ORAL TOLERANCE

FOR DELAYED TYPE HYPERSENSITIVITY CONTRIBUTION OF LOCAL AND PERIPHERAL MECHANISMS

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Abstract

Oral tolerance is a physiological immune mechanism which controls the outcome of deleterious hypersensitivity reactions to environnemental antigens absorbed through the gastrointestinal tract, and maintains homeostasis. Using a mouse model of oral tolerance of delayed type hypersensitivity to contact allergens, i.e. haptens, we have examined the mechanisms involved in the induction of oral tolerance, both locally within the gut mucosa and systemically. We showed that intestinal epithelial cells from hapten-fed mice exert a bystander inhibition of hapten-primed T cell proliferation, through the release of anti-inflammatory cytokines, which may contribute to down regulation of local immune responses to soluble antigens.

Furthermore, the lack of oral tolerance induction in MHC class II or Ii knock-out mice or in normal mice treated with a depleting anti-CD4 mAb, and the ability of these mice to mount a skin DTH following oral immunization with the hapten demonstrate that MHC class II restricted regulatory CD4+T cells play a central role in oral tolerance.

Introduction

Immunological tolerance is defined as the physiological mechanism by which the immune system controls untoward immune responses to self antigens. This homeostasis ensures the maintenance of the integrity of the host tissues by preventing any harmful immune responses that could generate lesions. tolerance to outoantigens, is viewed as the ability of the immune system to discriminate between self and non self and is almost exclusively mediated by deletion of outoreactive T or B cells (negative selection) in the thymus or the bone marrow respectively. This process is irreversible, although some autoreactive effector cells which may escape central tolerance could be eliminated by a similar process in peripheral lymphoid organs (peripheral tolerance).

Oral tolerance represents one example of peripheral tolerance, and is a paradigm to the self non self discrimination theory of tolerance. Indeed, daily ingested foreign exogenous antigens, including dietary proteins and haptens which remain immunogenic after transcytosis through the intestinal epithelium, do not normally induce deleterious inflammatory responses.

Alternatively, inflammatory bowel diseases (including Crohn's disease and Ulcerative Colitis) as well as Celiac disease have been attributed to a breakdown in oral tolerance leading to hyperresponsiveness to luminal antigens. (as yet undefined in the case of IBD and identified as gliadin in the case of Celiac disease). Oral tolerance is defined both as the lack of local and peripheral delayed type hypersensitivity (DTH) after intake of soluble antigens and as the inhibition of T cell-mediated immunity (DTH and CTL) to a given soluble antigen induced by prior intragastric administration of the same antigen (for review see 1).

Oral tolerance of DTH to haptens

We have described a in vivo assay of oral tolerance to haptens in mice which measures the inhibition of skin DTH to strong contact sensitizing haptens induced by a single oral administration of the hapten (2). Mice received a single intragastric administration of a non toxic dose of DNCB 7 days before skin painting with DNCB. Skin DTH was measured 5 days later by the ear swelling 24.48 hr after an ear challenge with the relevant hapten. Haptens are low molecular weight chemicals with covalently bind to distinct amino acid residues on exogenous and self proteins. Processing of haptenated proteins generates class I and class II peptides, including hapten-modified peptides which may be recognized by CD8 and CD4 T cells, respectively.

Intestinal epithelial cells contribute in oral tolerance:

We have previously shown that a single intragastric administration of the hapten, DNCB, 7 days before epicutaneous skin sensitization, abrogates the DTH to DNCB (2).

Feeding with the fluorescent hapten FITC revealed hapten capture by that intestinal epithelial cells (IEC) in the mucosal villi of the duodenum and by cells of dendritic/macrophage morphology located in the Peyer's patches and in the lamina propria. In order to determine whether IEC and PP lymphoid cells participate in oral tolerance, we tested the ability of IEC and PP lymphoid cells from DNCB or vehicle fed mice to inhibit the proliferation of T cells from peripheral lymph nodes of mice sensitized by skin painting with DNCB. IEC isolated shortly after hapten feeding are unable to activate hapten-specific T cells from peripheral lymph nodes of skin sensitized mice, but completely blocked their proliferation in response to stimulation by syngeneic haptened spleen cells used as APC(2). IEC from control vehicle fed mice partially inhibited T cell proliferation. The spontaneous inhibitory effect of IEC was mediated by IL-10 secretion; while complete suppression induced by DNCB-IEC was due to TGF-β, inasmuch as T cell responses could be restored by neurtalizing anti-IL-10 and anti-TGF-β, respectively. In contrast to epithelial cells, Peyer's patch cells from hapten-fed mice are able to activate hapten specific T cells in vitro, in th absence of exogenous hapten, indicating that they have captured the hapten in vivo and can serve as efficient APC for T cell activation. These results suggested that oral hapten is captured both by APC within PP and the villi, and that epithelial cells may block hapten specific T cell proliferation (generated either in the PP or in the villi) through the release of inhibitory cytokines. Although bystander suppressive effect of IEC through IL-10 and/or TGF-β may contribute to local homeostasis controlling T cell responsiveness to luminal antigen, it does not explain how oral hapten can suppress skin DTH responses mediated by T effector cells primed in peripheral lymph nodes.

The MHC class II/CD4 pathway is essential in the induction of oral tolerance

In order to examine the mechanisms that operate in orally induce tolerance to skin DTH, we investigated whether MHC class II molecules were required in oral tolerance. We took advantage of the fact that skin DTH to DNFB in C57BL/6 mice is mediated by class I-restricted CD8+T effector cells (3). We observed that MHC class II knockout C57BL/6 ($A\beta^{\circ/\circ}$) mice (which are deficient in class II-restricted CD4+T cells)(4), as well as invariant chain knockout ($Ii^{\circ/\circ}$) mice (which

have residual class-II restricted CD4+ T cells) (5), could not be tolerized by hapten feeding and develop a skin DTH of similar intensity as that generated by skin sensitization without prior feeding. Likewise, inhibition of skin DTH by DNFB feeding coule not be induced in normal C57BL/6 mice that were depleted in CD4+ T cells by treatment with anti-CD4 mAb. Moreover, a single oral odministratiion of DNFB in either Aβ°/°, Ii °/° or anti-CD4-treated mice, without skin sensitization, resulted in the induction of a skin DTH (6). These data indicated that the hapten presentation by MHC class II-expressing cells is required for the induction of oral tolerance to DNFB which appears mediated a subset of regulatory CD4+T cells. Studies as underway to identify which class II+APCs are involved in the induction of regulatory CD4+T cells and to determine the mechanism of their inhibitory effect.

Concluding remarks

We postulate that local inhibitory mechanisms mediated by resident epithelial cells in the intestine may be responible for the maintenance of homeostasis by preventing expansion of activated T cells and intestinal damage. IL-10 and TGF-β secretion by IEC and possibly other cells could mediate such bystander suppression, resulting in inhibition of effector T cells that may emigrate from the PP or primed in situ in the villi. Alternatively, the efficiency of oral antigen to prenent systemic T cell responsiveness relies on other mechanisms, mediated by class II + APC and a regulatory CD4+T cell subset, which may seed peripheral lymph nodes. It remains to be determined whether the intestine contains a particular APC type able to activate this regulartory CD4+T cell population and wether IEC contribute to this inhibitory pathway by providing the appropriate milieu of cytokines essential for this inhibitory pathway.

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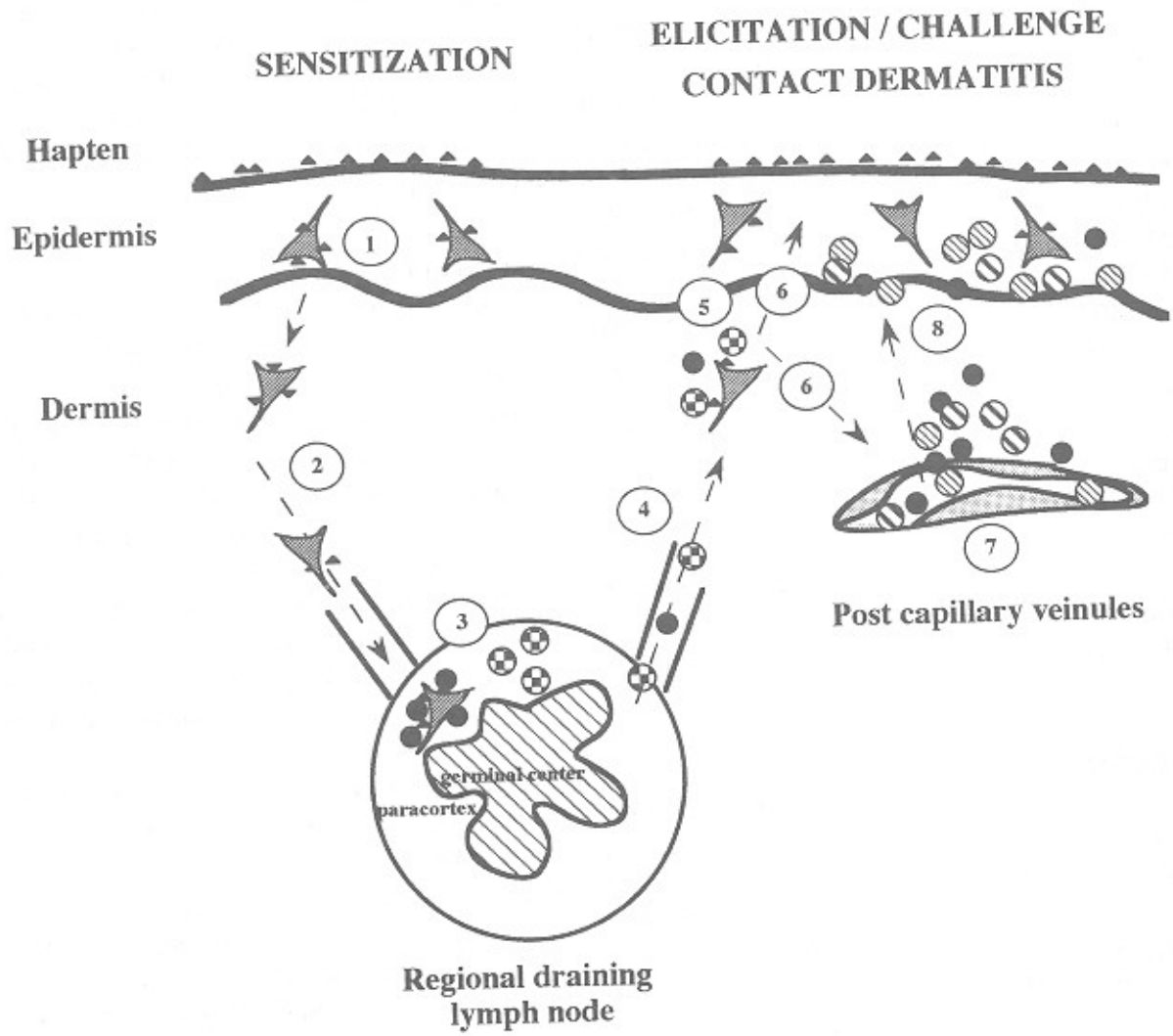
SENSITIZATION
ELICITATION / CHALLENGE
CONTACT DERMATITIS
Hapten
Epidermis
Dermis
1
2
3
4
5
6
6
7
8
germinal center
paracortex
Post capillary veinules
Regional draining
lymph node