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Autoimmune disorders – analysis by study of T cells, implications and speculations

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Introduction

Tissues undergoing an autoimmune reaction are infiltrated with lymphocytes, chiefly T cells. Many of these are activated and express Interleukin-2 receptors. The surrounding tissues often express a much higher level of HLA class II antigens than is normal, and often on cells such as thyrocytes or synovial cells which do not normally express them. Since HLA class II antigens are essential for the activation of T helper cells, this raises the question as to whether the tissue cells are contributing to the perpetuation of the disease by acting as antigen presenting cells [1] or whether the class II expression is just a consequence of local T cell activation and the presence of Interferon γ, without any pathological relevance.

In the past 3 years we have established that in autoimmune hyperthyroidism (Graves' disease) the antigen presenting function of the thyroid epithelium is of relevance, as these cells can stimulate autoreactive T cells cloned from the thyroid tissue. In this paper, we wish to discuss this concept with respect to other autoimmune diseases that we are studying, in particular Hashimoto's thyroiditis (hypothyroidism) and Rheumatoid Arthritis.

Results and discussion

There is conclusive evidence from our work that thyrocytes can present antigen, both synthetic peptides and intrinsic autoantigen (2, 3). So it would be anticipated that in Hashimoto's thyroiditis, the target tissue could present to autoreactive T cells. However the pathology is different from Graves', as there is widespread tissue destruction and eventual loss of thyrocytes. What accounts for the differences between the two?

There is evidence that the specificity and titres of autoantibodies differ between the two diseases, with the anti TSH receptor antibodies being restricted to Graves, microsomal antibodies and antithyroglobin can be found in both, but at much higher titres in Hashimoto's thyroiditis.

It was thus a question of interest to determine whether the activated T cells in Hashimoto's thyroiditis were different from those in Graves, which may be anticipated, in view of the differences in pathology. Due to the infrequency of obtaining Hashimoto's operative specimens, this has been difficult to test, but 2 have been obtained in the past 2 years and the lymphocytic infiltrate cultured as lines in mitogen free IL-2 (recombinant). The uncloned lines were phenotyped after 3–4 weeks and it was found that most of the T cells were $T8^+$, with also a substantial proportion of NK cells. Analogous cultures of Graves' lymphocytes yielded a preponderance of T4 and no NK type cells. These results are compatible with the notion that the lymphocytic infiltrate is intimately involved, and may determine the pathological features (Londei et al., manuscript in preparation).

In this context it is highly relevant to determine what lymphokines these cells produce, which may

be relevant to the pathology. For this purpose the ideal material to study would be the infiltrating lymphocytes directly, but regrettably the techniques available are not adequate. In situ hybridization with cDNA probes is not yet reliable with lymphokine messages, immunostaining does not work with most antibodies, taking the supernatant of small numbers of infiltrating cells does not exclude the presence of mediators. The latter technique has revealed IFN_γ and LT/TNF in the supernatant of Graves' disease, and also in Hashimoto's and Rheumatoid Arthritis (RA).

A more sensitive, but perhaps less representative evaluation of the products produced during the autoimmune process is to measure what clones or lines derived from the infiltrating lymphocytes can produce. This has the potential pitfall that it ignores inhibitory influences which may have masked the production or effect of a mediator *in vivo*.

It has been found that T cell clones derived from the activated T cells in RA and Hashimoto's thyroiditis (HT) produce IFN_γ, LT and TNF. The quantitative aspects of IFN_γ production have been assessed, and it is of interest that higher IFN_γ production is found in RA clones that in HT. In view of the fact that IFN_γ is intimately involved in the pathogenesis of HT, these results suggest, but do not prove, the possibility of IFN_γ may have a part to play in the joint pathology (Londei et al., manuscript in preparation).

Speculations

1. What do T cells recognize?

The demonstration of autoreactive $T4^+$ non killer cells which make IL-2 and IFN_γ, presumably T helper cells can recognize autologous thyrocytes bearing HLA class II raises questions about what antigens they recognize. At the moment this is not known. We have performed simple preliminary experiments which have not been conclusive. Thus extracts of normal thyroid tissue used with autologous irradiated blood antigen presenting cells did not restimulate autoreactive T cell clones.

Allegedly DR 'compatible' (but note that serological matching at DR and even DQ does not ensure the presence of the correct restriction elements) HLA class II expressing thyrocytes did not restimulate autoreactive clones.

Given with these results, it is possible to suggest that the thyroid recognizing autoreactive clones could recognize:

1. Thyroid specific autoantigens, e.g. TSH receptor, thyroglobulin, microsomal/microvillar antigen.
2. Epithelial cell autoantigens – specificity for thyroid does not exclude other epithelial reactivity, which cannot be easily tested in human systems.
3. Extrinsic antigen, possibly a virus. In view of retrovirus involvement in autoimmune diseases, possibly a lentivirus [4].
4. Thyroid specific alloantigen.
5. HLA antigen
   - a tissue specific variant
   - recombinant molecule
   - rarely expressed moiety (e.g. DO, DZ, DX).

Considerable insights into the nature of the disease would accrue if the specificity could be unravelled. Personal preferences lean towards 1 and 3.

2. Why do the normal immunological control systems fail in autoimmune diseases?

It is axiomatic that the immunoregulatory systems are not effective enough in autoimmune diseases, even if they are functioning to some extent. One possibility, which we consider unlikely is that autoimmune diseases always eventuate if the process of local class II expression on cells which normally do not express aberrant class II is initiated. The reasons for considering it unlikely is that various studies have found that many normal individuals have autoantibodies detected by routine clinical tests, this proportion is higher in females, and increases with advancing age [5]. The presence of many clinically normal individuals with autoantibodies suggests that the process of autoimmunization takes place rather often. What may be rarer is the progression to clinical manifestations, and the role of suppressor cells in this progression is obviously a question of interest. In view of some evidence that 'DR3' individuals have abnormal suppressor function [6] and have a very wide spectrum of autoimmune disorders [7] it would be of interest to know the distribution of autoantibodies in healthy $DR3^+$ compared to individuals lacking DR3 (and the associated extended haplotype).

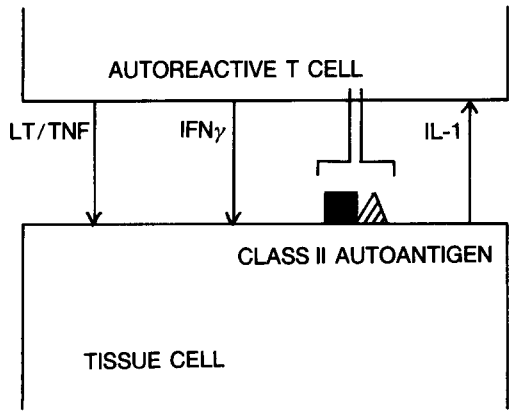

Figure 1
Scheme of cell interactions in autoimmunity. The tissue cell presents HLA class II and auto antigen to autoreactive T cells, which maintain class II expression by release of IFN$_\gamma$ and LT/TNF.

We consider that in responses against certain rare tissue specific autoantigens, normally only expressed on cells lacking class II antigens, the mechanisms needed to induce immunological tolerance or suppressor cells (corecognition with class II) do not come into play. Nor do they need to, as unless class II is induced, no immune response could result. The induction of tolerance or suppressor cells to a given antigen/HLA complex effectively causess the production of a 'hole in the repertoire' potentially seen by T cells, and the need to recognize extrinsic pathogens effectively suggests that the number of 'holes' created in the repertoire needs to be kept within bounds. This argument implies that one form of self non reactivity is based upon 'immunological ignorance', lack of recognition in the absence of class II, a state of no immunity no tolerance or suppression. It is possible that this mechanism underlies the fact that there are frequent autoimmune disorders directed against relatively rare cells, e.g. endocrine tissues seem to be involved in autoimmunity at an inordinately high frequency.

3. What is the role of IL-2 in autoimmunity?

There have been speculations that lack of IL-2 by itself favours the autoimmune state, e.g. in RA. This speculation has been difficult to understand. Lack of IL-2 would hinder the development of all types of T cells, helper, killer and suppressor and so would probably be a relatively neutral event. In fact the available data is contradictory. *In vitro* it was found that helper cell induction can persist despite anti IL-2 receptor antibody blocking of cell proliferation, perhaps suggesting that suppressor cells are more sensitive to IL-2 [8]. However *in vivo* studies with mice or rats bearing heart allografts indicates that anti IL-2 receptor antibody prevents rejection, and spares suppressor cell function, perhaps suggesting that suppressor cell function may require less IL-2 than helper or DTH cells [9].

Other considerations cast doubt on the possible importance of major or intrinsic IL-2 defect in autoimmune diseases, e.g. lack of IL-2 would predispose to infections. It appears more likely that experiments suggesting lack of IL-2 may be due to technical problems, e.g. inability to restimulate joint T cells to make IL-2 could be due to the fact that they have recently been activated, or that synovial fluid contains inhibitory material.

4. The presentation of antigen by cells which do not normally express class II may fail to activate the suppressor pathway

There is evidence from Peter Erb's laboratory [10] and our own [11] that antigen presenting cells may be selective in their capacity to induce various T cell functions. Thus while almost all antigen presenting cells can induce T cells to proliferate, with dendritic cells the most efficient, they did not all induce T helper cells. Only macrophages and astrocytes (but not B cells, dendritic cells) induce T cell help. The situation with T cell suppression is not clear, but old evidence indicates that their requirement for accessory cells is different from that of helper cells [12]. All the above clues are from murine experiments, as we are not aware of any clear data in human systems.

If, for example, HLA class II expressing thyroid epithelial cells can activate $T4^+$ helper cells but not $T4^+$ suppressor inducers, then a distortion of the normal pattern of the response will occur, and lead directly to an exaggerated response, and the autoimmune disease could follow.

The available evidence is compatible with this concept but does not prove it. Thus in our work in presenting influenza antigen by thyrocytes to T cell clones, a helper clone was used [2] and the

autoreactive $T4^+$ clones in Graves recognizing thyrocytes are probably helper cells.

We have not yet ascertain whether human T cells of the suppressor inducer phenotype ($T4^+$ and $Leu8^+/TQ1^+/2H4^+$) can be triggered by non classical antigen presenting cells such as thyrocytes.

A variant of this concept is that the pattern of lymphokines elicited by stimulation by non classical antigen presenting cells is different, and the pattern is inimical to the suppressor pathway.

This possibility is supported by recent experiments indicating that lymphokines can interfere with the tolerance pathway in T cells (Essery et al., in preparation).

The validity of this concept, that antigen presentation by non classical presenters leads directly to an excessive response by failing to activate suppressor inducer cells can be tested experimentally, in various ways.

Examination of tissue sections from autoimmune diseases such as rheumatoid arthritis, demonstrates a lack of cells bearing the suppressor inducer phenotype [13] lending some support to our idea that failure to activate suppressor inducers may be an important step in the generation of pathogenic autoimmune reactions. However there is evidence that the cell surface markers currently used to define suppressor inducer cells (Leu8, 2H4, TQ1) may not be stable markers, and their expression alters with activation. Thus the lack of $Leu8^+$ $T4^+$ expression in sections or in clones of cells derived from joints of suppressor inducer function, and so further functional analysis is essential.

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