

# Major differences in specificity among naturally occurring human IgG-subclass anti-IgE autoantibodies

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**Background:** The specificity of naturally occurring IgG-subclass anti-IgE autoantibodies (a-IgE Ab) was studied by ELISA inhibition assay with a highly purified IgE-DES myeloma protein as a solid-phase antigen. Because the IgG isotypes differ in effector functions, detailed specificity studies of IgG subclass anti-IgE antibodies might clarify their putative role.

**Methods:** Selected sera containing a high concentration of a single IgG a-IgE Ab subclass were allowed to react with purified immunoglobulins of all five classes including five different IgE myeloma proteins and papain-derived Fab $\epsilon$  and Fc $\epsilon$  fragments of IgE-DES.

**Results:** The inhibition results indicated that IgG1, IgG2, and IgG3 a-IgE Ab reacted in a low-affinity reaction with IgE myeloma protein-restricted determinants because only IgE-DES was inhibitory. Moreover, the reacting epitopes were heat resistant (2 hours, 56° C) and localized in the Fab $\epsilon$ -DES fragment. In sharp contrast, IgG4 a-IgE Ab reacted with high affinity to all five IgE myeloma proteins involving heat-susceptible epitopes confined to the Fc $\epsilon$  fragment.

**Conclusions:** It seems that a majority of IgG a-IgE Ab have a specificity for nonisotypic myeloma-restricted determinants whereas IgG4 a-IgE Ab are mainly isotype specific. These findings ought to be taken into account in the consideration of physiologic implications of IgG a-IgE Ab and in the interpretation of previous investigations in which intact IgE myeloma proteins have been used to detect IgG a-IgE Ab. (*J ALLERGY CLIN IMMUNOL* 1995; 95:1011-9.)

**Key words:** Anti-IgE, assay, atopic, autoantibody, IgE, IgG4, IgG subclass, specificity

Naturally occurring anti-IgE autoantibodies (a-IgE Ab) have been demonstrated by many investigators, and they may belong to the IgM, IgA, or IgG class of immunoglobulins.<sup>1,2</sup> The IgM and IgA a-IgE Ab seem to be of limited physiologic interest because of low affinity, restricted specificity, and common prevalence (C. G. M. Magnusson, unpublished observations).<sup>1-4</sup> In contrast, IgG a-IgE Ab have characteristics suggesting a putative role in IgE-mediated diseases. They are isotype specific, form circulating immune complexes with IgE, and

## Abbreviations used

|                        |                                          |
|------------------------|------------------------------------------|
| a-IgE Ab:              | Anti-IgE autoantibodies                  |
| BSA:                   | Bovine serum albumin                     |
| Mab:                   | Monoclonal antibody                      |
| NIP:                   | 5-Iodo-4-hydroxy-3-nitro-phenacetyl acid |
| OD <sub>405 nm</sub> : | Optical density at 405 nm                |
| PBS:                   | Phosphate-buffered saline solution       |

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Supported by the Swedish Medical Research Council; the National Association for Prevention of Asthma and Allergies; Herman Krefting; Konsul Th. C. Berghs; Tore Nilson; and Karolinska Institute funds.

Received for publication Dec. 13, 1993; revised Aug. 24, 1994; accepted for publication Oct. 13, 1994.

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0091-6749/95 \$3.00 + 0 1/1/62174

are most prevalent in persons with atopic disease.<sup>1,5-8</sup> Although IgG a-IgE Ab are suggested to elicit histamine release both in vitro and in vivo,<sup>8,9</sup> and to be involved in immunoregulation,<sup>10</sup> their physiologic implications are still obscure and mainly speculative.<sup>1,2,10</sup> However, any role of these IgG a-IgE Ab depends on both the specificity and the subclass, because the four human IgG subclasses are structurally different and demonstrate differences in various effector functions such as binding to C1q and Fc $\gamma$ R receptors.<sup>11</sup>

To our knowledge there is no systematic study

on the specificity of a-IgE Ab of all four IgG subclasses, but available studies of IgG a-IgE Ab suggest a reactivity with epitopes confined to the heat-susceptible D $\epsilon$ 2 (C $\epsilon$ 3 and C $\epsilon$ 4 domains) region of IgE.<sup>1, 5, 6, 8</sup> IgG a-IgE Ab titers increase after heat treatment of serum, which is interpreted as a disruption of immune complexes between a-IgE Ab and heat-susceptible D $\epsilon$ 2 determinants.<sup>12</sup> Moreover, the F(ab')<sub>2</sub> fragment lacking the D $\epsilon$ 2 determinants was unreactive with IgG a-IgE Ab.<sup>6, 13</sup> In a recent report the specificity of IgG1 a-IgE Ab was found to be to the C $\epsilon$ 2 and C $\epsilon$ 4 domain of IgE by means of ELISA inhibition, solid-phase recombinant F $\epsilon$ c fragment, and a panel of recombinant IgE fragments.<sup>14</sup> However, by agglutination assay we have also previously noticed the existence of another category of IgG a-IgE Ab that, like IgM a-IgE Ab, lacked isotypic specificity.<sup>4</sup> Hence there seems to be a heterogeneity in the specificity of IgG a-IgE that might be related to the different IgG subclasses.

The aim of this study was to investigate the specificity of naturally occurring IgG a-IgE Ab belonging to all four human IgG subclasses.

## METHODS

### Antibodies and proteins

Monoclonal antibodies (Mab) against the IgG1 (HP6012), IgG2 (HP6014), and IgG3 (HP6076) subclasses were those previously tested in the World Health Organization-International Union of Immunological Societies study of IgG-subclass reagents.<sup>15</sup> Mab against human  $\kappa$  light chains (clone 573), IgG (L12), and IgG4 (L13) were gifts of Dr. T. Karlsson, Kabi-Pharmacia AB, Uppsala, Sweden.

Rabbit anti-mouse immunoglobulin antibody in the form of F(ab')<sub>2</sub> fragment (gift from Leif Norin, Dakopatts AB, Stockholm, Sweden) was conjugated to alkaline phosphatase (from calf intestine, Boehringer-Mannheim Scandinavia AB, Stockholm, Sweden) by glutaraldehyde in a one-step procedure as described.<sup>16</sup>

Chimeric IgG-subclass antibody against the 5-iodo-4-hydroxy-3-nitro-phenacetyl acid (NIP) hapten and NIP conjugated to bovine serum albumin (BSA) were gifts of Dr. L. Hammarström, Clinical Immunology, Huddinge Hospital, Huddinge, Sweden. The chimeric antibodies consist of the same mouse light chain and a heavy chain assembled from different human IgG-subclass constant regions, each joined to the same mouse variable region specific for NIP.<sup>17</sup>

Purified IgE-DES( $\kappa$ ), IgE-ND( $\lambda$ ), IgE-PS( $\lambda$ ), IgE-UD( $\kappa$ ), and IgE-WT( $\lambda$ ), and papain-derived Fabe and F $\epsilon$ c fragments of IgE-DES, were previously described.<sup>3, 4</sup> Polyclonal IgG ( $\gamma$ -globulin) was from Kabi, Stockholm, Sweden. Purified myeloma proteins (i.e., IgG1-HO( $\kappa$ ), IgG1-LO( $\lambda$ ), IgG2-ISS( $\lambda$ ), IgG2-BI( $\kappa$ ), IgG3-BU( $\lambda$ ),

IgG3-HÅ( $\kappa$ ), IgG4-STE( $\lambda$ ), IgG4-STÖ( $\kappa$ ), IgG4-BRU( $\kappa$ ), IgA1-DEL( $\kappa$ ), IgA2-LOU( $\lambda$ ), IgD-BER( $\lambda$ ), and IgM-VIR( $\kappa$ )) were from different sources.<sup>3, 4, 15</sup> The purity of the immunoglobulin myeloma proteins was estimated to be at least 90% on the basis of several methods (e.g., sodium dodecyl sulfate-polyacrylamide gel electrophoresis in gradient gels (4% to 15%) under nonreducing and reducing conditions (Phastsystem, Pharmacia AB, Uppsala, Sweden) and immunoglobulin class- and immunoglobulin subclass-specific immunoassays. The purity of the Fabe-DES and F $\epsilon$ c-DES fragments was also estimated to be at least 90% by inhibition ELISA with Mab specific for the  $\kappa$  chain and for the D $\epsilon$ 0, D $\epsilon$ 1, and D $\epsilon$ 2 regions of IgE-DES.<sup>18</sup> The extinction coefficient at 280 nm was assumed to be 14 for all proteins at 10 mg/ml.

Heat treatment of immunoglobulin myeloma proteins and Fabe-DES fragment was performed at 56° C for 2 hours in a water bath. Likewise, polyclonal IgG was heated at 63° C for 20 minutes.

### Serum samples

Consecutive serum samples referred to our clinic for in vitro allergy testing were used in the screening to select sera with suitable titers of IgG-subclass a-IgE Ab for the specificity studies. All sera were kept at -20° C pending analyses.

### IgG-subclass anti-IgE ELISA

Serum samples were diluted in a diluent consisting of 1 volume of horse serum (Prist-diluent, Pharmacia AB) and 1 volume of phosphate-buffered saline solution (PBS)-BSA (PBS, 50 mmol/L [pH = 7.4]; 0.2% [wt/vol] of BSA). The dilutions varied depending on the IgG subclass as follows: IgG1, IgG2, and IgG3 at 1:50 or greater, and IgG4 a-IgE Ab at 1:10 or greater. Microtiter plates (Maxisorp, NUNC, Roskilde, Denmark) were coated overnight at room temperature with 100  $\mu$ l/well of highly purified IgE-DES protein (10  $\mu$ g/ml in carbonate-bicarbonate buffer, 0.1 mol/L, pH = 9.6). All incubations (100  $\mu$ l, room temperature) except color development were followed by four washings with PBS-Tween-20 (0.05% vol/vol) in an automatic plate washer (SLT Easywasher II; Ekoscann, Upplands-Väsby, Sweden). Microtiter plates were incubated overnight with sample (100  $\mu$ l) followed by anti-IgG-subclass Mab for 6 hours and alkaline phosphatase-conjugated rabbit F(ab')<sub>2</sub> anti-mouse immunoglobulin antibody overnight before color development (1 hour) with 1 mg/ml *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, Mo.) in 1 mol/L diethanolamine buffer containing 1 mmol/L MgCl<sub>2</sub> and 0.02% (wt/vol) NaN<sub>3</sub> (pH = 9.8). The appropriate dilutions of Mab in the form of ascitic fluid (1:1000 to 1:5000) or purified protein (2  $\mu$ g/ml), and secondary antibody (1:500 to 1:800) were determined in preliminary experiments. Optical densities at 405 nm (OD<sub>405 nm</sub>) were recorded in an ELISA reader (SLT Easyreader 330 or Anthos 2001, Ekoscann).

ELISA inhibition experiments were performed by incubating overnight different concentrations of inhibi-

tors with sera prediluted to contain IgG-subclass a-IgE Ab titers, giving an OD<sub>405 nm</sub> of 1.2 to 1.8 after 1 hour of color development. Typically the serum dilutions used in these inhibitions were 1:50 to 1:200 for IgG1, 1:50 to 1:100 for IgG2 and IgG3, and 1:10 to 1:20 for IgG4 a-IgE Ab. The inhibition results are expressed as a percentage according to the following equation

$$\frac{(\text{Preinhibitory OD}_{405 \text{ nm}} - \text{Postinhibitory OD}_{405 \text{ nm}})}{\text{Preinhibitory OD}_{405 \text{ nm}}} \times 100$$

after correction for background OD<sub>405 nm</sub>, which never exceeded 0.07 when all reagents except sample were added. An inhibition of 8%, which represents the mean of 36 inhibition experiments +3 SD with sample diluent alone as inhibitor, was considered significant.

## RESULTS

### Specificity of anti-IgG-subclass Mab

The specificity of the anti-IgG-subclass Mab used in this study was verified by direct ELISA. Two purified myeloma proteins of each of the four IgG subclasses, one of the  $\kappa$  and one of the  $\lambda$  light chain, and one IgG4b protein of the  $\kappa$  light chain, were used to coat microtiter plates at fivefold dilutions (0.8 to 2500 ng/ml). The assay was then conducted exactly as described for the measurement of IgG-subclass a-IgE Ab, with diluent as sample in all wells. In agreement with the International Union of Immunological Societies-World Health Organization study,<sup>15</sup> the selected Mab demonstrated a high specificity for their corresponding subclass (Table I).

### Screening and subclass typing of a-IgE Ab

Serum samples from consecutive patients referred to our clinic for in vitro allergy testing were screened for the presence of high titers of IgG a-IgE Ab with microtiter plates coated with IgE-DES myeloma protein. Subclass typing of such samples revealed the presence of a-IgE Ab belonging to all four IgG subclasses, although the most common was IgG1. A more extended screening was necessary to find sera with sufficiently high levels of IgG2, IgG3, and IgG4 a-IgE Ab for inhibition purposes. To avoid a putative influence of the different IgG subclasses on each other, samples that had preferential representation of one IgG-subclass a-IgE Ab activity over the others were selected for study of specificities. The results of the subclass typing of eight sera confirmed the specificity of the Mab also in the assay of IgG-subclass a-IgE Ab (Fig. 1).

### Specificity of IgG-subclass a-IgE Ab

The specificity of the selected IgG-subclass a-IgE Ab was first tested by inhibition experi-

**TABLE I.** Specificity of anti-IgG-subclass Mab in ELISA

| IgG myeloma coat | Specificity (%)    |                    |                    |                 |
|------------------|--------------------|--------------------|--------------------|-----------------|
|                  | Anti-IgG1 (HP6012) | Anti-IgG2 (HP6014) | Anti-IgG3 (HP6076) | Anti-IgG4 (L13) |
| IgG1 (n = 2)     | 100                | <2                 | <1                 | <0.4            |
| IgG2 (n = 2)     | <1                 | 100                | <1                 | <0.4            |
| IgG3 (n = 2)     | <1                 | <2                 | 100                | <0.4            |
| IgG4 (n = 3)     | <1                 | <2                 | <1                 | 100             |

\*Figures are calculated by generating standard curves from coated IgG-subclass proteins at fivefold dilutions (0.8 to 2500 ng/ml) and reading responses of other three IgG subclasses on corresponding curves (e.g., IgG1, IgG2, or IgG3 coat of 2500 ng/ml gives IgG4 content of <10 ng/ml [ $<10 \div 2500 \times 100 = <0.4\%$ ] as calculated from coated IgG4 standard curve obtained with anti-IgG4 Mab).

ments in which all five classes of immunoglobulins were allowed to compete with solid-phase IgE-DES for a fixed concentration of a-IgE Ab, giving an OD<sub>405 nm</sub> of 1.2 to 1.8 (see Fig. 1). All experiments were performed on at least four different sera for each IgG-subclass a-IgE Ab, but because similar inhibition patterns were obtained within a subclass, only some examples of each subclass are normally illustrated. The results clearly show that at the high final concentrations tested, neither polyclonal IgG (100  $\mu$ g/ml), nor monoclonal IgA1, IgA2, IgM, and IgD myeloma proteins (each at 250  $\mu$ g/ml) gave any significant (i.e., >8%) inhibitions. On the contrary, IgE-DES clearly inhibited all four IgG-subclass a-IgE Ab at both 10 and 100  $\mu$ g/ml (Fig. 2). However, inhibition experiments performed with an additional four IgE myeloma proteins (100  $\mu$ g/ml) (i.e., IgE-ND[ $\lambda$ ], IgE-PS[ $\lambda$ ], IgE-WT[ $\lambda$ ], and IgE-UD[ $\kappa$ ]) revealed that none of the IgG1, IgG2, and IgG3 a-IgE Ab tested reacted significantly with any of these IgE proteins. On the contrary, all IgG4 a-IgE Ab were inhibited nearly 100% by all five IgE myeloma proteins.

Inhibition curves indicated that the final concentration of IgE-DES necessary for 50% assay inhibition varied considerably both between and within subclasses. Thus in the case of IgG4 a-IgE Ab, only 0.1 to 0.3  $\mu$ g/ml was necessary for 50% inhibition whereas for IgG1, IgG2, and IgG3 a-IgE Ab the figures were 4 to 140  $\mu$ g/ml (i.e., 40 to 1000 times higher) (Fig. 3). These findings cannot be explained by any major difference in sensitivity among the IgG-subclass a-IgE assays. In fact, similar sensitivities were obtained for all four IgG

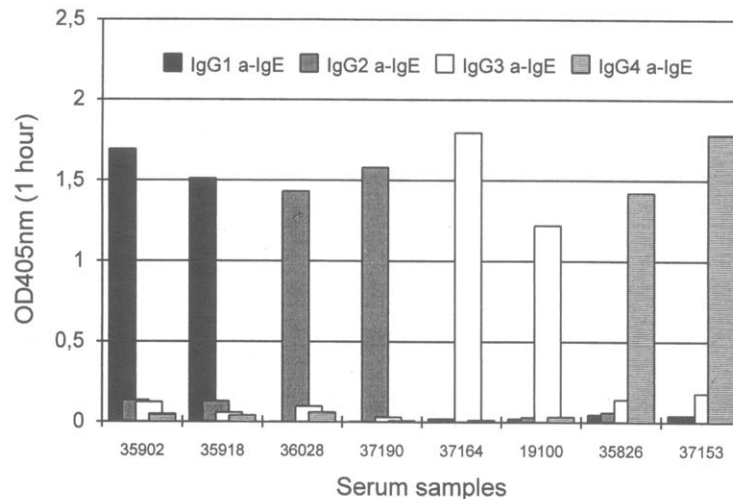


**FIG. 1.** Typing of IgG-subclass a-IgE Ab activity in eight different sera by ELISA with solid-phase IgE-DES. Serum samples were diluted 200-fold, 50-fold, 50-fold, and 20-fold for assay of IgG1, IgG2, IgG3, and IgG4 a-IgE Ab, respectively.

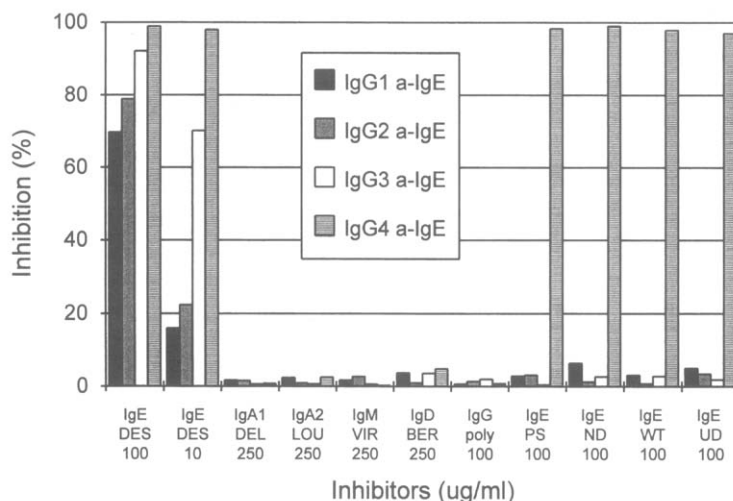


**FIG. 2.** Specificity of IgG-subclass a-IgE Ab as tested by inhibition ELISA with solid-phase IgE-DES and different purified immunoglobulin myeloma proteins as inhibitors. Calculation of inhibitions after background subtraction is explained in detail in Methods.

subclasses when the protocols for the assay of IgG-subclass a-IgE Ab were applied in the determination of calibrated chimeric IgG subclass anti-NIP antibody with BSA-NIP conjugate (10  $\mu$ g/ml) as coating antigen (data not shown). Furthermore, comparison of six different sera with IgG1 a-IgE Ab also revealed a heterogeneity in the inhibitions within a subclass because the 50% inhibitory range of IgE-DES differed 20-fold (i.e., 4 to 90  $\mu$ g/ml).

#### Characterization of reacting IgE epitopes

The reacting epitopes were characterized by heating IgE to 56° C for 2 hours before the inhibi-

tion experiments. Heat is considered to destroy the determinants in the De2 region (Ce3 and/or Ce4 domains) whereas those in the De0 region (Ce1 domain) and De1 region (Ce2 domain) are resistant.<sup>12</sup> In the case of IgG1, IgG2, and IgG3 a-IgE Ab the inhibitory capacity of IgE-DES, taken at 50% assay inhibition, increased by a factor of two to 21 after heat treatment whereas for IgG4 a-IgE Ab the inhibitory capacity decreased by a factor of two to five (Table II). Heat treatment of the other four IgE myeloma proteins did not make them inhibitory (100  $\mu$ g/ml) with IgG1, IgG2, or IgG3 a-IgE Ab, nor did any of the four IgG-subclass

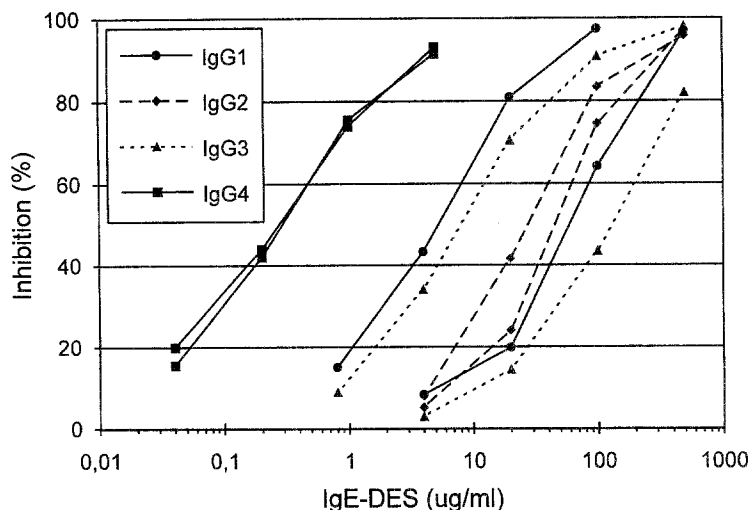


FIG. 3. Inhibition curves between IgE-DES and IgG-subclass a-IgE Ab as tested by inhibition ELISA. Two examples for each subclass are illustrated.

a-IgE Ab significantly react with heat-treated IgG, IgA1, IgA2, IgD, or IgM (data not shown).

In addition to the selected sera with high titers, 23 sera with moderate to low titers of IgG1 a-IgE Ab and 13 sera with various titers of IgG4 a-IgE Ab were tested in inhibition experiments with IgE-DES and IgE-PS. All sera with IgG1 a-IgE Ab were almost completely inhibited by heated IgE-DES (200  $\mu$ g/ml), whereas unheated IgE-PS (200  $\mu$ g/ml) gave no significant inhibitions (Fig. 4). These results further underscore the restricted specificity of IgG1 a-IgE Ab. Conversely, all IgG4 a-IgE Ab were inhibited in a parallel manner by both unheated IgE myeloma proteins tested at 20  $\mu$ g/ml (Fig. 5). Minor differences in the inhibitions may be due to background, incomplete inhibition, or low amounts of IgG4 antibody having the specificity of IgG1 antibody (e.g., sera 745 and 373) or vice versa (e.g., sera 79 and 80 in Fig. 4).

The specificity of IgG a-IgE Ab, with three in each subclass, was also studied in inhibition experiments with two papain-derived fragments of IgE-DES. The results indicate that all IgG1, IgG2, and IgG3 a-IgE Ab reacted only with the Fab<sub>e</sub> fragment whereas all three IgG4 a-IgE Ab reacted only with the Fc<sub>e</sub> fragment (Fig. 6). There was no increase in the inhibitory activity of heat-treated Fab<sub>e</sub>-DES fragments with IgG1-, IgG2-, and IgG3-subclass a-IgE Ab as was noted for heated intact IgE. Instead, a minor decrease of a few percent was observed (data not shown).

TABLE II. Effect of heat treatment (2 hours, 56° C) on inhibitory activity of IgE-DES with different IgG-subclass a-IgE Ab as measured by ELISA

| A-IgE Ab<br>IgG subclass | Inhibitory concentration<br>( $\mu$ g/ml)* |                   | Unheated/<br>heated<br>ratio† |
|--------------------------|--------------------------------------------|-------------------|-------------------------------|
|                          | Unheated<br>IgE-DES                        | Heated<br>IgE-DES |                               |
| IgG1                     | 19                                         | 6.0               | 3.2                           |
| IgG1                     | 55                                         | 5.0               | 11                            |
| IgG1                     | 4.4                                        | 1.4               | 3.1                           |
| IgG1                     | 70                                         | 13                | 5.4                           |
| IgG1                     | 42                                         | 2.0               | 21                            |
| IgG1                     | 90                                         | 15                | 6.0                           |
| IgG2                     | 75                                         | 20                | 3.8                           |
| IgG2                     | 23                                         | 6.0               | 3.8                           |
| IgG2                     | 51                                         | 21                | 2.4                           |
| IgG2                     | 30                                         | 10                | 3.0                           |
| IgG3                     | 8.0                                        | 3.4               | 2.4                           |
| IgG3                     | 140                                        | 25                | 5.6                           |
| IgG3                     | 125                                        | 9.0               | 14                            |
| IgG3                     | 72                                         | 12                | 6.0                           |
| IgG4                     | 0.27                                       | 0.85              | 0.32                          |
| IgG4                     | 0.30                                       | 0.75              | 0.40                          |
| IgG4                     | 0.17                                       | 0.68              | 0.25                          |
| IgG4                     | 0.15                                       | 0.75              | 0.20                          |

\*At 50% assay inhibition between a-IgE Ab and solid-phase IgE-DES.

†At 50% assay inhibition. Ratio greater than 1 indicates gain whereas ratio less than 1 indicates loss in inhibitory activity after heat treatment.

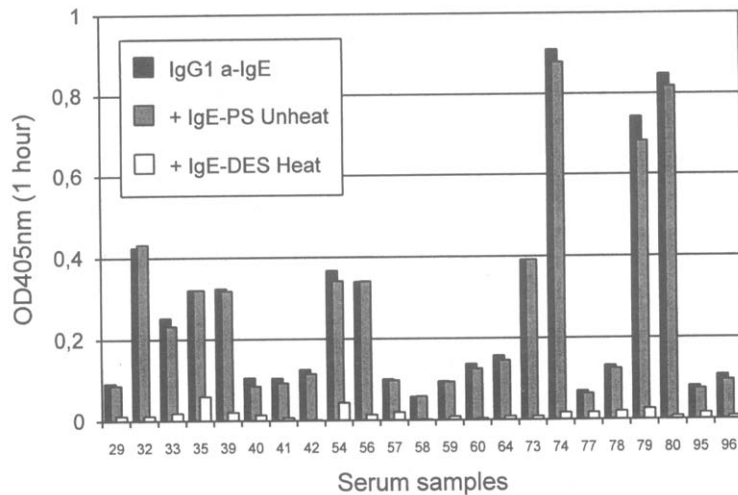


**FIG. 4.** Comparison of heated (2 hours, 56° C) IgE-DES (200  $\mu$ g/ml) and unheated IgE-PS (200  $\mu$ g/ml) as inhibitors of IgG1 a-IgE Ab activity among 23 sera with low to moderate titers. Samples were assayed at 1:50 final dilution by inhibition ELISA with solid-phase IgE-DES.

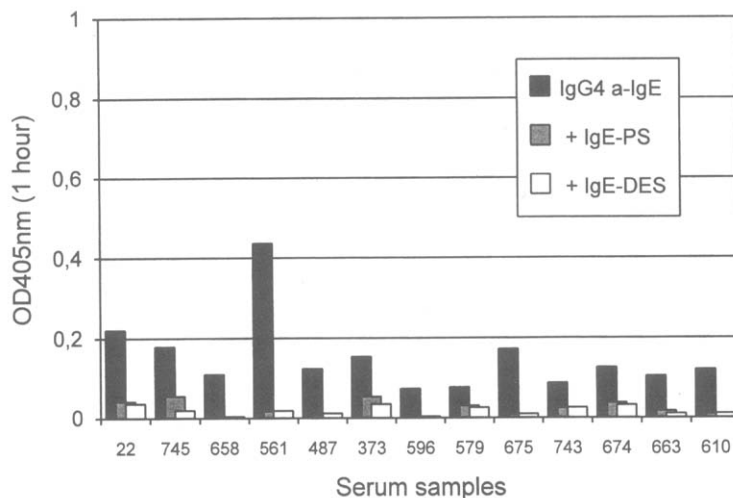


**FIG. 5.** Comparison of IgE-DES (20  $\mu$ g/ml) and IgE-PS (20  $\mu$ g/ml) as inhibitors of IgG4 a-IgE Ab activity among 13 sera with various titers. Samples were assayed at 1:10 final dilution by inhibition ELISA with solid-phase IgE-DES.

## DISCUSSION

We confirm here with IgG subclass-specific Mab, solid-phase IgE-DES, and ELISA that IgG a-IgE Ab may belong to all four IgG subclasses.<sup>19-24</sup> Inhibition ELISA revealed that all IgG subclass a-IgE Abs studied were inhibited by IgE-DES myeloma protein, whereas none was inhibited by IgA1, IgA2, IgD, or IgM myeloma protein or by polyclonal IgG. However, it was clear that all a-IgE Ab of the IgG1, IgG2, and IgG3 subclasses tested had a specificity toward epitopes restricted to IgE-DES (i.e., the protein also used for detection of a-IgE Ab). None of four additional IgE my-

eloma proteins representing both light chain types was reactive. In sharp contrast, all IgG4 a-IgE Ab tested demonstrated isotypic specificity because all five IgE myelomas were inhibitory.

In addition to this apparent difference in specificity, our results further distinguish the cluster of IgG1, IgG2, and IgG3 a-IgE Ab from that of IgG4 a-IgE Ab. First, the former antibodies seem to be of lower affinity because concentrations of IgE-DES necessary for 50% assay inhibition were 40 to 1000 times higher than those needed for IgG4 a-IgE Ab. Minor differences in the sensitivity of the IgG-subclass a-IgE Ab assays cannot account

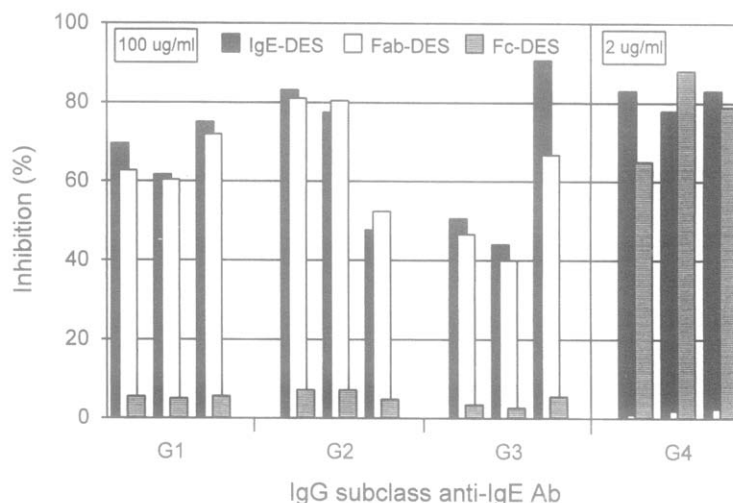


**FIG. 6.** Reactivity of IgG-subclass a-IgE Ab with papain-derived Fab and Fc fragments of IgE-DES by inhibition ELISA with solid-phase IgE-DES. Note differences in inhibitor concentrations for IgG1, IgG2, IgG3 (100  $\mu$ g/ml), and IgG4 a-IgE Ab (2  $\mu$ g/ml).

for this. Second, the IgG1, IgG2, and IgG3 a-IgE Ab clearly reacted with heat-resistant epitopes whereas IgG4 a-IgE Ab reacted with heat-susceptible ones. Third, the former group was directed against myeloma-restricted determinants in the Fab fragment whereas IgG4 a-IgE Ab reacted with isotypic epitopes pertaining to the Fc fragment. The observed twofold to 20-fold increase in inhibitory activity of heat-treated IgE with IgG1, IgG2, and IgG3 a-IgE Ab could be due to neoantigens but is more likely an effect of both epitope resistance and aggregation, which together would increase the avidity of the low affinity reaction. The limited influence of heat on the inhibitory capacity of Fab fragments, which are less prone to aggregate, is in line with this interpretation. On the other hand, heated IgE lost up to 80% of its inhibitory activity with IgG4 a-IgE Ab. This figure is close to the ones found for the antigenic heat susceptibility of determinants in the De2 region<sup>12</sup> and compatible with previously deduced specificities of IgG a-IgE Ab.<sup>1,5,6</sup> It is physiologically interesting why isotype-specific IgG a-IgE Ab are within IgG4, a subclass normally representing but a small percentage of serum IgG. However, it is functionally monovalent, forms small complexes, and does not activate complement but can modulate complement-activating properties of other immunoglobulins,<sup>25</sup> which makes it a putative blocking or scavenger antibody.

If the specificity of IgG a-IgE Ab for IgE myeloma-restricted epitopes seems as common as reported here, this must affect the interpretation of

most previous investigations in which IgE myeloma proteins have been used.\* The problem of putative anti-Fab antibody in a-IgE Ab assays has been considered before but has been found negligible,<sup>7</sup> probably because F(ab')<sub>2</sub> fragments of polyclonal IgG were used instead of that fragment of myeloma IgE. Furthermore, our study clearly demonstrates the restricted specificity of IgG1, which is considered the main source of IgG a-IgE Ab,<sup>19,24</sup> and it is conceivable that a major part of previous results is due to IgG a-IgE Ab of restricted specificity instead of isotypic specificity. This possibility is further illustrated by other studies. The concentrations of IgE necessary for 50% inhibition have been in the order of 30 to 100  $\mu$ g/ml,<sup>13,22,27</sup> which are figures close to ours for IgG1, IgG2, and IgG3 a-IgE Ab, but far from those of IgG4 a-IgE Ab. Moreover, specificity studies are few<sup>5,8</sup>; some have been performed with the homologous IgE myeloma protein only,<sup>13,27</sup> whereas others were only illustrated in "typical" experiments,<sup>26</sup> which were sometimes unclear and repeatedly published.<sup>13,20,22</sup> On the other hand, the extent of contribution from low-affinity antibodies may depend on the heterogeneity in Fab determinants including glycosylation<sup>30</sup> and on various assay-inherent factors<sup>31</sup> such as assay design, assay reaction times, sample dilutions, and the amount of coating antigen. Thus caution must be exercised when IgG a-IgE Ab results obtained with myeloma IgE proteins are interpreted.

\* References 1, 5, 8, 13, 19-22, 26-29.

Although clear-cut specificities were found in the present study, it is highly probable that isotype-specific a-IgE Ab of the IgG1, IgG2, and IgG3 subclasses also exist.<sup>23</sup> However, there is an apparent risk that such antibodies are overshadowed by the relatively abundant concentration of "bulk" a-IgE Ab having myeloma-restricted specificity. Conversely, IgG4 a-IgE Ab with myeloma-restricted specificity may also exist. One way to avoid bulk IgG a-IgE Ab and also to expand the isotypic specificities would be to use appropriate enzymatic or recombinant fragments of IgE.<sup>31</sup> Recently the specificity of IgG1 a-IgE Ab was studied with solid-phase recombinant Fc $\epsilon$  fragment, ELISA inhibition, and a set of different recombinant fragments of IgE.<sup>14</sup> The reacting epitopes were mapped to the C $\epsilon$ 2 and C $\epsilon$ 4 domains of IgE, although for unknown reasons native IgE was a poor inhibitor. In view of these data it is clear that further studies on both the fine specificity and prevalence of  $\epsilon$ -specific IgG-subclass a-IgE Ab are warranted.

We have previously shown that IgM a-IgE Ab also react with epitopes restricted to individual IgE myeloma proteins<sup>4</sup> and that these epitopes sometimes seem to be of carbohydrate origin.<sup>1</sup> We speculated that carbohydrate-rich organisms such as bacteria, fungi, and parasites could induce such antibodies, which subsequently crossreact with oligosaccharide structures in IgE myeloma proteins.<sup>1,4</sup> Anticarbhydrate antibodies are commonly found in normal sera,<sup>32,33</sup> and carbohydrates are both heterogeneous<sup>30</sup> and heat resistant. We have also recently demonstrated that IgA a-IgE Ab of the IgA1 subclass exist<sup>1</sup> (C. G. M. Magnusson, unpublished observations) and that they have a similar specificity to the IgG1, IgG2, and IgG3 a-IgE Ab described here. Whether some of these IgA- and IgG-subclass a-IgE Ab are also specific for carbohydrate moieties awaits further investigations. Polysaccharides are known to elicit mainly IgG2 antibody,<sup>34</sup> whereas IgA1, IgG1, and IgG3 may react with proteinaceous epitopes within the Fc $\epsilon$  fragment. The wide distribution in the inhibitory concentrations of both unheated and heated IgE-DES suggests that these antibodies represent a multitude of specificities. Similar anti-Fab antibodies against myeloma IgA proteins and polyclonal IgG have previously been described in healthy donors<sup>35</sup> and in patients,<sup>36</sup> but neither specificity nor a physiologic role have yet been shown for such antibodies. Perhaps some of these anti-Fab antibodies have some immunoregulatory function (e.g., in the idiotypic network).<sup>37</sup>

In summary, we have shown that four major

differences exist between two main groups of a-IgE Ab. One group, represented here by IgG1, IgG2, and IgG3, interact with low affinity to heat-resistant epitopes that are restricted to individual IgE-myeloma proteins and confined to the Fc $\epsilon$  fragment. It is doubtful whether these antibodies are of any interest in IgE-mediated disorders. The other group, represented by IgG4, is of higher affinity and reacts with heat-susceptible isotype-specific epitopes located in the Fc $\epsilon$  fragment. These antibodies are of potential interest in IgE-mediated diseases. The importance of these findings both with respect to reevaluation of previous studies on IgG a-IgE Ab and their physiologic implications are matters for further investigations.

I thank Mrs. M. Magnusson for technical assistance, Dr. T. Karlsson for supply of monoclonal antibodies, Dr. L. Hammarström for providing chimeric antibody, and Mr. L. Norin for providing F(ab')<sub>2</sub> fragments of rabbit anti-mouse immunoglobulin antibody.

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