Induction of Oral Tolerance

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INTRODUCTION

Oral immunologic tolerance or oral tolerance may be described as a state of systemic unresponsiveness to parenteral immunization that is induced by prior antigen feeding (1). Historically, the hypersensitivity to poison ivy was reportedly modulated by the intake of poison ivy leaves by American Indians, but sufficient documentation for this effect is lacking (2). The term "oral tolerance" was first coined by Chase (3), who described a reduction of cutaneous hapten sensitization in mice by prior feeding of the hapten. Oral tolerance has been demonstrated convincingly in rodents fed a variety of antigens such as bacteria (Streptococcus mutans) and heterologous blood cells or soluble protein antigens such as ovalbumin (4–6). However, species differences do exist, for example, the rabbit does not seem to develop oral tolerance (7). Also, certain antigens such as cholera toxin do not induce oral tolerance and may abrogate oral tolerance to unrelated antigens (8). Oral tolerance has been described in both the humoral and the cellular immune systems. Interestingly, antigen feeding at low doses suppressed cellular immunity but left the humoral responsiveness intact (9).

In general, immune tolerance may occur by clonal deletion, clonal anergy (10), or suppression (Table 1). With regard to oral tolerance, local and systemic suppressor T cell circuits may be operating (11–13). Also, the digestion and absorption of antigen by the intestine have been implicated in the production of tolerogenic forms of the antigen in the circulation (14,15). At least for protein antigens, a major mechanism appears to be the accumulation in Peyer's patches of antigen-specific suppressor T cells, which later populate systemic lymphoid tissues such as the spleen (11). A month or so after antigen feeding of the animals, suppressor T cells cannot be identified, but the animals remain unresponsive to the fed antigen, suggesting the operation of different mechanisms such as clonal deletion or anergy. A secretory immune response has been described despite systemic tolerance, but only a few studies have looked directly at the secretory immune response (16). The levels of salivary antibodies were low, so a secretory immune response may not be an obligatory event.
In humans, oral tolerance has only been studied to a limited extent (17–19), partly based on obvious ethical limitations. In this chapter, we concentrate on the induction of oral tolerance in humans and on disease models which are of immediate relevance for future clinical studies.

HUMAN STUDIES OF ORAL TOLERANCE

Based on the observation (20) that antibodies to foods tend to decrease with age, Korenblatt et al. (17) studied the antibody levels to bovine serum albumin (BSA). Human adult volunteers ingested BSA and then had intradermal immunization with BSA. The subjects without serum antibody to BSA prior to feeding were unresponsive to both oral and parenteral challenge, whereas the subjects with a measurable antibody level tended to show increased antibody levels after the challenge. These data are consistent with either anergy or a suppressive mechanism occurring in the subjects with low antibody levels.

The experiments of Chase (3) were directly applied to humans in a study of 2:4-dinitrochlorobenzene (DNCB) applied to the buccal mucosa of male prisoners (18). The response was measured as the reaction to DNCB later applied to the skin. The cutaneous reaction to DNCB was distinctly lowered in the study group that received more than 20 mg of DNCB by buccal application. This study clearly suggests that oral tolerance may be present in humans in the T cell immune system.

We undertook a study to determine whether the ingestion of a protein antigen by human volunteers results in either oral tolerance or oral immunization (19). Keyhole limpet hemocyanin (KLH) was chosen as a model antigen, because it is a strong immunogen that has previously been used to test for immunocompetence in humans and is not commonly eaten, in contrast to most food antigens. Out of a total of 16 adult volunteers, eight subjects were given ten doses (days 1 to 5 and 15 to 19) of oral KLH for a total dose of 0.5 g and then immunized parenterally (days 25 and 36), and eight subjects were parenterally immunized only. T cell responses were assessed in vitro by a proliferation assay and in vivo by delayed-type skin reactions. B cell immune responses to KLH of the three major immunoglobulin (Ig) classes were assessed in blood by the enumeration of antibody-forming cells with the enzyme-linked immunospot (ELISPOT) technique and antibody levels (IgG, IgM, IgA, and IgE) were determined by enzyme-linked immuno-sorbent assay (ELISA). IgA class
TABLE 2. Delayed-type skin test to KLH in eight subjects fed KLH and subsequently immunized parenterally and eight subjects given parenteral immunization only

<table>
<thead>
<tr>
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<th>24 hours</th>
<th>48 hours</th>
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<tr>
<td></td>
<td>Mean (range)</td>
<td>Number of positive/total</td>
</tr>
<tr>
<td>KLH-fed</td>
<td>1.2 (0–10)</td>
<td>1/8</td>
</tr>
<tr>
<td>Controls</td>
<td>11.9 (0–23)</td>
<td>7/8</td>
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p values

antibodies to KLH were likewise determined in salivary and intestinal secretions before and after the feeding and after parenteral immunization with KLH.

Feeding alone did not induce significant antibody levels in serum or secretions, nor were any anti-KLH-producing cells detected, as determined by the ELISpot technique (21). The subsequent subcutaneous immunizations induced significant levels of circulating anti-KLH-producing B cells of the three major isotypes. The levels of IgG and IgM antibody spot-forming cells were significantly higher in the group fed KLH than in the controls. The titers of the IgG, IgA, and IgM anti-KLH antibodies were likewise significantly higher in the group fed KLH. No IgE antibodies to KLH were detected.

As assessed by the proliferation assay, there was significant T cell reactivity to KLH after the KLH feeding, but a subsequent reduction to near baseline levels after the parenteral immunization. The T cell reactivity to KLH was significantly lower for the KLH-fed group than for the controls. Also, delayed skin test responses to KLH (Table 2) were significantly depressed as compared to controls. Intestinal and salivary secretions contained detectable anti-KLH levels as measured by ELISA after oral and parenteral immunization. This is in keeping with studies in experimental animals (16), where salivary antibodies were observed in low titers.

In conclusion (Table 3), our study indicated that oral tolerance to a soluble protein does occur in the human T cell system. Our study also demonstrated priming for serum and secretory antibodies by feeding. The dichotomy between the depression of the T cell and augmentation of the B cell response by the oral immunization could be the result of low zone tolerance. Only tiny amounts of antigen are usually absorbed from the gastrointestinal tract, in the order of micrograms per liter (μg/liter) or in the order of 1:10^5 of the amount ingested (22), so very small amounts of antigen may

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<tr>
<td>T cell reactivity in vitro and in vivo ↓</td>
<td></td>
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<tr>
<td>Systemic B cell responses ↑</td>
<td></td>
</tr>
<tr>
<td>Detectable local antibody production</td>
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TABLE 3. Conclusions from a study of oral tolerance in humans (19)
be presented to the systemic immune system. Low zone T cell tolerance after injecting antigen is well described in classical experiments in mice (23). Also, a more recent study in transgenic mice showed that small amounts of autoantigen released spontaneously in vivo rendered T cells, but not B cells, tolerant (24). Our study underscores the importance of the T cell system in the generation of oral tolerance as a physiological event of the human immune system.

### ANIMAL DISEASE MODELS

Oral tolerance has been exploited successfully as a therapeutic measure in several animal disease models of autoimmunity (Table 4). In addition, these experiments have yielded valuable information as to the mechanism of oral tolerance itself.

Experimental allergic encephalomyelitis has been the most intensely studied disease model. Both disease incidence and severity were suppressed after the oral administration of myelin basic protein and of encephalitic and nonencephalitic fragments of myelin basic protein (25,26). The data of Weiner and his research group (30) further indicated that suppression of experimental allergic encephalomyelitis was

![](image)
INDUCTION OF ORAL TOLERANCE

TABLE 4. Autoimmune animal disease models modified by the induction of oral tolerance

<table>
<thead>
<tr>
<th>Disease model</th>
<th>Species</th>
<th>Antigen</th>
<th>Effects</th>
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<tbody>
<tr>
<td>Experimental allergic encephalomyelitis (refs. 25, 26)</td>
<td>Lewis rat</td>
<td>MBP</td>
<td>Incidence ↓</td>
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<td></td>
<td></td>
<td></td>
<td>Delayed onset</td>
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<td>Collagen-induced arthritis (refs. 27, 28)</td>
<td>WA/KIR rat</td>
<td>Collagen II</td>
<td>Incidence ↓</td>
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<td>DBA/1 mouse</td>
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<td>Delayed onset</td>
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<td></td>
<td>Serum antibody ↓</td>
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<td>T cell reactivity ↓</td>
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<td>Serum antibody ?</td>
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<td></td>
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<td></td>
<td>T cell reactivity ↓</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence ↓</td>
</tr>
<tr>
<td>Experimental autoimmune uveoretinitis (ref. 29)</td>
<td>Lewis rat</td>
<td>S-antigen</td>
<td>Incidence ↓</td>
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<td></td>
<td></td>
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<td>Delayed onset</td>
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<td>Serum antibody ↓</td>
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<td></td>
<td></td>
<td></td>
<td>T cell reactivity ↓</td>
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MBP, myelin basic protein.

mediated by T cells specific for myelin basic protein (MBP), which were of the CD8⁺ phenotype, and that the suppression was obtained by transfer of MBP-specific CD8⁺ cells into naive mice (Fig. 1) (30). In vitro, these cells produced the cytokine transforming growth factor-β (TGF-β) and addition of antibody to TGF-β abrogated the suppression both in vitro and in vivo (Fig. 2). TGF-β accumulated in the brain in the animals fed MBP (31). This is the first demonstration of a cytokine involved decisively in the regulation of oral tolerance.

HUMAN DISORDERS

The suggestive evidence (19) of oral tolerance in the T cell system as a physiologic mechanism in humans and the results in animal disease models (25-30) may imply a break in oral tolerance as a putative pathogenic mechanism in autoimmune and allergic disorders.

A recent study of diabetes (32) indicated that antibodies to BSA in diabetic individuals cross-react with p69, a protein found on the β islet cells of the pancreas. These antibodies may be of pathogenic importance in the development of diabetes, and these data may explain why breast-fed babies more rarely become diabetic later in life than formula-fed babies.

Furthermore, the induction or reestablishment of oral tolerance could be a new type of treatment of great importance in autoimmune and allergic disorders. Clinical studies of the oral administration of autoantigens are under way (33) in patients with multiple sclerosis, rheumatoid arthritis, and uveitis. A series of studies have been performed with the oral administration of allergens to patients with asthma and allergic rhinoconjunctivitis with variable success. Several double-blind and placebo-controlled studies (34) have been performed with this purpose, some indicating an effect of the oral immunotherapy, whereas other studies did not show any beneficial
FIG. 2. The role of TGF-β: inhibition of in vitro suppression by anti-TGF-β antibody. Spleen cells from MBP-fed and control animals were incubated with MBP and antibody to TGF-β, interferon-γ (IFN-γ), or tumor necrosis factor-α/β (TNF-α/β). Supernatants were collected and antibody was removed with polymer beads. The supernatants were then added to an MBP-specific T cell line. Suppression was inhibited with increasing doses of anti-TGF-β. (From Miller A, Lider O, Roberts AB, Sporn MB, Weiner HL. Proc Natl Acad Sci USA 1992; 89: 421-5. Reproduced with permission.)

clinical effect. Doses and modes of administration may be changed to obtain more effective treatment regimens and perhaps definite answers to this question.

REFERENCES


DISCUSSION

**Dr. Bienenstock:** How do you explain the antigen specificity of the TGF-β in the model described by Howard Weiner? I follow the system and I understand TGF-β, but how do you get antigen specificity in that system?

**Dr. Husby:** Obviously the CD8+ T cells are activated in an antigen-specific way by myelin basic protein. It is a weak point in the hypothesis that TGF-β seems to be released in the general environment. However, the effect may be related to the microenvironment in which the TGF-β exerts its action. Indeed, a bystander suppressive effect has been noted (1).

**Dr. Brandtzaeg:** What do we know about retention of antigen on the antigen-presenting cells in the gut? How long do you think your antigen will be retained there? I am thinking of your results. Could they be explained by T cell sequestration as occurs in celiac disease when gluten is eaten, when it is very difficult to find gluten-reactive T cells in the circulation? Your suppression of delayed-type hypersensitivity could be explained by sequestration of specific cells in the gut if antigen is retained after it is eaten. Have you thought about that possibility?

**Dr. Husby:** Antigen taken up from the gut usually disappears within 8 hours. Antigen-reactive cells may be sequestered and remain in the gut. However, we are talking about weeks (approximately 20 days). The possibility seems unlikely, but in theory this could be an explanation, yes.

**Dr. Brandtzaeg:** You present animal models where you have suppression of delayed-type hypersensitivity peripherally. Have you any way of checking that T-cell-type hypersensitivity is also suppressed locally in the gut?

**Dr. Strobel:** You can induce a T-cell-mediated response in the gut by abrogating tolerance in several ways: by feeding the antigen to a neonatal animal and rechallenging without immunization, or by eliminating T suppressor cells with cyclophosphamide, or by stimulating antigen-presenting cells with an adjuvant. All these measures, when applied at the time an antigen is given, will lead to intraepithelial lymphocyte proliferation in the gut when the animals are rechallenged. If you continue, you might get a moderate degree of hyperplastic villous atrophy, but usually you only get an increase in crypt cell production rate and a slight decrease in villous height. So, there is evidence that these so-called systemic delayed-type hypersensitivity responses could be active at the gut level.

**Dr. Hill:** I am trying to think of some examples in clinical practice and, in particular, in food allergy, which might correspond to the sort of phenomenon you are investigating. We know from children with IgE-mediated milk allergy followed sequentially into clinical tolerance that there appears to be no difference in the IgE and IgG isotype responses to milk proteins. It is my impression that skin test reactivity diminishes in some of those children after they have developed tolerance to cow’s milk. Have you done any studies on children with food allergy, looking at that particular phenomenon?

**Dr. Husby:** Certainly, there is a tendency for clinical tolerance to develop in infants with cow’s milk allergy. In a longitudinal study I did with Arne Høst, we looked at IgE and IgG subclass antibodies and there was a tendency, though not statistically significant, for a decrease in the antibody levels, both of the IgE and IgG class.

**Dr. Hill:** We are looking at children over a 2-year period, half of whom have developed clinical tolerance to cow’s milk and half of whom have not. The interesting thing is that the children who developed clinical tolerance to cow’s milk had lower IgE responses to cow’s milk at the time they developed tolerance when compared to the children who had persistent
disease, but they had already set their IgE response at that level 2 years earlier. There was no significant difference between the initial and final antibody levels, so it seems almost as if there is a high responder trait and a low responder trait.

Dr. Bock: It seems to me that what we are interested in here is an experiment of nature that already exists. We just have to figure out how to dissect it and how to select the patients. We see children who are not sensitized, they tolerate certain food proteins, they become sensitized, they have clinical reactions—allergy. I think if we can figure out the molecular biology in these children, we could presumably apply it to those children who don’t outgrow their allergy.

REFERENCE