

Naturally occurring human IgA autoantibodies against IgE-DES myeloma protein

Prevalence and specificity

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The prevalence and specificity of naturally occurring human IgA anti-IgE autoantibodies (a-E Ab) were studied by ELISA with anti-IgA monoclonal antibodies (mAb) and a purified myeloma IgE as solid-phase protein, i.e., IgE-DES(κ). Such detected IgA a-E Ab were common among adults, and significantly increased geometric means (GM) were found in patients with atopy ($P = 0.006$; $n = 41$; GM = 79.3 arbitrary units (AU)/ml) and filariasis ($P = 0.02$; $n = 41$; GM = 75.9 AU/ml), as compared with nonatopic controls ($n = 42$; GM = 48.8 AU/ml). No such difference was observed between age-matched nonatopic ($n = 22$; GM = 36.7 AU/ml) and atopic ($n = 22$; GM = 38.6 AU/ml) children. Children had significantly ($P < 0.001$) lower IgA a-E Ab concentrations than adults, probably as a result of age, because IgA a-E Ab concentrations and age of children were significantly correlated ($n = 44$; $P < 0.05$; $r_s = 0.30$). IgA a-E Ab concentrations were very low in cord serum ($n = 32$; median < 0.1 AU/ml). Sex did not influence IgA a-E Ab concentrations in any study group. The specificity of IgA a-E Ab in nine sera was studied by ELISA inhibition assay using IgE-DES myeloma as solid-phase protein and inhibitory proteins of the IgG, IgM, IgD, and IgE classes, including five different IgE myeloma proteins, as well as three enzymatic fragments of IgE-DES. The inhibitions indicated that all IgA a-E Ab tested reacted in a low-affinity reaction with determinants restricted to IgE-DES, i.e., the solid-phase protein. These epitopes were heat-resistant (2 h; 56°C) and located in the Fab₂-DES fragment. No isotype-specific IgA a-E Ab were found because none of the four other IgE proteins were inhibitory. Subclass typing indicated that most IgA a-E Ab belonged to the IgA1 subclass. It is unlikely, for reasons of restricted specificity, low affinity, and common prevalence, that such IgA1 a-E Ab are connected with IgE-mediated disorders. The study also raises questions on the definition of anti-IgE antibodies.

Key words: anti-IgE; assay; atopic; autoantibody; children; cord serum; filariasis; IgA; IgE.

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Since the first report on anti-IgE autoantibodies (a-E Ab) in the early 1970s (25), many studies have confirmed their existence (reviewed in 18, 23). Thus, a-E Ab have been shown to belong to the IgM (14) and IgG isotypes including all four IgG subclasses (11, 26). IgM a-E Ab show low affinity, common prevalence, and restricted specificity for individual IgE myeloma proteins (14, 15) and seem therefore to be of limited interest in IgE-mediated disorders. On the other hand, some IgG a-E have been shown to be of high affinity, isotype-specific, immune com-

plexed to serum IgE, and most prevalent in allergic disorders, all of which are features indicating a potential physiologic role (6, 18, 21, 23). It has been suggested that some of these IgE a-E Ab elicit histamine release from basophils and mast cells both *in vivo* (21) and *in vitro* (5, 19), as well as affecting IgE synthesis (11, 24).

We have previously found a few serum samples with demonstrable IgA a-E Ab activity (18). Interestingly, in one case, the IgA Ab reacted with three different IgE myeloma proteins in an equimolar man-

ner, suggesting isotypic specificity (18), while in the other case the specificity was myeloma-restricted, as described for IgM a-E Ab (15). Because IgA is the main antibody involved in mucosal immunity, it seemed to be interesting to follow up these preliminary data.

The aim of the present study was to investigate IgA a-E Ab in more detail in terms of both prevalence and specificity.

Material and methods

Serum samples

Consecutive serum samples referred to our clinic for *in vitro* allergy testing were used to screen and select sera containing high titers of IgA anti-IgE antibodies (IgA a-E Ab) for the specificity studies. The prevalence of IgA a-E Ab among adults (>17 years old) was investigated in serum samples from 40 patients considered to be "nonatopic" because of total serum IgE below 25 IU/ml and a negative radioallergosorbent test (RAST; Pharmacia AB, Uppsala, Sweden) to three common allergens (birch, cat, and timothy), from 40 patients considered to be atopic because of total serum IgE above 500 IU/ml and a positive RAST (score $\geq 3+$) to at least one of the common allergens above, and from 40 patients with lymphatic filariasis (sera kindly provided by Dr J-Y Cesbron, Lille, France). The geometric means (GM) of total serum IgE as analyzed by PRIST (Pharmacia AB) in these three study groups were 8.8, 917, and 8103 IU/ml, respectively.

In addition, IgA a-E Ab concentrations were analyzed in sera from two groups of age-matched children, 2–10 years old. Twenty-two children (mean age = 5.1 years) were considered to be nonatopic because they had a total serum IgE within 1 SD of the GM for the age (9) and a negative RAST Phadiatop Pediatric (Pharmacia AB), whereas another 22 children (mean age = 5.8 years) were considered to be atopic because of a total serum IgE above 2 SD of the GM for the age (9) and a positive RAST Phadiatop Pediatric. The GM of total serum IgE in these two groups of children were 5.5 and 239 IU/ml, respectively.

Umbilical cord serum samples were taken from 32 consecutive neonates. All sera were kept at -20°C pending analyses.

Antibodies and proteins

Mouse monoclonal antibodies (mAb) against IgA (2F4), IgA1(1A7), and IgA2(3B7) were kindly provided by Dr J-P Vaerman (Brussels, Belgium); they have been described previously (2). Rabbit anti-mouse Ig Ab in the form of F(ab')_2 fragment (kind gift of Leif Norin, Dakopatts, Sweden) was conju-

gated with alkaline phosphatase (ALP; EC 3.1.3.1., calf intestine, Boehringer-Mannheim Scandinavia AB, Stockholm, Sweden) by glutaraldehyde in a one-step procedure essentially as described (1).

Polyclonal IgG (gamma globulin) was obtained from Kabi, Stockholm, Sweden. Purified IgE-DES(κ), IgE-ND(λ), IgE-PS(λ), IgE-WT(λ), and IgE-UD(κ), as well as the pepsin- $(\text{Fc}'')_{\epsilon}$ and papain- $(\text{Fab}_{\epsilon}$ and $\text{Fc}_{\epsilon})$ derived fragments of IgE-DES were those previously described (15). Purified IgG1-HO(κ), IgA1-DEL(κ), IgA1-BLA(λ), IgA2-LOU(λ), IgA2-GIR(κ), IgD-BER(λ), and IgM-VIR(κ) myeloma proteins, and polyclonal colostral s-IgA were obtained from various sources (14, 15; Mestecky et al., WHO/IUIS study on a-IgA mAb). The purity of the proteins was estimated to be at least 90% (w/w) by agarose gel electrophoresis and SDS-PAGE in gradient gels (4–15%) under non-reducing and reducing conditions (Phastsystem, Pharmacia AB, Uppsala, Sweden), as well as by Ig- and Ig-subclass-specific immunoassays. The extinction coefficient for all proteins was assumed to be $\text{E}_{280\text{ nm}, 1\%}$ 14.

Heat treatment of all monoclonal proteins and enzymatic fragments of IgE-DES was performed at 56°C for 2 h in a water bath, while polyclonal IgG was heated at 63°C for 20 min.

IgA anti-IgE ELISA

Serum samples from children and adults were diluted 100-fold, or as appropriate, in sample diluent consisting of 1 volume of horse serum (PRIST diluent, Pharmacia AB, Uppsala, Sweden) and 1 volume of PBS-BSA (phosphate-buffered saline (PBS) 50 mM, pH = 7.4; containing 0.2% (w/v) bovine serum albumin (BSA)). Cord-blood serum samples were assayed undiluted and at fivefold dilution.

Microtiter plates (Maxisorp, NUNC, Roskilde, Denmark) were coated overnight at room temperature with 100 μl /well highly purified IgE-DES protein (10 $\mu\text{g}/\text{ml}$ in carbonate-bicarbonate buffer 0.1 M; pH = 9.8). All the following incubations (100 μl , room temperature) were followed by four washings with PBS-Tween (0.05% v/v) in an automatic plate washer (SLT Easywasher II, Ekoscann, Sweden). Microtiter plates were incubated overnight with sample (100 μl), followed by anti-IgA or anti-IgA subclass mAb for 6–7 h and rabbit ALP-conjugated F(ab')_2 antimouse Ig Ab overnight before color development (1 h) with 1 mg/ml *p*-nitrophenyl phosphate (tablets; Sigma Chemicals, St Louis, MO, USA) in 1 M diethanolamine buffer (pH = 9.8) containing 1 mM MgCl_2 and 0.02% (w/v) NaN_3 . Optical densities at 405 nm ($\text{OD}_{405\text{ nm}}$) were recorded in an ELISA reader (SLT Easyreader 330, Ekoscann, Sweden). The results, mean of duplicates, were

transformed into arbitrary units (AU/ml) from a standard curve consisting of a serum with a high titer of IgA a-IgE Ab (1000 AU/ml). Suitable concentrations of mAb (2 µg/ml) and secondary Ab (1/500) were established in preliminary pilot experiments.

Inhibition experiments were done by preincubating overnight different concentrations of inhibitors with adult sera prediluted to contain IgA a-IgE Ab titers giving an OD 405 nm of 1.3–1.8 after 1-h color development. The inhibition results were expressed in percentage after background subtraction (never exceeding 0.090 for all reagents except sample) according to the formula:

$$\frac{(\text{OD}_{405\text{nm}}_{\text{preinhib}} - \text{OD}_{405\text{nm}}_{\text{postinhib}})}{\text{OD}_{405\text{nm}}_{\text{preinhib}}} \times 100$$

An inhibition of 8% was considered to be significant; this represented the mean of 36 inhibition experiments plus 3 SD with sample diluent alone as inhibitor.

Statistics

Results between groups were compared with non-parametric statistical methods including Kruskal-Wallis one-way analysis, Mann-Whitney U-test, and Spearman rank correlation. A two-tailed value of $P < 0.05$ was considered to be significant.

Results

IgA a-E Ab assay

In preliminary experiments, five sera containing high IgA a-E Ab levels were tested at four dilutions to assess the parallelism of the assay. The concentration of IgA a-E Ab in four of the sera was calculated with the fifth serum as standard curve. The normalized results corrected for dilution factors and expressed in percentage ranged from 87 to 113% ($n = 12$; mean = 100%; coefficient of variation (CV) = 6.8%). Hence, any of the five sera could be used to construct standard curves for the IgA a-E Ab assay. One serum was chosen and considered to contain 1000 AU/ml. The mean (± 1 SD) of six standard curves run in duplicate on six different microtiter plates is illustrated in Fig. 1. Interassay precision was also evaluated by including a 1/100- and a 1/400-dilution of two control sera. The CV were 7.2 and 6.8% for a mean IgA a-E Ab concentration of 67 and 563 AU/ml, respectively.

Prevalence

No significant differences in the concentration of IgA a-E Ab could be found between the nonatopic ($n = 22$; GM = 36.7 AU/ml; range: 8.11–119) and

atopic ($n = 22$; GM = 38.6 AU/ml; range: 8.29–109) children (Fig. 2). On the other hand, a significant difference was found among the nonatopic ($n = 42$; GM = 48.8 AU/ml; range: <0.4–348), atopic ($n = 41$; GM = 79.3 AU/ml; range: 13.3–440), and parasite-infested ($n = 41$; GM = 75.9 AU/ml; range: 32.5–830) adults (Kruskal-Wallis; $P < 0.01$; Fig. 2). This resulted from significantly lower levels among nonatopic than atopic (Mann-Whitney; $P = 0.006$) and filariatic (Mann-Whitney; $P = 0.017$), subjects, respectively. There was no significant difference between the last two groups. Children had significantly lower IgA a-E Ab concentrations than adults ($P < 0.001$) when the atopic and nonatopic groups were considered together. The range of IgA a-E Ab concentrations varied about 10- and 50-fold among children and adults, respectively, with one exception. In a nonatopic adult, no IgA a-E Ab (<0.4 AU/ml) could be detected. However, subsequent assay of this serum by ELISA revealed that it contained <10 µg/ml of IgA, suggesting that this patient was IgA-deficient.

Very low levels of IgA a-E Ab were found in the 32 cord-blood sera tested (range: <0.1–0.29 AU/ml). This was up to 1000-fold less than in the adults, and significant levels (i.e., >0.1 AU/ml) were detected in six (19%) cases only.

Sex did not influence IgA a-E Ab concentrations in any of the study groups.

Correlations

A weak but significant correlation was found between age and IgA a-E Ab concentration among the children taken together ($n = 44$; $r_s = 0.30$; $P < 0.05$). No such relation was found among the adults.

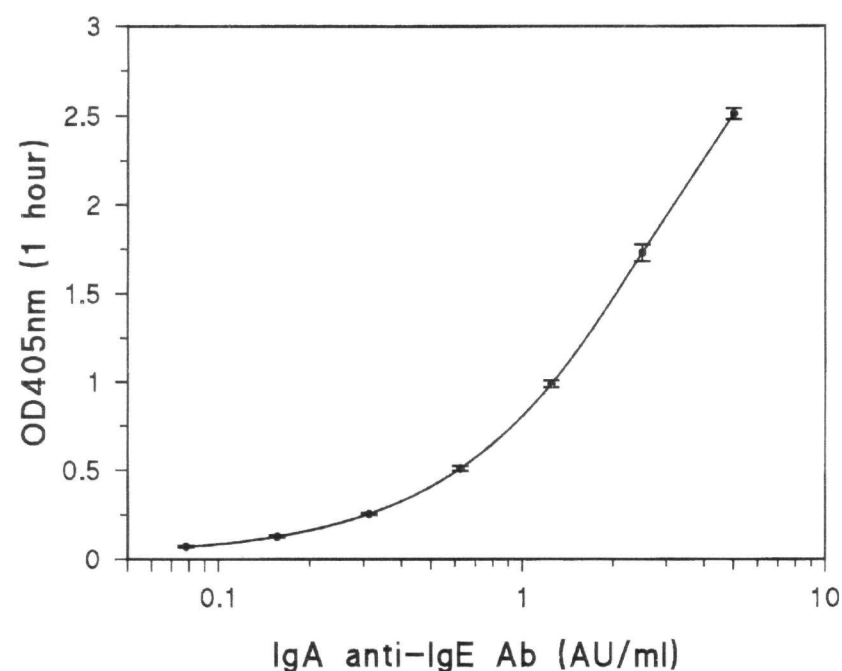


Fig. 1. Standard curve in arbitrary units/ml (AU/ml) for IgA a-E Ab determination by ELISA with solid-phase IgE-DES. Bars indicate ± 1 SD from mean of six standard curves run in duplicate in six different microtiter plates.

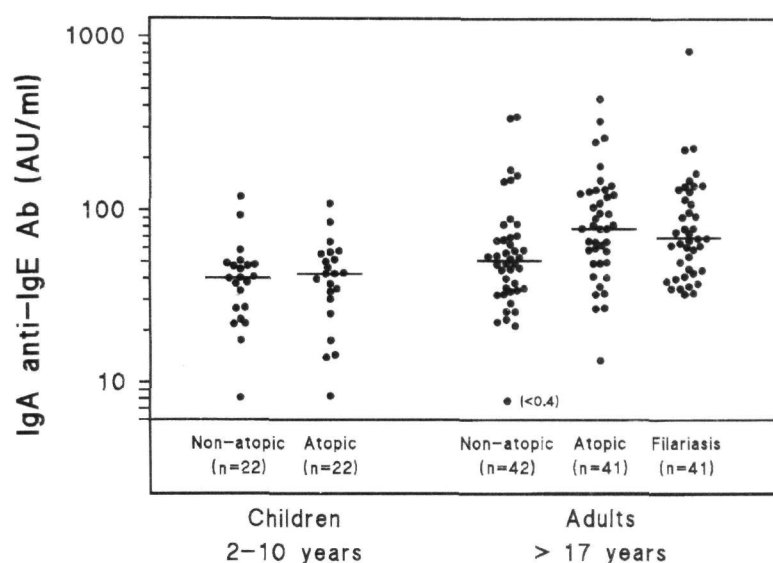


Fig. 2. Concentration of IgA a-E Ab (AU/ml) in sera from children and adults determined by ELISA. Horizontal bars indicate medians.

There were no significant correlations between IgE and IgA a-E Ab concentrations in any of the study groups analyzed separately. However, significant correlations were observed when all the adults were pooled into one group ($n = 124$; $r_s = 0.2$; $P = 0.012$) or when just nonatopic and atopic subjects were pooled ($n = 83$; $r_s = 0.34$; $P = 0.003$).

Specificity

The specificity of IgA a-E Ab in nine different sera, five with high and four with low-to-moderate titers, was tested by inhibition assay in which various inhibitors were allowed to compete with solid-phase IgE-DES for a fixed amount of IgA a-E Ab.

None of the nine different sources of IgA a-E Ab tested reacted significantly (i.e., $>8\%$) with either polyclonal IgG (100 $\mu\text{g/ml}$) or myeloma proteins of the IgG1, IgM, and IgD class (each at 250 $\mu\text{g/ml}$). IgE-DES clearly inhibited, although to different degrees, all sources of IgA a-E Ab at both 100 and 10 $\mu\text{g/ml}$ and even sometimes at 1 $\mu\text{g/ml}$ (Fig. 3). Since all the inhibition patterns were similar, only a few of them are shown. Further experiments with four additional IgE myeloma proteins (100 $\mu\text{g/ml}$) showed that it was only IgE-DES, the homologous protein also used for detection of IgA a-E Ab, that significantly inhibited the reaction between IgA a-E Ab and solid-phase IgE-DES (Fig. 3).

Inhibition curves indicated that the concentration of IgE-DES necessary for 50% inhibition varied almost 100-fold among the nine sera tested, i.e., 7–675 $\mu\text{g/ml}$.

Characterization of reacting IgE epitope

The influence of heat on the interacting epitope of IgE-DES with IgA a-E Ab was studied. Heat is

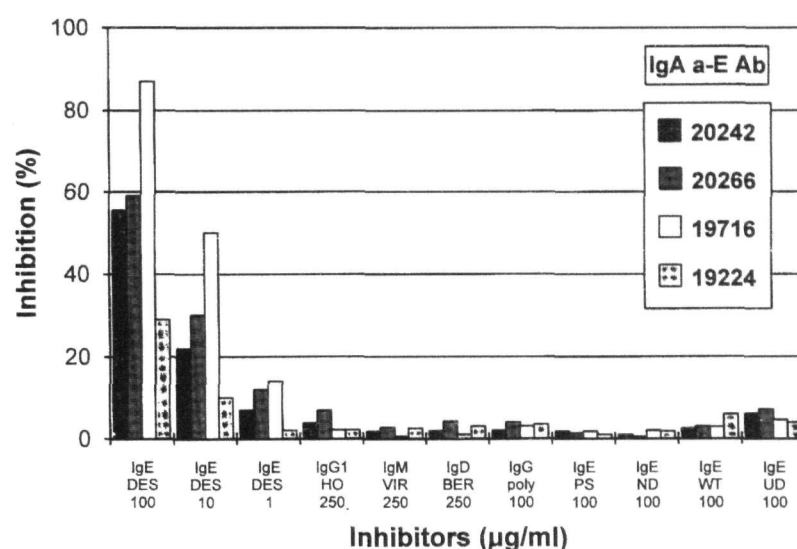


Fig. 3. Specificity of IgA a-E Ab from four different sera, as tested by inhibition ELISA with solid-phase IgE-DES and various inhibitory proteins ($\mu\text{g/ml}$). Results are expressed in percent inhibition, as explained in Material and methods.

considered to destroy the determinants in the $D_{\epsilon}2$ -region ($C_{\epsilon}3$ - and $C_{\epsilon}4$ -domains), while those in the $D_{\epsilon}0$ -region ($C_{\epsilon}1$ -domain) and $D_{\epsilon}1$ -region ($C_{\epsilon}2$ -domain) are resistant (3). Thus, comparison of nine inhibition curves of unheated and heated IgE-DES (2 h; 56°C) with IgA a-E Ab revealed a 10–70-fold increase in inhibitory activity after heat treatment. Five of the inhibition curves are shown in Fig. 4; in one case, only 0.5 $\mu\text{g/ml}$ of heated IgE-DES was needed for 50% inhibition, as compared with 35 $\mu\text{g/ml}$ before heating. Heat treatment of the other four IgE-myeloma proteins or the other immunoglobulin classes, i.e., IgG, IgM, and IgD (2 h; 56°C), or polyclonal IgG (20 min; 63°C), did not make any of them significantly inhibitory at 100 $\mu\text{g/ml}$ (data not shown).

The specificity of IgA a-E Ab from six sera was further studied with three enzymatic fragments of IgE-DES, i.e., the F_c'' (pepsin), F_c (papain), and Fab (papain) fragments constituting the $C_{\epsilon}2$, $C_{\epsilon}234$, and $C_{\epsilon}1$ domains of IgE, respectively. The purity of these fragments was estimated to be higher than 95%, as judged from inhibition assays with mAb specific for the κ -chain as well as the $D_{\epsilon}0$ -, $D_{\epsilon}1$ -, and $D_{\epsilon}2$ -regions of IgE-DES (16). The results showed that, beside intact IgE-DES, it was only the papain-derived Fab_{ϵ} fragment (50 $\mu\text{g/ml}$) that significantly reacted with IgA a-E Ab (Fig. 5). The papain-derived F_c_{ϵ} fragment and the pepsin-derived $F_c''_{\epsilon}$ -fragment showed no significant inhibitions at 50 $\mu\text{g/ml}$. The inhibitory capacity of the Fab_{ϵ} fragment paralleled that of IgE-DES and was not significant in one case in which IgE-DES also was a poor inhibitor, i.e., no. 19169 (Fig. 5). In contrast to the case with intact IgE, there was no increase in the inhibitory activity of the Fab_{ϵ} fragment after heat treatment (2 h; 56°C), as tested on three sera. Instead, a slight decrease of

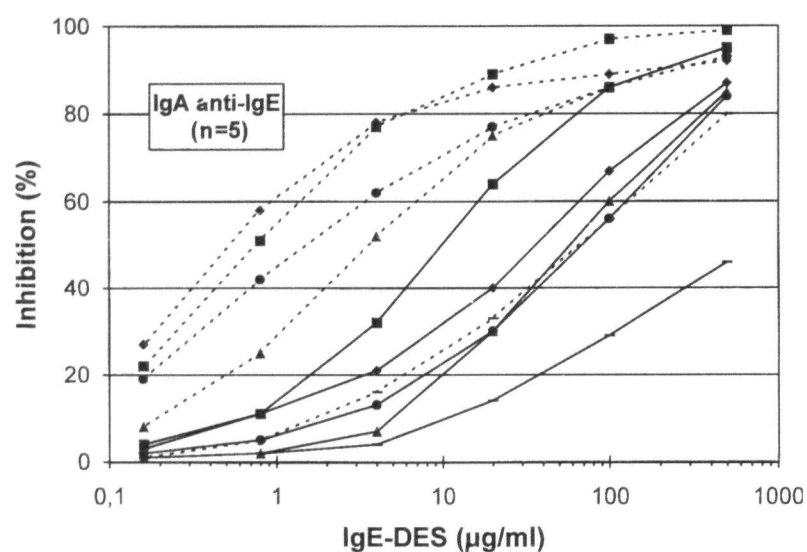


Fig. 4. Effect of heat (2 h; 56°C) on inhibitory activity of IgE-DES ($\mu\text{g/ml}$) with IgA a-E Ab from five sera, as tested by inhibition ELISA. Unheated IgE-DES (solid line); heated IgE-DES (dashed line).

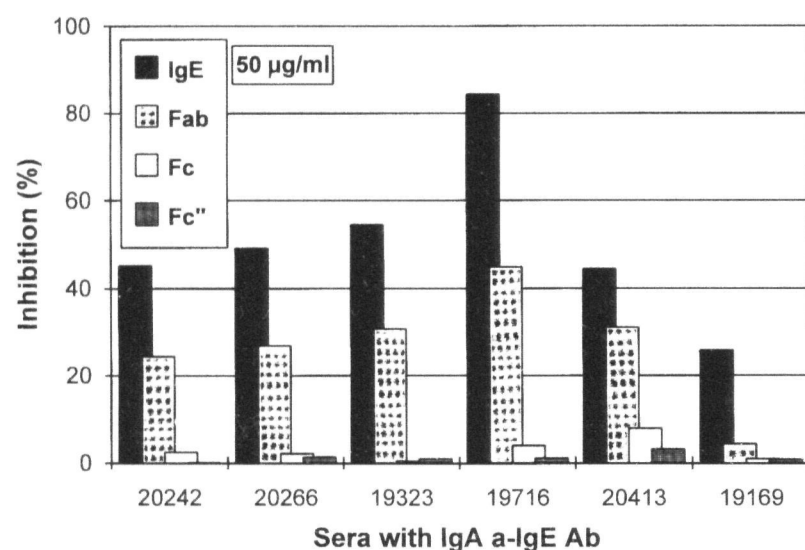


Fig. 5. Specificity of six different sources of IgA a-E Ab, as determined by inhibition ELISA with three different enzymatic fragments of IgE-DES, i.e., pepsin-derived Fc''_{E} fragment and papain-derived Fab_{E} and Fc_{E} fragments. All inhibitors at 50 $\mu\text{g/ml}$.

a few percentage points was registered (data not shown).

IgA a-E Ab subclass

Before subclass typing of IgA a-E Ab, the specificity of the a-IgA mAb used in this study was verified by direct ELISA. Polyclonal colostral secretory IgA, containing approximately equal concentrations of IgA1 and IgA2, and monoclonal IgA1(κ), IgA1(λ), IgA2(κ), and IgA2(λ) myeloma proteins were used to coat microtiter plates at fivefold dilutions (0.8–500 ng/ml). The assay was then performed exactly as described for the measurement of IgA a-E Ab except that only sample diluent was used in all wells. Performance of this assay protocol indicated that both a-IgA subclass mAb reacted with secretory IgA and were specific for the IgA1 and IgA2 subclasses,

respectively. The cross-reactivity was estimated to be less than 1% for both mAb (Table 1).

Thus, subclass typing of IgA a-E Ab from six donors of each adult patient category ($n = 18$) with subclass-specific mAb revealed that almost all IgA a-E Ab activity could be attributed to the IgA1 subclass (Fig. 6) because the two IgA-subclass assays had similar sensitivity. Minor reactivities with anti-IgA2 mAb could have been due to background or minor cross-reactivity to IgA1.

Discussion

To our knowledge, this is the only report on IgA a-E Ab beside our preliminary data on two serum samples that contained IgA a-E Ab (18). In one case, such activity was inhibited by three different IgE myeloma proteins, indicating a putative isotypic specificity, while the other case showed an IgE-myeloma-restricted specificity. In the present study, we have extended our initial observation by studying the specificity of many sera with IgA a-E Ab, as well as the prevalence of such a-E Ab among sera from adults, children, and neonates.

Our results on nine sera with various concentrations of IgA a-E Ab clearly demonstrate the restricted specificity of these Ab. The only myeloma protein capable of inhibiting the reaction between solid-phase IgE-DES and IgA a-E Ab was IgE-DES itself. Thus, none of the other classes of Ig nor any of the four other IgE myeloma proteins tested showed significant inhibition despite a sometimes more than 100-fold molar excess of these inhibitors over IgE-DES. Inhibition curves showed that the concentrations of IgE-DES necessary for 50% inhibition were quite high and differed 100-fold, suggesting IgA a-E Ab of low affinity and heterogeneous specificity. In addition, the reacting epitopes of IgE-DES were heat-resistant and located in the papain-derived Fab_{E} fragment. In fact, heat treatment dramatically increased the inhibitory activity of IgE-DES, probably as a consequence of both epitope resistance and aggregation, which would increase the avidity of the reaction. All these characteristics are in sharp contrast to those of isotype-specific IgG a-E Ab, which

Table 1. Specificity of anti-IgA subclass mAb in ELISA

IgA-myeloma coat	a-IgA1 1A7	a-IgA2 3B7
IgA1 ($n=2$)	100%	<1%*
IgA2 ($n=2$)	<1%	100%

* Figures were calculated by generating standard curves from coated IgA subclass proteins at fivefold dilutions (0.8–500 ng/ml) and reading responses of other IgA subclass on corresponding curves; e.g., IgA1-coat of 500 ng/ml gives IgA2 content of <5 ng/ml ($<5/500 \times 100 = <1\%$), as calculated from coated IgA2 standard curve obtained with a-IgA2 mAb.

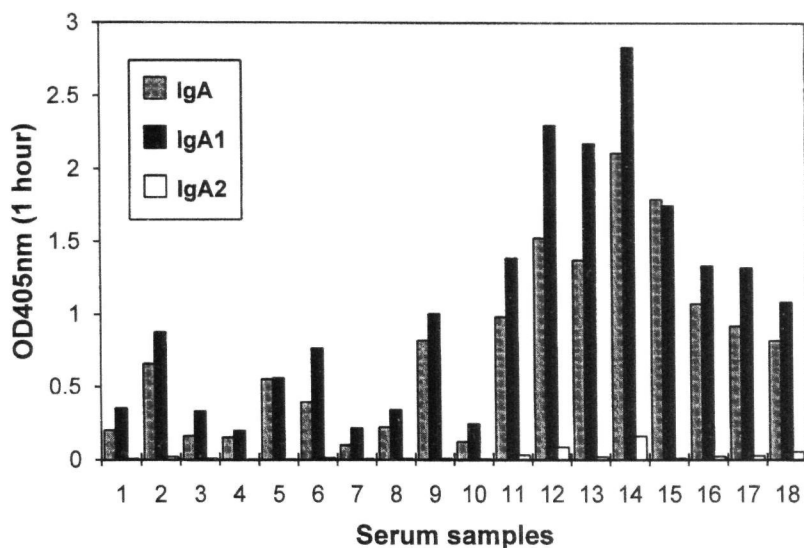


Fig. 6. Subclass typing of IgA a-E Ab from 18 different sera (six from each adult group) with ELISA using mAb specific for IgA1 and IgA2 subclasses.

are directed against heat-susceptible epitopes in the Fc_ϵ fragment of IgE (6, 18, 21).

The whole reaction pattern of IgA a-E Ab for myeloma protein IgE-DES shown here is quite similar to our previous findings on IgM a-E Ab (14, 15, 18) and our recent ones on some IgG subclass a-E Ab (Magnusson, paper submitted). The common characteristics of most of these a-E Ab are that they are heterogeneous, of low affinity, and preferentially directed against heat-resistant, myeloma-restricted epitopes mainly pertaining to the Fab_ϵ fragment. All these findings are compatible with carbohydrates as target antigens, as sometimes seemed to be the case with IgM a-E Ab (18). Anticarbohydrate Ab are heterogeneous and common and may be induced by infectious agents such as bacteria, fungi, and animal parasites (12, 13). However, epitopes of proteinaceous nature are probably also involved. Although the idea is highly speculative, both kinds of a-E Ab could play an immunoregulatory role because some polyclonal IgE in multivalent form may possess reacting epitopes that could participate in finely tuned phenomena at the cellular level, e.g., in the idiotypic network (4). Similar anti- Fab Ab against both polyclonal IgG (22) and myeloma IgA (8) have previously been described in both patients and healthy subjects, although without any evidence of fine specificity or physiologic function.

The IgA a-E Ab studied here seem to have a common prevalence very much like that of IgM a-E Ab (14). The subclass typing suggests that a major part of IgA a-E Ab belongs to the IgA1 subclass. However, it does not exclude the existence of IgA2 a-E Ab because no effort was made to screen specifically for this subclass. Interestingly, atopic and parasite-infested subjects had significantly higher IgA a-E Ab concentrations than nonatopic subjects, and significant correlations were obtained in pooled adult groups between IgE and IgA a-E Ab concen-

trations. However, despite these findings, it is unlikely that this kind of IgA a-E Ab is involved in IgE-mediated disorders because of their restricted specificity, low affinity, and common prevalence. The findings are instead a reflection of a hyperactive immune system, as seemed to be the explanation for IgM a-E Ab (14, 15). The two patient groups, atopic and filariasis-infested, both have IgE levels much higher than those of the nonatopic group. By their inflammatory state, the former groups are also more exposed to various agents which in a polyclonal manner may induce cross-reacting Ab of low affinity. IgA a-E Ab were also common among nonatopic and atopic children, although the concentrations were significantly lower than in adults. This difference is probably related to age because young children have lower IgA concentrations than adults (10), and this might also be reflected in a polyclonal response. Moreover, although weakly, IgA a-E Ab concentration and age correlated among children. However, in contrast to the adults, there was no difference in the concentration of IgA a-E Ab between atopic and nonatopic children, a finding which might be related to the ontogeny of the immune system. Not surprisingly, cord sera contained hardly any detectable IgA a-E Ab; this stems from the fact that IgA is very low in cord serum because it does not cross the placental barrier (10).

Although the results presented here suggest that IgA a-E Ab are mainly directed against nonisotypic epitopes, one cannot exclude the existence of IgA a-E Ab with isotypic specificity. They could be of the IgA2 subclass or be "hidden" by the bulk of the nonisotypic ones described here. They may also be engaged in immune complexes with IgE (6, 18, 21) and thereby escape detection. In contrast to the IgA a-E Ab described here, such putative isotype-specific IgA a-E Ab are more likely to have some physiologic function, e.g., as harmless scavengers for IgE, because IgA is a poor activator of complement (7) and various inflammatory cells express Fc_α -receptors (20). The restricted specificity of IgA a-E Ab shown here also raises questions about the terminology of a-E Ab. Logically, one should only use the term "a-E Ab" for antibodies that are isotype-specific. Further studies on IgA a-E Ab have to await the development of refined assays to avoid the detection of "bulk" nonisotypic IgA a-E Ab. The use of well-designed enzymatic or recombinant fragments of IgE may perhaps solve this problem (17) and thereby allow relevant investigation of the role of IgA a-E Ab.

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