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Innate lymphoid cells and cancer

Nicolas Jacquelot[1], Cyril Seillet[2,3], Eric Vivier[4,5,6] and Gabrielle T. Belz[7] ✉

The innate lymphoid cell (ILC) family is composed of natural killer (NK) cells, ILC1, ILC2 and ILC3, which participate in immune responses to virus, bacteria, parasites and transformed cells. ILC1, ILC2 and ILC3 subsets are mostly tissue-resident, and are profoundly imprinted by their organ of residence. They exhibit pleiotropic effects, driving seemingly paradoxical responses such as tissue repair and, alternatively, immunopathology toward allergens and promotion of tumorigenesis. Despite this, a trickle of studies now suggests that non-NK ILCs may not be overwhelmingly tumorigenic and could potentially be harnessed to drive anti-tumor responses. Here, we examine the pleiotropic behavior of ILCs in cancer and begin to unravel the gap in our knowledge that exposes a new horizon for thinking about modifying ILCs and targeting them for immunotherapy.

The distinction between the innate and adaptive arms of the immune system has been pivotal in shaping our understanding of immune protection and immunosurveillance.

Immunotherapies have revolutionized the treatment of cancer over the last two decades. It has focused our strategies on harnessing the body's own immune system, with the promise of eliminating tumor cells by utilizing pathways and evolutionarily selected mechanisms governing the molecular wiring of white blood cells. T cells were the first immune cells to be targeted by immunotherapy following the identification of potent immunosuppressive pathways impairing T cell function in tumors. Monoclonal antibodies that target specific receptors and act as immune checkpoint inhibitors were later designed to specifically target these immunological brakes to reinstate strong and long-term anti-tumor immunity[1]. More recently, autologous chimeric antigen receptor (CAR) T cells have been developed to treat leukemia and lymphoma, and have sparked enormous interest as a highly productive approach to eliminating tumors. Despite the unprecedented success of this first wave of cancer immunotherapies based on immune checkpoint inhibitors and CAR T cells, many people fail to benefit from such treatments, or alternatively, the benefit is not enduring. There is thus a need to expand next-generation cancer immuno-oncology treatment. Along this line, the central role of T cells against cancer should not obscure the fact that T cells are not autonomous. They intrinsically depend on the rapid delivery of signals from other cells, such as innate cells, to guide their responses. Thus, if we are to be successful in cancer eradication and immunotherapy, developing and effectively harnessing the innate system as well as the adaptive system is likely to be crucial to overcome the gap in therapeutic efficacy that currently exists.

Innate lymphoid cell subsets

ILCs are lymphocytes that do not express antigen-specific receptors but react to infection or insults through the generation of cytokines and secreted proteins, which direct and enhance immune responses on the front line of attack. They form five distinct subsets, including NK cells and lymphoid-tissue inducer (LTi) cells together with group 1, 2 and 3 ILCs (ILC1, ILC2 and ILC3, respectively)[2]. While ILCs have the capacity to migrate, ILC1–ILC3 mostly reside within tissues.

ILC1s and NK cells have several features in common, including their expression of the transcription factor T-bet (encoded by *TBX21*), the natural cytotoxicity receptor NKp46 and the capacity to secrete interferon-γ (IFN-γ). While NK cells are widely recognized for their role in immunosurveillance, ILC1s diverge significantly, as illustrated by their weaker cytotoxic activity[3–5]. ILC2s are a relatively homogenous population and form stable long-lived populations in most adult tissues at steady state. They depend on the transcription factor GATA3 for their development and maintenance and are constitutive sources of type 2 cytokines, such as IL-5 and IL-13, which are secreted in response to IL-33, IL-25, TSLP or IL-18, depending on the tissue[6]. All ILC3s depend on the transcription factor RORγt (encoded by *RORC*), but are grouped into more complex subsets on the basis of their expression of the natural cytotoxicity receptor (NCR) NKp46 in mice and humans[7] or NKp44 in humans[8]. Helper-like ILC3s can be divided into two main subsets, namely NCR^- and NCR^+ ILC3s. In contrast, LTi cells are considered to be a distinct lineage of ILC3 as they do not depend on the transcription factor promyelocytic leukemia zinc finger (PLZF) (encoded by the transcription factor Zinc finger and BTB domain containing 16, *ZBTB16*) for their development[9]. This subset initiates the formation of secondary lymph nodes (LNs) and Peyer's patches during embryogenesis, and the absence of LTi cells is associated with a loss of LN formation. Despite this, LTi cells and NCR^- ILC3s exhibit a highly similar transcriptome, with only a very small number of differentially expressed genes between the subsets[10,11]. NCR^+ ILC3s arise from NCR^- ILC3 cells through Notch2-dependent signaling and the upregulation of T-bet[12]. In adulthood, ILC3s and LTi cells constitutively secrete IL-22 that acts on the mucosal epithelium to induce the production of antibacterial peptides[13] and expression of tight-junctions[14], and to improve nutrient uptake[15]. IL-22 promotes colonization of the gut by beneficial commensal bacteria that protect against intestinal inflammation[16].

Recruitment of ILCs to the tumor microenvironment

The recruitment of immune cells into tumors is crucial to constrain tumor growth and effectively eliminate cancer cells. Trafficking of immune cells from the blood or lymph to tissues is finely controlled by the expression of myriad chemokines that form local gradients to guide ingress to tissues. Positioning and retention in tissues then depends on a combination of integrins and selectins, whose expression is often regulated by inflammatory signals.

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ILCs were initially identified as 'tissue-resident cells,' but later evidence has shown that ILCs, like other immune cells, have the capacity to migrate within and between organs under inflamma-tory conditions[17–20]. Similar to T cells or NK cells, ILCs express many chemokine receptors[21], integrins[22] and selectins[23], allowing them to appropriately traffic within the body and migrate specifi-cally towards inflammatory sites where they mediate their function. ILC trafficking has been observed following allergic or cytokine challenge and pathogen infection[17–20]. Indirect evidence, such as increased frequencies of circulating ILCs in people with cancer compared with those in healthy donors, may suggest that ILCs traf-fic to tumors. Indeed, increased ILC1 and ILC2 frequencies in the blood of people with colorectal[24] and gastric[25] cancers, melanoma or chronic lymphocytic leukemia[26] have been reported. Recruitment of ILC3s from the circulation into mammary tumors in mice has been shown to rely on mesenchymal-stromal-cell-derived CXCL13 expression[27]. These studies show that ILC may traffic from distant tissues to tumors, but the underlying mechanisms remain to be fully understood. It is also likely that expansion of ILC can occur via in situ ILC proliferation although the mechanism for ILC amplification in tissues is not known. Thus, accumulation of tumor-infiltrating ILCs in tumors likely reflects a combination of both recruitment and local expansion of tissue-resident ILCs[28,29].

Innate cells exhibit plasticity in response to inflammation and the tumor microenvironment

The tissue-specificity of ILC1s allows them to adapt to their micro-environment, a feature that has challenged our capacity to define the phenotypic and functional features of ILC1s, and to distinguish them from NK cells, particularly in inflammatory and tumor set-tings. For example, transforming growth factor beta (TGF-β) has been shown to control the development of salivary gland ILC1s[30], and a lack of TGF-β signaling was associated with a loss of ILC1-associated markers such as CD49a, CD103 and CD69 (ref. [30]). In the tumor microenvironment, which is enriched for TGF-β, NK cells can be diverted into ILC1-like cells and down-regulate the expression of the transcription factor EOMES[5]. These ILC1-like cells were unable to restrain fibrosarcoma growth and metastasis[5]. Such plasticity between NK cells and ILC1s is not limited to tumoral settings. It has also been reported in parasite infections, in which IL-12 drives conversion of NK cells into Eomes$^-$ ILC1-like cells[31] (Fig. 1), and in non-alcoholic fatty liver disease, in which it was associated with reduced cytotoxicity[4]. Whether ILC1s can transdifferentiate into cytotoxic NK cells is currently unclear. Ectopic expression of EOMES in ILC1s[32] promotes upregulation of NK-cell-associated markers, such as CD11b or KLRG1; how-ever, this alone does not transition cells into terminal mature NK cells. ILC1s, in the presence of IL-1β and IL-23, however, have been shown to convert into ILC3s[33]. This plasticity was observed in peo-ple with squamous cell carcinoma[34], highlighting the capacity of the ILC1 lineage to adapt to localized tumor cues.

During inflammation, a distinct subset of ILC2s becomes read-ily detectable in the circulation[19]. Thus, ILC2 are divided into two main subsets, (1) natural ILC2s (nILC2s) that reside within tis-sues at steady-state and (2) inflammatory ILC2s (iILC2s) that can be mobilized from blood and lymph during inflammation[17,19,35]. nILC2 are defined as CD90^{hi}ST2$^+$KLRG1$^-$ cells, while iILC2s are characterized by low expression of CD90, absence of ST2 and high expression of KLRG1 (ref. [35]). Activated circulating ILC2s can also be distinguished in humans from tissue-resident ILC2s by the absence of prostaglandin D2 receptor 2 CRTH2 and reduced CD127 expression[36].

ILC2s, similar to NK cells and ILC1s, exhibit plasticity and can transdifferentiate into ILC1s (refs. [37–40]) and ILC3-like cells[36,41–43] in both humans and mice. iILC2s preferentially respond to IL-25, which induces expression of RORγt and IL-17 following fungal infection[35]. In response to IL-1β and IL-12, ILC2s downregulate GATA-3, which reduces their capacity to produce IL-5 and upregulate T-bet and their IFN-γ production (Fig. 1). These 'ex-ILC2s' develop under chronic inflammation. Ex-ILC2s occur in the lungs of people with chronic obstructive pulmonary disease (COPD)[37,39] and in the gut of people with Crohn's disease[40]. Interestingly, while the conversion of ILC2s into ILC1-like cells can be reversed by IL-4 (ref. [39]), transdifferentiation of ILC1s into ILC2-like cells has not been reported. Human ILC2s can convert into RORγt-expressing, IL-17-producing ILCs in response to IL-1β and IL-23 (ref. [36]). ILC2 conversion into ILC3-like cells involves TGF-β signaling, which induces IL-23 receptor expression on ILC2s, allowing them to trans-differentiate into IL-17-producing cells[36]. Given the role of TGF-β in the tumor microenvironment and in driving ILC plasticity, detailed evaluation of its impact on ILC2 conversion into ILC3-like cells in tumors is warranted.

As NCR$^+$ ILC3s express T-bet, they can also transdifferentiate into IFN-γ-producing ILC1s under IL-12 signaling[33]. The balance between NCR$^-$ and NCR$^+$ ILC3 subsets depends on the upregula-tion of T-bet in response to Notch2 ligand signaling[12]. However, it seems pivotal that this pathway also appears to rely on TGF-β for NCR$^-$ ILC3s to differentiate into NCR$^+$ ILC3s. In the absence of TGF-βRII, NCR$^+$ ILC3s accumulate, indicating an inhibitory role of TGF-β signaling in the development of these cells[10]. Following bacterial infection or in response to IL-12, NCR$^+$ ILC3s downregu-late RORγt and transdifferentiate into IFN-γ-producing ILC1s. At steady state, the transcription factor c-MAF limits T-bet and IFN-γ expression in NCR$^+$ ILC3s, which normally maintains ILC3 iden-tity[44,45]. In humans, ILC3s can convert into cells resembling NK cells in response to IL-12 and IL-15 (ref. [46]). These ex-ILC3s upregulate

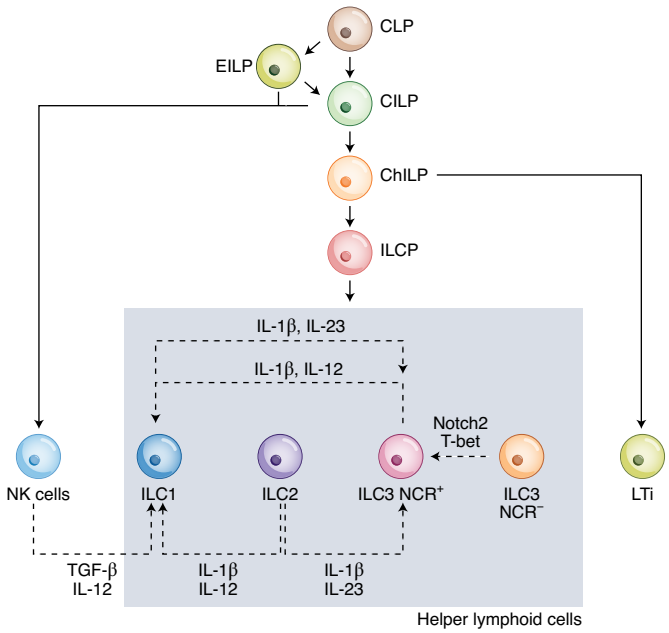

Fig. 1 | The ILC family. All ILCs develop from the CLPs (common lymphoid progenitors), which give rise to the EILPs (early ILC progenitors) and CILPs (common innate lymphoid progenitors). These progenitors can differentiate into NK cells or ChILPs (common helper innate lymphoid progenitors), which give rise to LTi cells and ILCPs (innate lymphoid cell precursors). ILCPs generate ILC1s, ILC2s and ILC3s. Under inflammatory conditions, ILCs can transdifferentiate into different subsets. NK cells can be converted into ILC1s under the influence of TGF-β and IL-12. IL-1β and IL-23 can induce the transdifferentiation of ILC1s and ILC2s into ILC3s, while IL-1β and IL-12 can convert ILC2s and ILC3s into ILC1s.

EOMES and T-bet and can even acquire cytotoxic activity, but are generally restrained by the expression of the aryl hydrocarbon receptor (AHR), which maintains ILC3 identity by repressing NK-cell-related genes[47]. Overall, while ILCs have been classified into multiple subsets, it is now clear that ILCs are highly plastic cells and can rapidly respond to microenvironmental signals (Fig. 1). This feature is likely to play a crucial role in shaping the phenotype and functions of ILCs in the tumor microenvironment. It is intriguing that, with the exception of LTi's, all ILC subsets can become ILC1s or acquire ILC1-like features. In contrast, the transdifferentiation of ILC1s into NK or ILC2s has not been reported so far, indicating differences in plasticity capabilities of ILC subsets.

Local immunosurveillance and the cancer–immune equilibrium

The body is constantly challenged with biological (for example, infection) and physical (for example, radiation) threats that may induce cellular damage that compromises genome integrity and increase the risk of cancer. Cell-intrinsic and cell-extrinsic tumor-suppressor mechanisms are established to successfully limit the growth of cancer cells and suppress cancer development. When nascent cancer cells evade intrinsic mechanisms, the immune system constitutes the last barrier to detect and eliminate cancer cells, preventing individuals from the development of a clinically detectable tumor[48]. Antigen-specific T cells play a major role in limiting tumor formation and provide an effective line of defense through immunosurveillance. However, settings in which T cells are absent (for example, recombination-activating gene (Rag)-deficient mice, which lack adaptive immune cells, or *Rag2*$^{-/-}$*Il2gc*$^{-/-}$ mice, which lack both adaptive and innate lymphoid immune cells), antibody depletion to remove T cells in the host or modulation of the ability for antigen-specific T cells[49] highlight that other lymphocytes such as ILCs[28,50] must also have a role in restricting tumor cells through immunosurveillance-like mechanisms. The appraisal of immune-cell migratory capacities using parabiosis, an experimental setting in which two mice share immune cells through their circulation, has documented the diversity of tissue-resident cells[51]. This experimental system revealed the presence of myeloid and lymphoid cell subsets, particularly ILCs, in tissues for extensive periods of time, potentially positioning them to be poised for tissue repair and protection against infection and to recognize and eliminate nascent cancer cells to control tumor development[52,53].

NK cells can be viewed as the innate counterpart of CD8$^+$ T cells. Distinct from killer T cells, they are wired to detect changes in 'self' major histocompatibility complex (MHC) class I expression and alterations in stress-induced ligands, empowering them to keep abnormal cells in check, such as in virally infected and transformed cells. NK cells are particularly attuned to identifying poorly immunogenic cells. Functionally, NK cells induce target-cell death predominantly through perforin and granzymes (Fig. 2) and are armed with a diverse array of tightly regulated activating and inhibitory receptors, which allow them to integrate 'self' signals and eliminate tumorigenic cells. Multiple exciting approaches building on much of the T cell blueprint to harness NK cells are currently underway and contribute to the emergence of a second wave of cancer immunotherapy[54]. As these approaches have recently been extensively reviewed[54], we will focus predominantly on the function and possibilities for ILC1, ILC2 and ILC3.

Villains, or simply misunderstood non-NK ILC1s?

Traditionally, all immunosurveillance functions of the Lin$^-$NKp46$^+$ cells have been attributed to NK cells, oblivious to the notion that these cells represent a heterogeneous population composed of both NK cells and non-NK ILC1s. The identification of group 1 ILCs as comprising conventional NK cells and less cytotoxic helper-like ILC1s has left a question over the contributions of each of these cell types in tumor immunity, particularly in the liver, which is home to both subsets in comparable numbers. The use of multiple and complementary mouse models deficient either in liver ILC1s or NK cells has revealed that ILC1s are essential to control the metastatic seeding of tumor cells, whereas NK cells limit tumor growth[55]. These results indicate a non-redundant function between these two cell types, and suggest that complementarity may exist within group 1 ILCs in local tumor immunosurveillance. Adding to the complexity, detailed analyses of a spontaneous mouse model of breast tumor formation (polyoma middle T, PyMT) revealed the presence of two innate cell types, referred to as type 1-like innate lymphoid cells (ILC1ls) and type 1 innate-like T cells (ILTC1s)[56]. These cell types are both NK1.1$^+$CD49a^{hi}Grzmb$^+$CD103$^+$. The ILC11s expressed CD8 but lacked PD-1 expression, while ILTC1s also expressed T cell receptor. Both cell types localize predominantly within tissues tethered by the retention molecules CD49a and CD103 and expand in response to tumor cell development (Fig. 2). This phenotype is distinct from conventional NK cells. Their development relies on the cytokine IL-15, but unlike for NK cells, the transcription factor NFIL3 is not required[56]. Early descriptions of ILC1s suggested that they lacked strong cytolytic activity, exhibited impaired immunosurveillance activities and, indeed, even contributed to the development of tumors such as hepatocellular carcinoma[5,57,58]. In people with melanoma, blood and LN metastases are enriched in ILC1s as compared with those of healthy donors, although these ILC1s are functionally suppressed by the tumor microenvironment[59]. Higher proportions of circulating ILC1s were found before ipilimumab treatment in individuals that failed to control disease[60], suggesting that ILC1s can be associated with a dismal prognosis and a poor response to immunotherapy (Table 1).

The behavior of group 1 ILCs depends largely on the cytokine IL-15. IL-15 is well known for its ability to drive differentiation and activation of conventional NK cells and the formation of different subsets of CD8$^+$ T cells[61]. In addition, IL-15 signaling drives NK cell conversion into intraepithelial like ILC1 in head and neck tumors. These converted NK cells express CD49a and CD103 and higher levels of IFN-γ and show greater anti-tumor function than do CD49a$^-$ NK cells[62]. Maintenance of expression of the IL-15 receptor by NK cells is indeed pivotal for their survival and reactivity and their transdifferentiation into killer ILC1-like cells in head and neck cancer[61,63]. Whether this is the case for other innate immune cells is not yet clear but could explain differences in tumor microenvironmental prevalence and function of distinct NK or ILC1 subsets.

Friends or foes in the ILC2s?

The presence of ILC2s within tumors is often associated with impaired anti-tumor responses and poor prognosis in many cancer types[64–66]. Dysregulated IL-13-producing ILC2 drive the recruitment and differentiation of monocytes into myeloid-derived suppressor cells (MDSCs) (Fig. 2), which are associated with increased recurrence rates and poor survival in acute myeloid leukemia, prostate and bladder cancer[64–66]. In addition, ILC2s can promote tumor growth by driving tissue repair, sustained cancer-cell proliferation or the recruitment and function of tumor regulatory T cells (Table 1).

Conversely, there is increased evidence that ILC2s can also promote anti-tumor responses through the recruitment of dendritic cells, anti-tumor eosinophils and antigen-specific CD8$^+$ T cells. Systematic analyses of primary and metastatic tumor cell lines and in vivo mouse systems revealed that ILC2s potentiated anti-tumor responses through innate and adaptive immune-cell tumor recruitment and activation and identified the dependence of ILC2s on IL-33 catalyzing anti-tumor responses[28,67] (Fig. 2 and Table 1). Tissues engineered to express IL-33 exhibit a strong delay in the establishment of tumors, and ILC2s were essential for this IL-33-dependent

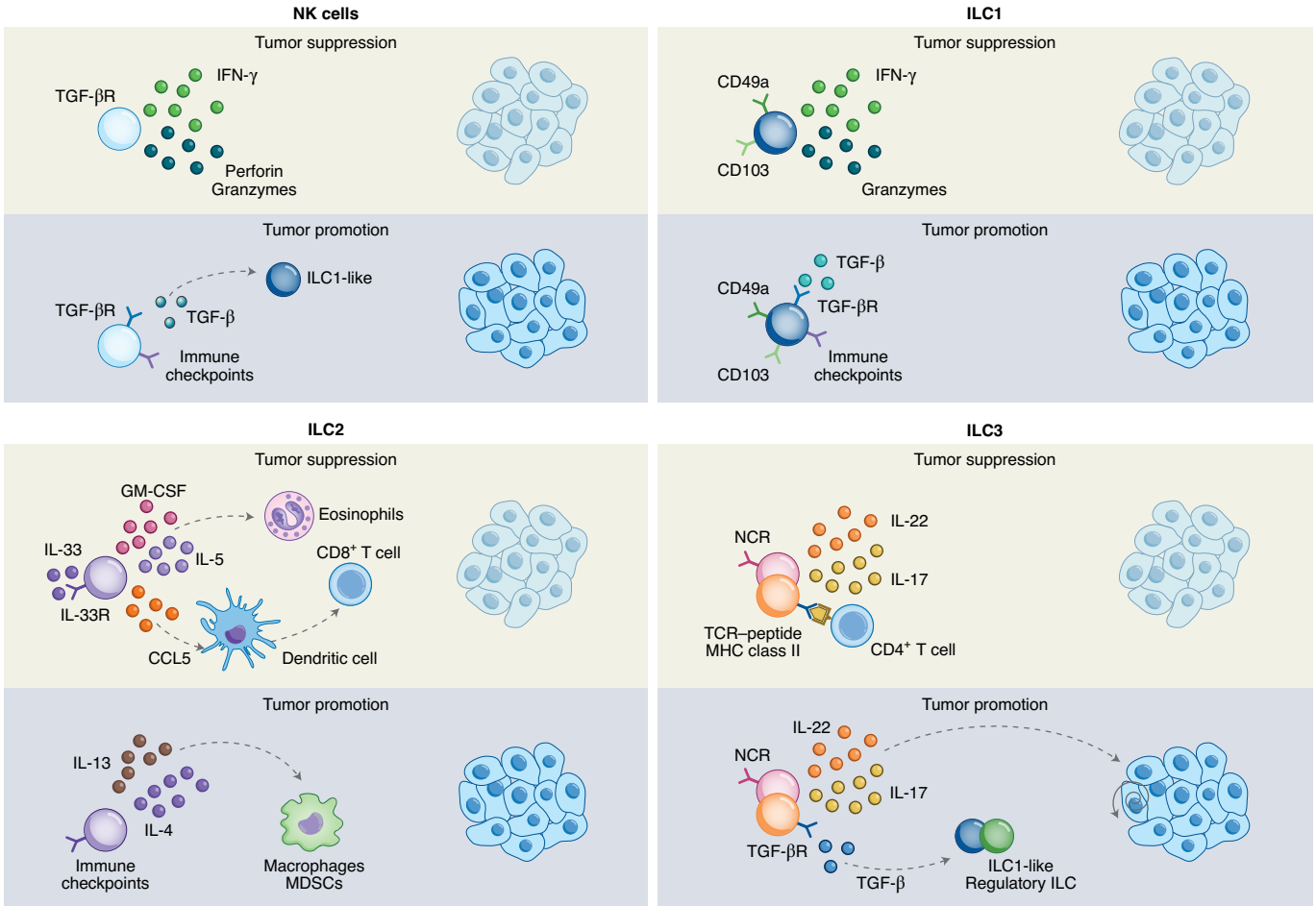

Fig. 2 | ILCs and tumor immunity. Tumor-infiltrating ILCs can use various mechanisms to suppress or promote the growth of cancer cells. NK cells express IFN-γ, perforin and granzymes to stimulate anti-tumor immunity and lyse tumor cells. Conversely, NK cells can express immune checkpoints, such as NKG2A, which inhibit their anti-tumor function. Furthermore, NK cells can transdifferentiate into poorly anti-tumorigenic ILC1s under the influence of the TGF-β signaling. Like NK cells, ILC1s can suppress tumor growth through the production of IFN-γ and granzymes, while the expression of immune checkpoints at their surface and the accumulation of TGF-β in the tumor microenvironment can impair their function, favoring tumor growth. ILC2s express a large variety of cytokines and chemokines that differently influence tumor outcomes. For example, IL-33 stimulation mainly drives the secretion of large amounts of IL-5, GM-CSF and CCL5, which allows the activation and recruitment of anti-tumorigenic eosinophils, dendritic cells and $CD8^+$ T cells. Conversely, the expression of immune checkpoint molecules, for example PD-1, and cytokines IL-4 and IL-13 dampen ILC2 function and promote the recruitment of macrophages and myeloid-derived suppressor cells (MDSCs), which together facilitate tumor progression. ILC3s express the cytokines IL-17 and IL-22, which drive both pro- and anti-tumorigenic responses depending on the tumor type. In addition, ILC3s can prime and stimulate $CD4^+$ T cells through the expression of MHC class II molecules, favoring anti-tumor responses. In contrast, under the influence of the TGF-β signaling, ILC3s can transdifferentiate into ILC1s or regulatory ILCs, impairing anti-tumor immunity.

protection[67]. In pancreatic ductal adenocarcinoma, ILC2s were shown to infiltrate tumors and activate tissue-specific immunity, including dendritic cells and $CD8^+$ T lymphocytes (Fig. 2). Their capacity to amplify protective responses could be mediated using anti-PD-1 blockade[68]. Similarly, in melanoma, ILC2s dominated the ILC tumor infiltrate and coordinately recruited eosinophils through their production of the cytokines IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF)[28] (Fig. 2). Like in pancreatic cancer, blockade of PD-1 expression induced by IL-33 to drive expansion of ILC2s significantly enhanced their capacity to limit primary melanoma growth. Paralleling this model system, analyses of human tumors showed a strong correlation between expression of signature genes for a type 2 immune profile associated with GM-CSF expression and an enhanced prognostic outcome[28].

Our current ability to distinguish between pro- and anti-tumorigenic ILC2s is very limited. Recent advances in mouse models and multi-omics approaches have uncovered unprecedented complexity within the ILC2 subset, with a continuum of states associated with particular functions and prognostic values[6,69]. Despite our increased understanding, the factors regulating ILC2 functions in cancers and how they can be manipulated to take advantage of these tissue-resident innate lymphocytes in our oncological armamentarium are yet to be fully realized. While multiple pieces of evidence implicate ILC2s in promoting tumor development[65,66], these recent studies highlight how harnessing early responses and prognostic relationships might play an important part when integrated with therapeutic approaches.

Roles beyond the intestinal mucosa for ILC3s?

ILC3s are particularly abundant in the intestine, where they participate in immunological protection against infection. Paradoxically, initial analyses using mouse models of colorectal cancer have shown that dysregulated ILC3 responses associated with IL-17 and IL-22 production promoted gut inflammation and sustained tumor

Table 1 | ILCs as biomarkers of prognostic outcomes

ILC subset	Prognostic indicator	Disease	Mouse/Human	Prognostic	Prognostic relationship
ILC1	Intraepithelial ILC1-like cells	Breast, head and neck cancer	Mouse	Good	IFN-γ (refs. [56,62])
ILC1	SlamF1	Colorectal cancer	Human	Good	High SlamF1 (ref. [97])
ILC1	ILC1	Metastatic seeding of the liver	Mouse	Good	Limit the seeding of tumor cells[55]
ILC1	Circulating ILC1	Melanoma	Human	Poor	Higher proportions in individuals who didn't respond to ipilimumab treatment[60]
ILC1	Converted cells into ILC3-like cells	Lung cancer	Human	Poor	Associated with tumor growth and reduced patient survival[34]
ILC2	GM-CSF	Melanoma	Mouse and human (TCGA)	Good	High GM-CSF[28]
	IL-5/GM-CSF- driven eosinophils			Good	High eosinophils[28]
	ILC2			Good	High ILC2 (ref. [28])
ILC2	IL-33	Metastatic melanoma	Human (TCGA)	Good	High IL-33 (ref. [98])
ILC2	ILC2	Colorectal cancer	Mouse and human (TCGA)	Good	High ILC2 (refs. [97,99])
ILC2	IL-33 and eosinophils	Melanoma	Human (TCGA)	Good	High *IL33* and *SIGLEC8* expression[100]
	ILC2	Melanoma	Mouse	Good	High ILC2 (ref. [100])
ILC2	ILC2	Pancreatic cancer	Mouse and human	Good	High ILC2 (ref. [68])
	IL-33			Good	High IL-33 expression[68]
ILC2	ILC2	Acute myeloid leukemia and prostate cancer	Human	Poor	High IL-13 production[66]
ILC2	ILC2	Bladder cancer	Human	Poor	High IL-13 secretion[65]
ILC3	NKp44$^+$ ILC3	NSCLC	Human	Good	High NKp44$^+$ ILC3 correlates with tertiary lymphoid structure density[76]
ILC3	ILC3	Squamous cell lung carcinoma	Human	Poor	High ILC3 (ref. [34])
ILC3	NKp44$^+$ ILC3	Colorectal cancer	Human	Good	ILC3 positively correlates with tertiary lymphoid structure density (ref. [82])
ILC3	ILC3	Pancreatic cancer	Human	Poor	High ILC3 (ref. [73])

progression[70,71]. In addition, it has been reported that ILC3s may transdifferentiate into regulatory ILCs secreting IL-10 following TGF-β signaling, negatively influencing disease outcomes[69] (Fig. 2). Tumor-infiltrating ILC3s can also transdifferentiate into ILC1-like cells and display cytotoxic activity in humans and suppress melanoma tumor growth in a mouse model[72], suggesting that ILC3s may drive anti-tumor protection and positively influence therapeutic outcomes in some circumstances (Fig. 2 and Table 1).

While the role of ILC3s in intestinal tumorigenesis and responses to therapy are beginning to emerge, their impact in tumor development and treatment outcomes in other tissues is largely unknown. Our current understanding is solely restricted to sporadic studies in pancreatic cancer[73], hepatocellular carcinoma[74] and breast cancer[27], in which ILC3s have been shown to promote tumor development and metastasis. Within these RORγt-expressing ILCs, little is known about the function of LTi cells and their impact in cancer prognosis. This is despite the fact that they are essential to the generation of secondary lymphoid tissues during embryogenesis, thus potentially identifying them as key drivers of the formation of tertiary lymphoid structure in tumors, which is associated with improved prognosis[75]. In lung cancer, for instance, the accumulation of NKp44$^+$ ILC3s in tumor lesions correlated with the density of tertiary lymphoid structures[76], possibly contributing to improved disease outcomes. These results offer a rationale to amplify LTi cell function though specific targeting to enhance anti-tumor immune responses and improve clinical outcomes.

Paradox of ILC protection versus damage in cancer reflect a key adaptation program that responds to the microenvironment

A major confounder in understanding the role of ILCs in responses to tumors is the divergent clinical outcomes. Multiple factors have contributed to the development of the current picture, including: (1) ILC sampling is often a snapshot and occurs late in disease development, (2) ILCs exhibit significant plasticity, allowing them to respond to extrinsic factors that reshape their apparent phenotype but increasing the complexity of the response, and (3) a major role of ILCs is in maintaining immune homeostasis, which may involve unrecognized immunosurveillance mechanisms in addition to tissue repair. Thus, the capability of ILCs to temporally alter their phenotype and function allows them to adapt to the tumor microenvironment (that is, tumor type, stage of disease and inflammatory milieu) and likely has a profound impact on ILC-mediated responses to cancer.

ILCs maintain immune homeostasis. This depends on balancing the generation of effector responses in the face of inflammation and their capacity to drive tissue repair and remodeling[7,77–81]. Inflammation restores normality in response to insults. Unregulated inflammation results in acute or chronic disruption of tissue architecture and behavior, potentially leading to tumorigenesis through the growth of new blood vessels and the induction of genomic instability in cells. ILCs have been implicated in multiple inflammatory disorders, including asthma, inflammatory bowel disease, Crohn's

disease and psoriasis and systemic and autoimmune diseases[43,82]. They are distributed widely throughout the body, which positions them to respond to disruptions that occur in tissues. In mice, ILC subsets are differentially enriched in different tissues; in humans, anatomically distinct compartmentalization is far less apparent[83]. ILCs are actively recruited to sites of inflammation and can be found early in the development of tumorigenesis[59,82]. ILC1s are enriched in breast and gastrointestinal cancers (including oesophageal, gastric, colonic and rectal cancer)[84], multiple myeloma[85], melanoma lymph node metastases[59], chronic lymphocytic leukemia and acute myeloid leukemia[26,64]. ILC2s are enriched in primary melanoma lesions[28], acute promyelocytic leukemia[66], breast cancer[84] and non-invasive bladder cancer[65] and are mobilized in lung tumors[50]. ILC2s can infiltrate pancreatic tumors as members of the tumor-infiltrating lymphoid population where they promote T-cell-mediated antitumor responses[68]. ILC3s are found in the lymphoid infiltrate of human non-small-cell lung cancer (NSCLC)[76] and in colorectal cancer in mouse models[70] and human tumors[82]. Thus, early, mild and more advanced inflammation activates the recruitment pathway for ILCs and influences their phenotype and function and disease outcomes. It is possible that differences in lineage markers and flow cytometry staining panels between studies may influence the measurement of the size of each ILC subset in tumors, resulting in potential discrepancies between investigations. A harmonized lineage staining panel and marker set to identify helper-like ILC subsets will be necessary to standardize results and to allow comparisons between different studies[86]. Nevertheless, these studies demonstrate that tumor-infiltrating ILCs (also known as TILCs) are players to consider in our dissection of the mechanisms leading directly or indirectly to coordinating tumor immune responses and to sustain tumor-specific adaptive responses, particularly in the context of immunotherapy.

Engineering innate cells to harness antitumor activity

Since their discovery in the early 1970s, NK cells have attracted the attention of immunologists and oncologists for their ability to kill a wide variety of tumor cells. Several modalities to harness the anti-tumor function of NK cells have been investigated in preclinical and clinical settings. NK cell therapies include the infusion of NK cells and the modulation of NK cell function by antibodies[54].

Cell therapy approaches can be divided into infusions of pre-activated autologous or allogeneic (including haploidentical) mature NK cells, stem-cell-derived NK cells and CAR NK cells[87–89]. All these protocols have shown that adoptive NK cell therapies are well-tolerated, and promising results have been obtained in malignant hemopathies such as acute myeloid leukemia (AML). The safety profile is an important and favorable difference between adoptive NK cell and T cell therapies. Indeed, most of the repertoire of activating receptors expressed on NK cells is selected in the species and not in the individuals, in contrast to the selection of the T cell repertoire[90]. As a consequence, whereas adoptive transfer of T cells can be harmful even in fully MHC-matched settings, the adoptive transfers of NK cells present a manageable safety profile. This is particularly key for the development of off-the-shelf products, which represent the ultimate goal of adoptive cell therapies. In terms of efficacy, the infusion of HLA-mismatched anti-CD19 CAR NK cells derived from cord blood has shown rapid responses in the majority of a small cohort of 11 people with relapsed or refractory CD19-positive cancers (non-Hodgkin's lymphoma or chronic lymphocytic leukemia)[91].

Biologicals based on monoclonal antibodies have been designed to increase NK cell activity, and include immune checkpoint inhibitors and NK cell engagers. NK cells express the inhibitory receptor NKG2A, which interacts with the non-classical MHC class I molecule HLA-E. The blocking of this inhibitory interaction by the immune checkpoint inhibitor candidate monalizumab has been shown to unleash the anti-tumor functions of NK cells in pre-clinical models and has led to the development of positive phase 2 clinical trial and ongoing phase 3 clinical trial in individuals with head and neck cancer[92]. In addition, multispecific NK cell engagers based on engagement of the activating Fc receptor CD16, co-engagement of CD16 with NKp46 or co-engagement of the NK cell activating receptors with the IL-2R and IL-15R signaling pathways have proven to have remarkable efficacy in pre-clinical models[93,94]. Other NK cell engagers based on engagement of CD16 or NKG2D are also being tested in clinical trials[93,94].

The next step for harnessing NK cells in cancer therapies includes the development of settings against solid tumors. This could be achieved by the combination of NK cell infusions and antibodies designed to increase NK cell activity, such as immune checkpoint inhibitors and NK cell engagers. Along this line, the combination therapy associating the infusion of cytokine-activated adult blood or cord blood NK cells and the tetravalent bispecific antibody AFM13 that binds CD30 on cancer cells and CD16 has shown promising results in $CD30^+$ hematologic malignancies[95].

Besides the harnessing of NK cells in cancer therapies, the development of ILC-based cell therapies has not started. The tissue residency of ILCs and their capacity to produce cytokines are interesting features that prompt pre-clinical investigations on the interest of their manipulation in the context of innovative treatments. The recent development of protocols that lead to the generation of ILCs from hematopoietic progenitors will be helpful in this matter[96]. Despite the relative rarity of ILC subsets compared with T cells, identification of molecular markers that correlate with survival outcomes has already been encouraging[28,59,68] suggesting that fine mapping of ILCs in the tumor microenvironment offers the possibility of building a prognostic toolkit to stratify patients and treatment approaches.

Conclusions

The past decade has witnessed an explosion of knowledge around the identification, developmental programs and functions of ILCs. This has uncovered unexpected contributions in both their protective and non-protective actions in disease, firstly in inflammation and more recently in cancer. Regarding the latter, there is much more to learn about the involvement of ILCs in the tumor microenvironment. Controversy still exists around the identity of different ILC subsets and the types of effector functions they might exhibit in vivo in the context of the tumor microenvironment.

A number of questions, however, are beginning to be answered. These include,

1. Do ILCs accumulate in tumors? It is now clear that ILCs are present in tumors, but it remains to be resolved whether they originate from bone marrow progenitors or develop in situ, or the degree to which they persist over time. Similarly, the mechanisms that drive the influx or local proliferation of ILCs are yet to be uncovered, although factors released from dying cells, such as IL-33, could play a critical role in local activation and/or expansion.
2. Do ILCs have prognostic or predictive value in assessing responses to immunotherapy? Reports describing the potentially positive impact of ILCs in the anti-tumor response continue to emerge, at least in some situations. Using modeling, it has been demonstrated that there can be very strong correlations or predictive ability of ILCs in tumors. More detailed assessment at different stages of disease is ultimately required to understand how temporally robust such predictors might be and whether the outcomes may be tumor specific. In addition, it is tantalizing to consider whether allied cells, such as eosinophils, recruited into the setting might serve as a 'biomarker' for tumor progression or treatment efficacy following immunotherapy.

3. What is the role of ILCs in the antitumor adaptive immune response? Innate and adaptive immune responses are highly complementary and act to inform each other in the context of the tumor microenvironment. It is possible that activation of ILCs to secrete cytokines (for example, IL-4 and IL-9) lays the foundation for activation and the function of adaptive cells, such as B cell isotype switching or $CD8^+$ T cell activation. Indeed, changes in MHC class II, ICOS and OX-40 ligands on ILCs modulates $CD4^+$ T cell responses in both the lung and gut, suggesting that ILCs have a significant impact on the tumor microenvironment and can choreograph their interplay between ILCs and with adaptive cells to determine tumor fate outcomes.
4. Does the tumor microenvironment change ILC phenotype and function? How does the stage of the disease, degree of local inflammation and associated tumor milieu shape the functions of ILCs? Precisely how these factors impact ILC plasticity, phenotype and function in tumors remains to be fully defined.

While increasing examples emerge of important roles for ILCs in the tumor microenvironment, understanding in greater detail how their molecular programing is orchestrated in the tumor context is urgently required to further take advantage of these unique innate immune cells in our immunotherapeutic armory.

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