

# The Emerging Protumor Role of $\gamma\delta$ T Lymphocytes: Implications for Cancer Immunotherapy

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## Abstract

Tumor-infiltrating lymphocytes are key mediators of tumor immune surveillance and are important prognostic indicators in cancer progression. Among the various lymphocyte subsets implicated in protection against cancer are  $\gamma\delta$  T lymphocytes, which can kill tumor cells and secrete potent antitumor cytokines. By contrast, recent reports have revealed an unexpected series of protumor functions of  $\gamma\delta$  T cells in mouse models and human patients. In particular, specific  $\gamma\delta$  T-cell subsets are

capable of recruiting immunosuppressive myeloid populations, inhibiting antitumor responses, and enhancing angiogenesis, thus promoting cancer progression. A common mediator of such functions appears to be the cytokine IL17, whose pathogenic effects can override the antitumor immune response orchestrated by IFN $\gamma$ . Here, we review these studies and discuss their implications for the manipulation of  $\gamma\delta$  T cells in cancer immunotherapy. *Cancer Res*; 75(5); 798–802. ©2015 AACR.

## Longstanding Protective Roles of $\gamma\delta$ T Cells in Cancer Surveillance

The hallmarks of cancer have been recently revised to include an immune escape component that presumably overrides surveillance by tumor-infiltrating lymphocytes (TIL). Since the pioneering work of Girardi and colleagues (1) on chemically induced tumors in mice, rapidly followed by other studies on transplantable, transgenic, and even spontaneous tumors (reviewed in ref. 2),  $\gamma\delta$  T cells have been seen as prototypic antitumor TILs. These protective functions derive from T-cell receptor (TCR)- and natural killer (NK) receptor-mediated tumor cell recognition and  $\gamma\delta$  T-cell activation, cytotoxicity and production of cytokines like TNF and IFN $\gamma$  (2). Consistent with these properties, mice lacking  $\gamma\delta$  T cells have been shown to develop faster and bigger tumors in models such as the transplantable B16 melanoma (3, 4). It should be noted, however, that  $\gamma\delta$  T cell subsets and their functional attributes differ significantly between mice and humans, which should qualify the conclusions drawn from mouse tumor models.

In humans, both major subsets of  $\gamma\delta$  T cells, either V $\delta$ 1<sup>+</sup> (predominant in tissues) or V $\delta$ 2<sup>+</sup> (predominant in the circulation), are also cytolytic against solid and hematologic tumors, and produce high amounts of IFN $\gamma$  and TNF upon activation. This has fuelled a series of clinical trials targeting  $\gamma\delta$  T cells, more specifically the blood-abundant V $\gamma$ 9V $\delta$ 2 population, either endoge-

nously or upon adoptive cell transfer. The most promising results (with objective responses up to 33%) were obtained upon *in vivo* administration of aminobisphosphonates, like zoledronate or pamidronate (5, 6). These FDA-authorized drugs are known to selectively stimulate V $\gamma$ 9V $\delta$ 2 T cells through metabolic accumulation of intracellular isopentenyl pyrophosphate, an agonist of the V $\gamma$ 9V $\delta$ 2 TCR (7).

We and others have also studied V $\delta$ 1<sup>+</sup> T cells, which have been found in increased numbers and with enhanced antitumor functionality (when compared with V $\delta$ 2<sup>+</sup> cells) in melanoma and leukemia (8–10). Despite these interesting findings, the therapeutic potential of V $\delta$ 1<sup>+</sup> T cells is still to be tested in the clinic.

## Emerging Pathogenic Roles of $\gamma\delta$ T Cells in Cancer Progression

### Tumor-promoting functions of $\gamma\delta$ T cells in mice and humans

Despite the well-established concept of  $\gamma\delta$  T cells as potent antitumor TILs, a study in 2007 on human breast cancer surprisingly revealed a potential protumor function (11).  $\gamma\delta$  T cells isolated from breast cancer biopsies were reported to inhibit the function of several immune cell populations *in vitro*, and consequently suppress their antitumor responses (11). Consistent with these observations, the presence of  $\gamma\delta$  T cells was shown to positively correlate with advanced tumor stages and inversely correlate with patient survival (12). Of note,  $\gamma\delta$  T cells represented the most significant independent prognostic factor for assessing severity of breast cancer (12).

In mice, two distinct populations of  $\gamma\delta$  T cells were shown to play opposite roles in melanoma progression. Whereas the V $\gamma$ 4<sup>+</sup> cells displayed several antitumor properties, the V $\gamma$ 1 subset were able to suppress V $\gamma$ 4<sup>+</sup> cells promoting tumor escape (13). These findings raised the interesting question as to whether different  $\gamma\delta$  T-cell subsets could exert strikingly distinct antitumor versus protumor roles.

The relatively recent identification of an additional functional subset of  $\gamma\delta$  T cells, able to produce IL17 rather than IFN $\gamma$ , fostered

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the exploration of its functions in homeostasis and disease (14). We have shown that commitment to production of IL17 or IFN $\gamma$  is determined in the thymus at epigenetic and transcriptional levels and is maintained in peripheral responses to several infectious microbes (14, 15). This dichotomy of cytokine secretion segregates with CD27, the expression of which is associated with IFN $\gamma$  production, whereas IL17 production is restricted to CD27<sup>-</sup>  $\gamma\delta$  T cells (14–16).

In 2010,  $\gamma\delta$  T cells were described as the main IL17-producing population in a murine fibrosarcoma model, in which they were suggested to promote tumor growth (17). Subsequently, in 2011,  $\gamma\delta$  T cells were similarly described as the main producers of IL17 in a lung metastasis model with detrimental effects for the tumor-bearing host (18). Recent reports using TCR $\delta$ <sup>-/-</sup> mice have also demonstrated the protumor effects of  $\gamma\delta$  T cells in hepatocellular carcinoma and ovarian cancer models, again correlated with IL17 production (19, 20).

In contrast with the significant emerging literature describing the potential protumor effector function of IL17<sup>+</sup>  $\gamma\delta$  T cells in murine models, reports on their human counterparts are still scarce. In 2011, a pioneering study showed that human  $\gamma\delta$  T cells produced IL17 in response to bacterial meningitis, an extreme inflammatory condition (21). Recently, IL17<sup>+</sup>  $\gamma\delta$  T cells were also shown to accumulate in human colorectal cancer as compared to normal tissue (22). In this context,  $\gamma\delta$  T cells were the main source of IL17, and unlike CD4<sup>+</sup> Th17 cells, appeared to positively correlate with advanced stages of disease (22). Furthermore, IL17<sup>+</sup>  $\gamma\delta$  T cells have been also observed to be elevated in peripheral blood, as well as in the tumor tissue, of patients with gallbladder cancer (Dr. S.V. Chiplunkar, Tata Memorial Centre, Mumbai, India, personal communication).

Collectively, these reports establish a new perspective on the contribution of  $\gamma\delta$  T cells to tumor development, which highlights the importance of studying the full range of  $\gamma\delta$  T-cell effector functions in preclinical animal models. Various different mechanisms have been proposed to mediate the protumor behavior of  $\gamma\delta$  TILs in several tumor models (Fig. 1), which will be described below.

#### Inhibition of antitumor responses

Tumor-infiltrating  $\gamma\delta$  T cells have been described to inhibit the response of several immune cell populations against tumors. Human V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells isolated from breast cancer biopsies inhibit proliferation of naïve T cells as well as IL2 production by effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells (11). Interestingly, tumor-derived V $\delta$ 1<sup>+</sup> cells are also able to inhibit the proliferation of V $\delta$ 2<sup>+</sup> cells, which potentially could exert potent antitumor responses. Furthermore, V $\delta$ 1<sup>+</sup> cells also impair the maturation and T-cell priming capacity of dendritic cells (DC; ref. 11). The molecular cues that mediate the suppressive functions of V $\delta$ 1<sup>+</sup> cells in breast cancer remain to be discovered. However, this suppressive behavior can effectively be reverted by TLR8 ligands, which may be important for translation into the clinic (11).

In the mouse B16 melanoma model, V $\gamma$ 4<sup>+</sup> and V $\gamma$ 1<sup>+</sup> subsets of  $\gamma\delta$  T cells were reported to play opposing roles in tumor progression. The protective immunity of V $\gamma$ 4<sup>+</sup> cells is mediated by both IFN $\gamma$  and perforin and controlled by the transcription factor eomesodermin (23). By contrast, tumor-infiltrating V $\gamma$ 1<sup>+</sup> cells are polarized toward a Th2 type of response, with characteristic production of IL4 and expression of Stat3 (13). Furthermore, this IL4 production by V $\gamma$ 1<sup>+</sup> cells is able to reduce the protective effect

of V $\gamma$ 4<sup>+</sup> cells, via transcriptional downregulation of IFN $\gamma$ , NKG2D, and perforin (13).

#### Promotion of angiogenesis

$\gamma\delta$  T cells were first suggested to promote angiogenesis via IL17 production in the murine CMS-G4 fibrosarcoma model (17). Using IL17<sup>-/-</sup> mice, the authors demonstrated that IL17 is a key factor for optimal tumor growth *in vivo*. In the absence of IL17, fibrosarcoma tumors grow slower and with a reduced number of blood vessels (17). Moreover, *in vitro* provision of IL17 increased the expression of the angiogenic-related genes *ang-2* and *vegf* in CMS-G4 cells (17). Similarly, in the ID8 ovarian cancer model, tumors grown in IL17<sup>-/-</sup> and TCR $\delta$ <sup>-/-</sup> mice express lower levels of *ang-2* and *vegf* when compared with tumors grown in wild-type animals (20). IL17<sup>+</sup>  $\gamma\delta$  T cells also support the mobilization of proangiogenic Tie2-expressing macrophages into the peritoneal cavity (20). In an oncogenic KRAS<sup>G12D</sup>-driven pancreatic cancer model, IL17 produced by both Th17 and  $\gamma\delta$  T cells is associated with increased IL6/Stat3 signaling, which was previously shown to upregulate prosurvival and proangiogenic genes (24, 25).

#### Recruitment of protumor myeloid populations

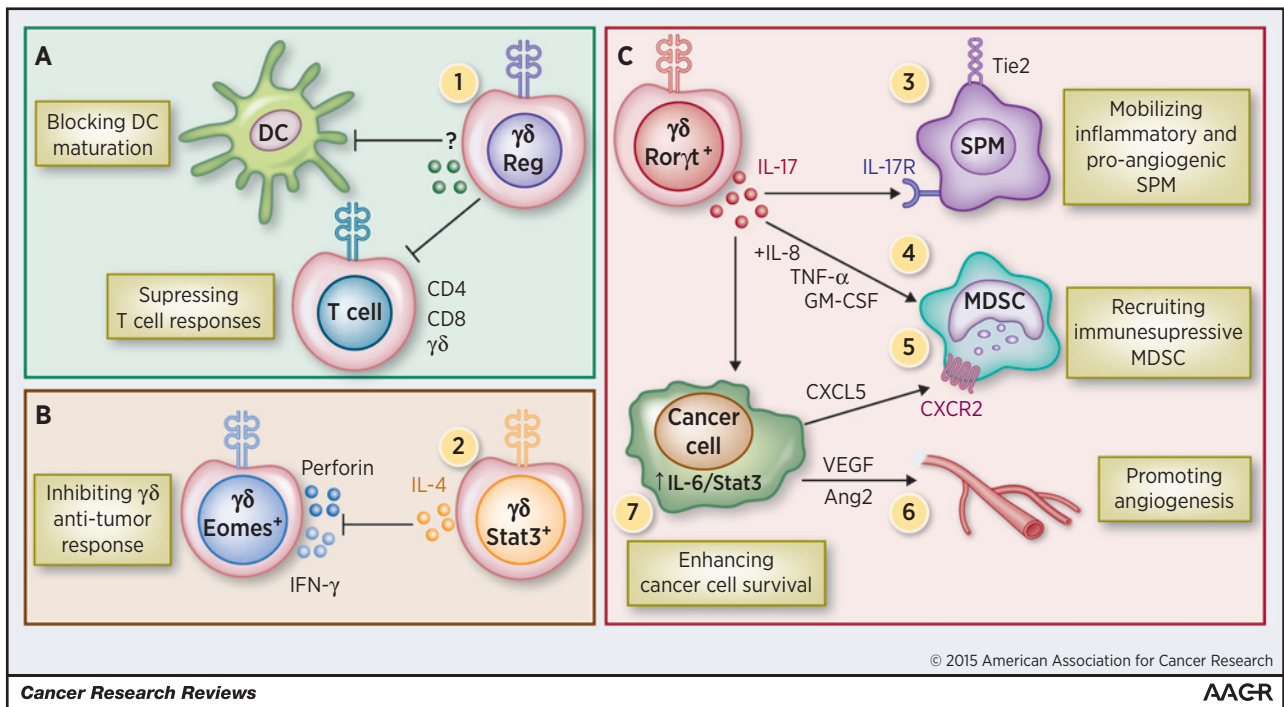
IL17<sup>+</sup>  $\gamma\delta$  T cells were demonstrated to mediate the mobilization of myeloid-derived suppressor cells (MDSC), which subsequently reduce cytotoxic responses by CD8<sup>+</sup> T cells, in a mouse model of hepatocellular carcinoma (19). Experiments *in vitro* suggest that IL17 upregulates CXCL5 expression on a murine hepatoma cell line that increases the migration of MDSC through interaction with CXCR2 (19). IL17 was also shown to enhance the immunosuppressive functions of MDSC (19). Interestingly, a similar capacity of IL17<sup>+</sup>  $\gamma\delta$  T cells to recruit MDSC was described in human colorectal cancer (22). In this study,  $\gamma\delta$  T cells promoted *in vitro* migration, proliferation, and survival of MDSC via production of IL17, IL8, GM-CSF, and TNF $\alpha$  (22).

Our recent work has demonstrated that IL17<sup>+</sup>  $\gamma\delta$  T cells also promote the mobilization of blood-derived small peritoneal macrophages (SPM) into the peritoneal cavity upon ID8 ovarian cancer cell transplantation (20). SPMs have a proinflammatory and proangiogenic gene-expression profile and are able to directly enhance ID8 tumor cell growth *in vitro* (20).

#### The key role of IL17 in the protumor functions of $\gamma\delta$ T cells

IL17 is a common mediator of the  $\gamma\delta$  T-cell protumor function in several of the aforementioned studies. Interestingly, in some of these, IFN $\gamma$ -producing  $\gamma\delta$  T cells are also reported to accumulate in the tumor environment (20, 22, 24). A dominant effect of IL17 over IFN $\gamma$  was previously reported, and may explain the net protumor effect of  $\gamma\delta$  T cells when IL17 is produced (25). For example, in murine models of melanoma and bladder cancer, the slow growth of tumors in double knockout IL17<sup>-/-</sup>.IFN $\gamma$ <sup>-/-</sup> mice resembles the IL17<sup>-/-</sup> phenotype rather than the fast growing phenotype of IFN $\gamma$ <sup>-/-</sup> animals (25). Expression of IL22 was also observed in tumor-infiltrating  $\gamma\delta$  T cells in a murine fibrosarcoma model as well as in human pancreatic cancer. However, IL22 transcripts were not detected in human colorectal carcinoma-infiltrating  $\gamma\delta$  T cells (17, 22, 24). However, the relevance of this cytokine or other Th17 cytokines, such as IL17F, that may be produced by  $\gamma\delta$  T cells in cancer has been poorly addressed.

IL17 production seems to be restricted to specific subsets of  $\gamma\delta$  T cells, which are preferentially expanded in the tumor



**Figure 1.** Mechanisms mediating the pathogenic roles of tumor-infiltrating  $\gamma\delta$  T cells in cancer progression. A, "regulatory"  $\gamma\delta$  T cells can suppress antitumor immune responses by inhibiting DC maturation and effector functions of  $CD4^+$ ,  $CD8^+$ , and  $\gamma\delta$  T cells. The molecular factor that mediates this inhibitory role is still unknown. B, Stat3-expressing  $\gamma\delta$  T cells produce IL4 that inhibits secretion of the antitumor cytokine IFN $\gamma$  and the cytotoxic molecule perforin, by Eomes $^+$   $\gamma\delta$  T cells. C, IL17 secreted by Ror1t $^+$   $\gamma\delta$  T cells exerts protumor effects via mobilization of inflammatory and proangiogenic, Tie2-expressing SPM; recruitment of immunosuppressive MDSC (also promoted by IL8, TNF $\alpha$ , and GM-CSF produced by  $\gamma\delta$  T cells); upregulation of CXCL5 in cancer cells that subsequently recruit MDSC through interaction with CXCR2; increase in expression of the proangiogenic factors VEGF and Ang-2 by cancer cells; and increase in IL6/Stat3 signaling in cancer cells that favor their survival. These mechanisms have been described in the following murine models or human cancers: 1, human breast cancer; 2, murine melanoma model; 3, murine ovarian cancer model; 4, human colorectal cancer; 5, murine hepatocellular carcinoma model; 6, murine fibrosarcoma model; and 7, murine pancreatic cancer model.

environment. In a hepatocellular carcinoma model, the majority of IL17 $^+$   $\gamma\delta$  T cells express the V $\gamma$ 4 chain and have the typical ROR1t $^+$ , CD27 $^-$ , CCR6 $^+$  phenotype (19). By contrast, the IL17 $^+$   $\gamma\delta$  T cells that accumulate in the ID8 ovarian cancer model express the V $\gamma$ 6 chain (20). In human colorectal cancer, tumor-infiltrating IL17 $^+$  V $\delta$ 1 $^+$  T cells are CD45RO $^+$ , CD161 $^+$ , CCR6 $^+$ , and display an effector memory (CD45RA $^-$  and CD27 $^-$ ) phenotype (22).

Tumor microenvironment-derived cytokines have been shown to trigger IL17 polarization in  $\gamma\delta$  T cells. In a fibrosarcoma model, inhibition *in vivo* of the Th17-polarizing cytokines IL6, TGF $\beta$ , and IL23 using blocking antibodies partially reduced the percentage of tumor-infiltrating IL17 $^+$   $\gamma\delta$  T cells (17). In a lung metastasis model, IL1 $\beta$  was shown to recruit and polarize  $\gamma\delta$  T cells toward IL17 production (18). In a murine hepatocellular carcinoma model, depletion *in vivo* of MDSCs reduced tumor-infiltrating IL17 $^+$   $\gamma\delta$  T cells, possibly as a result of the absence of MDSC-derived IL1 $\beta$  and IL23 (19). Similarly, in human colorectal cancer, DC-derived IL23 was proposed to polarize  $\gamma\delta$  T cells toward IL17 production (22). In an ovarian cancer model, a combined effect of IL7, IL6, and IL1 $\beta$  was suggested to drive the selective expansion of IL17 $^+$   $\gamma\delta$  T cells (20). Furthermore, oncogenic KRAS $^{G2D}$ -expressing pancreatic cells were shown to recruit IL17-producing  $\gamma\delta$  T cells into the pancreatic malignant environment via IL6 production, which accelerated the initiation and progression of pancreatic cancer (24). Experiments *ex vivo* suggest that TCR $\gamma\delta$  signaling

may also be required for IL17 production by  $\gamma\delta$  T cells (17, 26). Moreover, the preferential expansion in several models of IL17 $^+$   $\gamma\delta$  T cells with a limited receptor repertoire (19, 20, 22), suggests that an additional TCR-mediated mechanism may also be involved.

### Future Perspectives for $\gamma\delta$ T-cell-Based Cancer Immunotherapy

The therapeutic implications of the pleiotropic biologic interactions between  $\gamma\delta$  T cells and tumors still remain unclear. Nonetheless, the recent discovery of protumor roles for particular  $\gamma\delta$  T-cell populations may create new challenges for future therapeutic approaches. Thus, for both stimulation *in vivo* and activation *in vitro* (for adoptive transfer), it will be important to evaluate the stable functional polarization of effector  $\gamma\delta$  T cells. On the basis of the most available data, we argue that clinical protocols should maximize IFN $\gamma$  production and minimize IL17 secretion. However, it should be noted that murine IL17-producing  $\gamma\delta$  T cells have also been implicated in protective responses in some particular tumor scenarios. For example, they seemingly contributed to the therapeutic effect of doxorubicin in various transplantable models of epithelial tumors *in vivo* (27). The authors proposed a link between IL17 and the priming (in the draining lymph nodes) of an IFN $\gamma$  response by CD8 $^+$  T cells,

which depended on drug-induced "immunogenic tumor cell death." Future research should clarify the contexts in which IL17 production by  $\gamma\delta$  T cells may be potentially beneficial rather than detrimental for the host.

The balance between IFN $\gamma$ <sup>+</sup> versus IL17<sup>+</sup>  $\gamma\delta$  TILs is likely to vary considerably over time. For example, in the murine ID8 model, IFN $\gamma$ <sup>+</sup>  $\gamma\delta$  T cells increased only transiently before returning to baseline, whereas IL17<sup>+</sup>  $\gamma\delta$  T cells accumulated at later stages of tumor progression, concomitantly with the so-called angiogenic switch (20). Such dynamics may have important implications when trying to manipulate the tumor microenvironment, particularly as the clinical scenario will more often correspond to advanced stages of disease.

In humans, both V $\delta$ 1<sup>+</sup> and V $\delta$ 2<sup>+</sup> T cells are naturally biased toward IFN $\gamma$  (in the blood of healthy individuals). In fact, IL2 or IL15 stimulation of naïve  $\gamma\delta$  thymocytes results in the differentiation of producers of IFN $\gamma$  but not IL17 (28). Indeed, IL17 expression by human  $\gamma\delta$  T cells *in vitro* appears to require their activation in a highly inflammatory milieu (21). It may, therefore, be possible to manipulate  $\gamma\delta$  T-cell polarization *in situ* by targeting cytokines such as IL1 $\beta$ , IL6, IL23, and TGF $\beta$  (required for IL17 expression) or IL2, IL12, IL15, and IL18 (which promote IFN $\gamma$  production). Such a strategy would affect, not only on  $\gamma\delta$  T cells, but importantly also on CD4<sup>+</sup> T helper cell subsets, which can determine the course of the immune response to tumors (2).

From the perspective of adoptive  $\gamma\delta$  T-cell transfer, it seems logical to differentiate IFN $\gamma$ <sup>+</sup>, IL17<sup>-</sup> effector cells devoid of immune suppressive properties. Along these lines, activation of naïve V $\gamma$ 9V $\delta$ 2 T cells with pyrophosphate agonists plus IL2 leads to strong IFN $\gamma$  but no IL17 production (29). Moreover, we have characterized a population of V $\delta$ 1<sup>+</sup> cells expressing natural cytotoxicity receptors that also produces abundant IFN $\gamma$  but no IL17, and displays enhanced antileukemia cytotoxicity compared with

V $\gamma$ 9V $\delta$ 2 cells (9). Another interesting property of V $\delta$ 1<sup>+</sup> cells (especially for the adoptive cell transfer approach) is their tissue tropism and CCL2-mediated chemotaxis toward tumors (4). These characteristics may explain their enrichment (relative to V $\delta$ 2<sup>+</sup> cells) in various solid tumor types (10, 11). We are therefore particularly interested in exploring the therapeutic potential of V $\delta$ 1<sup>+</sup> cells, particularly as no clinical trial has yet focused on this  $\gamma\delta$  T-cell subset.

Of note, the success of  $\gamma\delta$  T-cell-based immunotherapy is also likely to depend on avoiding activation-induced cell death and exhaustion of the  $\gamma\delta$  T-cell compartment, and on overcoming inhibitory mechanisms as those posed by PD-1 expression (30) or the suppressive functions of regulatory T cells (31, 32).

Finally, we think it will be important to evaluate the prognostic value of  $\gamma\delta$  T-cell subsets (such as IFN $\gamma$ <sup>+</sup> vs. IL17<sup>+</sup>) in biopsies of various cancer types to assess their added value to the so-called immunoscore (33) and its capacity to inform oncologists and immunologists toward efficient immunotherapies against malignant tumors.

### Disclosure of Potential Conflicts of Interest

B. Silva-Santos is a consultant/advisory board member of LymphAct S.A. No potential conflicts of interest were disclosed by the other authors.

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