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Epithelial Expression of HLA Class II Molecules: A New Pathogenic Factor in Organ-Specific Autoimmunity

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Although much remains to be learnt about effector mechanisms in organ-specific autoimmunity, still less is known about the precise aetiology of autoimmune diseases. It is apparent, that the causes are complex, with contributions from both genetic and environmental factors.

Considering the intricacies of regulation which maintain the balance of the immune system, one can envisage two categories of phenomena which could be involved in the generation of autoimmune disease. One of these embodies 'permissive' mechanism which would allow autoimmune stimulation by default: breakdown of tolerance of aberrations of suppressive regulation (mediated by suppressor T cells or idiotypic interactions) fall within this category. The other type of phenomena are those which would actively stimulate autoimmune attack. It is well established that immunogenic presentation of antigen to helper T cells is normally achieved in the context of Class II molecules expressed on the surface of conventional antigen-presenting cells (1). Thus, if one could demonstrate an 'inappropriate' expression of Class II products by epithelial cells one could postulate that they may then be able to present as autoantigens their own specialized, tissue-specific cell surface molecules, which under normal circumstances would not be presented to the immune system in any significant amounts (2). In this way, the cells which are the target of an autoimmune attack could play an active role in promoting their own recognition and subsequent destruction.

The feasibility of this mechanism with regard to the thyroid was demonstrated by the finding that normal, Class II-negative human thyrocytes were induced to express HLA Class II molecules upon culture with plant lectins (3). But of more direct significance to autoimmune thyroid diseases (ATD) was the observation that thyrocytes in frozen sections of glands from Hashimoto's and most Graves' disease patients are strongly Class II-positive as well as having enhanced expression of HLA Class I (4). These initial data in thyroid have been confirmed by a number of groups (5, 6), and have also been extended by the findings of inappropriate Class II expression by epithelial of a variety of organs when subject to autoimmune attack (7). This indicates the potential importance of antigen presentation by target cells in a wide range of diseases.

Is epithelial HLA Class II expression functional?

In order to determine whether HLA Class II^+ thyrocytes can really function as antigen-presenting cells, their ability to stimulate a cloned human T cell line was investigated in collaboration with Drs M. Londei and M. Feldmann. The line employed, HA1.7, is specific for a defined peptide fragment (p20) of the influenza A haemagglutinin molecule, which it recognizes in association with DQwl (8). Class II^+ thyrocytes from a Graves' disease patient of the appropriate HLA type were indeed found to stimulate proliferation of HA1.7 in the presence of p20, but not an irrelevant peptide of haemagglutinin, and this presentation was blocked by monoclonal anti-Class II antibodies (9). However, unlike autologous monocytes, the thyrocytes were unable to present the whole virus (fixed or live), suggesting that they cannot process complex antigens for presentation at the cell surface. This did not necessarily mean that thyroid cells would be unable to present their own surface molecules as autoan-

tigens. Indeed, this was demonstrated in subsequent experiments which showed that autoreactive cloned T cell lines, derived from the activated lymphocytes infiltrating Graves' disease thyroids, proliferated upon exposure to autologous thyrocytes, but were not stimulated by either autologous peripheral blood mononuclear cells or allogeneic thyrocytes (10). The interaction with autologous thyrocytes could again be blocked with monoclonal anti-Class II antibodies. This experiment thus convincingly demonstrated that thyrocytes are capable of directly presenting their own autoantigens in an MHC Class II-restricted, tissue-specific fashion to autoreactive T cells infiltrating the diseased thyroid. Others have also shown the ability of human Class II-positive thyrocytes to stimulate autologous T cells (11), and have produced similar thyroid-specific autoreactive T cell lines (e.g. 12). Results consistent with the above have also been obtained in a murine model of T cell stimulation by syngeneic thyrocytes (13).

If HLA Class II expression by thyrocytes really does play an important role in the pathogenesis, then as well as permitting demonstrations, of direct interactions between thyrocytes and T cells, the occurrence of this inappropriate expression would be expected to correlate with other features of the autoimmune pathology. In this regard, we have analysed a large series of patients and found a significant relationship between HLA Class II expression by thyrocytes and the occurrence of circulating autoantibodies to thyroglobulin and thyroid microsomal antigen (14, 15).

A more detailed analysis of a similar type was performed in Graves' disease patients in whom we examined expression of the HLA-D subregions DR, DQ and DP. The incidence and intensity of Class II subregion expression by thyrocytes was found to vary between patients, with DR being most expressed, followed by DP, and DQ least expressed. In this analysis, the most significant relationships were observed between high serum titres of thyroglobulin autoantibodies and thyrocyte expression of HLA-DQ, and between autoantibodies to microsomal antigen and HLA-DR (14 and submitted). This type of analysis is necessarily indirect, and one cannot presume there to be a cause-and-effect relationship between the parameters being compared. However, the results are consistent with the notion that different HLA-D subregion products expressed by thyrocytes could be dominant in stimulating responses to different thyroid autoantigens.

Regulation of epithelial Class II expression

In view of its potential role in autoimmune pathogenesis, it was important to investigate the regulation of HLA Class II expression in the epithelial cells expressing these molecules in autoimmune diseases. What has clearly emerged is that such regulation is complex, with a variety of factors modulating Class II expression, with differences in susceptibility depending on the cell type. These principles are best exemplified by our studies on thyrocytes in relation to thyroid autoimmunity, and on pancreatic islet cells in relation to Type I diabetes mellitus.

Interferon (IFN)-gamma has been found to induce or enhance Class II expression by a variety of cell types and, indeed, we found that recombinant human IFN-gamma induced strong surface and cytoplasmic expression of Class II molecules in cultured normal human thyrocytes (16). By contrast, IFN-alpha and IFN-beta did not induce Class II expression although, like IFN-gamma, they did enhance HLA Class I expression by the thyrocytes. IL-2 had no effect on thyrocyte expression of either Class I or Class II. These findings suggest that IFN-gamma may well be an inducer of the Class II expressed by thyrocytes in ATD: the production of this lymphokine by activated autoreactive T cells infiltrating the gland could enable the spread of Class II expression by the epithelial cells, and hence the propagation of the autoimmune process.

All cells are responsive to a variety of regulatory stimuli, and it would be naive to assume that IFN-gamma is the only stimulus relevant to thyrocyte Class II expression, particularly

given the complexity of signals known to regulate other functional properties of these cells. With this in mind, we found that thyroid stimulating hormone (TSH) enhances IFN-gamma-induced Class II expression by cultured thyrocytes, and similar activity was mediated by the second messenger analogue dibutyryl cyclic AMP (dbcAMP) (12).

On the other side of the coin, we have found that epidermal growth factor (EGF) suppresses Class II induction in thyrocytes cultured with IFN-gamma, with or without TSH (17). In this context, it should be noted that mechanisms of down-regulating epithelial Class II expression could be just as important as stimulating mechanisms in determining the overall level of Class II and the course of autoimmunity. However, the effect of a particular factor appears to depend primarily on the type of cell responding: for example, although EGF suppresses Class II expression by thyrocytes, it has been found to enhance Class II expression by human monocytes (18).

Recent investigations in Type I diabetes highlight the importance of other factors possibly involved in triggering or perpetuating Class II expression on human pancreatic islet cells. In this disease, the insulin-producing beta cells, which are the target of the pathogenic process, aberrantly express Class II molecules, although the other islet endocrine cells and the exocrine cells of the pancreas remain Class II^- (19, 20). However, when experiments were performed 'in vitro', only a small proportion of cultured human beta cells were induced to express Class II by rIFN-gamma, although the exocrine and ductal cells in these cultures become strongly Class II^+ (21). But induction of Class II in cultured islet cells can be achieved with a combination of IFN-gamma and tumour necrosis factor (TNF) or lymphotoxin (LT), although TNF or LT alone have no effect (22, and submitted). Unlike the in vivo pathological situation, IFN-gamma plus TNF/LT led to induction of Class II in the glucagon cells and exocrine/ductal cells as well as in the beta cells of the pancreatic cultures. This raises the possibility that synergism between IFN-gamma and TNF or LT is not the mechanism initiating beta cell Class II expression in diabetes, although it could have a potentiating effect. Alternatively, effective beta cell specificity could be explained by localized release of these mediators in vivo, with their short half-life limiting their sphere of action, possibly together with yet another signal acting specifically on beta cells. In any case, it is clear that our in vitro systems do not fully reproduce the in vivo situation as yet.

In view of the complexity of Class II regulation in different epithelial cell types, the obvious question arises: are there non-immune stimulators able to switch on Class II genes in epithelial cells? One possibility is that certain viruses might directly induce Class II. This is supported by recent experiments in our laboratory in which epithelial cell lines were derived from thyroid monolayers by transfection with a plasmid containing the early region of SV40 viral DNA: a proportion of the cells in these lines showed constitutive Class II expression (23, and in preparation). A different example is provided by the finding that rat astrocytes express Class II following non-infective interaction with a murine neurotropic coronavirus (24).

Another possibility is that factors other than IFN-gamma, derived from non-lymphocytic cells (e.g. macrophages or endothelial cells), might activate Class II genes. Precedents for this include the production of a novel form of interferon by macrophages infected with lentiviruses (25) and the production of an Ia-inducing factor by a murine macrophage tumour cell line treated with IFN-gamma (26).

What attracts lymphocytes to the target tissues?

In order for Class II-positive target cells to effectively present their autoantigens, autoreactive T cells must come into close contact with them. Lymphocytic infiltration of the target tissues is clearly necessary for this to occur. An important part in permitting or promoting this

infiltration could be played by the capillary endothelial cells, which physiologically constitute a discrete and selective barrier between the blood and the tissues.

In organs affected by autoimmunity the capillaries are hypertrophic and strongly Class II-positive: this is apparent in the thyroids of ATD patients (4), but is most marked in diabetic pancreases (19, 20). In the latter, these changes are observed in seemingly healthy endothelium around and inside islets (with or without infiltration), whereas the capillaries in the exocrine tissue are unaffected. The 'activated' endothelia could play a role in facilitating the 'homing' of lymphocytes, including those which are potentially autoreactive (27) and/or could possibly be involved in the presentation of antigens cross-reactive with those expressed by the target endocrine cells (28). However, regardless of the details, these observations, together with those of inappropriate Class II expression by the epithelial cells, highlights the major contribution of the target tissues to the stimulation of autoimmune pathogenesis.

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