Correlation of platelet FcγRIIA polymorphism in refractory idiopathic (immune) thrombocytopenic purpura

YVONNE WILLIAMS,1 SARA LYNCH,1 SHAUN MCCANN,2 OWEN SMITH,2 CONLETH FEIGHERY1 AND ALEX WHELAN1
Departments of 1Immunology and 2Haematology, St James’s Hospital, and 3Kevin Street College of Technology, Dublin, Ireland

Received 29 January 1998; accepted for publication 17 April 1998

Summary. FcγRIIA, a low affinity receptor for IgG, is a polymorphic molecule: FcγRIIA-HH131, FcγRIIA-HR131 and FcγRIIA-RR131. This polymorphism influences the efficiency of the receptor to bind with IgG2. Recent reports on altered distribution amongst individuals with heparin-induced thrombocytopenia (HIT) prompted us to examine the FcγRIIA polymorphism in a cohort of patients with refractory idiopathic (immune) thrombocytopenic purpura (ITP), in whom severe disease had required them to undergo splenectomy. 29 post splenectomy ITP individuals and 61 normal controls were investigated. Polymerase chain reaction (PCR) and a Southern blotting technique were used to determine the FcγRIIA polymorphism. Although the distribution of the allotypes of FcγRIIA in the control population was similar to that found in other European studies of Caucasian populations (15 (25%) HH131; 35 (57%) HR131; 11(18%) RR131), the patient group was skewed towards the RR131 allotype which has least efficiency for IgG2 binding (3 (10%) HH131; 12 (42%) HR131; 14 (48%) RR131; P < 0.005). These findings suggest that FcγRIIA polymorphisms may be implicated in the pathophysiology of ITP or may be responsible for modulating the immune response in this heterogenous autoimmune disease.

Keywords: Fc gamma receptor IIA, polymorphism, idiopathic (immune) thrombocytopenic purpura, platelets, autoimmunity.

Idiopathic thrombocytopenic purpura (ITP) is an immune mediated disorder of unknown aetiology. Autoantibodies are produced to platelets which results in removal of the opsonized platelets by the reticuloendothelial system (RES). Two forms of the disease exist: acute ITP, which predominately occurs in children and accounts for approximately 90% of ITP in childhood, and the chronic form which is commonly seen in adults. The vast majority of childhood ITP is self limiting: resolution within 6 months is normal whether or not therapy is instituted. Spontaneous remission is rare in adult ITP and therapeutic intervention, mainly splenectomy, is the rule (George et al, 1996).

The pathophysiological mechanisms responsible for immune thrombocytopenia are beginning to be understood. The target platelet proteins involved in the binding of the anti-platelet antibodies have been identified over the past decade to include: GPIIb–IIa (16.7–83.3%), GPIb–IX (13.7–83%), GPIb (3.3–47.1%), and GPIIIa (21.6–33.3%) with other platelet glycoproteins (GPIa–IIa, GPIV and GPV) having a less important role to play. Over 90% of ITP sera react with more than one of these structures (Zhen-Yi & Zhi-Xiang 1997). The destruction of antibody sensitized platelets is in part due to receptors for the Fc portion of IgG (FcγR), present on macrophages in the RES, and perhaps in part due to FcγR on the platelets themselves (Rubinstein et al, 1995).

Three different families of FcγR exist: FcγRI, FcγRII and FcγRIII, which are quite diverse in both their structure and function. Fc receptors are glycoproteins and are members of the immunoglobulin superfamily. They are found on many different cells (neutrophils, macrophages, lymphocytes, platelets) and form a critical link between the humoral and cellular immune responses. FcγRI has a very strong affinity for monomeric IgG, whereas FcγRII and FcγRIII will only bind effectively to IgG in the form of immune complexes. FcγRII has three genes A, B, C; the gene for FcγRIIA (the only FcγR on platelets) is polymorphic giving rise to two co-dominantly expressed alleles, FcγRIIA-H131 and FcγRIIA-R131. The polymorphic variation of FcγRIIA is due to a single base substitution at nucleotide position 494. The nucleotide adenine (A) is substituted for nucleotide guanine (G). This results in a change at amino acid 131 (histidine (H131) → arginine (R131)) which is on the second extracellular domain of the FcγRIIA molecule. This polymorphism
results in differing abilities to bind to subclasses of IgG, with the H131 allele being the only FcR to bind efficiently to IgG2. IgG2 is the most common subclass involved in antibacterial polysaccharide responses, and a skewed distribution of the FcγRIIA polymorphisms towards RR131 has been reported in patients with recurrent bacterial infection and autoimmune diseases (van de Winkel & Capel, 1993).

Recent reports on altered distribution amongst individuals with heparin-induced thrombocytopenia (HIT) (Brandt et al., 1995; Arepally et al., 1997) prompted us to examine the FcγRIIA polymorphism in a group of refractory ITP patients and to ascertain whether a particular allotype may be associated with disease severity.

PATIENTS AND METHODS

Subjects. Blood samples were taken from 30 refractory ITP patients who had volunteered to be part of the study. All the patients had undergone splenectomy for disease control. The post-splenectomy interval at the time of the study varied between 1 and 13 years with an average of 4 years. The male to female ratio was 1:2 and the mean age was 38–4 years (7–75 years). There were 60 normal controls consisting of laboratory staff and medical students with a similar sex ratio as the patient group. The median age was 20 years with a range from 20 to 55 years. The citrated blood samples were stored at −70°C, then thawed and the DNA extracted.

PCR and Southern blotting. The base change in the FcγRIIA gene was detected using a PCR protocol adapted from that described by Osborne et al. (1994). The sense primer (p63) is found upstream of the FcγRIIA polymorphism and does not distinguish between FcγRIIA, B or C. The antisense primer (p52) downstream of the polymorphism contains nine bases unique to FcγRIIA. These amplify the section containing the codon effecting the polymorphism. The gene product of 1000 base pairs was separated out by electrophoresis through an agarose gel (Fig 1) and then transferred to nitrocellulose by Southern blotting. The DNA was then baked onto the nitrocellulose membrane at 80°C in a vacuum. Using digoxigenin (DIG) labelled allelic specific oligonucleotide (ASO) probes, the blots were hybridized at 41°C (for adenine) or 47°C (for guanine). The blots were stained with an alkaline-phosphatase conjugated anti-DIG antibody and the nucleotides visualized following incubation with nitroblue tetrazolium salt (NBT) and X-phosphate (Fig 2). A known FcγRIIA-HH131 individual and a known FcγRIIA-RR131 individual phenotyped by alternative methods were used as positive and negative controls with each of the ASOs.

Staining occurring on both blots indicated that the individual was heterozygous (HR131) for the allele, whereas homozygous individuals were positive in either the ‘A’ blot (HH131) or the ‘G’ blot (RR131) only.

Statistics. The statistics used were Chi-squared test for independence. The null hypothesis for this test is that there was no association between the rows and the columns. The Fisher exact test was carried out for each individual allotype which was more suitable for comparing only two columns. Statistics were performed with the aid of ‘InStat 2.03’ (the statistic package for Macintosh).

RESULTS

The frequency of FcγRIIA polymorphism among ITP patients

Fig 1. Polymerase chain reaction (PCR). Electrophoresis of PCR product through an agarose gel. The amplified FcγRIIA gene product is indicated at 1078 bp.
and normal controls is shown in Table I. In the control group, 57% (35) had the FcγRIIA-HR131 allotype, whereas the HH131 and RR131 allotypes were present in 25% (15) and 18% (11) respectively. This distribution is similar to that shown in other Caucasian studies (Salmon et al, 1996). However, 14 (48%) of the patients had the RR131 allotype, and three (10%) were of the HH131 and 12 (42%) of the HR131 allotypes. Using the Fisher Exact test, and three (10%) were of the HH131 and 12 (42%) of the HR131 allotypes. Using the Fisher Exact test, χ² analysis was carried out and found to be $P < 0.005$. These results indicated that patients with severe ITP showed statistically significant skewing towards the RR131 allotype.

### Table I. Distribution of FcγRIIA allotypes among ITP patients and normal controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>FcγRIIA-HH131</th>
<th>FcγRIIA-HR131</th>
<th>FcγRIIA-RR131</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITP patients</td>
<td>10%, P = 0.16* (n = 3)</td>
<td>42%, P = 0.18* (n = 12)</td>
<td>48%, P &lt; 0.005* (n = 14)</td>
</tr>
<tr>
<td>Controls</td>
<td>25% (n = 15)</td>
<td>57% (n = 35)</td>
<td>18% (n = 11)</td>
</tr>
</tbody>
</table>

ITP: idiopathic (immune) thrombocytopenic purpura; H, histidine; R, arginine.
* Fisher Exact test.

**DISCUSSION**

FcγRIIA polymorphism has been associated with various clinical conditions, including HIT (Brandt et al, 1995; Arepally et al, 1997), SLE (Salmon et al, 1996), meningococcal septicaemia (Bredius et al, 1994) and childhood recurrent bacterial infections (Sanders et al, 1994). Apart from HIT, where it has been reported (Brandt et al, 1995) that the FcγRIIA-HH131 allotype occurs more frequently amongst patients who have a more severe form of HIT (although this finding is disputed by others (Arepally et al, 1997)), the disease associations have been mainly with the FcγRIIA-RR131 genotype. In the study presented here we show a shift towards the FcγRIIA-RR131 genotype in patients with ITP. Thus, although the expected patient frequency based on our control population was five, 14/29 patients were homozygous for the FcγRIIA-RR131 genotype. Moreover, the FcγRIIA-HH131 genotype appears to be somewhat protective, in that the number of patients with this genotype was three, whereas the expected frequency was seven. All patients in our study group had undergone splenectomy, which could suggest that the group was preselected by their disease.

The aetiology of ITP is unknown, although an increased incidence in childhood ITP has been reported following infections. Fc receptors on phagocytic cells play a fundamental role in eliminating microbial infections and the FcγRIIA-HH131 receptor is the only receptor to bind IgG2 efficiently. As IgG2 is commonly synthesized in response to polysaccharide antigens, it is possible that in the absence of the appropriate Fc receptor, clearance through phagocytic mechanisms is inappropriately dealt with, and the infection is prolonged, resulting in residual levels of antigen. Low density of antigen expression has been reported to initiate a T helper 2 (Th2) response, i.e. CD4+ T cells that stimulate B-cell activation (Pfeiffer et al, 1991). The Th2 response may cause a further increase in IgG2 antibodies and the possibility of molecular mimicry to crossreactive antigens, some of which may be on platelets. Thus inappropriate clearance of bacterial antigens may predispose individuals to ITP, which may be the case in the FcγRIIA-RR131 group. Moreover, the FcγRIIA polymorphism may influence the clinical outcome and the severity of thrombocytopenia seen in the ITP patient. Consequently, depending on the patient’s IgG subclass response to the platelet antigens as well as their FcγRIIA polymorphism, platelets may be cleared at different rates by their phagocytic system. For example, patients homozygous for FcγRIIA-HH131 producing IgG1, IgG2 or
IgG3 platelet antibodies may have a more rapid clearance of their platelets compared to patients homozygous for FcγRIIA-R131. Thus, although patients with the FcγRIIA-H/H131 genotype may be less susceptible to ITP, they may have a more aggressive clinical outcome if they have this genotype.

Recently, evidence of a crucial role for FcγRIIA in ITP has been demonstrated by McKenzie et al. (1997). Human FcγRIIA transgenic mice were shown to have a more severe form of experimentally induced immune-mediated thrombocytopenia than similarly treated wild-type mice, indicating that FcγRIIA contributes to the clearance of platelets. In humans, however, the allotype of FcγRIIA must also be considered, and only one type (R131) may be involved in more severe disease, whereas the other (H131) may be protective of disease.

Thus, Fc receptor mediated clearance of platelets may be influenced to a lesser or greater extent by the subclass of platelet antibodies produced and the patient’s Fc receptor polymorphism.

ACKNOWLEDGMENTS

We are very grateful to the people who took the time to take part in the study and, in particular, special thanks to Catriona Duggan, who liaised with the patients and organized sample collection.

REFERENCES


