Severe Hypertension in the Spanish Population
Association with Specific HLA Antigens

MANUEL LUQUE OTERO, NIEVES MARTELL CLAROS, LEOPOLDO LLORENTE PÉREZ, CARMEN FERNÁNDEZ PINILLA, AND ARTURO FERNÁNDEZ-CRUZ

SUMMARY There is now increasing evidence for immunological changes in essential hypertension. Immunological response is determined in part by genes linked to the HLA system. It has been reported a positive association between HLA B15 and the risk for cerebral events in essential hypertensive (EH) patients. We studied the distribution of HLA antigens in 128 EH (age range, 13–85 years) and 1000 normotensive controls. EH were classified in accordance with the World Health Organization (WHO) criteria: in WHO Stages I and II, there were 100 patients; in WHO Stage III, there were 28 patients. HLA-A and B antigens of peripheral blood lymphocytes were studied according to the microlymphocytotoxicity test. The results were compared by chi-square analysis, and the p value was multiplied by the number of antigens studied at each locus, to avoid overestimation of an association. Frequency of HLA-BW 22 was higher in EH compared with controls (5.4% vs 1.2%, \( p < 0.01 \)). Frequency of HLA-B12 in EH with WHO Stage III hypertension (64.2%) was significantly increased compared either with EH in WHO Stage I or II (29%, \( p < 0.01 \)) or the control group (26.9% \( p < 0.001 \)). The incidence of HLA-B15 antigen in the whole hypertensive group was 3.1%, lower than in normotensive controls (6.4%, \( p < 0.8 \)). None of the patients with WHO Stage III hypertension had the HLA-B15 antigen. In conclusion, the results seemed to indicate that the Spanish population had an association between HLA-B12 and severe hypertension.

(KEY WORDS • essential hypertension • HLA A and B antigens)

THERE is increasing evidence of immunological changes in essential hypertension (EH). In 1970, Ebringer and Doyle\(^1\) demonstrated raised levels of immunoglobulins in EH patients. The same findings were reported by Olsen et al. in 1973\(^2\) and Kristensen in 1978.\(^3\) On the other hand, Kristensen and Anderson\(^4\) reported a higher frequency of autoantibodies in EH patients compared with a normal control population. In addition, Gudbrandsson et al.\(^5\) found increased T-lymphocyte reactivity to human arterial antigen in EH patients compared with control subjects.

Immunological response is determined in part by genes linked to the HLA system.\(^6\) It has been reported that HLA-B8 and HLA-B15 increased in patients with a positive history of hypertension.\(^7\) Moreover, it has been suggested that EH patients with HLA-B15 may have a greater risk of developing vascular complications than hypertensives without this antigen.\(^8\) From a preliminary study\(^9\) we found in Spanish EH patients that the frequency of HLA-B12 seemed to be increased in WHO Stage III hypertensives.

The aim of this study was to investigate the distribution of HLA antigens among Spanish hypertensive patients and to examine the possibility that a gene of the HLA system might be a predetermining factor in the severity of hypertension.

Subjects and Methods

Patients

We studied 128 EH patients, whose diagnosis we established after a full investigation, including intravenous urography, to rule out secondary forms of hypertension. We classified 28 patients (16 men and 12 women, aged 32 to 85 years) as Stage III in accordance with the World Health Organization (WHO) criteria;\(^10\) 13 patients had retinal hemorrhages and exudates, 11 had cerebrovascular diseases, and four had coronary heart diseases. All were receiving antihypertensive...
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TABLE 1. Frequency of HLA-A Locus Antigens in 128 Essential Hypertensive Patients and 1000 Controls

<table>
<thead>
<tr>
<th>HLA-A locus antigen</th>
<th>Frequency in hypertensives (%)</th>
<th>Frequency in controls (%)</th>
<th>pu</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>16.4</td>
<td>22.0</td>
<td>NS</td>
</tr>
<tr>
<td>A2</td>
<td>46.8</td>
<td>46.8</td>
<td>NS</td>
</tr>
<tr>
<td>A3</td>
<td>16.4</td>
<td>17.4</td>
<td>NS</td>
</tr>
<tr>
<td>A9</td>
<td>23.4</td>
<td>18.3</td>
<td>NS</td>
</tr>
<tr>
<td>A10</td>
<td>7.0</td>
<td>11.1</td>
<td>NS</td>
</tr>
<tr>
<td>A11</td>
<td>10.9</td>
<td>12.0</td>
<td>NS</td>
</tr>
<tr>
<td>A25</td>
<td>3.9</td>
<td>5.0</td>
<td>NS</td>
</tr>
<tr>
<td>A26</td>
<td>3.1</td>
<td>8.1</td>
<td>0.02</td>
</tr>
<tr>
<td>A28</td>
<td>3.9</td>
<td>3.6</td>
<td>NS</td>
</tr>
<tr>
<td>A29</td>
<td>6.2</td>
<td>11.7</td>
<td>0.02</td>
</tr>
<tr>
<td>AW24</td>
<td>7.8</td>
<td>8.1</td>
<td>NS</td>
</tr>
<tr>
<td>AW30</td>
<td>10.1</td>
<td>6.8</td>
<td>NS</td>
</tr>
<tr>
<td>AW31</td>
<td>0.7</td>
<td>0.7</td>
<td>NS</td>
</tr>
<tr>
<td>AW32</td>
<td>6.2</td>
<td>3.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

pu = p uncorrected.

drugs, including beta-adrenoceptor blocking agents (15 patients), hydralazine (15), furosemide (20), propranolol (12), chlorthalidone (5), and methyldopa (10). In addition, two HLA-B12-positive patients were excluded because they were found to have renovascular hypertension.

We classified 21 patients as WHO Stage I and 79 patients as WHO Stage II (41 men and 59 women, aged 13 to 75 years). The normotensive control group consisted of 1000 healthy men and women aged 21–59 years. This population of unrelated individuals from Madrid was representative of the population of Spain as a whole since Madrid has been an important migration center.

Methods

The HLA-A and HLA-B typing was carried out by a standard microlymphototoxicity technique. Anti-HLA sera were obtained from the Behring Institute, West Germany. At least two antisera defined each HLA specificity, and all had been closely matched to serum sets used in the various histocompatibility workshops. We studied 30 HLA antigens, 14 determined by genes belonging to HLA-A locus and 16 by genes of HLA-B locus.

Statistics

Statistical comparison of HLA antigen frequencies among diseased and healthy subjects was done by chi-square test with Yate's correction introduced for discontinuity in small samples. The p value thus calculated was corrected by multiplication with the number of tested antigens studied at each locus, to avoid overestimation of an association.

Results

Frequency of HLA-A antigens in EH patients and normotensive control subjects is shown in table 1. The frequency of two antigens, HLA-A26 and HLA-A29, was lower in EH patients compared with normotensive controls (3.1% vs 8.1%, pu < 0.02, and 6.2% vs 1.7% pu < 0.02 respectively).

The frequency of HLA-B antigens in EH patients and normotensive controls is shown in table 2. The frequency of two antigens, HLA-B12 and HLA-BW22, was higher in EH patients than in normotensive controls (36.7% vs 26.9%, pu < 0.02, and 5.4% vs 1.2%, pu < 0.001 respectively). On the other hand, the frequency of HLA-B15 was lower in EH patients compared with controls (3.1% vs 6.4%, pu < 0.05).

When the p values for these antigens were multiplied by 14 and 16, i.e., the number of antigens identified, the number of antigens identi-
HLA ANTIGENS IN SEVERE HYPERTENSION/Otero et al.  

TABLE 4. Frequency of HLA-B12 and HLA-B15 Antigens in 1000 Controls, 100 Hypertensive Patients (WHO Stages I and II), and 28 Hypertensive Patients (WHO Stage III)

<table>
<thead>
<tr>
<th>HLA locus antigen</th>
<th>Frequency in controls (%)</th>
<th>Frequency in hypertensives Stages I and II (%)</th>
<th>Frequency in hypertensives Stage III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>26.9</td>
<td>29—pc&lt;0.01—64.2</td>
<td></td>
</tr>
<tr>
<td>B15</td>
<td>6.4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

pc = p corrected.

Discussed at locus A and B respectively, the decreased incidence of HLA-A26, HLA-A29, and HLA-B15 in EH patients compared with controls was not statistically significant (table 3), nor was the increased frequency of HLA-B12 in EH patients. On the other hand, the increase in frequency in EH patients compared with the controls remained significant in the case of HLA-BW22 antigen (p corrected < 0.01).

Table 4 shows the frequency of two HLA-B locus antigens, HLA-B12 and HLA-B15 in controls, EH patients (WHO Stage I and II), and severe EH (WHO Stage III). The frequency of HLA-B12 was significantly increased in severe EH patients (64.2%) compared with controls (26.9%, p corrected (pc) < 0.001) and with WHO Stage I and II hypertensives (29%, pc < 0.01). Conversely, and surprisingly, not one patient with severe EH showed any presence of the HLA-B15 antigen.

Comparison of clinical characteristics of B12-positive and B12-negative WHO Stage III patients (table 5) showed no differences with respect to age, blood pressure, and male/female ratio. The known duration of the hypertension as well as the time required to reach Stage III varied greatly. Again, no significant differences were found between B12-positive and B12-negative patients.

TABLE 5. Clinical Characteristics in Relation to the Presence of HLA-B12 in 28 Patients with WHO Stage III Essential Hypertension

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HLA-B12-positive</th>
<th>HLA-B12-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>9/9</td>
<td>6/4</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>55 ± 12</td>
<td>60 ± 12</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>193 ± 32</td>
<td>194 ± 29</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>112 ± 17</td>
<td>111 ± 15</td>
</tr>
<tr>
<td>Known duration of hyperten-</td>
<td>14 ± 10</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>Stage III (yrs)</td>
<td>3.3 ± 6.9</td>
<td>5.8 ± 7.2</td>
</tr>
</tbody>
</table>

Values are means ± sd.

Our results show that certain HLA antigens have a higher frequency in Spanish hypertensive patients than in normotensive controls. Except for HLA-BW22, these differences were not statistically significant when the p values were corrected for the number of HLA antigens examined, as was the case in previous studies that were critically analyzed recently. In our study we found a statistically significant increase in the frequency of HLA-BW22 among hypertensives. This antigen had been found to be associated with multiple sclerosis among Japanese people. However, the frequency of this antigen in the Spanish population is low, 5.4% in hypertensives and 1.2% in normotensives. A similar frequency in normotensives has been reported in our city by Arnaiz-Villena et al.

Most interesting, our data demonstrated that in WHO Stage III hypertensives there was a statistically significant higher frequency of HLA-B12 than in both WHO Stage I and II hypertensive patients and normotensive controls. It seems that HLA-B12-positive patients might have a significantly higher possibility of developing a severe complication of hypertension than HLA-B12-negative patients. Similar results have been reported in Northern European populations, showing that the HLA-B15 antigen might be a marker for severe hypertension, especially for an increased risk of cerebrovascular diseases. This association may be of interest because HLA-B15 is also associated in these countries with Type I diabetes mellitus, another disease with an increased risk of vascular complications.

Our results show that in Spanish EH patients there is a nonsignificant decrease in the frequency of HLA-B15 antigen. Moreover, strikingly, not one of the WHO Stage III hypertensives had HLA-B15. Until we finish our research into families with several members affected by hypertension we will not be able to establish the significance of this result.

Our results do not agree with those that suggest a possible HLA-linked connection between severe EH and Type I diabetes mellitus. The increase of HLA-B15 antigen reported was mainly found in Northern Europe, while HLA-B18 was found increasingly in Southern Europe. A preliminary report suggested that HLA-B18 is more frequent than HLA-B15 in our Spanish diabetic population. On the other hand, the frequency of HLA-B18 in our whole hypertensive population was 13.2%, and when apportioned among WHO groups was 15% in WHO Stage I and II hypertensives, 7.1% in WHO Stage III hypertensives, and 13.9% in normotensive controls. None of these differences was statistically significant. The frequency of HLA-B15 in our control group was 6.4%, smaller than that reported in Northern European countries such as Sweden, 23%, and Denmark, 19%.

In our WHO Stage III EH patients, HLA-B12 was found in six of 11 patients who had experienced cerebral events, in eight of 13 who had severe retinopathy, and in all four suffering from coronary heart diseases. Thus, in our study we have not found a significant positive association between the HLA-B12 antigen
and the cardiovascular pathology (i.e., cerebrovascular diseases and severe retinopathy) that is specifically related to hypertension as did Kristensen\textsuperscript{8} for the HLA-B15 antigen. We found no significant differences among the clinical characteristics of B12-positive and B12-negative Stage III hypertensives. We did find that B12-positive patients tended to present a hypertension of longer duration and to reach Stage III earlier than B12-negative patients.

In conclusion, this study suggests that, in the Spanish population, HLA-B12 essential hypertensive patients represent a subgroup with a higher risk of vascular complications. Long-term studies are needed to determine whether HLA-B12 might serve as a predictor for vascular complications.

References
Severe hypertension in the Spanish population. Association with specific HLA antigens.
M Luque Otero, N Martell Claros, L Llorente Pérez, C Fernández Pinilla and A Fernández-Cruz

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