

Mucosal immunity in relation to the development of oral tolerance/sensitization

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The mucosal immune system has evolved specifically to protect the mammalian host against pathogens from the outside environment, and for this purpose employs a wide range of mechanisms for sampling, processing, and finally presentation of antigens derived from these pathogens to the T-cell system. However, the mucosal immune system is faced with a major logistical problem, that of “classification” of antigens into specific categories, which in turn dictate the type of immune response which is required.

The nature of the problem is essentially low signal-to-noise ratio, “signal” equating to genuine pathogenic antigen, and “noise” equating to non-pathogenic soluble proteins that are ubiquitous in the natural environment. The most common of these are antigens present in foodstuffs. Clearly, if these all evoked immune responses equivalent to viruses or parasites, chronic gastrointestinal inflammation would be the rule as opposed to the exception.

The evolutionary solution to this problem is encapsulated in the mechanism(s) of oral tolerance (OT). Thus, as first described in immunologic terms by Wells & Osborne in 1911 (1), inert protein antigens fed to immunologically naive animals elicit initial immune responses which are now recognized as Th2-polarized (a subset of their animals died during exposure from anaphylaxis [1]), but with continuing exposure these responses are self-limiting, and are supplanted by a state of antigen-specific unresponsiveness or “tolerance”. Thus, the antigen can in future be ingested with

impunity; i.e., it has been classified successfully by the immune system as an inert, nonpathogenic antigen, and hence acceptable as a dietary component.

Failure(s) in this OT mechanism are believed to lead to a variety of enteropathies, including classical food allergy (2). A comparable mechanism is operative at the level of the airway mucosa, for regulation of immune responses to nonpathogenic airborne antigens (3, 4).

Oral tolerance (OT): a multifaceted process

Qualitative aspects of the tolerance process

From experimental models, it is clear that OT is readily inducible for a wide range of soluble and particulate antigens, with the proviso that antigen supplies are not limiting (5). It is additionally clear, however, that a distinct hierarchy exists within the immune response with respect to ease of OT induction. At the extremes, the IgE component of the immune response appears most sensitive, and secretory IgA most resistant (reviewed in References 2, 4); IgG production falls between the latter two, but is less readily tolerized than DTH.

Host factors related to susceptibility to OT

Genetic background, at least insofar as genes determining IgE responder phenotype are concerned, does appear to influence susceptibility to

downregulation of IgE responses to fed antigens, as demonstrated in studies in both rats and mice (6). However, data are available only for low-dose/chronic exposure models, which (as detailed below) may employ mechanisms different from those operative at high-dose exposure.

Hormonal status may also influence OT susceptibility (7), but more important is age. It is clear from studies in mice that OT mechanisms are inefficient in newborn animals and are not fully functional until weaning (8, 9). This is likely to be of major importance in man with respect to regulation of IgE responses to dietary allergens (see below).

Environmental factors

A range of environmental factors influence this process; in particular, inflammatory agents which can affect the integrity of the gastric mucosa. These observations stem originally from studies with inflammatory adjuvants (10, 11). In addition, agents which produce transient systemic immunosuppression can also inhibit OT development (10–13).

Factor(s) released by the gastrointestinal microbial flora have been thought to affect the efficiency and longevity of OT, but these findings have not proven to be reproducible in all systems (5). However, they do appear to play a major role in relation to the tonus of mechanism(s) which regulate the most sensitive part of the immune response; i.e., IgE. In particular, recent evidence indicates that the presence of a conventional gut flora appears central to the normal postnatal maturation of Th2-suppressive aspects of OT, during the period from birth to weaning (14).

Cellular mechanisms in experimental OT

The nature of the key mechanism(s) regulating OT have been the subject of considerable controversy over the last 25 years, but a cautious consensus is now emerging with respect to broad aspects of the process.

In particular, it is generally agreed that fundamental mechanistic differences exist between OT induced by low and high doses of ingested antigen (5). Thus, it appears that at low-level exposure the dominant mechanism is immune deviation, in contrast to T-cell anergy and T-cell deletion at progressively higher exposures (15–18). OT induced by low-level exposures is most readily transferred by cytokine-secreting T cells, and both TcR α/β^+ and TcR γ/δ^+ and CD4 $^+$ as well as CD8 $^+$ effector cells have been demonstrated in various models (5, 19). However, it is not clear whether the role of these

cells is in the induction or the maintenance of the tolerant state, given that OT can still develop in CD4 and CD8 knockout mice (5, 19).

The precise role of antigen-presenting cells in OT induction is yet to be determined. However, it appears highly likely that both mucosal dendritic cells and gastrointestinal epithelial cells play important roles in the process (5).

Tolerance in comparison with “desensitization”

There is intense interest in the exploitation of OT mechanisms for inhibition (or tolerization) of various effector cells in disease states via the use of high doses of specific antigen, and/or appropriate adjuvant (19). However, under conditions of more natural exposure, OT can usually be developed only in an immunologically naive host (2), and it is extremely difficult to suppress or immune deviate pre-existing mature immune responses. Thus, in relation to OT to dietary antigens in man, the key period would appear to be at or around infancy and early childhood, during which most dietary antigens are first encountered.

Does OT in man exist?

Experimental OT

There is convincing evidence from several studies (20) that classical OT to rare proteins such as KLH can be readily induced by feeding to naive adults.

OT to common dietary antigens

Food allergy is comparatively rare in the adult population, indicating overall that highly efficient mechanisms must exist for downregulation of immune responses to dietary antigens. However, most adults produce significant levels of IgG subclass antibodies, and IgA, against these antigens, suggesting selective suppression of IgE and DTH reactivity, as in the animal models (reviewed in References 4, 21).

The nature of the underlying immunoregulatory mechanisms in man remains to be elucidated, but recent developments in the paediatric allergy literature have provided exciting new insights into the overall process.

Food antigen-specific T-cell responses during infancy and early childhood

Recent studies from a range of laboratories suggest that immunologic events during early life are major

determinants of the allergen-responder phenotype in adulthood.

Employing lymphoproliferation, it has been shown that T-cell reactivity to airborne antigens increases progressively during childhood, becoming polarized toward Th1 or Th2 cytokine phenotypes in nonatopics and atopics, respectively. In contrast, responses to food antigens typically peak in infancy and then decline to levels that are most frequently just at or below the levels of detection with conventional T-cell assays (22). These divergent patterns of immune maturation within responses to different classes of environmental antigens suggest the underlying operation of qualitatively different types of control mechanisms; viz., immune deviation (for inhalant allergens) in contrast to T-cell deletion/anergy (for dietary allergens [23]).

More recent studies from our group reinforce this view. Thus, we have shown that weak Th2-polarized priming against dietary antigens is common during late gestation, and T-cell epitope analysis indicates recognition of a broad range of T-cell epitopes in antigens such as ovalbumin (OVA) (24). During early postnatal life, these responses are "driven" by direct (dietary) exposure, and typically peak with respect to antibody titre/affinity during the first few years of life; low-level but significant IgE antibody production is common during this period (4, 21). However, the responses then decline (especially IgE [4, 21]) and by the age of 5 years, food antigen-specific reactivity is rare as measured by lymphoproliferation and cytokine production (25, 26); epitope analysis of the rare OVA responders indicates that recognition has shrunk by this time to usually one epitope (25).

Analysis of the cytokine responses of 5-year-olds to inhalant allergens indicates universal reactivity, compartmentalized as classical Th2 in skin prick test positive subsets (interleukin [IL]-4, IL-5, IL-6, IL-9, IL-10 and IL-13) as opposed to weak Th1 (interferon-gamma only) in nonresponders. In contrast, cytokine responses of peripheral blood mononuclear cells to dietary allergens such as OVA are restricted to monocyte-derived IL-6 (presumably generated via FcR-mediated activation), and some residual IL-13 reactivity (26). The significance of these latter cytokines in the maintenance of OT remains to be established. However, it is noteworthy that the *in vitro* IL-6 responses are extremely high, and if reflected *in vivo* in the gut-associated lymphoid tissues, they may play a regulatory role in such processes as IgA switch regulation.

Conclusions

The phenomenon of OT, first described in animal models, has a clear counterpart in man. It typically

develops during early postnatal life, and is likely to play an important role in limiting the development of food allergy.

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