Human γδ T cells: a nonredundant system in the immune-surveillance against cancer

Marina Ferrari, Elisabetta Ferrero, Lorenzo Dagna, Alessandro Poggi and Maria Raffaella Zocchi

Down-regulation of expression of MHC alleles, as well as tumor-specific antigens, is observed frequently during tumor progression, resulting in an impairment of MHC-restricted, αβ-T-cell-mediated, tumor-specific immunity. Given the unique set of antigens recognized and the lack of requirement for classical antigen-presenting molecules, γδ T cells might, therefore, represent a nonredundant system in anticancer surveillance, as proposed for the immune response against pathogens. Evidence that γδ and αβ T cells make distinct contributions to anticancer surveillance has been provided recently in mice. Here, we discuss the potential role played by resident Vδ+ and circulating Vδ2 T cells in the defense against solid tumors and hematological malignancies.

The role of tumor-infiltrating lymphocytes (TILs) in antitumor immunity, as well as their potential for cancer immunotherapy, has been investigated extensively. Besides αβ T cells [1], T lymphocytes bearing the γδ T-cell receptor (TCR) can infiltrate solid cancers, and they exhibit a selective lytic activity against a variety of tumors [2]. γδ T cells are a minor fraction of the T lymphocytes in peripheral blood and are found rarely in lymph nodes and spleen; by contrast, they are abundant in the intestine, skin, tongue, esophagus, trachea, lungs and genital epithelia [3,4]. Two main subsets of γδ T cells have been described: one, expressing the TCR variable regions Vγ9 and Vδ2, represents the majority of peripheral-blood γδ T lymphocytes [3]. γδ T cells of this subset play a role in the defense against intracellular pathogens and hematological malignancies [5–7]; indeed, activated Vγ9-Vδ2 lymphocytes are capable of in vitro killing of myeloma and lymphoma cell lines [6,7]. Vδ2 T cells have been found also with high frequency in disease-free survivors of acute leukemia after bone-marrow transplantation [2]. By contrast, the second subset of Vδ1 T cells is resident mainly within epithelial tissues, where these cells might provide a first line of defense against infections or malignancies [3–6]. We and others have reported that γδ TILs from epithelial tumors belong to the resident population, exert an in vitro antitumor activity and recognize antigens that are over-expressed at the tumor site[8–10].

How do γδ T cells localize to the tumor site?
The attraction of leukocytes to tissues is an essential step for the development of an immune response; the process is controlled by the interactions between chemokines (chemotactic cytokines) and their specific receptors [11]. With regard to γδ TILs, we have shown that they are responsive to chemotactic factors produced by autologous tumor cells in lung cancers [12]. In vitro, the migration of human γδ T cells in response to inflammatory chemokines – in particular, macrophage inflammatory protein 1α (MIP-1α), MIP-1β and regulated upon activation, normal T-cell expressed and secreted (RANTES) – has been reported [13]. Vγ9-Vδ2 T cells express a wide range of CC-chemokine receptors, including CCR1 and CCR5 [14], which are the receptors for the aforementioned cytokines. A remarkable increase in the number of T cells expressing the Vγ9-Vδ2 TCR has been found in the spleen of patients with hairy-cell leukemia [15]. This suggests that circulating γδ T cells might be susceptible, in vivo, to chemotactic or haptotactic gradients and thus, can be targeted to damaged tissues. Little is known, however, about the expression of chemokine receptors by resident γδ T cells. Our preliminary observations indicate that Vδ1-γδ T cells infiltrating lung cancers express CXC-chemokine receptor 3 (CXCR3), the receptor for inflammatory protein 10 (IP-10) [11], and CCR5 (M. Ferrari et al., unpublished). Expression of CXCR3 has been demonstrated recently in intrathymic γδ T cells and this receptor has been shown to mediate γδ T-cell migration specifically in response to IP-10 in vitro [16]. By contrast, we could not detect CCR3 (M. Ferrari et al., unpublished) – the receptor for monocyte chemotactic protein 2 (MCP-2), MCP-3, MCP-4 and eotaxin – which is present on eosinophils, basophils and T helper 2 (Th2) lymphocytes.

'Seeking to extrate, migrate throughout the extracellular matrix and reach the tumor site in response to specific stimuli. Of note, intraepithelial γδ T cells can, themselves, produce a number of chemokines, including RANTES and MIP-1β [17], thereby contributing possibly to the recruitment of circulating lymphocytes, including other γδ T cells.'
To extravasate, activated leukocytes use a number of adhesion molecules, which interact with endothelial counter-receptors and/or components of the subendothelial matrix. We have reported that γδ T cells with a ‘resident’ phenotype, including those infiltrating lung tumors, express the neural-cell adhesion molecule (N-CAM/CD56) and use it to bind to endothelial cells and subendothelial cells [18]. These Vδ1 TILs can use N-CAM for the invasion of heparan-sulfate matrices and reverse transendothelial migration (i.e. in the basal to apical direction) (A. Poggi et al., unpublished). Thus, N-CAM might contribute to the regulation of both extravasation of circulating γδ T cells and recirculation of resident γδ TILs. Interestingly, leukocyte function-associated antigen 1 (LFA-1), a β₂-integrin involved in the adhesion of leukocytes to endothelial cells, which is the first step of extravasation, has a low level of expression on intraepithelial γδ T cells [19] and is expressed variably by γδ TILs (A. Poggi et al., unpublished). Therefore, it is conceivable that N-CAM can substitute for LFA-1 in the transendothelial migration of γδ TILs expressing this integrin weakly, as we have reported for T cells from a patient with leukocyte adhesion deficiency syndrome, a genetic disease resulting from mutations in the β₂-subunit of LFA-1 [20].

Finally, circulating Vδ2 T cells express selectively the natural-killer-cell receptor protein 1A (NKRP1A), a type I membrane glycoprotein containing a C-type lectin domain, which might function as an adhesion molecule; furthermore, these lymphocytes use the receptor to transmigrate across endothelial cells in the absence of chemotactic stimuli [21]. Thus, the NKRP1A molecule might be important in driving the localization of circulating γδ T cells with potential anticancer activity to the tumor site. Because the expression of NKRP1A is regulated by interleukin-12 (IL-12) [21], potential release of IL-12 at the level of the damaged tissue might modulate the recirculation of NKRP1A-γδ T cells.

**Antigen-specific versus -nonspecific recognition by γδ T cells: implications for the antitumor response**

γδ T cells display a unique repertoire of antigen specificity. In particular, human Vδ2 T cells, which are involved also in the immune response against several pathogens [2,4,7], recognize cell-surface antigens that have been exposed to low molecular-weight, phosphate-containing, nonpeptide molecules. These include natural ligands – such as phosphorylated uridine- and thymidine-containing compounds, isopentenyl- or prenylpyrophosphate derivatives and certain bacteria-specific intermediates of the isoprenoid biosynthesis pathway – as well as synthetic phosphate analogs [22–27]. This recognition is TCR-mediated and does not require antigen processing and presentation by conventional MHC molecules [4,28]. Notably, nonpeptide antigens expressed by Vδ2 T cells are shared by microbial and mammalian cells; in particular, phosphorylated thymidine-related products, thought to be involved in a salvage pathway in nucleic-acid synthesis and repair, might be overexpressed by damaged or ‘stressed’ cells [22]. A similar link between the recognition of microbial pathogens and hematopoietic tumors by Vδ2 T cells might be provided by microbial prenyl-pyrophosphate intermediates, which are also present in mammalian cells [23]. Finally, certain synthetic aminobisphosphonates expand populations of Vγ9-Vδ2 T cells exhibiting cytotoxic activity against myeloma cells, both in vitro and in vivo [6].

In mice, γδ T cells have been shown to recognize protein antigens, including MHC class II and nonclassical MHC class I molecules, and a herpes simplex virus (HSV) glycoprotein (reviewed in Ref. [29]). In contrast to αβ T cells, all of these proteins are recognized directly by γδ T cells, with no requirement for antigen processing [29,30], suggesting an Ig-like recognition by these lymphocytes [29]. The specificity of recognition is influenced greatly by post-translational modifications of the protein, such as N-linked glycosylation [31]; interestingly, glycosylation patterns are altered substantially by malignant transformation or infection, further supporting the concept that γδ T cells recognize ‘stress’ signals.

‘...γδ T cells with a “resident” phenotype... express the neural-cell adhesion molecule (N-CAM/CD56) and use it to bind to endothelial cells and subendothelial matrix.’

A similar pattern of protein recognition has been proposed also in humans. Vδ2 T cells recognize, through their TCR, an as yet unknown antigen(s) expressed by HSV-infected cells and possibly, representing a self-molecule modified as a result of infection [32]. Moreover, earlier studies have suggested that Vγ9-Vδ2 T cells recognize proteins belonging to the heat-shock protein family (HSPs), which might be over-expressed by ‘stressed’ or transformed cells [4–6]. Also, we reported an association between the expression of HSP72 by lung tumor cells and the presence of γδ TILs belonging to the Vδ1 subset [9]. Direct recognition of HSPs by either Vδ2 or Vδ1 T cells has never been demonstrated convincingly; however, recent studies provide evidence that Vδ1 T cells recognize the human MHC-class-I-related molecules MICA and MICB [10,33]. These are stress-induced antigens, expressed under the control of the heat-shock-responsive promoters by intestinal epithelia and epithelial tumors, including carcinomas of the lung, kidney and colon. Notably, the same tumors were infiltrated by Vδ1 T cells [10]. Although the killing of MICA- and/or MICB-expressing cancers by Vδ1 TILs was TCR-mediated, a direct interaction between
MICA and/or MICB and the γδ TCR has not been demonstrated. Thus, it is possible that the latter recognizes as yet unknown molecules on tumor cells. The natural-killer-cell receptor NKG2D, identified as a ligand for MICA and MICB [4,34], has been found also on γδ T cells, in addition to natural killer (NK) cells, and has been shown to modulate both their antiviral and antitumor activity [4,35]. Therefore, it is conceivable that the activation of γδ TILs by MICA- and/or MICB-expressing tumors might be achieved through two different pathways. Indeed, enhancement of TCR-mediated effector functions upon MICA–NKG2D interaction has been demonstrated recently for Vδ2 T cells [36]. A nonredundant role for resident skin-associated γδ T cells in the immune response against cutaneous malignancies has been substantiated in experimental models using TCRγδ−/− mice, which were more susceptible to chemically induced or transplanted tumors than wild-type congenics [37]. In these models, γδ T cells were shown to kill tumor cells through the interaction between NKG2D and the murine equivalent of human MICA and MICB molecules (i.e. Rae-1) [37].

Other MHC-class-I-related molecules, such as CD1c, are recognized specifically by Vδ1 T cells and have been proposed to present self-derived lipid antigens [38]. However, we could not find evidence for an involvement of CD1 molecules in the recognition of lung tumor cells by Vδ1 TILs [8]. This is in keeping with the observation that CD1c is expressed mainly by professional antigen-presenting cells (APCs), rather than normal or neoplastic epithelial cells, in which MICA and/or MICB molecules are, conceivably, the surface structures responsible for the engagement of TCR and/or NKG2D on Vδ1 TILs.

Finally, consistent with their preferential distribution in epithelial tissues, Vδ1 T cells can interact with molecules expressed by epithelial cells. We have reported that γδ TILs in infiltrating lung cancers can recognize tumor cells expressing the monomeric laminin receptor (MLR) [9]. This receptor, usually localized to the baso-lateral surface of normal epithelia, is distributed along the whole cell membrane in cancer cells and is involved in tumor invasion and metastasis [9]. In our study, we found that only Vδ1 TILs were able to selectively lyse MLR+ autologous tumor cells; this cytotoxicity could be inhibited by masking the MLR, but not the TCR. These data further confirm the hypothesis that multiple pathways are used by γδ T cells—in particular, those localized in epithelial tissues—to recognize and clear tumor cells.

**γδ T cells as antitumor effectors**

Generally, it is accepted that perforin and interferon γ (IFN-γ) are key effector molecules in the immune response against cancer (reviewed in Ref. [39]). Perforin-mediated cytotoxicity has been reported for Vδ2 and Vδ1 T cells [3,38]. Moreover, the two subsets up-regulate their expression of Fas ligand (FasL) following TCR engagement [38,40], and therefore, are able to kill Fas-sensitive tumor cells. However, Fas–FasL interaction is implicated not only in killing tumor targets but also, in the activation-induced cell death (AICD) of effector cells. Long-term activated, Fas-sensitive Vγ9–Vδ2 cells undergo Fas-mediated apoptosis upon TCR-mediated recognition of Daudi lymphoma cells; this phenomenon can be prevented partially by the use of specific caspase inhibitors [41]. It is conceivable that, upon chronic recognition of persistent antigens, γδ TILs are deleted as well, resulting in an additional mechanism of immune escape for the tumor.

Both Vδ1 and Vδ2 T cells secrete cytokines, in particular IFN-γ [2,4,38]. In agreement with a proposed role for Vδ1 TILs in the immune response against solid cancers, Vδ1 T-cell clones from ovarian tumors released IFN-γ upon challenge with MICA+ tumor cells [10]. Also, we found evidence for the production of IFN-γ by Vδ1 TILs from lung adenocarcinoma (M. Ferrarini et al., unpublished).
Given that both the TCR and NKG2D can be triggered by MICA- and/or MICB-expressing tumors, it is tempting to speculate that the predominant engagement of either pathway is able to shape the effector function of γδ TILs in terms of cytotoxic activity or cytokine release. In addition, the engagement of NKG2D might provide a costimulatory signal to Vδ1 TILs, enhancing their antigen-dependent effector function, in a similar manner to that described for Vδ2 T cells [36].

Finally, γδ T cells might orchestrate the development of an immune response through the secretion of soluble factors [42]. Indeed, both circulating and resident γδ T cells secrete chemokines, including MIP-1α, MIP-1β, RANTES and IL-8 [14, 17, 43], well-known chemoattracants for activated lymphocytes, professional APCs and neutrophils [11]. Furthermore, the production of chemokines by endothelial cells and/or stromal cells might be induced by cytokines released by activated γδ T cells, thus providing an amplification loop in the recruitment of leukocytes during inflammation [44]. The evaluation of chemokine secretion by human γδ TILs in epithelial malignancies will help to clarify whether these cells can contribute also to tumor-associated inflammation and the recruitment of effector leukocytes, through the release of soluble factors.

Concluding remarks

Recent advances in the characterization of the functional capabilities of resident Vδ1 and circulating Vδ2 T cells indicate that they display unique features in terms of antigen specificities, requirements for antigen recognition and tissue distribution (Fig. 1), which make them suitable candidates as anti-tumor effectors. In particular, given their lack of antigen-processing requirements and ability to recognize nonconventional MHC-like molecules, γδ T cells might complement the MHC-restricted tumor-specific immune response mediated by γδ T cells. Moreover, the expression of NK-like receptors, such as NKG2D and killer inhibitory receptors [6, 36], provides γδ T cells with additional effector mechanisms in the defense against tumors that bear ‘stress’-induced molecules or have down-regulated expression of MHC class I molecules, a frequent occurrence during cancer progression.

‘...all of these proteins are recognized directly by γδ T cells, with no requirement for antigen processing’

These characteristics can be exploited to potentiate anti-tumor immunity. First, fusion proteins and/or humanized monoclonal antibodies might be designed to trigger γδ T cells through a TCR-independent pathway, such as that mediated by NKG2D. Second, manipulation of the cytokine and/or chemokine milieu might be envisaged, to enhance the antineoplastic function(s) of γδ TILs and promote the recruitment of circulating γδ T cells to the tumor site. Finally, phosphate antigens might be considered as potential vaccines to activate the responding Vδ2 γδ T-cell population in hematological malignancies.

References

15. van deCorput, L. et al. (1997) TCR γδ cells expressing V(γδ)2, which normally predominate in the blood, are found in the spleens of patients with hairy-cell leukemia. Leukemia 1, 106–109
Can nerve damage disrupt neuroendocrine immune homeostasis? Leprosy as a case in point

Graham A.W. Rook, Stafford L. Lightman and Cobi J. Heijnen

The crucial clinical problem in leprosy is the occurrence of acute inflammatory episodes that lead to nerve damage, even after the infecting organisms have been killed by antibiotics. We suggest that the instability of these inflammatory sites is attributable to a disturbance of the role that nerves play in the regulation of inflammation. The destruction of sensory C fibers and sympathetic innervation will remove anti-inflammatory feedback circuits. Moreover, diminishing levels of neuropeptides and changes in the cytokine profile will affect the cortisol-sensitivity of infiltrating T cells, and modulate the cortisol–cortisone shuttle so that the inflammatory site becomes resistant to physiological levels of anti-inflammatory adrenocortical steroids.

Although the incidence of new cases of leprosy is not falling, treatment of the underlying infection with antibiotics is efficient and therefore, the prevalence of the infection has been reduced sharply.

Unfortunately, the killing of Mycobacterium leprae is not a complete solution to the clinical problem, because a specific feature of leprosy is that the bacilli invade Schwann cells and axons. The presence of mycobacterial antigens exposes the nerves to damage during the acute inflammatory episodes that occur in or around the nerves during, or even long after, completion of the antibiotic treatment [1]. Type-1 reactions [also known as reversal reactions (RR)] might be due to an increase in cell-mediated responses to M. leprae, characterized by the activity of T helper 1 (Th1) lymphocytes expressing interleukin-2 (IL-2) and interferon-γ (IFN-γ) [2]. By contrast, type-2 reactions [also known as erythema nodosum leprosum (ENL)] are characterized by the infiltration of neutrophils, with increased expression of IL-6, IL-8 and IL-10, and sustained production of the Th2 cytokines IL-4 and IL-5 [3]. Both types of reaction are accompanied by increased release of the proinflammatory cytokines tumor necrosis factor α (TNF-α) and IL-1 [4,5]. The nerve damage caused by these episodes is the major clinical problem in leprosy.

‘…in the absence of raised circulating cytokine levels, the presence of inflammation is signaled to the CNS by the peptidergic sensory C fibers…’

Why, then, is the inflammation in leprosy lesions so unstable? Most studies have focused on the immunological and cytokine networks operating in the leprosy lesion, but regard the nerve as an inert bystander that happens to get damaged by the inflammation. We consider this view to be too narrow.