

Aluminium Phosphate but Not Calcium Phosphate Stimulates the Specific IgE Response in Guinea Pigs to Tetanus Toxoid

TCHAVDAR L. VASSILEV

Institute for Infectious and Parasitic Diseases,
Medical Academy, Sofia, Bulgaria

The ability of aluminium phosphate and calcium phosphate to stimulate the synthesis of tetanus toxoid-specific anaphylactic and antitoxic antibodies is compared. The observed differences in the kinetics of IgG_{1a}, IgG_{1b}, and antitoxines are quantitative—titres after application of aluminium phosphate are higher. Booster immunization with aluminium but not calcium phosphate-adsorbed toxoid leads to a prolonged synthesis of specific IgE. It is hypothesized that the regular application of aluminium compound-containing vaccines on the entire population could be one of the factors leading to the observed increase of allergic diseases.

Key words: guinea pig IgE; mineral adjuvants; tetanus toxoid.

Accepted for publication 25 March 1978

The adjuvant action of aluminium compounds was first described by Glenny, and nowadays they are the most widely used stimulators of the immune response in immunoprophylaxis. At the same time not all investigators agree on their absolute safety for human use (2).

In recent years many authors have found that aluminium adjuvants were potent stimulators of IgE synthesis in laboratory animals (for review 17). Aalberse et al. (1) and Nagel et al. (8) discovered that booster injection of humans with tetanus toxoid adsorbed on aluminium gels led to the appearance or increase of specific IgE antibodies in their sera. At the same time aluminium adsorbed antigens are widely used in immunotherapy of allergic persons.

French authors (14,16) suggested replacing aluminium compounds by calcium phosphate, especially in vaccines for immunization of allergic persons. They pointed

out that as the latter was a normal constituent of the body it ought to cause weaker local reactions at the site of injection. The application of calcium phosphate with several types of vaccines did not lead to an increase of the total serum level of IgE in healthy and allergic persons (15).

Traditionally, the anaphylactogenic properties of vaccines are tested on guinea pigs. Three types of anaphylactic antibodies mediate immediate hypersensitivity reactions—IgG_{1a}, IgG_{1b}, and IgE (11, 13, 19). The mechanisms which control IgE synthesis in this species have been less well studied than those in inbred strains of mice and rats. We found that after the booster immunization of guinea pigs with aluminium hydroxide-adsorbed tetanus toxoid a prolonged synthesis of specific IgE antibodies was observed (18). In the present communication we compare the kinetics of anaphylactic antibody production in guinea

pigs immunized with tetanus toxoid adsorbed on aluminium and calcium phosphate.

MATERIAL AND METHODS

Forty male, outbred guinea pigs weighing 250–300 g at the time of the first immunization were used. They were fed with oats and carrots and kept under standard conditions. The experiment was performed during the summer period (May–October).

The guinea pigs were immunized twice, with 1 month's interval, with 7 Lf units tetanus toxoid lot No. 353 1160 Lf/mg PN (Institute for Infectious and Parasitic Diseases, Sofia, Bulgaria). The antigen was adsorbed on 2 mg aluminium phosphate or 10 mg calcium phosphate. In a preliminary experiment we found that 5 mg calcium phosphate did not stimulate a satisfactory antibody response. Adsorption was performed as described by Raynaud et al. (16). Briefly, 1 vol. 0.14 M solution of Na_2HPO_4 containing 28 Lf units tetanus toxoid per millilitre was passed through a Seitz-filter and mixed with 1.1 vol. sterile 0.14 M solution of CaCl_2 (in order to eliminate all free phosphate ions from the final product). The adsorption of toxoid was always complete.

The animals were bled from the retroorbital plexus under ether anaesthesia and blood from each group was pooled. Sera were immediately used for determination of IgE, after which they were frozen and kept at -20°C until used.

Haemagglutinating antibodies were detected by the microtitre technique using sheep red blood cells coated with the antigen (4). 500 Lf units tetanus toxoid was mixed with 0.6 ml tanned sheep erythrocytes, and phosphate-buffered saline (PBS) pH 7.2 was added to a final volume of 5 ml. After incubation for 30 min at 37°C the erythrocytes were washed three times with PBS containing 1% normal heat-inactivated rabbit serum and used as a 1% suspension in the same diluent. Haemagglutination titres were converted to

antitoxic units/ml (AU/ml) by comparison with the results of a standard tetanus antitoxin 6 AU/ml (Institute "Tarasevitch", Moscow, U.S.S.R.).

Passive cutaneous anaphylaxis (PCA) was performed on female albino guinea pigs weighing 300 g (10, 11, 19). Sensitized animals were challenged with 100 Lf units tetanus toxoid in a 0.5% solution of Evans blue. The maximal dilution of the tested serum which gave a blue spot of 5 or more millimeters in diameter was considered a titre. For determination of the titres of all three types of anaphylactic antibodies three recipient animals were used and geometric mean value was determined. For determination of IgG_{1a} a sensitization period of 4 h was used, for IgG_{1b} - 2 days, and for IgE - 7 days (11, 19).

RESULTS AND DISCUSSION

Fig. 1 shows curves characterizing the kinetics of anaphylactic and haemagglutinating antibodies of guinea pigs immunized twice with tetanus toxoid adsorbed on two different mineral adjuvants. With respect to IgG_{1a} and IgG_{1b} the observed differences are quantitative - titres after application of aluminium phosphate are higher. The same is true for the antitoxic titres. The most interesting finding is the lack of specific IgE antibodies in the sera of animals immunized with tetanus toxoid with calcium phosphate as adjuvant. Higher doses of calcium phosphate were not used nor were the animals hyperimmunized - may be this would also have led to stimulation of IgE response (13). In the present experiment we used only doses of adjuvants and immunization schedules which were as close as possible to those used for humans.

One must be extremely cautious when transferring animal results to humans but we believe that it is justified to study anaphylactogenic properties of vaccines on guinea pigs if: (1) a suitable strain of animals is available which is able to develop a specific IgE response to the investigated antigen and (2) IgE antibodies are looked for

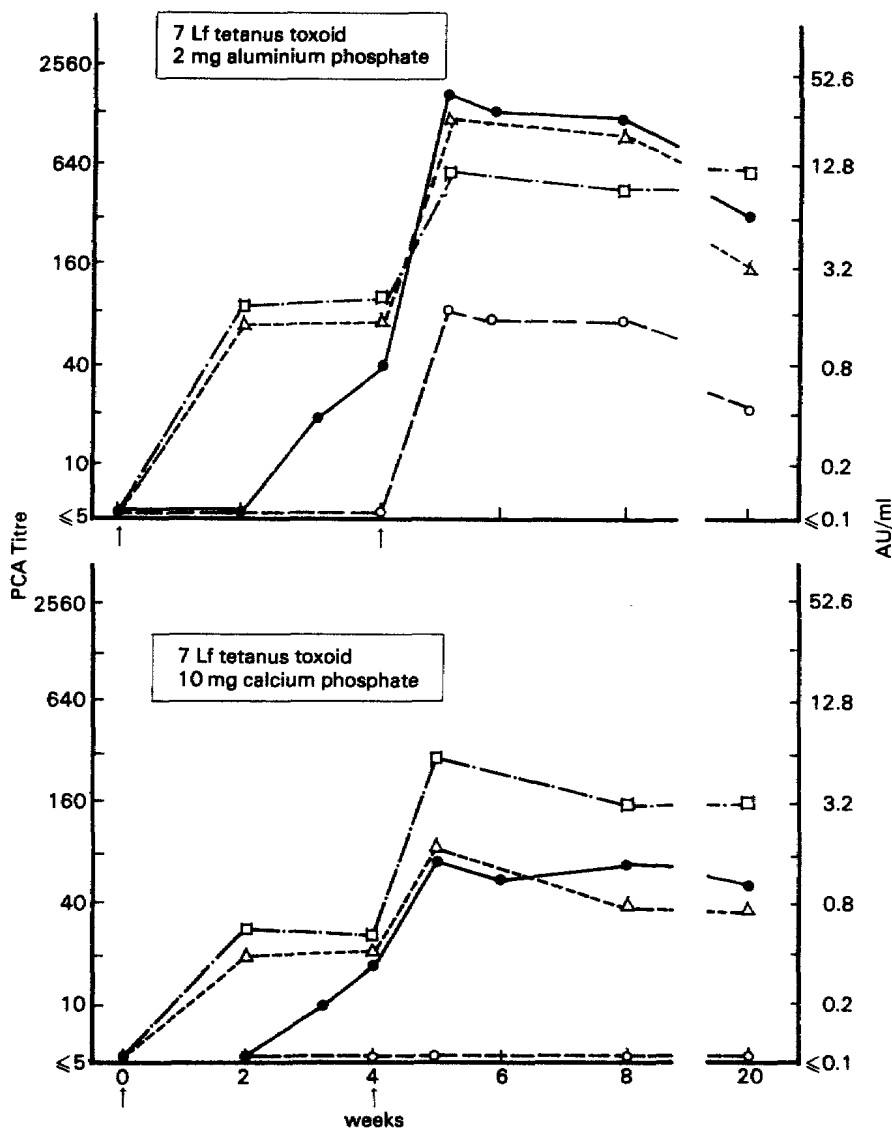


Fig. 1. Immune responses of guinea pigs to tetanus toxoid adsorbed on aluminium phosphate and calcium phosphate. Arrows point to day of first, and booster immunization. O—O IgE; Δ—Δ IgG_{1a}; □—□ IgG_{1b}; ●—● antitoxins.

purposefully. If the kinetics of tetanus toxoid-specific IgE in humans (7) and in guinea pigs are compared it will be seen that they are much alike – the titres remain stable

for a long time and a rapid reduction is not observed.

Guinea pig IgE differs from the other types of anaphylactic antibodies by its long

persistence in the skin of a recipient animal (up to 3 weeks and more) and its thermolability. In a series of preliminary investigations it was found that a serum which had a PCA titre of 1:160 retained a PCA titre of 1:20 after heating at 56° for 4 h (sensitization period—7 days). The PCA titre of an unheated serum determined by using a sensitization period of 3 weeks was usually two or three dilutions lower than when determined with a sensitization period of 1 week. Most probably this was because a small quantity of IgG_{1b} persisted not only up to the 7th day (19) but a few days beyond (12,13). This problem will be finally solved when an anti-guinea pig IgE antiserum is made available. It is too early to exclude the possibility that a heat-stable IgE subclass exists in this species (12). In our present communication we assume that IgE is one of those anaphylactic antibodies which persists in the skin of the recipient for 7 days (19) at the same time keeping in mind that in this case the PCA reaction is also due to a small quantity of IgG_{1b}.

There may seem to be a contradiction between the high frequency with which specific IgE after immunization with aluminium adjuvant-adsorbed tetanus toxoid, is synthesized in laboratory animals and humans, and the rarity of local and general anaphylactic reactions after its use. It is evident that nonanaphylactic antibodies decrease the possibility of injected antigen meeting the cell-fixed IgE and IgG₁ (1). Secondly, one must have in mind that applying the antigen in an insoluble adsorbed form does not favour the manifestation of an immediate hypersensitivity reaction. In all cases the presence of specific IgE antibodies in human serum must be regarded as a potential danger which can manifest itself after booster immunization, especially of hyperimmunized and allergic persons.

Practicing allergologists agree that recently in most countries there has been an increase in the number of allergic diseases. Although atopy is regarded as a disease state whose

pathogenesis is not only connected with an abnormal IgE synthesis (3), it is evident that in most cases this class of antibodies plays an important role. Many authors (5, 6, 9) associate the increasing frequency of allergic diseases with the intensive mass immunization programmes without regard for the possible mechanisms.

If it is assumed that aluminium compounds stimulate the synthesis of IgE in man as they do in laboratory animals and that their action is mediated by soluble circulating factors (7) it is justified to suppose that these adjuvants could stimulate an IgE response to other immunogenes which come into contact with the immune system of the body at the same time. This might result in the development of an IgE response to different environmental antigens and in some cases lead to clinically manifested reactions of hypersensitivity. According to us the periodical use of aluminium compound-containing vaccines on the entire population could be one of the factors leading to the observed increase of frequency of allergic diseases.

Only careful comparative studies on humans can give a definitive answer to the possible advantages of using calcium phosphate instead of aluminium compounds in the most widely used vaccines—tetanus and diphtheria toxoids and pertussis vaccine.

ACKNOWLEDGMENTS

The author is grateful to Dr. V. Dentchev and Dr. Z. Ovary for helpful discussion, and Dr. K. Solomonova for the gift of tetanus toxoid.

REFERENCES

1. Aalberse, R. C., Hoorweg, E. M. & Reering-Brongers, E. E.: IgE antibodies as a regular component of the anti-tetanus immune response (Abstract). 1975 European Immunology Meeting, Amsterdam, 1975.
2. Aprile, M. A. & Wardlaw, A. C.: Aluminium compounds as adjuvants for toxoids and vaccines: a review. *Can. J. Public Health* 57, 343–354, 1966.
3. Berrens, L.: The atopen-a rehabilitation. *Ann. Allergy* 36, 351–361, 1976.

4. Herbert, W. J.: Passive hemagglutination with special reference to the tanned cell technique. In Weir, D. M. (ed): Handbook of experimental immunology, vol. 1. Blackwell, Oxford, 1973.
5. Hubscher, T. T.: Immune and biochemical mechanisms in the allergic disease of the upper respiratory tract; role of antibodies, target cells, mediators and eosinophils. *Ann. Allergy* 38, 83-93, 1977.
6. Khantchourin, A. H.: Editorial. In Khantchourin, A. H. (ed): Postvaccinal allergic reactions and allergens. Ministry of Health, Moscow, U.S.S.R. (in Russian), 1974.
7. Munder, P. G., Farber, E., Modolell, M. & Fischer, H.: The influence of various adjuvants on the metabolism of phospholipids in macrophages. *Int. Arch. Allergy* 36, 117-128, 1969.
8. Nagel, J., Svec, D., Waters, T. & Fireman, P.: IgE synthesis in man. I. Development of IgE antibodies after immunization with tetanus-diphtheria toxoids. *J. Immunol.* 118, 334-341, 1977.
9. Nossov, S. D.: Effect of immunizations on child health. *Pediatrics* No. 5, 15-20. (In Russian), 1969.
10. Ovary, Z.: Passive cutaneous anaphylaxis. In Ackroyd, J. F. (ed): Immunological methods, pp. 259-283. Blackwell, Oxford, 1964.
11. Ovary, Z., Kaplan, B. & Kojima, S.: Characteristics of guinea pig IgE. *Int. Arch. Allergy* 51, 416-428, 1976.
12. Ovary, Z.: Personal communication, 1977.
13. Parish, W. E.: Homologous serum passive cutaneous anaphylaxis in guinea pigs mediated by two γ_1 or γ_2 -type heat-stable globulins and a non- γ_1 heat-labile reagin. *J. Immunol.* 105, 1296-1298, 1970.
14. Ralyveld, E. H., Henocq, E. & Raynaud, M.: Etude sur la vaccination antidiphtérique des sujets allergiques avec une anatoxine pure adsorbée sur phosphate de calcium. *Bull. WHO* 30, 321-325, 1964.
15. Ralyveld, E. H., Lavergue, M. & de Rudder, J.: Taux d'immunoglobulines chez les sujets normaux et allergiques après vaccinations divers (Abstract). *Rev. Fr. d'Allergol.* 4, 219, 1974.
16. Raynaud, M., Ralyveld, E. H., Turpin, A. & Mangalo, R.: Preparation d'anatoxines diphtérique, tétanique et staphylococcique de haute pureté adsorbées sur phosphate de calcium (brushite). *Ann. Inst. Pasteur* 96, 60-71, 1959.
17. Tada, T.: Regulation of reaginic antibody formation in animals. *Progress in Allergy*, pp. 122-194, vol. 19, S. Karger, Basel, 1975.
18. Vassilev, T.: Experimental study of immediate type hypersensitivity to tetanus toxoid. *Epidemiol. Microbiol. Infekt. Bol. No. 2*, 127-131 (in Bulgarian), 1977.
19. Watanabe, H. & Ovary, Z.: Antigen and antibody detection by *in vivo* methods; A reevaluation of passive cutaneous anaphylactic reactions. *J. Immunol. Methods* 14, 381-390, 1977.

Address:

Dr. Tchevdar L. Vassilev
 Institute for Infectious and
 Parasitic Diseases
 boul. VI. Zaimov 26
 Sofia
 Bulgaria

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.