The aim of my research is to clarify the pathogenesis of autoimmune endocrine diseases especially of Graves’ disease, Hashimoto’s thyroiditis, and insulin-dependent diabetes mellitus. My main interest is “what destroys the mechanism for self-nonself discrimination?” In other words, “what triggers the autoimmune reaction against the self?”

At the early stage of our study, we paid attention to major histocompatibility complex (MHC) class II antigens, since this antigen plays a key role in presentation of nonself antigens to helper T-cells. Nonself or foreign antigens such as viruses are first incorporated into antigen-presenting cells, and, after modification of the foreign antigen, it is presented to helper T-lymphocytes. The most important point at this stage is that helper T-cells can recognize foreign antigens only in the context of MHC class II antigens. However, the textbook of basic immunology says that MHC class II antigen is detected only in a limited type of cells in the body. They are B-lymphocytes, macrophages, dendritic and other antigen presenting cells and endothelial cells.

Other types of cells normally do not express MHC class II antigen. In the cryostat section of normal thyroid tissue stained with monoclonal anti-HLA-DR, the positive cells were only macrophage-like cells and endothelial cells, confirming the textbook of basic immunology. However, when we collected frozen blocks of thyroid tissue from patients with autoimmune thyroid diseases and when we cut and stained the sections with anti-HLA-DR, we were surprised, excited, and fascinated by bright green fluorescence for HLA-DR antigens on thyroid follicular cells (Figure 1). This was the first demonstration of expression of HLA-DR antigen on target cells in autoimmune endocrine diseases. This unforgettable slide was the start of our DR story.

After confirming that thyrocytes of patients with Graves’ disease and Hashimoto’s thyroiditis express DR antigen in vivo, we then analyzed “what factor or factors induce this DR expression?” To answer this question, we first cultured normal thyroid cells negative for DR. The cells expressing DR in this culture were again only macrophage-like cells. In the attempt to induce DR expression in these DR-negative thyrocytes, we added various factors in the culture medium. When we added lectins or gamma-interferon in this culture, bright green dots could be detected on the surface of thyroid cells using monoclonal anti-DR,
indicating that DR antigen was newly expressed by lectins or gamma-interferon in thyroid cells.\(^5\)

These findings lead us to study whether the same phenomenon could be seen in another possibly autoimmune-mediated endocrinopathy, that is, insulin-dependent diabetes mellitus (IDDM). Before studying islets of human IDDM patients, we first looked for expression of I-A antigen, one of mouse MHC class II antigens, in the islets of NOD mice, an animal model for insulitis-associated IDDM.

![Image of thyroid section](image)

**Fig. 1.** Cryostat section of the thyroid obtained from a patient with Graves' disease and stained with monoclonal anti-HLA-DR. Positive fluorescence is detected on the follicular cells.

Double staining for I-A and insulin in the islet of an NOD mouse showed that insulin-containing beta-cells were positive for I-A. Another double staining for I-A and glucagon revealed that glucagon-containing alpha-cells were virtually negative for I-A. Thus, it is concluded that NOD mouse islet cells express MHC class II antigen and the expression is beta-cell specific.\(^3\)

We then studied which type of lymphocytes are responsible for the induction of insulitis in the NOD mouse. This experiment showed that Lyt2\(^+\) cell-depleted fraction, that is L3T4\(^+\) helper T-cells were necessary for the transfer of insulitis in NOD mice.\(^4\) This result is interesting, since L3T4\(^+\) helper T-cells can recognize antigens only when associated with class II MHC molecules. Thus, it could be possible that L3T4\(^+\) helper T-cells recognize beta-cell-specific antigen together with I-A antigen to initiate an immune reaction against beta-cells.
NOD mice have provided a useful model for IDDM, but still human IDDM is different in various aspects from animal models. And we still do not know whether autoimmunity is the central mechanism for destroying beta-cells, and thus, we still do not know whether the trial of immunosuppressive therapy in human IDDM is reasonable or not.

After several years of careful consideration and discussions, we decided to perform pancreas biopsy under laparoscopy in newly diagnosed IDDM patients. The main purpose of the test is to select patients for immune intervention therapy by directly detecting lymphocyte infiltration in the islets. We are lucky enough to have in our department excellent hepatologists who have more than 6000 experiences of laparoscopy.

We were first disappointed to see that islets obtained by pancreas biopsy from a newly diagnosed IDDM patient were negative for HLA-DR, although endothelial cells surrounding these islets showed increased expression of DR antigen. However, the counterstaining of these islets with anti-glucagon antibody revealed that these islets are consisted mainly of glucagon-positive alpha-cells.

After cutting many sections from one end of several biopsy blocks to the other, and after searching a number of sections for insulin-containing beta-cells, we finally found out an islet. The insulin staining confirmed that this particular islet is consisted mainly of beta-cells. And the counterstaining of the same islet confirmed that this islet is also positive for HLA-DR antigen. So, now, the evidence of MHC class II antigen expression in target cells

![Diagram](image)

**Fig. 2.** Scheme for the possible induction of autoimmune reaction against the 'self' endocrine cells through the newly expressed MHC class II antigen (right panel) compared with the normal immune response against the 'nonself' or 'foreign' antigen (left panel).
was obtained, first, in autoimmune thyroid diseases including Graves’ and Hashimoto’s thyroiditis, second, in NOD mice, and finally, in human IDDM. Given the fact that target cells of autoimmune endocrinopathies express class II antigens, the next question is obvious: what does it mean?

Our hypothesis to answer this question is illustrated in Figure 2. As shown in the very first part of my talk, helper T-cells can recognize nonself or foreign antigens when MHC class II antigen is also present. If so, it is now reasonable to speculate that autoreactive helper T-cells can recognize endocrine cell specific self antigens, provided newly expressed MHC class II antigen is present together with endocrine cell specific antigens on the surface of endocrine cells.\(^1\)

The direct demonstration of the presence of both MHC class II antigen and endocrine cell specific antigen on the surface of endocrine cells is clearly shown in the thyroid monolayers cultured with gamma-interferon and double-stained for HLA-DR and thyroid cell specific microsomal antigen, now thyroid peroxidase. DR antigen and microsomal antigen are both detected on the surface of the same thyroid cells. These are the two components necessary and sufficient to activate helper T-cells. Once helper T-cells are triggered, then, further immune reactions take place; such as activation of cytotoxic T-cells to kill target cells or activation of B-lymphocytes to produce antibodies. And the final outcome is inevitable. That is the destruction of target cells followed by the occurrence of autoimmune endocrine diseases.

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References