

ABROGATION BY SUBSEQUENT FEEDING OF
ANTIBODY RESPONSE, INCLUDING IgE,
IN PARENTERALLY IMMUNIZED MICE*

By SERGYL LAFONT, CLAUDE ANDRÉ,‡ FRANÇOISE ANDRÉ, JOHN GILLON,
AND MARIE-CLAIRE FARGIER

*From the Groupe d'Immunopathologie Digestive, INSERM U 45, Hôpital Edouard Herriot,
69003 Lyon, France*

The immune response to numerous antigens administered parenterally can be suppressed or reduced by prior oral treatment with the same antigen. This applies to both particulate (1, 2) and soluble (3-5) antigens. The immune tolerance thus produced is specific and can be shown to affect cell-mediated hypersensitivity (6) as well as the production of specific antibody, including reaginic antibody (7, 8).

Little is known about the effects of antigen ingestion after immunization by the parenteral route with the same antigen (9). The few published data suggest that the hypersensitive state is not altered (6), although there may be a booster effect on IgE production (10). We show here that ingestion of antigen after immunization can prevent the secondary antibody response and interrupt reaginic antibody production. These findings may have important clinical applications.

Materials and Methods

Antigen. Ovalbumin recrystallized five times (Serva, Heidelberg, Federal Republic of Germany) was used for all immunization procedures.

Animals and Immunization. We used 3-mo-old mice of both sexes of the strains AKR, DBA/2, and C3H supplied by IFFA-CREDO (L'Arbresle, France). Intraperitoneal immunization was carried out by injecting 1 mg of ovalbumin (OVA) in 0.5 ml of 0.15 N saline, without adjuvant. Oral administration of antigen was performed by gavage, the dose being 20 mg OVA in 0.2 ml 0.15 N saline.

Antibody Determinations. Antibodies to OVA were measured by passive haemagglutination, using sheep erythrocytes coated with OVA as described by Avrameas et al. (11). Reaginic antibodies of both IgE and IgG1 types were detected by passive cutaneous anaphylaxis using male AKR mice at least 12 wk old (12).

Results

Abrogation of the Secondary Antibody Response by Repeated Oral Administration of OVA to Mice Previously Immunized Parenterally (Figs. 1-3). Variable results were obtained when OVA was given orally during the interval between two intraperitoneal immunizations 1 mo apart, the effect being a function of the number of oral doses given. No attenuation of the secondary responses was seen after only one oral administration

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‡ To whom reprint requests should be addressed at U 45 INSERM, Pavillon H, Hôpital Edouard Herriot, 69374-Lyon Cédex 2, France.

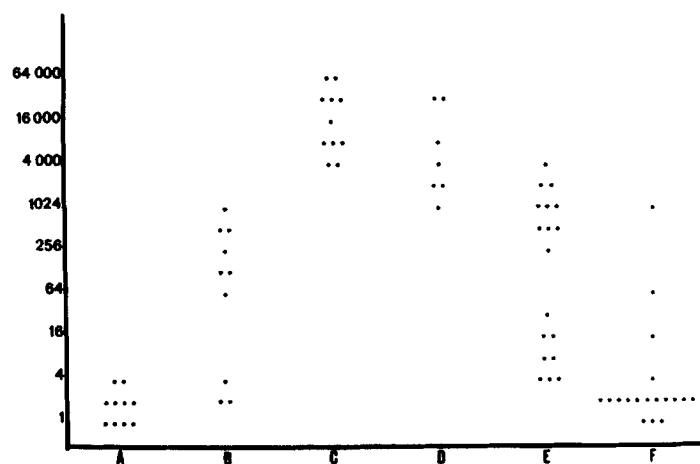


FIG. 1. Antibodies to ovalbumin in AKR mice as measured by passive haemagglutination in the following groups: (A) sacrificed 11 d after primary immunization with 1 mg OVA i.p. (B) 20 mg orally on day 1, 1 mg OVA i.p. on day 15, and killed on day 26. (C) 1 mg OVA i.p. on days 1 and 30, and killed on day 41. (D) 1 mg OVA i.p. on days 1 and 30, 20 mg orally on day 15, and killed on day 41. (E) 1 mg OVA i.p. on days 1 and 30, four consecutive doses of 20 mg OVA orally on days 15-18, and killed on day 41. (F) 1 mg OVA i.p. on days 1 and 30, 20 mg OVA orally on each of days 15-19 and 21-25 (total 10 doses), and killed on day 41. Differences between groups: C vs. D, not significant; C vs. E or F, $P < 0.001$; E vs. F, $P < 0.02$ (Student's t test).

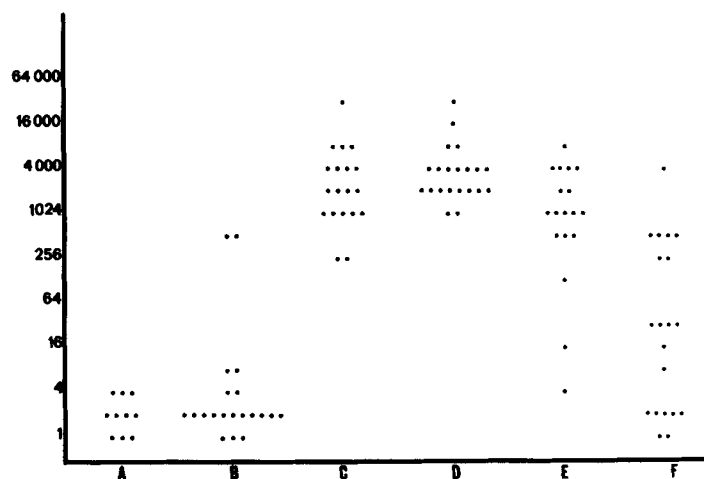


FIG. 2. Antibodies to ovalbumin in DBA/2 mice as measured by passive haemagglutination in the groups A through F (groups as in Fig. 1). Differences between groups: C vs. D or E, not significant; C vs. F, $P < 0.01$; E vs. F, $P < 0.001$.

(group D), regardless of the strain. AKR mice showed definite reduction in the secondary antibody response when given four oral doses of OVA on days 15-18 after primary immunization (group E). This was not seen in DBA/2 and C3H mice. When OVA was given on 10 occasions (group F) after primary immunization (days 15-19 and 21-25), the secondary antibody response was almost completely abolished in AKR and C3H mice, and a clear reduction in the response was observed in DBA/2.

Amplification of the Antibody Response to Intraperitoneal Immunization by Prior Administration

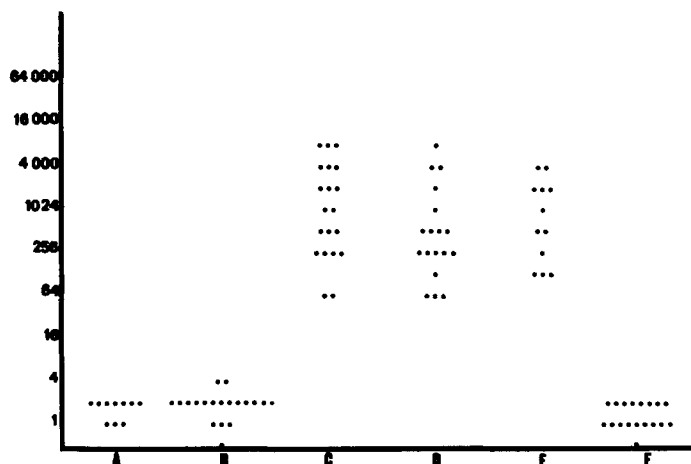


FIG. 3. Antibodies to ovalbumin in C3H mice as measured by passive haemagglutination in groups A through F (groups as in Fig. 1). Differences between groups: C vs. D or E is not significant; C vs. F and F vs. E, $P < 0.01$.

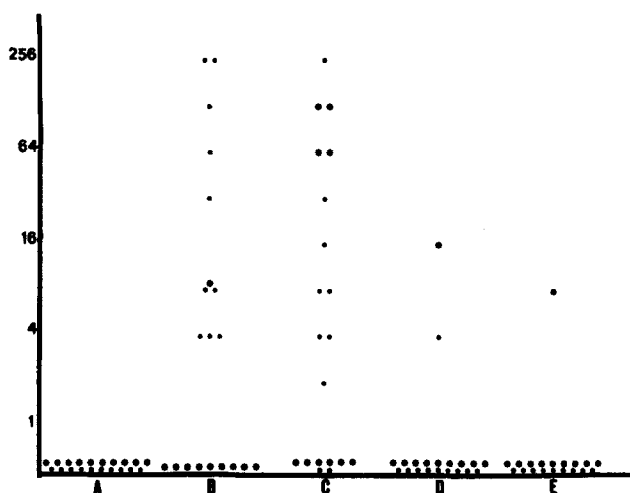


FIG. 4. Reagenic antibody titers (IgE (·) and IgG1 (*)) to OVA in AKR mice as measured by passive cutaneous anaphylaxis. (A) titer on day 15 after immunization with 20 mg OVA orally; (B) titer on day 30 after immunization with 20 mg OVA orally; (C) titer on day 30 after immunization with 1 mg OVA i.p. and one oral dose of 20 mg on day 15; (D) titer on day 30 after immunization with 1 mg OVA i.p. alone and 4 consecutive oral doses of 20 mg OVA on days 15-18; (E) titer on day 30 after immunization with 1 mg OVA i.p. and 10 oral doses of 20 mg OVA given on days 15-19 and 21-25. IgE level differences between groups: B vs. C, not significant; B vs. D and D vs. E, $P < 0.05$. IgG1 level difference between groups B and C, $P < 0.05$.

of One Oral Dose of OVA (Figs. 1-3). When AKR mice were given 20 mg OVA orally 15 d before intraperitoneal immunization with 1 mg OVA (no adjuvant), the antibody response was greatly augmented in the majority of cases (group B). This did not occur in DBA/2 or C3H mice.

Repeated Administration of Oral OVA Diminishes Reagenic Antibody Production in Sensitized Animals (Fig. 4). AKR mice showed an IgE reagenic antibody response to one

intraperitoneal injection of 1 mg OVA without adjuvant (group B). A single oral dose of 20 mg OVA 15 d after intraperitoneal sensitization did not suppress antibody production (group C) and occasionally induced IgG1 synthesis. However, 4 oral doses of OVA on days 15–18 (group D), or 10 doses on days 15–19 and 21–25 (group E) abolished both IgE and IgG1 responses 30 d after parenteral immunization. A single oral dose of OVA did not provoke a reagenic antibody response in AKR mice.

Discussion

It is difficult to induce immune tolerance after an initial immunization procedure (10, 13). Here we show that repeated oral administration of antigen (OVA) is necessary to prevent secondary antibody responses in mice previously parenterally immunized. This is in contrast with other studies (4–8), and is at variance with the recent studies that failed to demonstrate suppression of the secondary response (10, 13). They did not study the effect of repeated oral administration of antigen. It is, however, well established that repeated stimulation of Peyer's patch suppressor T cells with OVA is necessary to suppress the effect of primary parenteral immunization (8, 14–17). The possibility of antibody blocking by increased intestinal absorption of the antigen may be ruled out for two reasons. (a) Local antibodies produced after oral administration of antigens markedly decrease its absorption (18). (b) The oral administration of larger doses (25 mg/d) and for a longer period (14 d) of OVA in C3H mice resulted in a nonsignificant amount of circulating antigen when assessed by radioimmunoassay in a time protocol very similar to our own (4).

Our results varied with the strain of mouse used, as previously described (19). Inconsistent results were obtained with DBA/2 mice, good suppression was seen in the C3H strain, and the best results were obtained using AKR mice, in which a clear reduction in the secondary response occurred with four doses of oral antigen. We have also noticed that suppression of a primary response by prior ingestion of antigen is difficult to obtain in DBA/2 mice, but occurs readily in C3H and AKR (unpublished data). It therefore appears that induction of tolerance is genetically determined and is identical for both primary and secondary responses.

Our interest in the reagenic antibody response in AKR mice was prompted by the observation that all the animals were in anaphylactic shock for at least 1 h after the second intraperitoneal dose of OVA at day 30. However, when one oral dose was given 15 d after sensitization, 4 out of 10 mice went into shock, 3 of 10 died, and 3 remained healthy. We found a substantial IgE response 30 d after primary immunization, although the dose of OVA was quite high (1 mg) and none of the usual adjuvants necessary to elicit reagenic responses were used. An oral dose of 20 mg does not provoke reagenic response (Fig. 4, group A). Other authors (20) have found reagenic responses with minute doses of OVA by the oral route. We have shown that one dose of oral OVA at day 15 after intraperitoneal sensitization does not diminish the IgE response at day 30, and may occasionally induce IgG1. However, when four oral doses of OVA are given on consecutive days (days 15–18), reagenic antibodies become almost undetectable in the serum at day 30. Similar results are obtained with 10 oral doses on days 15–19 and 21–25. A new intraperitoneal sensitization at day 30 does not provoke any shocks in AKR mice after 4 or 10 oral doses.

We have confirmed, as previously suggested (21), that oral ingestion of antigen could reduce anaphylactic reactions in sensitized subjects. Recently, it has been shown

that repeated intraperitoneal injections of OVA can reduce reaginic antibody titers 1 mo after sensitization (22). It therefore seems possible that desensitization occurs as a result of repeated exposure to relatively small amounts of absorbed antigen.

The possible reduction in intensity of a secondary humoral response by repeated ingestion of antigen has important clinical implications. That this is more than a theoretical possibility has been demonstrated by Bierme et al. (23), who reported the beneficial effect of feeding rhesus antigens to extremely immunized mothers.

Summary

We have investigated the possibility of oral administration of ovalbumin (OVA) to prevent a secondary antibody response and interrupt reaginic antibody production. Repeated feeding seems necessary for both. Quality of results was dependent on the number of ingestions. Differences in abrogation of antibody response between mice strains were observed. Best results were obtained with AKR mice, good suppression was seen in C3H strain, and inconsistent results were obtained with DBA/2. 10 OVA oral doses were necessary to prevent a secondary antibody response in parenterally immunized mice, but 4 doses interrupted reaginic production in sensitized AKR mice. These results demonstrate that antigen feeding can prevent a secondary antibody response and interrupt reaginic antibody production.

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