Recent advances in IL-22 biology

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Abstract

Several cell types, in particular epithelial cells, express the receptor for the cytokine IL-22 and upon its recognition produce molecules that are active both locally and systemically. Many different types of lymphocyte secrete IL-22. Th17 cells produce IL-22 although the optimal conditions for secretion of IL-17 or IL-22 by Th17 cells differ, as do the transcription factors involved. Aryl hydrocarbon receptor is required for IL-22 production by Th17, Th22 and γδ T cells. Th22 cells produce IL-22 in response to IL-6 and tumor necrosis factor α (TNF-α), particularly in the skin, whereas γδ T cells produce IL-22 in response to IL-23, particularly in the lung. NK cells produce IL-22 in response to IL-12 and IL-18 or IL-23. Retinoic acid-related orphan receptor-y-positive innate lymphoid cells, including lymphoid tissue inducer (LTI) and LTI-like cells express IL-22 with IL-23 again enhancing expression. IL-22 is known to be expressed in many chronic inflammatory conditions, including psoriasis and rheumatoid arthritis, and its up-regulation often correlates with disease activity. IL-22 is known to be protective in the gastrointestinal tract in inflammatory bowel disease but may mediate either harmful or helpful inflammatory responses in different models of intestinal infection. Finally, IL-22 may also play an important role in tissue repair.

Introduction

IL-22 is a cytokine that is important for the modulation of tissue responses during inflammation. Through activation of Stat3-signaling cascades, the cytokine induces proliferative and anti-apoptotic pathways, as well as anti-microbial molecules, that help prevent tissue damage and aid in its repair. IL-22 is expressed by both the adaptive arm of the immune system, such as CD4 T-cell subsets, as well as by innate lymphocytes, including NK cells and LTI-like cells. IL-22 plays an important role in inflammation, including chronic inflammatory diseases and infectious diseases. This review focuses on new findings on the cell subsets that express IL-22 and its functional role during inflammation.

Cells that respond to IL-22

IL-22 is recognized by a heterodimeric receptor consisting of IL-22R and IL-10Rβ (1, 2); the expression of IL-22R is mainly confined to epithelial cells, thus providing signaling specificity to tissues (3). The best characterized IL-22-target cells include keratinocytes, hepatocytes and colonic epithelial cells. Binding of the cytokine to this receptor leads to activation of Stat3 signaling cascades, as well as Akt and mitogen-activated protein kinase pathways (4). This in turn results in induction of various tissue-specific genes, including serum amyloid A (SAA), anti-microbial proteins (β-defensin, Reg3γ, lipocalin-2), and mucins (1, 3, 5–7). These molecules have localized effects in the tissues (i.e. anti-microbials) but also have more generalized effects due to systemic induction (i.e. SAA in sera). IL-22 also induces proliferative and anti-apoptotic pathways in responsive cells allowing for tissue preservation (8).

The expression of IL-22 in cells of the immune system

IL-22 is expressed by many different types of lymphocytes, including both those of the innate and adaptive immune system. This includes CD4 T cells, most notably T17 cells, and γδ T cells, NK cells, LTI cells and LTI-like cells. Regulation of IL-22 expression by these different subsets has some common qualities, such as similar activation receptors and transcription factors, but there are also unique mechanisms.

T17 cells

IL-22 was originally thought to be a T17-associated cytokine but with the discovery of T17 cells has since been found to be most highly expressed by this particular subset (9). IL-22 expression differs from that of IL-17A and other T17-associated cytokines. The ideal in vitro culture conditions for generating IL-17A-expressing cells, mainly the presence of transforming growth factor-β (TGF-β) and IL-6, does not lead to optimal IL-22 expression; this is because TGF-β is inhibitory to IL-22 expression (10–12). IL-17A production was just revealed to be able to occur independently of TGF-β signaling by differentiating naive T cells in the presence of IL-6, IL-23 and IL-1β and...
this cytokine milieu also leads to IL-22 expression (11). Furthermore, in vivo IL-22 expression during infections is dependent on IL-23, but IL-17A expression is less so (13, 14).

IL-17A is highly dependent on the nuclear hormone receptor transcription factors retinoic acid-related orphan receptor γt (RORγt) and RORα for its expression; IL-22 is less so (15, 16). In contrast, IL-22 expression requires the ligand-dependent transcription factor aryl hydrocarbon receptor (AHR) (16). Ligands include environmental toxins, such as halogenated aromatic hydrocarbons, and endogenous natural ligands, such as the breakdown products of aromatic amino acids (16, 17). Addition of these AHR ligands during in vitro culture or in vivo during an immune response augments IL-22 expression. Furthermore, Notch signaling induces production of endogenous AHR ligands, leading to greater IL-22 expression (18).

**T17,22 cells**

In humans, a subset of CD4 T cells that specifically expresses IL-22 and is mainly found in tissues has been identified. Termed T17,22 cells, these cells express the chemokine receptor CCR6, and the skin-homing receptors CCR4 and CCR10, allowing for localization to the skin (19, 20). They do not express IFN-γ or IL-17A, or the T17- and T17-associated transcription factors T-bet or RORγt, respectively (19). Like that described for murine T17 cells, AHR is an important transcription factor for the expression of IL-22, but not IL-17A (20).

Inflammatory cytokines, namely IL-6 and tumor necrosis factor α (TNF-α), promote priming of T17,22 cells and addition of active vitamin D (found in the sun-exposed skin) enhances IL-22 expression. Langerhans cells, specialized professional antigen-presenting cells found in the epidermis, are able to induce T17,22 cells (21), as were plasmacytoid dendritic cells (19). Th22 T-cell clones appear to be quite stable; if cultured in T17,2- or T17- or Treg-polarizing conditions, the T17,22 clones continue to express IL-22 and not the other cytokines associated with these T17 subsets (22). T17,22 cells appear to be important for skin homeostasis and in inflammation as the T17,22 cell population is increased in psoriasis patients (23).

**γδ T cells**

The γδ T-cell subset comprises innate T cells that have rapid and immediate effector functions upon recognition of unknown ligands. Unlike conventional CD4 T cells, these cells constitutively express IL-23R, and therefore immediately respond to IL-23 stimulation to express both IL-17A and IL-22 (24). As for T17,17 and T17,22 cells, AHR is an important transcription factor for the expression of IL-22, but not IL-17A (24). IL-22-expressing γδ T cells are particularly important in pulmonary immune responses (25).

**NK cells**

NK cells are innate lymphocytes that lack somatically rearranged cell surface antigen-specific molecules and develop independently of the transcription factor RORγt. Upon activation, conventional NK cells rapidly express IFN-γ, perforin and granzymes that play an important role in the control of viruses, intracellular bacteria and tumors. These cells can be activated by a synergy between two cytokines, IL-12 and IL-18, presumably secreted by macrophages activated by antigen/innate sensor encounter. This activation by IL-12 and IL-18 can also induce NK cells to secrete IL-22 (26, 27). Additionally, stimulation with the cytokine IL-23 leads to induction of IL-22, but not IFN-γ, in NK cells (27). These NK1.1+ IL-22-expressing cells contribute to IL-22 expression during inflammatory bowel disease (IBD) (27), as well as in influenza infection (28). Other studies have reported IL-22-secreting NK cells termed NK22 cells (29); however, as it currently stands, these cells are now better characterized as the LTi-like IL-22-expressing cells discussed in the following section.

**RORγt-positive innate lymphoid cells**

LTi cells were originally discovered in the fetal tissue of mice (30). These cells express lymphoty whole-α1β2 and other factors that are important for the stromal re-organization and lymphocyte recruitment required for lymph node development. More recent work has shown that LTi cell development is dependent on the transcription factor RORγt and also needed for formation of Peyer’s patches in the small intestine (31). Although discovered in mice over 10 years ago, only recently has a corresponding cell subset been identified in human fetal lymph nodes and spleens (32). In discovery of this cell subset, it was also revealed that these cells express considerable amounts of IL-22, as well as IL-17A (32).

LTi-like cells also express IL-22 and share expression of NK-cell and LTi markers; they express the NK-cell surface marker Nkp46 and the LTi cell transcription factor RORγt (33–35). There are strong lineage relationships between NK cells and LTi-like cells. Both NK cells and LTi-like cells are dependent on expression of the transcription factors ID2 and TOX for their development (36, 37). However, these cells are dependent on different downstream transcription factors; NK cells are dependent on E4bp4/Nfil3 (38, 39), whereas LTi-like cells are dependent on RORγt (35). Additionally, NK cells require IL-15 for their development, and IL-7 is important, but not essential, for LTi-like cells (33, 35). An elegant new study has performed a lineage relationship analysis of RORγt-expressing innate lymphocytes (40). These cells arise from distinct fetal liver RORγt precursors and the data suggest that LTi cells are required in the fetus for lymph node development and after birth they undergo a programmed population change to what has been termed LTi-like cells, to migrate to the gastrointestinal (GI) tract to sustain intestinal homeostasis.

LTi-like cells are CD3– and express CD127, a component of the IL-7 receptor, and IL-23R, and in mice the cells are also CD4+. Unlike other cell subsets that only express IL-22 upon activation, LTi-like cells express low constitutive levels of IL-22 that are highly induced upon activation by IL-23 stimulation. They also express IL-17A, but not IFN-γ, perforin or granzymes (33–35, 41). In addition to being localized to the small intestine, these cells have also been found in lymphoid tissues such as the spleen (42).

**IL-22 function**

**Chronic inflammatory diseases**

IL-22 is highly expressed in several different chronic inflammatory conditions, including psoriasis, IBD and rheumatoid...
arthritis (43–45). This up-regulation of IL-22 is a correlation with disease; it cannot be inferred if IL-22 is a cause of the inflammation and/or a result of it. As such, small animal disease models have been employed to help elucidate the role of IL-22 in inflammation. Using either gene-deficient mice or administration of neutralizing antibodies, investigators have studied the role of IL-22 in various inflammatory conditions. These models have identified both inflammatory and protective roles for IL-22.

The best studied function for IL-22 is within the skin; IL-22 is inflammatory during skin inflammation. Transgenic mice engineered to over-express IL-22 have an aberrant skin phenotype that resembles psoriasis (46). The IL-22 transgenic pups are born with shiny and stiff skin and die several days post-birth. Histological analysis of the skin reveals epidermal thickening and that the dermal layer contains infiltrating macrophages. Using IL-22-deficient mice, Zheng et al. showed that in the absence of IL-22, IL-23-mediated dermal inflammation was reduced (10). Another group has also shown that IL-22 is inflammatory in a T-cell-mediated model of psoriasis (47).

These data are consistent with in vitro studies in which IL-22 was found to mediate keratinocyte proliferation and epithelial hyperplasia (46, 48). Furthermore, using three-dimensional epidermis culture systems, Wolk et al. showed that IL-22 is important for epidermal remodeling (46). Thus, though induction of proliferation, IL-22 induces keratinocyte migration, leading to the hyperplasia of keratinocyte layers, and results in a thickening of the epidermis.

In addition to psoriasis, an inflammatory role for IL-22 has been found in rheumatoid arthritis (49). Using an experimental model in which mice are immunized against collagen generating an autoimmune response in the joints, mice deficient in IL-22 had decreased incidence of arthritis and pannus formation (49). IL-22 may promote osteoclastogenesis leading to bone erosion in rheumatoid arthritis.

IL-22 also has a protective role in inflammation. The dual nature of this cytokine, protective versus inflammatory, likely depends on the inflammatory context, which includes, but is not limited to, the duration and amount of IL-22 present, the overall cytokine milieu and the tissues involved. IL-22 has shown to be protective during hepatitis (8, 50). In hepatocytes, IL-22 activates anti-apoptotic and pro-survival pathways, which allows for their enhanced survival (8). IL-22-expressing T\(_{h}17\) cells transferred to mice before induction of hepatitis ameliorated liver injury (50). IL-22 also plays a positive role in liver regeneration and in liver lipogenesis and hepatic steatosis induced by a high-fat diet or ethanol (51–53).

IL-22 is also protective during IBD. Genome-wide linkage analysis of IBD patients has identified mutations in the genes encoding IL-22 and the IL-10R\(_{β}\) subunit of the IL-22 receptor complex (54, 55). The IL-22 receptor complex is highly expressed within the GI tract and in the inflamed colon, IL-22 is expressed by CD4 T cells, likely T\(_{h}17\) cells, and innate lymphocytes such NK cells and LTI-like cells (27). Using different experimental models of IBD—DSS-induced colitis which is thought to be mainly driven by innate immune response cells and CD4+ CD45RB\(^{hi}\) T-cell-mediated colitis in which naive T cells devoid of regulatory T cells are transferred into T-cell-deficient mice where they proliferate unimpeded leading to colitis—IL-22 has been shown to be protective in both cases (5, 27). Furthermore, Sugimoto et al. showed that IL-22 can be therapeutic to IBD; gene therapy transfer of the il22 gene into the colons of already inflamed mice resulted in amelioration of the inflammation (5).

Several molecules appear to be important for the mechanism of how IL-22 provides protection within the GI tract. IL-22 induces expression of several anti-microbial molecules in the GI tract, including Reg3\(γ\), lipocalin-2 and β-defensins (3, 6, 7). These proteins may be important in the control of pathogenic micro-organisms within the gut. IL-22 also induces expression of mucins, a large heavily glycosylated family of proteins that forms a protective layer in the GI tract allowing for separation of commensal and pathogenic flora from the epithelium and hereby minimizing the immune response (5). Furthermore, IL-22 has direct effects on the colonic epithelium allowing for its proliferation and thereby contributing to its integrity.

IL-22-expressing γ\(δ\) T cells are important for protection from lung fibrosis (25). Repeated antigen stimulation in the lung leads to collagen deposition and the development of fibrosis. In the absence of IL-22 expression, mice had significantly more lung fibrosis than control mice, and the administration of recombinant cytokine further reduced the production of collagen. These data suggest that IL-22 plays a protective role in the development of fibrosis.

**Infectious diseases**

IL-22 also has a function in inflammation mediated by pathogens. IL-22 can be inflammatory and mediate disease in the GI tract. When infection by the parasite Toxoplasma gondii is introduced via the peritoneal cavity or the bloodstream, IL-22 does not play a detectable role in its pathogenesis, including parasite loads in the brain and liver lesions (56). In contrast, oral inoculation of the parasite into IL-22-deficient mice results in less disease pathology in the small intestine compared with wild-type controls (14). Thus, IL-22 is pathogenic in the course of T. gondii infection in the GI tract, but not other tissues.

By contributing to the maintenance of epithelial barriers, IL-22 helps prevent dissemination of pathogenic bacteria, such as Klebsiella pneumoniae in the lung (57), or enteropathogens, including Citrobacter rodentium and Salmonella enterica serotype Typhimurium, in the GI tract, thereby limiting bacterial growth (6, 7). Additionally, IL-22 aids in the elimination of pathogens by inducing different anti-microbial proteins (6, 7).

Although human data indicating a role for IL-22 in infection are sparse, besides correlative reports showing induction of IL-22 during diseases, there is an interesting report that suggests that IL-22 plays a role in human infection. Patients with autoimmune polyendocrine syndrome type I have a high rate of chronic mucocutaneous candidiasis (58). In that study Puel et al. showed that these patients have high levels of auto-antibodies to IL-17A and IL-22, in effect leading to cytokine neutralization and suggests that these cytokines are important for controlling yeast infections. In animal models of candidiasis, there are conflicting reports as to the importance of IL-22 to pathogenesis (59, 60).

**Tissue repair**

In addition to inflammation induced by autoimmune or infectious diseases, IL-22 also plays a role in tissue repair after
wounding. Pickert et al. showed delayed healing of mucosal colonic biopsies of IL-22-deficient mice compared with controls (61). IL-22 is also important for the regeneration of liver tissue after partial hepatectomy and repair from alcohol-induced damage (52,53). In vitro assays have shown that IL-22 treatment of keratinocytes quickens repair after wounding (22). Thus, IL-22 has many roles in aiding tissue repair.

Concluding remarks

Many outstanding questions regarding IL-22 and inflammation exist. Most in vivo studies have not elucidated which IL-22-expressing cell subset mediates the observed effects. For example, T\(_h\)17 cells have been shown to be able to provide protection against hepatitis (50) but the study did not show if this subset was required for protection. Other studies have distinguished between innate and adaptive immune system-derived IL-22 by comparing models between immunocompetent mice and mice with deficient adaptive immune responses (i.e. Rag-deficient or scid) (6, 27). Further examination pin-pointing the role of different IL-22-expressing subsets will allow us to better understand this cytokine.

Second, the role of IL-22 not under inflammatory conditions, but instead during homeostasis needs to be more closely examined. IL-22 is expressed constitutively by I-Le-like cells within the small intestine, a tissue that is under the careful immune balance between inflammation and tolerance. Gaining a better understanding of the expression and role of IL-22 in health and disease is important for development of IL-22 as a potential drug target.

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