Published in final edited form as:
Curr Opin Oncol. 2013 November ; 25(6): 637–645. doi:10.1097/CCO.0000000000000006.

Current status of IL-10 and regulatory T-cells in cancer

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Abstract

Purpose of review—Tumor growth elicits antigen-specific cytotoxic as well as immune suppressive responses. Interleukin-10 (IL-10) is a key immune-suppressive cytokine produced by regulatory T-cells (Tregs) and by helper T-cells (TH). Here we review pleiotropic functions of IL-10 that impact the immune pathology of cancer.

Recent findings—The role of IL-10 in cancer has become less certain with knowledge of its immune stimulatory functions. IL-10 is needed for T-helper cell functions, T-cell immune surveillance, and suppression of cancer-associated inflammation. By promoting tumor specific immune surveillance and hindering pathogenic inflammation IL-10 is emerging as a key cytokine in the battle of the host against cancer.

Summary—IL-10 functions at the cross roads of immune stimulation and immune suppression in cancer. Immunological mechanisms of action of IL-10 can be ultimately exploited to develop novel and effective cancer therapies.

Keywords

IL-10; Tregs; immune surveillance; inflammation; cancer

Introduction: IL-10 and popular view of tumor immune surveillance

With the discovery of tumor antigens [1-4] cancer immunology entered a new era where efforts to control cancer could be focused on the mechanistic principles of improving tumor specific T-cell responses. The concept of T-cell help [5] and natural tolerance induced by regulatory T-cells [6] were both established after the discovery of tumor antigens, and provided additional knowledge of mechanisms that promote or hinder cancer immune surveillance. Interleukin-10 (IL-10) was first recognized as a T-Helper-2 (TH2) cytokine that modulates the growth and/or differentiation of innate immune cells, keratinocytes, and endothelial cells and suppresses the activation and effector functions of T cells [7]. Simplistically, protective cytotoxic responses require $CD4^+$ T-cell help [8-11] and are driven

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by the T-helper (TH)-1 cytokines INF-γ and IL-12. By contrast, inflammatory responses produced by the TH2 committed CD4+ T-cells are a diversion if not an obstacle to effective transplant and tumor rejection by cytotoxic T-cells [12-14]. IL-10 and TGFβ that are produced by regulatory T-cells (Tregs) are expected to be major obstacles to $CD8^+$ T-cell mediated tumor lysis [15] and cancer immune therapy [16]. Defying this expectation, protective roles for IL-10 in cancer are emerging that stimulate a fresh look at the functions of this cytokine and of its cellular sources [17]. IL-10 is now recognized not only as the most potent anti-inflammatory cytokine but also for enabling cancer immune surveillance and tumor rejection. This review addresses the controversial functions of IL-10 in cancer and their potential value for cancer immune therapy.

Text of Review

1. Cellular sources of IL-10

Many cell types have the ability to produce IL-10, including $CD25^+Foxp3^+$ [18,19], Tr1 $CD4^+$ T cells [19-23], B cells [24-28], macrophages, mast cells, eosinophils, and dendritic cells (DCs) [29-31], as well as $CD8^+$ T cells [32]. Interestingly, while IL-10 inhibits expression of TH1 cytokines, IL-10-producing TH1 cells have been described in both mice and humans [33-42]. The balance between IL-10 and IFN-γ secreted by TH1 cells is thought to achieve a healthy equilibrium that permits effector responses while preventing autoimmunity [43-45]. The source as well as the spatial and temporal availability of IL-10 may differ between tissues and depend on the immune status of the organism. Macrophages are a major source of IL-10 in the intestine/colon of immune deficient $Rag1^{-/-}$ mice [46]. In immune competent mice T-cells are the major source of IL-10 in the intestine and colon [19], and are solely responsible for the increase in levels of IL-10 during polyposis [47]. IL-10 deficient mice without exception develop microbial dependent colitis [48]. Ablating IL-10 expression in T-cells alone is sufficient to cause colitis, revealing the importance of T-cells as a source of IL-10 and in controlling microbial induced inflammation in the colon [47]. The fact that the small intestine is not affected by IL-10 deficiency shows that the impact of IL-10 on immune homeostasis is tissue environment specific (Dennis, *et al* 2013, manuscript submitted).

2. IL-10 and suppression of immune response

Interleukin-10 (IL-10) was initially heralded as a major immune suppressive factor that was critical for induction of tolerance through inhibition of TH1 immune response and T-cell cytotoxic activity [49-52]. IL-10 was shown to impair the proliferation, cytokine production and migratory capacities of effector T cells [50]. Elevated levels of IL-10 obstructed cytolytic activity in grafted tumors [53-56], and conversely the blockade of IL-10 in animal models facilitated rejection of transplanted tumors [57-60]. The suppressive activity of IL-10 was reported to be direct, based primarily on *in vitro* experiments [49,61-66]. However, there is evidence suggesting that much of the suppression attributed to IL-10 is indirect and cell mediated [67].

Professional antigen presenting cells, also known as dendritic cells (DC), are important targets of action of IL-10. In earlier studies IL-10 down-regulated expression of MHC class

II and co-stimulatory molecules CD80/B7-1 and CD86/ B7.2, and Th1 cytokines including IL-12 by DCs [50,68,69] (reviewed in [70]). T cells that were activated in the presence of IL-10 or DC previously treated with IL-10 failed to respond to re-stimulation, and were described as anergic [64,71]. Tolerogenic DCs produced IL-10 [21,72,73] and autocrine activation of the IL-10 receptor (IL-10R) signaling helped to maintain DCs in an immature tolerogenic state [50,74]. IL-10 expressing DCs were shown to generate Tregs and Tr1 cells, which were also IL-10 producing cells [75-78]. Furthermore, IL-10 contributed to sustained expression of Foxp3 [46,79], TGFβ-Receptor-2 [80] and TGFβ [81,82] by recently activated Tregs, thus stabilizing Treg phenotype and functions[67,81]. IL-2 enhanced the expression of IL-10 by Tregs in a STAT5 dependent manner [83]. Tregs in turn catalyzed the generation of Tr1 cells through secretion of IL-27 [84]. IL-27, a member of the IL-12 cytokine family, induced both Th1 development and production of IL-10 by $CD4^+$ T cells [84-86]. Tr1 cells were also generated through the direct actions of IL-10 and INF-α [87,88] or through antigen presentation by tolerogenic IL-10 producing DC [72,73,89]. These observations showed that much of the immune suppression that is attributed to IL-10 *in vivo* can be accounted for by the generation and the complex immune modulatory mechanisms of action of Tregs and Tr1.

The impact of IL-10 on immune homeostasis is spatially and temporally controlled. Naïve CD4 T-cells were shown to be more sensitive than memory T-cells to IL-10, explained by down-regulation of IL-10 receptor (IL-10R) upon T cell activation [50,69,90]. For example L. major vaccination produced more robust TH1 responses when IL-10 was limiting at the time of antigen priming [91]. Also, neutralization of endogenous IL-10 with anti-IL-10 mAb inhibited the development of insulin dependent diabetes mellitus when performed early in mice life (priming phase) [92], while the same treatment had no influence on the disease when given to older animals (memory phase) [93]. IL-10 could also compromise immune surveillance by changing immunogenicity of the antigen presenting cell through down-regulation of Transporter Associated with Antigen Processing (TAP1/2) and therefore antigen presentation by MHC class I / HLA class I [94,95]. In fact, both TH17 and TH2 cells express IL-10 and there is good reason to expect IL-10 to work in a negative feedback loop to control activation of T-helper cells [96].

Mechanistically, ligation of IL-10R on DC triggers phosphorylation of janus kinases (JAK) that in turn activate the signal transducer and activator of transcription 3 (Stat3) [97-99]. STAT3 is critical for the expression of IL-10 but is also known to activate the expression of pro-inflammatory cytokines including IL-6. Interestingly, the IL-10 mediated activation of STAT3 is anti-inflammatory. This is achieved through IL10R signaling through Lymphocytic Activation Molecule (SLAM), Src Homology 2 Domain-containing Protein tyrosine phosphatase-1 (SHP-1), and Suppressor of Cytokine Signaling 3 (SOCS3) [100]. SLAM activates SHIP-1 that dephosphorylates and inactivates the co-stimulatory receptors CD28, ICOS, and CD2 [101,102]. Dephosphorylation inhibits the recruitment of phosphatidylinositol-3-kinase (PI3K) and blocks co-stimulatory signaling [90,103-107](for reviews see: [17,80]). Simultaneously, SOCS3 suppresses Stat-dependent signaling of inflammatory cytokines IL-6, [108] TNF-α, and IL-1β [109]. SOCS3 also suppresses signaling through the IL-23R and the expression of IL-17 in inflammatory Th17 T cells

[110]. Inhibition of pro-inflammatory cytokines is critical for generating functional extrathymic Tregs, since exposure of Tregs to IL-6 alone can compromise their lineage commitment and ability to suppress inflammation functions [111-113]. Thus, IL-10R signaling utilizes STAT3 but avoids the inflammatory consequences of action STAT3.

3. IL-10 and immune stimulation

The immune suppressive action of IL-10 was so attractive that it overshadowed the almost concomitant discovery of its stimulatory effects on thymocytes, B cells, and mast cells [24,114,115], and the interesting fact that the human IL-10 gene was first cloned from a T-cell line that also secreted IFN-γ [116]. A growing literature is revealing pleiotropic functions of IL-10 that include T-cell activation and tumor rejection. It has been even argued that many of the attributes of IL-10 that were associated with immune suppression are equally likely to enhance immune activation [67].

Stimulatory effects of IL-10 on T-cells and NK are known. IL-10 in combination with IL-2 substantially increased the frequency of $CD8^+$ T cells and augmented their cytolytic activity [117]. In the OVA specific OT1 model, exposure of CD8 T-cells to IL-10 during priming increased T-cell survival and numbers but not proliferation [118]. IL-10 in combination with IL-18 increased the frequency and cytolytic activity of natural killer (NK) cells [119]. These immune stimulatory functions of IL-10 had biologically meaningful consequences. Expression of IL-10 by tumor cells or antigen-presenting cells was protective in tumor transplant models [120,121]. Ectopic expression of IL-10 in mouse B16 melanoma cells induced NK cell mediated shrinkage of the transplanted tumors [122]. Similarly, expression of IL-10 reduced growth of the mouse TSA mammary adenocarcinoma cells upon transplantation [123]. Polyposis prone $APC^{\Delta 468}$ mice that were rendered IL-10 deficient had a significant delay in the onset of small intestine polyposis that coincided with increased intrapolyp cytotoxic activity at the onset of polyposis, suggesting improved priming of polyp-specific T-cells (Dennis *et. al.* 2013, manuscript submitted). There are also reports of protective benefits of systemic delivery of IL-10 [124,125]. A comprehensive study of the anti-tumor properties of IL-10 used multiple mouse tumor models including both chemically induced tumors and transplanted tumor cells [126]. In this study IL-10 induced several essential mechanisms of antitumor immune surveillance: infiltration and activation of intra-tumoral tumor-specific cytotoxic $CD8^+$ T cells, expression of IFNγ and granzymes by $CD8^+$ T cells, and enhancement of intratumoral antigen presentation. Consequently, IL-10 deficiency weakened tumor immune surveillance whereas transgenic overexpression of IL-10 or treatment with pegylated IL-10 protected mice from carcinogenesis[126]. Thus, IL-10 stimulated immune responses by modulating the quality of antigen presentation and influencing lymphocyte differentiation and lineage commitment. The need or IL-10 in TH1 lineage commitment suggests that it may have a role in establishing CD8 T-cell memory. Our observations in a mouse model of hereditary polyposis support these findings. In contrast to IL-10 competent mice that have prolonged intra-polyp cytotoxicity and never developed invasive lesions, IL-10 deficient mice rapidly lost their intra-polyp cytotoxicity and their lesions progressed into large invasive cancers, suggesting an abortive immune response that did not lead to establishment of T-cell memory (Dennis *et. al.* 2013, manuscript submitted).

The immune stimulatory functions of IL-10 were corroborated by studies of human T-cells, in which IL-10 enhanced IL-2-stimulated proliferation of both human $CD4^+$ and $CD8^+$ T cells by increasing cell division after activation, and augmented IL-2 but not IL-15-induced cytotoxicity of intestinal lymphocytes against colon cancer [127]. IL-10 in combination with IL-2 consistently increased the cytotoxic activity of human papillomavirus E7-specific $CD8^+$ cytolytic T-cells, while combinations of IL-2 and IFNγ or IL-12 or TGF-β failed to do the same [128]. In addition, while IL-10 inhibited the ability of DCs to generate allospecific cytotoxic activity, IL-10 in combination with IL-2 and anti-CD3 activated and promoted growth of human $CD8^+$ T-cells [129]. Finally, IL-10 effector $CD4^+$ T-cells with no immune regulatory functions have been described that are generated by treatment of naïve $CD4^+$ T-cells with TGF-β and IL-4 [130].

Altogether these observations support the provocative view that IL-10 is essential for TH1 and CD8 cytotoxic T-cell responses. They also raise questions as to the mechanisms by which IL-10 stimulates immunity, how these differ from mechanisms by which IL-10 suppresses immunity, which of these mechanisms of action is direct and which indirect result of IL10R signaling in effector T-cells, and to what extent the tissue environment determines the immunologic outcome of the action of IL-10.

4. IL-10 and suppression of Inflammation at environmental interfaces

Focal inflammation can improve antigen uptake and presentation leading to better specific $CD8^+$ T-cell responses and effective anti-tumor immune surveillance [131-136]. However among the T-cells that infiltrate the intestines the cytolytic status of CD8 T-cells in unknown while there is evidence suggesting that the intestine infiltrating $CD4^+$ cells can be cytolytic [137]. The majority of $CD8^+$ T-cells that infiltrate the intestines are either anergic or immune suppressive [138]. The intestines are also the major extrathymic sites of generation of anti-inflammatory Tregs [139] as well as pro-inflammatory T-helper -17 (TH17) cells [140]. Here, a fine balance between anti- and pro-inflammatory T-cells critically maintains microbes at bay while providing growth and repair functions [141]. Deregulation of inflammation in the colon predisposes to inflammatory bowel diseases [142-145]. Inflammation is an inherent component of sporadic colon cancer progression [146,147]. TH17 cells and cytokines are elevated in human colon cancer tumors [148] and negatively correlate with patient survival [149]. Animal modeling has demonstrated that inflammatory responses in intestinal cancers start in aberrant crypts and early adenomatous polyps [150-152]. Inflammation correlated with focal accumulation of microbes [47] and with the breakdown of epithelial barrier functions [153,154]. Much of this inflammation was driven by TH17 cytokines [113,155] whose genetic ablation (of IL-6, IL-23, TNFα) in bone marrow derived cells hindered polyp growth with varying efficacies [148]. Interestingly, control of inflammation in mice with polyposis also enhanced polyp specific TH1 response, increased intra-polyp cytolytic activity, and provided long term protection[148]. TH17 inflammation in polyposis is microbial driven and we recently showed that colonization of the mouse colon with beneficial microbiota can increase Treg expression of IL-10, protect against inflammation, and hinder polyposis [156].

TH17 responses are suppressed by IL-10 producing Tregs [140,157-159]. The adoptive transfer of IL-10 proficient Tregs from healthy donor mice to recipient mice that have polyposis or are otherwise prone to intestinal carcinogenesis suppressed inflammation and protected mice from intestinal carcinogenesis [155,160]. Only IL-10 competent Tregs were effective, and IL-10-deficient Tregs failed to prevent colitis [161] and carcinogenesis [155,162]. T-cell-specific ablation of IL-10 in mice exacerbated microbe driven inflammation and polyposis in the colon [47]. Treg specific ablation of IL-10 and germ-line deletion of IL-10 produced similar colonic inflammations emphasizing the role of Tregs as a major source of IL-10 in the control of microbial inflammation [159]. These observations define a central role for IL-10 expressing Tregs in control of pathogenic inflammation at environmental interfaces. In line with these findings, in a range of inflammation driven cancers including colon [163-167], gastric [168], and head and neck [169,170] as well as $HER2^+$ breast cancer[171], infiltration of tumors with Tregs is associated with less aggressive tumors and can be an independent predictor of longer patient survival. Altogether, these observations indicate that Treg expression of IL-10 is protective against colon cancer, by suppresing cancer-associated inflammation and improving anti-tumor immune surveillance.

TH17 cells are responsible for orchestrating microbial induced inflammation [172,173] and are intimately linked with the control of microbiota [174]. Expression of IL-17, is contingent upon activity of the transcriptional factors RORγt [175], RORα [176] KLF4 [177]. Expression of IL-10 by Tregs is elevated in the intestines to control TH17 inflammation [19,45,178]. However, during polyposis in mice expression of IL-10 is down-regulated and Tregs acquire TH17 like characteristics including expression of the signature TH17 transcription factor RORγt as well as IL-17 [155]. Similarly, both circulating and tumor infiltrating Tregs in human colon cancer have compromised expression of IL-10 and while remaining potently T-cell suppressive are unable to control inflammation [113,148]. These characteristics are shared by Tregs derived from lung cancer and pancreatic cancer patients (our unpublished observations). Mouse modeling has demonstrated that the IL-10 deficient Treg subset significantly contributes to disease outcome [113,148,155], since ablation of RORγt in the bone marrow derived cells or even just in Tregs was sufficient to recover expression of IL-10 and protect mice against polyposis [148]. These findings provide evidence for a central role of IL-10 in control of inflammation and immune surveillance (Figure 1), and open new possibilities for improving protective immunity at environmental surfaces through manipulation of this crosstalk.

Protective anti-inflammatory roles of IL-10 in cancer are gleaned from studies with pre-clinical models of cancer immune therapy. Therapies aimed at blocking the CTLA-4 inhibitory checkpoints, have promoted antitumor immunity in mouse models of cancer and objective clinical benefits in cancer patients [179]. In mouse models CTLA4 blockade expanded IL-10 producing $CD4^+FoxP3^+$ Tregs in a dose-dependent fashion [180]. In a mouse model of colitis anti-CTLA-4 treatment induced $IL\text{-}10^+$ Tregs that expressed the cell surface receptor inducible co-stimulator ligand (ICOS) and had potent IDO-dependent anti-inflammatory properties [181]. These observations are particularly interesting as expression of ICOS is an immunologic marker that correlates with clinical responses in prostate cancer patients who receive anti-CTLA-4 therapy [182]. Mouse modeling has revealed that

expression of ICOS is intimately related to production of IL-10 by Tregs [96,183] and Tr1 cells [184]. Expression of ICOS and IL-10 were in turn related to protective anti-tumor immunity. Furthermore, $Foxp3^+$ precursors isolated from the human thymus that co-expressed ICOS preferentially upregulated IL-10 expression in response to stimulation with DCs that express ICOS-L [185]. Together these observations suggest that protective mechanisms of CTLA4 blockade involve induction of IL-10 and suppression of inflammation by Tregs.

Concluding paragraph

Until recently, IL-10 was regarded as an immune suppressive cytokine that hindered anti-tumor immunity. It is becoming evident that IL-10 is essential for T-helper-1 cell function and anti-tumor cytolytic activity. The potent anti-inflammatory functions of IL-10, which are mainly indirect and cell mediated, synergize with its immune stimulatory functions to improve tumor specific immune surveillance. Knowledge gained thus far implicates IL-10 in the protective arm of immune response to at least those cancers that appear at environmental interfaces and are driven by microbial instigated inflammation (Figure 1). This knowledge provides new opportunities or immune intervention in cancer.

Acknowledgments

Funding:

NIH1R01 1R01CA160436-01**(PI: Khazaie)**

Circle of Service, Robert H. Lurie Comprehensive Cancer Center (PI: Khazaie)

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Key Bullet Point Summary

1. Regulatory T-cells (Tregs) and helper T-cells (TH) are major cellular sources of interleukin-10.
2. IL-10 is needed for T-helper cell functions, T-cell immune surveillance, and suppression of inflammation instigated by microbes and by transformed cells.
3. Mechanism of action of IL-10 is mostly indirect and mediated by T-cells.
4. IL-10 is a protective in cancers that occur at environmental surfaces.
5. IL-10 can be ultimately exploited to develop novel and effective cancer therapies

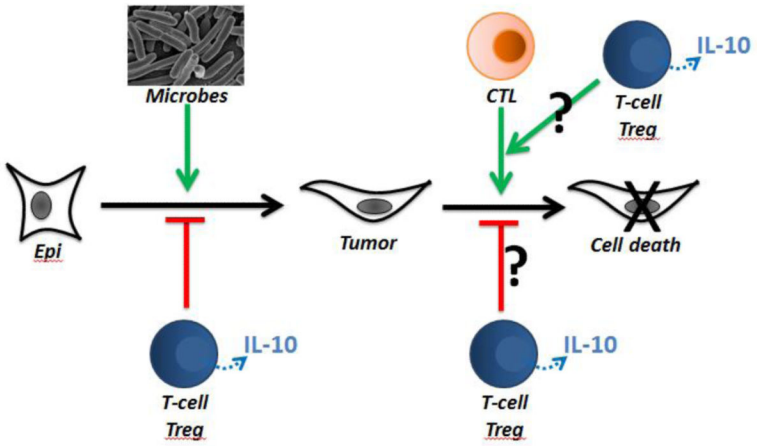

Figure 1. Overarching hypothesis

Colon cancer is driven by microbial associated. Typically this is a TH17 inflammation that is controlled by IL-10-expressing T-cells and Tregs. There is also a general consensus that cancer is controlled by immune surveillance and TH1 response. Ultimately, disease outcome is the product of both control of inflammation and effective immune surveillance. IL-10 is essential for both processes.