asparagine concentrations return toward normal much faster when care is taken to inhibit ex vivo enzyme activity.\textsuperscript{4} We sent a blinded set of our samples to Dr Asselin, and she confirmed the asparagine values we reported. Liver perfusion studies and modeling of asparaginase treatment suggest that there is a high rate of asparagine input into the circulation from diet and the tissues.\textsuperscript{5,6} An equilibrium between the rate of asparagine input and the asparagine activity can result in a low (nonzero) steady-state asparagine concentration.

Vassilios I. Avramis and John S. Holcenberg

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To the editor:

Idiopathic thrombocytopenic purpura, \textit{Helicobacter pylori} infection, and HLA class II alleles

Recently, Emilia et al\textsuperscript{1} reported a high prevalence of \textit{Helicobacter pylori} infection in patients with idiopathic thrombocytopenic purpura (ITP) and a significant increase in platelet count after bacterium eradication. In this study, we analyzed the correlation between \textit{H pylori} infection and HLA class II alleles in 39 ITP patients (median age, 48.9 years; range, 21-82 years; 17 males, 22 females; M/F ratio, 0.8) observed at our department between December 1998 and April 2002. We compared the frequency of the HLA-DR/DQ antigens in these patients with that of 150 healthy bone marrow donors, matched for sex and age (Table 1).

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So far, there is little evidence of an association between major histocompatibility complex class II and ITP.\textsuperscript{2,3} A higher prevalence of

Table 1. Comparison of HLA-DRB1/-DQB1 frequencies of ITP patients and controls and relationship with \textit{Helicobacter pylori} infection

<table>
<thead>
<tr>
<th>HLA class II alleles</th>
<th>DRB1</th>
<th>DQB1</th>
<th>Controls (%), n = 150</th>
<th>ITP patients (%), n = 39</th>
<th>Controls (%), n = 150</th>
<th>ITP patients (%), n = 39</th>
<th>Controls (%), n = 150</th>
<th>ITP patients (%), n = 39</th>
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<tbody>
<tr>
<td></td>
<td>*01</td>
<td>*02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 (20.5)</td>
<td>17 (43.6)</td>
<td>32 (21.3)</td>
<td>NS</td>
<td>5 (20.8)</td>
<td>3 (20.0)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*15</td>
<td>*03</td>
<td>7 (17.9)</td>
<td>10 (25.6)</td>
<td>30 (20.0)</td>
<td>28 (18.6)</td>
<td>12 (10.2)</td>
<td>4 (2.6)</td>
</tr>
<tr>
<td></td>
<td>*16</td>
<td>*04</td>
<td>5 (12.8)</td>
<td>6 (15.4)</td>
<td>30 (20.0)</td>
<td>21 (14.0)</td>
<td>28 (18.6)</td>
<td>12 (10.2)</td>
</tr>
<tr>
<td></td>
<td>*03</td>
<td>*05</td>
<td>10 (25.6)</td>
<td>6 (15.4)</td>
<td>30 (20.0)</td>
<td>21 (14.0)</td>
<td>30 (20.0)</td>
<td>12 (10.2)</td>
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<tr>
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<td>*11</td>
<td>6 (15.4)</td>
<td>12 (30.8)</td>
<td>15 (10.0)</td>
<td>68 (45.3)</td>
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</tr>
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</tr>
<tr>
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<td>*13</td>
<td>*08</td>
<td>6 (15.4)</td>
<td>9 (23.1)</td>
<td>28 (18.6)</td>
<td>6 (4.0)</td>
<td>26 (17.3)</td>
<td>6 (4.0)</td>
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<tr>
<td></td>
<td>*07</td>
<td>*0910</td>
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<td>6 (4.0)</td>
<td>28 (18.6)</td>
<td>6 (4.0)</td>
</tr>
<tr>
<td></td>
<td>*08</td>
<td>*02</td>
<td>0</td>
<td>17 (43.6)</td>
<td>48 (32.0)</td>
<td>6 (4.0)</td>
<td>48 (32.0)</td>
<td>6 (4.0)</td>
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<td>94 (62.6)</td>
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<tr>
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<td>*04</td>
<td>*05</td>
<td>3 (7.7)</td>
<td>17 (43.6)</td>
<td>7 (4.6)</td>
<td>17 (43.6)</td>
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<tr>
<td></td>
<td>*05</td>
<td>*06</td>
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<td>0</td>
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<td>50 (33.3)</td>
<td>6 (4.0)</td>
<td>50 (33.3)</td>
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<tr>
<td></td>
<td>*06</td>
<td>*06</td>
<td>0</td>
<td>17 (43.6)</td>
<td>48 (32.0)</td>
<td>6 (4.0)</td>
<td>48 (32.0)</td>
<td>6 (4.0)</td>
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</tbody>
</table>

The \(\chi^2\) method with Yates correction and Fisher exact test were used for data analysis. NS indicates not significant.

*ITP patients versus healthy controls.

†

\(H pylori\)-positive patients versus \(H pylori\)-negative patients.

References


other class II alleles and ITP patients has been described in some human races, although other studies failed to demonstrate a statistically significant association. By contrast, in our study the HLA class II allele pattern seems to identify 2 groups of ITP patients with a different incidence of *H pylori* infection and, possibly, with different pathogenetic mechanisms.

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Mauro Krampfera, and Massimo Franchini

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Response:

Idiopathic thrombocytopenic purpura, *Helicobacter pylori* infection, and the HLA system

The interesting letter of Veneri et al gives us the opportunity for some remarks. First of all, the authors confirm the high prevalence of *Helicobacter pylori* infection in idiopathic thrombocytopenic purpura (ITP) patients. In a recent careful review of the literature, including an update of our case series, we found 112 *H pylori*–infected patients out of a total of 193 ITP patients studied so far. Thus, by including the 24 *H pylori*–infected, out of the 39 ITP patients reported by Veneri et al, the prevalence of *H pylori* infection in ITP is 58.6%. Of course, this important association between ITP and a bacterium infection may prompt to investigate whether host genetic factors are involved in the pathogenesis of the disease and in susceptibility to infection. With respect to this, the study of human leukocyte antigens (HLAs) seems to be appropriate.

Veneri et al found that a low frequency of HLA-DRB1*11* and -DQB1*03 alleles characterizes the ITP patients compared with healthy controls. Moreover, a low frequency of such alleles seems to be typical of *H pylori*–negative patients. This is a suggestive finding, but we would like to introduce a note of caution. The complexity of HLA system, the variability of *H pylori* strains, and the yet not well defined pathophysiology of ITP make this type of in vivo investigations very complicated.

In particular, in the literature there is a very large amount of reports dealing with possible correlations between diseases with underlying immune mechanisms and HLA system, with a great many alleles that correlate to certain features of the disease, while other alleles do not. For example, the HLA-DRB1*11* allele has been found at low frequency in hepatitis C virus (HCV)–positive Turkish patients, whereas allele has been found at high frequency in French HCV-positive patients with mixed cryoglobulinemia and vasculitis. In HIV-positive patients the -DR*4*11 allele seems to represent a risk factor for the AIDS development and poor prognosis, but some reports have failed to confirm such an association between HLA and the disease. In aplastic anemia, HLA-DR2 has been found at increased frequency, particularly in patients with associated paroxysmal nocturnal hemoglobinuria. The findings about *H pylori* infection and HLA are contradictory. Some HLA alleles, like -DQB1*0602, have been found at high frequency in *H pylori*–positive patients favoring gastric cancer occurrence, whereas -DQB1*0301 seems to be protective against cancer in Taiwanese patients. In contrast, other alleles were found to be not associated with *H pylori* infection and cancer risk in Italian (*DQA1* and -DQB1) and German (*DQA1*, -DQB1, and HLA class II) patients, respectively, whereas -DRB1*1501, -DQA1*01021, and -DQB1*0602 seem to be protective against *H pylori* infection in Japanese patients. Similarly, other different alleles were found at high or low frequency in Caucasian, Chinese, Spanish, Greek, and Japanese patients infected with *H pylori*. Similarly, as reported by Veneri et al, the few studies on ITP and *H pylori* infection revealed contradictory findings concerning a correlation with HLA alleles.

A better reassessment of these data and their possible clinical relevance should await for further rigorous studies on specific HLA alleles on very large series of ITP patients, which could necessarily take into account the racial differences of the population groups tested and also the *H pylori*–strain differences.

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References


To the editor:

**ABO blood group also influences the von Willebrand factor (VWF) antigen level in heterozygous carriers of VWF null alleles, type 2N mutation Arg854Gln, and the missense mutation Cys2362Phe**

The levels of von Willebrand factor (VWF) in plasma are influenced by several variables, such as age, blood group, pregnancy, hormones, and smoking. The VWF levels may vary greatly on repeated laboratory testing in the same subject. About 60% of the variation in plasma VWF is caused by genetic factors, with ABO blood group accounting for only 30% of this. Indeed, in the normal population VWF levels are 25%-35% lower in individuals with blood group O than in individuals with non-O blood groups. Based on the results of epidemiologic studies, blood group–specific reference ranges have been suggested for diagnosis in von Willebrand disease (VWD). This biologic variation in VWF concentration may cause pitfalls when diagnosing mild cases of VWD.

There are no reports on the effect of ABO blood group in subjects heterozygous for VWF null alleles or heterozygous for some specific missense mutations. The influence of the ABO blood group in these individuals would be worthy of reporting since in several of these cases bleeding is rare despite the presence of clearly reduced VWF levels in plasma.

We have compared the VWF antigen (VWF:Ag) levels, according to blood group, of the heterozygous subjects from our families with recessive VWD. These individuals are heterozygous for VWF null alleles (splice site mutation, stop codon, frameshift, gene deletion), the type 2N mutation Arg854Gln, or the recessive missense mutation 7085G (Cys2362Phe) in exon 42 of the VWF gene (and unpublished data, 1999). Cys2362Phe is a true recessive mutation since it causes bleeding in compound heterozygous or homozygous states only.

All heterozygous subjects carrying blood group O have significantly lower VWF:Ag levels regardless of the type of mutation (Table 1). Interestingly, subjects heterozygous for Cys2362Phe showed VWF:Ag values almost indistinguishable from those observed in carriers of a null allele. Subjects with the type 2N mutation Arg854Gln seem to have values roughly similar to those of healthy individuals. The same analysis was carried out also for factor VIII procoagulant activity (FVIII:C), but no significant differences in FVIII:C between subjects with O and non-O blood groups, according to the different types of mutations, were observed (data not shown).

Zhang et al reported phenotypic data in 3 groups of subjects heterozygous for null alleles (2430delC, Arg1659Xaa, and Arg1853Xaa). Subjects with Arg1853Xaa showed significantly lower VWF:Ag levels than did subjects heterozygous for 2430delC. But an excess of subjects with a non-O blood group (21, versus 9 with blood group O) was present in the group of subjects with 2430delC, compared to the group of subjects with Arg1853Xaa (1, versus 5 with blood group O). Thus the discrepant VWF:Ag levels between the 2 groups could be simply due to the different prevalence of blood groups. We have calculated VWF:Ag levels of subjects heterozygous for null alleles reported by Zhang et al. The mean VWF:Ag value for blood group O subjects (n = 17) was 32.3 ± 17.3 U/dL, in comparison with 48.8 ± 25.9 U/dL in non-O blood group subjects (n = 25; P = .05), thus confirming our results. As a further confirmation of the present findings, also in the study of Zhang et al, no differences were observed as to FVIII:C.

It has been recently suggested that VWF levels in carriers of blood group O are presumably lower due to increased clearance of VWF. The present results show that blood group–associated VWF difference still exists despite the fact that individuals with null alleles (and possibly Cys2362Phe) have a clear defect in synthesis. This defect causes a 50% reduction of VWF which is larger than the blood group–dependent variation. The fact that in addition to the synthetic defect of the null allele, the VWF synthesized by the second normal allele still shows variation due to blood group may suggest that the effect of blood group is independent of the synthesis and occurs after VWF is secreted into bloodstream. This hypothesis is supported also by the demonstration that platelet VWF content, which reflects endothelial synthesis and storage of VWF, is similar in subjects with O and non-O blood groups.

In conclusion, blood group significantly influences VWF:Ag level not only in normal subjects but also in heterozygous subjects with a null allele, Arg854Gln, or Cys2362Phe missense mutation. The contribution of blood group may explain in itself, at least in part, the heterogeneity of plasma phenotypes observed in heterozygous carriers for type 3 VWD. A puzzling, still unanswered question is why subjects with absolute reduction of VWF in plasma associated with heterozygosity for null alleles or, for instance, Cys2362Phe mutation are almost asymptomatic.

**Table 1. Von Willebrand factor antigen levels in subjects heterozygous for the Cys2362Phe mutation, null alleles, or Arg854Gln (type 2N) according to blood group**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean VWF:Ag ± SD, U/dL</th>
<th>Range, U/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys2362Phe*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O blood group (n = 8)</td>
<td>35.2 ± 16.2</td>
<td>25–55</td>
</tr>
<tr>
<td>Non-O blood group (n = 15)</td>
<td>61.5 ± 26.6</td>
<td>30–140</td>
</tr>
<tr>
<td>Null allele†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O blood group (n = 15)</td>
<td>43.2 ± 10.8</td>
<td>30–66</td>
</tr>
<tr>
<td>Non-O blood group (n = 15)</td>
<td>61.3 ± 23.6</td>
<td>25–98</td>
</tr>
<tr>
<td>Arg854Gln*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O blood group (n = 10)</td>
<td>75.4 ± 31.4</td>
<td>50–142</td>
</tr>
<tr>
<td>Non-O blood group (n = 7)</td>
<td>105.3 ± 24.3</td>
<td>74–153</td>
</tr>
</tbody>
</table>

*Values determined by Mann-Whitney test and indicate the differences between blood groups.

*P = .01.

†P = .03.

‡P = .003.
Idiopathic thrombocytopenic purpura, *Helicobacter pylori* infection, and HLA class II alleles

Dino Veneri, Michele Gottardi, Elisabetta Guizzardi, Carla Zanuso, Mauro Krampera and Massimo Franchini