

Immune response versus mucosal tolerance to mucosally administered antigens

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Abstract

The enhancement of protective mucosal responses to infectious agents sought by vaccinologists, and the desired suppression of immune responses to autoantigens, allergens and food antigens induced by mucosal exposure to relevant antigens, may seem paradoxical. However, such outcomes are not mutually exclusive because of a hierarchy in the quality of immune responses: mucosal immunity manifested by the appearance of secretory antibodies and systemic tolerance manifested by diminished cell-mediated responses may be induced concomitantly. We have demonstrated that the ingestion, or nasal application, of a neo-antigen–keyhole limpet hemocyanin (KLH)-primes human volunteers for subsequent humoral mucosal and systemic responses induced by systemic immunization but suppresses cell-mediated responses (T cell proliferation and delayed-type hypersensitivity). Furthermore, extended ingestion of selected food antigens diminished, but did not totally suppress, cell-mediated immunity and levels of serum antibodies while salivary antibodies were easily detectable. Most importantly, the humoral and cellular immune responses induced by systemic immunization with KLH could not be suppressed by subsequent extended ingestion of large doses of the same antigen. Thus, ongoing systemic responses are refractory to the induction of mucosal tolerance in humans. This finding is of considerable importance for the development of and sequence of immunization with mucosally administered vaccines.

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1. Introduction

The induction of preventive and protective immune responses to mucosal infectious agents, and to inert food antigens and environmental allergens that would limit their absorption is the most emphasized functional aspect of the mucosal immune system. Yet, recently revived interest in oral or nasal (mucosal) tolerance, i.e., the induction of systemic unresponsiveness to antigens applied first by the mucosal route, has directed the attention of immunologists working in the fields of autoimmunity, transplanta-

tion, and hypersensitivity to the exploitation of this fundamental principle. Although limited in its clinical success, the phenomenon of mucosal tolerance is an essential feature and critical functional component, which efficiently prevents over-stimulation of the entire immune system by the common environmental antigens represented by food and the indigenous bacterial microbiota. The goals of enhancement of protective mucosal responses to infectious agents sought by vaccinologists on the one hand, and the suppression of systemic immune responses to autoantigens and transplantation antigens on the other hand, may seem paradoxical. Yet, such outcomes are not mutually exclusive due to the compartmentalization of immune responses induced by mucosal immunization. Antigen feeding can concomitantly induce both mucosal immunity manifested by the appearance of secretory antibodies and systemic tolerance manifested by diminished cell-mediated responses. Thus, the

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fundamental objectives of the mucosal immune system is containment of the vast onslaught of environmental antigens without compromised integrity of mucosal barriers and prevention of over-stimulation of the systemic compartment which are achieved by concerted interactions of lymphoid and non-lymphoid cells, epithelial cells in particular, and their respective products as a mucosal internet of communication.

2. Does mucosal tolerance exist in humans?

Induction of immune response and mucosal tolerance by mucosally administered antigens depends on many variable factors including the species, genetic background, and age of the animal, dose, form (soluble versus particulate, and live versus inactivated), and the route and sequence of antigen administration [1]. Moreover, the use of adjuvants may accentuate either immunity or tolerance [1]. Understandably, almost all experiments concerning the induction of mucosal tolerance have been performed in rodents such as mice and rats, using classical tolerogens such as ovalbumin (OVA), bovine serum albumin (BSA) or bovine gamma-globulin (BGG), as well as antigens that are relevant in animal models of autoimmune diseases. In the latter case, extensive experiments performed in animals convincingly demonstrate that oral or nasal exposure to antigens such as myelin basic protein (MBP) or collagen Type II with adjuvants (e.g., cholera toxin B subunit [CTB]) induces a state of unresponsiveness in the systemic compartment upon subsequent systemic immunization. In animal models of experimental allergic encephalomyelitis and arthritis, mucosal exposure to these antigens prevents the development of the disease [1,2]. Because of the obvious danger in overextending and extrapolating data based on animal models to humans, several more recent studies have addressed the induction of mucosal tolerance in humans. The above-mentioned classic tolerogens were not used due to pre-existing exposure to such antigens in ingested food; instead, a neo-antigen–keyhole limpet hemocyanin (KLH) was given by the oral or nasal route [3–5]. Surprisingly, extended ingestion or nasal administration primed the humoral arm of the systemic as well as mucosal compartments so that subsequent systemic immunization with KLH induced significantly higher serum and mucosal antibodies and circulating anti-KLH-secreting B cells in the KLH-fed group than in controls. In contrast, T cell-mediated responses, defined as *in vitro* antigen-driven proliferation and delayed-type hypersensitivity, were both suppressed. Thus, the tolerogenic effect of mucosally administered antigen was manifested exclusively by suppressed T cell responses (primarily Th1) with a boosting effect on humoral, particularly mucosal, responses (so-called split tolerance). These results have been fully confirmed and extended in a recent study by Kraus et al. [5]. Thus, mucosal tolerance can be induced in humans by ingestion of previously unencountered antigen.

3. The immune response to long-term antigen feeding

To prevent the over-stimulation of the entire immune system, the dominant response to daily ingested massive doses of food-derived antigens must be tolerance. Indeed, serological studies in children and adults revealed that humoral responses to BSA and α -lactalbumin decrease with age [6]. Recently, we evaluated the magnitude and quality of humoral and cellular responses to BGG, OVA, and soybean protein (SBP) in adult normal human volunteers as a surrogate for prolonged antigen feeding [7]. Humoral and T cell proliferation responses to these antigens were measurable but low, consistent with immune tolerance. There were limited correlations between responses in the systemic and mucosal compartments, and in general the responses to one dietary antigen could not predict the response to another. T cell proliferation to dietary antigens was increased significantly by the addition of recombinant human interleukin-2 (rhIL-2). Peripheral blood mononuclear cells stimulated with BGG or OVA expressed IL-2R α chain but not IL-2 mRNA, consistent with T cell anergy. Incubation with exogenous IL-2 alone did not restore T cell proliferation to BGG or OVA. In some individuals, T cell proliferation to an unrelated vaccine antigen was suppressed by the addition of BGG or OVA, but could be reversed with low doses of rhIL-2. We concluded that, in humans, anergy is the major mechanism of tolerance to chronic antigen feeding, and we proposed that such anergic, antigen-specific T cells actively contribute to maintenance of homeostasis in the intestine in the face of massive antigen challenge. The comparative levels (μ g of antigen-specific/mg of total) of anti-OVA, BGG, and SBP in sera and secretions indicated that salivary IgA levels were substantially higher than in serum; this finding is consistent with local mucosal synthesis rather than circulatory origin. Further analyses of serum and mucosal antibodies of major isotypes have revealed that the mucosal and systemic humoral responses are not regulated in parallel but display a considerable degree of mutual independence [7,8]. Important for vaccine application is the fact that despite the ingestion of large quantities of foreign proteins such as BGG, an average daily consumption in American diet is 0.5 g [9] (this translates to $\sim$ 180 g/year and $\sim$ 5 kg/30 years) mucosal IgA responses specific for ingested antigens remain high with no apparent signs of tolerance [8]. In fact, high levels of mucosal antibodies to environmental antigens were observed even in individuals in their late eighties [10].

4. The route and sequence of mucosal versus Systemic priming and boosting play essential roles in the induction of immunity versus tolerance

In animal models of autoimmune diseases such as experimental allergic encephalomyelitis, collagen Type II–induced arthritis, and autoimmune diabetes mellitus, initial mucosal exposure to the autoantigen prevents the development of dis-

ease otherwise induced by systemic immunization with the relevant antigen in adjuvant [1,2,11,12]. Because of these data, it was incorrectly assumed that feeding of the corresponding antigen to patients with an existing disease (multiple sclerosis and rheumatoid arthritis) would result in the improvement or at least arrest in the progression of the disease. Studies performed in animals [13] and humans [10,11,14,15] have demonstrated the futility of this approach: animals first systemically sensitized to the hapten could not be tolerized by subsequent antigen feeding and patients with delayed-type hypersensitivity reactions could not be treated by ingestion of the conjugated hapten [11–13]. In the same vein, a double-blind multi-center study of the treatment of patients with multiple sclerosis or rheumatoid arthritis by long-term ingestion of bovine MBP or cartilage collagen Type II, respectively, did not result in disease improvement [16]. Finally, Moldoveanu et al. [17] clearly demonstrated that volunteers first systemically immunized with KLH were not tolerized by subsequent long-term ingestion of the same antigen (KLH). Thus, systemically induced B and T cell responses are not suppressed by subsequent delivery of the relevant antigen alone to the mucosa. This finding is of basic importance for mucosally-delivered vaccines: pre-existing systemic immunity induced by natural infection or systemic immunization is likely to effectively prevent the induction of tolerance by mucosal immunization.

5. Concluding remarks

Mucosal tolerance is an inseparable and essential feature of the entire immune system: it prevents the over-stimulation with a multitude of antigens encountered in everyday life by the mucosal routes. However, it is highly unlikely that orally, nasally, or vaginally applied vaccines would induce mucosal humoral tolerance rather than a protective immune response. As demonstrated in several studies performed in humans and in animals, extended mucosal exposure to antigens induces decreased systemic responsiveness of Th1-mediated pathway but actually primes for subsequent humoral immune responses in both mucosal and systemic compartments. Considering the fact that all vaccines used in human medicine (irrespective of the route of administration) exhibit their protective effect through the induction of antigen-specific antibodies rather than cytotoxic T lymphocytes (CTL), the fear of inducing tolerance with vaccines is unwarranted. However, the effect of extended exposure of mucosal surfaces to inactivated viruses or killed bacteria on subsequent induction of CTL in the mucosal or systemic compartments by live attenuated microorganisms or infections has not been evaluated experimentally. Nevertheless, it is obvious that existing cellular and humoral systemic responses are refractory to the potential tolerogenic effect of mucosally administered antigens.

The parallel induction of mucosal immunity, manifested by the appearance of antigen-specific IgA antibodies in ex-

ternal secretions, and systemic T cell hyporesponsiveness as a consequence of mucosal exposure to antigens [18] appear to share a common regulatory pathway. The regulatory cytokines, TGF β and IL-10, produced by mucosal T cells and epithelial cells play an essential role in the switching and terminal differentiation of B cells into IgA-producing cells and, at the same time serve as central T cell-derived mediators of systemic unresponsiveness [19]. This cytokine-mediated pathway of B cell differentiation to the production of mucosal IgA with its non-inflammatory properties [20] and suppression of potentially deleterious systemic responses represents one of the most physiologically important aspects of the immune system.

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