Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. Vol. 82, November 1987/Page 274

TDR-7

INDUCTION OF AUTOIMMUNITY: IS THERE A ROLE FOR INFECTIOUS AGENTS?

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INTRODUCTION

As our understanding of the immune system deepens, it has become clear that 'autoimmunity' recognition of self antigens by the receptors of the immune system is not pathological. Both T cells and B cells are involved in self recognition of different types, and so a major topic of contemporary research is aimed at understanding why autoimmune diseases develop in certain individuals.

This chapter will review approaches and current concepts and will highlight the potential role of infectious agents.

PHYSIOLOGICAL AUTOIMMUNITY

The existence of autoreactive B cells has been known for quite some time, based on several lines of evidence. First autoantigen binding B cells were detected by using radiolabelled antigens (1). Second, mitogen stimulation, e.g. LPS leads to the production of autoantibodies. More recently it has been shown that B cells expressing the CD5 (Lyt1, T1) marker mostly make autoantibodies (2). Hybridomas produced from young mice (fetal liver, neonatal spleen) have a high degree of connectivity, reacting with a lot of other Ig idiotypes.

At the T cell level it is now clear that physiological 'autoreactivity' in terms of recognition of MHC structures is an essential part of the process of T cell recognition. Other evidence of 'autoreactivity' has been chiefly obtained in terms of the autologous mixed lymphocyte reaction (AMLR), envisaged as a reaction against autologous class II antigens. This response is noted solely in vitro and is experimentally very difficult to differentiate from a response to a serum protein or other medium constituent which is corecognised in association with class II antigens. Other examples have been found, e.g. T cells of normal individuals recognizing myelin basic protein.

PHYSIOLOGICAL MECHANISMS OF 'SELF TOLERANCE'

The relative importance of various mechanisms of 'self tolerance' are not currently clear. Possibly all of them have some role, with the relative importance differing with the antigen concerned, and the immune status of the host.

There is evidence that there is clonal deletion during thymic selection of immune competent cells. This is suggested by a variety of thymus chimera experiments, in which the T cell repertoire was clearly influenced, and more recently by analysis of T cell receptor expression in immature and mature thymocytes. Using a monoclonal to Vβ17, Kappler and Marrack's group found that whereas immature thymocytes in certain strains, expressing I-E were $V\beta17^+$, the mature ones lacked Vβ17, indicating failure of this set of T cells to mature (3).

Suppressor cells have been implicated in the mechanism of self tolerance. One line of evidence comes from mice, e.g. SJL that have deficiencies in suppressor cell function. These are particularly prone to develop experimental autoimmune diseases, if challenged appropriately e.g. rat RBC leads to an anti mouse red cell response. Another line of evidence comes from the effects of T cell depletion, which favours the development of autoimmunity.

A variety of immune deficiency states in man are also associated with an enhanced frequency of autoimmune diseases e.g. immunodeficiency of chronic mucocutaneous candidiasis.

There is the possibility that anti-idiotypic antibodies may be involved in immuneregulation and self tolerance. How this may occur is not clear, but there is recent evidence that anti idiotypic antibodies may influence the T cell repertoire, altering low responder to high responder phenotype (4) and also that antireceptor antibodies may be involved in clonal deletion by bridging CD4 and CD8 cells sharing Vβ regions (de Berardinis et al, manuscript in preparation).

WHAT HAPPENS DURING THE AUTOIMMUNE DISEASE PROCESS AT THE LOCAL SITE

Considerable new insights into the autoimmune process have come from looking at the local tissue in human autoimmune diseases. Immunohistological approaches have identified the presence of numerous activated lymphocytes, chiefly T cells in autoimmune sites, e.g. rheumatoid arthritis, thyroiditis. Equally striking are the changes in local MHC expression. HLA class I is augmented, but HLA class II is very markedly augmented, both in cell types which normally express class II but most significantly in cells which in health do so. The expression of class II on tissue cells, e.g. thyrocytes and pancreatic islet cells led to the concept that the autoimmune process was perpetuated by the target cells acting as antigen presenting cells to autoreactive T cells, which produce mediators which induce class II expression on the tissue cells - a self perpetuating positive feedback loop. Due to the activation of autoreactive T helper cells, the various effector mechanisms became engaged - with DTH cells, B cells, killer cells, NK, LAK activated macrophages all being possible contributors to the tissue damage that eventually ensues (5).

While this overall concept fits the facts i.e. over expression of class II in human autoimmune sites is found in all local autoimmune diseases except myasthenia gravis (see Table 1) it leaves many questions unanswered, both about the early events, and also about the mechanisms of tissue damage.

Additional experimental support for this concept is listed in Table 2.

EARLY EVENTS

The events which antedate the chronic phase are not easy to study in human diseases. Family studies in diabetes have suggested that there is a long prodromal phase in which autoantibodies to islets can be detected. However it has become clear that the presence of anti islet cell antibodies does not inevitably lead to the disease, suggesting that the homeostatic immunoregulatory pathways may overcome the autoimmune process.

Analysis of animal models which resemble human autoimmune diseases, such as BB rats and NOD mice which become diabetic have shown features that differ from the human disease. The expression of HLA class II on islet cells is not nearly as clear in these situations as it is in diabetes, even though in animals it is possible to study time courses. 'Insulitis' - infiltration of lymphocytes is more marked in BB rats. Thus it is possible that the pathogenesis of these animal diseases may not reflect that of the human counterpart.

Attempts to study the early events of human diseases have been restricted due to obvious inadequate of tissue samples, and so very limited data is available. Immunohistological analyses have been made of the lesions in diabetes, to attempt to address the question as to whether the tissue class II expression precedes the lymphocytic infiltration or vice versa. Foulis has performed extremely detailed analysis of 16 pancreata from newly diagnosed diabetics that died soon after diagnosis, by serial sectioning. His results indicate that in many islets, where the insulin producing islet cells (but not glucagon and somatostatin cells) and endothelial cells are expressing class II, there are no lymphoid cells visible (6). This data is most easily interpretable as suggesting that class II induction may precede lymphocytic infiltration, but other interpretations may be possible, e.g. diabetic ketoacidosis may cause the loss of lymphoid cells. Foulis has also studied other early events, e.g. class I expression was augmented on all islets which still contained insulin producing cells. If these were absent the islets were class I, negative. These results emphasize the central role of the target cell in the disease process, and the close correlation between class II expression and disease.

In BB rats, no evidence for class II expression prior to lymphocytic infiltration has been seen (7), nor in alopecia areata.

If the only signals that induce class II are T cell derived then lymphocyte activation somewhere close by must precede class II expression. However there is beginning to accrue evidence for a role of viral infections in regulating class II expression. Thus JHM virus can induce class II expression in astrocytes *in vitro* (8). SV40 transfected thyrocytes can also express class II (9), and in this context it is interesting to note that diabetic islets make IFNα, a product made in response to viral infection (Foulis, personal communication). It is likely that the endothelial cells which

are activated in diabetes may be significant contributors to the mediators produced which may be of importance, e.g. TNF which is a coinducer of class II in islet cells (10).

Currently it is not possible to make any definitive statements about the early events in any human autoimmune disease (or spontaneous animal autoimmune disease) as too little information is available.

CLASS II EXPRESSION ON TARGET CELLS APPEARS NECESSARY, IS IT SUFFICIENT TO ACCOUNT FOR AI?

It is of interest that specific cell surface structures of relatively uncommon cells are often the target of autoimmune attack, e.g. TSH receptor of thyrocytes in Graves' disease, the acetylcholine receptor of muscle in myasthenia gravis. This raises the issue as to whether the mechanisms of self tolerance are as fully engaged with this type of rare, tissue restricted antigen, which would not be accessible to maturing thymocytes, and that T cells could not recognize in the absence of class II induction in the tissues.

Current concepts indicate that tolerance induction *in vivo* (and *in vitro*) is MHC restricted, i.e. involves corecognition of MHC (11), and so is suppressor cell function. Thus it is not evident that the mechanisms of self tolerance would function adequately for these antigens, and it is possible that since self tolerance involves a functional (or actual) loss of self recognition capacity, there has to be a limit to the efficiency of self tolerance in order to leave a functional T cell repertoire. Thus for rare antigens, it is possible that the major mechanism of self non responsiveness is 'immunologic ignorance' and that the price paid for a larger repertoire is a propensity to develop autoimmunity towards our smaller organs and rarer antigens e.g. endocrine tissue. Thus it could be that class II expression is both necessary and sufficient for the development of autoimmunity (discussed 12).

We have attempted to investigate this question using a disease which has a few autoimmune features, non toxic goitre (NTG) Grubeck-Loebenstein has reported that some NTG patients do express class II on their thyrocytes (13) and it has been known for some time that some of these patients also have thyroid autoantibodies.

Thus it was of interest to explore whether NTG was in fact an autoimmune disease, of a mild type, or whether class II expression occurred without any consequent autoimmunity.

The antigen presenting capacity of IFNγ treated and hence class II expressing NTG thyroid follicular cells was compared to that of Graves' disease. These induced an MLR equally, verifying that NTG thyroid can act as APC.

However when the lymphocytes of NTG were cultured to look for autoreactive T cells, none were detectable, in contrast to results obtained in Graves' disease, performed in parallel.

Thus our current concepts is that class II expression is necessary but not sufficient. So what else contributes to the autoimmune diathesis?

A number of possibilities emerge, based on genetic family and other studies.

(a) Presence of appropriate polymorphic class II antigen.
(b) Presence of the appropriate T cell receptor genes.
(c) Inadequate function of suppressor cells.
(d) Loss of tolerance mediated by lymphokines.
(e) Presence of environmental agents as triggers.

OTHER FACTORS INVOLVED IN AUTOIMMUNITY

Family and twin studies have highlighted the importance of both genetic and non genetic factors in the development of autoimmune diseases. Twin studies in diabetes and RA, indicate that <50% (~ 30-40%) of identical twins are concordant (e.g. 14), whereas in non identical twins the concordance rate is not much different from siblings in general. These results emphasize the fact that several genetic systems are involved, and also since non identical twins are substantially discordant, the role of non-inherited factors. These could (and are likely to) be environmental but could involve other somatic events, such as the gene rearrangements involved in the generation of functional T or B cell receptors (15). Concerning environmental agents, it would be expected that identical twins, reared together would be exposed to agents in a virtually identical manner. Hence there may be events apart from infection and genotype which may also predispose. Perhaps random events, intercurrent infections which may affect the suppressor pathways may be involved.

All autoimmune diseases (plus a few other, e.g. narcolepsy) are associated with the HLA class II genes (16), apart from the HLA-B27 cluster. These studies, both on populations and families, indicate that the polymorphic structures have a role. The nature of that role is not clear, as there is increasing evidence from sequencing the genes that the expressed class II sequences are the same in diseased as in normal individuals.

However it is still conceivable that the class II products on patients differ from that of normals by:

(1) abnormal complementation, e.g. DRα with DQβ.
(2) DQα from one haplotype associating with DQβ from another.
(3) Expression one of usually silent products, e.g. DX, DO, DZ

My own prejudice is that overabundance of class II on the wrong cells is the key feature, which would of course predispose to the above especially if there was unbalanced synthesis of class II molecules to permit unusual pairings.

The function of T cell receptors is to recognize entities in close association with HLA antigens. If polymorphism of T cell receptor genes exist, these may be of importance in the predisposition to autoimmune disease (15). However the function of T cell receptor genes is to generate a very substantial expressed repertoire and it is not clear if that would differ significantly between normals and patients.

RFLP analysis has revealed that there are genetic differences between patient and normal populations, with RFLP differences detected in several diseases (e.g. 17). How these RFLP findings may relate to the expressed and functional T cell repertoire is not clear.

Inadequate function of suppressor cells has been speculated to be of relevance to the development of thyroid autoimmunity by Volpe's group (18). Regrettably the current paucity of methods for analysing suppressor cells has made it difficult to adequately perform the appropriate experiments to pursue the role of suppressor cells in more depth.

Modulation of self tolerance mechanisms by products of an immune response may be of relevance to the development of self non responsiveness. It has been reported that IL-1 may interfere with the development of B cell tolerance (19). We have studied T cell induction of non responsiveness or tolerance to high concentrations of peptide antigen. In that system IL-2 can either prevent the induction of non responsiveness, or reverse it, once induced, whereas IL-1 and IFNγ had no effect (20).

ENVIRONMENTAL TRIGGERS OF AUTOIMMUNITY

There is no doubt that environmental agents can trigger off autoimmune responses. This is most clearly seen in drug induced reactions, for example drug induced lupus. In these diseases, a clear genetic influence as noted, as in the 'traditional' autoimmune diseases without known precipitating cause. In drug induced lupus two genetic influences have been defined, HLA association and acetylator status, which is relevant to the rate of drug metabolism and hence effective tissue concentrations. It is of interest that drugs which cause lupus, e.g. hydrazines can conjugate to C4A and reduce its activity (21).

Of relevance here is the question whether infectious agents are capable of inducing autoimmune diseases. The evidence from animal models is convincing, but that from human diseases is largely circumstantial, but merits further analysis, as it is evident that in chronic infectious diseases, e.g. schstosomiasis, autoantibodies are usually generated.

In mice, JHM virus induces several types of demyelination. One of these is clear dependent on an autoimmune response closely resembling experimental allergic emcephalitis - myelin reactive T cells are found, astrocytes express class II

antigens, and occurs preferentially in certain strains of mice (8).

In ungulates, lentiviruses are capable in inducing a number of autoimmune diseases which closely resemble human autoimmune diseases. Thus sheep get a disease, maedi-visna which involves a lentivirus infection of macrophages. This spreads to the central nervous system and causes a relapsing paralytic disease with a course like multiple sclerosis. Certain features of this diseases resemble human autoimmunity, e.g. there is excessive class II induction in the tissues, there is the production of an atypical Interferon which is not Interferon α, β, γ (e.g. 22). Such atypical Interferon have been reported in the serum of patients with rheumatoid arthritis, SLE and scleroderma and also in AIDS patients (23).

Goats and certain strains of sheep get a chronic arthritis which is due to a lentivirus, caprine encephalitis- arthritis virus. Thus arthritis produces lesions which resemble rheumatoid arthritis by immunohistology and by clinical course, e.g. erosion and pannus are formed (24). Type C virus particles have been commonly noted in mouse and dog models of SLE, and Schwartz and others searched for retrovirus involvement in SLE in the mid 70's.

A Scandinavian group has reported that immune complexes derived from patients with ankylosing spondylitis contain antibodies against a retrovirus protein (25).

Epidemiological data is compatible with a role of extrinsic, probably infectious agents in a number of human auto-immune diseases. In multiple sclerosis, there is a clear geographic incidence mapping closely to Northern latitudes, which is imprinted upon the population before the age of 15 - migration after 15 does not reduce the risk. In islands, e.g. Farves, there have been 'epidemics' of cases following entry of immigrants into closed societies (26).

Insulin dependent diabetes has epidemiological features resembling infectious disease: a young age distribution, seasonal incidence, marked local variations (reviewed 27).

It is thus of interest that diabetic mice, both obese and non obese diabetic (NOD), harbour retroviruses in their pancreatic islet cells (28).

The question of interest is how could infectious agents contribute to the development of autoimmunity. Several concepts have been proposed. The most popular is 'antigen mimicry', in which determinants are shared between viruses/ bacteria and autoantigens. Exposure to the viruses/bacteria induces a response to this determinant, and the expanded population of cells can then recognize the autoantigen also. There is no doubt from sequence analysis that there is quite a widespread potential for antigen mimicry. A well documented example is the cross reaction of a gluten peptide with adenovirus (29). However the antigen mimicry is clearly restricted by homeostatic systems, as in most instances

exposure to the antigen does not lead to diseases. Antigen mimicry could explain HLA associations, if it was shown that the appropriate epitope was presented better in the HLA haplotypes most prone to that disease. This has not yet been performed.

Ankylosing spondylitis has been proposed by Geczy (e.g. 30) and others (31) to be due to a cross reaction of Klebsiella with HLA-B27. Not just any Keebsiella, but those carrying a certain plasmid. Thus rabbit antisera to Klebsiella are alleged to lyse only HLA-B27 cells from spondylitic individuals. There has been widespread interest in these observations, which unfortunately are difficult to reproduce by assays other than Geczy's.

It is possible that infectious agents may cause polyclonal activation, e.g. bacterial lipopolysaccharide LPS, staphylococcal toxins or viruses with transforming potential such as Epstein-Barr Virus. These could cause disease and Steinberg for example is a champion of the idea that lupus syndromes may be due to polyclonal activation of B cells (32).

My colleagues and I have championed the notion that any local infection, by activating T cells to produce mediators such as Interferonγ and tumour necrosis factor or lymphotoxin could induce class II expression on cells which do not normally express it, e.g. thyrocytes. In certain individuals this process leads to the activation leading to disease. Unlike antigen mimicry, there are no limitations on the type of agent, more on its distribution and capacity to activate T cells to produce mediators. The capacity of Interferonα treatment in vivo, which does cause class II expression in vivo (in man and mice) to precipitate autoimmune disease in a high percentage of cancer patients treated with IFNα suggests that this is a plausible model; as there would be no antigen 'mimicry' (e.g. 33).

CONCLUSIONS

There is evidence that infectious agents can cause autoimmune reactions, in several systems, in man and experimental animals. However it is not clear if the common and severe human autoimmune disease, e.g. rheumatoid arthritis, diabetes and so on are precipitated by infections. My prejudice is that it is likely that they are; sufficiently likely that more work is eminently justified; especially as if infectious agents are identified, vaccination may be possible to eradicate these diseases.

TABLE I

Autoimmune Diseases

WITH EXCESS CLASS II ON TARGET
Graves', Hashimoto's, insulin dependent diabetes, Rheumatoid arthritis, Sjogren's, biliary cirrhosis, coeliac, multiple Sclerosis
WITHOUT EXCESS CLASS II ON TARGET
Myasthenia gravis
? Haemopoietic (unassessable)

TABLE 2

Experimental evidence supporting HLA class II overexpression concept of AI

1. Target cells of AI can be induced to express class II by T cell products e.g. IFNγ for thyrocytes (Todd et al, 1985)
   IFNγ + LT or TNF for islet cells (Pujol Borrell et al, 1987 Nature, 326; 304-306)

2. Target cells can act as APC
   Thyrocytes (Londei et al, 1984, Nature, 312, 639-641)

3. Infiltrating T cells can be restimulated by class II expressing tissue cells, e.g. in Graves' disease (Londei, Bottazzo and Feldmann, 1985, Science, 228; 85-89)

4. Injection of IFNα induces autoimmune side effects at a high frequency (Fentiman et al, 1985, Lancet i, 1166)

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This publication received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.