

Immunoglobulin E and G antibodies two years after a booster dose of an aluminium-adsorbed or a fluid DT vaccine in relation to atopy

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Immunoglobulin E and G levels to diphtheria and tetanus toxoids were investigated two years after a DT booster with either an adsorbed or a non-adsorbed, fluid vaccine, given at 10 years of age. Although IgE levels had declined, detectable IgE to diphtheria and tetanus toxoids were still found in 82% and 67% of samples, respectively, to be compared to prebooster levels of 3–14% and postbooster levels of 92–94%. The IgG levels had also declined but remained at equal and high levels in both the adsorbed and the fluid vaccine groups. The prevalence of allergic symptoms was similar in the two vaccination groups. Thus, the study showed an unexpected, long duration of the IgE responses elicited by a booster dose of DT vaccine. The booster dose also induced a durable, high IgG antibody response to both the adsorbed and the fluid vaccine.

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The aim of general vaccination programmes is to induce long lasting immunity with as few adverse effects as possible. It is well-known that diphtheria-tetanus (DT) toxoid vaccines and diphtheria-tetanus-pertussis (DTP) vaccines often cause local reactions. Studies have suggested a reactogenic role to high amounts of antigen in the vaccines, to impurities and/or to the aluminium adjuvant (1–3). Relatively high levels of antigen are needed both in primary and booster vaccinations in order to induce and maintain adequate levels of immunity to toxin-mediated diseases. Therefore, decreasing side effects to vaccination by studying the role of other possible reactogens seems to be a logical first step.

We have previously found that the aluminium-adjuvanted DT vaccine, which has been used since 1979 in Sweden for primary immunisation in infancy, is the likely cause for the increase of local reactions after a DT booster given 10 years later in comparison to children who had received a nonadsorbed DTP for priming in the previous programme (4). In an attempt to decrease the local side effects of the DT booster given at 10 years of age, we then compared the adverse reactions and immune responses of 10 year-olds, who were randomly given either

aluminium-adsorbed or nonadsorbed (fluid) DT vaccines (5). Immediate IgG immune responses were high to both vaccines, as well as the rate and the levels of IgE antibody responses to the toxoids (6). No significant differences were noted in the rates of local side effects. Thus, aluminium appeared not to be a necessary adjuvant to the booster vaccination, nor did a non-adsorbed vaccine decrease the number of adverse reactions.

Since the long-term IgG responses to non-adsorbed DT vaccines are largely unknown and since the duration of IgE responses to vaccination have not previously been studied, the aim of this study was to follow up the immunoglobulin responses to the two vaccine alternatives. Furthermore, the IgE-antibody responses to the vaccines raised the question of a possible connection to allergy and atopy. Therefore, a questionnaire regarding the presence of allergic symptoms was included.

Materials and methods

Subjects

The 104 school-children, 12 years of age, who two years previously participated in a randomised dou-

ble blind vaccine trial comparing aluminium-adsorbed and fluid DT vaccines, were invited to participate in the present study. The families were given written information regarding the purpose of the study and gave a written informed consent. The children were asked for a new (third) blood sample and the parents were requested to complete a questionnaire on allergic symptoms in their children.

Of the original DT booster study population, 76/104 (73%) accepted a third blood sample two years later within the frames of the present study. A total of 82 (79%) children/parents answered a structured questionnaire concerning atopy and allergic symptoms, of which 73 were amongst the children who had also accepted blood sampling. Of the 76 children with a third blood sample, 36 had been vaccinated with a regular, adsorbed DT vaccine and 40 children with a non-adsorbed (fluid) DT vaccine.

Questionnaire

The structured questionnaire, employed by ISAAC (7), contained 20 questions on current or previous occurrence of airway, skin or nose-eye problems of possible allergic origin. Each focal manifestation was answered successively; if the pupil had experienced "any breathing problems", "itching eruptions" or "sneezing or running nose without having a cold" ever in life and, if so, had the symptoms appeared during the previous 12-month period. The parents were also asked specifically if their child ever have had "asthma", "eczema" or "hay fever".

Serological assays

The modified Phadebas RASTTM (Pharmacia, Uppsala, Sweden) was used to measure IgE antibodies to diphtheria and tetanus toxoids, employing a similar procedure as that described in detail previously using pertussis toxin as antigen (8). Briefly, each toxoid was coupled to CNBr-activated discs by the Special Allergen Service, Pharmacia, Uppsala. Sera were diluted 1:2 and tested in duplicate, extending the incubation time to 24 h under continuous shaking. Values of 0.1 Phadebas RAST Units (PRU) per ml and above were regarded as positive. The IgG antibody responses to diphtheria toxoid were analysed by the microneutralisation test in Vero cells and to tetanus toxoid by toxin-binding inhibition test (9, 10).

Statistical analysis

Frequency of allergic and atopic symptoms in the two vaccine groups was studied by the Chi-square test using the contingency table analysis. Compari-

son between immune responses was made by Mann-Whitney U rank sum test. Correlation between antibody concentrations was investigated by simple regression analysis.

Ethical considerations

The study was approved by the Ethics Committee at the Medical Faculty of the University of Göteborg.

Results

Two years after the booster injection, immunoglobulin E antibodies of >0.1 PRU/ml were still detectable in the samples of 62/76 (82%) children to diphtheria toxoid and in 51/76 (67%) to tetanus toxoid. These rates are to be compared to prebooster levels of 3–14% and postbooster levels of 92–94% (6). The number of IgE seropositive individuals was similar in the adsorbed and the fluid vaccine groups. The decline in the magnitude of the IgE responses to the two toxoids from the high immediate postbooster levels to the 2-year follow up samples was also similar in the two groups (Figure 1, upper part). For the whole group, the median IgE titer immediately after vaccination was 1.7 PRU/ml to diphtheria toxoid, declining to 0.32 PRU/ml, and 0.40 PRU/ml to tetanus, declining to 0.17 PRU/ml two years later. None of the differences between the aluminium-adsorbed and the non-adsorbed vaccine groups were statistically significant.

Neutralising antibodies to diphtheria and tetanus toxoids were still at high levels 2 years after vaccination, with a lower range of titers of 0.4 IU/ml to diphtheria and of 0.25 IU/ml to tetanus toxoids, respectively. For the whole group, the median titer to diphtheria toxoid had declined from 26 IU/ml to 6.4 IU/ml and to tetanus toxoid from 32 IU/ml to 4 IU/ml. The tendencies towards higher titers in the non-adsorbed vaccine group to both diphtheria and tetanus toxoid ($p=0.15$ and 0.08 , respectively) had disappeared two years later (Fig. 1, lower part).

There was no significant correlation between IgG and IgE levels to either tetanus or diphtheria toxoids immediately after vaccination (p values of 0.65 and 0.35 , respectively), nor in the 2-year follow up samples for diphtheria toxoid ($p=0.63$). A correlation between IgG and IgE to tetanus toxoid was, however, noted in the 2-year samples ($r=0.32$, $p=0.006$).

Of the 79 children/parents who answered the structured questionnaire concerning allergic symptoms, 39 (49%) had at some time experienced airway, skin or nose/eye problems. The 12-month prevalence was 33% (26/79). When specifically asked whether they had ever had asthma, eczema or hay fever, 29 (37%) reported one or more of these

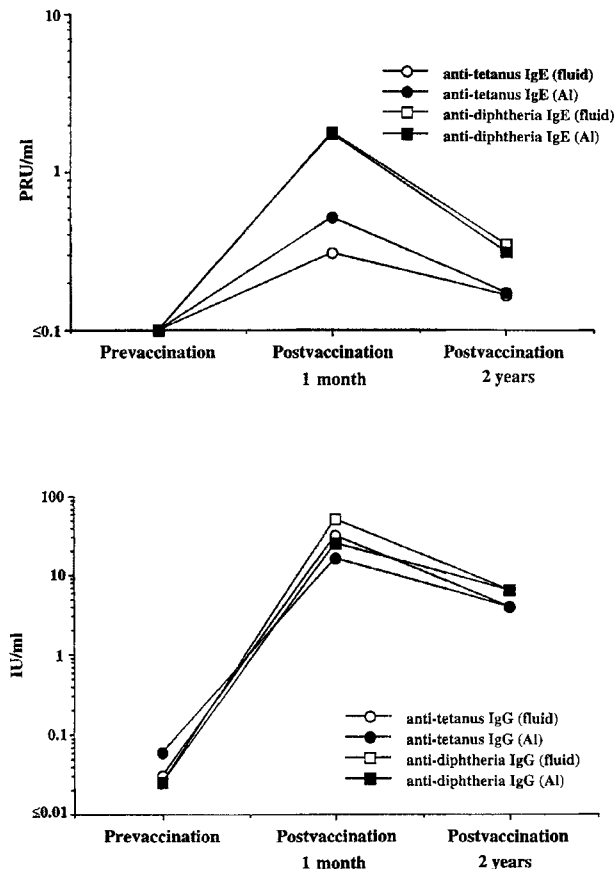


Fig. 1. Antibody responses to a booster dose of a regular, adsorbed DT vaccine (filled symbols) or an experimental, fluid DT vaccine (open symbols) given as fourth dose at 10 years of age. The samples were drawn immediately prior to, one month after and two years after vaccination. The upper part of the figure shows the kinetics of the IgE responses and the lower part shows the kinetics of the IgG responses to diphtheria toxoid and to tetanus toxoid.

diseases. Asthma was reported by four (5%), eczema by 20 (26%) and hay fever by ten (13%).

Symptoms suggestive of allergy were more common among the children in the aluminium-adsorbed 24/38 (63%) than in the non-adsorbed group 15/41 (37%) ($p=0.018$). The 12-month prevalence in the two groups was 54 and 46% respectively ($p=0.47$). Asthma, eczema or hay fever tended to be more common in the adsorbed vaccine group than in the non-adsorbed vaccine group (62% and 38% respectively, $p=0.058$).

The IgE antibody levels to both toxoids, but especially to tetanus toxoid, tended to be higher in the immediate postvaccination and in the two-year samples of children with allergic problems but not significantly so. Children reporting either asthma, eczema or hay fever also tended to have higher IgE

antibody levels to tetanus toxoid and to diphtheria toxoid in the two-year follow up samples.

Discussion

Two years after a booster dose of either an adsorbed or a non-adsorbed DT vaccine, high IgG antibody levels to both diphtheria and tetanus toxoids were maintained in both vaccine groups. The median neutralising antibody titers of 6.4 and 4.0 IU/ml to diphtheria and tetanus toxoids respectively were satisfactory, as was the fact that the titers were still well above protective levels in all children (11). Thus, the aluminium adjuvant was not necessary for a durable IgG-response, as the fluid vaccine induced an equally long-lived immune response as the adsorbed vaccine.

The long duration of the IgE responses to diphtheria and tetanus toxoids was a somewhat unexpected finding. Over 90% of the immediate DT-postbooster samples had been positive both to diphtheria and tetanus toxoids (6). In the 2-year follow-up samples, there were still measurable IgE-titers (>0.1 PRU/ml) to the diphtheria-tetanus-antigens in 82% and 67%, respectively.

High rates of possible allergic and/or atopic symptoms were reported by parents as assessed by the ISAAC questionnaire. Some airway, skin or nose/eye problems – not associated with common colds – were recalled by 49%, while 37% stated such problems in answer to specified questions on asthma, eczema or hay fever. The 12-month prevalence of airway, skin and nose/eye problems was 33%. These rates are similar to current Scandinavian prevalence figures in children (12, 13). Although the children receiving the adsorbed vaccine had a higher cumulative incidence of symptoms as compared to the group given fluid vaccine, i.e. 63% vs. 37%, the 12-month prevalence after the vaccination was similar in the two vaccine groups. This indicates that more allergic individuals had been randomly recruited to the former group, rather than an increased risk for allergy after vaccination with the aluminium adsorbed vaccine. This conclusion is supported by the results of a very recent 3-year follow-up of over 700 infants immunised with adsorbed and non-adsorbed pertussis vaccines, showing similar incidences of allergy and sensitisation in all the vaccination groups (14).

The significance of IgE responses during infections and after vaccination remains unclear and needs to be further studied. Immunoglobulin E responses to several microbial antigens have been reported and induction of sensitisation to environmental allergens cannot be excluded (15–21). A long-lasting IgE response to immunising agents would not seem to be wanted and possible steps to de-

crease such responses should be considered. One such measure would be to review whether an aluminium adjuvant is truly necessary for every antigen and in every part of an immunisation programme, since the IgE-inducing effect of aluminium is well known (3). Some antigens, which are adjuvanted by routine, would probably be equally immunogenic without aluminium, as shown in the present study for diphtheria and tetanus toxoids.

Another approach to decrease the IgE reactivity could be to use more highly purified antigens in vaccines. The amount of impurities vary between toxoid vaccines from different manufacturers, with the purest toxoids obtained by purification of the toxins prior to toxoiding. Highly purified toxoids give less local side effects and, since IgE responses co-varied with local reactions in some studies, purification would probably also decrease IgE responses (22–25). The need for repeated boosters in order to maintain adequate immunity to toxin-mediated diseases and the increasing number of antigens administered in combined vaccines give this issue a wider perspective.

In conclusion, the present study shows the persistence of high IgG levels to diphtheria and tetanus toxoids two years after a booster dose with both a regular, adsorbed DT vaccine and an experimental, non-adsorbed (fluid) DT vaccine. Less encouraging was the finding that the IgE levels to the toxoids also remained at detectable levels in a majority of the children.

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