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Oral Tolerance in Experimental Animals

Key Words

Oral tolerance · Immune regulation · Experimental animals · Review

Background

There is a continuous influx of intact macromolecules and peptide fragments of various sizes from the gut lumen across the epithelium [1–3]. These antigenic molecules can be presented directly or at least processed by epithelial cells [4, 5] and then further presented by 'professional' antigen-presenting dendritic cells [6, 7], abundant in the basal epithelial region, to a numerous T cell population in the villi [8]. It is interesting to note that CD8⁺ T cells are morphologically closest to the epithelium, whilst CD4⁺ T cells are situated more centrally in the villi [8]. This morphological arrangement provides a cellular basis for regulating the immune response to soluble antigens entering from the gut lumen.

Early works proposed that antigen feeding gives rise to suppressor T cells which cause hyporesponsiveness in orally tolerant animals [9–11]. More recently, other studies have demonstrated that suppressor T cells of the CD8⁺ phenotype are triggered by antigen, but mediate their effects mainly by secreting the non-specifically-acting cytokine TGF- β [12–14]. A subpopulation of the CD8⁺ T cells bearing the $\gamma\delta$ T cell receptor has been shown to mediate antigen-specific suppression of the IgE response when transferred from aerosol-tolerised to naive recipient mice, possibly by production of IFN- γ [15].

Bystander Tolerance

As a result of the lack of specificity in the effects mediated by cytokines, immune responses to another antigen occurring in the close vicinity and presumably presented by

the same antigen-presenting dendritic cells can also be suppressed – a situation termed 'bystander suppression' [13, 16]. We have recently demonstrated a similar mechanism for exogenous antigens [17]. The induction of suppressor cells has earlier been shown to be both MHC class I and class II dependent [18], which has caused some confusion. However, some light was shed upon this when McMenamin and Holt [19] showed that induction of class-I-restricted suppressor T cells required help through IL-2 secretion from class-II-restricted CD4⁺ Th cells.

CD4⁺ T Cells and Peripheral Tolerance

Lately, a role of CD4⁺ T cells in the induction of oral tolerance has emerged [20–22] with clear links to the revised view on the induction and maintenance of tolerance to auto-antigens, where regulatory CD4⁺ T cells play a dominant role [23]. Several recent papers have described a subpopulation of IL-2R α -chain (CD25) expressing CD4⁺ T cells which are necessary to maintain peripheral tolerance to auto-antigens [24–27]. Our preliminary data also show that such cells are important in tolerance to fed heterologous antigens, as described below.

Intestinal Antigen Processing and MHC Class II

The importance of CD4⁺ T cells also makes a strong case for the importance of the abundant MHC class II⁺ vacuoles in the small intestinal epithelial cells and the intensely MHC class II⁺ dendritic cells just beneath, in the lamina propria. Luminal antigens are processed both in the gut

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1018–2438/97/1133–0219 \$12.00/0

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lumen and by the epithelial cells, and it appears from immunohistological examinations [unpubl. observations] that these MHC-peptide-complex-containing vacuoles are transported into the professional antigen-presenting dendritic cells. The dendritic cells then either present to local memory T cells or to naive T cells after migration to draining lymph nodes [6, 28]. Such an arrangement would overcome the relatively poor ability of dendritic cells to process soluble protein antigens [29] and would explain the necessity for luminal degradation and MHC class II expression by the enterocytes for the induction of oral tolerance, as discussed above.

Active Suppression or Anergy?

The dose of the fed antigen has been claimed to determine whether active suppression or anergy will occur, with a high dose causing anergy and a low dose giving rise to active suppression [30, 31]. However, we have shown that other parameters, like the timing of the feeding and the age of the animal, might be more important for the outcome of tolerisation [32]. In a situation of abrupt early weaning, with feeding of high doses of heterologous antigen, T and B cell anergy might be the dominant outcome. Such a state of anergy, induced in the neonate by sudden large intake of food antigen, is of relatively short duration and seems to impair the development of active suppression [33]. This fact could be of potential interest in infant feeding, since the breakdown of anergy might jeopardise the homeostasis of the mucosal immune system in the response to dietary antigens later in life.

The molecular characteristics of dietary proteins after digestion are usually ignored, which complicates interpretation of the immune responses to them. The general consensus is that, in the adult, only a very small amount of an ingested protein is absorbed in a form recognisable by antibodies and that feeding a soluble protein antigen usually induces immunological tolerance [34]. However, the induction of oral tolerance seems to be dependent both on the maturation of the immune system, with special reference to the expression of MHC class II molecules in the intestinal epithelial cells [32, 35, 36], and the ability to generate immunogenic peptides of adequate size [30, 31, 37].

Gut Processing and Generation of Tolerogenic Serum Factor

The existence of a tolerogenic factor transferable with serum from an antigen-fed donor has previously been described in the mouse system [38, 39]. We have obtained similar results in our rat system, with the additional observation that the transferred serum factor was capable of inducing an actively suppressed immune response in the recipient, as shown by a profound bystander tolerance to the bystander antigen human serum albumin (fig. 1). We are currently investigating whether this tolerogenic factor consists of pre-fabricated MHC class II peptide complexes deriving from intestinal epithelial cells. We are also investigating whether these complexes can be transferred to the neonate via the milk. Such complexes might overcome the impaired proteolysis and lack of MHC class II in the gut epithelium of the neonatal rat until 5 weeks of age [40], thus allowing early development of active dominant tolerance to environmental antigens in this period.

Role of CD25+ T Cells

The abrogation of the inflammatory swelling of the lymph node draining the site of immunisation with Freund's complete adjuvant and the bystander antigen human serum albumin by including the previously fed antigen (OvA) in the adjuvant mixture clearly demonstrates the existence of an active suppressor mechanism (fig. 2). Interestingly, there is an initial limited but significant swelling of the draining lymph node of OvA-tolerant animals not seen in the control animals. This may indicate an early infiltration of suppressor cells in the tolerant animals. We are currently investigating the longitudinal cytokine profile of these lymph nodes with immunohistochemistry and quantitative PCR. Our preliminary data [41] show the existence of TGF- β -producing cells from at least day 7 after immunisation in the tolerant animals. Such cells were absent in the controls.

In a different set of experiments, we have tried to focus the response to the fed antigen (OvA) back to the intestine first by parenteral immunisation and second by colonising the animals with *Escherichia coli* genetically engineered to produce OvA. Surprisingly, the tolerant animals showed a marked increase of IL2-R α -chain positive (CD25) T cells in the lamina propria, but no signs of inflammation in these animals [42]. Furthermore, in a similar experiment using cholera toxin as a mucosal adjuvant we have shown that by depleting CD25+ T cells from an in vitro culture of mesenteric lymph node cells from tolerant rats, we could restore

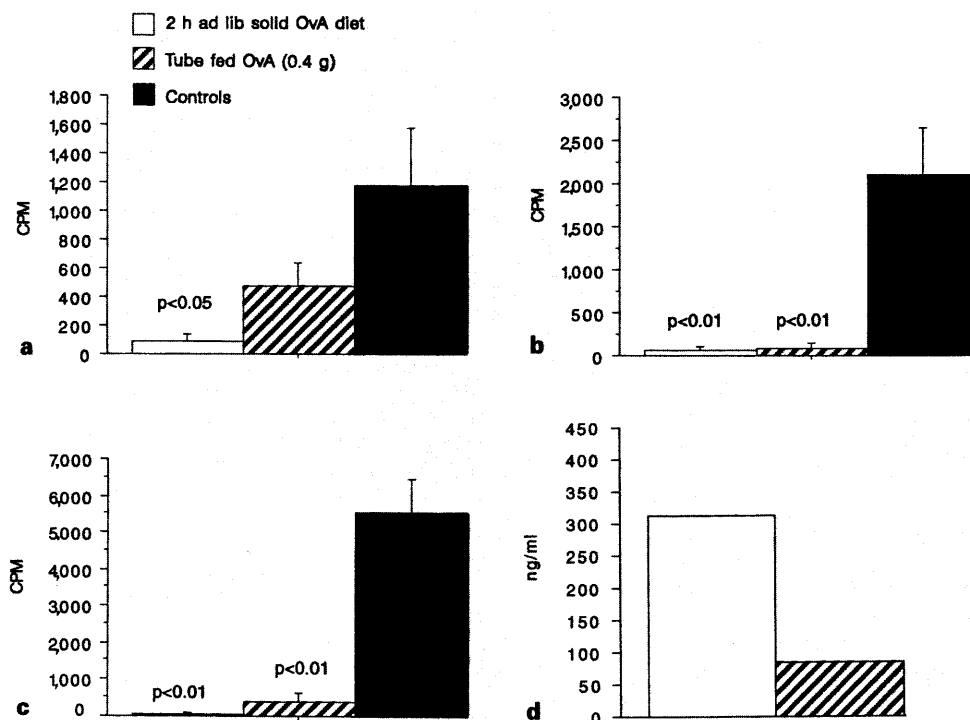


Fig. 1. After an overnight fast, rats were fed an OVA-containing diet ad libitum for 2 h or OVA by a stomach tube, 0.4 g/rat in PBS. Control rats were fed a normal diet or PBS only by a stomach tube. Thereafter, the rats were sacrificed and the blood was collected by heart puncture. The obtained serum was injected intraperitoneally into new sets of rats the same day. One week after serum transfer, the recipient rats were immunised subcutaneously in the hind legs with OVA and/or human serum albumin (HSA) mixed in Freund's complete adjuvant. Blood samples were collected weekly during the experiment. Seven weeks after serum transfer, the rats were killed and an in vitro lymphocyte transformation test in response to OVA (a), HSA (b), and both HSA and OVA (c) was performed on isolated spleen cells. d Serum concentration of OVA in the donor rats.

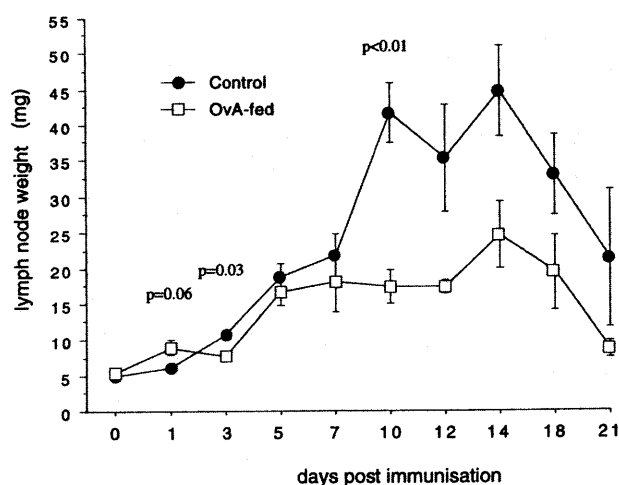
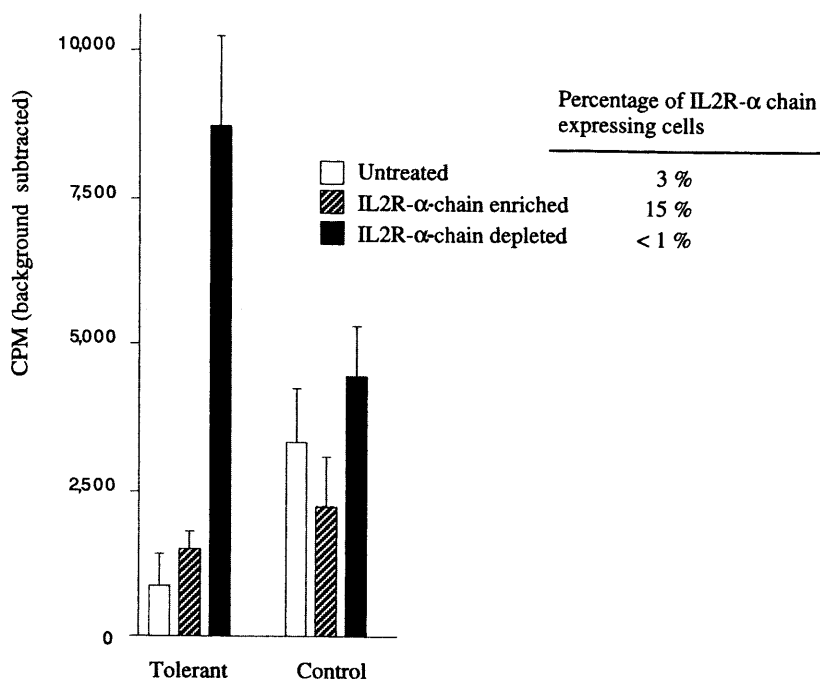


Fig. 2. Adult Wistar rats were made tolerant to OVA by feeding them with OVA-containing pellets for 2 weeks (from 6 weeks of age); the estimated intake of OVA was 0.8 g/day. The control group of rats was given a standard diet without OVA throughout the experiment. All rats were immunised with OVA and human serum albumin mixed with complete Freund's adjuvant in the hind legs. The popliteal draining lymph nodes were dissected out and weighed at regular intervals after immunisation.

Fig. 3. Adult Wistar rats were made tolerant to OvA by feeding them with OvA-containing pellets for 2 weeks (days 28–14); the estimated intake of OvA was 0.8 g/day. The control group of rats was given a standard diet without OvA throughout the experiment. All rats were immunised perorally on days 0, 6 and 11 with a mixture of 20 µg cholera toxin, 20 mg OvA and 20 mg human serum albumin in PBS. On day 12, the rats were sacrificed and cell cultures from the mesenteric lymph nodes were prepared and stimulated with OvA. Depletion of IL-2 receptor α -chain expressing (CD25+) cells were made using a monoclonal anti-rat CD25 in an MACS separation system.



the otherwise suppressed proliferative response to OvA (fig. 3). These results suggest a suppressive activity by a CD25+ CD4+ subpopulation of antigen-specific T cells. A population of T cells with this phenotype has recently been shown to be important for the maintenance of peripheral tolerance to self-antigens [24–27]. These experiments are currently followed up by cell transfer studies.

Concluding Remarks

Oral administration of protein antigens has proven very effective in inducing a profound antigen-specific tolerance including both cellular and humoral immune responses. The intestinal mucosa is the major interface between the organism and its environment and is also the major lymphoid

organ harbouring the majority of the lymphocytes in the body. The structural organisation of the intestinal immune system shows similarities with the thymus and may serve to supply regulatory T cells for maintaining immunological homeostasis to harmless environmental antigens. The regulatory capacity of this system may well be functionally connected to an overall system for the control of peripheral antigens including self-antigens. Our understanding of these systems will help us to explain the development of untoward reactions to environmental antigens as seen in allergies, but may also provide a modified view of the maintenance of tolerance to peripheral self-antigens. These systems may also provide a safe and natural way for therapeutic intervention using pre-defined suppressor peptides of the original antigen.

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