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B L Zuraw, C H O'Hair, J H Vaughan, D A Mathison, J G Curd, D H Katz

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## Research Article

A solid-phase radioimmunoassay was developed to detect immunoglobulin (Ig)E antibodies that bound to human IgG. IgE-rheumatoid factor activity was found in the serum of 18 of 20 patients with seropositive rheumatoid arthritis, 1 of 4 patients with seronegative rheumatoid arthritis, 3 of 32 patients with seronegative rheumatoid arthritis, 3 of 32 patients with asthma, and in 1 patient with hypocomplementemic vasculitis and iodide sensitivity. Immunopathologic implications of IgE-rheumatoid factor are discussed.

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# Immunoglobulin E-Rheumatoid Factor in the Serum of Patients with Rheumatoid Arthritis, Asthma, and Other Diseases

BRUCE L. ZURAW, CONSTANCE H. O'HAIR, JOHN H. VAUGHAN, DAVID A. MATHISON,  
JOHN G. CURD, and DAVID H. KATZ, *Departments of Molecular Immunology and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California 92037*

**ABSTRACT** A solid-phase radioimmunoassay was developed to detect immunoglobulin (Ig)E antibodies that bound to human IgG. IgE-rheumatoid factor activity was found in the serum of 18 of 20 patients with seropositive rheumatoid arthritis, 1 of 4 patients with seronegative rheumatoid arthritis, 3 of 32 patients with asthma, and in 1 patient with hypocomplementemic vasculitis and iodide sensitivity. Immunopathologic implications of IgE-rheumatoid factor are discussed.

## INTRODUCTION

Rheumatoid factors (RF)<sup>1</sup> of the  $\mu$ -,  $\gamma$ -, and  $\alpha$ -isotypes have been well documented in rheumatoid arthritis (RA) and other conditions (1-3). Interest in the possible existence of RF of the  $\epsilon$ -isotype is due to the ability of IgE antibodies to bind to and trigger mast cells and basophils. Suggestive evidence that IgE-RF might exist was presented in 1969 by Broder et al. (4) who described an apparent immune complex in the serum of many patients with RA that caused the release of histamine from isolated perfused guinea pig lung.

IgE-RF then could focus an array of inflammatory mediators at a site containing basophils or mast cells and an appropriately high epitope density of IgG. To look for IgE-RF, we have developed a solid-phase radioimmunoassay (RIA) for its detection. In this paper we present evidence of the specificity of our assay, and results of the screening of sera from patients with RA, asthma, and several other diseases possibly mediated by immunologic mechanisms, some of which indeed contained IgE-RF activity.

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<sup>1</sup>Abbreviations used in this paper: BBS, borate-buffered saline; GAHE, goat anti-human IgE; NCS, newborn calf serum; RA, rheumatoid arthritis; RF, rheumatoid factor; RIA, radioimmunoassay.

## METHODS

**Reagents and materials.** The following reagents were purchased as indicated: newborn calf serum (NCS; Microbiological Associates, Walkersville, Md.); bovine lactoperoxidase (Worthington Biochemical Corp., Freehold, N.J.); 96-well flexible polyvinyl plates (Dynatech Laboratories, Inc., Alexandria, Va.); Sepharose 4B (Pharmacia, Div. Pharmacia Fine Chemicals, Piscataway, N.J.); Na<sup>125</sup>I (New England Nuclear, Boston, Mass.); Bence Jones  $\kappa$  and  $\lambda$  proteins (Tago Inc., Burlingame, Calif.); RA-test (Hyland Diagnostics Div., Travenol Laboratories, Costa Mesa, Calif.); Tandem IgE kit (Hybritech Inc., La Jolla, Calif.); and human Cohn fraction II globulins (Sigma Chemical Co., St. Louis, Mo.).

Monoclonal human IgE protein (kindly provided by Dr. O. R. McIntyre, Dartmouth Medical School and Dr. K. Ishizaka, Johns Hopkins Medical School) was isolated from the sera of a patient with IgE myeloma (5); this is designated IgE(PS) which has  $\lambda$ -light chains. Cohn fraction II IgG, IgE, and IgA myeloma proteins, and IgM macroglobulins were isolated from sera as previously described (6).

**Sera.** Serum was obtained from patients followed at Scripps Clinic and Research Foundation. Normal serum was obtained from laboratory employees and from the clinical pathology laboratory.

**RIA for total IgE.** Total IgE in serum was measured using the Tandem IgE kit. Results were expressed as IU/ml.

**RIA for IgE-RF.** Affinity purified <sup>125</sup>I-labeled goat anti-human IgE (GAHE) was prepared as described (7). 19.7 mg of Cohn fraction II protein were depleted of any contaminating IgE by passage three times at 4°C through a 4-ml GAHE-Sepharose column (containing 13 mg GAHE; 8). 17.6 mg were recovered in the effluent, and the absence of IgE was confirmed by the lack of binding of <sup>125</sup>I-labeled GAHE to plates coated with the Cohn fraction II preparation.

RIA were performed in 96-well flexible polyvinyl plates coated with 100  $\mu$ l of IgE-depleted Cohn fraction II solution (10  $\mu$ g/ml) overnight in a humidified box at 4°C. After washing the plates with 250  $\mu$ l of borate-buffered saline (BBS) per well three times, the wells were filled with 200  $\mu$ l of 5% NCS in BBS for 2 h at 4°C (to occupy available unbound sites) and washed again with BBS three times. To measure IgE-RF, 100  $\mu$ l of serum diluted 1:10 in 5% NCS-BBS was added to each well. After an overnight incubation at 4°C in a humidified box, the plates were washed five times with 250  $\mu$ l of BBS and the amount of IgE protein bound to each well detected by the addition of 100  $\mu$ l of affinity-purified <sup>125</sup>I-labeled GAHE at a

concentration of 200 ng/ml. After a final 3-h incubation at room temperature, the plates were washed 10 times, the wells cut, and the radioactivity of the individual wells counted on an automatic gamma counter.

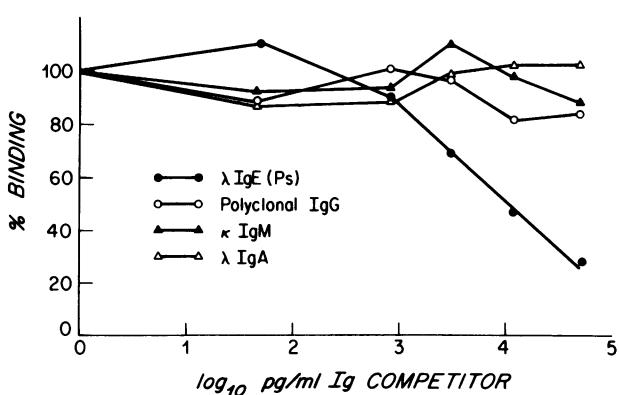
**Analysis of results.** All values are arithmetic means unless stated otherwise. Statistical analyses were performed by Student's *t* test with logarithmically transformed data. Correlation coefficients were generated by least-squares regression analysis using logarithmically transformed data.

## RESULTS

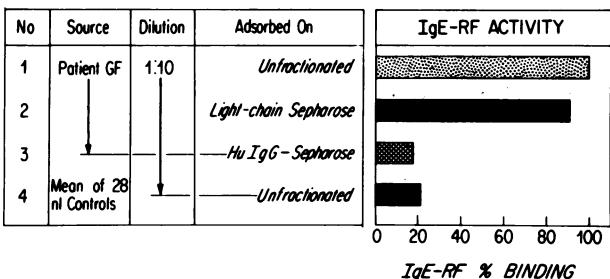
**Specificity of the RIA.** The specificity of the GAHE antibody has been described (7), and was confirmed for this assay. The binding of  $^{125}\text{I}$ -GAHE in the last step of the RIA was specifically inhibited by the addition of IgE(PS) but not by IgG, IgA, or IgM protein (Fig. 1). IgE (For), which has a  $\kappa$ -light chain, also inhibited the binding (data not shown). Furthermore, serum shown to contain IgE-RF activity was passed three times over either a GAHE-Sepharose column (to deplete the IgE) or a Bence Jones light chain-Sepharose column (as a control). The IgE-RF activity was totally removed in the GAHE-Sepharose column effluent, but was not significantly changed in the light chain-Sepharose column effluent compared to the original serum. Latex titers in both column effluents were unchanged from the original serum.

The specificity of the binding of IgE-RF to IgG is shown in Fig. 2. Serum incubated with light chain-Sepharose (group 2) retained 91% of the IgE binding to IgG. When incubated with HuIgG-Sepharose, however, only 18% of the binding to IgG remained—an amount that was equivalent to the nonspecific binding from the normal controls.

IgE-RF activity was independent of total IgE concentration. Serum IgE (IU/ml) was plotted against IgE-RF activity (counts per minute) for 67 patients,



**FIGURE 1** IgE specificity of the assay: IgE protein competes with IgE-RF for the binding of anti-IgE antibodies. Increasing amounts of purified immunoglobulins were incubated with  $^{125}\text{I}$ -GAHE overnight at 4°C, added to triplicate wells containing IgE-RF, and the assay completed in the usual manner. The 100% binding value was 2,293 cpm.



**FIGURE 2** IgE-RF activity can be removed by adsorption on IgG-Sepharose. 50  $\mu\text{l}$  of serum containing IgE-RF was added to 450  $\mu\text{l}$  of either HuIgG-Sepharose or light chain-Sepharose. The tubes were gently rotated at room temperature for 3 h, spun at 250 g for 1 min and the supernatant gently aspirated and assayed for IgE-RF activity. The 100% binding value was 1,718 cpm.

and no correlation was observed ( $r = 0.05$ ). Similarly, adding IgE(PS) protein to the assay wells in amounts corresponding to serum IgE levels ranging from 0.4 to 430 IU/ml (9) did not increase the counts per minute above background. These results additionally exclude any possible contribution to the assay of GAHE and/or GAHE-IgE complexes that might have leaked from Sepharose columns used to purify IgG.

**Distribution of IgE-RF.** Several populations of donors were studied for the prevalence of IgE-RF (Table I). Because there is no IgE-RF reference standard with which to absolutely quantitate the results, all the results in Table I are from a single assay. However, it should be emphasized that all of the samples have been assayed many different times, and the relationships between background, control, and patient sera have been very consistent. Results were considered positive for IgE-RF if the counts per minute

**TABLE I**  
*IgE-RF Levels in Several Populations*

Population	No.	IgE-RF		
		% Positive*	% Negative	Latex Test
Normal control	28	3.6	96.4	0
RA, seropositive	20	90	10	100
RA, seronegative	4	25	75	0
Asthma	32	9.4	90.6	22
Angioedema/urticaria	7	0	100	43
SLE	3	0	100	67
Iodide sensitivity	3	33	67	100
Sjogren's	1	0	100	100
Mixed cryoglobulinemia	1	0	100	100

\* Positive IgE-RF cut-off taken as the mean counts per minute of the control group + 2.33 times the SD = 1,264 cpm in this assay.

† Positive latex test taken as a titer of  $\geq 1:80$ .

were greater than the mean plus 2.33 times the standard deviation of the control group (confidence level of 99% in a one-tailed test). 1 control donor out of 28 was classified as positive by this criterion; she was a healthy employee with a negative latex test, an elevated serum IgE level (196 IU/ml), and a strong family history of asthma.

Of 20 sera from patients with seropositive RA, 18 were positive for IgE-RF activity. This was a highly significant difference compared with the control group ( $P < 0.001$ ). One of four patients with seronegative RA was positive for IgE-RF. Fig. 3 shows these sera as well as the control sera plotted as IgE-RF activity vs. latex titer. There was a positive correlation between IgE-RF levels and the latex titers ( $r = 0.776$ ), although there were instances of positive latex titers and negative IgE-RF levels, and vice versa.

The independence of IgE-RF from IgM-RF was confirmed using sera from three patients with RA and high IgE-RF levels. Each aliquot of serum was depleted of IgE by passage three times over a GAHE-Sepharose column, then diluted with exogenous IgE protein. In all cases the IgE-RF activity in the effluent was removed by the adsorption, and was not recovered by adding back either IgE(PS) protein or IgE containing serum from either a normal donor or an asthmatic with elevated serum IgE. Furthermore, the eluants contained all of the original IgE-RF activity, and were negative in the latex test.

3 of 32 patients with asthma were positive for IgE-RF—all 3 had low levels of latex positivity, 2 of the 3 appeared to have an allergic component to their asthma and polyarthralgias. One patient with hypocomplementemic vasculitis, iodide sensitivity, and polyarthralgias also had an elevated IgE-RF level. The remaining patients tested, including SLE, Sjögren's syndrome, urticaria/angioedema, and mixed cryoglobulinemia, were all negative for IgE-RF activity.

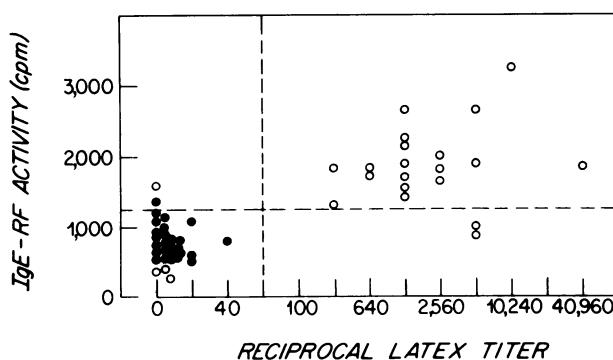


FIGURE 3. IgE-RF binding activity vs. latex titer in normal controls (●) and patients with RA (○). The horizontal dashed line represents the lowest IgE-RF binding activity considered positive; the vertical dashed line represents the lowest latex titer considered positive.

## DISCUSSION

Results presented in this paper clearly document the existence of IgE-RF. The specificity of the assay in detecting only IgE, as well as the specificity of the binding of the IgE-RF to IgG, were shown. IgE-RF activity was shown to be independent of total serum IgE concentration.

Two recent papers have reported IgE-RF (10, 11). IgE concentrations in serum are four to five orders of magnitude less than that of IgM or IgG, and so great care has to be used to prove that the assay does in fact measure IgE; however, neither of the prior papers established the specificity of their assays. One group (10) estimated IgE-RF by measuring total IgE levels before and after adsorption with insolubilized IgG in normal employees receiving regular gamma globulin injections, but the error inherent in their indirect assay was close enough to their results to make their conclusions tentative. The other group (11) used a direct assay to measure IgE-RF in patients with RA, but their assay was 1,000 times less sensitive than our assay. This is reflected in their detecting IgE-RF in only 4 patients out of 109 with RA.

We found 18 of 20 patients with RA to have IgE-RF. The two negative results were from patients with minimally active disease. One patient of four with seronegative RA had IgE-RF (similar to the percentage of IgG- and IgA-RF reported in seronegative RA) (12), and she had severe erosive disease.

3 of 32 patients with asthma were found to have IgE-RF as was 1 patient with hypocomplementemic vasculitis and iodide sensitivity. All four of these patients had complaints of polyarthralgias and low levels of latex positivity. Seven patients with angioedema/urticaria, three with SLE, and one each with Sjögren's syndrome and mixed cryoglobulinemia were negative for IgE-RF activity despite variable and often high latex titers.

The synovium contains large numbers of mast cells (13) and is freely exposed to circulating basophils because of its vascularity. In RA self-aggregating IgG complexes are found in the synovial tissue and space (14, 15). We hypothesize therefore that IgE-RF could bind to the aggregated IgG and be bound to receptors on the mast cell/basophil. The resulting release of mediators would lead to an intense inflammatory reaction and favorable conditions for fibroblast proliferation in the joint.

The etiology and pathogenesis of rheumatoid arthritis have eluded any simple unifying explanation (16, 17). The demonstration that RF of the IgE class are frequently present in patients with RA provides another incremental advance in our understanding of the disease. Because of the unique ability of IgE antibodies to trigger release of mast cell and basophil mediators, IgE-RF may prove to have an important role in the immunopathogenesis of RA.

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