Major histocompatibility complex class II and complement polymorphisms in postpartum thyroiditis

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The objective was to re-evaluate the association between class II HLA-DR and DQ MHC antigens and postpartum thyroiditis (PPT) and to determine the prevalence of the class III complement allotypes of Properdin factor B (Bf), C4A and C4B in this condition. Two hundred and sixty-five (of 2897) pregnant women screened positive for thyroid autoantibody activity took part. Further blood samples were obtained for HLA class II (185) and complement (193) typing. The severity of the ensuing PPT was assessed by measuring thyroid function during the postpartum year. The HLA-DR and DQ phenotypes were assigned from restriction fragment length polymorphism analysis, and Bf, C4A and C4B allotypes were determined by immunofixation with anti-Bf or anti-C4 antibodies after electrophoresis. A weak association between the HLA class II antigens and PPT, as indicated by a reduced frequency of DR15 and DQ6 together with an increased frequency of DR5 and DQ7, was confirmed. However, only the change in DR5 frequency remained significant after correction (corrected p < 0.05). Postpartum thyroiditis was also associated with frequency disturbances in Bf and C4A allotypes but not C4B allotypes. Whilst this study has not provided evidence of a strong marker gene for PPT, it does not preclude the involvement of the MHC in this condition. These data show disturbances in complement allotype frequencies. suggesting that the class III region may provide a useful focus for further study of this pathology.

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Postpartum thyroiditis (PPT), a destructive autoimmune thyroiditis associated with raised circulating thyroglobulin levels (1), elevated iodine excretion (2) and changes in thyroid echotexture on ultrasonographic examination (3), results in thyroid dysfunction in 50% of women with circulating antithyroid peroxidase (TPO) antibodies (4) and an increase in depressive symptomatology (5) in 30% of women with elevated circulating thyroid antibodies.

Studies have shown that, in common with other forms of thyroid autoimmunity, PPT is associated variably with HLA-DR3, DR4 and DR5 (6–14). However, in many of these reports the numbers of women studied have been relatively small. We have re-evaluated the association of PPT with HLA-DR and DQ specificities, accurately defined using a DNA restriction fragment length polymorphism (RFLP)-based method, and studied the prevalence of the class III region complement polymorphisms of Properdin factor B (Bf), C4A and C4B in a large thyroid antibody-positive group of postpartum women. Frequency comparisons have been made with a population of normal blood donors.

Methods

Study groups

The women described in this paper took part in a study of the incidence, prevalence and clinical consequences of PPT in a South Wales community carried out between 1983 and 1989 (5). Ethical approval for the study was obtained from the Mid Glamorgan Health Authority, South Wales and all participants gave their written informed consent. During this time blood samples were obtained from 2897 women at the antenatal booking clinic (16 weeks gestation), at delivery or on both occasions. These samples were screened for the presence of circulating thyroid autoantibodies by ELISA (15) and were considered positive if TPO antibody activity was in excess of 19.4 kIU/l. Of these women 265 had elevated thyroid autoantibodies (Ab pos) and were recruited into the study together with a group of 314 age-matched thyroid antibody-negative
women (Ab neg). The DNA samples for RFLP were available from 185 Ab pos women and 314 Ab neg controls for HLA class II analysis. Suitable blood samples for complement (Bf, C4A and C4B) typing were available from 193 Ab pos women and 326 Ab neg controls. A group of 1010 local blood donors was also used as a random control population.

Thyroid function was assessed in both groups of women at monthly intervals during the first year postpartum. Free thyroxine (FT₃) and free triiodothyronine (FT₄) were measured using the Amerlex M methods (Amersham International PLC, Chalfont, Bucks, UK) and thyrotrophin (TSH) by an enhanced luminescence immunoassay using the Amerlite TSH (monoclonal) reagents (Amersham International PLC). The functional sensitivity of this assay was 0.04 µU/l (16). The reference ranges for thyroid function tests used in this study (FT₃, 4.2–7.7 pmol/l; FT₄, 8–19 pmol/l; TSH, 0.5–3.6 µU/l) were derived from the analysis of serum samples from antibody-negative subjects included in the trial. An episode of hyperthyroid PPT was indicated by suppressed TSH together with FT₄ greater than 19 pmol/l or FT₃ greater than 7.7 pmol/l, or elevated FT₃ and FT₄. Hypothyroid PPT required the serum TSH level to be greater than 3.6 µU/l together with FT₄ less than 8 pmol/l or FT₃ less than 4.2 pmol/l. A diagnosis of hypothyroid PPT was also made if the TSH level was greater than 10 µU/l. During the course of the postpartum year 90/193 (86/185) Ab pos women showed one or more episodes of PPT. Hyperthyroid PPT was always transient but hypothyroid PPT, although normally a transient phenomenon, was still present in 20 of the PPT women at 1 year postpartum. These women were considered to have a persistent PPT.

HLA class II (DR and DQ) typing

The HLA-DR and DQ phenotypes were determined by RFLP analysis using Taq1-digested genomic DNA and DRB, DQA and DQB cDNA probes (17).

Complement (Bf, C4A and C4B) allotyping

The Bf allotypes (BfS, Bff, Bf0.7, Bff1) were determined by high-voltage agarose gel electrophoresis of serum followed by immunofixation with specific goat anti-Bf (18, 19). The C4A (C4A1–A6 and C4AQ0) and C4B (C4B1–B5 and C4BQ0) allotypes were defined by agarose gel electrophoresis of neuraminidase- and carboxypeptidase B-digested EDTA plasma followed by immunofixation with antisera to human C4 (20). In addition, a haemolytic gel overlay was used to distinguish between the gene products of C4A and C4B (21).

Statistical analysis

Haldane’s modification (22) of Wool’s method (23) was used to calculate relative risk (RR) and to assess the significance of differences in frequencies between the various patient groups and the control population. Frequency differences were considered of interest when significant at the 1% level (uncorrected) and p values were then corrected for the 25 comparisons undertaken (21, 24). Heterogeneity in the distribution of allotypes controlled from a single locus was evaluated by an analysis of the distribution of p values (25) (multiplex analysis (26)).

Results

Analysis of the HLA-DR and DQ phenotype frequencies in the groups of postpartum women and the random control population is shown in Table 1. These data show a weak association between HLA class II specificities and the development of PPT. This association was indicated by a lower frequency of HLA-DR2, more specifically with the DR15 subtype, and DQ6 (which are in tight linkage disequilibrium) and an increased frequency of DR3 (DR17) and DR5 (both DR11 and DR12 subtypes of DR5) and DQ7 with PPT. However, only one of these associations, that of DR5 (DR11 and DR12 combined) with PPT, remained statistically significant after correction of the p values. A similar pattern of association was seen when the analysis was performed using the group of women who showed persistent PPT and those women whose PPT included a hypothyroid phase. HLA-DR7 was reduced in patients with persistent thyroid dysfunction (p < 0.05; p corrected, NS).

The frequencies of Bf and complement C4A and C4B allotypes in the various study groups were compared with the random control population. Postpartum thyroiditis was associated with a reduced frequency of BfS (p < 0.05), whilst the frequency of Bff1 was increased from 2.7 in the control group to 7.8 in the women with PPT (p < 0.001) and to 10% in the women with persistent PPT (p < 0.05). There was an increase in the frequency of C4A2 and C4A5 in the total PPT group together with a decreased frequency of the null allele C4AQ0 and C4BQ0 (p < 0.05). Elevated frequencies of C4A1, C4A2 and C4B5 were also associated with the persistent PPT cases (p < 0.05). However, none of these disturbances in allotype frequencies were significant after correction of the p values. No significant differences (p < 0.05) were found for the allotypes of Bf in the two groups of euthyroid women.

Multiplex analysis of the complement allotyping data (Table 2) showed significant variations in the frequencies of Bf and C4A (with and without the C4AQ0 and C4BQ0 data) and the development of PPT. These changes were also apparent in the hypothyroid PPT and persistent PPT groups of patients. There was no difference in the frequency of the allotypes of C4B between either the total PPT...
group or the hypothyroid PPT group. However, a difference in C4B allotype frequencies was seen in the persistent PPT cases, whether or not C4BQ0 was included in the analysis.

Discussion

In this study, we have re-evaluated the association between PPT and 20 class II specificities (13 DR and 7 DQ), using a DNA RFLP-based typing technique, together with the allotypes of three complement proteins (Bf, C4A and C4B), all relevant to autoimmunity (4), from the class III region.

Table 1. Selected HLA-DR and DQ frequencies (%) (and relative risk) in women with postpartum thyroiditis (PPT) and in euthyroid antibody-positive and antibody-negative women compared to the local blood donor population.

<table>
<thead>
<tr>
<th>HLA</th>
<th>Women with PPT</th>
<th>Euthyroid women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All women (N = 86)</td>
<td>Persistent thyroid dysfunction (N = 20)</td>
</tr>
<tr>
<td>DR2</td>
<td>15.0 (0.48)*</td>
<td>15.0 (0.53)</td>
</tr>
<tr>
<td>DR5</td>
<td>37.2 (1.68)*</td>
<td>45.0 (2.18)</td>
</tr>
</tbody>
</table>

* The significance of the relative risk is: *p < 0.05. **p < 0.01 and *p < 0.001.

Corrected p values.

HLA class II antigen associations with autoimmune thyroid disease have been widely studied and suggest an association between certain HLA antigens and PPT, in particular HLA-DR3, DR4 and DR5 (14). Farid et al. (6) reported an increased frequency of HLA-DR3 and DR5, and later DR4 (8), in women with transient hypothyroid PPT. Lervang et al. (7) and Jansson et al. (9) also observed higher frequencies of DR4 in women with PPT. Pryds et al. (10) and Vargas et al. (11) both showed a relatively strong association with DR5. This association with DR5 has been confirmed in this study. However, in contrast to our present findings, our previous study (13) identified an association between PPT and DR3. Both studies, however, found a negative association between PPT and HLA-DR2. Tachi et al. (12) also reported a negative association between PPT and HLA-DR2 and an increased frequency of DR3. The frequency of HLA-DQ7 was also raised in the PPT women in this study, although this was not significant after correction. This is likely to be a consequence of the increase in DR5, because both DR11 and DR12 are in linkage disequilibrium with DQ7.

Based on their interpretation of HLA-DR frequencies seen in PPT, Pryds et al. (10) suggested that PPT may in fact be an early manifestation of Hashimoto's thyroiditis. This would appear quite plausible as Hashimoto's thyroiditis has variously been associated with an increased frequency of HLA-DR3, DR4 and/or DR5, an increased frequency of HLA-DQ7 and a negative association with HLA-DR2 (27--33) and is in accord with our hypothesis, based on clinical and laboratory findings, that PPT and Hashimoto's thyroiditis may share a common aetiology (34).

Table 2. Multiplex analysis for Bf, C4A and C4B allotypes in the various postpartum thyroiditis (PPT) study groups compared with the local blood donor population (N = 1010). 

<table>
<thead>
<tr>
<th>Allotype</th>
<th>All PPT women (N = 90)</th>
<th>Persistent PPT (N = 20)</th>
<th>Biphasic or hypop PPT (N = 67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bf</td>
<td>X² = 13.2</td>
<td>X² = 12.9</td>
<td>X² = 14.5</td>
</tr>
<tr>
<td>p = 0.01</td>
<td>p = 0.01</td>
<td>p = 0.006</td>
<td></td>
</tr>
<tr>
<td>C4A</td>
<td>X² = 23.4</td>
<td>X² = 29.5</td>
<td>X² = 26.7</td>
</tr>
<tr>
<td>p = 0.001</td>
<td>p = 0.0001</td>
<td>p = 0.0004</td>
<td></td>
</tr>
<tr>
<td>No C4AQCQ</td>
<td>X² = 15.5</td>
<td>X² = 24.3</td>
<td>X² = 18.5</td>
</tr>
<tr>
<td>p = 0.02</td>
<td>p = 0.0005</td>
<td>p = 0.005</td>
<td></td>
</tr>
<tr>
<td>C4B</td>
<td>X² = 7.3</td>
<td>X² = 14.3</td>
<td>X² = 6.17</td>
</tr>
<tr>
<td>p = 0.3</td>
<td>p = 0.03</td>
<td>p = 0.4</td>
<td></td>
</tr>
<tr>
<td>No C4BQCQ</td>
<td>X² = 3.0</td>
<td>X² = 12.4</td>
<td>X² = 4.2</td>
</tr>
<tr>
<td>p = 0.7</td>
<td>p = 0.03</td>
<td>p = 0.5</td>
<td></td>
</tr>
</tbody>
</table>

* Degrees of freedom = 4 for Bf, 7 for C4A and 6 for C4B (6 and 5, respectively, for no C4AQCQ and C4BQCQ).
A positive association between an HLA antigen and disease pathology may generally be interpreted in one of two ways. Firstly, it may indicate that the disease locus is in linkage disequilibrium with the HLA locus. When interpreted in this way the data reported here and elsewhere (6–13) are relatively weak, although this does not exclude the possibility. Alternatively, the HLA antigen itself may have a direct role in the disease process.

A complex antigen will produce a number of antigenic peptides when processed within antigen-presenting cells (APC), and not all these will be physically compatible with the peptide groove of particular HLA molecule. Thus, the polymorphic forms of the HLA may be one factor influencing the ability of an APC to present a particular species of antigenic peptide and so modulate disease susceptibility. This may be of particular importance in autoimmune thyroid disease where, despite the large number of potential antigenic peptide sequences within the three known protein antigens, only a very restricted set of antigenic epitopes are known to exist (35, 36).

The class III area of the MHC contains the coding for several proteins that are important in the pathogenesis of the autoimmune diseases (viz. TNF-α, HSP70, complement). In this study we have measured the allotype frequency of three complement proteins Bf, C4A and C4B and found that there were significant differences in their overall frequency distribution in PPT women. These findings are similar to those reported by Ratanachiyavong et al. (37) in Graves’ disease. an autoimmune thyroid disease that is also associated with an increased frequency of HLA-DR3. This may indicate linkage disequilibrium with candidate genes in the class III region but could equally be interpreted on a functional basis. because the pathogenesis of PPT is known to be linked to activation of the complement system (4, 38, 39).

In conclusion, whilst we have shown an association between PPT and HLA-DR. DQ and complement polymorphisms, we, like others, have failed to identify any strong candidate gene for this disorder.

References

8. Thompson P, Farid NR. Post-partum thyroiditis and goitrous (Hashimoto’s) thyroiditis are associated with HLA-DR4. Immunol Lett 1985:11:301

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