Interruption of recently induced immune responses by oral administration of antigen

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Abstract

Interest in oral tolerance has been renewed in the last few years as a possibility of intervention in human autoimmune diseases. An obstacle in this direction is that, although easily induced in animals virgin of contact with the antigen, oral tolerance becomes hard to induce in previously immunized animals. The present results show that there is an early period after primary immunization in which prolonged oral exposure to the antigen may arrest ongoing immune responses. Beyond this period, oral exposures to the antigen become ineffective and may actually boost immune responses. The end of the susceptible period coincides with the emergence of free specific antibodies in serum. However, the previous administration of purified anti-ovalbumin antibodies (40 µg) was unable to block the induction of oral tolerance to ovalbumin in normal mice.

Key words
- Suppression
- Antibody response
- Oral tolerance
- Ovalbumin

The intestinal mucosa is the largest surface exposed by the organism to its environment and is in contact with a plethora of antigenic materials. Small amounts of incompletely degraded or intact proteins regularly penetrate the circulation and antibodies to dietary antigens are a common finding in normal individuals (1,2). However, another and possibly more frequent consequence of feeding is the development of oral tolerance - an apparent inhibition of specific immune responses to previously ingested proteins (3,4).

Easily induced in animals virgin of previous exposure to the antigen, oral tolerance becomes difficult to induce in primed or immune animals (5,6). Nevertheless, the development of ways of blocking ongoing immune responses by oral tolerance would be particularly important in the therapy of many forms of allergy (7) and autoimmune diseases (8,9).

In the present study, we show that, early in their induction, ongoing immune responses in the mouse may be interrupted by intensive and prolonged ingestion of, or gavage with solutions containing the antigen. After a period of two weeks, concomitantly with the emergence of specific antibodies in serum, immune responsiveness is no longer reversed by oral contacts with the antigen.

We used 6- to 8-week-old B6D2F1 (C57BL/6 x DBA/2J) F1 mice of both sexes. Crystallized hen ovalbumin (Ova) was used as antigen. Ova V (grade V; Sigma Chemical Co., St. Louis, MO) was used for immunization...
Figure 1 - Secondary anti-Ova immune responses (ELISA*) in B6D2F1 mice gavaged with 20 mg Ova at different days before, during or after parenteral (sc) immunization. Controls were gavaged with either 20 mg Ova (tolerant control) or 0.5 ml saline (immune control) on day -7 and received tap water thereafter. Parenteral (sc) immunizations: primary (day 0) 10 µg Ova + 1 mg Al(OH)3; secondary (day 14) 10 µg soluble Ova. Bleeding: day 21. Data are reported as means ± SEM for N = 4-6. *P<0.05 compared to saline controls (Scheffé (ANOVA) test).

Specific anti-Ova antibodies were purified by affinity chromatography of pooled B6D2F1 anti-Ova sera collected after ip immunization with 100 µg Ova in complete Freund’s adjuvant (Difco, Detroit, MI), by passage through an Ova-Sephadex column with a peristaltic pump overnight at 4°C. Immunoglobulins not bound to the column were washed with PBS (0.15 M, pH 7.5). Anti-Ova antibody was eluted with glycine-HCl buffer, pH 2.8, and the pH of the eluate was buffered with Tris-HCl, pH 8.5. The protein concentration of the solutions was determined by absorbance at 280 nm.

As shown in Figure 1, a single gavage with 20 mg Ova performed either 7 days or 1 day (24 h) before immunization (sc) with Ova plus Al(OH)3 resulted in marked oral tolerance. The ingestion of egg white for 7 days starting 0 or 3 days after primary immunization inhibited secondary anti-Ova responses.
Interruption of ongoing immune responses (Figure 2), whereas ingestion starting at day 10 was on the fringe of effectiveness. The result of ingestion of egg white for only 3 consecutive days, starting on the same days, was less impressive. Ingestion of egg white for 7 days starting 14 days after primary immunization had no effect on the magnitude of secondary antibody responses. Gavages with 20 mg Ova for 7 consecutive days starting 21 days after primary immunization was also ineffective (data not shown).

The passive transfer of specific antibodies has been previously shown to interfere with the induction of oral tolerance (5,6); transfer of low doses (10 µg) of antibodies was effective, probably because only trace amounts of antigenically intact ovalbumin are absorbed (1,2). In the present experiments, however, the transfer of 40 µg of affinity-purified mouse polyclonal anti-Ova antibodies 24 h before, concomitantly or 24 h after gavage with Ova failed to block the development of oral tolerance. This passive transfer of antibodies also had little or no effect on the magnitude of antibody responses of control mice gavaged with saline.

Whether oral contacts with antigens will result in oral tolerance, “local” or “systemic” immunization, i.e., circulating antibody formation, depends on several factors, related to the antigen and/or the organism (5,6,10, 11). Oral tolerance may occur concomitantly with local slgA production (12); or, both systemic and local inhibition of slgA production may occur (13); or, the intestinal IgA response may be blocked even more effectively than systemic responses (14). Which type of immunological consequences will result from oral exposures to antigen is as yet unresolved. Previous parenteral exposures to antigen interfere with subsequent mucosal IgA responses to the antigen (15), a phenomenon which is the mirror image of oral tolerance, and concomitant oral/parenteral exposures to antigen may block oral tolerance (Lahmann WM and Vaz N, unpublished observations).

Oral exposure of previously immunized animals to antigen fails to induce tolerance (10-12) and may result in secondary serum and/or mucosal antibody responses (5,6), although prolonged exposures have been shown to block the formation of IgE antibodies (16) or delayed type hypersensitivity (17). Regardless of these pending issues, several empirical attempts have been and are presently being made to treat human autoimmune diseases by intensive and prolonged feeding of proteins considered to be relevant immunogens in triggering these diseases (9).

Specific antibodies become detectable in serum (as determined by ELISA or radioimmunoassays) at 9 to 10 days after primary immunization of B6D2F1 mice with small (microgram) doses of Ova in Al(OH)3 (5,10,11). The avoidance of voluntary ingestion of Ova-containing solutions by Ova-immunized mice, the most sensitive clue of active immunization with low doses of protein, emerges between 10 and 14 days after primary immunization, concomitantly with the rise of detectable antibody titers in serum (18).

Intestinal dendritic cells containing orally administered antigens and able to stimulate T cells may be found in the lymph of the thoracic duct a few hours after antigen ingestion (19) and the presentation of peptides may have been irreversibly determined at this point.

In conclusion, the present results show that intensive and prolonged exposure to the antigen by direct feeding is only effective in...
blocking the effects of parenteral immunization when initiated shortly after primary immunization. Recently developed techniques of antigen delivery in the gastrointestinal tract by coupling with cholera toxin B subunit or micro-encapsulation (20) are potentially able to modify this situation.

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References


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References