FcγRIIa polymorphism in patients with rheumatoid arthritis

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ABSTRACT

Objective. *Polymorphism of phagocyte IgG receptor FcγRIIa may modulate immune complex mediated inflammation, particularly when immune complex contain IgG2.*

Methods. *FcγRIIa genotyping in 82 patients with rheumatoid arthritis (RA) and 148 healthy subjects was performed using the polymerase chain reaction technique with allele specific primers.*

Results. *No significant relation between FcγRIIa genotypes and susceptibility to RA was observed, but extraarticular complications with high frequency were revealed in patients with R/R131 genotype.*

Conclusion. *The results suggest that the FcγRIIa polymorphism is not a risk factor for RA.*

Introduction

The chronic tissue-destructive process in patients with rheumatoid arthritis (RA) has been attributed to an ongoing immune response in which immune complexes play an important role. Immune complexes containing IgG may be a major pathogenic factor, responsible for the initiation and persistence of the inflammatory cascade and its resulting destructions in the cartilage (1, 2). IgG complexes interact with synovial cells via cellular receptors for IgG. The most widely distributed class of IgG Fc receptors is receptor FcγRIIa, which mediates a variety of physiological functions upon clustering by immune complexes. An allelic polymorphism in the human FcγRIIa receptor gene that consists of a single base G-A substitution at nucleotide 494 results in an amino acid change from arginine R to histidine H at position 131 (3). This substitution alters the affinity of the receptor for at least three IgG subclasses: murine IgG_1, human IgG_2 and IgG_3. The polymorphism was defined by differences in the binding of murine IgG_1 anti CD3mAB to FcγRIIa of human monocytes and T cell. The allelic form with arginine as amino acid 131 expressed high affinity for murine IgG_1 whereas the form with histidine showed low affinity (4). Recent data suggest that this polymorphism may be relevant to FcγRIIa function. Polymorphonuclear neutrophils homozygous for H/H131 show greater capacity to phagocytose bacteria or erythrocytes opsonized with IgG_2 than those homozygous for R/R131 (5). This polymorphism may also be implicated in the susceptibility to heparin-induced thrombocytopenia and lupus erythematosus, especially lupus nephritis (6, 7).

The aim of the study was to determine the FcγRIIa polymorphism in patients with rheumatoid arthritis

Materials and methods

Materials

We examined 82 patients (56 women, 26 men, age 16-79 years, mean 54.2) with rheumatoid arthritis diagnosed according to the criteria of American College of Rheumatology. The disease duration was 1-20 years (mean 14.3). Patients were recruited from the outpatient and inpatient populations of the Department of Rheumatology, University Hospital in Szczecin, Poland. All subjects were Caucasian from the Pomeranian region of Poland.

Subjects involved in the study underwent routine biochemical blood analysis and when required anticardiolipin antibodies, antinuclear antibodies and immunologic complexes. In all patients the x-ray of chest, hands and feet and when required other joint were performed. The evaluation of subjects included physical examinations with attention to pattern of joint involvement, presence of nodules and other extraarticular features such as vasculitis, anaemia, sicca syndrome, amyloidosis, organ involvement, and laboratory features such as erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF). The subcutaneous nodules without other extraarticular manifestations in 19 patients were diagnosed. The group with other extraarticular manifestations included 15 patients: 3 with anaemia, 2 with anaemia and nodules, 1 with vasculitis and nodules, 6 with vasculitis, 1 with vasculitis and amyloidosis, 2 with sicca syndrome and amyloidosis. Amyloidosis was diagnosed by histomorphology (skin- and bowel- or duodenum biopsy), vasculitis by histomorphology (skin biopsy) and angiogram.

The group with severe joint manifestations included patients with more than 6 swollen joints and radiologically diagnosed erosions. In the group with severe RA was 15 patients with particularly severe disease progress, resistant to second-line therapy. The control group consisted of 148 healthy subjects (86 women and 62 men, age 19-75 years, mean 48.7). The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Isolation of genomic DNA

Genomic DNA was extracted manually (precipitation with trimethylammonium bromide salts from leukocytes contained in 450 μL of venous blood with ethylendiaminetetraacetic acid (EDTA) as an anticoagulant. DNA was then precipitated in 95% ethanol dissolved in distilled water, and stored at -20°C until analysis (8). The chemicals for the extraction were purchased from Sigma Company (St Louis, Mo).

FcγRIIA genotyping

We performed 25 μL PCRs containing 2.5 μL of genomic DNA (approx. 100 ng), 2.5 μL of 10 x PCR buffer (contains 15 mM $MgCl_2$, Gibco BRL), 200 μM of each dNTP (Gibco BRL), and 0.5 U of Taq polymerase (Gibco BRL). We used 0.5 μM H131–specific sense primer (5`-ATCCCAGAAATTCTCCCA-3`) from the second extracellular domain or 0.5 μM R131-specific sense primers (5`-ATCCCAGAAATTCTCCCG-3` (all primers were synthesized by Gibco BRL) and 0.5 μM common antisense primer from an area of the downstream intron where the sequences for FcγRIIa, FcγRIIb, and FcγRIIc diverge (5`-CAATTTTGCTGCTATGGGC-3`). The resulting fragment was 253 bp in length. As internal control, we used 0.125 μM human growth hormone (HGH)–I primer (5`-CAGTGCCTTCCCAACCATTCCCTTA-3`) and 0.125 μM HGH-II primer (5`-ATCCACTCACGGATTTCTGTTGTGTTTC-3`), which resulted in a 439-bp fragment. We used a thermal cycler (Mastercycler Eppendorf) to perform a hot-start PCR as follows: 5 minutes at 95°C, 10 cycles of 1 minute at 95°C, 2 minutes at 57°C, and 1 minute at 72°C; thereafter, to enhance the sensitivity, we used 22 cycles of 1 minute at 95°C, 2 minutes at 54°C, and 1 minute at 72° C and a final extension step for 5 minutes at 72°C. Each PCR analysis was performed with control samples for R/R, H/R and H/H genotypes. The PCR amplification products were separated on 1.5% agarose and visualized by using ethidine bromide (9).

Statistical analysis

Frequencies of FcγRIIa alleles were given with 95% confidence intervals (95%CI). The distribution of FcγRIIa alleles in patients with RA was compared with healthy subjects and statistically evaluated by use of the $\chi 2$ test with Yates correction for small groups.

Results

The distribution of FcγRIIa genotypes in 148 Polish healthy subjects did not differ from that of Caucasian populations (Table I). The genotypes R/R131, R/H131 and H/H131 were found in 33 (22.3%), 71 (48.0%) and 44 (29.7%) subjects respectively and there was no difference in distribution between the male and female controls. For the total group of 82 RA patients the distribution was: R/R131:19 subjects (23.2%); R/H131: 36 (43.9%); and H/H131: 27 (32.9%) and did not significantly differ from the control group (Table I).

Subcutaneous nodules without other extraarticular manifestations were detected among 6 (31.6%), 7 (19.5%), 6 (22.2%) of patients with genotypes R/R131, R/H131 and H/H131 respectively. The differences were not statistically significant (Table II). Other extraarticular manifestations were diagnosed in 6 (31.6%) patients with R/R131 genotype, in 5 (13.9%) with R/H131 and in 4 (14.8%) with genotype H/ H131. The odds ratio for the risk of extraarticular complications in individuals with R/R131 genotype was 2.65 greater, (95% CI 0.88 – 9.63) than in subjects with genotype H/H131. The frequency of extraarticular involvement was not high enough to yield statistical significance. A larger cohort of RA patients with extraarticular manifestations will be required to confirm that association. Among subjects with R/R131 genotype magnitude joint involvement in 8 patients (42.1%) was diagnosed, but in subjects with genotypes H/R131 and H/H131 in 16 (44.4%) and 11 (40.7%) respectively. The risk of severe joint manifestations was similar in particular FcγRIIa genotypes (OR for R/R131 vs H/H131 - 1.06) (Table II). Moreover the correlation between FcγRIIa polymorphism and diseases activity and response under therapy was revealed. In subjects with R/R131 genotype 14 patients (73.7%) with active RA were found, in subjects with H/R131 26 (72.2%) in H/H131 19 (70.4%). The risk of active of RA not differed depending on FcγRIIa genotypes (OR for

Table I. Distribution of FcγRIIa genotypes in control group and RA patients.

	FcγRIIa R/R 131		FcγRIIa R/H 131		FcγRIIa H/H 131		p value*
	n	%	n	%	n	%	
Control group n - 148	33	(22.3)	71	(48.0)	44	(29.7)	NS
RA patients n - 82	19	(23.2)	36	(43.9)	27	(32.9)	NS

* RA patients vs control group.

Table II. The distribution of FcγRIIa genotypes in RA patients.

	FcγRIIa R/R 131 n - 19		FcγRIIa R/H 131 n - 36		FcγRIIa H/H 131 n - 37		P value*	OR	95% CI
	n	%	n	%	N	%			
Patients with nodulosis without other extra-articular manifestations	6	(31.6)	7	(19.5)	6	(22.2)	NS	1.62	0.56 – 7.48
Patients with extraarticular manifestations	6	(31.6)	5	(13.9)	4	(14.8)	NS	2.65	0.82 - 14.15
Patients with positive RA	14	(73.7)	21	(58.3)	15	(59.3)	NS	1.93	0.46 – 8.41
Patients with severe joint involvement	8	(42.1)	16	(44.4)	11	(40.7)	NS	1.06	0.27 – 4.11
Patients with active RA	14	(73.7)	26	(72.2)	19	(70.4)	NS	1.18	0.27 – 5.34
Patients with severe RA	4	(21.0)	7	(19.4)	4	(14.8)	NS	1.52	0.26 – 8.96

* Patients with R/R 131 genotype vs H/H 131.

R/R131 vs H/H131- 1.18).

There was also no correlation between FcγRIIa polymorphism and response of the disease under therapy. Among subjects with R/R131 genotype were 4 patients (21.0%) resistant to second-line therapy, in H/R131 and H/H131 7 (19.4%) and 4 (14.8%) respectively. The risk was similar for R/R131, H/R131 and H/H131 genotypes (OR for R/R131 vs H/H131 - 1.53).

Among patients with R/R131 genotype rheumatoid factor in 14 subject (73.7%) was diagnosed, for R/H131 genotype in 21 (58.3%), for H/H131 genotype in 16 (59.3%). There was no statistically significant difference in frequency of rheumatoid factor between studied groups (Table II).

Additionally, there was no correlation between the FcγRIIa polymorphism and age of first occurrence of clinical symptoms among patients with RA, what may suggest that specific FcγRIIa alleles are not associated with disease susceptibility (Table III).

Discussion

This study examined the hypothesis that FcγRIIa polymorphism may be a heritable factor influencing RA susceptibility and its extraarticular manifestations.

The distribution of FcγRIIa genotypes in control population was similar to that obtained in other European populations (10, 11). Comparing the distribution of FcγRIIa genotypes among RA patients with controls there was no significant difference between these two groups.

The Fcγ receptors for IgG constitute a family of hemopoetic cell surface molecules that can stimulate cellular responses upon binding of antibody-antigen complexes and mediate antibody–dependent phagocytosis (12, 13). FcγRIIa receptors play an essential role in the clearance of immune complexes (14). Impaired handling and removal of immune complexes by the mononuclear phagocyte system results in its deposition and inflammatory processes in organs and tissues. However, the underlining basis of such defective immune complex clearance and its relation to the observed clinical manifestation has not been defined. Down regulation of FcγRIIa expression and function effects on signal transduction and anti Fcγ antibodies have been implicated as factor in aberrant Fcγ functioning (15, 16).

Indeed, there is increasing evidences that the FcγRIIa R/R131 genotype is a risk factor for the manifestation of immune complex mediated diseases. The impaired phagocytosis of immune complexes seen in patients with systemic lupus erythematosus can be correlated to the presence of R/R131 allele (17,18). The SLE patients with R/R131 genotype develop at a higher rate lupus nephritis, haematological abnormalities, sicca syndrome, antinuclear antibodies and hypocomplementemia (19). Additionally, FcγRIIa polymorphism is associated with heparin-induced thrombocytopenia (20, 21). Furthermore, the FcγRIIa polymorphism has an impact on the susceptibility to bacterial infections (5,22). Phagocytosis of IgG-opsonized bacteria is less effective in individuals with R/R131 allotype and these patients exhibit a higher susceptibility to infection by encapsulated bacteria. Accordingly, a low incidence of infection with encapsulated bacteria has been noted in the Japanese population where the prevalence of H/H131 allotype was observed (11, 23).

Several lines of evidence have impli-

Table III. The mean age of first occurence of clinical symptoms among patients with RA according to FcγRIIa genotypes.

	FcγRIIa R/R 131		FcγRIIa R/H 131		FcγRIIa H/H 131		p value *
Mean value (years) ± SD	44.9	(± 8.66)	46.22	(± 15.21)	49.59	(± 10.75)	0.15

*Patients with genotype R/R 131 vs H/H 131.

cated Fc receptors, in particular IgG binding receptors in RA pathogenesis (24). FcγRIII was detected on synovial intima in normal and arthritic human joints and on invading macrophages. An FcγRIII gene polymorphism has been correlated with RA susceptibility and extraarticular manifestations involvement (25, 26).

The results of our study suggest that FcγRIIa polymorphism does not represent a genetic risk factor for the RA occurrence and disease progress. Thought the risk of some extraarticular manifestations was higher in subjects with R/R131 genotype, there ware no correlations with joints manifestations, disease activity, response under therapy.

It seems that RA pathogenesis may be associated with FcγRIII polymorphism but FcγRIIa polymorphism is involved particularly in immune complexes mediated diseases such as SLE.

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