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REVIEW

Group 3 innate lymphoid cells: regulating host–commensal bacteria interactions in inflammation and cancer

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Abstract

A delicate balance exists between the mammalian immune system and normally beneficial commensal bacteria that colonize the gastrointestinal tract, which is necessary to maintain tissue homeostasis. Dysregulation of these interactions between the host and commensal bacteria is causally associated with chronic inflammation and the development of cancer. In contrast, recent reports have highlighted that commensal bacteria also play an essential role in promoting anti-tumor immune responses in several contexts, highlighting a paradox whereby interactions between the host and commensal bacteria can influence both pro- and anti-tumor immunity. Given the critical roles for group 3 innate lymphoid cells (ILC3s) in regulating inflammation, tissue repair and host–microbe interactions in the intestine, here we discuss new evidence that ILC3s may profoundly influence the development, progression and control of tumors. In this review, we provide an overview of recent advances in understanding the impact of commensal bacteria on tumorigenesis, discuss recent findings identifying ILC3s as critical regulators of host–microbe interactions and highlight the emerging role of this immune cell population in cancer and their potential implication as a therapeutic target.

Keywords: cancer, commensal bacteria, innate lymphoid cells, intestinal homeostasis

Introduction

Inflammation occurs in response to perturbed tissue homeostasis and is necessary to orchestrate immunity to pathogens and wound healing (1). In addition, chronic inflammation is causally associated with the initiation and progression of cancer (2, 3). In mouse models, tumor development can result from prolonged inflammation that is either aseptic or triggered by chronic infection by pathogenic bacteria, and in patient populations there are well-defined associations of chronic bacterial infection with an increased risk of cancer (3). In contrast to pathogenic micro-organisms, the human body contains trillions of harmless commensal bacteria collectively referred to as the 'microbiota' that play a critical role in many fundamental aspects of our physiology: comprising nutrition, metabolism, organ morphogenesis and the development of the immune system (4–9). This complex interaction between the host and its resident microbes necessitates a tight and dynamic regulation of the microbiota by the immune system and perturbation of these interactions is associated with the onset and exacerbation of multiple chronic inflammatory diseases, including inflammatory bowel disease (IBD), HIV infection and autoimmune disease (4).

Recent studies have highlighted that disruption of interactions between the host and commensal bacteria also influences tumorigenesis (10) and immune responsiveness to anti-cancer therapies (11). Therefore, a better understanding of the role and regulation of the microbiota in the context of cancer may drive the development of novel therapeutic strategies to limit tumor progression and enhance anti-tumor immunity. In line with this prospect, a recently appreciated family of innate immune cells, termed innate lymphoid cells (ILCs), has been described to play a major role in mucosal immunity, inflammation and tissue repair (6, 12–14). In particular, group 3 ILCs (ILC3s) have been identified as key regulators of host–microbiota interactions and mucosal tissue repair (6, 15, 16), and dysregulation of ILC3 responses has recently been implicated in chronic mucosal inflammation and cancer (17–28). In this review, we summarize the impact of the microbiota on pro- versus anti-tumor immunity, discuss the

central role of ILC3s in regulating host–microbe interactions and chronic inflammation and address the implications for the development and progression of cancer.

Inflammation, cancer and the microbiota: a new triumvirate

First hypothesized over 2000 years ago by the Greek physician Galen, the existence of an intrinsic connection between cancer and inflammation is an ancient concept (29, 30). Inflammation can promote carcinogenesis via multiple pathways, including the production of reactive oxygen and nitrogen species that induce DNA damage and drive genomic instability, as well as production of cytokines and growth or angiogenic factors that favor tumor development (3, 30, 31). Once a tumor is established—regardless of whether tumorigenesis was promoted by local inflammation or not—the tumor microenvironment exhibits pro-inflammatory features that contribute to tumor progression (3). The tumor-promoting consequences of inflammation are now recognized as a fundamental aspect or 'hallmark' of cancer and are supported by numerous experimental, epidemiological and clinical studies (2, 3, 30).

Many inflammatory disorders (e.g. IBD, chronic infections, obesity) are associated with an increased cancer incidence, which is supportive of the ability of chronic inflammation to drive a tumor-permissive milieu (3, 30). Among them, bacterial and viral infection has gradually been accepted as a major driver of inflammation-induced tumorigenesis and ~16% of human cancers worldwide are related to infectious agents or infection-associated chronic inflammation (32). In contrast, bacterial infection can also promote anti-tumor effects, as first famously demonstrated with the 'Coley's toxin' where injection of bacterial components in sarcoma patients elicited a protective anti-bacterial immune response that concomitantly induced tumor regression (33, 34).

Thus, the relationship between cancer and bacteria appears complex as bacteria can influence cancer via many distinct—and sometimes opposite—effects: by promoting tumor development, by promoting cancer-associated complications or by promoting immune responsiveness to tumors (10). Given the role of chronic pathogenic bacterial infections in influencing cancer development, recent studies have begun to explore the role of the abundant commensal bacteria, which constitutively colonize mammalian barrier surfaces, in tumor-promoting inflammation (31).

One seminal observation illustrating the influence of the microbiota on cancer susceptibility demonstrated a reduced incidence of intestinal tumors in germ-free (GF) rodents with a genetic susceptibility to cancer, as compared with control animals with a normal microbiota (35). Accordingly, in different chemically induced and genetically susceptible experimental mouse cancer models, GF mice, or mice treated with wide-spectrum antibiotics, exhibited a significant reduction of tumor development in various organs including the colon, skin, liver, breast and lung (30, 36–46). Subsequently, it was also identified that deficiencies in pathways that recognize microbes, such as TLR-mediated pathways, are also associated with a protective effect in multiple colorectal cancer (CRC) mouse models (47).

In contrast to pathogenic bacteria, the host microbiota is generally characterized by temporal stability, resilience and the lack of inflammatory properties (48). However, disruption of this equilibrium can lead to dysbiosis, a state of altered microbial composition that results in the preferential outgrowth of species with increased inflammatory potential, known as 'pathobionts' (4). In most cases, dysbiosis is associated with increased inflammation and has been recently linked with tumor development (10, 11). One of the first studies linking intestinal dysbiosis with cancer was performed in mice deficient in both *Tbet* and *Rag2* genes (TRUC mice) that spontaneously develop innate cell-driven colitis that can progress to CRC in a microbiota-dependent manner (49). Interestingly, inflammation and cancer susceptibility were found to be transmissible between mice, and a similar phenotype was also observed in mice deficient in NOD2 (50) and NLRP6 (51, 52), demonstrating that dysbiotic microbiota can be a transmissible driving force of cancer. Further, perturbation of the microbiota in a selective location (e.g. the intestine) can also influence distal cancer development, including sarcoma, breast, ovarian and hepatocellular carcinoma (46, 53, 54).

Moreover, the tumor microenvironment is a permissive medium for microbial growth as it harbors oxygen and nutritional niches. Thus, inflammation and tumor progression can induce a microbial shift that will influence cancer progression (55, 56). For example, in colitis-susceptible mice that lack the immunoregulatory cytokine interleukin-10 (*Il10*$^{-/-}$ mice), inflammation and cancer progression are associated with the expansion of *Escherichia coli* harboring the polyketide synthase (*pks*) genotoxic island, a gene cluster encoding a bacterial genotoxin that induces DNA damage and genomic instability and subsequently promotes tumorigenesis (57, 58). In addition, *Fusobacterium nucleatum*, a rare constituent of the gut microbiota in the healthy human population, is enriched in adenoma and adenocarcinoma tissues as compared with non-tumoral colonic tissue and promotes intestinal tumorigenesis in a mouse model of spontaneous CRC (59). In particular, *F. nucleatum* potentiates tumor immune escape by inhibiting NK-cell and T-cell cytotoxicity (60).

Although most studies to date have reported a tumor-promoting effect of the host microbiota, recent findings demonstrated that the presence of commensal bacteria is also required to promote protective anti-tumor immunity in response to chemotherapy or immunomodulatory drug treatments (11, 61–63). Delivery of immunotherapeutic CpG-oligodeoxynucleotides combined with neutralization of IL-10 results in regression of transplanted subcutaneous tumors in mice, which was dependent upon the presence of commensal bacteria, revealing a microbiota-dependent anti-tumor mechanism (62). This effect was primarily due to the priming of tumor-infiltrating myeloid-derived cells by the microbiota, which induced inflammatory cytokines (TNF, IL-12) and nitric oxide production—ultimately resulting in hemorrhagic tumor necrosis and regression (62). Further, administration of antibiotics decreases the early genotoxic effects of the drug oxaliplatin (62), demonstrating that commensal bacteria also directly modulate the efficacy of chemotherapy drugs. In particular, it has been well demonstrated that chemotherapy treatment can induce an immunogenic cell death that elicits

an anti-tumor immune response, but a role for the microbiota in this pathway is poorly understood (64). It has been recently reported that the efficacy of the chemotherapeutic drug cyclophosphamide is also reduced in GF or antibiotic-treated tumor-bearing mice (61). Cyclophosphamide treatment alters the intestinal microbiota composition, which is associated with translocation of Gram-positive bacteria to the draining lymph nodes (LNs) and induction of effector T_{h17} cell and memory T_{h1} cell anti-tumor immune responses (61). Thus, dysbiosis or microbial translocation induced by therapeutic interventions like chemotherapy can positively influence the development of an anti-tumor immune response (61, 62). Consequently, the role of commensal bacteria in modulating the response to therapeutic interventions like chemotherapy and immunotherapy may have strong implications for the novel design and improvement of immunotherapeutic strategies.

Taken together, these studies demonstrate that the interaction between immune cells, cancer and the microbiota is tightly regulated, reciprocal and complex (Fig. 1). Nevertheless, the specific pathways influencing the pro- versus anti-tumor effects of interactions between the host and commensal bacteria remain poorly defined and an increased understanding of these pathways is needed to inform novel strategies to both prevent and treat cancer. Significant progress toward our understanding of the regulation of host–microbe interactions has been recently achieved with the appreciation of a new subset of innate immune cells termed ILC3s. In the second part of this review, we will summarize the role of ILC3s as key regulators of mucosal immune homeostasis and discuss how these cells may influence interactions between the host and commensal bacteria and consequently tumor progression.

ILC3s: critical regulators of host–microbe interactions

Recent studies in mice and humans have characterized rare populations of innate lymphocytes, known as ILCs and identified these cells as critical regulators of intestinal immunity, inflammation and tissue homeostasis (6, 12, 65–67). ILCs have been subdivided into three groups based on their cytokine and transcription factor expression profiles (65, 68, 69).

In particular, ILC3s expressing the transcription factor retinoic acid-related orphan receptor γt (RORγt) are critical regulators of mucosal barrier tissue homeostasis and modulate the interactions between the mammalian host and commensal bacteria (6, 12, 14, 15). ILC3s at barrier tissue sites, including the intestine and associated lymphoid tissues, are largely composed of two related, yet distinct, subsets that are defined in mice by their expression of the NK-cell associated receptor NKp46 and the transcription factor T-bet (T-bet$^+$ or NCR$^+$ ILC3s) or the chemokine receptor CCR6 [lymphoid tissue inducer (LTi)-like ILC3s]. These ILC3 subsets play critical roles in influencing barrier homeostasis, tissue inflammation, commensal bacteria colonization and innate immunity against invading pathogens, in part through the production of the effector cytokines IL-17A, IL-17F and IL-22 (6, 13, 14, 70–77).

IL-22, in particular, is critical in mediating the effects of ILC3s in barrier tissues. Following activation by dendritic cell-derived cytokines such as IL-1β, IL-6 and IL-23, ILC3s produce robust levels of IL-22 that acts on non-hematopoietic cells expressing the IL-22R, including epithelial cells in the intestine that physically separate the mammalian immune system from the microbiota (78, 79). In particular, epithelial cell IL-22R signaling results in the expression of mucins and antimicrobial peptides, including RegIIIβ, RegIIIγ, β-defensins and S100A family members, which inhibit bacterial growth proximal to the epithelial barrier and establish physical separation between the host immune system and the microbiota. This physical separation has been termed the 'demilitarized zone' (80) and is essential to limit commensal bacteria-dependent chronic inflammation (78, 81–86). Production of IL-22 by ILC3s is required for protective immunity to bacterial pathogens, such as *Citrobacter rodentium*, and mice lacking ILC3s or IL-22 quickly succumb to infection with this enteric pathogen (87, 88).

IL-22-producing ILC3s have also been implicated in tissue inflammation, homeostasis and tissue repair (78, 79). In line with a tissue protective role for ILC3s and IL-22, mice lacking IL-22 develop severe intestinal inflammation following administration of dextran sodium sulfate (DSS) and IL-22 can enhance tissue repair by inducing epithelial cell proliferation and survival (78, 89, 90). Further, it has been demonstrated that ILC3-derived IL-22 induces fucosylation of small intestine epithelial cells, an important mechanism that maintains host–microbial interactions during pathogen-induced stress (91–93). In contrast, IL-22 has also been demonstrated to have pro-inflammatory roles in murine models of infection and tissue inflammation, with this seemingly paradoxical role ascribed to the inflammatory milieu and the presence of other cytokines such as IL-17A (78, 79, 94, 95).

In line with a central role for ILC3-derived IL-22 in the containment of commensal bacteria, *Rag1*$^{-/-}$ mice in which ILC3s were depleted or IL-22 was neutralized demonstrated dissemination of commensal micro-organisms to peripheral organs including the liver and spleen and outgrowth of segmented filamentous bacteria, which unlike many other commensal bacteria strains can form intimate interactions with the surface of epithelial cells (96–98).

ILC3s also have the capacity to orchestrate adaptive immune responses (15). The canonical example of this is exemplified by the involvement of LTi-like ILC3s in the development of secondary and tertiary lymphoid tissues (99, 100). During embryonic development, LTi cells initiate the development of secondary LNs and tissue-associated lymphoid structures such as the Peyer's patches, through the expression of lymphotoxins (e.g. $LT\alpha_1\beta_2$) (100). Interactions of surface-bound $LT\alpha_1\beta_2$ on ILC3s with stromal cells result in the production of chemokines that promote the recruitment and anatomical organization of antigen-presenting cells, T cells and B cells in lymphoid structures, which is necessary for optimal induction of antigen-specific adaptive immune responses and antibody production (99).

In the intestine, LTi-like ILC3s expressing $LT\alpha_1\beta_2$, or the secreted $LT\alpha_3$ trimer, regulate production of IgA by intestinal plasma cells, which influences the composition of commensal bacteria and neutralizes potential pathobionts (101). ILC3s further orchestrate B-cell affinity maturation and antibody production in the spleen by providing signals, including BAFF, CD40L and the NOTCH2 ligand delta-like 1 (DLL1), which

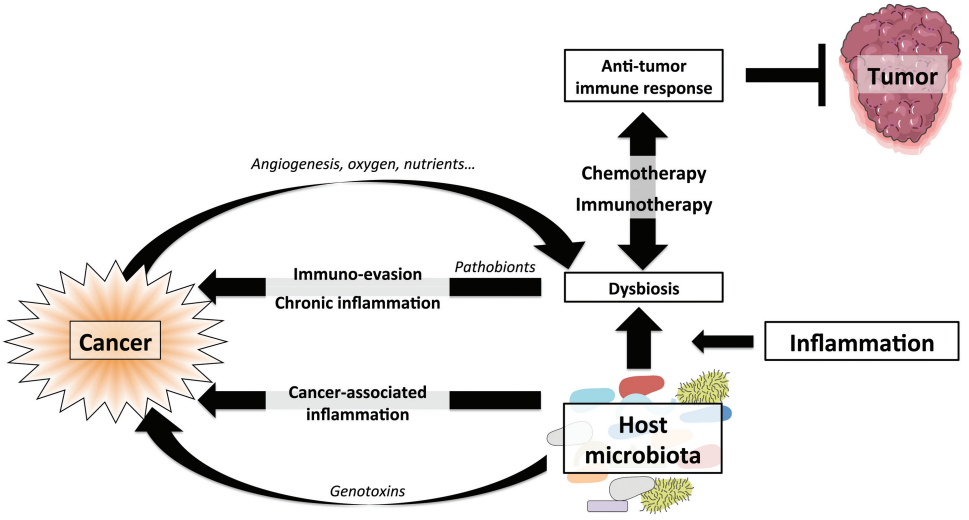

Fig. 1. The complex interplay between inflammation, cancer and the microbiota. The interactions between inflammation, cancer and the microbiota are reciprocal and complex. Microbiota can promote cancer via multiple mechanisms, for example, by producing genotoxins, by promoting chronic inflammation and cancer-associated inflammation and by eliciting immunosuppression. The tumor can induce a microbial shift that will influence cancer progression. The microbiota can modulate the efficacy of chemotherapy and immunotherapy to potentiate anti-tumor immune responses.

induce innate-like B-cell class switching in the marginal zone of human spleen (102).

In addition, emerging evidence suggests that LTi-like ILC3s in adult mice and humans directly modulate the CD4$^+$ T-cell response. LTi-like ILC3s express MHC class II (MHCII), which allows for presentation of antigenic peptides to CD4$^+$ T cells (15). In agreement with a central role for ILC3s in regulating interactions between the host and commensal bacteria at barrier surfaces, deletion of ILC3-intrinsic MHCII resulted in a failure to control CD4$^+$ T cells specific for commensal bacteria and thus resulted in the onset of spontaneous inflammation and tissue damage (25, 26). Similarly, MHCII expression was reduced in intestinal ILC3s from pediatric IBD patients, suggesting that dysregulation of this pathway in humans results in a failure to prevent commensal bacteria-dependent intestinal inflammation (26). ILC3s may also indirectly influence tolerogenic responses to commensal bacteria via the production of GM-CSF, which promotes the recruitment and differentiation of macrophage subsets that in turn induce *de novo* generation of immunosuppressive FoxP3$^+$ T_{reg} cells in the intestine (103).

Although ILC3s largely lack canonical co-stimulatory molecules such as CD80 and CD86 (25, 26), they express several other molecules that enable modulation of adaptive immune responses, including CD30L and OX40L (104, 105). Mice lacking both CD30L and OX40L are unable to sustain germinal center formation and antibody production and ILC3s expressing these molecules were found to cluster in the interfollicular zone and form interactions with memory T cells following bacterial infection (106–108), suggesting that ILC3s may prevent inflammatory responses while also supporting memory cell responses necessary for optimal immunity. Taken together, these studies support a role for ILC3s in supporting B-cell class switching and antibody production and suggest that ILC3 modulation of CD4$^+$ T-cell responses may be dependent upon the quality of the T-cell response, the local inflammatory milieu and the context of antigen delivery.

In line with the involvement of ILC3s in regulating inflammation, tissue repair and immunological homeostasis (6, 12, 13, 15), recent findings have implicated ILC3s in the development of cancer (17). In the last part of this review, we will summarize and discuss the potential implications of ILC3s and related pathways in the promotion or control of cancer.

ILC3s: an emerging role in cancer

ILC3s have been recently reported to infiltrate tumors and influence cancer development and progression (17, 18, 24, 95, 109, 110). In line with the multiple functions and context-dependent roles during inflammatory processes, ILC3s appear to exert both pro- and anti-tumor roles in cancer depending on the context, the type and the stage of the disease. As discussed above, dysregulated ILC3 responses can promote a chronic inflammatory state, which could subsequently influence tumorigenesis (95). However, the specific contribution of ILC3s in cancer currently remains poorly defined.

As previously discussed, LTi-like ILC3s orchestrate lymphoid organogenesis and neogenesis (99) and the transfer of LTi-like cells alone is sufficient to induce lymphoid neogenesis in non-lymphoid tissues (111–113). In mice, lymphoid neogenesis within the tumor microenvironment can be induced using an antibody–LTα fusion protein that subsequently provokes the recruitment of naive T cells and their differentiation into effector cells and is associated with the generation of tumor-specific T cells and tumor rejection (114–117).

Further, an increasing number of studies have reported the presence of tertiary lymphoid-like structures in human tumors, which in most cases are associated with significant

lymphocyte infiltration, coordination of local adaptive immune responses and a favorable prognostic outcome for patients (118–123), thus suggesting that LTi-like ILC3s may control tumor development by promoting lymphoid neogenesis via lymphotoxin (Fig. 2). However, it should be mentioned that lymphoid neogenesis pathways independent of the presence of LTi cells and lymphotoxin have also been identified in mice, and the cells that trigger lymphoid neogenesis within the human tumor microenvironment remain undefined (99, 124). Although not exclusive, LTi-like ILC3s are an important candidate as an initiator of lymphoid neogenesis within the tumor microenvironment. Thus, participation to the formation and maintenance of tumor-associated lymphoid-like structures that can drive local adaptive immune responses against the tumor may represent an unappreciated anti-tumor property of ILC3s and a new target to optimize immunotherapeutic strategies.

Similar to adaptive immune cells, ILC3 functions can be modulated by cytokine-based therapy to modulate the anti-tumor immune response. In an implantable melanoma model with B16.F10 cells, the expression of IL-12 by B16.F10 cells or the co-administration of IL-12 stimulated NCR$^+$ ILC3-like cells, which induced a local anti-tumor immune response and resulted in tumor eradication (110). NCR$^+$ ILC3-like cells were sufficient to induce tumor suppression in the absence of adaptive immune cells and acted in part by promoting adhesion molecule expression on the tumor vasculature, resulting in subsequent leukocyte infiltration within the tumor microenvironment (110). In contrast, in a CCL21-expressing B16.F10 melanoma model, CCR7-dependent recruitment of ILC3s is associated with a tolerogenic switch in the host immune response that favors tumor growth (109). Interestingly, CCR7 has been shown to be important for the recruitment of MHCII$^+$ ILC3s that promote tolerance to commensal bacteria (108). Thus, the pro- and anti-tumor effects of ILC3s may be modulated by exogenous cytokine signals; however, additional investigation is required to define whether this represents a potential therapeutic approach.

IL-22, a cytokine derived primarily from ILC3s, has been recently implicated in cancer (17, 79, 125). In relation with the dual nature of this cytokine, IL-22 has been reported to exert paradoxical effects in mouse models of cancer. On one hand, IL-22 activates the STAT3 cascade, which is considered pro-tumorigenic because it leads to downstream activation of pro-inflammatory, mitogenic, pro-survival and anti-apoptotic genes (79, 125). In contrast, the tissue-repair properties of IL-22 can limit the risk of carcinogenesis during an inflammatory episode by maintaining barrier function and tissue homeostasis. An elegant demonstration of this 'double-edged sword' effect was reported using mice lacking the IL-22 decoy receptor IL-22BP that exhibit an increased incidence of colitis-associated CRC after treatment with DSS and the pro-carcinogen azoxymethane (AOM) (126). Surprisingly, *Il22*$^{-/-}$ mice also developed a higher tumor load compared

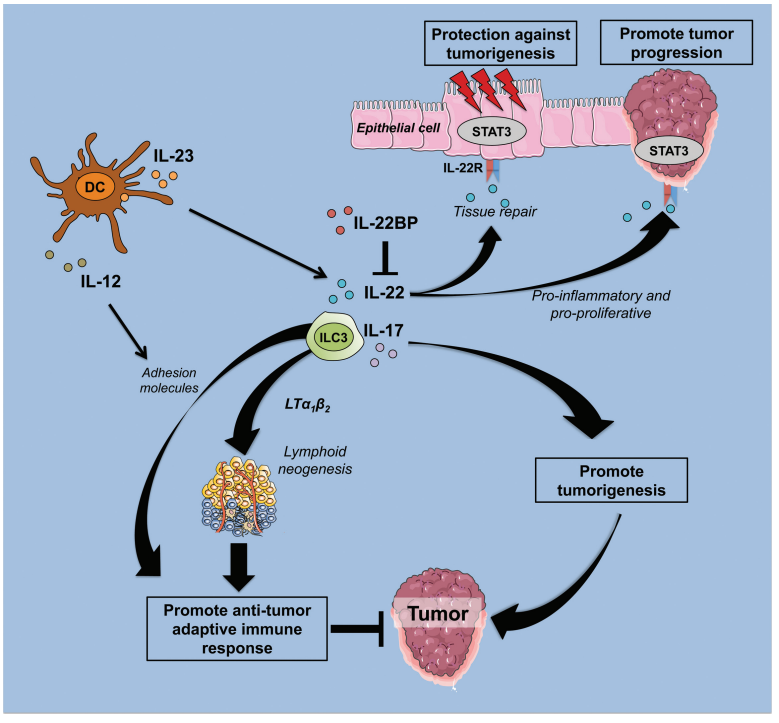

Fig. 2. The role of ILC3s in the context of cancer. ILC3s can promote tumorigenesis and influence tumor progression. In line with the multiple and context-dependent roles of ILC3s during the inflammatory process, ILC3s may exert pro- or anti-tumor properties in cancer. LTi-like ILC3s can promote formation of ectopic lymphoid-like structures in the tumor microenvironment that can drive a protective adaptive anti-tumor immune response. Delivery of the cytokine IL-12 can potentiate the anti-tumor functions of NCR$^+$ ILC3s by inducing adhesion molecule expression and subsequent leukocyte recruitment to the tumor microenvironment. Stimulation of ILC3s by IL-23 can promote tumorigenesis, implicating the IL-17 pathway. IL-22 production by ILC3s can prevent tumorigenesis during intestinal damage by promoting tissue repair; however, IL-22 also exerts pro-inflammatory and pro-proliferative properties that can promote tumor progression after tumor establishment.

with wild-type mice in the DSS/AOM model as well as in the APC^{min} spontaneous CRC mouse model (126). In particular, blockade of IL-22 with a neutralizing antibody results in exacerbated tumor growth when administrated at the peak of inflammation, whereas it limits tumor growth when administered during the recovery phase (126). Thus, IL-22 appears to limit tumorigenesis during the tissue-damage phase by promoting intestinal repair but supports tumor development during the recovery phase by mediating pro-inflammatory and proliferative properties (126), revealing that the pro- or anti-tumor effect of IL-22 is context dependent.

As ILC3s have been identified as a major cellular source of IL-22 in the intestine (70), we suggest that they may be one of the critical determinants orchestrating the pro- and anti-tumor effect of IL-22 in the DSS/AOM model. In support of this idea, a critical role of IL-22-producing ILC3s for transition from colitis to CRC was reported in an innate colitis-associated CRC model driven by *Helicobacter hepaticus* infection and AOM injection in 129SvEv.*Rag*$^{-/-}$ mice (18). In this bacteria-induced CRC model, tumor development is associated with colonic accumulation of IL-17$^+$ and IL-22$^+$ ILC3-like cells and progression to CRC is impaired after depletion with neutralizing anti-IL-22 or anti-Thy1 (which could exhibit non-specific effects) monoclonal antibodies, suggesting a critical role of both ILCs and IL-22 (18). The neutralization of IL-22, and not of IL-6 or IL-17, abrogated STAT3 phosphorylation, resulting in decreased epithelial proliferation and reduced tumor growth, demonstrating that activation of the STAT3 pathway by IL-22 is the dominant driver of tumor progression in this model (18). Although IL-22 promotes tumor progression in these inflammation-related tumor models, it was not shown to exert any causative role in malignant transformation, suggesting that IL-22 mainly influences tumor growth by delivering a trophic signal that sustains tumor progression.

Adaptive immunity plays a major role in tumor control (2, 127), and it should therefore be considered that most studies investigating a role for ILC3s were performed using immunocompromised mouse models or non-specific depletion strategies (anti-Thy1). Thus, the specific impact of ILC3s in an immunocompetent tumor model remains poorly defined and should be extensively questioned in the future. Indeed, it should be noted that a significant amount of IL-22 is also produced by CD4$^+$ T cells (78) and increased frequencies of circulating or tumor-infiltrating IL-22-producing CD4$^+$ T cells have been reported in pancreatic, lung, gastric and colorectal human cancer patients (128–132). Thus, the relative contribution of ILC3s and CD4$^+$ T cells in IL-22-dependent pro-tumor growth remains to be fully elucidated.

It is well known that the IL-23 pathway (that promotes IL-17 production in T cells and ILC3s) can provide an inflammatory environment that supports tumorigenesis (30, 127). IL-23 has been directly implicated in promotion of IBD (133, 134) and contributes to the development of various tumors types (24, 133, 134). These findings are supported by the observation that IL-23 expression is up-regulated in human colorectal tumors (133, 134). In a mouse model with transgenic overexpression of IL-23, IL-17-producing Thy-1$^+$Sca-1$^+$IL-23R$^+$ ILC3s were identified as a potential initiator of colorectal tumorigenesis independently of any other pro-tumorigenic factors (24). Surprisingly, early tumorigenesis consistently occurred before recruitment of inflammatory infiltrates, suggesting that ILC3s promote tumorigenesis in an IL-23–IL-17-dependent manner that occurs prior to the initiation of inflammation (24). Thus, ILC3s appear to be sufficient to drive tumorigenesis in this model, raising questions as to the potential implication of this immune subset as a tumor initiator in other tumor models linked to IL-23–IL-17 pathway.

The presence of an IL-17-dependent signature in tumors has been associated with a poor clinical outcome in CRC patients (135–137), which was primarily due to the infiltration of T_{h17} cells in the tumor microenvironment. Currently, the participation of ILC3s remains undefined. Further, the presence of IL-22$^+$ cells was recently reported in human CRC tumors (18, 136) and IL-22 signaling can induce activation of cancer 'stemness genes' in human cancer cells, which is associated with poor survival in patients (in healthy subjects stemness genes are expressed in stem cells but not differentiated cells) (136). IL-22 was reported to be predominantly expressed by memory CD4$^+$ T cells in a cohort of human CRC patients (136), but the presence of IL-22$^+$ ILC3-like cells was also detected in human CRC samples (18), suggesting that tumor-infiltrating ILC3s may influence human CRC progression. The so-called tumor 'immune contexture', which is defined as the quantity, quality and functional orientation of the immune microenvironment in primary and metastatic tumor sites, is a powerful prognostic indicator in human cancer (127). Thus, defining and integrating the specific impact of ILCs among this intricate network currently represents a major challenge. The impact of ILC3s in human cancer remains largely unexplored and extensive investigation is needed to uncover their role in tumor progression, to determine their potential association with patients' prognosis and to determine the possible interactions of ILCs with therapeutic protocols like chemotherapy, radiotherapy and immunotherapy.

Conclusion

Unraveling the complex connections between microbiota, inflammation and cancer appears to be one of the major challenges for the next decade in order to delineate the pro- versus anti-tumor effects of interactions between the host and commensal bacteria, as well as the potential role for ILC3s in modulating these interactions. In particular, future studies are required (i) to decipher how the microbiota influences anti-tumor immunity during the different steps of tumor development and progression, (ii) to determine to what extent the microbiota can interfere with or potentiate the efficacy of therapeutic protocols, (iii) to evaluate the potential of the microbiota as a biomarker for cancer diagnosis and (iv) to determine how we can translate these findings into new therapeutic strategies based on the manipulation of the microbiota or host–microbe interactions.

ILC3s play a critical role in regulating adaptive immune responses and maintaining mucosal homeostasis. In line with the major impact of microbiota and adaptive immune cells in anti-tumor immunity, the influence on these pathways during tumor development provokes a need for further investigation. A majority of reports of ILC3s in cancer development concerns mucosal (notably intestinal) sites; however, their potential influence on non-mucosal tumor development should also

be intensively questioned. In particular, the co-stimulatory functions of ILC3s may also modulate adaptive anti-tumor immune responses and support systemic or memory T-cell responses to prevent tumor dissemination and relapse. Finally, in complement with recent breakthroughs of immunotherapy, deciphering the mechanisms by which ILC3s promote or restrain tumor development may provoke the development of new or combinatorial therapeutic strategies that modulate pro- versus anti-tumor outcomes.

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