Clin. exp. Immunol. (1988) **72,** 32–36

Role of IgM-rheumatoid factor interference in the determination of total serum IgE and IgE-containing circulating immune complexes

L.S. DE CLERCK, P.L. GIGASE, C.H. BRIDTS & W.J. STEVENS *Laboratory of Immunology, University of Antwerp, Antwerp, Belgium*

(Accepted for publication 28 October 1987)

SUMMARY

IgM-rheumatoid factor (RF) interference in the determination of total serum IgE and IgE-containing circulating immune complexes (IgE-CIC) was studied by inhibition experiments *in vitro* comparing a new ELISA technique free of IgM-RF interference with more widely used RIA methods. It was shown that a considerable overestimation of the IgE content in CIC can exist when high levels of IgM-RF are present in the same serum. The clinical part of this study revealed a dramatic fall in prevalance of IgE-CIC in patients with rheumatoid arthritis (RA) with the ELISA technique, compared with the more conventional RIA method (respectively 1/20 compared to 12/20 positive for IgE-CIC). In these patients, there was a good correlation between the level of IgM-RF and the amount of IgE detected in the CIC by the RIA method ($r=0{\cdot}87$) whereas the RF-interference free ELISA method showed no correlation between these two parameters ($r=0{\cdot}06$). Total serum IgE determination with a solid phase RIA was also influenced by IgM-RF interference, whereas the PRIST method was not affected by the presence of IgM-RF. In conclusion, in patients with rheumatic diseases, IgE-assays using polyclonal rabbit or sheep anti-IgE antibodies are not appropriate and monoclonal anti-IgE antibodies that have been proved not to interfere with IgM-RF should be advocated.

Keywords IgE rheumatoid factor immune complexes

INTRODUCTION

The exact pathogenic mechanisms leading to rheumatoid arthritis (RA) are still unknown. However, the presence of autoantibodies (e.g. rheumatoid factors (RF), antinuclear antibodies) and circulating immune complexes (CIC), including IgE-CIC (Meretey *et al.*, 1982; 1984; De Clerck *et al.*, 1985), suggests a role for immunological events.

In an earlier study, it was found that elevated IgE levels and IgE-CIC are often present in RA patients, even more frequently than in patients with allergic asthma (De Clerck *et al.*, 1985).

A major theoretical drawback in the determination of IgE-CIC in RA patients might be the interference from IgM-RF in the test. Indeed, most techniques for the detection of IgE-CIC use a rabbit or sheep anti-human IgE antibody (Brostoff, Philips & Stanworth, 1977; Meretey *et al.*, 1979; Permin, Wiik & Djurup, 1984; Stevens & Bridts, 1984). This antibody, being a whole IgG molecule, could act as the antigen for RF, leading to falsely high values for the amount of IgE present.

In order to evaluate the practical implications of this RF interaction, we first developed an ELISA technique for the determination of IgE, that proved to be free of IgM-RF interference: a mouse monoclonal anti-IgE not interfering with IgM-RF was used. Although the use of $F(ab')_2$ anti-IgE antibodies would also avoid this interference, this technique was not considered here since no commercially available $F(ab')_2$ anti-IgE antibodies exist and since a technique to produce these antibodies is far more elaborate. Moreover, it has been shown that most mouse monoclonal antibodies do not interfere with IgM-RF (Teitson & Valdimarson, 1984).

Next the results of total serum IgE and IgE-CIC determination by this technique were compared with the more widely used PRIST and other RIA methods in a population of RA patients with a high prevalence of IgM-RF in comparison with a population of asthma and/or rhinitis (A/R) with a low prevalence of IgM-RF.

Correspondence: W.J. Stevens MD, PhD, Laboratory of Immunology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium.

MATERIALS AND METHODS

Patients and controls

Sera from 20 patients with classical or definite RA according to the criteria of the American Rheumatism Association (Ropes *et al.*, 1959) and from 49 patients with A/R were collected. Control sera from 31 healthy individuals were obtained.

General ELISA procedure

Polystyrene microtitreplates (Costar, Cambridge, USA) were coated overnight at 4°C with 100 μl/well of a 10 μg/ml solution of antigen or antibody in carbonate buffer (0·1 M, pH = 9·6). Free binding sites were blocked with 150 μl of a 1% bovine serum albumine (BSA, Sigma, St Louis, USA) in carbonate buffer solution for 90 min at 37°C. Next, plates were washed five times with physiological saline solution containing 0·1% Tween 20 (PT) solution in an automated microplate washer (Titertek, Flow Laboratories, Belgium). Subsequently 100 μl of sample at an appropriate dilution in TRIS buffer containing 1%BSA and 0·1% Tween 20 (TBT) were added to each well for 150 min at 37°C. After washing five times with PT the antibodies were detected by incubating the wells with 100 μl of diluted peroxidase or biotin conjugated antibody in TBT for 120 min at 37°C. For biotinylated antibodies a supplementary incubation of the wells with 100 μl of streptavidin-peroxidase (Amersham International, Amersham, UK) diluted 1/1000 in TBT for 60 min at room temperature was performed. After five washings with PT, 100 μl of a 0·4 mg/ml orthophenylenediamine (Sigma, St Louis, USA) in citrate-phosphate buffer (0·15 M, pH = 5·0) was added for 5–15 min at room temperature. The colour reaction was stopped with 50 μl of a 2 M H_2SO_4 solution. Optical densities were measured with a microtitreplate photometer (Titertek, Flow Laboratories, Belgium) at 492 nm with a reference absorbance at 690 nm. The calculation of the calibration curve and the samples was performed with a computerized four parameter logistic model on an Olivetti M24-PC (Gigase *et al.*, 1987).

Serum IgE

Serum IgE was determined with three different techniques: firstly the PRIST (Pharmacia Belga, Brussels, Belgium) according to the instructions of the manufacturer, secondly a solid phase RIA, and thirdly an ELISA, according to the ELISA procedure. For the solid phase RIA and the ELISA assays, microtitre plates were coated with rabbit antihuman IgE (Dakopatts, Glostrup, Denmark). Sera diluted 1/20 were added and IgE was quantified with either radiolabelled rabbit antihuman IgE (Pharmacia, Brussels, Belgium) or murine monoclonal antihuman IgE, kindly provided by Dr Peltre from the Institut Pasteur (Paris, France), and biotinylated with biotin-N-hydroxysuccinimide (Sigma, St Louis, USA) according to the method of Subba Rao (Subba Rao, McCartney-Francis & Metcalf, 1983). The tests were calibrated with Pharmacia IgE reference preparations.

IgE-CIC

Precipitation of CIC. Sera were diluted 1/2 in phosphate-buffered saline (PBS) to which an equal volume of 5% polyethylene glycol (PEG) in PBS was added to obtain a final 2·5% PEG concentration. After incubation for 60 min at 4°C, sera were spun at 3000 *g* (4°C) for 60 min. The precipitates were washed with 10 times the serum volume of 2·5% PEG, and IgE was quantified in the precipitate with either a RIA (RIA-IgE-CIC) or an ELISA (ELISA-IgE-CIC).

RIA-IgE-CIC. This assay was performed as described earlier (Stevens & Bridts, 1984). Briefly, radiolabelled antihuman IgE was added overnight to the 2·5% PEG precipitate. After washing, the bound anti-IgE was precipitated with 2·5% PEG and counted.

ELISA-IgE-CIC. This assay was carried out according to the general ELISA procedure. Briefly, rabbit antihuman IgE was coated on microtitreplates. Polyethylene glycol precipitates (2·5%) were resuspended to the original volume in TBT and added to the microtitre plates. Next, IgE was detected with biotinylated monoclonal antihuman IgE and calibrated with the Pharmacia IgE reference preparations. This assay had a detection limit of 0·08 kU/l IgE.

IgE-CIC levels were considered as elevated when they exceeded the mean + 2 s.d. of controls.

IgM-RF

IgM-RF was also determined with an ELISA according to the general ELISA procedure. Briefly, chromatographically purified rabbit IgG (Cappel, Worthington, USA) was coated on microtitreplates. Sera diluted 1/200 were added and IgM-RF was detected with peroxidase conjugated goat $F(ab')_2$ antihuman IgM (Tago, Burlingame, USA). The test was standardized with a reference preparation containing 100 kIU/l of IgM-RF (Behring, Marburg, FRG).

Statistics

Variables that were not normally distributed were transformed logarithmically in order to obtain a ln-normal distribution. Linear regression analysis was performed with determination of the Pearson correlation coefficient.

RESULTS

In vitro inhibition of the binding of radiolabelled rabbit antihuman IgE in the RIA-IgE-CIC

Increasing amounts of rabbit IgG were added simultaneously with the radiolabelled antihuman IgE in the detection of IgE-CIC with the RIA technique. If IgM-RF interference would be present in the RIA-IgE-CIC, one would expect that, for IgM-RF positive samples, the added rabbit IgG would compete with the radiolabelled antihuman IgE for the binding places on IgM-RF (Fig. 1), but not for IgE-isotype specific epitopes. Results are presented for three IgM-RF-positive and two IgM-RF-negative samples (Fig. 2). A significant decrease was observed in the patients with high IgM-RF levels. For the IgM-RF negative samples, there was no influence from the addition of rabbit IgG.

Comparison of RIA-IgE-CIC with ELISA-IgE-CIC in patients and controls

The two techniques for the quantification of IgE-CIC were compared in patient and control groups, and results were correlated with serum IgM-RF levels. With the RIA technique (Table 1) we found 12 out of 20 RA patients and 12 out of 49 A/R patients positive for IgE-CIC. However, there was a significant correlation between IgM-RF levels and the amount of RIA-IgE-CIC ($r = 0{\cdot}82$, $P < 0{\cdot}001$).

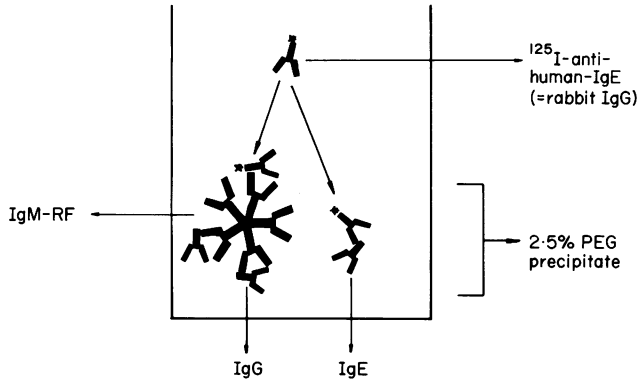

Fig. 1. Schematic representation of RIA-IgE-CIC with possible IgM-RF interference.

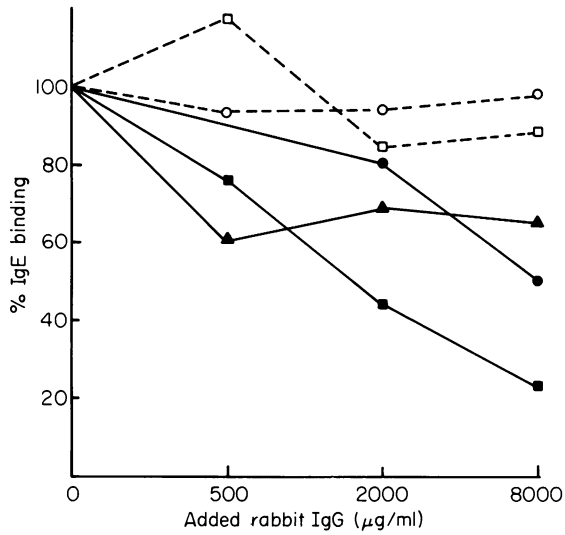

Fig. 2. Inhibition of the binding of radiolabelled antihuman IgE in the RIA-IgE-CIC by addition of increasing amounts of rabbit IgG, in 3 IgM-RF positive (+———+) and 2 IgM-RF negative (+----+) samples. Results are expressed as the percentage of the initial RIA-IgE-CIC values, before the addition of rabbit IgG.

Table 1. Numbers of individuals in the different groups with negative (−ve) and positive (+ve) IgE-CIC with RIA and ELISA technique

	RIA-IgE-CIC		ELISA-IgE-CIC	
RIA-IgE-CIC	−ve	+ve	−ve	+ve
Controls	28	3	31	0
RA	8	12	19	1
A/R	37	12	37	12

With the ELISA technique (Table 1) only one out of 20 RA patients and 12 out of 49 A/R patients had IgE-CIC. No correlation was observed between IgM-RF levels and amount of ELISA-IgE-CIC ($r=0{\cdot}06$, $P>0{\cdot}50$).

In vitro inhibition of the binding of radiolabelled antihuman IgE in the solid phase RIA for the quantification of total serum IgE

Total serum IgE levels were measured by the solid phase RIA and the ELISA technique. IgM-RF interference was examined by addition of increasing amounts of rabbit IgG simultaneously with the detecting anti-IgE antibody. If IgM-RF interference would exist in the solid phase RIA and not in the ELISA, one would expect the RIA-total serum IgE levels to be higher than the ELISA-total serum IgE levels for IgM-RF positive samples. Moreover, the addition of rabbit IgG would decrease these RIA-total serum IgE values towards those approximating to the ELISA-total serum IgE levels. The results are presented for three IgM-RF positive sera and for two IgM-RF negative sera (Fig. 3). Higher RIA-total serum IgE levels were observed in the IgM positive sera, and the addition of rabbit IgG lowered these levels approximating the ELISA results. In contrast, the ELISA-total serum IgE levels were not influenced by the addition of rabbit IgG. In the two IgM-RF negative samples RIA-total serum IgE and ELISA-total serum IgE levels were comparable and there was no influence of the addition of rabbit IgG on either RIA or ELISA.

Comparison of PRIST-total serum IgE with ELISA-total serum IgE in patients and controls

The two techniques were used to determine total serum IgE in 10 RA patients with elevated IgM-RF and eight patients with A/R without IgM-RF. The differences between PRIST and ELISA in the patient groups with and without IgM-RF were not significant. An excellent correlation between the two techniques was observed ($r=0{\cdot}997$, $P<0{\cdot}001$).

DISCUSSION

Not only an elevation of monomeric IgE has been reported in RA, but also a high prevalence of IgE-CIC, especially in the patients with vasculitis (Meretey *et al.*, 1982; De Clerck *et al.*, 1985) and Felty's syndrome (Meretey *et al.*, 1984; Permin, Wiik & Djurup, 1984). IgE-CIC could be able to activate mastcells and/or basophils, but also some monocytes and macrophages possessing IgE-receptors. This binding could lead to inflammatory cell activation, in organs rich in IgE-receptors such as skin, lung and also synovium (Grubner *et al.*, 1986). However, in patients with a high prevalence of RF such as RA and other systemic diseases, there might be an interference of IgM-RF in the IgE-assays. Indeed, the different IgE-CIC assays reported, mostly use an anti-IgE that is not a $F(ab)_2$-fragment but a whole immunoglobulin molecule containing an Fc part that can act as an antigen for IgM-RF (Teitsson & Valdimarsson, 1984).

In this study, we clearly demonstrate that, in contrast with the ELISA-IgE-CIC technique, the IgM-RF interference is indeed present in the routinely used RIA-IgE-CIC determinations: firstly this RF-interference is shown by competitive inhibition experiments where the addition of increasing amounts of rabbit IgG (that will bind with the IgM-RF) results in a progressive fall of the level of IgE measured in the CIC. Moreover, in the patients sera studied there was also a clear

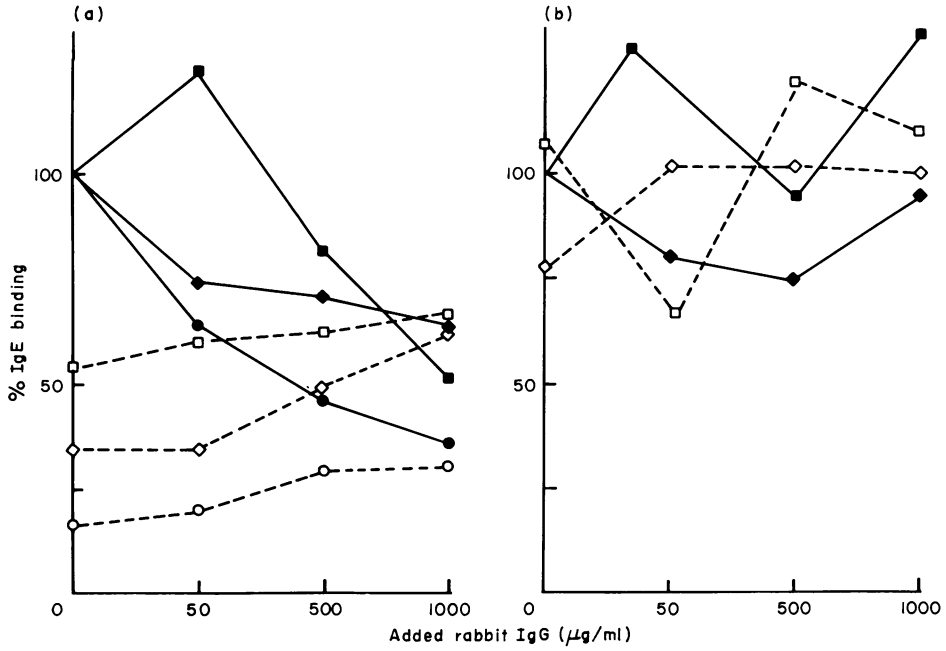

Fig. 3. Influence on total serum IgE values by the simultaneous addition of anti-IgE antibody and increasing amounts of rabbit IgG. Total serum IgE levels are expressed as the percentage of the initial RIA-total serum IgE values, before the addition of rabbit IgG. Results are given for (a) three IgM-RF-positive samples and (b) two IgM-RF negative samples tested with the RIA technique (———) and the ELISA technique (- - - -).

IgM-RF interference: indeed, in the 20 RA patients studied, there was a high correlation between the level of IgE-CIC as determined by the RIA assay and the amount of IgM-RF present in the same serum. On the contrary, for the ELISA-IgE-CIC technique there was no correlation. Our studies *in vitro* with a solid phase RIA for the determination of total serum IgE also demonstrated the interference of IgM-RF in the test, in contrast with the ELISA-technique. Thus in patients with high titres of IgM-RF one not only has to be aware of an interference of IgM-RF in the IgE-CIC determination, but also in other assays such as determination of monomeric serum IgE and IgE-RF. This could at least partly account for the elevated levels of IgE (Hunder & Gleich, 1974; Grennan & Palmer, 1979) and IgE-RF (Mizushima *et al.*, 1984) reported in patients with RA. It should be noted that with our IgM-RF free system, we could not demonstrate until now the presence of IgE-RF in RA patients (results not shown). However, total serum IgE determination with the PRIST method was not influenced by the presence of IgM-RF. This lack of RF-interference can be explained by the fact that addition of horse IgG before the anti-IgE in the PRIST technique, blocks the available IgM-RF binding sites.

In summary, we clearly demonstrated that IgM-RF interference is not only a theoretical but also a practical problem in the determination of IgE levels. This interference is especially important in populations such as RA, where the prevalence of IgM-RF is high. Studied concerning the prevalence and importance of IgE and IgE-CIC should therefore consider seriously this interference. In these assays, techniques using murine monoclonal anti-IgE antibodies that proved not to interefere with IgM-RF should therefore be advocated.

REFERENCES

De Clerck, L.S., Westedt, M.L., Cats, A., Vermeer, B.J., Weltevreden, E.F., Bridts, C.H. & Stevens, W.J. (1985) IgE-deposition in normal skin of patients with rheumatoid arthritis in relation to clinical and laboratory findings. *Ann. Rheum. Dis.* **44,** 772.

Gigase, P.L., Bridts, C.H., De Clerck, L.S. & Stevens, W.J. (1987) A simple method for determining class specific rheumatoid factors with computerised analysis of data. *J. immunol. Methods* in press.

Grennan, D.M. & Palmer, D.G. (1979) Serum IgE concentrations in rheumatoid arthritis: lack of correlation with gold toxicity. *Br. med. J.* **ii,** 1477.

Grubner, B., Poznansky, M., Boss, E., Partin, J., Gorevic, P. & Kaplan, A.P. (1986) Characterization and functional studies of rheumatoid synovial mast cells. Activation by secretagogues, anti-IgE and a histamine-releasing lymphokine. *Arthritis Rheum.* **29,** 944.

Hunder, G.G. & Gleich, G.J. (1974) Immunoglobulin E (IgE) levels in serum and synovial fluid in rheumatoid arthritis. *Arthritis Rheum.* **17,** 955.

Meretey, K., Böhm, U., Falus, A. & Bozsoky, S. (1979) Radioimmune double PEG precipitation technique for detecting complexed IgE. *J. immunol. Methods* **26,** 223.

Meretey, K., Falus, A., Bohm, U., Permin, H. & Wiik, A. (1984) IgE class immune complexes in Felty's syndrome: characterisation of antibody activities in isolated complexes. *Ann. rheum. dis.* **43,** 246.

Meretey, K. Falus, A., Erhardt, C.C. & Maini, R.N. (1982) IgE and IgE-rheumatoid factors in circulating immune complexes in rheumatoid arthritis. *Ann rheum. Dis.* **41,** 405.

Mizushima, Y., Shoji, Y., Hoski, K. & Kiyokawa, S. (1984) Detection and clinical significance of IgE rheumatoid factor. *J. Rheumatol.* **11,** 22.

Permin, H., Wiik, A. & Djurup, R. (1984) Phagocytosis by normal

polymorphonuclear leukocytes of immune complexes from serum of patients with Felty's syndrome and rheumatoid arthritis with special reference to IgE immune complexes. *Acta Pathol. Microbiol. Immunol. Scand. C* **92,** 37.

ROPES, M.W., BENETT, G.A., COB, S., JACOX, R. & JESSAR, R.A. (1959) Revision of diagnostic criteria in rheumatoid arthritis. *Bull. Rheum. Dis.* **9,** 175.

STEVENS, W.J. (1986) Immune complexes containing IgE: clinical considerations. In *Proceedings of the XII International congress of Allergology and Clinical Immunology* (ed. J. Pepys) p. 50. C.V. Mosby Company, St Louis.

STEVENS, W.J. & BRIDTS, C.H. (1984) IgG-containing and IgE-containing circulating immune complexes in patients with asthma and rhinitis. *Allergy Clin. Immunol.* **73,** 276.

SUBBA RAO, P.V., MCCARTNEY-FRANCIS, N.L. & METCALFE, D. (1983) An avidin-biotin microELISA for rapid measurement of total and allergen-specific human IgE *J. Immunol. Methods* **57,** 71.

TEITSON, I. & VALDIMARSON, H. (1984) Use of monoclonal antibodies and $F(ab')_2$ enzyme conjugates in ELISA for IgM, IgA and IgG rheumatoid factors. *J. immunol. Methods* **71,** 149.