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IL-22 in mucosal immunity

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Recent studies in infectious disease and autoimmune disease models have revealed that interleukin (IL)-22 might have both proinflammatory and anti-inflammatory functions. There is, however, lack of evidence for IL-22 directly repressing immune responses of leukocytes. We propose that IL-22 promotes innate immunity of tissues, as well as repairing and healing mechanisms during inflammation. Consequently, the restoration of tissue homeostasis helps to attenuate the inflammatory responses involving various immune cells.

IL-22 IN THE IMMUNITY OF THE TISSUE EPITHELIA

To control the invasion of various pathogens, the immune system has evolved two defense mechanisms: innate immunity and adaptive immunity. Innate immunity is primarily mediated by tissue epithelial cells, phagocytic cells, and natural killer cells, whereas adaptive immunity is mediated by antigen-specific B and T cells. Tissue epithelial cells, especially those covering mucosal surfaces, are important components of innate immunity. During normal homeostasis, epithelial cells form the first barrier that blocks the penetration of harmful pathogens into the body. Tissue epithelial cells can also produce various mucosal proteins and antimicrobial peptides that can sequester, inhibit, and kill invading microbes. Inflammatory responses are initiated if there is a breach of the integrity of the epithelial barrier. The recruitment and activation of immune cells in tissue epithelia are partially stimulated by the proinflammatory chemokines and cytokines produced by epithelial cells. Activated immune cells orchestrate both the innate and the adaptive immunities that fight and eliminate invading pathogens. In addition, immune cells also produce cytokines that enhance the innate responses of epithelial cells and restore the integrity of tissue barrier.

IL-22 is one of the key cytokines that mediates this cross talk. IL-22 is an IL-10 family cytokine, which is produced by various leukocytes, including $CD4^+$ and $CD8^+$ T cells, γδ T cells, natural killer cells, and myeloid cells.[1,2]. Recently, IL-22 has also been identified as an effector cytokine of the newly discovered T helper (Th)17 cells.[3,4] The regulation of IL-22 during autoimmune and infectious diseases remains to be fully elucidated. Recent data imply that IL-23 plays an essential role in induction of IL-22 from various cells both *in vitro* and *in vivo*[4] (**Figure 1**). In autoimmune encephalomyelitis, *Klebsiella pneumonia* infection in the lung, and *Citrobacter rodentium* infection in the colon, the upregulation of IL-22 is abolished in IL-23-deficient mice.[5–7] IL-22 receptor is composed of the IL-22R chain and the IL-10R2 chain. The IL-10R2 chain is broadly expressed, whereas IL-22R is preferentially expressed on epithelial cells.[2] IL-22 promotes the proliferation of various epithelial cells, including skin, colon, and lung epithelial cells. Furthermore, IL-22 induces serine proteases, matrix metalloproteinases, and some serpin family protease inhibitors from keratinocytes, supporting its role in wound healing, tissue remodeling, and re-epithelialization.[8–10] Taken together, IL-22 may help to restore the barrier function of damaged epithelia during inflammation. In addition, IL-22 enhances the innate immune responses of the tissues by stimulating the production of antimicrobial peptides from epithelial cells, which we will discuss further. It also activates epithelial cells to secrete proinflammatory chemokines and cytokines (**Figure 1**).[8–10] These data point toward a proinflammatory role for IL-22 in tissue immunity.

IL-22 IN MUCOSAL IMMUNE DEFENSES

The mucosal surfaces, such as the lung and gastrointestinal tract, are the key battle grounds for hosts to fight against the invasion of various microbes. Recent studies on the IL-22 pathway in infectious disease models have elucidated its essential protective role in mucosal immunity against bacterial pathogens.[6,7] During *K. pneumonia* infection of the lung, IL-17A and IL-22, presumably produced by Th17 cells, synergistically protect the integrity of the bronchial epithelial layer by inducing proinflammatory chemokines, cytokines, and antimicrobial peptides. The lack of either IL-17 or IL-22 significantly compromises the host defense against *K. pneumonia* invasion.[7] *C. rodentium* is an attaching and effacing bacterium that specifically attacks murine colon epithelial cells. The infection of *C. rodentium* in wild-type mice can result in infectious

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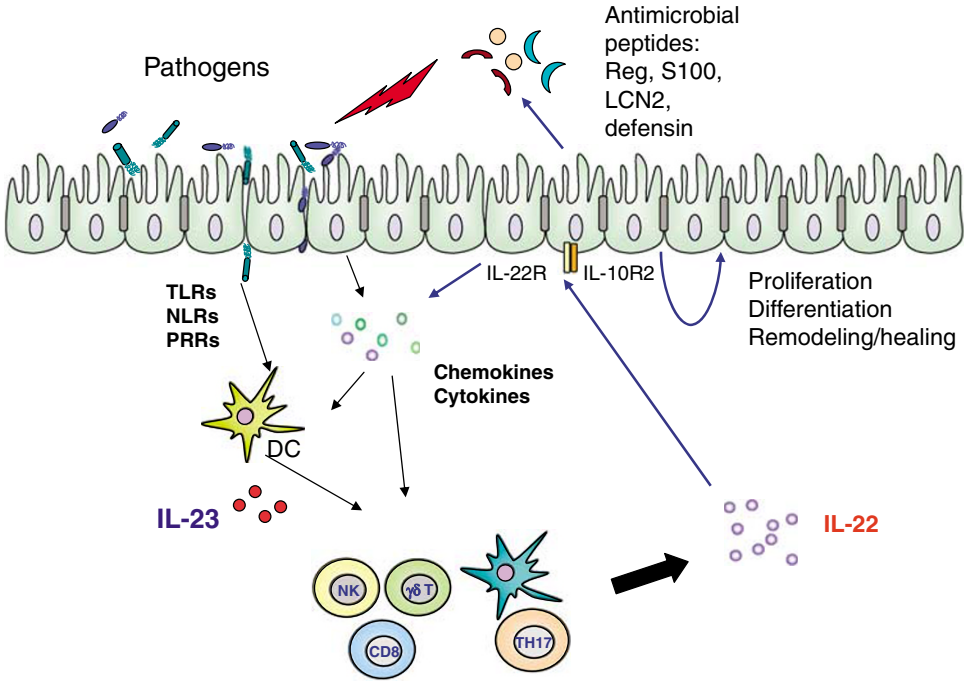

Figure 1 A proposed model for the functions of IL-22 in mucosal immunity. IL-22 produced by various leukocytes directly acts on tissue epithelial cells, enhances their innate immune responses, and helps to restore their integrity.

colitis in the first 2–3 weeks, but it is usually self-resolved after the development of the adaptive immune responses in the host, particularly the *C. rodentium*-specific antibody response. Interestingly, in the absence of IL-22, but not IL-17 signaling, *C. rodentium* infection causes much more severe damage to the colon epithelial layer, systemic dissemination, and mortality.[6] IL-22 directly targets colon epithelial cells and induces the expression of antimicrobial peptides, such as the Reg family proteins. In contrast to the production of IL-22 by T cells during *K. pneumonia* infection, the production of IL-22 during *C. rodentium* infection is independent of T and B cells. Innate immune cells, such as dendritic cells, are the major cellular sources of IL-22 during the early phase of infection.[6] These data support crucial protective functions of IL-22 in mucosal immunity during the infection by extracellular bacteria.

IL-22 IN IBD: PATHOGENESIS VS. PROTECTION

What are the functions of IL-22 in inflammatory bowel diseases (IBD)? Recently, genome-wide association studies linked IL-23R to human IBD.[11] The IL-23R chain is one component of the heterodimeric IL-23 receptor complex, which also contains IL-12β1. The IL-23 receptor binds to IL-23, a cytokine formed by binding IL-12p40 subunit to IL-23p19 subunit.[12] IL-23 shares a common p40 subunit with IL-12, which has its own p35 subunit. The IL-23 receptor is highly expressed on Th17 cells, and IL-23 is important for the development of Th17 cells *in vivo*.[13] Several different single-nucleotide polymorphisms in IL-23R locus are independently associated with human IBD. An uncommon coding variant of IL-23R (Arg381Gln) confers strong protection against Crohn's disease (CD).[11] A few other non-coding variants are linked with the susceptibility to both CD and ulcerative colitis. However, these genetic data do not provide further mechanistic information regarding the functions of the IL-23 pathway in the pathogenesis of IBD. On the other hand, studies with preclinical animal IBD models strongly support a pathologic role of IL-23 in the onset of IBD.[14,15] In the absence of IL-23 signaling, mice develop less severe disease in several preclinical models of IBD. Importantly, encouraging data from human clinical trials indicating amelioration of CD with a p40 antibody, which neutralizes both IL-23 and IL-12 pathways, further support an important role of IL-23 in human IBD.[16] Given the critical functions of IL-23 in driving IL-22 production from various immune cells, one might conclude that IL-22 also promotes pathogenesis of human IBD. The story, however, is more complicated.

Many preclinical studies thus far have suggested that IL-22 may play a protective role in the gut. In ulcerative colitis and CD patients, IL-22 has been found circulating in the blood and in $CD4^+$ T cells of the colon.[17–19] Expression of IL-22 is augmented upon progression of disease in dextran sulfate sodium-induced colitis,[18] *C. rodentium* infection-induced colitis,[6] and T-cell receptor-α knockout model of colitis.[20] In addition to its protective role in infectious colitis, as we discussed before, IL-22 also alleviates disease severity in both T-cell receptor-α knockout and dextran sulfate sodium models of colitis.[20] In these models, IL-22 helps to restore goblet cells and enhance mucus production. In summary, these data suggest that the augmented IL-22 in IBD may facilitate restoration of the normal homeostasis of intestinal epithelial cells. However, we cannot exclude the potential pathogenic role of IL-22 in other models of IBD.

As discussed before, IL-22 induces the production of various antimicrobial peptides from epithelial cells.[2] Genes induced by IL-22 in colon cells include the S100 and Reg families of proteins[6] (**Figure 1**). The functions of these antimicrobial proteins in IBD are still largely unclear. S100A8 and S100A9 are calcium-binding proteins produced by neutrophils, monocytes, and epithelial cells under inflammatory conditions.[21] S100A8 and S100A9 are components of the heterodimeric protein calprotectin. An increased expression of S100A8 and S100A9 is associated with human ulcerative colitis and CD, as well as mouse models of colitis. One postulation is that during inflammation, these proteins can bind endothelial cells and aid in leukocyte extravasation. Recent data, however, suggest that calprotectin can inhibit the growth of *Staphylococcus aureus* in abscesses by chelation of nutrient Mn^{2+} and Zn^{2+}. RegIIIγ and RegIIIβ are both induced during bacterial colonization in the gut and in an otherwise inflamed colon.[22] Expression of the RegIII proteins is also increased in human ulcerative colitis and CD, as well as in mouse models of IBD.[23] *In vitro*, RegIIIγ kills Gram-positive bacteria and RegIIIβ can aggregate *Escherichia coli*.[22] In the small intestine, the upregulation of RegIII is dependent on the colonization by commensal bacteria. In the colon, however, IL-22 is critical for the induction of Reg family protein expression during *C. rodentium* infection. Recombinant RegIIIγ can partially rescue mortality associated with the loss of IL-22 following *C. rodentium*-induced colitis.[6] Finally, lipocalin 2 is an antimicrobial protein expressed in colon epithelial cells and leukocytes in the CD colon.[24] The antibacterial function of lipocalin 2 is thought to be mediated by sequestering iron away from the pathogen. Given that IL-22 induced lipocalin 2 in human skin keratinocytes and mouse tracheal epithelial cells,[7,10] it is likely that IL-22 can also directly induce lipocalin 2 in the gastrointestinal tract, although this has not been experimentally demonstrated. Despite the convincing data regarding these antimicrobial proteins in controlling the invasion of pathogens in the gastrointestinal tract, there is a lack of conclusive data on their roles in human IBD. Future studies focusing on the role of these proteins in IBD will also facilitate our understanding of the biology of IL-22 in the pathogenesis of IBD.

In summary, it is important to determine the functions of IL-22 and its downstream mediators in human IBD, particularly if we want to design better strategies to treat human IBD by targeting the IL-23 pathways.

PROINFLAMMATORY AND ANTI-INFLAMMATORY ROLES OF IL-22

IL-22 not only shares structural homology with IL-10, but also uses the IL-10R2 chain as one component in its receptor complex. In addition, both IL-10 and IL-22 activate Stat3, as well as enhance the expression of SOCS3.[25] These data lead to the speculation that IL-22 may play a similar anti-inflammatory role as does IL-10 during immune responses. Indeed, as we previously discussed, in autoimmune colitis and *C. rodentium*-induced infectious colitis, lack of IL-22 resulted in exacerbated tissue inflammation.[6,20] Furthermore, IL-22 also protects against liver damage caused by the *in vivo* administration of concanavalin A.[26,27] IL-10 targets various immune cells and inhibits the production of many proinflammatory cytokines from these cells. Similar functions, however, have not been observed for IL-22, mainly because IL-22R is not expressed by immune cells.[2,25] An alternative hypothesis is that IL-22 exerts its anti-inflammatory functions on epithelial cells. This premise, however, contradicts the proinflammatory functions of IL-22 observed *in vitro*, in infectious diseases and inflammatory skin diseases.[2] It is possible that IL-22 plays distinct roles in different epithelial cells, for example, promoting immune responses in keratinocytes and repressing inflammation in hepatocytes. Further research is needed to provide solid data to support this hypothesis. On the other hand, the protective benefit observed in IBD and hepatitis may simply reflect the consequences of tissue protection and repair of IL-22. By restoring the tissue epithelial barrier, IL-22 may help to sequester leukocytes from the proinflammatory stimuli produced by pathogens or dying tissues and consequently block the further amplification of the immune responses. This premise may provide a coherent explanation for the convoluted results seen in the studies of IL-22.

In conclusion, in the classic Th1 and Th2 dichotomy model, Th1 cells and the downstream effector cytokines promote cellular immunity by activating CD8 cells, natural killer cells, and macrophages, all of which control infection by intracellular microbes.[28] Th2 cells, on the other hand, are essential for humoral immunity and are specialized to protect hosts from the invasion of parasites, such as helminths. The recently identified Th17 cells preferentially produce IL-17A, IL-17F, IL-22, and IL-21.[13] IL-22, as well as IL-17A and IL-17F, targets tissue epithelial cells. Th17 cells and their downstream cytokines, such as IL-22, fill a gap in our understanding of the role of CD4 T cells in the immune response toward extracellular bacteria, which is not explained by the Th1/Th2 paradigm. It is now clear that Th17 cells are essential for host defense against extracellular bacteria through the induction of proinflammatory mediators and antimicrobial peptides from tissue epithelia.

DISCLOSURE

All authors are employees of Genentech Inc.

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