The IL-23 to IL-17 cascade in inflammation-related cancers

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Received and accepted on August 28, 2015

Clin Exp Rheumatol 2015; 33 (Suppl. 92): S87-S90.

Key words: inflammation, colorectal cancer, IL-23, IL-17.

Funding: this work was supported by the Croucher Foundation and China Postdoctoral Science Foundation (20110490919) to K. Wang and NIH (AI043477; DK035108) and American Association for Cancer Research (07-60-21-KARI) grants to M. Karin, who is an American Cancer Society Research Professor and holds the Ben and Wanda Hildyard chair in Mitochondrial and Metabolic Diseases.

Competing interests: none declared.

ABSTRACT

Two inflammatory cytokines, IL-23 and IL-17A, produced by myeloid cells and different lymphocyte subsets, were found to play important pathogenic functions in several inflammation-related cancers. In colorectal cancer, elevated expression of IL-23, IL-23 receptor and IL-17A has been linked to adverse prognostic outcome and rapid progression to metastatic disease. In mouse models of colorectal tumourigenesis genetic or pharmacological inhibition of these cytokines attenuates tumour development and malignant progression. Collectively, such findings suggest that IL-23 and/or IL-17A inhibitors should be evaluated for their therapeutic and preventative potential in human cancers, especially in colorectal cancer.

Introduction

IL-23 is a heterodimeric cytokine composed of a common p40 subunit, that it shares with its relative IL-12, and a unique p19 subunit (1-3). IL-23 binds to a heterodimeric IL-23 receptor (IL-23R), whose engagement leads to activation of STAT3 and other signalling pathways (1-4). IL-23 is mainly produced by activated M1 macrophages in response to engagement of Toll-like receptors (TLRs), which act to induce its expression through the NF-κB and STAT3 transcription factors (5-7). IL-23 plays an important role in expression of another cytokine, IL-17A, by phenotypically stabilising and inducing the expansion of IL-17 producing Th cells (Th17 cells) or through activation of innate lymphoid cells (iLC) and γδ T cells together with IL-1 (8-11). There are six members of the IL-17 family, namely IL-17A, B, C, D, E and F (12). IL-17A and F are the closest members of this family, and both bind to IL-17 receptors A (IL-17RA) and C, whose engagement leads to activation of mitogen activated protein kinases (MAPK), NF-κB and C/EBP signalling pathways through the adaptor proteins Act1 and TRAF6 (13, 14). IL-17A and F are produced by Th17 cells, γδT cells, NKT cells, and other types of iLCs (13, 15, 16).

Although IL-23 and IL-17 have traditionally been studied for their roles in host defense, auto-immunity and chronic inflammatory diseases, elevated expression of these cytokines and their receptors has also been detected in various human cancers, including those of the colon, ovaries, lung, breast, stomach, skin, liver, and head and neck (17-19). Importantly, elevated expression of IL-23, IL-17 and IL-6 in stage 1 to stage 4 colorectal cancer has been linked to adverse prognosis and a more aggressive disease (20). Another study described an IL-23-Th17 gene signature, whose elevated expression in stage 1 and 2 early colorectal cancer predicted rapid progression to incurable metastatic disease (21).

IL-23 and IL-17 in mouse models of cancer

While the human studies described above suggest that elevated IL-23 and IL-17 expression has an important pathogenic function in cancer, strong and conclusive evidence that these cytokines indeed have a causal role in tumour development and progression came from mouse studies. The first study to show a role for IL-23 in cancer development had used the mouse two-step skin carcinogenesis model based on treatment of mice with the carcinogen DMBA and the tumour promoter TPA (17). That study has shown that IL-23 promotes the development of skin cancer by inducing the expression of MMP9 and other genes involved in angiogenesis while reducing the infiltration of $CD8^+$ T cells into skin tumours (17). Subsequent studies have shown that IL-17A also supports the development of skin cancer by activating STAT3 in tumour and stromal cells and promoting the infiltration of

myeloid cells into the tumour microenvironment (22, 23). These studies, however, did not show that IL-23 and IL-17A act in a cascade.

The IL-23 to IL-17 cascade in colorectal tumourigenesis

Although the studies described above demonstrated the importance of IL-23 and IL-17 in mouse models of cancer, they have not shown that IL-23 acts by inducing IL-17 expression, neither have they explained the cause of elevated IL-23 or IL-17 in the skin cancer models that were investigated. A much deeper understanding of the relationship between IL-23 and IL-17 in cancer and the mechanisms that control their expression has been gained from studies of colorectal tumourigenesis in mice. Using the Apc^{Min} model of spontaneous colorectal tumourigenesis, Wu and colleagues have shown that infection of mice with the human enterotoxigenic bacterium *Bacteroides fragilis* (ETBF) triggers colitis and accelerated tumour development that is dependent on the induction of IL-17A expression (24) . Neutralisation of IL-17A with a specific antibody prevented the ETBF-induced acceleration of colorectal tumour development (24). IL-17A is also important in the development of colitis associated colorectal cancer (CAC) induced by administration of the procarcinogen AOM and the irritant DSS (25-27). Although IL-17A and IL-17F belong to the same family and signal through the same receptors and effector mechanisms, IL-17F plays an opposite role from IL-17A and protects mice from CAC induction (27). The divergent roles of IL-17A and F cytokines in CAC may be explained by their distinct roles in autoimmune and chemically-induced inflammation, which is a critical step in CAC induction (28). Other studies have shown that genetic ablation of either IL-17A or IL-17F results in attenuation of tumour development in Apc^{Min} mice (29, 30).

However, the Apc^{Min} mouse does not provide the most accurate model for colorectal cancer as most tumours in these mice are microadenomas that develop in the small intestine rather than the distal colon. A more accurate model of colorectal cancer is provided by the so-called CPC-APC mouse in which one allele of the *Apc* tumour suppressor gene is deleted in the colon and loss of the second allele through loss-of-heterozygocity (LOH) results in development of large colonic adenomas that can progress to invasive carcinomas (31). Using this model, we found that colonic adenomas exhibit substantial upregulation of IL-23 and IL-17 expression relative to adjacent non-tumour tissue (18). The major site of IL-23 expression in colorectal tumours of CPC-APC mice are $CD11b^{+}$ tumour associated macrophages (TAM), whereas IL-17 is expressed in Th17 cells, γδ T Cells and iLC (18). Disruption of either the *Il-23p19* or *Il-23r* genes inhibited tumour development by 2.5- to 3-fold, mostly due to a decrease in tumour cell proliferation, rather than an increase in tumour cell apoptosis (18). In addition, ablation of either IL-23p19 or IL-13R inhibited adenoma to carcinoma progression. Biochemical analysis indicated that the blockade of IL-23 signalling resulted in inhibition of STAT3 activation in tumour epithelial cells but had no effect on activation of β-catenin, which is driven by the loss of *Apc* (18). Although IL-23R engagement can lead to STAT3 activation, there was no evidence that IL-23R is expressed on tumour epithelial cells. Furthermore, adoptive transfer experiments indicated that IL-23R signals to stimulate tumour development in haematopoietic derived cells, which unlike epithelial cells express high levels of this receptor. Indeed, IL-23 was found to control the tumoural expression of IL-17A and ablation of IL-17RA also inhibited tumour development and progression, suggesting the operation of a pro-tumourigenic cytokine cascade in which IL-23 controls IL-17A expression and IL-17A stimulates tumour development through engagement of IL-17RA (Fig. 1). Unlike IL-23R which is not expressed on tumour epithelial cells, IL-17RA is expressed on both tumour epithelial cells and various haematopoietic cell types.

What is responsible for the induction of IL-23 expression in TAMs and not in normal lamina propria macrophages? Adoptive transfer experiments revealed that IL-23 expression in TAM depends on expression of TLR2, 4 and 9 and their signalling adaptor MyD88 (18). Most likely, engagement of these TLRs leads to MyD88-dependent NF-κB signalling and transcriptional activation of both the p40 and p19 genes. Supporting a role for TLRs in induction of IL-23 expression in TAMs, treatment of CPC-APC mice with a cocktail of broad spectrum antibiotics that leads to a 99.9% decrease in the colonic commensal bacterial population, results in decreased IL-23 and IL-17 expression and leads to a reduction in colorectal tumour load in wild type, but not in IL-23R-deficient, mice (18). These results strongly suggest that commensal bacteria living in the colon or their disintegration products are responsible for tumoural IL-23 induction.

How do colonic bacteria lead to specific induction of IL-23 only within TAM and not in normal lamina propria macrophages? The answer is provided by the observation that tumour-bearing CPC-APC mice exhibit a marked increase in colonic barrier permeability. Introduction of fluorescent-labelled LPS into experimentally-generated colonic loops of tumour-bearing mice revealed specific penetration of LPS into the tumours but not into the normal colonic epithelium (18). Furthermore, within the tumours most of the LPS signal was located right next to TAMs. These findings suggested the specific deterioration of the intestinal epithelial barrier only in tumour areas. Indeed, colorectal tumours in CPC-APC mice were found to be devoid of mucin-producing goblet cells and as a result are not coated by the mucin layer that coats the normal colonic epithelium and prevents access of commensal microbes. In addition, colorectal tumours were found to be devoid of junctional-adhesion-molecule (JAM) expression and show other defects in tight junction formation. Importantly, absence of mucin coating, JAM deficiency and tight junction defects were also observed in human colorectal cancers (18). Furthermore, in both mouse and human colorectal tumours these defects are detected rather early in the tumourigenic

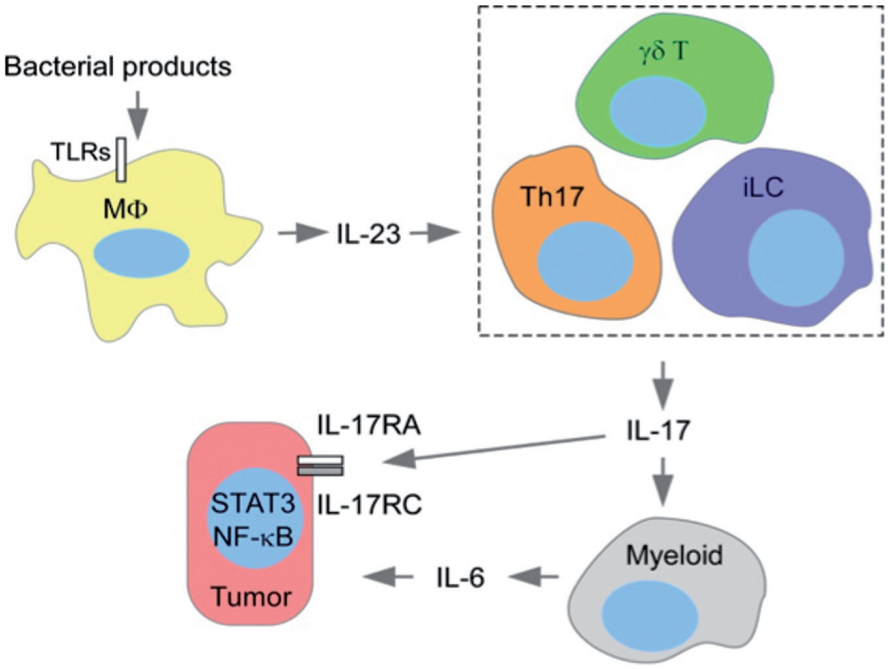

Fig. 1. The IL-23 to IL-17 cascade in inflammation and cancer.
Adenoma cells that arise upon loss of *Apc* expression in intestinal epithelial cells fail to form an effective surface barrier that prevents penetration of commensal bacteria and their products. As a consequence, bacterial products can access and activate tumour associated macrophages (MΦ) through engagement of Toll-like receptors (TLRs) and induce the production of IL-23. IL-23, in turn, promotes the production of IL-17 by Th17 cells, γδ T cells and innate lymphoid cells (iLC). IL-17 may signal in tumour infiltrating myeloid cells to induce the production of IL-6, which acts as a STAT3 activator in malignant epithelial cells. IL-17 may also engage its receptors expressed on malignant cells and promote their survival and growth in a more direct manner.

pathway, at the adenoma stage or even earlier. In addition to the barrier defects, both human and mouse early adenomas exhibit upregulation of both IL-23 and IL-17 and occasional presence of bacterial 16S RNA (18).

The IL-23 to IL-17 cascade as a therapeutic target.

The mouse studies reviewed above clearly indicate that the IL-23-IL-17 cytokine cascade is of great importance in the pathogenesis of colorectal cancer and may be instrumental in other cancers as well. These studies also suggest that agents that inhibit IL-23 or IL-17 production, receptor binding or receptor signalling may prove to be efficacious in the treatment or even prevention of colorectal cancer, one of the most common malignancies in the developed world and the third leading cause of cancer deaths. Such drugs are expected to be more effective in those patients whose tumours exhibit high levels of IL-23, IL-23R or IL-17A expression, especially in patients with colorectal cancer stage 1/2 that is highly positive for the IL-23-Th17 signature (21). Several types of agents should be considered for clinical development. First and foremost are the IL-23 and IL-17A neutralising antibodies already found to be effective and non-toxic in the treatment of various chronic inflammatory conditions such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease and psoriasis (32-36). Second, several small molecule inhibitors of Th17 differentiation were described (37-39) and amongst them RORγt antagonists are likely to inhibit IL-17 expression not only in Th17 cells but also in iLC. Such agents, however, need to be evaluated for safety and efficacy in both small animal models and humans before their use in cancer treatment and prevention can be considered. Another class of agents that merit consideration are molecules capable of restoring barrier function and thus may inhibit the induction of IL-23 within TAMs. However, such molecules remain to be identified and tested in the CPC-APC mouse model described above. Importantly, it is unlikely that any such agent will be able to exert an anti-tumour effect on its own and their use should only be considered in conjunction with more conventional chemotherapeutic drugs, such as 5-fluorouracil, oxaliplatin, anti-VEGF and anti-EGF receptor antibodies (40-42), which have already shown effectiveness in the treatment of colorectal cancer.

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