protein P2-C) or strategies to avoid contact with the environmental trigger (for example β casein in infant formula) in an at risk population could play an important role in reducing the incidence of the disease and both strategies deserve further attention.

In diabetes, other immune interventions have recently been tried with varying success. Treatment with immunosuppressive drugs, such as cyclosporin A and azathioprine, after clinical diagnosis is ineffective because 80–90% of the β cell mass is already destroyed at onset of the disease. Oral exposure to antigen at the time of sensitisation might direct the immune response from type 1 (cytotoxic) to type 2 (humoral), protecting against autoimmune disease. The American multicentre diabetes prevention trial is looking at the effect of immunomodulation with insulin in early diabetes.[27] BCG vaccination, if given in the prediabetic state or shortly after induction of diabetes is protective in mice. It possibly acts via a switch from a type 1 to a type 2 response.

However, epidemiological data failed to support a sustained protective role for BCG vaccination against juvenile onset IDDM.[28] Nicotinamide inhibits the generation of free radicals and nitric oxide and increases the intracellular NAD pool and energy supply of the cell. To test the preventive effect of nicotinamide against IDDM in high risk individuals, a prospective, randomised, placebo controlled, multicentre study (European nicotinamide diabetes intervention trial; ENDIT) was started in 1993.

The identification of high risk haplotypes HLA DQA1*0301 and DQB1*0302, and an increased risk of diabetes in first degree relatives with antibodies to islet cells, insulin, and GAD has already opened up possibilities for prevention and immunomodulation. Once the sensitivity and specificity of screening tests are improved to allow for population screening, prevention will become practical for the general population. Primary prevention of IDDM by vaccination or exposure prophylaxis, acting via the prevention of the induction of autoimmune disease by molecular mimicry, becomes an exciting possibility.

I acknowledge the helpful comments of Drs J Rennie, C Buchanan, J Mahr, and Professors G Mieli-Vergani and J Price during the preparation of this review.

1 Oldstone MB. Molecular mimicry and autoimmune disease. *Cell* 1987;**50**:819–20.
2 Staunton DE, Merluzzi VJ, Rothlein R, Barton R, Marlin SD, Springer TA. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* 1989;**56**:849–53.
3 McDougal JS, Nicholson JK, Cross GD, Cort SP, Kennedy MS, Mawle AC. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J Immunol* 1986; **137**:2937–44.
4 Baum H, Davies H, Peakman M. Molecular mimicry in the MHC: hidden clues to autoimmunity? *Immunol Today* 1996;**17**:64–70.
5 Seager K, Bashir HV, Geczy AF, Edmonds J, de-vere-Tyndall A. Evidence for a specific B27-associated cell surface marker on lymphocytes of patients with ankylosing spondylitis. *Nature* 1979;**277**:68–70.
6 Williams KM, Hirofuji T, Raybourne RB, Shook DL, Trucksess MW, Yu DT. Synthetic oligonucleotide probes complementary to the HLA-B variable region hybridize with Klebsiella pneumoniae. *Microb Pathog* 1989;**6**:277–86.
7 Forrest JM, Menser MA, Burgess JA. High frequency of diabetes mellitus in young adults with congenital rubella. *Lancet* 1971;ii:332–4.
8 Chicz RM, Urban RG, Gorga JC, Vignali DA, Lane WS, Strominger JL. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. *J Exp Med* 1993;**178**:27–47.
9 Honeyman MC, Cram DS, Harrison LC. Transcription factor jun-B is target of autoreactive T-cells in IDDM. *Diabetes* 1993;**42**:626–30.
10 Rabin DU, Pleasic SM, Shapiro JA, *et al.* Islet cell antigen 512 is a diabetes-specific islet autoantigen related to protein tyrosine phosphatases. *J Immunol* 1994;**152**:3183–8.
11 Szopa TM, Ward T, Dronfield DM, Portwood ND, Taylor KW. Coxsackie B4 viruses with the potential to damage beta cells of the islets are present in clinical isolates. *Diabetologia* 1990;**33**:325–8.
12 Jones DB, Crosby I. Proliferative lymphocyte responses to virus antigens homologous to GAD65 in IDDM. *Diabetologia* 1996;**39**:1318–24.
13 Hou J, Said C, Franchi D, Dockstader P, Chatterjee NK. Antibodies to glutamic acid decarboxylase and P2-C peptides in sera from coxsackie virus B4-infected mice and IDDM patients. *Diabetes* 1994;**43**:1260–6.
14 Hyoty H, Hiltunen M, Knip M, *et al.* A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Childhood diabetes in Finland (DiMe) study group. *Diabetes* 1995;**44**:652–7.
15 Lonnrot M, Hyoty H, Knip M, *et al.* Antibody cross-reactivity induced by the homologous regions in glutamic acid decarboxylase (GAD65) and 2C protein of coxsackie-virus B4. *Clin Exp Immunol* 1996;**104**:398–405.
16 Hiltunen M, Hyoty H, Knip M, *et al.* Islet cell antibody seroconversion in children is temporally associated with enterovirus infections. *J Infect Dis* 1997;**175**:554–60.
17 Virtanen SM, Saukkonen T, Savilahti E, *et al.* Diet, cow's milk protein antibodies and the risk of IDDM in Finnish children. *Diabetologia* 1994;**37**:381–7.
18 Cavallo MG, Fava D, Monetini L, Barone F, Pozzilli P. Cell-mediated immune response to beta casein in recent-onset insulin-dependent diabetes: implications for disease pathogenesis. *Lancet* 1996;**348**:926–8.
19 Paronen J, Vaarala O, Savilahti E, Saukkonen T, Akerblom HK. Soluble adhesion molecules and oral antigen feeding in infants. *Pediatr Res* 1996;**40**:276–9.
20 Karjalainen J, Martin JM, Knip M, *et al.* A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med* 1992;**327**:302–7.
21 Inman LR, McAllister CT, Chen L, *et al.* Autoantibodies to the GLUT-2 glucose transporter of beta cells in insulin-dependent diabetes mellitus of recent onset. *Proc Natl Acad Sci USA* 1993;**90**:1281–4.
22 Kagnoff MF, Paterson YJ, Kumar PJ, *et al.* Evidence for the role of a human intestinal adenovirus in the pathogenesis of coeliac disease. *Gut* 1987;**28**:995–1001.
23 Mantzaris G, Jewell DP. In vivo toxicity of a synthetic dodecapeptide from A gliadin in patients with coeliac disease. *Scand J Gastroenterol* 1991;**26**:392–8.
24 Ellis HJ, Doyle AP, Sturgess RP, Ciclitira PJ. Coeliac disease: characterisation of monoclonal antibodies raised against a synthetic peptide corresponding to amino acid residues 206–217 of A-gliadin. *Gut* 1992;**33**:1504–7.
25 Mahon J, Blair GE, Wood GM, Scott BB, Losowsky MS, Howdle PD. Is persistent adenovirus 12 infection involved in coeliac disease? A search for viral DNA using the polymerase chain reaction. *Gut* 1991;**32**:1114–16.
26 Carter MJ, Willcocks MM, Mitchison HC, Record CO, Madeley CR. Is a persistent adenovirus infection involved in coeliac disease? *Gut* 1989;**30**:1563–7.
27 Keller RJ, Eisenbarth GS, Jackson RA. Insulin prophylaxis in individuals at high risk of type I diabetes. *Lancet* 1993;**341**:927–8.
28 Parent ME, Siemiatycki J, Menzies R, Fritschi L, Colle E. Bacille Calmette-Guerin vaccination and incidence of IDDM in Montreal, Canada. *Diabetes Care* 1997;**20**:767–2.