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Short Communication

**INDUCTION OF IgE ANTIBODY PRODUCTION IN MICE WITH
DIFFERENT DPT-VACCINE PREPARATIONS**

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SUMMARY: Antibody production in mice after immunization with diphtheria-pertussis-tetanus (DPT) vaccine was investigated. Six lots of the vaccine produced in the same year by six manufacturers in Japan were chosen. Production of IgE antibody specific to either diphtheria or tetanus toxoid varied among vaccine preparations used, although there was no apparent difference in IgG antibody production after immunization. In addition, the level of IgE antibody specific to diphtheria toxoid was correlated with that of IgG1 antibody and inversely with that of IgG2 antibody. These results suggest that each vaccine preparation may induce a distinct pattern of antibody production and, therefore, the type of immune response induced by vaccination may vary among vaccine preparations.

Adverse reactions such as local swelling and immediate-type reaction upon vaccination have been reported (1). Aluminum hydroxide (alum) has been widely used as an adjuvant for human vaccination and is well known to induce produc-

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tion of IgE antibody participating in allergic reaction (1). A number of attempts have been made to diminish IgE production while retaining its neutralizing activity, mainly associated with the IgG isotype (2-4). Alum is still in use, however, for various vaccines because it is effective as an adjuvant to induce protective antibodies, easy to handle, and low cost. Diphtheria-pertussis tetanus (DPT) vaccine, one of the alum-adsorbed vaccines, is known to induce adverse reactions possibly by the production of IgE antibodies (5); however, the safety of vaccine preparations concerning allergic reactions upon vaccination has not been assessed. In this study, antibody productions in mice, including that of antigen-specific IgE antibody, after immunization with several lots of DPT-vaccines were investigated. The possibility of the use of experimental animals to control adverse reactions which may occur after vaccination in humans will be discussed.

BALB/c mice (female, 8 weeks of age) and Sprague-Dawley rats (female, 8 weeks of age) were purchased from SLC, Shizuoka, Japan. DPT-vaccines, produced in 1994 by different manufacturer in Japan, were obtained as clinical vials. Each mice were immunized with DPT-vaccine by injecting intraperitoneally 0.5-ml of vaccine solutions. After the immunization, mice were bled from tail vein. Anti-diphtheria IgG antibody was titrated by ELISA with peroxidase-labeled rabbit antibodies against mouse IgG or Ig subclasses (ZYMED Lab., San Francisco, CA). Purified preparations of diphtheria and tetanus toxoid used for coating the ELISA plates were kindly provided by the Research Foundation for Microbial Diseases, Osaka University, Osaka. Anti-diphtheria and anti-tetanus IgE antibodies were titrated by the passive cutaneous anaphylaxis (PCA) test in rats as described previously (4).

In a preliminary experiment, a significant level of anti-diphtheria and anti-tetanus IgG production was observed as early as one week after ip injection of BALB/c mice with 0.5 ml of DPT-vaccine and the level reached plateau in 3 weeks. In addition, dilution of vaccine in saline up to 8x did not affect the induction of IgG antibody production (data not shown). Therefore, in this study, mice were immunized with 0.5 ml of twofold diluted DPT-vaccine and three weeks after immunization, anti-diphtheria and anti-tetanus antibodies were titrated by ELISA and PCA assay. As shown in Fig. 1a, there was no apparent difference in the induction of either anti-diphtheria or anti-tetanus IgG antibody production in groups of mice immunized with six different lots of DPT-vaccine. Anti-diphtheria and anti-tetanus IgG titers converged in ranges from 8.6 to 9.8 and from 7.0 to 8.2, respectively. On the other hand, induction of antigen-specific IgE antibody production varied among DPT-vaccines (Fig. 1b).

Fig. 1. Anti-diphtheria (□) and anti-tetanus (■) IgG (Fig. 1a) and IgE (Fig. 1b) antibody response in mice three weeks after ip injection with 0.5 ml of 2x diluted vaccine. The data represent means and SEs of five mice per group. '*' in Fig. 1b shows a significant difference as compared with lot-1 (in anti-diphtheria IgE response) and lot-6 (in anti-tetanus IgE response) vaccine-injected group (pointed by arrows).

Fig. 2. Anti-diphtheria IgG subclass antibody titers of the serum samples assayed as shown in Fig. 1. The sera from mice three weeks after immunization with lot-1 (□) or lot-3 (■) vaccines were assayed. The data represent means and SEs of five mice per group.

The lot-1 DPT vaccine induced a significantly higher level of anti-diphtheria IgE production as compared with those in lot-2, -3, and -4 vaccine-injected groups ($p < 0.01$). On the other hand, anti-tetanus IgE production was induced most in lot-6 vaccine-injected group and the level was significantly higher than those in lot-2 and lot-4 vaccine-injected groups. In the next experiment, anti-diphtheria IgG antibodies were assayed on the sera of mice immunized with either lot-1 or lot-3 vaccine. These vaccines induced highest and the lowest anti-diphtheria IgE antibody titers, respectively. As shown in Fig. 2, lot-1 vaccine induced predominantly IgG1 antibody, while lot-3 vaccine induced IgG2a antibody. Thus, the level of IgE antibody was correlated with that of IgG1 and inversely with that of IgG2a antibody. No difference was observed in the levels of IgG2b and IgG3 antibodies between lot-1 and lot-3 vaccine-injected groups.

Induction of anti-diphtheria IgG and IgE antibodies in mice after immunization with DPT vaccine was investigated. All six lots of vaccine induced substantial IgG antibody responses. However, IgE antibody production by the vaccination varied among vaccine lots. At present, it is unclear whether this variation in the inducibility of IgE production reflects intra-manufacturing difference or inter-

manufactural difference. Because of the shortage of purified antigen, we failed to assess the pertussis toxin-specific IgE antibody production, although it may also vary among vaccine preparations. The level of antigen-specific IgE antibody was correlated with the level of IgG1, but inversely with that of IgG2a. In mice, the production of IgE antibody is known to be regulated reciprocally by two kinds of cytokines, IFN-gamma and IL-4 secreted by two types of T helper subsets, Th1 and Th2, respectively (6). IL-4 enhances synthesis of both IgG1 and IgE, whereas IFN-gamma stimulates IgG2a production counteracting the IL-4-mediated synthesis of IgG1 and IgE. Some of those vaccine preparations used in this study may have stimulated activation of Th1 cells predominantly and others stimulated activation of Th2 cells, resulting in the induction of varied IgE responses that varied from one vaccine lot to another. It is unlikely that the different extents of induction of IgE antibody production observed among vaccine preparations was caused by the alum preparations or by the pertussis component contained in DPT-vaccines since the inducibility of IgE antibody production against diphtheria and that against tetanus were different with the same vaccine preparation (Fig. 1b). These differences may have been caused by the difference of each toxoid preparations. Although it is unclear whether the inducibility of IgE production in mice correlates with that of adverse reaction occurring in humans, the observation of IgE antibody production in experimental animals was expected to be applicable to vaccine control for minimizing adverse reaction after vaccination in humans. Investigation to find if there is any correlation between the adverse reaction reported after human vaccination and the induction of IgE antibody production in experimental animals is now in progress.

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