MOLECULAR MIMICRY: BASIS FOR AUTOIMMUNITY

Sheshadri Narayanan

Department of Pathology, New York Medical College - Metropolitan Hospital Center, New York, New York 10029

ABSTRACT

Structural similarity between a viral protein and a self-component can trigger an autoimmune response, which is the basis of molecular mimicry. Alternatively an invading virus can induce an inflammatory response which in turn can initiate an attack by hitherto dormant T cells on a specific self-antigen, a phenomenon which is referred to as Bystander Activation. Several viruses share amino acid sequences with target self-proteins. A widely studied viral interaction is the structural mimicry of a small portion of coxsackie virus to a specific region of the enzyme glutamic acid decarboxylase (GAD) which is expressed by the β cells of the islet of Langerhans in the pancreas leading to the destruction of insulin producing cells and the onset of Type I insulin dependent diabetes mellitus (IDDM). Knowledge of specific epitopes in GAD susceptible to autoimmune attack can permit devising therapeutic strategies for the prevention and suppression of IDDM.

KEY WORDS: Molecular mimicry, bystander activation, coxsackie virus, GAD 65 epitope, IDDM, Th1 versus Th2-cell subset response.

INTRODUCTION

In 1968 George Snell first used the term molecular mimicry to explain persistent viral infections. An interaction between the major histocompatibility complex (MHC) which is a collection of genes on chromosome 6 coding for the human leukocyte antigens (HLA) and the viral protein with a similar amino acid sequence was suggested as the basis of molecular mimicry that apparently spares the virus from immune attack (1). In reality however, attack by a viral agent does trigger a specific immune response by the host which, in turn can cross-react with the structurally mimicked sequence in the host thus turning on the autoimmune response that ultimately leads to the destruction of host target cells and tissue.

Genesis of Molecular Mimicry

The primitive T cell produced by the bone marrow is processed by the thymus where only those T cells that can recognize and bind to the

peptide sequence presented by the MHC are selected while those cells which either do not recognize the MHC peptide or which are strongly self reactive are removed (2). This process of positive and negative selection of primitive T cells presented to the thymus results in barely 1% of the primitive T cells reaching maturity, while 99% of them are removed by apoptosis or programmed cell death. The mature T cell is equipped with a cell surface receptor containing the marker CD3 together with either CD4 or CD8 as an accessory molecule. The process of thymic selection still leaves some T cells that are self-reactive. These self-reactive T cells could conceivably be activated by a virus bearing structural sequences similar to the MHC derived peptide sequence. These activated T cells can mount an autoimmune response against the host target cells of tissue bearing structural similarity to specific regions of an invading pathogen (3). The interplay between 1.) MHC which selects T cells in the thymus, 2.) the invading pathogen such as viruses, bacteria, and foreign antigens such

as food, which triggers immune response and 3.) host target cell or tissue susceptible to attack by self reactive T cells represents a three way molecular mimicry between peptide sequences of these three groups (3).

Bystander Activation

An alternative concept of autoimmunity is based on events that are triggered subsequent to the inflammatory response induced by viral infection. Cytokines are released by antigen presenting cells such as macrophages which can activate dormant self reactive T cells to attack self antigens in target tissue that were previously ignored. The inflammatory process could facilitate this autoimmune attack by activated self-reactive T cells by the release of self-antigens by target tissue during the inflammatory process (4,5).

Microbial Sequence Mimics of Target Sequences

Several examples of microbial protein mimics with host target sequences have been reported in literature. For instance in ankylosing spondylitis the link between the microbial organism *Klebsiella pneumoniae* and HLA B27 is due to the identical amino acid sequence (QTDRED or glutamine, threonine, aspartic acid, arginine, glutamic acid, and aspartic acid) found in the nitrogenase protein of *Klebsiella pneumoniae* and the amino acids 72-77 in the HLA B27(6).

In celiac disease which is characterized by small intestinal muscle injury and malabsorption there is an 8 amino acid identity between Elb protein of adenovirus 12 present in the intestinal tract and the A-gliadin protein of wheat gluten (1).

The fact that the amino acid sequence 63-67 of β -casein is identical to residues 415-419 of the β cell specific glucose transporter-2 (GLUT-2) makes reduced breast feeding time and early feeding of cow's milk to babies a risk factor for diabetes. Indeed autoantibodies to GLUT-2 have been demonstrated in a great majority of patients with

recent onset insulin dependent diabetes mellitus (IDDM) (3).

Studies linking coxsackie virus and IDDM have revealed a relationship between the enzyme glutamic acid decarboxylase (GAD) which is expressed in the β cells of the pancreas and coxsackie viral protein (7,8). The function of the enzyme GAD which is also expressed in the brain is to catalyze the formation of the neurotransmitter γ amino butyric acid (GAB).

The 40-kD coxsackie virus P2-C protein (COX P2-C) which is involved in viral replication and the β cell 65 kD enzyme GAD (GAD 65) share a stretch of 6 identical amino acids with antibodies to the COX P2-C and GAD 65 cross reacting with each other (8). Indeed a three way molecular mimicry concept mentioned earlier exists between HLA DR4 which displays the processed peptide of the β chain of HLA DQ, coxsackie viral coat peptides and an enzyme carboxypeptidase H expressed within the β cells which serves as the target autoantigen (3,9).

Diabetes Model of Autoimmunity

Non-obese diabetic (NOD) mice have provided a model for the study of IDDM. Studies with NOD mice have implicated the T-helper 1 (Th1) response in the autoimmune attack against β -cells (10). The stimulation of the Th1 subset of T-cells begins when the macrophages or other antigen presenting cells process a β -cell protein and express it in a complex with MHC class II molecules on the macrophage surface. The processed β -cell protein in effect serves as an autoantigen (β -AG). Th1 subset of T-cells are primarily activated by β -Ag-MHC II complex and other accessory molecules such as B7 on the surface of the macrophage. In addition, the cytokine interleukin 12 (IL-12) elaborated by the macrophage activates Th1 cells. Th1 cells, in turn, produce the cytokines IL-2 and interferon γ (IFN- γ) which serves to inhibit the Th2-cell subset response and the generation of cytokines IL-4 and IL-10. Thus there is a cytokine imbalance in IDDM favoring the release of cytokines produced

by Th1-cell subset and inhibiting the release of cytokines produced by Th2-cell subset (10). Th1-cell subset derived cytokines IL-2 and IFN- γ activate cytotoxic macrophages to produce oxygen free radicals, nitric oxide and cytokines IL-1 and tumor necrosis factor- α (TNF- α) which attack β -cells. IL-2 and IFN- γ also activates the cytotoxic T-cell which in addition to secreting the cytokines TNF- β and IFN- γ which attack the β -cell also recognizes the β -cell autoantigen in complex with MHC-I on the surface of the β -cell leading to its destruction (10). Thus the hallmark of IDDM is an inflammatory infiltrate of pancreatic β cells producing a lesion called insulinitis (11). Contributing to this lesion are not only the products elaborated by the macrophages and Th1 cells but also other cells in the pancreatic islet area such as vascular endothelial cells which produce cytokines IL-1 and IL-6 and attract from the circulation macrophages and lymphocytes into the pancreatic islet by expressing adhesion molecules on its surface in response to inflammatory cytokines such as IL-1, TNF and IFN- γ (12). Even endothelial cells could function as antigen presenting cells and present β -cell autoantigen in conjunction with MHC II Complex to T cells (13). Thus the components that make up the insulinitis lesion are CD4 and CD8 positive T lymphocytes, macrophages, natural killer cells and a variable number of B lymphocytes (11). MHC Class II loci DRB1 and DQB1 on chromosome 6p21 are strongly linked to the genetic risk for IDDM (14). In more than 95% of childhood-onset IDDM Caucasian patients the HLA DRB1 alleles DRB103 or DRB104 or both (called DR3 and DR4) are present in contrast to its presence in only 45-55% in the general population. The heterozygote DR3/DR4 presents the greatest risk for IDDM (14). Alleles which code for an amino acid other than aspartate at codon 57 at the DQB1 locus represents an increased risk factor for IDDM in white Caucasians. The DR3 allele presents a risk for IDDM due to its association with DQA1 alleles that code for arginine at codon 52 such as DQA10501. The fact that DR4 alleles represent a risk is primarily related to its association in a haplotype with the DQB10302 allele that codes for alanine at codon 57 and is found in greater than 70% of DR4 haplotypes in juvenile-onset IDDM (14).

Therapeutic Strategies for IDDM

Since the activation of Th1-cells results in autoimmune attack on the pancreatic β cells the inhibition of the Th1-cell subset response by activation of the Th2-cell subset can spare the β -cells from autoimmune attack.

The fact that GAD 65 is a β -cell autoantigen which is attacked subsequent to activation of Th1-cells, the administration of GAD 65 is a therapeutic strategy to inhibit Th1-cell subset and turn on the Th2-cell subset response (10). The administration of GAD 65 in effect would suppress the autoimmune response by activating Th2 subset of T cells. The cytokines IL-4 and IL-10 elaborated by Th2-cells can suppress Th1-cell subset response and in turn the release of Th1-cell cytokines IL-2 and IFN γ . Without the latter two cytokines (IL-2 and IFN γ) the cytotoxic T-cell and macrophage functions are inhibited thus sparing the β cell from autoimmune attack. In addition IL-12 release by macrophages is inhibited by IL-10 thus further inhibiting Th1-cell activation (10). Thus the net effect of immunostimulatory intervention by the administration of GAD is to spare the β cell from an attack by the immune system by recognizing the β cell antigen as a self component.

Epitopes of GAD 65 differ in their ability to induce Th2-cell subset response in NOD mice and in turn prevent overt IDDM (15).

The administration of GAD epitopes corresponding to amino acids 217-236 (p217), 247-265 (p247), 290-309 (p290), and 524-543 (p524) to NOD mice before the onset of insulinitis were all effective in preventing the subsequent development of insulinitis and diabetes. However, in NOD mice which had developed extensive β cell autoimmunity only a mixture of p217 and p290 GAD epitopes were effective in preventing progression of insulinitis and the development of overt IDDM (15). However GAD epitope therapy was ineffective in NOD mice which had diminished IL-4 expression, since this cytokine elaborated by Th2-cells is critical to inhibiting the Th1-cell response that in turn will spare the β cell from autoimmune attack.

Assay for GAD 65

Since antibodies to GAD 65 are seen in nearly 80% of recent-onset IDDM patients long before the appearance of clinical symptoms an assay for this marker will be helpful to identify patients at risk for IDDM among patients with impaired glucose tolerance, so that they can be treated to prevent insulinitis and diabetes.

A immunoprecipitin assay has been used for the detection of GAD 65 autoantibodies in serum (16). In this double-antibody radioimmunoassay (RIA) ¹²⁵I labeled-recombinant (r) GAD protein was used as the antigen, and the immune complex formed when this antigen was incubated with GAD 65 autoantibody present in serum, was precipitated with an immunoprecipitant mixture of IgG and IgM. The radioactivity of the pelleted and washed immunoprecipitate was measured in a gamma counter. Since this assay was also positive in patients with thyrogastric cluster of autoimmune disorders such as Grave's disease, Hashimoto's thyroiditis and pernicious anemia, an assay for a pancreatic islet specific marker such as a putative tyrosine phosphatase would be a valuable adjunct to the GAD 65 antibody assay for identification of normal subjects at risk for IDDM (16).

CONCLUSION

Microbial amino acid sequence identity with human host proteins in target tissues lends credence to the microbial molecular mimicry concept of autoimmunity. However, recent studies using coxsackie virus indicate that the autoimmune response leading to IDDM could result from an inflammatory response from viral infection and tissue damage causing release of a sequestered β cell antigen. This could conceivably activate dormant self-reactive T cells to attack the host's β cells which is in line with the concept of bystander activation (4,5,17).

It is in the realm of future research to further elucidate the exact interplay between the pathogen and host tissue antigen in the genesis of autoimmune disease, so that appropriately refined therapeutic strategies can be devised for effective treatment.

Finally, whether there is also an autoimmune connection between the enzyme aldose reductase and the diabetic complications that it introduces is an exciting area to explore as well as the genesis of gestational diabetes (18,19).

REFERENCES

1. Oldstone, M.B.A. (1987) Molecular mimicry and autoimmune disease. *Cell* 50, 819-820.
2. Baum, H., Davies, H. and Peakman, M. (1996) Molecular mimicry in the MHC: hidden clues to autoimmunity? *Immunol. Today* 17, 64-70.
3. Leech, S. (1998) Molecular mimicry in autoimmune disease. *Arch. Dis. Child.* 79, 448-451.
4. Benoist, C. and Mathis D. (1998) The pathogen connection. *Nature* 394, 227-228.
5. Wekerle, H. (1998) The viral triggering of autoimmune disease. *Nature Med.* 4, 770-771.
6. Williams, K.M., Hirofuji, T., Raybourne, R.B., Shook, D.L., Trucksess, M.W. and Yu, D.T. (1989) Synthetic oligonucleotide probes complementary to the HLA-B variable region hybridize with *klebsiella pneumoniae*. *Microb. Pathog.* 6, 277-286.
7. Jones, D.B. and Crosby, I. (1996) Proliferative lymphocyte responses to virus antigens homologous to GAD 65 in IDDM. *Diabetologia* 39, 1318-1324.
8. Hou, J., Said, C., Franchi, D., Dockstader, P. and Chatterjee, N.K. (1994) Antibodies to glutamic acid decarboxylase and P2-C peptides in sera from coxsackie virus B4-infected mice and IDDM patients. *Diabetes* 43, 1260-1266.
9. Chicz, R.M., Urban, R.G., Gorga, J.C., Vignali, D.A., Lane, W.S. and Strominger, J.L. (1993) Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. *J. Exp. Med.* 178, 27-47.

10. Rabinovitch, A. (1994) Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Therapeutic intervention by immunostimulation. *Diabetes* 43, 613-621.
10. Ellis, T.M. and Atkinson, M.A. (1996) The clinical significance of an autoimmune response against glutamic acid decarboxylase. *Nature. Med.* 2, 148-153.
11. Bevilacqua, M.P. (1993) Endothelial-leukocyte adhesion molecules. *Annu. Rev. Immunol.* 11, 767-804.
12. Pober, J.S. and Cotran, R.S. (1991) Immunologic interactions of T lymphocytes with vascular endothelium. *Adv. Immunol.* 50, 261-302.
13. Horton, V., Stratton, I., Bottazzo, G.F., Shattock, M., Mackay, I., Zimmet, P., Manley, S., Holman, R. and Turner, R. (1999) Genetic heterogeneity of autoimmune diabetes: age of presentation in adults is influenced by HLA DRB1 and DQB1 genotypes (UKPDS 43). *Diabetologia* 42, 608-616.
14. Tisch, R., Wang, B. and Serreze, D.V. (1999) Induction of glutamic acid decarboxylase 65-specific Th2 cells and suppression of autoimmune diabetes at late stages of disease is epitope dependent. *J. Immunol.* 163, 1178-1187.
15. Walikonis, J.E. and Lennon, V.A. (1998) Radioimmunoassay for glutamic acid decarboxylase (GAD 65) autoantibodies as a diagnostic aid for stiff-man syndrome and a correlate of susceptibility to type I diabetes mellitus. *Mayo. Clin. Proc.* 73, 1161-1166.
16. Horwitz, M.S., Bradley, L.M., Harbertson, J., Krahl, T., Lee, J. and Sarvetnick, N. (1998) Diabetes induced by coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nature. Med.* 4, 781-785.
17. Horwitz, M.S., Bradley, L.M., Harbertson, J., Krahl, T., Lee, J. and Sarvetnick, N. (1998) Diabetes induced by coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nature. Med.* 4, 781-785.
18. Narayanan, S. (1993) Aldose reductase and its inhibition in the control of diabetic complications. *Ann. Clin. Lab. Sci.* 23, 148-158.
19. Narayanan, S. (1991) Laboratory monitoring of gestational diabetes. *Ann. Clin. Lab. Sci.* 21, 392-401.