

Oral tolerance to protein antigens

Oral tolerance is an active non-response to antigens delivered via the oral route. Mechanisms governing tolerance induction have been well characterized in mouse. Similar studies in man are lacking, although there is evidence that tolerance can be induced. In disease states, tolerance is altered and this may account for the presence of mucosal inflammation. In food hypersensitivity there is evidence that allergens may be handled differently and this may play a role in disease expression.

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For quite some time, investigators have recognized that the administration of foreign substances by mouth results in the absence of an immune response despite the presence of an overwhelming number of immunocompetent cells in the intestinal mucosa. While early on this observation was relegated to the level of empiricism, over the past three decades we have come to recognize this phenomenon as an active process of immune regulation called oral tolerance. H.G. Wells was one of the first investigators to document that orally administered antigens protected from hypersensitivity reactions but it wasn't until the 1970s that investigators established a role for lymphocytes and subsequently T cells in this process (1–6). This changed the definition of oral tolerance into one where there were active events resulting in immune unresponsiveness. Significant progress has been made in our understanding of this phenomenon along with the recognition that tolerance can be mediated by several mechanisms. What has been less well explored is the role that oral tolerance might play in various disease states. These include food allergy and inflammatory diseases of the bowel.

Overview of oral tolerance on mice

As alluded to above, initial studies in mice supported a role for regulatory T cells in the induction of oral tolerance. Several investigators demonstrated that following oral administration of various soluble protein

Ags (e.g. OVA), there was a marked reduction in T cell proliferative responses and antibody responses after systemic challenge (7). Initial studies using the transfer of whole splenocytes and subsequently CD8+ T cells documented that tolerance could be transferred to naïve animals (7–11). Tolerance was antigen specific and there was no evidence of generalized immunosuppression.

More recently, Weiner and his colleagues have identified an additional cell type involved in tolerance induction. He has described a CD4+ T cell population (Th3) present within Peyer's patches which promotes tolerance via the secretion of TGFβ and to a lesser extent IL-10 and IL-4 (12–14). Using specific oral feeding regimens he has been able to transfer tolerance by transferring Peyer's patch CD4+ T cells. These Th3 cells within the patch subserve other functions as well. TGFβ is the factor which promotes an isotype switch in B cells from IgM to IgA (15). Such cells, described initially by Kawanishi et al. (16–18), help to explain the unique phenomena recognized in the GI tract; selective production of secretory IgA, suppression of IgG and IgM secretion and the induction of oral tolerance.

However, tolerance is not the same in all species or in fact in all strains of mice, supporting a genetic component to tolerance induction (19, 20). Some strains are easy to tolerize (Balb/c), while others are not (DBA/2). Furthermore, there is a hierarchy in terms of tolerance induction; Th1 cells being more easy to tolerize than Th2 cells and B cells (the most resistant to

tolerance) (21, 22). We have also learned about other important facets of tolerance. As seen in Table 1, tolerance is dependent upon the nature of the antigen – soluble proteins induce tolerance, whereas particulate or globular Ags fail to do so. The dose of Ag is also important. Low doses of Ag activate regulatory T cells (Th3 cells), whereas high doses of antigen induce anergy or deletion (21–23).

The description of Th3 cells helped to explain another phenomenon seen with oral tolerance, that of bystander suppression (24–27). As mentioned earlier, oral tolerance is antigen specific. However, TGFβ is the most potent antigen nonspecific suppressor factor known. OVA-specific Th3 cells will only secrete TGFβ when activated by OVA peptides. If a second Ag is administered simultaneously, there is suppression of the response to that Ag as well. This phenomenon has been termed bystander suppression and has been used by several groups as a means to treat autoimmune disorders in mouse models. The concept is that feeding an animal an antigen which is expressed in the organ involved in the autoimmune process will result in the activation of Th3 cells in that organ, thereby non-specifically suppressing the activity of autoreactive T and B cells. While this has worked extremely well in mouse models, its efficacy in humans has been less compelling (28–31). This has led to the concept that oral tolerance may in fact be different in genetically outbred humans or even in genetically outbred mice.

Overview of oral tolerance in man

Despite anecdotal reports of tolerance like events in man, several studies have verified the presence of food antigen specific T and B cells in peripheral blood. Such a finding would suggest that tolerance may be very different in humans. However, it wasn't until a study by Husby et al. (32–35) that the ability to develop oral tolerance to a neoantigen in man was assessed. What this group showed was that tolerance in man was different than that described in mouse. Suppression of DTH responses was seen but there was no effect on Ab secretion. No measurement of cytokines was performed. There is also indirect evidence of skewing of the immune repertoire by oral Ag administration. Hafler and colleagues fed MBP to a group of patients with multiple sclerosis. MBP reactive T cell clones from those patients showed a skewed reactivity to an MBP

peptide 84–102, an encephalogenic peptide in mice. After feeding, the dominant reactivity was shifted to a non encephalogenic peptide 28–34. This was the first evidence that skewing of an immune response could be mediated by a change in the route of antigen administration ADDIN ENRfu (13). Such a finding suggests that the manner by which antigen is sampled may dictate the response generated.

Antigen uptake in the GI tract

A phenomenon probably linked to oral tolerance is that of controlled or physiologic inflammation in the gut. Despite the presence of roughly 10^{12} bacteria in the gut, normal individuals fail to develop T or B cell reactivity to this sea of antigens. However, there are activated T cells, B cells and macrophages in the lamina propria which do not change under normal conditions. The mechanisms underlying the phenomenon of controlled inflammation are not understood but clearly reflect some form of suppression. The key question is how this suppression is induced. The studies from Weiner's and Strober's group would suggest that Ag sampled by the epithelium overlying Peyer's patch would insure a Th3 response. However, soluble proteins are ineffectively taken up by these specialized cells called M cells. Studies from a number of groups have described soluble antigen uptake by surface or absorptive epithelium (36–42). Such uptake can result in presentation to T cells and selectively to CD8+ suppressor T cells (39, 43). If there was a defect either in the way Ag was sampled or in the activation of these regulatory cells, then disease might ensue. There have been some new findings in this area which may have implications for food allergy. Berin et al. (40) previously described the finding that in pre-immunized rats, feeding soluble Ags would promote subepithelial mast cell degranulation within 3–5' of exposure. Normally, Ag uptake by IECs is much slower, so it was proposed that novel mechanisms were triggered to facilitate antigen trafficking. More recently, this same group has shown that IL-4 induces CD23 (FcεRII) expression on the surface of enterocytes and that IgE/allergen complexes are rapidly internalized and transcytosed by this mechanism (44). Thus in a genetically predisposed individual, IL-4 production could be enhanced, resulting in greater CD23 expression on IECs. Food allergens encountering IgE within the lumen would be taken up by FcεRII and food allergic symptoms would result.

In other studies, we have been able to document that certain food allergens may display facilitated uptake in the absence of IgE or CD23. Previous studies from our group have demonstrated that Ag uptake by IEC is a reflection of solubility of the Ag and that neither size nor cytokines could alter the kinetics of uptake (e.g. OVA (43 kDa) and KLH (3500 kDa), are taken up with

Table 1. Factors affecting the induction of oral tolerance

Age	Newborn poor tolerance
Genetics	Different susceptibility to tolerance induction in distinct mouse strains
Nature of antigen	Soluble vs. particulate – soluble more tolerogenic
Dose of antigen	High dose – anergy/deletion Low dose – regulatory cells

similar kinetics) (45, 46). When we evaluated a series of food allergens – casein, α lactalbumin and β lactoglobulin – they were taken up with slow kinetics. However, crude peanut extract was internalized rapidly and exhibited rapid transcytosis in a colonic epithelial cell line. Thus it remains conceivable that the nature of the Ag will dictate the route and the type of immune response generated.

Oral tolerance in disease

Given defects in IECs defined in IBD, we undertook studies to define whether oral tolerance exists in patients with this disorder. Using a protocol similar to that of Husby et al. ADDIN ENRfu (35), we measured tolerance induction to KLH. At a low dose of Ag (5 mg $\times$ 10 doses) normal volunteers failed to become tolerant. At the same dose, however, in patients with quiescent Crohn's disease both T cell and antibody responses were elicited, even before systemic challenge. In ulcerative colitis no immune response was seen after feeding but primary systemic immunization resulted in

a brisk T cell and antibody response, suggesting that oral feeding had primed an immune response in these patients. Similar studies are underway in patients with cow milk allergy.

Conclusions

The literature reviewed as well as the studies presented support the concept that oral tolerance exists in man. While it is clear that oral tolerance is not the same as that described in mouse, these studies allow for the initiation of similar studies in food hypersensitivity. The hypothesis proposed, that the mode of antigen uptake dictates the type of immune response generated, is certainly a testable one.

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