

Prostaglandins and the induction of food sensitive enteropathy

Newberry RD, Stenson WF, Lorenz RG. Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. *Nat Med* 1999;5:900-6.

Abstract

Intestinal inflammatory diseases are mediated by dysregulated immune responses to undefined luminal antigens. Feeding hen egg-white lysozyme to mice expressing a transgenic T-cell receptor that recognizes hen egg-white lysozyme peptide 46-61 resulted in no intestinal pathology; however, simultaneous administration of cyclooxygenase-2 inhibitors and dietary hen egg-white lysozyme resulted in increased proliferation of lamina propria mononuclear cells and crypt epithelial cells, crypt expansion and villus blunting. Lamina propria mononuclear cells produce high levels of cyclooxygenase-2-dependent arachidonic acid metabolites, which act as immunomodulators in the immune response to dietary antigen. These findings establish that cyclooxygenase-2-dependent arachidonic acid metabolites are essential in the development and maintenance of intestinal immune homeostasis.

Comment

The role of antigen specific T lymphocytes in mediating food sensitive enteropathies such as coeliac disease has been suggested for many years,¹ but the link between specific T cell activation and intestinal pathology has been difficult to prove directly. This partly reflects the absence of suitable animal models in which mucosal T cells can be activated by dietary antigens, owing to the fact that immunological tolerance is the usual result of feeding dietary protein antigens to animals.^{2,3} Therefore the recent paper by Newberry *et al* describing the induction of small bowel enteropathy in mice fed hen egg lysozyme (HEL) as a representative dietary protein, is a welcome and interesting addition to the field.

Activation of mucosal CD4+ T cells by alloantigens during a graft-versus-host reaction (GvHR) in experimental mice,^{4,5} or by mitogens in explants of human fetal intestine *in vitro*^{6,7} can produce a pattern of small bowel pathology similar to that in coeliac disease, with crypt hypertrophy, epithelial cell hyperplasia and villus atrophy. However, these models clearly cannot reproduce local T cell responses to food proteins and, in both cases, the pathology often progresses to complete mucosal destruction, a feature not usually associated with coeliac disease. Similarly, although it is possible to induce crypt hyperplasia in the jejunum by oral challenge with ovalbumin (OVA) in mice in whom oral tolerance has been abrogated experimentally,^{3,8} the resulting intestinal pathology is mild,

with the hallmark feature of coeliac disease, villus atrophy, not being found. This may reflect the low proportion of food antigen specific T cells in normal animals.

In their paper, Newberry *et al* make use of T cell receptor (TcR) transgenic mice backcrossed on to a TcR α KO background. As this ensures that there are no T cells expressing endogenously rearranged TcRs, virtually all the animals' T cells (including those in the intestinal lamina propria) are CD4+ T cells specific for a class II MHC restricted peptide in HEL. Feeding these transgenic animals 2 mg/ml HEL for seven days induced a minor degree of crypt lengthening, but this phenomenon was increased dramatically if the mice were treated throughout with the cyclooxygenase (COX) inhibitor indomethacin. In parallel, the mice developed villus damage and fairly intense crypt hyperplasia, although neither of these features was quantified in the study. The authors then show that lamina propria lymphocytes (LPL) from mice with HEL induced enteropathy have very high levels of HEL specific proliferative activity *in vitro*, whereas mice fed HEL without indomethacin have lower proliferative responses than LPL from naive, unfed mice. This is consistent with the idea that even TcR transgenic mice have T cell tolerance to food antigens and that this has broken down following inhibition of COX. To investigate the effects of COX inhibition, the authors did a series of experiments to show that LPL from TcR transgenic (or normal) mice spontaneously produce prostaglandin (PG) E₂ in culture. This did not occur with other sources of mononuclear cells unless bacterial lipopolysaccharide (LPS) was present. Like PGE₂ itself, supernatants from LPL cultures significantly inhibited the HEL specific proliferation of spleen cells or LPL and, using lamina propria cells from COX-1 and COX-2 knockout mice, PGE₂ production was shown to be dependent on the COX-2 isoform of the enzyme which is inducible mainly in macrophages. Finally, the *in vivo* role of COX-2 was confirmed by the fact that enteropathy could also be induced in HEL-fed TcR transgenic mice treated with the COX-2 selective inhibitor, NS-398. Thus, the authors conclude that harmful T cell mediated immune responses to food proteins are normally held in check via the COX-2 dependent production of PGE₂ by local macrophages and that this is a central physiological mechanism which prevents food hypersensitivity.

A number of important issues are raised by this work. Firstly, the pathology in TcR transgenic mice fed antigen was restricted to the upper small intestine and there was no evidence of transmural inflammation, ulceration, or granuloma formation. Thus, the present model is quite distinct from other murine models of intestinal immunopathology, in which aberrant T cell responses to bacterial flora produce an inflammatory bowel disease (IBD), mostly confined to the colon.⁹ These differences mirror the different clinical pictures of IBD and coeliac disease and are particularly interesting when one considers the prevailing view that both conditions are dependent on γ -interferon producing Th1 T cells.^{9,10} The authors do not tackle the pathological mechanisms involved in their model, but sug-

gest that the absence of colonic pathology could reflect the fact that fed proteins do not gain access to the distal small intestine or colon. Alternatively, the large and small bowel may have differential requirements for COX-2 dependent regulatory mechanisms. This would be consistent with the fact that administration of COX inhibitors did not induce colonic pathology in TcR transgenic mice, despite the presence of intestinal bacteria. However, this may not have occurred in the present study, because the transgenic mice used should have no T cells capable of recognising bacterial antigens. As COX inhibitors can exacerbate IBD in humans,¹¹ it would be instructive to examine their effect on some of the animal models of IBD now available.

The second issue concerns the nature of the COX dependent regulatory processes revealed in the study. The authors show that T cell proliferative responses were inhibitable by PGE₂, an effect which could reflect either of two known properties of PGE₂: direct suppression of effector T cells, or upregulation of the production of inhibitory mediators such as interleukin (IL) 10.¹² This latter idea would certainly be consistent with the evidence that IL-10 plays an important regulatory role in some models of experimental oral tolerance^{2 13} and in the prevention of IBD in mice.¹⁴ This IL-10 could be derived from regulatory T cells (Th3 or Tr1), or could be from mucosal dendritic cells (DC), as proposed recently.¹⁵ Together, these findings predict the existence of a homeostatic pathway in which LPS and other products from intestinal bacteria stimulate the production of PGE₂ by macrophages, leading to the induction of IL-10 from T cells or DC, or both, with subsequent polarisation of T cell responses against local antigens towards a non-pathological or regulatory phenotype.¹⁴ A suppressive microenvironment in the normal intestinal mucosa has been an issue of interest for some time, but the initiating factors and the pathways leading to T cell down-regulation have remained unclear. An antigen specific model of enteropathy such as that described by Newberry *et al* offers one possible means of exploring these processes.

Finally, it is necessary to consider the relevance of this model to the pathogenesis of conditions like coeliac disease. The pattern of epithelial cell pathology and apparent activation of lamina propria cells are remarkably similar to what is seen in coeliac disease. However, the authors do not indicate whether there were also increased numbers of intraepithelial lymphocytes. This is a cardinal feature of the clinical disease and it would be important to reproduce experimentally. As noted above, Newberry *et al*'s study also did not examine which immunological mechanisms might be responsible for the enteropathy. Although previous studies have focused on Th1 dependent cytokines as the mediators of this form of intestinal immunopathology in humans and animals,^{5 6 10 16 17} other mechanisms have also been implicated, including IL-4,^{18 19} Fas-Fas ligand interactions,²⁰ and keratinocyte growth factor.²¹ The ability to induce appropriate pathology with immunologically well defined antigens and specific T cells therefore now opens the door to a wide range of avenues of exploration. Lastly, is the message of this intriguing study that the induction of coeliac disease simply requires an immunoregulatory defect together with the presence of a sufficient proportion of antigen specific CD4+ T cells?

The authors propose that the COX inhibitors which induce enteropathy *in vivo* act by interrupting a cascade of PGE₂ dependent immunoregulatory mechanisms. Nevertheless, COX inhibitors themselves cause alterations in intestinal permeability and function.^{11 22} Although it is tempting to speculate that this may be secondary to inflammation induced by local antigens in the absence of PGE₂ mediated immune suppression, primary defects in the epithelial barrier can predispose to IBD in experimental animals.^{9 23} Thus it will be important to determine how the various actions of COX inhibitors can contribute to the development of antigen specific enteropathy.

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