Induction of Tolerance in Humans

Effectiveness of Oral and Nasal Immunization Routes[a]

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INTRODUCTION

The diversity (humoral and cellular response in the mucosal and systemic immune compartments) and dichotomy (specific response versus tolerance) of the immune responses induced by the mucosal route of antigen exposure are essential for survival of an individual in an environment rich in foreign antigens and infectious agents. Decreased or abolished antigen-specific systemic unresponsiveness achieved by preceding ingestion of the same antigen has far-reaching consequences for human health. Successful suppression of humoral as well as cellular systemic responses to environmental allergens (*e.g.*, tree and grass pollens), autoantigens (*e.g.*, myelin basic protein and collagen type II), and alloantigens (*e.g.*, transplantation antigens) by ingestion of the corresponding antigens could alleviate health problems of large populations of patients with atopic and autoimmune diseases or, possibly, organ transplantation recipients.[1-6] By contrast, induction of tolerance by ingestion of oral vaccines could impair protective immune responses in mucosal tissues—the most frequent portal of entry of infectious agents.[7,8] The factors that influence the balance between the desired (protective immunity) and undesired (suppression) response to oral immunization with microbial antigens are not yet known. Why does one soluble protein antigen given orally induce systemic tolerance (*e.g.*, ovalbumin), whereas another induces neutralizing antibodies in external secretions (*e.g.*, glucosyltransferases of *Streptococcus mutans*)?[1,2,8] A partial answer to this paradox was obtained from studies

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of mucosal and systemic immune responses in mice orally immunized with soluble and particulate antigens.[9] These studies suggested that systemic immune unresponsiveness may coexist with active mucosal responses, manifested by the presence of antigen-specific secretory IgA antibodies, and stressed the relative independence of the systemic and mucosal compartments of the immune system.[7,10,11] Further compartmentalization has been observed even within the mucosal immune system: an allergen encountered by the respiratory route may induce an immediate-type hypersensitivity, and yet the same allergen given orally desensitizes an individual to a subsequent respiratory tract challenge.[1,5,6] Although some factors, including the nature and dose of an antigen, species, age, and genetic background of the individual, have a profound effect on the end result of an antigen encounter at a mucosal surface, many other factors remain unexplained.[1,2] Furthermore, abundant literature concerning oral tolerance deals, with a few exceptions, with animal models.[1,2]

ORAL TOLERANCE IN HUMANS

Erroneous interpretation of Dakin's[13] results from 1829 have led to a perpetuation of a story of decreased skin reactivity to poison oak and ivy by chewing leaves of these plants. Nevertheless, several investigators (for review see ref. 14) have achieved a substantial reduction in skin reactivity after a long-term ingestion of initially minute but progressively increasing doses of extracts of fresh or dry leaves. Analogous suppression of contact hypersensitivity has been observed by the feeding of low doses of haptens to volunteers;[15] specific serum and mucosal antibodies were not evaluated. However, in a typical protocol for induction of oral tolerance in animals, large doses of protein antigens are necessary to induce systemic unresponsiveness.[1,2] Thus, human newborns and adults were fed with bovine serum albumin (BSA), and sera were examined for anti-BSA antibodies.[16,17] The response varied with the age of the individuals; surprisingly, newborns were immunized rather than tolerized. However, in adults, despite continued oral stimulation with BSA, the levels of serum antibodies decreased progressively with age. Although there were marked variances in levels of serum and secretory antibodies to dietary antigens,[18] most adults usually had low levels, and the titers did not increase upon parenteral immunization despite prolonged ingestion. These results suggest that an extended exposure to common dietary antigens may indeed lead to a diminished systemic responsiveness reminiscent of an oral tolerance.

To address the question of basic importance—the induction of systemic unresponsiveness in humans by mucosal exposure—groups of volunteers were first exposed to a protein antigen by the intestinal or nasal route and subsequently immunized by the systemic route; both humoral (serum and secretory antibody levels) and cellular (T-cell proliferation and delayed-type hypersensitivity) responses were measured.[19,20] Studies carried out in humans are usually hampered by difficulties with the selection of an antigen that has not been encountered previously as a component of the diet. To avoid this problem, we used keyhole limpet hemocyanin (KLH), a potent systemic immunogen in humans and animals, and an excellent oral tolerogen in animals.

Oral Immunization[19]

A group of eight volunteers was administered 500 mg KLH orally divided in 10 doses given on days 1-5 and 15-19; subcutaneous immunization (100 μg KLH) was performed on days 26 and 36. Levels of serum, salivary, and intestinal IgG, IgM, and IgA anti-KLH antibodies (measured by ELISA) were determined on days 0, 10, 26, 36, and 44; antibody-

secreting cells (ASC) were enumerated (by ELISPOT) on days 0, 26, and 44; T-cell proliferation was determined on the same days. A control group of eight volunteers received only two subcutaneous doses of KLH (100 μg).

Ingestion of 500 mg KLH did not induce significant levels of IgA, IgG, and IgM antibodies in sera or external secretions, or anti-KLH ASC in peripheral blood. However, feeding followed by systemic immunization resulted in significantly higher levels of serum and secretory antibodies and ASC in KLH-fed and systemically immunized volunteers, compared to those receiving only systemic immunization. Thus, mucosal immunization had a priming effect, manifested by higher humoral immune responses (FIG. 1). This priming effect of oral immunization was not seen when T cell-mediated responses were evaluated. Instead, T-cell proliferation and delayed-type hypersensitivity testing (10 μg KLH, intracutaneously) revealed that KLH-fed and systemically immunized volunteers displayed lower stimulation indexes (FIG. 2) and skin reactivities than volunteers who received only systemic KLH injections (TABLE 1).

These studies demonstrated that KLH given orally at this dose and by this schedule altered the outcome of a subsequent systemic immunization: a priming effect was seen in humoral serum and secretory antibody responses, but a tolerogenic effect was seen in T-cell responses (*in vitro* T-cell proliferation and *in vivo* skin reactivity). Thus, the induction of tolerance was restricted to the T-cell compartment.

Intranasal Immunization[20]

Although frequently used for stimulation of mucosal responses to viral vaccines,[7,8,21] rarely has the intranasal immunization route been considered for induction of tolerance in animal models.[12]

In humans, intranasal immunization with tetanus toxoid induced an increase of serum IgA antibodies to a subsequent intramuscular immunization.[22] Because almost all volunteers had been exposed to tetanus toxoid by previous systemic immunization, the priming effect of intranasal exposure was difficult to evaluate. Therefore, we used KLH in aerosol spray given as three to four doses (100 mg each) on days 0, 14, 28, and 42. Three months after the final intranasal immunization, the four volunteers involved in this study received 100 μg KLH subcutaneously. A control group comprised eight volunteers immunized only subcutaneously (100 μg KLH). In contrast to oral immunization, intranasal exposure to KLH resulted in the appearance of ASC of IgA, IgM, and IgG isotypes in peripheral blood 7-11 days (peak on day 9) after the first immunization. Interestingly, this response was not boosted by subsequent intranasal immunizations. Anti-KLH antibodies of IgA, IgG, and IgM isotypes were detected in sera and secretory IgA in nasal secretions after two to three intranasal immunizations (FIG. 3).

Subcutaneous immunization (100 μg KLH) carried out after a three-month hiatus resulted in an appearance of IgA and IgG ASC in peripheral blood in both experimental and control groups of volunteers. However, a boosting effect of mucosal priming seen in orally immunized individuals was not present in intranasally immunized individuals. Although not statistically significant, intranasally and subcutaneously immunized volunteers displayed a lower IgG and IgA ASC response than only subcutaneously immunized individuals. This was also reflected in lower serum IgG and IgA antibodies to KLH in the experimental group.

In agreement with orally immunized volunteers, intranasal immunization resulted in an equally profound and statistically significant decrease in delayed-type hypersensitivity, as evaluated by skin testing (TABLE 1).

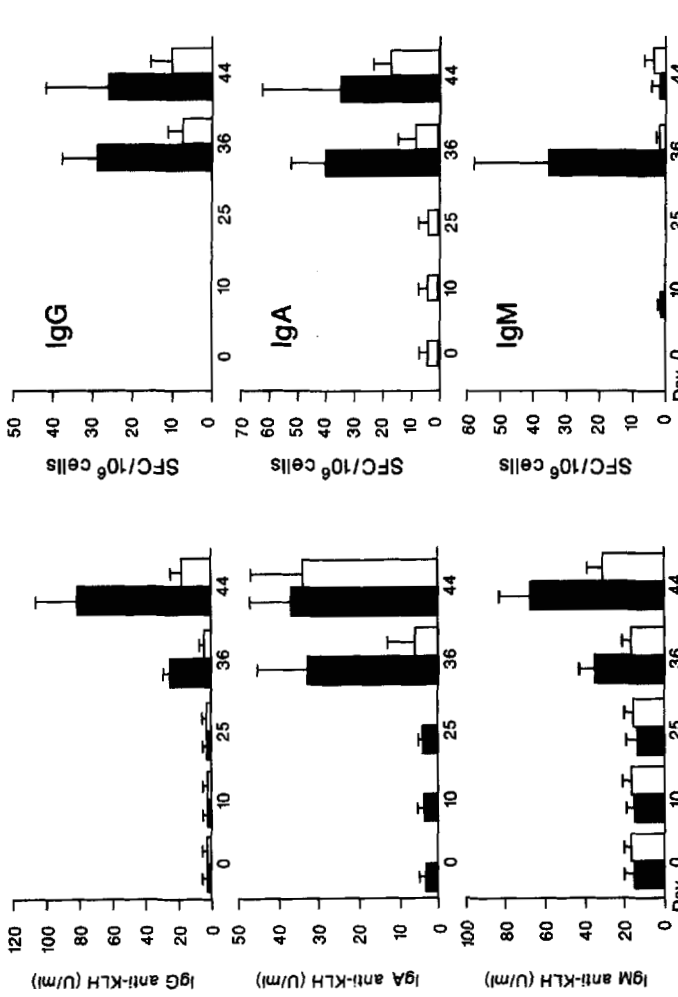

FIGURE 1. Left: IgG, IgA, and IgM anti-KLH antibodies in sera of KLH-fed and sc immunized (■) and control (□, sc immunized only) groups of volunteers. Right: Antibody (spot)-forming cells (SFC) in the peripheral blood of KLH-fed and control group volunteers. Oral immunization was administrated on days 1-5 and 15-19; subcutaneous immunization was on days 26 and 36.

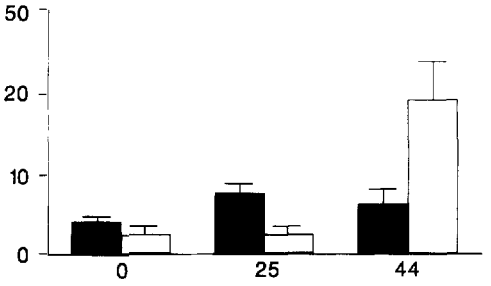

FIGURE 2. KLH-induced proliferation (SI) of peripheral blood T cells from KLH-fed ■ and control □ group volunteers.

DISCUSSION

Despite the differences in the immunization protocols, our studies have demonstrated that systemic cellular immune responses to KLH were markedly altered by previous oral or intranasal immunization with the same antigen.[19,20] Delayed-typed hypersensitivity reactions were consistently diminished in both experimental groups when compared to only systemically immunized volunteers. The most notable difference was seen in the appearance of serum antibodies. Although oral immunization with a total dose of 500 mg KLH primed volunteers for a subsequent systemic response, serum antibodies as well as ASC in peripheral blood were not induced by oral immunization. By contrast, a single intranasal dose of 100 mg KLH resulted in the appearance of both serum antibodies and peripheral blood ASC. This variance may have been due to a relatively low absorption of undigested KLH from the intestinal tract, and a marked, but not fully appreciated, difference in the compositions of immunocompetent cells in the intestinal and respiratory tracts.[23,24] Furthermore, repeated intranasal immunizations did not result in increased numbers of ASC in peripheral blood, and did not prime, in contrast to orally immunized individuals, for subsequent systemic boosting. Therefore, it appears that the primary intranasal immunization with a protein antigen in humans is perhaps even more effective in the induction of systemic tolerance than the oral administration of such antigens. It must be strongly emphasized, however, that these are only initial studies performed with a rather limited number of volunteers, a single antigen (KLH) without extensive determination of optimal doses and the most effective immunization frequencies, and evaluation of antigen-delivery systems that would induce the most profound systemic unresponsiveness.[25] Nevertheless, with these limitations, it is apparent that oral, or perhaps more correctly mucosal, tolerance defined by systemic unresponsiveness to mucosally

TABLE 1. Delayed Hypersensitivity Test Responses to Intradermally Injected KLH (10 μg) of Orally or Nasally Immunized Volunteers

Group	Mean (Range)	Number of Positive/Total
KLH oral	0 (0-0)	0/8
KLH nasal	0.05[a] (0-0.2)	1[a]/4
Controls	11.9 (0-23)	7/8

[a] Redness but not induration.

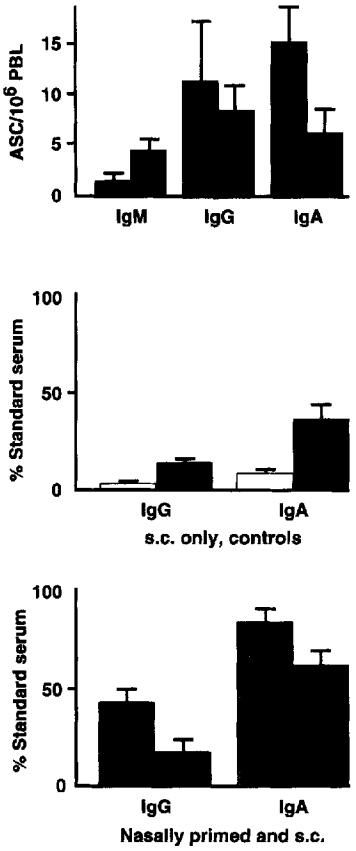

FIGURE 3. Antibody-secreting cells (ASC) and serum antibodies to KLH, nasally and sc immunized (■), and control (□, only sc immunized) groups of volunteers. Top graph: ASC were determined one week after systemic immunization (week 17 after the beginning of intranasal immunization on weeks 0, 2, 4, and 6). PBL, peripheral blood lymphocytes. Middle graph: Serum anti-KLH responses: □, preimmune; □, one week after systemic (sc) immunization. Bottom graph: Serum anti-KLH responses (□) after intranasal immunization; ■, intranasal and sc immunizations.

administered antigens can be induced not only in species such as mice and rats but also in humans.

UNRESOLVED PROBLEMS AND FUTURE STUDIES

The molecular and cellular mechanisms involved in the induction of oral tolerance in humans remain unclear;[1,2] current concepts that are beyond the scope of this communication are discussed elsewhere in this volume. Because of the enormous potential medical importance of mucosal tolerance in the prevention and treatment of immediate as well as delayed-type hypersensitivity reactions[1,5,6,14,15] and some autoimmune diseases (*e.g.*, multiple sclerosis,[3] rheumatoid arthritis,[4] and possibly others), further studies are warranted. Specifically, (1) the relative effectiveness of the oral[19] versus nasal,[20] or rectal immunization routes should be compared; (2) the types and forms of relevant antigens for optimal tolerization should be further examined; (3) optimal antigen-delivery systems, such as covalent linkage to cholera toxin B subunit,[25] should be evaluated in humans to maximize the tolerance and at the same time minimize the doses necessary to make mucosal tolerance

applications economically feasible; and (4) immunization protocols should be explored that would selectively enhance or suppress the type of desired immune response. The last point deserves a particular emphasis in studies of orally or mucosally delivered microbial vaccines whose development is currently promoted by national and world health agencies.[7,8] Although, as expected, mucosally delivered viral and bacterial vaccines induce mucosal and often serum antibodies, T cell-mediated protective responses and induction of cytotoxic T cells in humans have not been adequately studied.

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