The expression of the beta 1 integrin CD29 and the beta 2 integrin CD11b is decreased in peripheral blood lymphocytes from Graves’ disease patients

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Abstract

We have prospectively examined the percentage of peripheral blood lymphocytes which expressed adhesion molecules in untreated Graves’ disease patients. Eighteen patients with Graves’ disease, twenty-four patients with Hashimoto’s thyroiditis and thirty-two sex- and age-matched healthy control subjects were studied. The expression of the lymphocyte adhesion molecules beta-1 integrin CD29, beta-2 integrin CD11b and L-selectin Leu8 (CD62L) was analyzed by cytofluorometry. A decreased percentage of CD29+ and CD11b+ lymphocytes was observed in hyperthyroid patients in comparison with Hashimoto’s thyroiditis patients and healthy controls. However, there was no difference in the percentage of CD62L+ lymphocytes in the three groups. Percentages of lymphocyte activation markers, hyperthyroid status, presence or absence of ophthalmopathy or serum levels of antithyroid antibodies were not related to the proportions of CD29+ or CD11b+ lymphocytes. Four Graves’ patients required radical therapy but after the treatment, there was no modification in the percentages of CD29+ and CD11b+ lymphocytes compared with those determined at diagnosis. Our findings suggest that the decrease in beta-1 and beta-2 integrins could be a predisposing marker of development of Graves’ disease.

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Introduction

Graves’ disease (GD) and Hashimoto’s thyroiditis (HT) are autoimmune thyroid diseases characterized by the presence of various degrees of lymphocyte infiltration in the thyroid gland (1). The density of infiltrating lymphocytes and the proportion of those with a cytolytic function are lower in the thyroid glands of GD patients compared with those of HT patients (2–5).

The infiltration of the thyroid is the result of the regulation of the expression and function of a set of adhesion receptors on both leukocytes and endothelial cells. Three main groups of adhesion receptors are involved in these interactions: the integrin and selectin and the immunoglobulin supergene families (6). Integrins constitute a family of widespread alpha-beta heterodimeric adhesion receptors, grouped in four distinct subfamilies based on beta subunit utilization (7). Members of the beta-1 subfamily contain a common beta-1 chain (CD29). The beta-2 integrin subfamily consists of the association of either CD11a, CD11b or CD11c with the-beta-2 subunit (CD18). Both beta-1 and beta-2 integrins interact with counter-receptors from the immunoglobulin supergene family, such as the intercellular adhesion molecules (ICAM-1, ICAM-2). The selectins (L-selectin, P-selectin, E-selectin) are predominantly implicated in the adhesion to high endothelial venules of lymphatic nodes (8, 9).

Recently, increased attention has been focused on adhesion molecules in autoimmune thyroid diseases (10–17). The expression of ICAM-1 has been shown to be upregulated on thyroid peril follicular endothelial cells (10), fibroblasts (10) and thyrocytes (11), although the expression in Graves’ thyroid glands is lower than that in HT (12). P-selectin and E-selectin expression has been detected on thyroid peril follicular endothelial cells (13, 14).

Analyses of the expression of adhesins on the surface of peripheral blood lymphocytes are scarce. A decreased expression of lymphocyte function-associated antigen-1 (LFA-1), a member of the beta-2 integrin family (CD11a), has been demonstrated in peripheral blood lymphocytes from patients with GD, but not in HT (15). Likewise, the percentage of thyroid-infiltrating LFA-1+ lymphocytes in patients with GD is lower than that observed in the glands of patients with HT (5, 12).

In this study, we examined the expression of the beta-1 integrin marker CD29, the beta-2 integrin CD11b and
the L-selectin Leu8 (CD62L) on the peripheral blood lymphocytes in untreated patients with GD and HT, before and after the euthyroid state was obtained.

**Patients and methods**

**Patients**

We prospectively studied 18 patients with GD (2 men, 16 women; mean age: 33.8 ± 15.2 years, range: 15–62 years), 24 patients with HT (all of them women, mean age: 36.6 ± 16.9 years, range: 14–67 years) and 32 sex- and age-matched healthy control subjects with no personal or familial history of thyroid or autoimmune disease (2 men, 32 women; mean age: 35.1 ± 14.5 years, range: 18–55 years). The diagnosis of GD was established on the presence of thyrotoxicosis, low levels of thyrotropin (TSH) (0.07 ± 0.09 mU/l), high thyroid hormone levels (free thyroxine (T4): 57.1 ± 16.5 pmol/l, free tri-iodothyronine (T3): 19.9 ± 13.4 pmol/l) and increase of gammagraphic 99mTc or 131I uptake (18). Every patient had a goiter and at least one of the three antithyroid antibodies tested (antibodies against receptor for thyroid-stimulating hormone (TBII) present in 12 cases, 66.7%, mean titer: 425.5 ± 65.2 IU/l; antithyroid peroxidase (anti-TPO) antibodies present in 14 cases, 77.8%, mean titer: 2091.9 ± 2416.5 IU/ml or antithyroglobulin antibodies present in 8 cases, 44.4%, mean titer: 425.5 ± 551.0 IU/l). Infiltrative ophthalmopathy, defined according to classical criteria (18), was detected in 6 patients (37.5%). The diagnosis of HT was based on commonly accepted cytological criteria (18). Anti-TPO antibodies were present in all the HT patients (mean titer: 3153.0 ± 2287.1 IU/l). Of 24 patients with HT, 12 were euthyroid and 12 were hypothyroid (7 with subclinical and 5 with clinical hypothyroidism).

The patients had to comply with the following conditions: (i) the absence of clinical evidence of disease, other than thyroid autoimmune disease and (ii) the absence of previous or actual treatment. Informed consent was obtained from patients and controls.

**Thyroid hormones and autoantibodies**

Serum free T4 levels (FT4), serum free T3 levels (FT3) and TSH were measured with radioimmunoassay kits from Diagnostic Products Corporation (Los Angeles, CA, USA). The serum levels of TBII, anti-TPO or antithyroglobulin antibodies were measured using radioimmunoassay kits from Henning Berlin GmbH (Berlin, Germany).

**Immunological marker analysis**

Peripheral blood samples were analyzed by direct immunofluorescence, as previously described (19). Monoclonal antibodies directed against membrane antigens were employed to characterize the following molecules: anti-CD3 (pan-T), anti-CD25 (CD25 is the p55 subunit of the interleukin-2 receptor), anti-CD45RA (naive cells), anti-CD45RO (memory cells), anti-CD29 (anti-beta-1 integrin), anti-CD11b (anti-beta-2 integrin), and anti-Leu8 (anti-L selectin). All of them were purchased by Becton-Dickinson (Mountain View, CA, USA).

**Study schedule**

Analysis of lymphocyte markers was performed at the first visit. Then, GD patients were treated with methimazole, 15 mg/day, until euthyroidism was achieved. Levothyroxine was added if hypothyroidism appeared. Patients were followed-up for 24 months after the diagnosis. Fourteen patients achieved control of hyperthyroidism by receiving methimazole for 12 months and remained euthyroid after the suppression of treatment (GD patients with favorable outcome). At the 12th week, when a euthyroid state had been obtained, lymphocyte subpopulations were re-evaluated. The rest of the patients (4 cases) required radical treatments (131I in three cases: surgery in one case) because of clinical and analytical manifestations of hyperthyroidism despite antithyroid therapy (GD patients with unfavorable outcome).

**Table 1**

Proportions of peripheral blood lymphocyte subsets in healthy controls, and in patients with Graves’ disease and Hashimoto’s thyroiditis. Values are means ± s.d. and are expressed in cells/mm³ (for total lymphocytes) and in percentages (for lymphocyte subsets).

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 32)</th>
<th>Graves’ disease (n = 18)</th>
<th>Hashimoto’s thyroiditis (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lymphocytes</td>
<td>2103.7 ± 414.9</td>
<td>2354.3 ± 857.0</td>
<td>1965.0 ± 574.0</td>
</tr>
<tr>
<td>CD3 + CD29+</td>
<td>48.2 ± 8.9</td>
<td>39.1 ± 8.5*</td>
<td>46.6 ± 8.9</td>
</tr>
<tr>
<td>CD3 + CD11b+</td>
<td>23.9 ± 8.5</td>
<td>16.2 ± 7.1†</td>
<td>23.9 ± 8.1</td>
</tr>
<tr>
<td>CD3 + CD62L+</td>
<td>36.6 ± 10.5</td>
<td>33.3 ± 5.6</td>
<td>34.0 ± 6.9</td>
</tr>
<tr>
<td>CD3 + CD25+</td>
<td>5.2 ± 6.5</td>
<td>8.7 ± 4.8†</td>
<td>10.6 ± 2.4†</td>
</tr>
<tr>
<td>CD3 + CD45RA+</td>
<td>34.9 ± 7.0</td>
<td>39.2 ± 6.0</td>
<td>38.3 ± 6.3</td>
</tr>
<tr>
<td>CD3 + CD45RO+</td>
<td>36.9 ± 7.9</td>
<td>33.2 ± 6.8</td>
<td>32.0 ± 7.5</td>
</tr>
</tbody>
</table>

*P < 0.01, indicating significant differences between Graves’ disease and both healthy controls and Hashimoto’s thyroiditis; †P < 0.01, indicating significant differences between both Graves’ disease and Hashimoto’s thyroiditis and healthy controls.
outcome); these patients were also re-evaluated three months after radical therapy, with euthyroidism achieved in every case.

**Statistical analysis**

Comparison of the percentages of adhesins-expressing lymphocytes in the different groups was carried out using the Mann-Whitney U test. Significance of parameters within each group was tested by the Wilcoxon matched-pairs signed rank test. For correlation studies, linear-regression analysis with the least squares method was used. A P value <0.05 was considered to indicate a significant difference between groups.

**Results**

The percentage of both CD29+ and CD11b+ lymphocytes was lower in GD patients in comparison to the HT and healthy control groups. There was no significant difference between the proportions of both lymphocyte markers in HT, either euthyroid or hypothyroid, and healthy controls. The percentage of CD62L+ lymphocytes was similar in the GD, HT and healthy control subjects (Table 1, Fig. 1).

The analysis of expression of activation antigens on T cells from patients and controls showed that the percentage of CD3+CD25+ lymphocytes was increased in GD and HT patients. The percentages of naive (CD45RA+ cells) and memory (CD45RO+ cells) lymphocytes were similar in patients and controls (Table 1). No significant correlation was detected between the proportions of lymphocytes expressing adhesion molecules and those of lymphocytes expressing CD25 (relationship between CD25+ and CD29+ lymphocytes: r = 0.43; between CD25+ and CD11b+ lymphocytes: r = 0.44; between CD25+ and Leu8+ lymphocytes: r = 0.13; P > 0.05 in each case) or CD45RO antigens (relationship between CD45RO+ and CD29+ lymphocytes: r = 0.54; between CD45RO+ and CD11b+ lymphocytes: r = 0.45; between CD45RO+ and Leu8+ lymphocytes: r = 0.17; P > 0.05 in each case).

In GD patients, the euthyroidism induced by methimazole or radical therapy was not linked to significant changes in CD29+, CD11b+ or CD62L+ lymphocytes percentages in GD (Table 2). Also, in HT patients with clinical hypothyroidism, similar values of lymphocyte-expressing adhesins were detected prior to and after treatment with l-thyroxine (Table 2).

No correlation was detected between the percentages of lymphocyte subsets and serum levels of FT4, FT3, TSH, TBII, anti-TPO and antithyroglobulin antibodies (Table 3).

There was no statistical difference between the percentages of CD29+, CD11b+ or CD62L+ lymphocytes in patients with or without infiltrative ophthalmopathy (CD29+: 39.9 ± 9.5 vs 37.2 ± 6.0; CD11b+: 17.3 ± 7.6 vs 15.8 ± 4.9; Leu8+: 42.2 ± 5.7 vs 45.9 ± 4.9; P > 0.05 in each case).

At diagnosis, there was no statistically significant difference in the proportions of cells expressing adhesins

**Figure 1** Percentages of CD3+ lymphocytes expressing (A) CD29, (B) CD11b and (C) CD62L antigens in healthy controls (●), Graves’ disease patients (●) and Hashimoto’s thyroiditis patients (▲). Significant differences were detected between the means of the percentages of CD29+ and CD11b+ lymphocytes from Graves’ disease patients and both healthy controls and Hashimoto’s thyroiditis patients (P < 0.01).
in groups with favorable or unfavorable outcome, although a tendency for lower percentages of CD29+ and CD11b+ lymphocytes was detected prior to treatment in the group of patients requiring more definitive therapy. After radical or medical therapy, patients were re-evaluated for their percentages of CD29+, CD11b and CD62L+ lymphocytes. There were no significant changes in the proportions of cells expressing these antigens when compared with the percentages obtained at diagnosis of the entity (Table 2).

Discussion

Our results have demonstrated a decreased percentage of lymphocytes expressing the beta-1 integrin marker CD29 and the beta-2 integrin CD11b, but not the L-selectin Leu8, in GD patients. The different expression of L-selectin and integrins in GD patients is not surprising: studies analyzing monocyte adhesion to vascular endothelium have demonstrated the involvement of selectins without initial contribution of integrins (20), suggesting a different modulation of these adhesins. Leu8 expression is linked to the lymphocytes’ ability to migrate to lymphatic nodes. After activation, lymphocytes lose Leu8+ expression (acquiring different receptors, such as some integrins). Leu8—lymphocytes have the capacity to migrate to extralymphatic territories (8, 9). The normality of Leu8+ lymphocytes (and alternatively of Leu8—lymphocytes) supports the ability of these to link to counter-receptor antigens on thyroid endothelium. Although GD and HT are organ-specific autoimmune diseases, characterized by intrathyroidal lymphocytic infiltration (1), our studies were performed on peripheral blood. Nevertheless, disturbances in GD and HT are not solely limited to the thyroid gland (21). Moreover, data from thyroid tissue are representative of that group of GD patients who require surgical therapy because of either uncontrolled hyperthyroidism or a relapse of GD. However, they are not representative of the GD or HT patients overall. Previous studies have reported an increased percentage of intrathyroidal lymphocytes expressing LFA-1 and CD11b molecules in autoimmune thyroid disorders when compared to normal controls, more marked in HT than in GD glands (4, 5, 12, 22–24). These findings imply that the decreased integrin expression in peripheral blood lymphocytes could probably be attributed to an increase in migration of CD29+ and CD11b+ to the thyroid gland.

In healthy subjects expression of these integrins is associated with cytokine-mediated lymphocyte activation and acquisition of a memory (CD45RO) phenotype (25, 26). Neither the percentage of activated (CD25+) nor of memory (CD45RO+) CD3+ lymphocytes was lower in GD patients. This finding suggests an abnormal regulation of the expression of integrin molecules in GD.

Peripheral blood cells expressing LFA-1, a member of the beta-2 integrin family (CD11a), are fewer in patients with GD, but not in patients with HT (15). CD29, CD11a

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Proportions of peripheral blood lymphocytes expressing adhesins in patients with Graves’ disease and Hashimoto’s thyroiditis, pre- and post-treatment. Values are means ± S.D. and are expressed in cells/mm³ (for total lymphocytes) and as percentages (for lymphocyte subsets).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graves’ disease (n = 16)</td>
<td>Hydrothyroid (glycol-T4)</td>
</tr>
<tr>
<td>Favorable prognosis</td>
<td>Unfavorable prognosis</td>
</tr>
<tr>
<td>Hypothyroid (pre-MMI)</td>
<td>Hypothyroid (post-MMI)</td>
</tr>
<tr>
<td>Total lymphocytes</td>
<td>2344.3 ± 667.8</td>
</tr>
<tr>
<td>CD3+</td>
<td>40.0 ± 7.8</td>
</tr>
<tr>
<td>CD3+ + CD29+</td>
<td>17.3 ± 6.9</td>
</tr>
<tr>
<td>CD3+ + CD11b+</td>
<td>30.0 ± 4.8</td>
</tr>
<tr>
<td>CD3+ + CD62L+</td>
<td>33.0 ± 7.1</td>
</tr>
<tr>
<td>MMI, methimazole; L-T4, levothyroxine.</td>
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</tr>
</tbody>
</table>
and CD11b antigens mediate the binding of leukocytes to endothelial cells via ICAM-1 (12). Taken together, these deficits could be implicated in the decreased lymphocyte infiltration in the thyroid gland of GD patients compared with HT patients (2, 5, 22). It is possible to hypothesize that the decreased expression of integrins is a predisposing condition to GD after an adequate antigenic stimulation of the immune system. The absence of modifications in the percentages of CD29+ and CD11b+ lymphocytes after the elimination of putative antigens supports this theory. Moreover, it has been observed that the congenital defect of CD11b and LFA-1 expression is associated with autoimmune thyroid diseases (27).

**Acknowledgements**

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