Association of Heart Failure in Homozygous β-Thalassemia
With the Major Histocompatibility Complex

Dimitrios T. Kremastinos, MD; Panagiota Flevari, MD; Maria Spyropoulou, MD; Helen Vrettou, MD; Dimitrios Tsiapras, MD; Catherine G. Stavropoulos-Giokas, MD

Background—In β-thalassemia major, heart failure primarily affecting left ventricular systolic function is the most common complication and cause of death. Apart from iron deposition, it has been recently reported that myocarditis might be another contributing factor in the pathogenesis of acute or chronic heart failure, acting possibly through an autoimmune mechanism. In an attempt to assess the role of immunogenetic factors in the development of heart failure associated with β-thalassemia major, we studied the frequency of major histocompatibility antigens/alleles A, B, DR, and DQ in homozygous β-thalassemic patients with and without heart failure primarily affecting the left ventricle.

Methods and Results—Forty-five consecutive unrelated Greek patients with homozygous β-thalassemia and left-sided chronic heart failure were studied. Fifty-eight unrelated Greek patients with homozygous β-thalassemia without heart failure and 130 unrelated Greek healthy controls were also studied. In all subjects, class I HLA-A and -B typing was performed by the complement-mediated lymphocytotoxicity assay, whereas class II HLA-DR and -DQ typing was performed by polymerase chain reaction. HLA-DRB1*1401 allele frequency was significantly increased in patients with β-thalassemia major without left-sided heart failure compared with those with heart failure (corrected \( P \) \( =0.02 \), odds ratio 0.1) and healthy controls (\( P \) =0.001). HLA-DQA1*0501 allele frequency was increased in patients with heart failure compared with patients without heart failure (\( P \) =0.04, odds ratio 14) and healthy controls (\( P \) =0.004).

Conclusions—Differences exist in the immunogenetic profile between homozygous β-thalassemic patients with and without left-sided heart failure, raising the possibility that genetically defined immune mechanisms may play an important role in the pathogenesis of heart failure in β-thalassemia. (Circulation. 1999;100:2074-2078.)

Key Words: genetics ■ heart failure ■ immune system ■ cardiomyopathy

The increased frequency of infections associated with β-thalassemia major seems to be related to abnormalities of the immune system.1–3 Cardiac disorders and, most notably, left-sided heart failure are considered the most common causes of death in patients with β-thalassemia major.4 We have recently reported that apart from iron overload, myocarditis is another contributing factor in the pathogenesis of heart failure.5 Myocarditis can cause acute or chronic left ventricular systolic dysfunction and dilatation, which appear to be mediated by predominantly immunologic mechanisms rather than viral infection and replication.6,7 In this respect, apart from iron deposition itself, myocardial dysfunction might be related to a late autoimmune process in a manner similar to that observed in idiopathic dilated cardiomyopathy due to acute myocarditis.5–11

The predisposition to autoimmune diseases is under the control of immune response genes, which play a central role in the presentation of antigens to the immune system.12 In dilated cardiomyopathy, immune-related disorders show preferential associations with HLA genes.13 In β-thalassemia major, left ventricular dysfunction attributed to myocarditis seems to be related to immune system dysregulation. Thus, to examine whether the development of left-sided heart failure in β-thalassemia major might be under immunogenetic control, we investigated the frequency of major histocompatibility antigens/alleles A, B, DR, and DQ among patients with β-thalassemia major with and without left-sided heart failure.

Methods
Forty-five unrelated Greek patients with homozygous β-thalassemia with left-sided chronic heart failure were studied: all had been referred by the first symptoms and/or signs of heart failure and were consecutively examined in our department. Heart failure was diagnosed according to the Framingham study criteria and New York Heart Association functional classification. Left-sided heart failure was diagnosed on the basis of the following: (1) symptoms/signs of raised pulmonary venous pressure and (2) echocardiographic examination indicative of left ventricular dilatation and systolic dysfunction with right ventricular dimensions within normal limits. Another group consisting of 58 unrelated patients was derived during the same 5-year period from a consecutive series of Greek patients with homozygous β-thalassemia without heart failure, as assessed by the lack of symptoms and signs of heart failure. For these patients, the age, sex, mean serum ferritin, and total blood units received were

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From the 2nd Department of Cardiology (D.T.K., P.F., H.V., D.T.), Onassis Cardiac Surgery Center, and the Department of Immunology & National Tissue Typing Center (M.S., C.G.S.-G.), Athens General Hospital, Athens, Greece.
Correspondence to Dimitrios T. Kremastinos, MD, Onassis Cardiac Surgery Center, 356 Sygrou Ave, 17674 Athens, Greece. E-mail elbee@ath.forthnet.gr
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comparable to those of heart failure patients. One hundred thirty apparently normal unrelated subjects of the same ethnic origin were used as controls regarding HLA frequencies.

Each patient received a transfusion every month to maintain hemoglobin levels between 10 and 13 g/dL. In all patients, transfusion therapy had started before the age of 5 years. The mean serum ferritin level in each patient was derived as the mean of 30 values obtained at 2-month intervals over the past 5-year period. The mean hemoglobin and hematocrit levels were derived in the same way. Clinical cardiac evaluation and echocardiographic examination were performed 48 hours after the last transfusion, and hemoglobin/hematocrit levels were determined before and after transfusion in all patients. There were no alterations in blood transfusion management or chelation therapy of patients, whether they were in heart failure or not.

The echocardiographic examination of β-thalassemic patients was carried out 48 hours after blood transfusion. The entire study population underwent 2D and M-mode echocardiograms by use of instruments with a 3-MHz transducer. A 2D study was first performed to identify the overall cardiac anatomy and motion. Four- and 2-chamber apical views were used to estimate left ventricular systolic and diastolic volumes, which were calculated by the disc method.14 Left ventricular ejection fraction was calculated as follows: ((end-diastolic volume−end-systolic volume)÷end-diastolic volume)×100. Long-axis views at the midventricular level were used to derive M-mode measurements of left ventricular end-diastolic diameter and right ventricular cavity dimensions, according to the recommendations of the American Society of Echocardiography.15

In β-thalassemic patients, venous blood for HLA typing was drawn before transfusion therapy. HLA typing was also carried out in the 130 healthy controls.

**HLA Typing**

**Class I HLA-A and -B Typing**

Class I HLA-A and -B typing was performed on purified T-lymphocyte suspensions by use of the complement-mediated lymphocytotoxicity assay as previously described.16

**Class II HLA-DRB1* Typing**

**Cell Lines**

DNA from HLA-D homozygous B lymphoblastoid cell lines, fully defined during the XIIth International Histocompatibility Workshop, was used as a reference.

**DNA Extraction**

DNA was prepared from anticoagulated venous blood by use of a salting out method.17

**Amplification of Class II HLA Alleles**

DNA amplification was carried out by the polymerase chain reaction (PCR) method according to the method of Saiki et al.18 The PCR reaction mixture consisted of 1 µg genomic DNA, PCR buffer (100 mM Tris [pH 8.5], 500 mM KCl, 20 mM MgCl₂, and 0.1% gelatin), 0.2 mM of each dNTP, 1 ng of each primer, (100 mM Tris [pH 8.5], 500 mM KCl, 20 mM MgCl₂, and 0.1% gelatin), 0.2 mM of each dNTP, 1 ng of each primer, with each primer designed to amplify the second exon of DRB1, DQA1, and DQB1 genes (primers and probes were defined by the XIIth International Histocompatibility Workshop19). and 2.5 U Taq DNA polymerase (Perkin-Elmer/Cetus). The mixture was covered with an equal volume of liquid paraffin. Then the mixture was submitted to 30 cycles by use of the Perkin-Elmer/Cetus thermocycler model. Specifically, the following primer pairs were used, according to the XIIth International Histocompatibility Workshop: for the DRB region, 2DRBAMP-A and 2DRBAMP-B; for DR4 subtyping, 2DRBAMP-4 and 2DRBAMP-B; for DR2 subtyping, 2DRBAMP-2 and 2DRBAMP-B; for the DQA1 region, 2DQAAMP-A and 2DQAAMP-B; and for the DQB1 region, 2DQBAMP-A and 2DQBAMP-B.

**Determination of Amplified DNA**

Amplified DNA samples were denatured and dot-blotted onto positively charged nylon membranes. The respective membranes were hybridized with 29 sequence-specific oligonucleotide (SSO) probes for DRB, with 10 SSO probes for DQA1, and with 17 SSO probes for DQB1, allowing relatively high resolution of the known alleles.19 SSO probes were 3‘-end labeled with digoxigenin-dideoxyuridine triphosphate and DNA deoxynucleotide transferase. The membranes were hybridized for 1 hour at 54°C in 3 mol/L tetramethylammonium chloride (TMACL) solution (5× SSPE, 5× Denhardt’s solution, 0.1% SDS, and 3 mol/L TMACL) with the end-labeled SSO probe and then washed in 3 mol/L TMACL buffer (50 mmol/L Tris HCl [pH 8], 2 mmol/L EDTA, 0.1% SDS, and 3 mol/L TMACL) for 15 minutes at either 58°C (18mer), 59°C (19mer), or 60°C (20mer). Membranes were incubated with a sheep Fab antidigoxigenin IgG fragment conjugated to alkaline phosphatase, and the detection was performed by using substrate-producing chemiluminescence after enzymatic reaction with (3′-2′-spiroadamanate)-4-methoxy-(4′-3′′-phosphoryloxy)-phenyl-1,2-(AMPDD). Dots were visualized after exposure for 15 minutes to 2 hours to Kodak x-ray films at room temperature, according to the appearance of positive and negative controls.20

**Statistical Analysis**

A χ² test with Yates correction was used for comparisons between antigen/allele frequencies. P values were corrected for the number of antigens/alleles tested in each locus, and associations were considered significant at P<0.05 (where P is the corrected P value). Odds ratios (ORs) and 95% confidence intervals were calculated as statistically significant differences regarding HLA frequencies. Unpaired t tests were used for comparisons between quantitative variables. A model of multivariate logistic regression has, finally, been implemented (SAS System for Windows, version 6.12). Concerning t tests and logistic regression analysis, a value of P<0.05 was considered statistically significant.

**Results**

**Clinical and Echocardiographic Characteristics of Study Population**

The clinical and echocardiographic profiles of the 2 groups of β-thalassemic patients are presented in Table 1. No difference was noted in age, mean serum ferritin, hemoglobin level, and blood units received between patients with β-thalassemia with heart failure and patients with β-thalassemia without heart failure. Nineteen (42%) of the 45 patients with heart failure had a history of acute inflammatory heart disease, presenting as perimyocarditis (P=NS). This was diagnosed according to the following criteria: typical precordial chest pain, fever, flulike symptoms, ECG changes, increased creatine kinase-MB levels, and typical echocardiographic findings. Significant differences were observed between β-thalassemic patients with and without heart failure regarding left ventricular ejection fraction (30±8% versus 60±8%, P<0.0001) and left ventricular end-diastolic diameter (55±4% versus 41±4 mm, P<0.0001). No difference was observed between the 2 groups of patients regarding right ventricular diameter.

**HLA Frequencies in the 2 Groups of β-Thalassemic Patients**

HLA-A and HLA-B antigen frequencies were similar in the 2 groups of patients. HLA-DRB1*1401 allele frequency was higher in β-thalassemic patients without heart failure compared with β-thalassemic patients with left-sided heart failure.
TABLE 1. Clinical and Echocardiographic Profiles of \(\beta\)-Thalassemic Patients

<table>
<thead>
<tr>
<th></th>
<th>Left-Sided Heart Failure (n=45)</th>
<th>No Heart Failure (n=58)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>28/17</td>
<td>33/25</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>24±4</td>
<td>26±5</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ferritin,* ng/mL</td>
<td>3010±1500</td>
<td>3080±1600</td>
<td>NS</td>
</tr>
<tr>
<td>Total blood units†</td>
<td>535±103</td>
<td>548±97</td>
<td>NS</td>
</tr>
<tr>
<td>Hb, g/dL</td>
<td>8.7±0.7</td>
<td>8.8±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDd, mm</td>
<td>55±4</td>
<td>41±4</td>
<td>0.0001</td>
</tr>
<tr>
<td>RVd, mm</td>
<td>23±5</td>
<td>23±6</td>
<td>NS</td>
</tr>
<tr>
<td>2D EF, %</td>
<td>30±8</td>
<td>60±8</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are mean±1 SD. Ranges are shown in parentheses. Hb indicates hemoglobin; EF, ejection fraction; LVEDd, left ventricular end-diastolic diameter; and RVd, right ventricular diameter.

*Mean serum ferritin value in each patient derived as the mean of 30 values obtained at 2-month intervals over the last 5-year period.

†Mean total number of blood units from initiation of transfusions to inclusion in the study.

TABLE 2. HLA-A, HLA-B, HLA-DR, and HLA-DQ: Differences Between \(\beta\)-Thalassemic Patients With and Without Heart Failure

<table>
<thead>
<tr>
<th>HLA Allele</th>
<th>Left-Sided Heart Failure (n=45)</th>
<th>No Heart Failure (n=58)</th>
<th>(P)</th>
<th>(\text{OR} P_{c})</th>
<th>(95% \text{CI})</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*1401</td>
<td></td>
<td></td>
<td></td>
<td>0.0017</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.4</td>
<td>18</td>
<td>31.0</td>
<td>0.10 (0.023–0.47)</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>97.7</td>
<td>44</td>
<td>75.9</td>
<td>0.04</td>
</tr>
</tbody>
</table>

TABLE 3. HLA-DRB1*1401 Allele Frequency in Patients With \(\beta\)-Thalassemia Major With and Without Heart Failure vs Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n=130)</th>
<th>Heart Failure (n=45)</th>
<th>No Heart Failure (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA DRB1*1401</td>
<td>10 (7.7%)</td>
<td>2 (4.4%)</td>
<td>18 (31.0%)</td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P_{c})</td>
<td></td>
<td></td>
<td></td>
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</table>

Discussion

This is the first study assessing the importance of the major histocompatibility complex in the expression of heart disease associated with homozygous \(\beta\)-thalassemia. The most notable finding of the present study was the increased frequency of the HLA-DRB1*1401 allele in patients with \(\beta\)-thalassemia without heart failure compared with those of similar age and iron loading with left-sided heart failure and with healthy controls. This implies that the HLA-DRB1*1401 allele may have a protective effect in the pathogenesis of heart failure in \(\beta\)-thalassemia. A second finding was the increased frequency of the HLA-DQA1*0501 allele in patients with \(\beta\)-thalassemia major with heart failure compared with those without heart failure and with healthy controls; this increased frequency was possibly related to an increased risk for heart failure development among patients positive for this allele. Multivariate analysis of the results showed that the DRB1*1401 allele is more important than DQA1*0501 in heart failure pathogenesis and possibly offers a protective effect. In addition, we found that 26.3% of patients without heart failure who were positive for the HLA-DQA1*0501 allele were also positive for HLA-DRB1*1401, whereas the same was observed in only 5.1% of the patients with heart failure. This may indicate that the coexistence of HLA-DRB1*1401 and HLA-DQA1*0501 genes does not alter the substantial protection conferred by the HLA-DRB1*1401 allele.

Heart Failure in \(\beta\)-Thalassemia Major

Heart failure is the leading cause of death in patients with \(\beta\)-thalassemia major (63.6%). Although biventricular heart...
failure in homozygous β-thalassemia has been traditionally attributed to iron overload,21,22 it has been recently documented that the pathophysiology of heart failure is poorly understood and multifactorial in etiology.23 β-Thalassemia major itself is not a true hemochromatosis; it is a secondary hemochromatosis, which is based on the combination of chronic hemolytic anemia, iron intestinal hyperabsorption, and multiple blood transfusions that the patients receive.

Left- and right-sided heart failure seems to be independent clinical entities with different pathogenetic mechanisms and survival rates. The majority of heart failure patients (82.7%) develop left-sided heart failure at a younger age, along with left ventricular dilatation and systolic dysfunction. Right-sided heart failure appears in an older and more hemosiderotic population (17.3%), characterized by right ventricular dilatation and normal left ventricular systolic function (unpublished data from our institution, 1999).

Engle at al24 first reported that 10 (30.5%) of 26 β-thalassemic patients with heart failure also had a history of pericarditis. In the present study, there was evidence of perimyocarditis in 19 (42%) of 45 patients with left-sided heart failure and in 20 (34%) of 58 patients without heart failure. In addition, we have recently reported2 that in a well-documented acute myocarditis population with β-thalassemia major, 11 (23.4%) of 47 patients developed acute heart failure and 13 (27.6%) of 47 patients developed chronic heart failure within $3 \pm 3.1$ years after the acute phase. All heart failure patients presented with left ventricular systolic dysfunction and dilatation.

Apart from myocarditis, which may lead to immune-mediated chronic left ventricular dysfunction and failure, other factors, acting through immunologic or genetically defined mechanisms, might also affect the development of left-sided heart failure. Multiple transfusions represent a repetitive antigenic stimulus together with iron chelation therapy itself. This is supported by the increased IgA neutral antibody activity found in the sera of Greek patients with homozygous β-thalassemia major.25 Iron loading, apart from its toxic effect, might contribute to heart failure development through immune-mediated mechanisms.26 In this respect, we have also recently found a significantly higher frequency of the ApoE e4 allele in patients with β-thalassemia major with left-sided heart failure.27 This allele is related with a decreased antioxidant activity and may represent an important genetic risk factor for the development of myocardial damage caused by iron myocardial deposition or myocarditis.28–30

Among the most widely recognized functions of the major histocompatibility complex is the presentation of antigens to the immune system and the determination of antigen immunogenicity. It is involved in numerous immune-mediated processes, one or more of which might be related to heart failure pathogenesis in β-thalassemia major. Apart from iron loading itself, infectious myocarditis seems to play an important role in the pathogenesis of heart failure, possibly acting in a way similar to that observed in idiopathic dilated cardiomyopathy. Myocarditis may initiate an autoimmune reaction13,12 in which HLA molecules seem to play a central role, through multiple pathogenetic mechanisms. Increased HLA antigen expression has been found in cardiac myocytes of patients with idiopathic dilated cardiomyopathy.33,34 Circulating autoantibodies35–37 and altered T-cell function38–41 induced by the major histocompatibility complex may lead to immune-mediated heart disease. Similarities between microbial antigens and self HLA molecules may result in autoimmune reaction after infections.26 It is reasonable to hypothesize that one or more of the HLA-mediated mechanisms mentioned above, culminating in the development of full-blown idiopathic dilated cardiomyopathy, might also be important in the pathogenesis of the left-sided heart failure observed in β-thalassemia major. In this regard, a demonstrable immune-mediated process would make the contribution of these molecules to heart failure very important.

In conclusion, the differential expression of DRB1*1401 and DQB1*0501 alleles between homozygous β-thalassemic patients with and without left-sided heart failure found in the present study supports the hypothesis of immunogenetic involvement in the pathogenesis of left-sided heart failure. Multiple blood transfusions, hemochromatosis, repetitive iron chelation therapy, and myocarditis may interact with the underlying immunogenetic background, which plays a regulatory role, either conferring protection or leading to irreversible left ventricular myocardial dysfunction and failure.

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References

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