Abstract. Several studies demonstrated a relationship between HLA-B8 and -DR3 and the early course of thyroid function after treatment of thyrotoxicosis. However, the association between certain DR antigens and the outcome of thyroid function years after radioiodine treatment for Graves' disease remains unclear.

We therefore determined the HLA pattern in 2 groups of female patients with different severity of hypothyroidism. From a total of 45 patients, 27 had developed pre-clinical hypothyroidism (normal serum levels of T₄, FT₄ and T₃, normal or elevated basal TSH levels, but an exaggerated TSH response to TRH, Group A). Mean follow-up was 111 months (range 36–360 months) for this group. Eighteen patients had become overtly hypothyroid (T₄ and FT₄ levels in the hypothyroid range and an elevated basal TSH concentration, group B) after a mean interval of 51 months (range: 4–132 months) following treatment. Eighty-seven healthy blood donors served as controls. Positive plasma antibody titres (tanned red cell haemagglutination technique) were observed in 67% of all patients with a preponderance in group B (83% versus 56% in group A, n.s.). The whole group of Graves' disease patients showed the antigens B8, DR3 and DRw6 in 37.8%, 33.3% and 35.6%, respectively (P < 0.02, < 0.05, and < 0.04 vs controls). In patients with pre-clinical hypothyroidism there was a significantly increased prevalence of antigen B8 (P < 0.01) and DR3 (P < 0.05) compared to the control group. In contrast, the overt hypothyroid group showed an augmented frequency of HLA-DRw6 (P < 0.04). Antibody positivity was related to the antigens B8 and DR3 (P < 0.005, < 0.05, respectively). Thus, the presence of B8/DR3 represents a genetic pattern possibly protecting against the development of overt hypothyroidism, at least over several years after ¹³¹I-treatment. The presence of DRw6 and the absence of DR3 were associated with an increased risk for overt post-irradiation hypothyroidism.

Autoimmune thyroid disease is related to organ specific deficiency in suppressor T-lymphocytes followed by an immune response to autoantigens (Strakosch et al. 1982; Mitchison 1985). The human leucocyte antigen (HLA) system seems to be involved in the control of such immune responses (Farid & Bear 1981; Mitchison 1985). The association between HLA-B8 and -DR3 with Graves' disease in Caucasoids is generally accepted (Farid & Bear 1981; Farid et al. 1975; Chopra et al. 1977; Bech al. et 1977; Lamberg 1979). Initially B8 and later DR3 were related to low remission rates of hyperthyroidism after antithyroid therapy (Bech et al. 1977; Farid et al. 1980; Davies et al. 1982; McGregor et al. 1980). This finding was, however, not observed in all centers (Dahlberg et al. 1981; McKenna et al. 1982; Allanic et al. 1983). Moreover, the prediction of outcome of treatment

HLA-DR3 and DRw6: prognostic factors for the incidence of hypothyroidism in Graves' disease after radioiodine treatment

B. Althaus¹, J. J. Staub¹, T. M. Neri³, M. Hauenstein, J. Müller-Brand², W. Berger¹ and N. R. Farid⁴

Divisions of Endocrinology and Metabolism¹, Department of Medicine and Nuclear Medicine², University Hospital of Basel; Institute of Immunology³, Basel, Switzerland and The Memorial University of Newfoundland⁴, St. John's, Canada

This work was presented in part at the 7th Int Congr Endocrinol, July 1984, in Quebec, Canada, and was published in Abstract form (abstract 93).
with $^{131}$I (Farid et al. 1980) could predominantly be related to presence of antibodies to TSH receptor rather than that of DR3 (Davies et al. 1982). It is open to debate whether there is a relationship between certain DR antigens and the outcome of thyroid function years after treatment for Graves’ disease.

The aim of the present study was to examine the HLA pattern in different grades of hypothyroidism (i.e. pre-clinical and overt hypothyroidism) in the long-term follow-up of radioiodine treated Graves’ disease. Our special interest was to determine whether there is any relation between the HLA pattern and different grades of hypothyroidism years after radioiodine treatment.

Patients and Methods

We examined 45 patients with Graves’ disease previously treated with radioiodine ($^{131}$I) (41 patients without any other form of treatment, 4 patients had additional subtotal thyroidectomy) for subsequent development of various degrees of hypothyroidism. In order to obtain a more homogeneous population we included only female patients in our study.

Initially the diagnosis of hyperthyroid Graves’ disease was made on the basis of the typical history, clinical signs of hyperthyroidism and elevated levels of thyroxine (T₄), free T₄ and triiodothyronine (T₃), measured by radioimmunoassay as previously described (Staub et al. 1978). In less severe cases an absent response to oral TRH was demonstrated. Most patients had a diffuse enlargement of the thyroid; in some subjects a palpable goitre was absent. The scintiscan showed homogeneous uptake of $^{131}$I in every case. Patients with nodular goitre and/or inhomogeneous appearance on scintiscan were excluded from the study. Thirteen patients had endocrine ophthalmopathy.

Group A

Twenty-seven patients developed pre-clinical hypothyroidism as defined previously (Staub et al. 1983). They showed normal serum levels of T₄, FT₄, and T₃, normal or elevated basal TSH levels but an exaggerated TSH response to oral TRH (normal peak TSH 3 h after oral TRH in 32 healthy female volunteers: xlog 16.8 mU/l; ± 2 SD log = 7.5–37.3 mU/l). These patients received no hormone replacement therapy.

Group B

Eighteen patients with overt hypothyroidism (T₄, FT₄ levels in the hypothyroid range, normal or diminished T₃ levels, and elevated basal TSH concentrations) received replacement therapy with thyroxine.

The mean age did not differ significantly in the two groups (56.8 ± 7 in group A vs 60 ± 10 years in group B, n.s.). The mean total dose of radioiodine administered was similar in both groups (6.0 ± 2 (range 4–10) vs 7.4 ± 3 (3–12) mCi; n.s.). The applied dose of $^{131}$I was calculated in each patient in order to achieve approximately 6000 rad in the thyroid tissue, taking into account the estimated weight of the thyroid gland, the effective half-life, and the maximal iodine uptake (Jackson 1975).

The volume of the thyroid was estimated by clinical palpation and by planimetry of the area of scintiscan. The grade of goitre size was classified following the criteria of the World Health Organization (Perez et al: WHO 1960): In group A 87% of patients had a goitre grade I or smaller, 13% showed a grade I to II. In group B 78% of patients had a goitre grade I or smaller, 14% a grade I to II and one patient showed a grade II goitre. The planimetry was performed using an automatic planimeter with 10 succeeding measurements calculating the mean of these 10 values. The mean area of scintiscan was quite similar in both groups of patients: the patients with pre-clinical hypothyroidism (group A) covered a mean area of 31.5 ± 8.7 cm² (range 17–50) vs 30.5 ± 8.7 cm² (range 12–41) in the group with overt hypothyroidism (n.s.).

The patients of group B developed overt hypothyroidism after a mean of 51 ± 36 months (range 4–132 months) following $^{131}$I-therapy compared to a mean follow-up time of 111 ± 65 months (36–360 months) in the group A ($P < 0.001$). Student’s t-tests were employed to make these comparisons.

HLA-typing

Fifty-six alleles of the 4 HLA loci A, B, C and DR were determined in each patient. A standard cytotoxicity method was used for typing of the loci A, B and C (Mittal 1978). DR antigens were tested on bone-marrow (B) derived lymphocytes after a prolonged cytotoxic incubation as previously described (Garotta & Neri 1981). Eighty-seven healthy blood donors of the same ethnical group and geographic area (Basle, Switzerland) served as controls (49 males, 38 females with an age range comparable to our patient groups). Antibodies to thyroglobulin (TGHA) and thyroid microsomes (MCHA) were determined by the tanned red cell haemagglutination technique (Thymone-T and -M Wellcome) in the laboratories of Drs E. and M. Viollier, Basel (Althaus et al. 1983). Determination of TSH receptor related antibodies was not available at the time of the study. Statistical analysis for HLA frequencies were done by the Fisher’s exact test (two-tailed). The relative risk (RR) was calculated according to the method of Woolf (in Svejgaard et al. 1974).
Results

1. Antibody frequency

Positive antibody titres were observed in 30 of the 45 patients (67%). Twenty-three patients showed only positive MCHA. Six patients had both elevated MCHA and TGHA titres. One patient had an augmented TGHA titre without MCHA.

In the group of patients with overt hypothyroidism after 131I-treatment we could not observe a significantly higher antibody frequency (83%) than in those with pre-clinical hypothyroidism (56%, P n.s.). The TGHA titres tended to be higher in overt hypothyroidism (28% with titres ≥ 1:160) compared to the pre-clinical hypothyroid subjects (4% with titres ≥ 1:160). MCHA titres did not differ in the two groups.

2. HLA pattern

Table 1A lists the antibody frequencies for B8, DR3 and DRw6 in all patients with Graves’ disease. The antigen B8 was found in 38% vs 16% in controls (P < 0.02), DR3 in 33% vs 16% (P < 0.05). DRw6 also showed an increased frequency when compared with the controls (36% vs 17%, P < 0.04). The relative risks for B8, DR3 and DRw6 were 3.2, 2.6 and 2.7, respectively.

DR3/DRw6 heterozygotes were not significantly

<table>
<thead>
<tr>
<th></th>
<th>Graves’ disease (N = 45)</th>
<th>Controls (N = 87)</th>
<th>P*</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>B8</td>
<td>17</td>
<td>38</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>DR3</td>
<td>15</td>
<td>33</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>DRw6</td>
<td>16</td>
<td>36</td>
<td>15</td>
<td>17</td>
</tr>
</tbody>
</table>

B) HLA-B8, DR3 and DRw6 in different grades of hypothyroidism in radio-iodine treated Graves’ disease (N = 45).

<table>
<thead>
<tr>
<th></th>
<th>Preclinical hypothyroidism (N = 27)</th>
<th>Overt hypothyroidism (N = 18)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>p*</td>
</tr>
<tr>
<td>B8</td>
<td>12</td>
<td>44</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DR3</td>
<td>10</td>
<td>37</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>DRw6</td>
<td>7</td>
<td>26</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

C) Frequencies of HLA-B8, DR3 and DRw6 in Graves’ disease according to thyroid antibody state.

<table>
<thead>
<tr>
<th></th>
<th>Antibody positive (N = 25)</th>
<th>Antibody negative (N = 20)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>B8</td>
<td>12</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td>DR3</td>
<td>10</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>DRw6</td>
<td>6</td>
<td>24</td>
<td>10</td>
</tr>
</tbody>
</table>

* P vs controls. RR = relative risk.
increased in patients with Graves' disease compared to controls.

When comparing patients with pre-clinical hypothyroidism to normal controls, we could observe significant differences in frequencies of antigens B8 (P < 0.01) and DR3 (P < 0.05) (Table 1B, Fig. 1). DRw6 was not significantly different.

In contrast the overt hypothyroid group had a significant elevation of DRw6 (P < 0.04), but the frequencies of the antigens B8 and DR3 did not differ compared to controls (Table 1B, Fig. 1).

The frequencies of HLA-B8, DR3 and DRw6 according to the thyroid antibody state are shown in Table 1C. We found a significant relationship between antibody positivity and antigens B8 and DR3 (P < 0.005, < 0.05, respectively vs controls). On the other hand, antibody negative subjects showed a clearly elevated DRw6 frequency (P < 0.01).

We could find no further significant relation between other DR antigens and Graves' disease. HLA-DR2 was slightly augmented in the whole group of patients and was most frequent in the antibody negative group (40% vs 22% in controls, n.s.).

There was no difference in the pattern of HLA antigens in the presence or absence of endocrine ophthalmopathy.

Discussion

Of our Graves’ disease patients 38% were positive for HLA-B8, which is in good agreement with earlier studies in caucasoid patients (Farid et al. 1975; Chopra et al. 1977; Bech et al. 1977; Thorsby et al. 1975). DR3 which is in linkage disequilibrium with B8 has a somewhat lower prevalence in our patients (33%) than reported by other investigators (Farid et al. 1979, 1980, 1981; Bech et al. 1977; Thorsby et al. 1975; Schleusener et al. 1983) and confers a relative risk of 2.6. The reasons for the less strong association of DR3 than B8 may be related to the fact that older patients are selected for treatment with radioiodine (Farid et al. 1980). Our new finding of a significant association between Graves’ disease and DRw6 (35.6% vs 17% of controls) has also to be interpreted in that light. As the purpose of the study was, however, to compare the effect of radioiodine on long-term thyroid function, this caveat should not invalidate our results.

The mean dose of 131I administered and the mean gland size estimated by planimetry of scinti-scan were not different for groups A and B, and thus could not have been responsible for the different outcome of thyroid function years after treatment. Moreover, the time of follow-up after radioiodine is not the cause for the different
degrees of hypothyroidism in the groups A and B: the group of patients who became overtly hypothyroid did so after a mean interval of 51 months, compared to a mean follow-up of 111 months in the pre-clinical hypothyroid group (P < 0.001). These observations indicate that the development of overt hypothyroidism after 131I could be related to immunological or genetic factors, as already suggested by other authors (Malone & Cullen 1976; Lamberg 1979). Whereas the influence of HLA-DR3 on the early course of 131I (Farid et al. 1980; Davies et al. 1982) or antithyroid drug-treated thyrotoxicosis (Bech et al. 1977; McGregor et al. 1980; Dahlberg et al. 1981; McKenna et al. 1982; Allanic et al. 1983) has been a subject of several studies, the predictive value of HLA for the long-term outcome of the treatment remains to be elucidated. Our data show that pre-clinical hypothyroid patients have an elevated frequency of HLA-B8 (P < 0.01) and of DR3 (P < 0.05). On the other hand, the overt hypothyroid group demonstrated an elevated prevalence of DRw6 (P < 0.04) and no statistical association with B8 or DR3. Thus, the presence of B8/DR3 could be a protective factor against the development of overt hypothyroidism, at least over several years after 131I-treatment, whereas DR3 negative/DRw6 positive patients have a higher risk for overt post-irradiation hypothyroidism. This finding is in keeping with the suggestion, that thyroid epithelial cells of DR3 positive Graves’ patients are inherently resistant to radioiodine. The significance of HLA-DRw6 in the course of Graves’ disease must be further investigated with larger numbers of patients in order to draw definite conclusions.

Acknowledgments

We wish to thank Drs E. and M. Viollier for the determination of thyroid autoantibodies and Mrs L. Eidenbenz and Mrs I. Nickler for preparing the manuscript.

This study was supported by the Swiss Research Foundation (Schweiz. Nationalfonds No. 3.995-0.82).

References


Received November 18th, 1985.
Accepted June 26th, 1986.

Prof Dr J. J. Staub,
Division of Clinical Endocrinology,
Department of Medicine,
University Hospital of Basel,
Kantonsspital,
CH-4031 Basel,
Switzerland.