

IgE Anti-IgG Antibodies in Patients with Juvenile and Adult Rheumatoid Arthritis Including Felty's Syndrome

HENRIK PERMIN and ELSE-MARIE EGESKJOLD

Laboratory of Immunology, University Clinic for Infectious Diseases, Rigshospitalet, Copenhagen, and the Rheumatism Research Unit of Aarhus University, Aarhus, Denmark

Anti-IgG antibodies (anti-IgG) of the IgE class were studied in sera from patients with juvenile rheumatoid arthritis (JRA), rheumatoid arthritis (RA) and patients with Felty's syndrome (FS) by use of an indirect immunofluorescence technique. Forty-two per cent of 26 patients with JRA had IgE anti-IgG in serum all in low titers. Positive reactions prevailed in patients with multiple joint involvement. Sixty-three per cent of 30 patients with RA and 80% of 20 patients with FS had IgE anti-IgG, the titers found in FS patients being significantly higher. In JRA and FS patients the IgE anti-IgG titers were correlated to the titers of anti-IgG of the IgG class, and for FS patients also with the IgM and IgA classes of anti-IgG. In six of 10 patients with RA the synovial fluid samples from both knees contained IgE anti-IgG. In four of these patients the titers of IgE anti-IgG were higher than in the corresponding serum sample, pointing to a local production. After G-200 Sephadex chromatography IgE anti-IgG were demonstrated in the void volume indicating the presence of these autoantibodies in immune complexes. IgE anti-IgG may be involved in the pathogenesis of JRA and RA by eliciting Type I and III reactions.

Key words: anti-IgG antibodies; antinuclear antibodies; Felty's syndrome; IgE; juvenile rheumatoid arthritis; rheumatoid arthritis; synovial fluid.

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Raised levels of serum IgE and eosinophils are known as hall-marks in atopic patients. IgE has also been demonstrated in the skin from this type of patients. IgE has a great affinity to basophilocytes and mast cells and is suggested to play a role in Type I reactions. Juvenile and adult rheumatoid arthritis (JRA and RA) may involve Type I allergic mechanisms. This supposition is based on the finding of IgE anti-IgG antibodies (anti-IgG) in the sera from JRA and RA patients (11, 12, 14). Histamine release from basophilocytes of RA patients by stimulation with nuclear antigens and aggregated IgG may reflect the functional role of these antibodies (13). Further, aggregated IgG, IgM and immune complexes (IC), the levels of which

may be raised in RA patients, may promote chemotaxis of eosinophils (5).

The purpose of the present study was to determine the prevalence of IgE anti-IgG in the sera from patients with JRA and RA and to investigate whether such antibodies may participate in IC formation.

MATERIAL AND METHODS

Twenty-six patients with JRA, fulfilling the proposed criteria for JRA (3), were examined. Eleven had pauci- and 11 polyarticular disease and four had the systemic type of JRA. In all except three patients the disease was estimated to be active at the time of study (12). Fifty

Table 1

Patients' age and disease duration, and the prevalence of IgE, IgG, IgM and IgA anti-IgG antibodies in the groups studied

Subjects	$\frac{\text{♀}}{\text{♂}}$ <i>n</i>	Mean age in years (range)	Duration of disease in years (range)	IgE anti-IgG		IgG anti-IgG		IgM anti-IgG		IgA anti-IgG	
				≥ 1	≥ 16	≥ 10	≥ 80	≥ 10	≥ 80	≥ 10	≥ 80
Juvenile rheumatoid arthritis	19/7	9 (2-16)	3 (½-15)	11 (42 %)	0	22 (85 %)	4 (15 %)	1 (4 %)	0	0	0
Rheumatoid arthritis without neutropenia	24/6	45 (25-67)	4 (½-10)	19 (63 %)	5 (17 %)	22 (73 %)	9 (30 %)	26 (86 %)	14 (47 %)	13 (43 %)	7 (23 %)
Felty's syndrome	14/6	64 (46-76)	16 (1-32)	16 (80 %)	10 (50 %)	16 (80 %)	10 (50 %)	18 (90 %)	11 (55 %)	17 (85 %)	9 (45 %)
Controls:											
Children	14/9	8 (5-12)		1 (4 %)	0	0	0	0	0	0	0
Adults	24/11	56 (25-78)		0	0	2 (6 %)	0	2 (6 %)	0	2 (6 %)	0

patients had classical or definite RA by the ARA criteria (15). Twenty of the patients had Felty's syndrome (FS) characterized by neutropenia (neutrophil count $< 1.5 \times 10^9/\text{l}$) and splenomegaly. Synovial fluid from both knees of 10 of the RA patients was obtained at the same time. The distribution of age, sex and disease duration of the patients is listed in Table 1. Patients with FS were selected from a large number of RA patients on the basis of neutropenia and enlarged spleen, whereas JRA and RA patients were randomly selected. Sera from 23 healthy children and 35 healthy adults served as controls (Table 1).

Anti-IgG antibodies of the IgE, IgG, IgM and IgA classes were examined with an indirect immunofluorescence technique previously described in detail (8, 9). The test samples were inactivated at 56°C for 30 min in order to destroy complement. This treatment did not result in undesirable formation of aggregated IgG able to consume soluble anti-IgG antibodies, as in a series of experiments identical titers were obtained with and without inactivating the sera.

Prior to examination for IgG anti-IgG dithiothreitol (Sigma Chemical Company, U.S.A.) in a final concentration of 0.01 M was added for 60 min at 37°C to destroy IgM anti-IgG, as IgG may be attached to IgM anti-IgG leading to false-positive reactions. Formalin-fixed sheep red cell IgG acted as antigen in this assay. For IgG, IgM and IgA anti-IgG a positive reaction in a 1:10 dilution of the sample was considered elevated, whereas IgE anti-IgG was considered positive when present in undiluted sample.

Granulocyte-specific and organ-nonspecific (GS- and ON-) ANA of IgE, IgG, IgM and IgA classes were tested by an indirect immunofluorescence technique previously described (12, 14, 17, 19). IgE ANA were tested in undiluted samples and IgG, IgM and IgA ANA were tested at dilution 1:16.

Conjugates: Rabbit antiserum specific for human ϵ chains was obtained from Behringwerke, West Germany. The IgG fraction was isolated from the antiserum by DEAE fractionation and labelled with fluorescein isothiocyanate (FITC) as

previously described (18). FITC-labelled rabbit IgG specific for human γ , μ and α chains were obtained from DAKO, Denmark. Antisera to IgG, IgM and IgA showed monospecific reactions with normal human serum in crossed immunoelectrophoresis. FITC-labelled antisera were tested for specificity by means of IgG, IgM, IgA and IgE monoclonal bone marrow specimens from patients with multiple myeloma and macroglobulinaemia. The fluorescein/protein ratios estimated by OD ratio 495/280 nm were 0.5–0.7. The slides were examined in a Leitz fluorescence microscope (8, 19).

In a series of experiments $F(ab)_2$ fragments of anti-IgE conjugate were used to avoid the interference of non-IgE anti-IgG which may bind the Fc portion of the antibodies. Equal results were, however, obtained with the two conjugates.

The total concentration of IgE was determined by paper radioimmunosorbent technique (PRIST) using Phadebas IgE kits from Pharmacia, Sweden. The concentrations were expressed in units according to WHO standard

serum (IU = 2.4 ngIgE). The total serum concentration of IgG, IgM and IgA was determined by quantitative immunoelectrophoresis (2). Monospecific rabbit antisera for DAKO were used. Normal ranges in the adults: IgE 1–265 $\times 10^3$ U/l, IgG 7.2–15.1 g/l, IgM 0.23–1.33 g/l and IgA 0.74–3.06 g/l. In children the normal ranges for IgE were in accordance with Kjellman et al. (9).

Synovial fluid samples were drawn into EDTA, and were centrifuged to remove cells and particulated material. Two sera from FS patients containing high titers of IgE anti-IgG were fractionated at 4°C on a G-200 Sephadex column at pH = 7.4 using Tris-HCl, $i = 0.10$, and at pH = 4.5 using acetate buffer, $i = 0.10$, and with human albumin 0.1%, respectively. The fractions were then dialysed against water and freeze-dried.

Statistics

Spearman's rank correlation test and rank sum test were used (6). Significance level 5%.

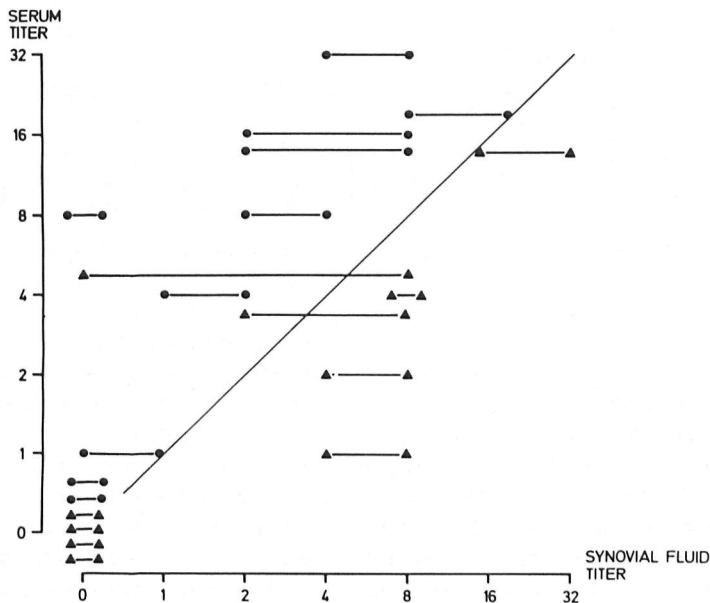


Fig. 1. Relation between the titers of IgE anti-IgG and IgE ANA in paired synovial fluids and in sera from patients with rheumatoid arthritis.

▲: IgE anti-IgG; ●: IgE ANA.

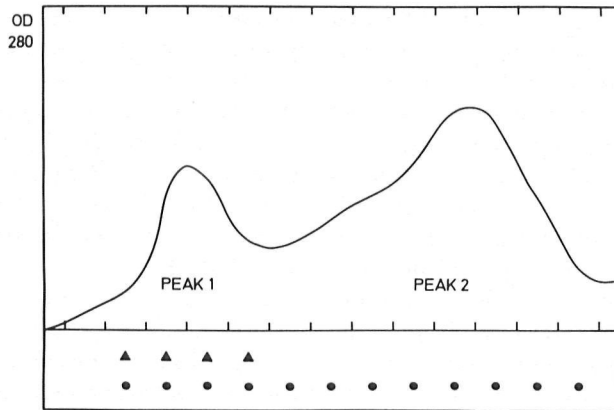


Fig. 2. 2.5 ml of serum from a patient with Felty's syndrome subjected to Sephadex G-200 gel filtration at pH = 7.4 in Tris-HCl buffered saline containing 0.1 % human albumin.

▲: designates IgE anti-IgG and ●: IgG anti-IgG antibody activity in the individual fractions. Abscissa: fraction No., ordinate: optical density at 280 nm.

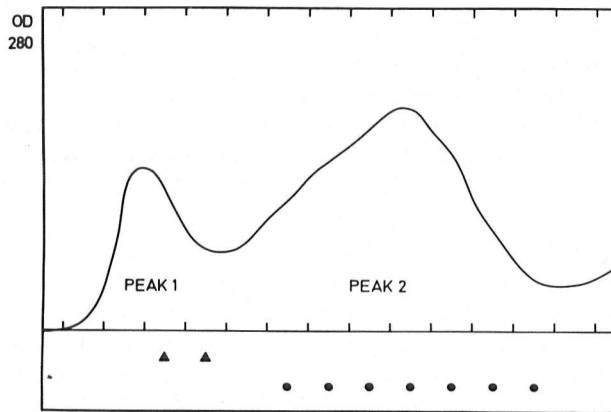


Fig. 3. 2.5 ml of the same serum subjected to Sephadex G-200 gel filtration at pH = 4.5 in acetate buffered saline containing 0.1 % human albumin.

▲: designates IgE anti-IgG, and ●: IgG anti-IgG antibody activity in the individual fractions. Abscissa: fraction No., ordinate: optical density at 280 nm.

RESULTS

The prevalence of IgE, IgG, IgM and IgA anti-IgG is listed in Table 1. IgE anti-IgG were found in 11 patients with JRA. Three of the positive patients had pauciarticular, seven had polyarticular, and one had the systemic type with polyarticular joint affection. These patients also had high titers of IgG anti-IgG ($P < 0.05$). IgE ANA were found in nine patients with JRA, five with pauciarticular and four with polyarticular joint involvement. There

was no correlation between IgE anti-IgG and the presence of IgE ANA. IgM anti-IgG were only found in one patient with JRA and none had IgA anti-IgG.

IgE anti-IgG were found in 63% of the patients with RA and in 80% of patients with FS. The titers of IgE anti-IgG were significantly higher in FS patients ($P < 0.05$). In patients with FS a significant correlation was found between IgE anti-IgG and the presence of IgA ($P < 0.001$), and IgG ($P < 0.01$) and IgM anti-IgG ($P < 0.01$), respectively. In patients with

Table 2
Serum concentrations of IgE, IgG, IgM and IgA in the groups studied

Subjects		IgE 10 ³ U/l	IgG g/l	IgM g/l	IgA g/l
Juvenile rheumatoid arthritis	median	7*§	17.0*	0.67*	1.55*
	range	1-210	9.0-42.0	0.16-1.49	0.48-2.88
Rheumatoid arthritis without neutropenia	median	41§	13.4§	0.80	2.31
	range	8-265	8.5-23.2	0.55-3.17	0.99-4.20
Felty's syndrome	median	35*	23.9*§	1.09*	2.91*
	range	2-577	5.3-38.0	0.37-4.48	0.34-11.50

* and §: $P < 0.01$.

RA the same tendency was found, but the correlations were not significant. IgE ANA, predominantly in the form of GS-ANA, were found in 14 patients with FS and in 10 with RA. In FS patients there was a covariation between IgE anti-IgG and IgE ANA ($P < 0.05$).

In the controls only five adult persons had anti-IgG, one had IgG, one IgM and two had IgA, and one had both IgG and IgM anti-IgG. All were in low titers ($\leq 1:20$). One child had IgE anti-IgG in undiluted serum.

Examination of synovial fluids from both knees of 10 patients with RA showed that six patients had IgE anti-IgG (Fig. 1). In four of these cases the titers in synovial fluids exceeded the titers found in the corresponding serum samples. In synovial fluids four had IgA and nine had IgM anti-IgG and the titers were also higher than in the corresponding serum samples. Six patients had IgE ANA in synovial fluid and the titers were lower than in the corresponding serum samples. No covariation was found between these and the IgE autoantibodies. Some discrepancy was found in titers of IgE autoantibodies as up to 4 titer-steps in difference could be obtained between paired synovial fluids (Fig. 1).

Sera from two patients with FS, showing high titers of IgE anti-IgG were fractionated by Sephadex G-200 gel filtration at neutral pH. IgE anti-IgG were detected only in peak 1, whereas IgG anti-IgG were found both in peak 1 and 2 (Fig. 2). The same two sera were frac-

tionated at acid pH. Now the IgE anti-IgG were found only at the back of peak 1, and IgG anti-IgG in peak 2 only (Fig. 3).

Total IgE concentrations in the sera were elevated in two patients with FS and within normal range in all RA patients and no significant difference was found between the two patient groups (Table 2). IgE concentrations were significantly higher in the adult patients than in JRA. In all JRA patients the serum IgE concentrations were within the normal range according to the age (9). Serum IgG concentrations were significantly higher in FS patients ($P < 0.01$) than in those with RA and JRA. Serum IgA and IgM were also higher in FS, although this trend was not statistically significant (Table 2).

DISCUSSION

The study demonstrated the presence of IgE autoantibodies in patients with arthritic disorders. In a previous study using the same assay it was found that atopic patients only had negligible amounts of IgE anti-IgG in serum whereas IgG anti-IgG were found in 88% (7). The occurrence and titers of IgE anti-IgG in FS patients were higher than in patients with RA and JRA (Table 1). IgE anti-IgG were found in 42% of JRA patients, indicating immunological similarities between the juvenile and the adult form of RA. FS patients were older of age and had a longer disease duration

than the RA patients. This may add to the explanation why autoantibodies were especially common in FS patients since old age seems to lead to a higher prevalence of anti-IgG as well as ANA (8, 17). Long duration of disease also has been shown to signify higher prevalence of anti-IgG (8).

IgE anti-IgG may be regarded as part of a broad polyclonal antibody response to IgG molecules, as FS patients generally exhibited simultaneous presence of IgE, IgG, IgM and IgA anti-IgG. The presence of a number of sera with high titers of these autoantibodies and absence of IgE anti-IgG and vice versa exclude the possibility of unwanted cross-reactivity between the different anti-IgG conjugates as an explanation for the observed covariation.

The higher titers of IgE anti-IgG in some synovial fluids compared to those in the corresponding serum (Fig. 1), and sometimes clearly different titers of these antibodies in paired synovial fluids may indicate a local production. However, other explanations may be valid as well, such as different ability of IgE anti-IgG to participate in synovial fluid IC formation in different joints.

In contrast to IgE anti-IgG the occurrence and titers of IgE ANA in synovial fluids were generally lower than those of the matched sera (Fig. 1), indicating a different pathogenesis.

There was found no relation between total serum IgE concentrations and IgE autoantibody levels which indicates that different trigger mechanisms may be responsible for those parameters. Total serum IgE concentrations were not elevated in RA patients whether extra-articular manifestations were present or not.

Previously, it has been demonstrated that IgE in sera may be involved in IC formation (10, 14, 16). Fig. 3 demonstrates a clear dissociation of IgG anti-IgG-containing complexes by acid treatment. Unexpectedly IgE anti-IgG are still found in the high molecular fraction. A possible explanation for this may be a stronger binding in the IgE-containing complexes. Further investigation of this is necessary. A role of IgE anti-IgG in the pathophysiology of rheumatic diseases may be a reaction with IgG on the surface of tissue mast cells, which are abundant-

ly present in rheumatoid synovia (1). The reaction may liberate vasoactive amines resulting in increased vascular permeability and a local IC deposition (13, 14, 16). The platelet-activating factor liberation from the mast cells may aggregate platelets and polymorphonuclear neutrophils (4), which are involved in the vascular inflammation.

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Address:

Henrik Permin

Laboratory of Immunology, M 7641

Rigshospitalet

Tagensvej 20

DK-2200 Copenhagen

Denmark

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