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A polymeric IgA response in serum can be produced by parenteral immunization

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SUMMARY

The magnitude and the kinetics of the serum-specific polymeric (p-) and monomeric (m-) IgA antibody responses were analysed following parenteral stimulation with tetanus toxoid (TT) vaccine in 10 volunteers, 5–20 years after a previous boost. A rapid marked serum IgA antibody response involving both the monomeric and polymeric components of IgA was observed: m-IgA and p-IgA antibodies reached a peak of serum activity at about 11 days, around 6 days before the peak of IgG antibody activity. At the peak of the IgA response, p-IgA accounted for approximately half of the anti-TT activity (median 54%, 25–79%). However, p-IgA antibodies rapidly disappeared from serum over a few weeks, whereas the serum m-IgA antibody response was maintained over a prolonged period of time. For one subject out of five, anti-TT IgA were also detected in saliva with a peak of activity earlier than in serum. Calculation of the albumin relative coefficient of excretion for anti-TT IgA in this saliva suggested a local synthesis of these antibodies. The present study indicates that a polymeric IgA antibody response in serum can be produced by parenteral immunization in primed individuals, and it raises the question of the mechanisms that control polymeric versus monomeric IgA production.

INTRODUCTION

In man, IgA can be found in relatively high concentrations both in mucosal secretions and in the serum (Mestecky *et al.*, 1986). The relationship between the IgA in these two compartments is not well understood; however, the differences in subclass distribution and molecular forms of the IgA in these two compartments suggest that they are derived from independent sources. Over 90% of the IgA in mucosal secretions is in the polymeric form (Tomasi *et al.*, 1965; Heremans, 1974), whereas only 5–15% of the IgA in serum is in this form (Heremans, 1974; Delacroix *et al.*, 1983a). Recent studies have demonstrated that lymphoid cells from the bone marrow spontaneously secrete large amounts of IgA (Kutteh, Prince & Mestecky, 1982). This IgA resembles serum IgA in that over 90% is in the monomeric form (Kutteh *et al.*, 1982) and the majority is of the IgA1 subclass (Skvaril & Morell, 1974), suggesting that this tissue may be the source of serum IgA. However, the source of polymeric IgA in the serum remains controversial. The aim of the present study was to characterize the molecular size distribution of the IgA antibodies appearing in serum and saliva following parenteral immunization with tetanus toxoid (TT). We have analysed the intensity and the kinetics of the serum-specific monomeric and polymeric IgA responses.

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MATERIALS AND METHODS

Subjects

Sera were obtained from 13 volunteers (median of age: 30 years, range: 22–59 years) who were injected intramuscularly with one dose of the tetanus vaccine (tetanus toxoid absorbed on aluminium hydroxyde: Smith Kline Rit, Rixensart, Belgium). Three of them had never been vaccinated for tetanus before, whereas the other 10 had been immunized 5–20 years previously. The first three individuals received a second injection 2 months after the first. Blood samples were obtained just before vaccination and thereafter on Days 8, 11, 17, 24 and 50. Whole unstimulated saliva was collected on the same days for eight individuals (three primary and five secondary immunizations).

Serum anti-tetanus toxoid assay

The serum titre of anti-tetanus toxoid (TT) antibodies was measured by solid-phase radioimmunoassay. Polystyrene cups were passively coated with tetanus toxoid (Pasteur Institute of

Brabant, Brussels), 10 μg/ml in phosphate-buffered saline, pH 7·2 (PBS). The remaining protein-binding sites were blocked with PBS supplemented, respectively, with 10% (v/v) heat-inactivated (56°) horse serum or 2% (v/v) human serum albumin. After washing with PBS, diluted sera were added and incubated overnight at room temperature. Sera were diluted 1:50–1:100 for IgA and 1:100–1:200 for IgG in PBS supplemented, respectively, with 10% (v/v) horse serum or 2% (v/v) human serum albumin. After washing the cups, ^{125}I-labelled IgG1 mouse monoclonal anti-human alpha-chains or ^{125}I-labelled affinity-purified goat anti-human gamma-chains were added. After incubation for 16 hr at 20° and a final washing, the radioactivity bound to the solid phase was counted. All samples were tested in parallel on blocked wells without TT and these non-specific counts were subtracted. Results were expressed in arbitrary units (a.u.) referring to standard curves obtained from the results of serial two-fold dilutions of a strongly positive serum.

Monomeric and polymeric IgA antibodies

The molecular size distribution of serum IgA antibodies was analysed by sucrose density gradient ultracentrifugation (SDGU) as previously described (Delacroix *et al.*, 1983a). Only the sera containing more than 40 a.u. of anti-TT IgA could be accurately analysed by this technique. Serum samples (60 μl diluted 1:5 in PBS) were applied on top of isokinetic 5–21% (w/w) sucrose gradients and run for 16 hr at 160,000 ***g***. Sucrose gradients were run in PBS; three of them were run in parallel in 0·1 M glycine–HCl buffered saline, pH 3·0, to permit dissociation of immune complexes. After centrifugation, 30 fractions of 0·4 ml were collected by downward elution of the gradients. The fractions were diluted 1/5 in PBS containing 10% horse serum, and anti-TT IgA was measured in each fraction using the assay described above. Proportions of p-IgA and m-IgA antibodies were measured by planimetry. In order to evaluate the possible over-estimation of p-IgA antibodies in our test, perhaps due to a more avid binding of p-IgA compared to m-IgA to the antigen-coated cups, a mild reduction-alkylation (4 mM DTT–8 mM iodoacetamide) was performed on all the fractions from three gradients. We have previously shown that, under these conditions, p-IgA was completely dissociated into m-IgA (unpublished data). Anti-TT IgA was measured in parallel in the fractions with and without reduction-alkylation. Finally, in three cases, the binding of antibodies to antigen-coated cups was measured in all gradient fractions with ^{125}I-labelled polyclonal anti-secretory component antibodies (Delacroix & Vaerman, 1982).

Salivary anti-tetanus toxoid assay

In order to measure the titre of salivary IgA anti-TT, we used the same assay as was used to measure the serum titres except that samples were diluted 1:5 and 1:10. In order to evaluate local synthesis versus passive diffusion of antibodies, we have calculated an albumin relative coefficient of excretion (RCE) for anti-TT IgA:

$$\mathrm{RCE} = \frac{\text{IgA anti-TT saliva (a.u.)/albumin saliva (}\mu\text{g/ml)}}{\text{IgA anti-TT serum (a.u.)/albumin serum (}\mu\text{g/ml)}}.$$

The RCE expresses a protein excretion rate relative to that of albumin which is derived entirely from plasma by passive diffusion. It corrects for differences in plasma protein concentration and in degree of dilution of the secretion (Dive & Heremans, 1974; Delacroix *et al.*, 1980).

RESULTS

Serum IgA and IgG antibody responses to tetanus vaccine

The three individuals who had never been vaccinated with TT developed no IgA and only a low IgG antibody response 25–50 days after the first injection. After the second injection only very low titres of IgA antibodies were detected compared to a marked serum IgG antibody response.

In contrast, all the individuals boosted with TT 5–20 years after a last injection showed marked serum IgG and IgA antibody responses. IgA activity peaked at 11 days after injection, whereafter antibody titres rapidly fell. In contrast, the highest IgG titres were recorded on Days 17 and 24, and they remained elevated on Day 50 (Fig. 1). Before re-immunization, low levels of anti-TT IgG were detected in six out of 10 individuals, and low levels of anti-TT IgA in five out of 10.

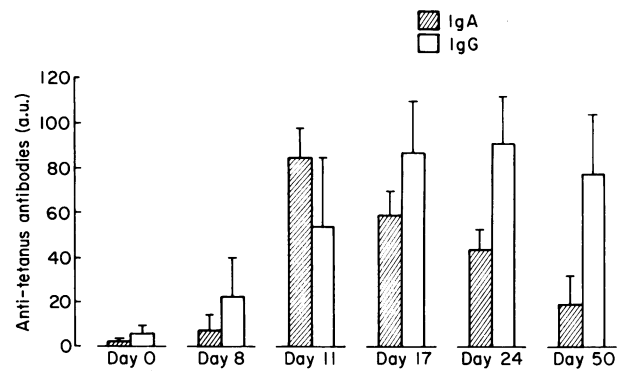

Figure 1. Kinetics of serum IgA and IgG anti-TT response in 10 individuals boosted with one dose of tetanus vaccine injected intramuscularly. Columns give geometric mean + SEM.

Serum monomeric and polymeric IgA antibody response to tetanus vaccine

Both monomeric and polymeric IgA contributed to the IgA responses to TT. When the antibody titre was high enough to allow IgA size characterization (>40 a.u.), m- and p-IgA antibodies appeared simultaneously (four out of 10 individuals tested at Day 8). At the peak of the IgA response (Day 11), p-IgA represented approximately half the IgA antibody activity for the 10 secondary anti-TT responses (median: 54%; range 25–79%) (Fig. 2). Most of these serum anti-TT p-IgA sedimented in the 10·2 S position, corresponding to dimeric IgA (Fig. 3). No change in the proportion of p-IgA antibodies was observed when SGDU were performed at pH 3·0 (Table 1), or when the anti-TT IgA activity was measured on the fractions after reduction-alkylation (Table 2). These results ruled out both the possibility of interference by immune complexes and the possible over-estimation of p-IgA compared to m-IgA due to differing avidities of these antibodies. These dimeric IgA antibodies were not complexed with secretory component as demonstrated by the absence of reaction when a labelled anti-

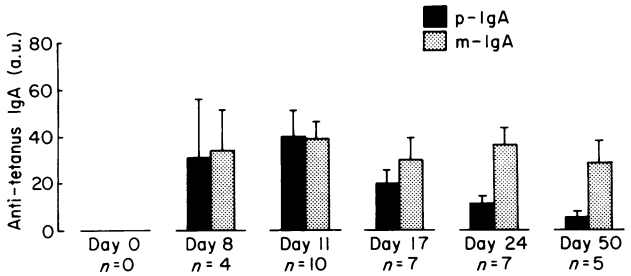

Figure 2. Kinetics of polymeric IgA (p-IgA) and monomeric IgA (m-IgA) anti-TT response in sera from boosted individuals containing more than 40 a.u. of anti-TT IgA. Columns give geometric mean + SEM.

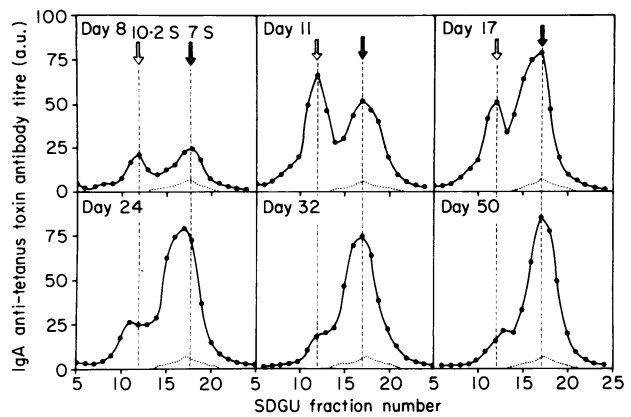

Figure 3. Molecular size profile of anti-tetanus toxoid IgA in sequential blood samples from an individual injected intramuscularly with one dose of tetanus vaccine 13 years after a last boost (dotted lines represent preimmunization sample). Open arrows: sedimentation position of 10·2 S dimeric IgA. Black arrows: sedimentation position of 7 S monomeric IgA.

Table 1. Influence of pH on serum anti-TT IgA fractionation by SDGU

Patient no.	Anti-TT p-IgA (% of total anti-TT IgA)	
	Neutral pH	Acid pH
1	59·2	58·3
2	27·6	27·8
3	26·2	33·0

secretory component antibody was used in the assay (data not shown). There were marked differences in persistence of the serum m- and p-IgA responses to tetanus vaccine (Figs 2 and 3): p-IgA responses were short-lived, whereas m-IgA responses persisted over a much longer period of time and accounted for over 90% of IgA antibodies at 50 days after injection of the vaccine.

Table 2. Influence of the molecular size of IgA on the anti-TT IgA titre detected by RIA

Patient no.	Anti-TT m-IgA (% of total anti-TT IgA)	
	Control	Reduction-alkylation*
1	50·4	48·1
2	22·0	22·2
3	30·2	27·1

* The anti-TT IgA titre was measured after reduction-alkylation of all the SDGU fractions.

Salivary anti-TT IgA response

No anti-TT IgA could be detected in saliva after primary vaccination with TT. After TT boost, anti-TT IgA response was clearly detected in only one out of five saliva samples corresponding to the serum with the highest IgA antibody titre. In this case, anti-TT IgA peaked earlier in saliva (Day 8) than in serum (Day 11) (Table 3). The albumin relative coefficient of excretion

Table 3. Salivary anti-TT IgA response in one boosted individual

Days	Anti-TT IgA (a.u.*)		RCE† for serum-derived anti-TT IgA	Anti-TT p-IgA/mg p-IgA	
	Saliva	Serum		Saliva	Serum
0	25·0	0	0	250	0
8	135·0	1,050	257·00	1,277	3,148
11	62·5	8,250	18·93	735	27,083
17	30·0	3,400	26·73	385	7,963
24	35·0	2,500	35·90	522	4,800

* Antibody titres are expressed in arbitrary units after correction of the different dilution factors used for serum and saliva.

† RCE: albumin relative coefficient of excretion:

$$RCE = \frac{\text{IgA anti-TT saliva (a.u.)}}{\text{IgA anti-TT serum (a.u.)}} \Big/ \frac{\text{alb. saliva } (\mu g/ml)}{\text{alb. serum } (\mu g/ml)}.$$

(RCE) calculated for anti-TT IgA in saliva was highest at Day 8 but remained elevated on Days 11–17 and on Day 24 (Table 3). This RCE was calculated after correction of the different dilution factors for testing anti-TT activity in serum and saliva. In order to evaluate the possible mucosal origin of serum anti-TT IgA of the polymeric form, we have calculated the anti-TT p-IgA antibody per mg of total p-IgA both in the mucosal and in the systemic compartments: it was always higher in serum than in saliva (Table 3).

DISCUSSION

The present study indicates that a polymeric IgA antibody response in serum can be produced by parenteral immunization in primed individuals, and it raises the question of the mech-

anisms that control polymeric versus monomeric IgA production. The difference in response between the three recently primed individuals and the other 10 suggests that the presence of memory T cells would be necessary to induce an IgA antibody response after parenteral immunization as no anti-TT IgA appeared during primary immune responses, compared to low IgG antibody responses. Nevertheless, undetectable levels of anti-TT IgA appearing together with IgG antibodies cannot be excluded.

Several studies have documented that gut lamina propria IgA B cells produce 60–70% p-IgA in contrast to the 90% m-IgA produced by bone marrow IgA B cells (Radl *et al.*, 1974; Kutteh *et al.*, 1982). These observations prompted some authors to investigate the pathophysiology of IgA nephropathy and dermatitis herpetiformis by analysing the molecular size of IgA involved in kidney or skin tissue deposits (Bene, Faure & Duheille 1982; Unsworth *et al.*, 1982). The presence of p-IgA was interpreted by some investigators to suggest a mucosal origin of these diseases. The present report shows that this interpretation may be misleading as a marked serum p-IgA antibody response may be induced by parenteral antigenic stimulation. The serum IgA antibody response to TT analysed in this study represents a model of secondary antibody response to a particulate antigen: anti-TT IgA appeared in serum only after a booster injection with TT absorbed on aluminium hydroxide. Intramuscularly injected antigens enter the regional lymph node through the afferent lymphatics. In the node, particulate antigens are phagocytosed by sinus-lining macrophages, which further present antigen to lymphocytes. This is followed by the formation of active germinal centres and the appearance of plasma cells in the medulla. Absorbing tetanus toxoid to aluminium hydroxide leads to an enhancement of antigen uptake by the antigen-presenting cells (Mannhalter *et al.*, 1985). Our results indicate that, at the peak of the IgA response, p-IgA accounted for nearly half of the anti-TT activity. This high proportion of p-IgA antibodies could not be accounted for by the presence of circulating IgA immune complexes nor by an over-estimation of p-IgA antibodies secondary to a possibly higher avidity of p-IgA compared to m-IgA. It is likely that this antigen-specific IgA was produced in regional lymph nodes draining the site of immunization. Kutteh *et al.* (1982) have demonstrated that up to 40% of the IgA secreted by cultured lymph node cells is in the polymeric form.

Our data showed that a secondary immunization with TT led at least in one case to an antigen-specific IgA response in saliva. This is in agreement with the results of Smith *et al.* (1986) reporting the presence of salivary anti-TT antibodies in a moderate percentage of children after immunization with TT. Although Brandzaeg, Fjellander & Gjeruldsen (1970) have shown that in whole saliva more than 10% of the IgA can be derived directly from serum, the serum origin of salivary anti-TT IgA appears unlikely. In the only saliva we could analyse the albumin relative coefficients of excretion (RCE) for anti-TT IgA calculated for the four sequential samples were all greater than values due both to passive diffusion (RCE = 1), or to selective transport from serum into saliva (mean RCE 4·23; range 2·15–10·45; Delacroix *et al.*, 1980). These RCE results, and the difference in the time of peak of response in serum versus saliva noted in this single individual, suggest a local synthesis of anti-TT IgA in salivary glands. As serum anti-TT IgA was probably produced in the lymph nodes, the synthesis of anti-TT IgA in the salivary glands might be secondary to the homing to the mucosal surfaces of some blast cells that left the node before their differentiation into plasma cells. Conversely, the presence of anti-TT IgA in the mucosa cannot account for anti-TT p-IgA in serum as mucosal IgA responses were not the rule, and the anti-TT p-IgA antibody titre per mg of total p-IgA was always higher in serum than in saliva in the positive case.

The differences in persistence between serum monomeric and polymeric IgA responses suggest the existence of different regulatory mechanisms for monomeric and polymeric IgA synthesis in serum. They cannot be explained solely by the more rapid plasma clearance of p-IgA compared to m-IgA since the differences in plasma half-life of monomeric and polymeric IgA in man is only about 2 days (mean $t_{1/2} = 4{\cdot}7$ days for m-IgA and 3·0 days for p-IgA; Delacroix *et al.*, 1983a). Compared to the important contribution of p-IgA to the anti-TT IgA response 11 days after the vaccine, anti-TT IgA were nearly entirely associated to the monomeric form of IgA 50 days after antigenic stimulation. It suggests that the presence and/or the intensity of the stimulating signal would play a more important role in p-IgA production than the route of antigenic exposure. This hypothesis could explain the higher proportion of polymeric IgA in the sera of babies who are exposed to many new antigens resulting in intense antigenic stimulation of their immune system (Delacroix, Leroux & Vaerman, 1983b).

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