INVITED COMMENTARY

Lymphocyte traffic and homing in autoimmune thyroid disorders

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Introduction

Human autoimmune thyroid disorders (AITD) – Graves’ disease (GD) and Hashimoto’s thyroiditis (HT) – are characterized by reactivity to self thyroid antigens, which may be expressed as destructive inflammatory or anti-receptor autoimmune diseases (1–3). In AITD, the autoimmune process is probably initiated by the activation of autoreactive T cells by an MHC II/self antigen complex and a co-stimulatory signal that enables full activation of T cells (1). T cell activation is followed by the expression of cell surface antigens and cell adhesion molecules that allows the homing of immune cells onto their target organs (1, 2). This invited commentary emphasizes the major events regulating lymphocyte traffic in AITD resulting in targeting of circulating lymphocytes to the target organs: the thyroid and the orbit. The role of adhesion molecules in regulating leukocyte localization in AITD will also be reviewed in a broader perspective.

Cellular immune response in AITD

Cellular immune responses have been studied in circulating and infiltrating lymphocytes in AITD. Intrathyroidal lymphocytes seem to play a prominent role in the pathogenesis of AITD not only through thyroid antigen recognition, but also mediating important inflammatory effects, such as the release of cytokines (1, 2). In addition, studies in circulating populations are also important because immunological disturbances in AITD are not solely limited to the thyroid gland, as the orbit and occasionally the skin can also be affected (1). This multiorgan involvement and the association of AITD with other autoimmune diseases suggest a generalized immune disregulation, not solely limited to the thyroid gland. Numerous and often non-concordant reports in the literature have described changes in the pattern of circulating and infiltrating lymphocytes in AITD. A possible explanation for these divergent results may be different methods used for the study, including flow cytometric analysis, immunohistochemical methods, production of thyroid-derived T cell lines or T cell cloning (4, 5). In addition, studies have been performed at different stages of active or inactive AITD: some studies have compared peripheral blood lymphocytes (PBL) from untreated patients with controls, whereas others have compared peripheral blood and thyroid cells from the same patients. Finally, as thyroid fine-needle aspiration and biopsies are very rarely performed in patients with AITD, the data available are mostly obtained from surgical tissue that probably does not reflect the situation present in vivo (6). However, some universal features have been described in the cellular immunity in AITD. PBL from untreated Graves’ patients usually show an activated phenotype. Activation of human T cells causes transient and sequential appearance of specific cell-surface structures, such as the early activation antigen CD69, MHC class II antigens (HLA-DR) and receptors for growth factors such as interleukin-2 (CD25) and transferrin (CD71) (7). Increased numbers of circulating HLA-DR+ and CD69+ T cells have commonly been found in patients with AITD of recent onset, but not in patients in remission (8–10). However, CD25 and CD71 structures have not been found to be increased in PBLs of these patients (9, 10). In addition, activated T cells directed against thyroidal antigens have been described in peripheral blood from patients with GD (11). Intrathyroidal lymphocytes, when compared with PBLs, expressed an increased percentage of HLA-DR and CD69 molecules (6, 12). These activated cells are found mainly in interstitial and intraepithelial locations. Not only do peripheral blood GD lymphocytes show an activated phenotype, but they also have a reduced percentage of blood CD8+ T cells (13). Hyperthyroidism may itself be partly responsible for this reduced CD8 percentage, due to a metabolic effect of thyroid hormones on T cells (13). In contrast, intrathyroidal lymphocytes show an increased fraction of CD8+ lymphocytes, mainly in interstitial and intraepithelial locations (12, 14).

Although previous assessments of natural killer (NK) populations in peripheral blood have been discrepant (6, 15), most reports agree that the percentage of NK cells is decreased in intrathyroidal lymphocytes (15). Lower percentages of CD16/56+CD3- cells have recently been reported at diagnosis in PBLs of GD patients who had an unfavorable response to antithyroid therapy, when compared with patients with a favorable prognosis.
The differential expression of CD45 isoforms has been used to characterize functionally distinct T cell subsets, termed naive (CD45RA+) and memory activated (CD45RO+) cells (17). T cells that have not yet encountered antigen are termed naive and migrate almost exclusively through lymph nodes and other secondary lymphoid tissue (18). This pattern of migration ensures that the initial contact of naive T cells with antigen takes place in the lymph node where professional antigen-presenting cells can present antigen optimally. After activation by specific antigen, the T cell switches from the resting state to a memory or effector cell, primed to respond rapidly to the same specific antigen the next time an encounter occurs. An increased percentage of naive CD45RA+ cells has been reported in PBLs in untreated GD and HT patients (9). By contrast, mononuclear cells infiltrating the thyroid and the orbit inAITD patients showed a marked increase in CD45RO antigen (6, 19–22). This memory phenotype in intrathyroidal lymphocytes could represent cells that have been previously activated, typically by exposure to antigen, and migrate to the specific target organ (18).

Human CD4+ lymphocytes may be functionally distinguished by the pattern of cytokine secretion (TH1 and TH2) (23). CD4+ TH2 cells stimulate a strong B cell response, whereas CD4+ TH1 cells activate cytotoxic mechanisms. Although only a small number of studies have been performed, there is some evidence that peripheral blood TH1 cells are predominant in HT, whereas T cells with a less restricted range of cytokines are present in GD (6, 24).

**Adhesion molecules in AITD**

Adhesion molecules regulate the transendothelial migration of leukocytes to lymphoid and non-lymphoid tissues. The process of leukocyte extravasation requires a cascade of sequential adhesive events between leukocytes and endothelial cells (EC). The initial leukocyte–EC interaction is mainly mediated by selectins which interact with carbohydrate moieties linked to mucin-like proteins. The selectin–mucin interaction causes the rolling of leukocytes along endothelium (25). The rolling leukocytes are stimulated by locally produced chemokines which cause a conformational change of the integrin adhesion receptors (activation) that increases the affinity by their ligands, members of the immunoglobulin superfamily (26). The action of integrins is a key event that results in the firm adhesion of leukocytes to the vessel wall. Finally, leukocytes squeeze themselves between ECs and move to the inflammatory foci (27, 28). Each one of these adhesive events is absolutely essential for the whole process of leukocyte extravasation. A key feature is that selectin–mucin, chemoattractant receptor, and integrin–immunoglobulin family interactions act in sequence, not in parallel. Combinatorial use of multiple adhesion and chemoattractant receptors with distinct distributions on leukocyte subsets could regulate the selectivity of leukocyte localization in vivo (29).

Research on adhesion molecule pathophysiology in AITD has focused on characterizing adhesion molecule expression, identifying adhesive interaction essential to development and progression, and determining whether modulation of adhesion molecule expression influences disease severity. Adhesion molecule data are frequently obtained after clinical disease is established, perhaps masking adhesive interactions responsible for initiating and promoting accumulation of autoreactive cells.

Intrathyroidal lymphocytes of AITD patients express an increased number of various adhesion molecules of the integrin and immunoglobulin superfamilies, including lymphocyte function-associated antigen (LFA)-1α, ICAM-1, VLA-1β, β1 (CD29), very late antigen (VLA)-1 (CD49a), -4 (CD49d), -5 (CD49e), intercellular adhesion molecule (ICAM)-1, and ICAM-3 when compared with the corresponding PBLs (19, 21). Not only thyroid infiltrating cells but also mononuclear cells infiltrating the orbit express LFA-1 (30). With regard to PBLs, untreated GD patients have significantly less LFA-1 (31, 32), CD18 and CD29 (32) expression than controls. These findings could be related to the increased migration of LFA-1+ cells from peripheral blood to the thyroid gland or the orbit. The differential expression of adhesion molecules in certain lymphocyte subsets may determine their homing to sites of inflammation where the autoimmune process takes place, the thyroid and the orbit in AITD.

ECs can also express adhesion molecules, which are central to the recognition of targets by autoreactive T lymphocytes. Thyroid ECs from patients with AITD bear an enhanced expression of various molecules of the immunoglobulin and selectin families including ICAM-1, vascular cell adhesion molecule (VCAM)-1, P-selectin and E-selectin (33–35). Vascular endothelium in retro-ocular tissues in Graves’ ophthalmopathy has also revealed a strong immunoreactivity for ICAM-1, LFA-3, E-selectin and VCAM-1, not present in normal retro-ocular tissues (22). In addition, ICAM-1 expression has been induced after in vitro exposure to several cytokines of retro-ocular fibroblasts of GD patients (30). All these findings together suggest that the LFA-1/ICAM-1, VLA-4/VCAM-1, and selectin adhesion pathways are probably predominant in leukocyte adherence to and migration through the endothelium of both the thyroid and the orbit in AITD (19, 21, 22).

Thyrocytes might function as antigen-presenting cells and present their own antigens via aberrant expression of human histocompatibility class II (36). Nevertheless, antigen presentation by class II molecules is not sufficient to activate autoreactive T cells, and concurrent expression of co-stimulatory signals is

(16). If these results are confirmed, NK cells could be used as potential markers of the response to antithyroid therapy.
necessary for T cell stimulation. In the absence of these signals class II expression may have a protective role, inducing T cell anergy. Although thyroid follicular cells inAITD express class II molecules, there is no conclusive evidence that they express co-stimulatory signals. In certain reports, expression of the adhesion molecules ICAM-1 and LFA-3 has been reported both in vivo and in vitro in thyrocytes from AITD patients, nevertheless, other reports have not confirmed these findings (37, 38). However, B7–1 and B7–2 co-stimulatory molecules have not been detected or induced on thyrocytes in AITD (39). Because thyrocytes constitutively express few adhesion molecules and have weak activity in antigen presentation to T cells, bystander professional antigen-presenting cells, such as dendritic cells (DC) or monocytes, could help thyrocytes to deliver co-stimulatory signals and synergistically amplify antigen presentation to T cells (3). DCs which present antigen more efficiently than any of the other antigen-presenting cells, are likely to help in perpetuating the autoimmune process. They have been identified as the first immune cells invading the thyroid gland in AITD (40). DCs have been found to express ICAM-1, VCAM-1 and E-selectin in AITD (33, 34). This pattern of expression, as well as their increased number suggest an active involvement in antigen presentation to intrathyroidal B and T lymphocytes in AITD (40, 41).

On the other hand, cytokines produced by infiltrating mononuclear cells could play a role in HT pathogenesis through induction of apoptosis. In this regard, interleukin-1, abundantly produced by infiltrating cells in HT glands, can induce Fas expression in normal thyrocytes. As the Fas ligand is constitutively expressed by thyrocytes, cross-linking of Fas with Fas ligand can induce a massive thyrocyte apoptosis (42).

Together these observations help us to understand the immune mechanisms responsible for tissue infiltration in GD and HT, and the possible role of adhesion molecules in directing T cell traffic to the thyroid gland and the orbit, not only involving its localization, but also their activation and proliferation, and the persistence of the immunologic reactivity. It is likely that interference of these mechanisms could provide new approaches in the therapy of AITD.

Concluding remarks

Although phenotypic studies on PBLs in AITD are often discordant, probably because in organ-specific diseases the most reliable reflection of the autoimmune disease state lies in the target organ, most studies have found an activated phenotype, reflecting a generalized immune disregulation. Studies of these abnormalities could prove useful in the future as prognostic indices of a future response to antithyroid therapy.

The endothelium, positioned at the interface between blood and the target tissues of the autoimmune process, probably plays a pivotal role in AITD, controlling the influx of inflammatory cells. The increased expression of adhesion molecules of the immunoglobulin superfamily within the vascular endothelium facilitates the recruitment of activated, memory phenotype lymphocytes with an upregulated expression of various adhesion molecules to the thyroid and the orbit. These lymphocytes mediate inflammatory and autoimmune reactions.

DCs, which are capable of presenting antigen more efficiently than any of the other antigen-presenting cells, could help thyrocytes in initiating and perpetuating AITD, contributing to homing of autoreactive lymphocytes.

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