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Fcγ RECEPTOR ALLOTYPES IN CHILDREN WITH BACTERIAL MENINGITIS*

A Preliminary Study

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SUMMARY: Tezcan İ, Berkel A.İ., Ersoy F, Sanal Ö, Kanra G. (Immunology and Infectious Diseases Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Fcγ receptor allotypes in children with bacterial meningitis. A preliminary study. Turk J Pediatr 1998; 40: 533-538.

IgG2 antibody is the essential subclass to protect against encapsulated bacteria FcγRIIa is the only Fcγ receptor that interacts with human IgG2. The two genetically determined allotypes of human FcγRIIa, FcγRIIa-R131 and FcγRIIa-H131 alleles have functionally different reactivities with IgG2 in vitro, and H/H-131 cells have markedly higher binding affinity for human IgG2. Homozygous FcγRIIIb-NA1/NA1 PMNLs show higher phagocytic capacity than FcγRIIIb-NA2/NA2 PMNLs. To evaluate in vivo significance of FcγRIIa and FcγRIIIb allotypes, we analyzed FcγR allotypes in children with bacterial meningitis due to Haemophilus influenzae type b, Streptococcus pneumoniae and Neisseria meningitidis. FcγRII and FcγRIIIb polymorphisms were determined by using quantitative flow cytometry. FcγRIIa were studied in 23 children with bacterial meningitis and 50 healthy Turkish controls, and FcγIIb in 18 and 43 such individuals, respectively. The distribution of FcγRIIa in the healthy Turkish control group was found to be significantly different from that in the Chinese and Japanese population ($p<0.05$), but similar to that of the white population in the USA and the Netherlands. No case (0%) had the FcγRIIa-H/H-131 FcγRIIIb-NA1/NA1 the corresponding figure in the controls was 4 (9.3%). Homozygous FcγRIIa-H/H-131 phenotype was underrepresented with borderline significance (p: 0.057) in patients below two years of age in comparison with the healthy subjects and with patients with meningitis over two years of age (p: 0.059). Although the study needs to be conducted in a large series of patients in order to draw a firm conclusion, FcγRIIa polymorphism may be a contributing factor to the increased susceptibility to meningitis with encapsulated bacteria in children below two years of age. *Key words: FcγR allotypes, bacterial meningitis.*

Encapsulated bacteria such as *Haemophilus influenzae type b, Streptococcus pneumoniae, Neisseria meningitidis* are the most common etiological agents in the bacterial meningitis of childhood[1]. Antibodies against bacterial polysaccharide capsules are of prime importance in the defense system. Opsonophagocytosis due to antipolysaccharide antibodies and an intact complement system play a role in protection against *Neisseria meningitidis.* Especially in people with a

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normal complement system, specific IgG2 antibodies are essential to protect against this microorganism[2,3]. Receptors for the Fc region of IgG, FcγR, provide an important link between cellular effector mechanisms and antibody-antigen interaction[4]. The neutrophils (PMNL) have two types of FcγRs including IIa and IIIb. FaγRIIa is the only Fc receptor with high binding affinity for human IgG2. The two genetically determined allotypes of human FcγRIIa, FcγRIIa-R131 and FcγRIIa-H131, have functionally different reactivities with IgG2. The polymorphism of FcγRIIa is attributable to one amino acid difference which is critical for IgG2 binding, a histidine (H) or an arginine (R) residue at position 131. FcγRIIa-H/H-131 cells have markedly higher binding affinity for human IgG2 than do R/R-131 cells, whereas heterozygous H/R-131 cells have intermediate affinity[5]. PMNL from homozygous FcγRIIa-R131 are less effective than FcγRIIa-H/H 131 at phagocytizing IgG2-opsonized H. influenzae type b and Staphylococcus aureus, in vitro[6].

The FcγRIIIb has an allotypic polymorphism referred to as neutrophil antigens (NA) 1 and NA2. PMNL with the NA2 allotypic form internalize Ig-opsonize particles less effectively than do PMNL with the NA1 form, in vitro[7,8].

In this study we evaluated the in vivo significance of FcγRII a and FcγRIIIb allotypes of FcγRs in children with bacterial meningitis due to *H. influenzae type b, S. pneumoniae and N. meningitidis* who did not have any primary immunodeficiency disorder.

Material and Methods

Twenty-three children who were diagnosed with bacterial meningitis by positive cerebrospinal fluid culture in Hacettepe University Children's Hospital were enrolled in the study. Of 23 children (14 male, 9 female), *S. pneumoniae* was isolated in 8, *H. influenzae type b* in 6, and *N. meningitidis* in 9. Their ages were between three months and 16 years (median age 2 years). A control group consisted of 50 healthy volunteers from different regions of Turkey.

Immunological studies including serum immunoglobulins, IgG subclasses and complement system were found to be normal.

Determination of FcγRIIa and IIIb allotypes: The FcγR allotypes were determined by quantitative flow cytometry[9]. For FcγRIIa allotyping, we used monoclonal antibody (MAb) 41H16 (provided by T. Zipft MD, Anderson Cancer Center, Tx USA), which only reacts with the FcγRIIa-R131 allotype, and MAb IV.3 (Medarex, Annandale, NJ, USA), which binds to both IIa-R131 and IIa-H131. Fluorescence was measured with a flow cytometer (FACScan, Becton Dickinson, San Jose, CA, USA).

The FcγRIIIb-NA1/NA2 allotype was also determined with immunofluorescence analysis using specific MAbs to the IIIb-NA1 (CLB/Gran 11: CLB) and IIIb-NA2 allotypes (GRM1: provided by M. Garrido, Granada, Spain).

Statistical analysis: The phenotypic distribution of FcγR allotypes between patients and controls was compared with the Fisher's exact test.

Results

FcγRIIa allotypes were determined in 23 patients and 50 healthy Turkish controls. FcγRIIIb allotypes were studied in 18 patients and 43 controls. The distribution of allotypes is given in Table I. There was no statistical difference between the patient and control groups.

We analyzed the combined phenotype frequency for FcγRIIa and FcγRIIIb. In the patient group, H/H-131 and NA1/NA1 combination (theoretically, the more resistant form in vitro) was found in none while the corresponding figure in the healthy control group was 4 (9.3%) ($p>0.05$) (data not shown).

H/H-131 phenotype was underrepresented with borderline significance (p: 0.057) in children with meningitis below two years of age in comparison with the healthy subjects and patients with meningitis over two years of age (p: 0.059, Table II).

Discussion

A number of studies have been performed from different countries on the phenotypic frequency of FcγRII and FcγRIIIb[10-13]. The distribution of FcγRIIa in a healthy Turkish group was found to be significantly different from that of Chinese

Table I: Distribution of FcγR Allotypes in Patients with Meningitis and Healthy Controls

	Patients		Controls	
	No.	%	No.	%
FcγRIIa				
H/H-131	6	(26)	18	(36)
H/R-131	8	(35	19	(38)
R/R-131	9	(39)	13	(26)
FcγRIIIb				
NA1/NA1	2	(11)	8	(19)
NA1/NA2	10	(56)	19	(44)
NA2/NA	6	(33)	16	(37)

Table II: Comparison of Controls and Patients According to Age Limit

FcgRIIa Allotypes	Patients		Controls
	<2 Years No. (%)	>2 Years No. (%)	No. (%)
H/H-131	1 (8.3)	5 (45.5)	18 (36)
Other allotypes	11 (91.7)	6 (54.5)	32 (64)

p: 0.059 p: 0.82

p: 0.057

and Japanese populations ($p<0.05$) but was similar to that of the white population of the Netherlands and the USA. No difference was found between our control group and other ethnic groups with respect to FcγRIIIb allotypes.

Although many publications mention genetic localization, molecular structure and in vitro function of FcγRs, their in vivo significance is not clear and the subject needs further clinical investigation. According to epidemiological studies performed in Japan, meningococcal disease and infections with H. influenzae are very rare; the H-131 phenotype was documented as 85 percent in the Japanese population versus 30 percent among Caucasians[10,14]. This finding suggests that FcγRIIa allotypes play a role in the resistance to infections due to encapsulated bacteria. Our study suggested that the Turkish population may have a similar susceptibility pattern to infections caused by encapsulated bacteria as observed in the white populations of the Netherlands and the USA[11,12].

Fijen et al.[13] showed a correlation between meningococcal disease and R/R 131-NA2/NA2 phenotype in adult patients with primary late complement component deficiency. Bredius et al.[15] reported that R/R-131 homozygosity was significantly higher in a group of children with fulminant meningococcal shock due to meningococcal disease in comparison with healthy controls. In our study, combined H/H-131, NA1/NA1 phenotype was not present in the patient group. This finding is rather remarkable although it was not statistically significant as a small study group.

We observed that the H/H-131 phenotype was underrepresented at a borderline significance (p: 0.057) in children with meningitis below two years of age, the age group with relatively low IgG2 levels and immature antibody response to

bacterial polysaccharides, in comparison with the healthy control subjects. The functional differences between H-131 and R-131 phenotypes may be more critical when IgG2 antibody levels are relatively low, and having the R/R-131 phenotype may be less important when IgG2 anticarbohydrate antibodies reach adult levels[16]. It is conceivable that the physiological increase of IgG2 levels may overcome the disadvantage of R/R-131 with low binding capacity for IgG2 type antibodies. Our results may support the theory that if high levels of IgG2 antibodies are used in vitro, the difference in the binding capacities between H/H/131 and R/R-131 phenotypes is decreased.

In summary, although the study needs to be conducted in a large series of patients to draw a firm conclusion, our findings suggest that FcγRIIa polymorphism may be a contributory factor in increased susceptibility to meningitis with encapsulated bacteria in children below two years of age.

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