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Kinetics and localization of IgE tetanus antibody response in mice immunized by the intratracheal, intraperitoneal and subcutaneous routes

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Summary. The heterologous adoptive cutaneous anaphylaxis system was used to determine the kinetics of appearance of IgE-producing cells in various lymphoid tissues of mice following intratracheal (i.t.), intraperitoneal (i.p.), or subcutaneous (s.c.) immunization with tetanus toxoid and *Bordetella pertussis* organisms. Immunization, i.t. and i.p., produced similar patterns of response with the bronchial lymph nodes quantitatively exceeding the responses in other lymphoid tissues. In both cases the splenic lymphocyte response was second only to the bronchial and both appeared to parallel the serum PCA antibody. It is suggested that both responses represent draining lymph node responses since the bronchial lymph node drains both sites of immunization. After s.c. immunization a primary response of low order was found in the draining popliteal lymph node but not elsewhere. Although a dissociation was seen between responses obtained in various lymphoid tissues following s.c. and i.p. or i.t. immunization, no real evidence for a local mucosal response, such as has been reported for IgA, was obtained. These results lend experimental support to the observations that intratracheal and intraperitoneal immunization routes are most effective in production of IgE antibodies.

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INTRODUCTION

The mammalian respiratory tract has long been recognized as a portal of entry of antigens for the effective induction of immune responses. In the last decade particularly, studies in man and animals have shown that systemic- as well as local mucosal-immune responses can be induced by inhalation or by instillation into the respiratory tract of a variety of antigens (Waldman, Wood, Torres & Small, 1970; Waldman & Henney, 1971; Nash & Holle, 1973). Production sites of various classes and subclasses of antibodies have been examined after respiratory immunization of animals (Nash, 1973; Kaltreider, Kyselka & Salmon, 1974). However, successful induction of IgE antibodies by aerosol has so far only been reported in rats (Van Hout & Johnson, 1972). Comparative analysis of IgE antibodies in serum, sputum (Ishizaka & Newcomb, 1970; Waldman, Virchow & Rowe, 1973), and nasal and tracheobronchial washings of human patients (Deusche & Johansson, 1974) has strongly suggested local IgE production in the nasal and tracheobronchial submucosa. Indeed, the major mass of IgE forming cells has been detected by immunofluorescence in studies of non-atopic patients' adenoids, tonsils, bronchopulmonary and mesenteric lymph nodes, and in the respiratory and gastrointestinal lamina propria (Tada & Ishizaka, 1970). Only few

IgE-positive cells were present in the spleen and the subcutaneous lymph nodes. Since the general mucosal distribution of IgE-forming cells was similar to that seen for IgA, local mucosal production of reaginic antibodies appeared likely.

In order to study the localization and kinetics of reaginic antibody production, we compared the IgE response in mice following hind foot pad (s.c.), intraperitoneal (i.p.), and intratracheal (i.t.) injection of tetanus toxoid. The choice of this antigen enabled us to compare the reaginic antibody response with previous studies of the haemagglutinating antibody response to various routes of immunization (Gerbrandy & Van Dura, 1972; Gerbrandy, 1973). It does induce PCA antibodies in mice and the IgE response can be localized in various mouse lymphoid tissues by the heterologous adoptive cutaneous anaphylaxis (HACA) technique (Kind & Macedo-Sobrinho, 1973; Kind & Malloy, 1974).

MATERIALS AND METHODS

Animals

Female, 8–12-weeks old, inbred (C57Bl/6 × DBA/2) F_1/J mice, from Jackson Laboratories, Bar Harbor, Maine, were used throughout. Male Sprague-Dawley or Wistar rats, obtained from Bio Breeding, Ottawa, Ontario, served as recipients for heterologous skin reactions.

Immunizations

Concentrated tetanus toxoid (3200 Lf u/ml) and heat-killed *B. pertussis* vaccine were supplied by Connaught Laboratories Ltd., Toronto, Ontario. Subcutaneous (s.c.) immunization was carried out by injecting 20 μl saline containing 5 Lf tetanus toxoid plus 5×10^7 *B. pertussis* cells into each hind foot pad For intratracheal immunization (i.t.) mice were anaesthetized with Na-pentobarbital and restrained in a supine position. Intratracheal immunization was performed by dispensing via a blunt 20-gauge needle mounted on a 0·5 ml Hamilton Syringe, 10 μl (2 Lf tetanus toxoid + 10^8 *B. pertussis* cells) perorally into the trachea. A median cervical incision and gentle separation of the submaxillary glands enabled visual inspection of the correct insertion of the needle. The incisions were closed with one or two sutures. For intraperitoneal (i.p.) immunization 2 Lf tetanus toxoid + 10^8 *B. pertussis* cells in 0·5 ml saline were injected. For secondary immunizations the same tetanus toxoid doses as for primary immunizations, but without the *B. pertussis*, were given.

Sera and cell suspensions

Mice were killed with ether or by an i.p. overdose of Na-pentobarbital and were bled out by heart puncture. For intermittent *in vivo* serum titrations, blood was obtained from the orbital plexus under ether anaesthesia.

Donor organs of test lymphocyte preparations were carefully dissected, washed in saline, and transferred to cold Hanks's balanced salt solution (HBBS) pH 7·4. Bronchial lymph nodes were dissected from the dorsal side of the right bronchus; in i.t. and i.p. immunized mice they were invariably visibly increased in size. 'Peripheral lymph nodes' consisted of pooled popliteal, axillary and branchial nodes, except in the case of s.c. immunization where popliteal lymph nodes were tested separately. Since the popliteal lymph nodes showed no reactivity in i.p. or i.t. immunized animals they were pooled with the peripheral lymph nodes to increase cell recoveries in these groups. All tissues were homogenized in Pyrex brand tissue grinders. Peyer's patch homogenates were in addition sieved through sterile gauze sponges. Cells were washed thrice in cold HBBS, counted in 0·1 per cent trypan blue and usually made up in suspensions of 100×10^6 white cells/ml. Lung lymphocytes were isolated by Ficoll–Isopaque centrifugation of pooled lung homogenates.

PCA and HACA titrations

Passive cutaneous anaphylaxis (PCA) and heterologous adoptive cutaneous anaphylaxis (HACA) reactions were measured as described by Kind & Macedo-Sobrinho (1973). For PCA titration 0·1-ml volumes of two-fold serially diluted mouse serum samples were intradermally (i.d.) injected into the shaven dorsal skin of Na-pentobarbital anaesthetized rats. For HACA titration standard quantities of 10^7 lymphocytes in 0·1 ml HBBS were similarly injected i.d. into three rats or in as many spots as cell recoveries permitted. Lymphocytes from single organs from a minimum of five mice were pooled and provided cells for each HACA experiment. For lung lymphocytes at least thirty animals provided cells for a single experiment.

The challenge procedure for HACA was the same as for PCA titration. Twenty-four hours after sensitization the rats were i.v. injected under ether

anaesthesia with 1 ml saline containing 100 Lf tetanus toxoid and 1 per cent Evans blue dye. One hour after challenge, blue spots on the reversed dorsal skin were measured in two perpendicular directions; both measures in mm were multiplied and the square roots of the average products are given as the mean HACA spot size. The final HACA results seen in the figures represent the mean of all values obtained. Spot sizes greater than 5 mm were arbitrarily considered positive.

RESULTS

Groups of 50–100 mice were injected s.c., i.p. or i.t. with antigen plus adjuvant dosages that had been shown previously (Gerbrandy, 1973) to induce strong primary and secondary haemagglutinating antibody responses via the three immunization routes. Four successive weeks after primary immunization, HACA reactions were measured in spleen, draining lymph nodes (i.e. popliteal and bronchial nodes), peripheral lymph nodes and Peyer's patches. PCA was performed on serum pools. The measurements were repeated once or more after the remaining mice had been boosted 4 weeks after primary immunization. Occasional HACA observations were made on lung lymphocytes.

Subcutaneous immunization

Primary IgE response to s.c. injected antigen (Fig. 1) was restricted to the draining popliteal lymph node only, apart from low and questionably positive reactions seen in Peyer's patch cells. Booster immunization caused a secondary IgE response which now extended to spleen and peripheral lymph nodes, including the bronchial nodes. Both the HACA and PCA responses were lower after s.c. immunization than with either i.p. or i.t.

Intraperitoneal immunization

I.p. immunization (Fig. 2) elicited a rapidly rising primary reagin response in all lymphoid cell populations. After a peak on day 7 the PCA titre gradually fell off to a minimum level on day 14 when only the bronchial lymph node showed HACA reactivity. By day 21 the PCA titre as well as HACA reactions of bronchial nodes, spleen and Peyer's patches appeared to be rising and by day 28 the peripheral lymph nodes had resumed IgE production. This biphasic course of the primary IgE response has been consistently reproducible. I.p. booster injection caused a generalized secondary response as reflected by the PCA titres on days 30 and 33, and strong HACA reactions on days 35 and 42. The

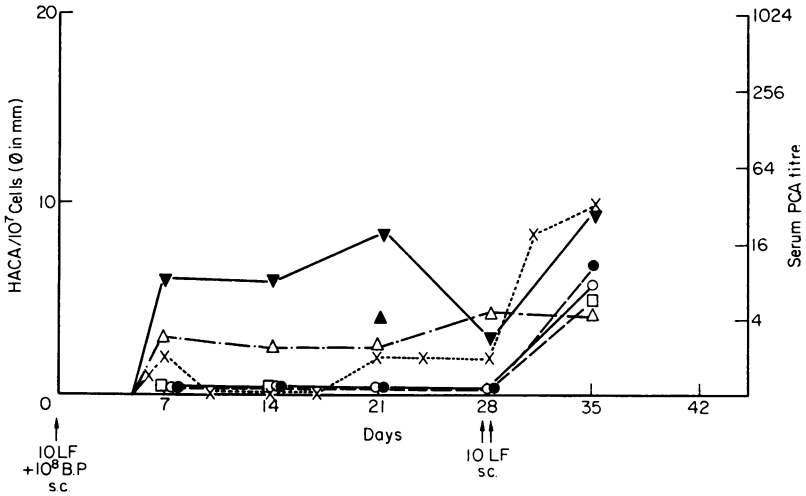

Figure 1. Reaginic tetanus antibody response of female B6D2Fl mice immunized via the subcutaneous route as determined by 24 h HACA and PCA reactions in Sprague–Dawley rats. (↑) Primary immunization: 5 Lf tetanus toxoid + 5×10^7 *B. pertussis* cells/hind foot pad. (↑↑) Secondary immunization: 5 Lf tetanus toxoid/hind foot pad. (○——○) Spleen; (●– – –●) bronchial lymph node; (□– – –□) peripheral lymph nodes; (△– · – · –△) Peyer's patches; (▲) lung lymphocytes, (× · · · · · ×) serum PCA; (▼——▼) poplited lymph nodes.

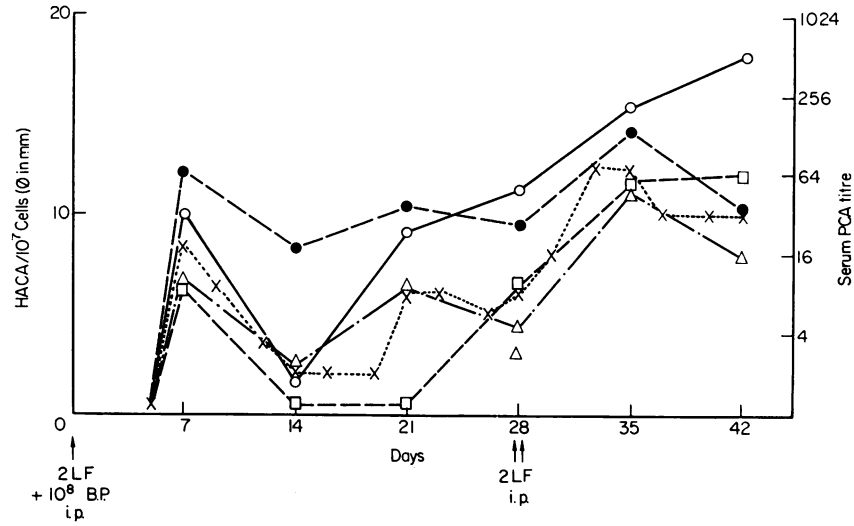

Figure 2. Reaginic tetanus antibody response of female B6D2Fl mice immunized via the intraperitoneal route as determined by 24 h HACA and PCA reactions in Sprague–Dawley rats. (↑) Primary immunization: 2 Lf tetanus toxoid + 10^8 *B. pertussis* cells i.p. (↑↑) Secondary immunization: 2 Lf tetanus toxoid i.p. (○——○) Spleen; (●– – –●) bronchial lymph node; (□– – –□) peripheral lymph nodes; (△– · – · –△) Peyer's patches; (▲) lung lymphocytes; (× . . . . . ×) serum PCA.

PCA titres of all serum samples could be reduced to zero by heating the sera for 2 h at 56°.

Intratracheal immunization

The primary response to i.t. immunization (Fig. 3) had the aspect of a slightly retarded i.p. response. A first PCA peak was reached in 9 days followed by a low reached between 17 and 21 days. Booster injection again induced a response which peaked after 4–7 days and was still substantially maintained 2 weeks after the booster. The sera of the i.t.-boosted mice showed residual PCA titres (16, 16 and 4 on days 32, 35 and 42 respectively) after 2 h incubation at 56°.

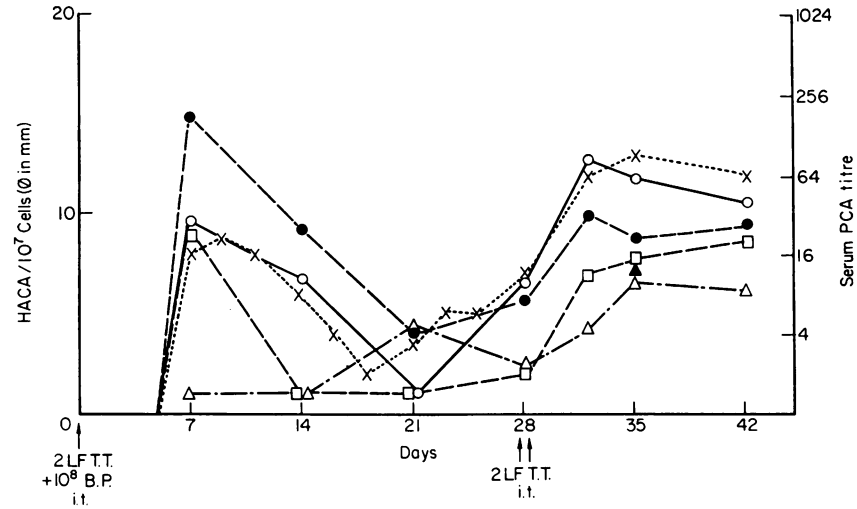

Figure 3. Reaginic tetanus antibody response of female B6D2F$_1$ mice immunized via the intratracheal route as determined by 24 h HACA and PCA reactions in Sprague–Dawley rats. (↑) Primary immunization: 2 Lf tetanus toxoid + 10^8 *B. pertussis*-cells i.t. (↑↑) Secondary immunization: 2 Lf tetanus toxoid i.t. HACA: (○——○) Spleen, (●– – –●) bronchial lymph node; (□– – –□) peripheral lymph nodes; (△– · – · –△) Peyer's patches; (▲) lung lymphocytes; (× · · · · ×) serum PCA.

Lung homogenates from i.t.-boosted mice (day 35) yielded an enriched lymphocyte fraction showing a clearly positive HACA reaction. In various other trials of lung lymphocytes very small HACA spots were found which we have considered non-specific. Peripheral blood lymphocytes were found to be negative.

DISCUSSION

We have used the heterologous adoptive cutaneous anaphylaxis technique (Kind & Macedo-Sobrinho, 1973) to follow the kinetics of the appearance of the IgE primary and secondary immune response following intratracheal, intraperitoneal and subcutaneous immunization with tetanus toxoid as antigen and *Bordetella pertussis* organisms as adjuvant. The specificity of this reaction seems assured at least in the primary response, since heating of sera to 56° for 120 min always totally abolished the respective PCA serum antibody titre in the same animal, and only mouse IgE appears to sensitize rat mast cells for the antigen specific release reaction (Mota & Wong, 1969; Kind & Macedo-Sobrinho, 1973). Since the HACA spot diameter was found to bear a logarithmic relationship to the number of cells transferred, the technique appeared to be a useful parameter of the IgE synthetic capacity of various lymphoid populations. In this sense the technique could be compared to the use of plaque forming cells as a method of assessment of production of immunoglobulin classes in the draining lymph nodes and bronchoalveolar cells of the lung (Nash, 1973; Kaltreider *et al.*, 1974).

Since Tada & Ishizaka (1970) have shown by immunofluorescence that much of the IgE forming lymphoid mass is either found in or related to the respiratory tract mucosa and Deuschl & Johansson (1974) have demonstrated that the majority of IgE found in respiratory tract secretions is locally synthesized, it is perhaps not surprising that following intratracheal immunization the major IgE response was contained within the draining bronchial lymph node. In man, after a variety of especially chronic infections of the lung the numbers of IgA and IgE containing cells rise disproportionately to other antibody classes in the bronchial lamina propria (Callerame, Condemi, Ishizaka, Johansson & Vaughan, 1971). Thus the demonstration at the peak of the response that there was an IgE response in lymphocytes obtained from the lung parenchyma was clearly compatible with local synthesis in the lung. It is not clear from these studies whether the IgE antibody was synthesized in the bronchial mucosa, in bronchiolar tissue by infiltrating cells, or cells infiltrating the alveolar walls.

By analogy with other forms of respiratory tract immunization, if IgE was considered to be a mucosal type of antibody locally synthesized in the lamina propria of the bronchial tract, we were expecting to find a difference between i.t. and i.p. immunization. However, i.p. immunization also showed a predominant IgE response in the bronchial lymph node with a somewhat similar pattern to that seen with i.t. immunization in the other lymphoid tissues. Since the drainage of the peritoneal cavity in mice as well as most of the lung parenchyma appears to be via the bronchial lymph nodes (Cuq, 1966), this response is perhaps not surprising and may explain the good IgE responses to immunization which other investigators have found via the intraperitoneal route. Indeed, instillation of India ink into the peritoneal cavity can be shown to drain directly to the bronchial lymph node (unpublished observations).

Both i.p. and i.t. immunization produced a somewhat similar time course of appearance in serum PCA antibody which was paralleled very closely by the response of splenic lymphocytes. The surprisingly high contribution of the spleen in IgE responses has been noted before by Kind & Macedo-Sobrinho (1973) but at first sight appears to be in some contradiction to the reported distribution of IgE in monkeys and humans (Tada & Ishizaka, 1970). However, others have shown effective adoptive transfer of IgE synthesis with splenic cells from primed mice (Okudaira & Ishizaka, 1974; Lee & Sehon, 1975).

After subcutaneous immunization only the draining lymph node showed any appreciable rise in HACA. This weak response could not be ascribed to the higher antigen dose as compared to the i.p. and i.t. experiments. Similar experiments over a dose range from 32 to 1/8 Lf units of antigen did not result in major differences in HACA in the draining lymph nodes; only the PCA titres were slightly higher with the higher antigen doses. No bronchial lymph node activity was found after primary s.c. immunization despite the higher dose.

In the primary IgE response it appears that the

draining lymph nodes contain the highest IgE production. This appears to be similar to the response seen with IgG and although to a degree, dissociation occurred between peripheral lymph node responsiveness following s.c. immunization, and bronchial lymph node responsiveness after either i.p. or i.t. immunization, the kinetics and the relative distribution of responses in various lymphoid tissues did not follow the kinetics generally seen with local mucosal IgA responses. These results either suggest a greater concentration of IgE precursors in the lung and draining lymph node, or a greater capacity to recruit from the IgE pool such precursors to the draining lymph node or to the respiratory tract lymphoid tissue.

Peyer's patch HACA activity was seen at a low level, and no conclusion can be drawn as to the involvement of lymphocytes in this mucosal lymphoid tissue as compared to mucosal or other lymphoid tissue elsewhere. Peyer's patches have been shown to contain IgA precursor cells destined for gut (Craig & Cebra, 1971) and other mucosal sites (Rudzik, Clancy, Perey & Bienenstock, 1975). Since immunofluorescent localization of IgE has been found with monkey Peyer's patches (Tada & Ishizaka, 1970) a comparison of the results reported here with oral feeding would be of interest.

The characterization of the secondary response was not easy since with both i.t. and i.p. immunization a biphasic primary immune response occurred in most positive lymphoid tissues as well as in the serum PCA response, which was on the upswing at the time chosen (28 days) for the booster. In a separate experiment, however, the secondary response could be clearly distinguished from a continued primary response. This biphasic response has been noted before with bronchial lymph node cells following intratracheal immunization (Nash, 1973) and following oral immunization (Werner, Lefèvre & Raettig, 1971; Dolezel & Bienenstock, 1971). Various explanations should be considered including the likely possibility of an extremely short half-life for mouse IgE molecules (on the basis of known half-lives for other mouse immunoglobulins (Bazin & Malet, 1969) and human IgE (Waldmann, 1969)) which would follow the kinetics of antibody forming cell proliferation, feedback regulation by IgG antibodies (Tada & Okumura, 1971), suppressor cell activity (Okumura & Tada, 1974), or a delayed proliferation of IgE precursor cells from a limited stem cell pool. Available data do not allow discrimination between these possibilities at present.

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