The Emerging Protumor Role of γδ T Lymphocytes: Implications for Cancer Immunotherapy

Margarida Rei1,2,3, Daniel J. Pennington2, and Bruno Silva-Santos1

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 γδ 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 γδ T cells in mouse models and human patients. In particular, specific γδ 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 IFNγ. Here, we review these studies and discuss their implications for the manipulation of γδ T cells in cancer immunotherapy.

Longstanding Protective Roles of γδ 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 (TILs). 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), γδ 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 γδ T-cell activation, cytotoxicity and production of cytokines like TNF and IFNγ (2). Consistent with these properties, mice lacking γδ 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 γδ T cells 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 γδ T cells, either Vδ1+ (predominant in tissues) or Vδ2+ (predominant in the circulation), are also cytolytic against solid and hematologic tumors, and produce high amounts of IFNγ and TNF upon activation. This has fuelled a series of clinical trials targeting γδ T cells, more specifically the blood-abundant Vγ9Vδ2 population, either endogenously 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 Vγ9Vδ2 T cells through metabolic accumulation of intracellular isopentenyl pyrophosphate, an agonist of the Vγ9Vδ2 TCR (7).

We and others have also studied Vδ1+ T cells, which have been found in increased numbers and with enhanced antitumor functionality (when compared with Vδ2+ cells) in melanoma and leukemia (8–10). Despite these interesting findings, the therapeutic potential of Vδ1+ T cells is still to be tested in the clinic.

Emerging Pathogenic Roles of γδ T Cells in Cancer Progression

Tumor-promoting functions of γδ T cells in mice and humans

Despite the well-established concept of γδ T cells as potent antitumor TILs, a study in 2007 on human breast cancer surprisingly revealed a potential protumor function (11): γδ 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 γδ T cells was shown to positively correlate with advanced tumor stages and inversely correlate with patient survival (12). Of note, γδ T cells represented the most significant independent prognostic factor for assessing severity of breast cancer (12).

In mice, two distinct populations of γδ T cells were shown to play opposite roles in melanoma progression. Whereas the Vγ4α T cells displayed several antitumor properties, the Vγ1 subset were able to suppress Vγ4α cells promoting tumor escape (13). These findings raised the interesting question as to whether different γδ T-cell subsets could exert strikingly distinct antitumor versus protumor roles.

The relatively recent identification of an additional functional subset of γδ T cells, able to produce IL17 rather than IFNγ, fostered...
the exploration of its functions in homeostasis and disease (14).
We have shown that commitment to production of IL17 or IFNγ 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 segre-
gates with CD27, the expression of which is associated with IFNγ
production, whereas IL17 production is restricted to CD27−γδ T
cells (14–16).

In 2010, γδ 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,
γδ 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 TCRδ−/− mice have also
demonstrated the protumor effects of γδ 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+ γδ T cells in
murine models, reports on their human counterparts are still
scarce. In 2011, a pioneering study showed that human γδ T cells
produced IL17 in response to bacterial meningitis, an extreme
inflammatory condition (21). Recently, IL17+ γδ T cells were
also shown to accumulate in human colorectal cancer as com-
pared to normal tissue (22). In this context, γδ T cells were the
main source of IL17, and unlike CD4+ Th17 cells, appeared to
positively correlate with advanced stages of disease (22).
Furthermore, IL17+ γδ 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. Chipulkar, Tata
Memorial Centre, Mumbai, India, personal communication).

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

Inhibition of antitumor responses

Tumor-infiltrating γδ T cells have been described to inhibit the
response of several immune cell populations against tumors. Human
Vδ1+ γδ T cells isolated from breast cancer biopsies
inhibit proliferation of naïve T cells as well as IL2 production
by effector CD4+ and CD8+ T cells (11). Interestingly, tumor-derived
Vδ1+ cells are also able to inhibit the proliferation of Vδ2+ cells,
which potentially could exert potent antitumor responses. Fur-
thermore, Vδ1+ cells also impair the maturation and T-cell prim-
ing capacity of dendritic cells (DC; ref. 11). The molecular cues
that mediate the suppressive functions of Vδ1+ cells in breast
cancer remain to be discovered. However, this suppressive behav-
ior can effectively be reverted by TLR8 ligands, which may be
important for translation into the clinic (11).

In the mouse B16 melanoma model, Vγ4− and Vγ1+ subsets of
γδ T cells were reported to play opposing roles in tumor progres-
sion. The protective immunity of Vγ4+ cells is mediated by both
IFNγ and perforin and controlled by the transcription factor
comesodermin (23). By contrast, tumor-infiltrating Vγ1+ cells are
polarized toward a Th2 type of response, with characteristic
production of IL4 and expression of Stat3 (13). Furthermore,
this IL4 production by Vγ1+ cells is able to reduce the protective effect
of Vγ4+ cells, via transcriptional downregulation of IFNγ,
NKG2D, and perforin (13).

Promotion of angiogenesis

γδ T cells were first suggested to promote angiogenesis via IL17
production in the murine CMS-G4 fibrosarcoma model (17).
Using IL17−/− 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−/− and TCRδ−/− mice express lower levels of
ang-2 and vegf when compared with tumors grown in wild-type
animals (20). IL17+ γδ T cells also support the mobilization of
proangiogenic Tie2-expressing macrophages into the peritoneal
cavity (20). In an oncogenic KRASG12D-driven pancreatic cancer
model, IL17 produced by both Th17 and γδ 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+ γδ T cells were demonstrated to mediate the mobili-
ization of myeloid-derived suppressor cells (MDSC), which
subsequently reduce cytotoxic responses by CD8+ 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+ γδ T cells to recruit
MDSC was described in human colorectal cancer (22).
In this study, γδ T cells promoted in vitro migration, prolifer-
ation, and survival of MDSC via production of IL17, IL8, GM-
CSF, and TNFα (22).

Our recent work has demonstrated that IL17+ γδ 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 γδ T cells

IL17 is a common mediator of the γδ T-cell protumor function in
several of the aforementioned studies. Interestingly, in some of
these, IFNγ-producing γδ T cells are also reported to accumulate in
the tumor environment (20, 22, 24). A dominant effect of IL17
over IFNγ was previously reported, and may explain the net
protumor effect of γδ 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−/−/IFNγ−/− mice
resembles the IL17−/−/IFNγ−/− phenotype rather than the fast growing
phenotype of IFNγ−/− mice (25). Expression of IL22 was also
observed in tumor-infiltrating γδ 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 γδ T cells (17, 22, 24). However, the relevance of this
cytokine or other Th17 cytokines, such as IL17F, that may be
produced by γδ T cells in cancer has been poorly addressed.
IL17 production seems to be restricted to specific subsets of
γδ T cells, which are preferentially expanded in the tumor
environment. In a hepatocellular carcinoma model, the majority of IL17γδ T cells express the Vγ4 chain and have the typical RORγt+, CCR6+, CD161+ phenotype (19). By contrast, the IL17γδ T cells that accumulate in the ID8 ovarian cancer model express the Vγ6 chain (20). In human colorectal cancer, tumor-infiltrating IL17γδ T cells are CD45RO+CD69+CD27−/C0+, and display an effector memory (CD45RA−CD62L−) phenotype (22). Tumor microenvironment–derived cytokines have been shown to trigger IL17 polarization in γδ T cells. In a fibrosarcoma model, inhibition in vivo of the Th17-polarizing cytokines IL6, TGFβ, and IL23 using blocking antibodies partially reduced the percentage of tumor-infiltrating IL17γδ T cells (17). In a lung metastasis model, IL1β was shown to recruit and polarize γδ T cells toward IL17 production (18). In a murine hepatocellular carcinoma model, depletion in vivo of MDSCs reduced tumor-infiltrating IL17γδ T cells, possibly as a result of the absence of MDSC-derived IL1β and IL23 (19). Similarly, in human colorectal cancer, DC-derived IL23 was proposed to polarize γδ T cells toward IL17 production (22). In an ovarian cancer model, a combined effect of IL7, IL6, and IL1β was suggested to drive the selective expansion of IL17γδ T cells (20). Furthermore, oncogenic KRASG12D–expressing pancreatic cells were shown to recruit IL17-producing γδ 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 TCRγδ signaling may also be required for IL17 production by γδ T cells (17, 26). Moreover, the preferential expansion in several models of IL17γδ T cells with a limited receptor repertoire (19, 20, 22), suggests that an additional TCR-mediated mechanism may also be involved.

**Future Perspectives for γδ T-cell–Based Cancer Immunotherapy**

The therapeutic implications of the pleiotropic biologic interactions between γδ T cells and tumors still remain unclear. Nonetheless, the recent discovery of protumor roles for particular γδ 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 γδ T cells. On the basis of the most available data, we argue that clinical protocols should maximize IFNγ production and minimize IL17 secretion. However, it should be noted that murine IL17-producing γδ 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 IFNγ response by CD8+ T cells,

---

**Figure 1.**

Mechanisms mediating the pathogenic roles of tumor-infiltrating γδ T cells in cancer progression. A, "regulatory" γδ (Reg) T cells can suppress antitumor immune responses by inhibiting DC maturation and effector functions of CD4+, CD8+, and γδ T cells. The molecular factor that mediates this inhibitory role is still unknown. B, Stat3-expressing γδ T cells produce IL4 that inhibits secretion of the antitumor cytokine IFNγ and the cytolytic molecule perforin, by Eomes−γδ T cells. C, IL17 secreted by Rorγt−γδ T cells exerts protumor effects via mobilization of inflammatory and proangiogenic, Tie2-expressing SPM; recruitment of immunosuppressive MDSC (also promoted by IL8, TNFα, and GM-CSF produced by γδ T cells); upregulation of CXCL5 in cancer cells that subsequently recruit MDSCs 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, IL17 production (18). In a murine hepatocellular carcinoma model, depletion of IL17γδ T cells was shown to recruit and polarize γδ T cells toward IL17 production (18). In a murine hepatocellular carcinoma model, depletion in vivo of MDSCs reduced tumor-infiltrating IL17γδ T cells, possibly as a result of the absence of MDSC-derived IL1β and IL23 (19). Similarly, in human colorectal cancer, DC-derived IL23 was proposed to polarize γδ T cells toward IL17 production (22). In an ovarian cancer model, a combined effect of IL7, IL6, and IL1β was suggested to drive the selective expansion of IL17γδ T cells (20). Furthermore, oncogenic KRASG12D–expressing pancreatic cells were shown to recruit IL17-producing γδ 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 TCRγδ signaling may also be required for IL17 production by γδ T cells (17, 26). Moreover, the preferential expansion in several models of IL17γδ T cells with a limited receptor repertoire (19, 20, 22), suggests that an additional TCR-mediated mechanism may also be involved.
which depended on drug-induced "immunogenic tumor cell death." Future research should clarify the contexts in which IL17 production by γδ T cells may be potentially beneficial rather than detrimental for the host.

The balance between IFNγ+ versus IL17+ γδ TILs is likely to vary considerably over time. For example, in the murine ID8 model, IFNγ+ γδ T cells increased only transiently before returning to baseline, whereas IL17+ γδ 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 Vδ1+ and Vδ2+ T cells are naturally biased toward IFNγ+ (in the blood of healthy individuals). In fact, IL2 or IL15 stimulation of naïve γδ thymocytes results in the differentiation of producers of IFNγ but not IL17 (28). Indeed, IL17 expression by human γδ T cells in vitro appears to require their activation in a highly inflammatory milieu (21). It may, therefore, be possible to manipulate γδ T-cell polarization in situ by targeting cytokines such as IL1β, IL6, IL23, and TGFβ (required for IL17 expression) or IL2, IL12, IL15, and IL18 (which promote IFNγ production). Such a strategy would affect, not only on γδ T cells, but importantly also on CD4+ T helper cell subsets, which can determine the course of the immune response to tumors (2).

From the perspective of adoptive γδ T-cell transfer, it seems logical to differentiate IFNγ+, IL17− effector cells devoid of immune suppressive properties. Along these lines, activation of naïve γδ T cells with pyrophosphate agonists plus IL2 leads to strong IFNγ but no IL17 production (29). Moreover, we have characterized a population of Vδ1+ cells expressing natural cytotoxicity receptors that also produces abundant IFNγ but no IL17, and displays enhanced antileukemia cytotoxicity compared with Vγ9Vδ2 cells (9). Another interesting property of Vδ1+ 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 Vδ2+ cells) in various solid tumor types (10, 11). We are therefore particularly interested in exploring the therapeutic potential of Vδ1+ cells, particularly as no clinical trial has yet focused on this γδ T-cell subset.

Of note, the success of γδ T-cell–based immunotherapy is also likely to depend on avoiding activation-induced cell death and exhaustion of the γδ 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 γδ T-cell subsets (such as IFNγ+ versus IL17−) 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.

References

17. Wakata D, Sumida K, Iwakura Y, Nishikawa H, Ohkuni T, Chamoto K, et al. Tumor-infiltrating IL-17-producing gamma/delta T cells support the

www.aacajournals.org Cancer Res; 75(5) March 1, 2015 801

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.

Acknowledgments

The authors thank Telma Lancã and Natacha Gonçalves-Sousa for helpful discussions; Capucine Grandjean for help in figure design; Dr. S.V. Chipulkar for her personal communication; and Fundaçãao para a Ciência e Tecnologia (M. Rei), The Wellcome Trust (D.J. Pennington) and European Research Council (StG_260352; B. Silva-Santos) for funding.

Received November 3, 2014; revised December 10, 2014; accepted December 10, 2014; published OnlineFirst February 6, 2015.
The Emerging Protumor Role of γδ T Lymphocytes: Implications for Cancer Immunotherapy

Margarida Rei, Daniel J. Pennington and Bruno Silva-Santos

Cancer Res 2015;75:798-802. Published OnlineFirst February 6, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-14-3228

Cited articles
This article cites 33 articles, 16 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/5/798.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/75/5/798.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.