Association of HLA Antigen BW35 with Severe Graves' Ophthalmopathy

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Human leukocyte antigens (HLA) in patients with Graves' disease were investigated according to the ophthalmic classification system of the American Thyroid Association. All prior HLA studies of Graves' disease have disregarded the patients' specific ophthalmic manifestations. Examination of 18 A and 34 B loci antigens disclosed an increased frequency (corrected \( P = 0.002 \), relative risk \( = 13.1 \)) of HLA-BW35 in patients improving with oral corticosteroids who have severe extraocular muscle and orbital inflammation (class 4-5) when compared to a geographically and racially matched control population. No statistically significant associations were found when patients without ophthalmic manifestations or with other categories of Graves' ophthalmopathy were compared to controls. The association of severe Graves' ophthalmopathy with HLA-BW35 may provide immunologic evidence to explain both the unpredictable association of the orbital and thyroid disturbances of Graves' disease as well as the unpredictable response of the orbital inflammation to oral corticosteroids. Invest Ophthalmol Vis Sci 24:124-127, 1983

The ophthalmic manifestations of Graves' disease may or may not be associated with clinical or biochemical evidence of thyroid gland dysfunction.1 It is enigmatic that some patients may develop only thyroid abnormalities, while others manifest only ophthalmopathy, and yet a third group demonstrate both thyroid and ophthalmic disturbances. Although Graves' thyroid disease and Graves' ophthalmopathy are most certainly immunologic disturbances, their unpredictable association and often divergent courses suggest strongly that immunologic distinctions should exist to correspond to these diverse clinical observations.2

Prior studies of human leukocyte antigens (HLA) have considered all patients with Graves' disease as a uniform group regardless of their varied ocular involvement.3-6 Because recent investigations have revealed distinct lymphocyte characteristics for the thyroid and ophthalmic disorders,7,8 we have reassessed HLA frequencies according to the specific ophthalmic manifestations of Graves' disease. In addition, since the most severe ophthalmic complications may indicate a heterogeneous collection of pathogenetic mechanisms or different phases of the same disease as judged by variable responses to therapeutic regimens,8 we have correlated HLA findings with the results of corticosteroid therapy.

Material and Methods

Patients

Ninety-eight white patients from the Wills Eye Hospital and Research Institute, Philadelphia, PA, comprised the study population. Two hundred fifteen...
white individuals without known immunologic disorders from the same geographic area served as controls. All patients and controls were unrelated.

Patients were classified by their ophthalmic manifestations according to the system of the American Thyroid Association (Table 1). Because of the lack of firm physical diagnostic criteria to separate classes 1 and 2, these patients were considered together (class 1-2). Patients in classes 4 and 5 were grouped together because their predominant problem was diplopia and restrictive ophthalmoplegia.

**HLA Typing**

The microdroplet lymphocyte cytotoxicity assay was used to define 18 A and 34 B loci antigens. All HLA typing was performed at the surgical research laboratory of the Mary Imogene Bassett Hospital, Cooperstown, NY. One investigator (CAS) performed the HLA determinations without knowledge of the patients' clinical status. HLA tissue typing plates were obtained from Dr. Paul Taraski, UCLA Medical Center, Los Angeles, CA.

**Statistical Analysis**

The statistical analyses comparing the HLA antigen frequencies in the different classes of Graves' ophthalmopathy and the control population was evaluated by Fisher's exact test. The resultant $P$ values were multiplied by two to make the determination two sided. The subsequent $P$ values were then multiplied by 52, the number of antigens tested to account for Type I error. This value is referred to as "corrected $P$," and any value less than 0.05 was considered statistically significant. Because the study extended over several years and HLA antigen designations changed during that time, we used the most recently available antigen determinations when a single locus was refined into two more precise loci. Relative risk (Rr) was determined by Woolf's method or Haldane's modification.

**Results**

Table 2 illustrates the important HLA statistical comparisons. Twenty-six of 215 individuals in the control population demonstrated HLA-BW35. In patients with thyrotoxicosis without ophthalmopathy (class 0), 7 of 30 individuals had BW35, a frequency not statistically significant. Of all 33 patients in class 4-5, 12 were positive for BW35 but this finding did not achieve statistical significance. However, when only class 4-5 patients responding favorably to oral corticosteroids (9 of 14) were compared to controls, the corrected $P$ value became 0.002, with a relative risk of 13.1. Only 1 of 13 class 4-5 corticosteroid resistant patients was positive for BW35. Six patients in class 4-5 were not treated with corticosteroids.

When all patients in class 6 were compared to controls, no statistical significance was computed. All seven class 6 patients with BW35 were corticosteroid

### Table 1. Abridged Classification of eye changes of Graves' disease *

<table>
<thead>
<tr>
<th>Class*</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No physical signs or symptoms</td>
</tr>
<tr>
<td>1</td>
<td>Only signs, no symptoms (signs limited to upper eyelid retraction, stare, and eyelid lag)</td>
</tr>
<tr>
<td>2</td>
<td>Soft tissue involvement (symptoms and signs)</td>
</tr>
<tr>
<td>3</td>
<td>Proptosis</td>
</tr>
<tr>
<td>4</td>
<td>Extraocular muscle involvement</td>
</tr>
<tr>
<td>5</td>
<td>Corneal involvement</td>
</tr>
<tr>
<td>6</td>
<td>Sight loss (optic nerve involvement)</td>
</tr>
</tbody>
</table>

* Each class usually, but not necessarily, included the involvements indicated in the preceding class.

### Table 2. Statistical comparisons among different classes of Graves' ophthalmopathy for HLA-BW35

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Number of patients with HLA-BW35</th>
<th>Significance (corrected $P$) and relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 0 vs controls</td>
<td>7/30</td>
<td>NS</td>
</tr>
<tr>
<td>Class 4-5 vs controls</td>
<td>12/33</td>
<td>NS</td>
</tr>
<tr>
<td>Class 4-5 (R) vs controls</td>
<td>9/14</td>
<td>$P = 0.002$</td>
</tr>
<tr>
<td>Class 4-5 (NR) vs controls</td>
<td>1/13</td>
<td>NS</td>
</tr>
<tr>
<td>Class 6 (R) vs controls</td>
<td>7/19</td>
<td>$P = 0.051889$</td>
</tr>
<tr>
<td>Class 6 (NR) vs controls</td>
<td>0/4</td>
<td>NS</td>
</tr>
<tr>
<td>Class 4-5-6 (R) vs controls</td>
<td>16/33</td>
<td>$P = 0.000478$</td>
</tr>
<tr>
<td>Class 4-5 (R) versus Class 4-5 (NR)</td>
<td>9/14</td>
<td>NS</td>
</tr>
<tr>
<td>Class 4-5-6 (R) vs Class 4-5-6 (NR)</td>
<td>16/33</td>
<td>NS</td>
</tr>
<tr>
<td>Class 6 (R) vs Class 6 (NR)</td>
<td>7/19</td>
<td>NS</td>
</tr>
<tr>
<td>Control population</td>
<td>26/215</td>
<td></td>
</tr>
</tbody>
</table>

R—corticosteroid responsive. 
NR—corticosteroid nonresponsive. 
Rr—relative risk. 
NS—not significant.
responsive, but when compared to the control population by the statistically conservative Fischer’s exact test combined with the two-sided antigen number adjustments, the corrected P was not less than the value (0.05) usually considered to be statistically significant.

Since corticosteroid responsive 4-5 and class 6 patients usually demonstrate similar peripheral blood T-lymphocyte characteristics,8 we combined these patients for further statistical analysis. Sixteen of 33 steroid responsive patients in class 4-5-6 possessed BW35, yielding a corrected P value of 0.000478 and a relative risk of 6.84 when compared to controls. Comparison of corticosteroid-responsive patients to corticosteroid resistant patients in class 4-5-6 failed to show a statistically significant difference for BW35, despite the fact that only one patient with BW35 was corticosteroid resistant and 16 of 33 responsive patients had BW35.

Analyzing patients and antigen frequencies according to euthyroid Graves’ ophthalmopathy (classes 1-2 and 4-5-6 without clinical or biochemical history of thyrotoxicosis) vs ophthalmopathy with documented thyrotoxicosis did not result in any statistically significant comparisons (data not shown).

Discussion

Re-examination of HLA frequencies according to the specific ophthalmic manifestations of Graves’ disease has disclosed a high association between BW35 and the steroid responsive, severe ophthalmopathy characterized by marked extraocular muscle and orbital inflammation. Others12-13 have reported an increased association with B8 and CW3 antigens but these investigators considered all patients under the general heading of “endocrine ophthalmopathy” without exactly defining the individuals according to the more specific American Thyroid Association classification (Table 1). When patients were simply classified as having “exophthalmos,” Bech and co-workers reported that 80.5% of their study population had B8.14 In contrast, two other studies, classifying patients under the broad heading of “exophthalmos,” failed to show a statistically significant association with B8 or any other antigen.6,15 Other investigations have failed to make any distinction regarding the ophthalmic signs.3-5 Our study is unique in that it employs precise neuro-ophthalmologic diagnostic criteria in combination with the American Thyroid Association classification system for Graves’ ophthalmopathy. This major difference in methodology is critical in our investigations of the immunology of Graves’ ophthalmopathy.7,8

Two studies of Japanese patients with Graves’ disease have found an increased frequency of HLA-W5 (since renamed HLA-BW35).16-17 Unfortunately, both these investigations did not include descriptions of the ophthalmic findings. There were no Japanese patients in our study.

We believe that two possible mechanisms may exist by which BW35 relates to the pathogenesis of Graves’ ophthalmopathy. First, BW35 may be closely linked to gene(s) controlling immunologic responsiveness to antigen(s) crucial to orbital inflammation. Or, a significant biochemical cross-reaction may exist with the causative agent and the structure of the BW35 antigen. In regard to this second possibility, it is interesting to recall the reported association of BW35 and subacute (de Quervain’s) thyroiditis, a disorder probably caused by a cytopathic virus.18,19

This association of BW35 antigen in patients with the most severe Graves’ ophthalmopathy provides a further immunologic distinction7,8 directed at explaining the unpredictable association of the orbital manifestations and the thyroid disturbances. Graves' ophthalmopathy and thyrotoxicosis, while frequently associated and sharing a common dysthyroid substrate, probably have more independent and heterogeneous immunopathogeneses than once believed. This statistically significant association between BW35 and the more severe inflammatory orbital manifestations of Graves’ disease indicate that certain thyrotoxic individuals have an increased immunogenetic risk to develop ophthalmopathy.

Key words: Graves’ ophthalmopathy, thyroid eye disease, HLA antigens, corticosteroids, thyrotoxicosis, lymphocytes

References

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