

Mesenteric lymph nodes at the center of immune anatomy

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The surface of the intestinal mucosa is constantly assaulted by food antigens and enormous numbers of commensal microbes and their products, which are sampled by dendritic cells (DCs). Recent work shows that the mesenteric lymph nodes (MLNs) are the key site for tolerance induction to food proteins and that they also act as a firewall to prevent live commensal intestinal bacteria from penetrating the systemic immune system.

Systemic tolerance to food antigens

It has been known for nearly a century that feeding rodents with a protein blunts subsequent responses to systemic challenge with the same protein (1). This phenomenon is termed oral tolerance and has been extensively studied using soluble proteins fed to rodents (Fig. 1), although it can be induced in other species including humans (2), with a variety of T-dependent antigens, and also via the nasal mucosa (3). The paper by Worbs et al. in this issue (p. 519) provides new insights into the anatomy of intestinal oral tolerance (4). They show that tolerance is induced exclusively in the MLNs after migration of antigen-loaded DCs from the intestinal mucosa.

Some of the battery of food antigens taken with every meal are absorbed as proteins or protein fragments and reach secondary lymphoid structures via lymph and blood (5). Oral tolerance can give us insight into the crosstalk between mucosal surfaces and the systemic immune system, in particular how hypersensitivity to food components is normally avoided. In oral tolerance models, cell-mediated and humoral (IgE, IgG, and IgM) responses are tolerized (6, 7). The resulting systemic hyporesponsiveness is dependent on CD4⁺ T cells, because CD4⁺ T cell depletion abrogates the effect (8), and adoptive transfer of these cells into a naive animal transfers the tolerance (9).

Tolerized T cells are unable to provide cognate B cell help (10), and B cells are tolerized by the absence of T cell help (11).

The mechanisms that produce T cell hyporesponsiveness after antigen feeding are complex and depend to some extent on experimental setup and antigen dose (3). The importance of DCs in tolerance induction has been shown in vivo: mice treated with the cytokine flt3 ligand (flt3L) to increase DC numbers are more susceptible to oral tolerance (12). Hyporesponsiveness can be the result of direct inactivation of lymphocytes (deletion or anergy) or modulation through regulatory T (T_{reg}) cell populations. If very high doses of antigen are given to T cell receptor transgenic mice, the antigen-specific T cells are clonally deleted, although how important this is in physiological circumstances is uncertain (13). T cell unresponsiveness (anergy) is induced in models where lower doses of antigen and lower frequencies of antigen-specific T cells are used (14, 15). CD4⁺ T_{reg} cells are also involved in oral tolerance: CD4⁺ T cells producing the suppressive cytokine TGFβ (T helper type 3 cells) (16) and CD4⁺ CD25⁺ FoxP3⁺ T_{reg} cells have both been reported to be induced by antigen feeding (17). The relative role that these different mechanisms play in physiological tolerance to normal food antigens is still unclear.

Oral tolerance can be induced in the systemic immune system

Although different experiments give different answers about the mechanisms of T cell hyporesponsiveness, perhaps reflecting several different methods of

tolerization, we still have the question of where tolerization is taking place. It is intuitive that oral tolerance should be induced locally in the mucosal immune system, but we must remember that the readout is an attenuated systemic immune response after parenteral immunization (Fig. 1). There is no reason why systemic tolerance cannot be induced by a food antigen that enters the vascular circulation through tributaries of the hepatic portal vein and reaches peripheral secondary lymphoid structures via the circulation (Fig. 2). Indeed, there is evidence that this happens, and we will first examine some of these experiments.

To pinpoint the site of tolerization, the earliest effects of fed antigen on T cells have been examined by transferring TCR transgenic T cells specific for ovalbumin and then giving an oral dose of antigen (18). These experiments showed that antigen-specific T cells in MLNs and peripheral lymph nodes divided with similar kinetics at early times during the tolerization protocol, indicating that oral antigen has early effects on T cells in the periphery as well as in the mucosa. In a follow-up study, the same workers assessed in vivo T cell behavior in lymph nodes in real time, during the induction of oral tolerance or priming (19). They found that oral tolerization led to smaller, more transient T cell clustering around the antigen-presenting cells (APCs) compared with the larger more stable clusters formed after immunization. Again, the effects of tolerization could be seen both in the mesenteric and peripheral lymph nodes with similar kinetics, suggesting that tolerance can be triggered both mucosally and systemically.

Two older observations indirectly suggest that oral tolerance can be induced outside mucosal lymphoid structures. First, transfer of serum from tolerized mice, presumably containing processed antigen, can induce tolerance in a recipient animal (20). The serum

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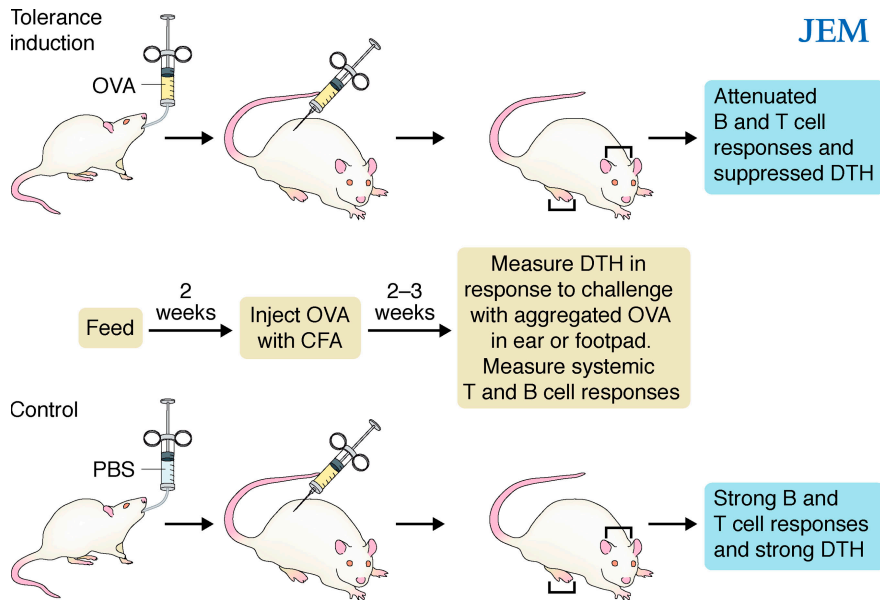


Figure 1. Induction of oral tolerance. To induce oral tolerance animals are fed with soluble protein (such as ovalbumin; OVA). Tolerance can be induced with single high doses, typically 100 mg, or several repeated low doses, typically 1–25 mg. Control animals are fed PBS. 2 weeks after feeding animals are injected subcutaneously with protein in adjuvant (such as OVA with complete Freund's adjuvant; CFA). 2–3 weeks after subcutaneous priming, animals are challenged with heat-aggregated protein in the ear or footpad. Changes in footpad or ear thickness are then recorded, usually 24 or 48 h after challenge (delayed type hypersensitivity; DTH). Tolerized animals exhibit reduced DTH reactions. Antibody responses are assessed after serum collection, and *in vitro* techniques, such as antigen-stimulated thymidine incorporation, are used to assess T cell responses. Using these readouts both B and T cell responses are attenuated in tolerized animals.

was injected into the peritoneum; thus, the antigen would mostly be cleared into the circulation, the spleen, and systemic lymph nodes. Follow-up experiments showed that oral tolerance could be blocked in these experiments by treatment of the recipients with the immunosuppressant cyclophosphamide after serum transfer, suggesting that tolerization was the result of an active immune response to gut processed antigen that had been absorbed into the blood and transferred with the serum (21). Second, it is well established that antigen given directly into the hepatic portal vein induces systemic tolerance (22).

MLNs are the key site for oral tolerance induction

Although some tolerization could take place systemically after antigen feeding, there has been no reason to doubt that it takes place largely within the local mucosal immune system. Of the local lymphoid tissues, it appears that the MLN

are the main site for oral tolerance induction. Presentation of fed antigens occurs preferentially in the MLN rather than the Peyer's patches, which are small lymphoid structures on the intestinal wall (23). Oral tolerance cannot be induced in mice lacking MLN (and peripheral lymph nodes), but it is unaffected in mice that lack only Peyer's patches (24). Furthermore, it has been shown that DCs constitutively traffic from the intestinal epithelium and Peyer's patches to the MLN, so there is a clear mechanism whereby antigen can be picked up at the intestinal epithelial surface and taken to the MLN (25), where T cell tolerization can occur.

The paper by Worbs et al. (4) elegantly shows that in clean mice the MLN are an obligatory and exclusive site of oral tolerance induction. TCR transgenic T cells transferred into naive mice were seen to proliferate at day 2 after antigen feeding in the MLN, but not until day 4 in peripheral lymph

nodes. Using either the drug FTY720 or surgical excision of the MLN to block T cell egress from MLN, they showed not only that antigen-specific T cells in systemic lymph nodes were the progeny of migrants from MLN, but also that oral tolerance to a systemic immunization challenge depends on MLN T cell migrants.

The authors then used two experimental systems to show that antigen-laden DCs migrating from the intestinal wall to the MLN can tolerize MLN-resident T cells. First, in an animal with an intestinal transplant in which the vascular supply is anastomosed to the host, but the lymphatic system is separate, an oral protein challenge caused T cells only in the host and not the graft MLN to be stimulated. Second, in mice deficient for the chemokine receptor CCR7, which have impaired DC migration to MLN, both T cell stimulation and functional oral tolerance were abrogated. These experiments allowed them to conclude that oral tolerance is exclusively generated in the MLN with antigen transported from the intestinal surface by DCs through the afferent lymphatics (Fig. 2).

Although Worbs et al. see no evidence for direct peripheral tolerization, they do show that intravenous protein at a dose 100-fold lower than that given for oral tolerance drives proliferation equivalently in peripheral lymph nodes and MLNs. The dose of antigen reaching the systemic circulation is not measured directly in most oral tolerance experiments, but it is reasonable to assume that antigen reaches the vasculature in some experimental scenarios (for instance, in the presence of low grade enteropathy accompanying a different hygiene status) to account for early systemic T cell proliferation seen in some studies. Oral tolerance induction is clearly MLN-centric in very clean mice; but the intestinal epithelial barrier remains thin and vulnerable, so some hepatic or systemic tolerization may compensate when antigen escapes into the blood stream.

MLNs preserve systemic ignorance to commensal bacteria

Food antigens are certainly not the only immunogenic load in the gut. The

nonpathogenic microbes in the lower intestine outnumber our body's own cells, and the dramatic increases of IgA-secreting plasma cells and lamina propria CD4⁺ cells after the colonization of germ-free animals with intestinal bacteria attest to the way in which the immune system adapts to help us live harmoniously with these organisms. Oral tolerance is not as effective for particulate antigens as it is for soluble proteins, and live bacteria are extremely immunogenic. Fortunately, despite the very high loads of commensal bacteria present in the intestine, the systemic immune system of clean mice is ignorant of these bacteria, although it can be very easily primed by small (10^4 – 10^6 colony-forming units) doses of live commensals given intravenously (26). This compartmentalized setup is useful because it achieves strong local mucosal responses without needing to tolerize the systemic immune system and hence suppress its ability to respond to systemic sepsis from a commensal or a closely related pathogen. MLNs are also a key part of this immune geography, but here they act as a firewall to preserve systemic ignorance of commensal organisms, rather than an obligatory site for induction of the adaptive response itself.

IgA⁺ B cells are induced by intestinal DCs that have sampled commensal intestinal bacteria (27). These B cells then recirculate through the lymph and blood to populate the intestinal lamina propria with IgA-secreting plasma cells. Induction of IgA⁺ B cells occurs mostly in the Peyer's patches, and mice which have had their MLN removed show normal *in vivo* IgA responses against intestinal doses of commensals. Although a small proportion of an intestinal challenge dose of commensal bacteria does get carried to the MLNs by intestinal DCs, commensal-laden DCs do not penetrate further into the thoracic duct lymph or reach the systemic circulation (Fig. 2). Thus, the MLNs are the firewall that stops constant systemic penetration and priming by these abundant intestinal microbes. Any bacteria released by dying DCs are probably very quickly eliminated by MLN macrophages. In animals without

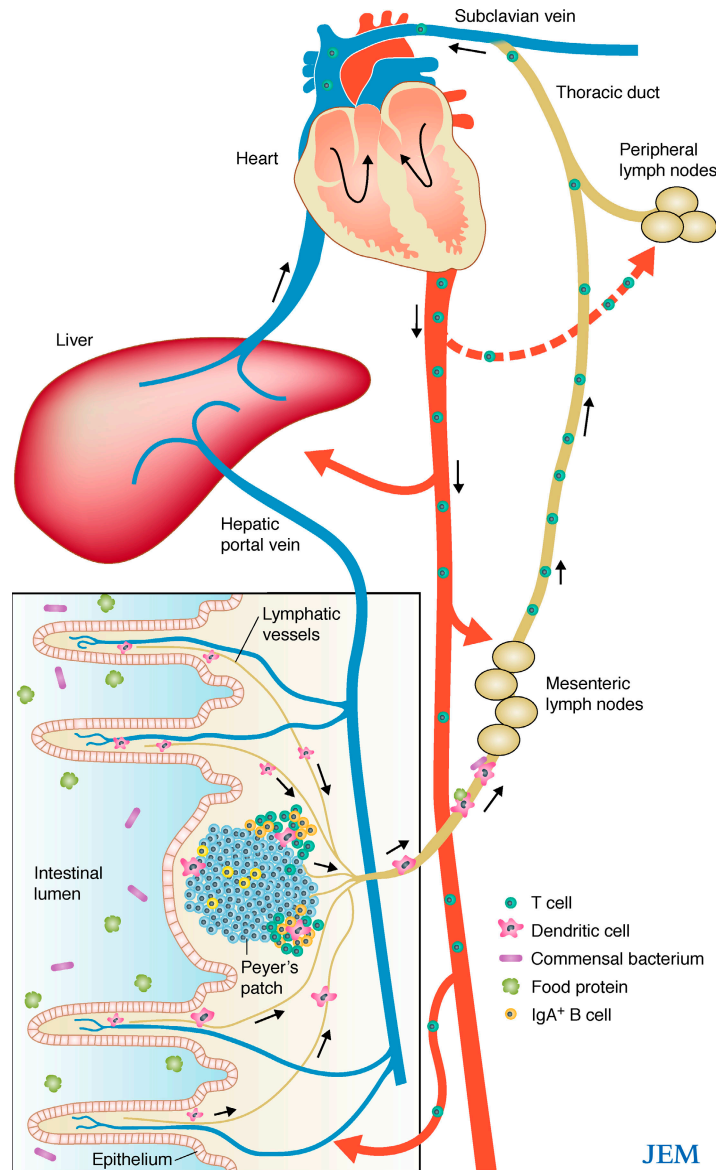


Figure 2. Functional anatomy of induction of immune responses by intestinal antigens.

Abundant protein antigens and live commensal bacteria are present in the intestine. Antigenic peptides can pass into the bloodstream through one of the tributaries of the hepatic portal vein or are taken up by DCs in the subepithelial region of the Peyer's patches and carried to the MLNs via the afferent lymphatics. Although it is possible for circulating peptides to tolerize T cells in the liver or peripheral lymph nodes, presentation in the MLNs is the dominant tolerogenic pathway. Commensal bacteria are also sampled by intestinal DCs and induce IgA responses in the Peyer's patches; although very small numbers of commensals can be carried to MLN by DC, systemic tolerance to these organisms is not induced. Because the commensal laden DCs do not penetrate further than the MLN, the systemic immune system is protected from unwanted priming reactions from live bacteria.

MLNs that are repeatedly challenged with intestinal bacterial, commensal-laden DCs pass into the thoracic duct and allow live bacteria to reach the circulation, causing systemic priming with massive splenomegaly and lymphadenopathy (27).

We generally perceive ourselves as free-living metazoa, occasionally assaulted by pathogens, but this is an illusion. We need to eat, and our bodies must live in harmony with our intestinal commensal microbes. The MLNs are central to this mutualism, providing a site of tolerance

induction to soluble proteins and a firewall against our commensal passengers. Most of the body's lymphocytes and antibody production are actually in the gut, so the MLNs are at a pivotal position in immune anatomy and immigration control, forming the border crossing between mucosal immunity and the remainder of immune system.

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