SCIENCE ALERT

Altering cytokine soups: a recipe for inflammatory bowel disease?

Watanabe M, Ueno Y, Yajima T, *et al*. Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. *J Exp Med* 1998;**187**:389–402.

Abstract

We have demonstrated that intestinal epithelial cells produce interleukin 7 (IL-7), and IL-7 serves as a potent regulatory factor for proliferation of intestinal mucosal lymphocytes expressing functional IL-7 receptor. To clarify the mechanism by which locally produced IL-7 regulates the mucosal lymphocytes, we investigated IL-7 transgenic mice. Here we report that transgenic mice expressing murine IL-7 cDNA driven by the SR α promoter developed chronic colitis in concert with the expression of SR α/IL-7 transgene in the colonic mucosa. IL-7 transgenic but not littermate mice developed chronic colitis at 4–12 wk of age, with histopathological similarity to ulcerative colitis in humans. Southern blot hybridization and competitive PCR demonstrated that the expression of IL-7 messenger RNA was increased in the colonic mucosal lymphocytes but not in the colonic epithelial cells. IL-7 protein accumulation was decreased in the goblet cell-depleted colonic epithelium in the transgenic mice. Immunohistochemical and cytokine production analysis showed that lymphoid infiltrates in the lamina propria were dominated by T helper cell, type 1 CD4+ T cells. Flow cytometric analysis demonstrated that CD4+ intraepithelial T cells were increased, but T cell receptor γ/δ T cells and CD8 α/α. cells were not increased in the area of chronic inflammation. Increased IL-7 receptor expression in mucosal lymphocytes was demonstrated in the transgenic mice. These findings suggest that chronic inflammation in the colonic mucosa may be mediated by dysregulation of colonic epithelial cell-derived IL-7, and this murine model of chronic colitis may contribute to the understanding of the pathogenesis of human inflammatory bowel disease.

Comment

The precise aetiology of chronic inflammatory bowel disease (IBD) has remained elusive despite many years of investigation. Both Crohn's disease and ulcerative colitis are characterised by chronic inflammation of the gastrointestinal tract, but the regions of intestine that can be affected and the histopathological features are quite different between the two forms of IBD. It has been hypothesised that the persistent intestinal inflammation seen in either case is likely to be the result of increased or aberrant immunological responsiveness to normal constituents of the gut lumen, or an overall immune dysregulation and imbalance. Are cytokines responsible for tipping the balance?

CYTOKINE DEFICIENCY CAN LEAD TO IBD SYMPTOMS IN MICE

The recent establishment of experimental animal models for studying the pathogenesis of intestinal inflammation has provided some insight into potential disease mechanisms and has had particular impact within the mucosal immunology field. The advent of genetically targeted mice bearing modified or disrupted immune systems, and the observation that spontaneous development of IBD is a prominent feature in many of these mice lacking T cells or cytokines, is highly suggestive that an immune imbalance is a critical factor in IBD pathogenesis.

IBD arises spontaneously in mice deficient for αβ+ T cells and in mice deficient for either Th1 or Th2 regulatory cytokines, such as interleukin (IL) 2 or IL-10.[1–4] The fact that mice deficient in either one of the contrary Th1 or Th2 type cytokines are equally susceptible to developing inflammation of the intestinal tract is hard to reconcile, but it tells us that the mucosal microenvironment is an exquisitely regulated system. Disrupting the homoeostatic cytokine balance in any way might thus lead to the development of an aberrant or uncontrolled inflammatory response in the gut.

HUMAN IBD CAN BE ASSOCIATED WITH INCREASED CYTOKINE CONCENTRATIONS

Although there is sufficient evidence from the mouse models to indicate that abrogation of regulatory cytokines can lead to intestinal inflammation, there is not much evidence that humans with IBD are unable to make cytokines such as IL-2 or IL-10. In contrast, there are many studies reporting that other inflammatory cytokines are increased at the mRNA and protein level in patients with IBD.[5–8] Many of the changes associated with increased cytokine expression in patients with IBD probably result from secondary non-specific inflammatory processes rather being than primary causes of disease. Or are they? This question has been almost impossible to answer owing to the lack of a suitable means for analysing whether the increase in cytokine concentration itself is the initiating factor for triggering intestinal inflammation, or merely the downstream result of perpetual inflammation. The recent observation that serum from patients with ulcerative colitis contains significantly increased concentrations of IL-7 has led to this particular cytokine being investigated as a potential mediator triggering IBD.[9] What evidence is there to suggest that IL-7 might be important in mucosal immunoregulation?

INTERLEUKIN-7 AND CELLS IN THE GUT

A role for IL-7 in mucosal immunoregulation has only recently begun to emerge. First described over 10 years ago

as a soluble mediator promoting growth of progenitor B cells,[10 11] IL-7 has evolved into a rather promiscuous cytokine affecting other cell lineages including T cells[12 13] and gastrointestinal epithelial cells.[14] Mucosal αβ+ and γδ+ T cells from both the intraepithelial and lamina propria compartments have been shown to be particularly responsive in culture with IL-7 alone,[15 16] although CD3 dependent proliferation of mucosal cells seems to be inhibited in the presence of IL-7.[15] The gut is home to many other cells that are capable of producing IL-7. Gastrointestinal epithelial, goblet[15] stromal cells also secrete IL-7.[10 11]

INTERLEUKIN-7 TRANSGENIC MICE

As sufficient evidence linked the pleiotropic cytokine IL-7 with intestinal immunoregulation, Watanabe and colleagues chose to generate a transgenic mouse line overexpressing the gene encoding IL-7 under the SRα promoter. Their observations certainly support the notion that widespread overexpression of IL-7 can lead to the development of chronic colitis in mice with a pathology similar to ulcerative colitis in humans.

The IL-7 transgenic mice develop signs of acute colitis at 1–3 weeks of age. This acute stage of intestinal inflammation is associated with an increase in neutrophils, CD4+/αβ+ T cells and γδ+ T cells infiltrating the lamina propria. By 6–8 weeks of age, many of the mice develop diarrhoea, weight loss, rectal prolapse, and intestinal bleeding. In the chronic stages of disease, erosions and neutrophil infiltration can be observed in the anal ring. Inflammatory cell infiltration and goblet cell depletion can be observed throughout the large intestine, and these features are most prominent in the rectum. Crypt abscesses, Paneth cell metaplasia and eosinophil and macrophage infiltration are also seen. In this more chronic stage of disease, infiltration by both αβ+ and γδ+ T cells into the lamina propria is evident, but only αβ+ T cell numbers seem to increase in the intraepithelial compartment. CD4+ cells from IL-7 transgenic mice seem to be biased towards Th1 type cytokine expression, producing significantly more IL-2 and interferon-γ than CD4+ cells from littermates.

The development of colitis in the IL-7 transgenic mice was concordant with active expression of the IL-7 transgene within colonic tissues. Although IL-7 mRNA was estimated to be increased up to 100-fold in the colonic tissues of transgenic mice with active inflammation, there were no observable differences in IL-7 mRNA expression in tissues without inflammation. Increased expression of IL-7 mRNA seemed to be restricted to lymphocytic populations. Colonic epithelial cells in transgenic mice had similar levels of IL-7 mRNA as wild type mice, suggesting that the increase in IL-7 mRNA expression in the inflamed transgenic gut is most likely a result of overexpression by the infiltrating immune cells.

IL-7 protein expression was analysed in normal and transgenic mice. Lymphocytes from the epithelium and lamina propria, as well as epithelial cells isolated from transgenic mice all produced increased amounts of IL-7 protein compared with cells from non-transgenic mice. However, when IL-7 protein expression was analysed in situ, surprisingly there seemed to be a decrease in inflamed tissues. As goblet cells are a known source of IL-7 protein, it is possible that the goblet cell depletion associated with chronic intestinal inflammation might be responsible for this decrease. However, the authors view is that IL-7 production per se is not decreased in the inflamed tissues of transgenic mice, rather the accumulation of IL-7 is decreased. IL-7 receptor expression was also analysed; expression was increased on mucosal lymphocytes in inflamed colonic tissues.

All of these data support the hypothesis that transgenic overexpression of IL-7 is directly related to chronic inflammation. However, the authors make a cautionary point "...it remains to be clarified whether increased expression of the transgene in the colonic mucosa induces chronic colitis or whether chronic inflammation in the colonic mucosa induces activation of the transgene".

IS IL-7 THE MISSING LINK IN THE PATHOGENESIS OF IBD?

The studies by Watanabe *et al* show that IL-7 is important for maintaining the homoeostatic balance of immunoreactivity in the intestine. IL-7 obviously regulates proliferative reactivity of intestinal lymphocytes; and intestinal epithelial cells can clearly be a source for IL-7 production. There are some tantalising pieces of information provided by Watanabe *et al* to suggest that IL-7 regulation really may be different in patients with ulcerative colitis. Unpublished observations cited by the authors indicate that IL-7 protein accumulation is decreased in intestinal disease, IL-7 receptor expression is notably different on mucosal cells from inflamed tissues, and IL-7 can inhibit growth of lymphocytes from inflamed mucosa, perhaps by regulating apoptotic mechanisms. Together, this knowledge definitely leads us to believe that dysregulation of IL-7 can be associated with the pathogenesis of colitis.

Investigation of the regulation of IL-7 in intestinal inflammation is certainly an avenue to pursue in IBD research, but it might be worth remembering from the knockout mouse models, that dysregulation of any one of a number of cytokines has the ability to upset the very complex microenvironment. What is the take-home message? Altering cytokine soups in any number of ways can be a recipe for spoiling the homoeostatic balance in the gut and initiating intestinal disaster.

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