ROLE OF GAMMADELTA T LYMPHOCYTES IN TUMOR DEFENSE

Maria Raffaella Zocchi [1] and Alessandro Poggi [2]

[1] Laboratory of Tumor Immunology and Department of Internal Medicine, H San Raffaele Scientific Institute, Via Olgettina 60, I-20132 Milano, Italy, [2] Laboratory of Immunology, National Institute for Cancer Research, L.go Rosanna Benzi 10, I-16132 Genova, Italy

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1. ABSTRACT

The effectors of mucosal and natural immunity (i.e. natural killer, NK, cells and NKT lymphocytes) are known to play an important role in host defence against tumors. Gammadelta T lymphocytes are the most represented cell populations in mucosal associated lymphoid tissue and share several characteristics of T and NK cells. Two main subsets of gammadelta T cells are known: one, expressing the Vdelta2 T cell receptor (TCR), is found in the peripheral blood, while T cells expressing Vdelta1 TCR are resident in epithelial tissues. The former subset is capable of killing myeloma and Burkitt lymphoma cells, while the latter has been implied in the defence against epithelial cancers. Furthermore, there is increasing evidence that alphabeta and gammadelta T lymphocytes make distinct contributions to anticancer surveillance. Indeed, unlike alphabeta T cells, gammadelta T lymphocytes are involved in the recognition of antigens that do not undergo the conventional major histocompatibility complex (MHC)-driven antigen presentation.

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, alphabeta-T-cell-mediated tumor-specific immunity. Given the unique set of antigens recognized and the lack of requirement for classical antigen-presenting molecules, gammadelta T lymphocytes might, therefore, represent a useful and potent system in anti-cancer surveillance, as proposed for the immune response against pathogens. Evidence that gammadelta and alphabeta cells make distinct contribution to anti-cancer surveillance have been recently provided in mice. Here, we discuss the potential role played by resident Vdelta1^{+} and circulating Vdelta2^{+} T lymphocytes in the defense against solid tumors and hematological malignancies.

2. INTRODUCTION

The majority of peripheral blood T lymphocytes, in humans, bear the alphabeta type of the T cell receptor (TCR), whereas gammadelta T cells represent less than 5% of circulating lymphocytes and are rarely found in the lymph nodes and spleen (1, 2); in turn, immunohistological studies have shown that the majority of gammadelta T cells are localized in the mucosal associated lymphoid tissue (MALT), and are abundant in the tongue, esophagus, trachea, lungs, genital epithelia and skin (3, 4). The alphabeta and gammadelta T cell populations contribute differently to the immune host defense, possibly due to their different tissue distribution, gammadelta T lymphocytes representing an important immune barrier against pathogens which attempt to enter the body through the mucosal surfaces, such as the respiratory and gastroenteric

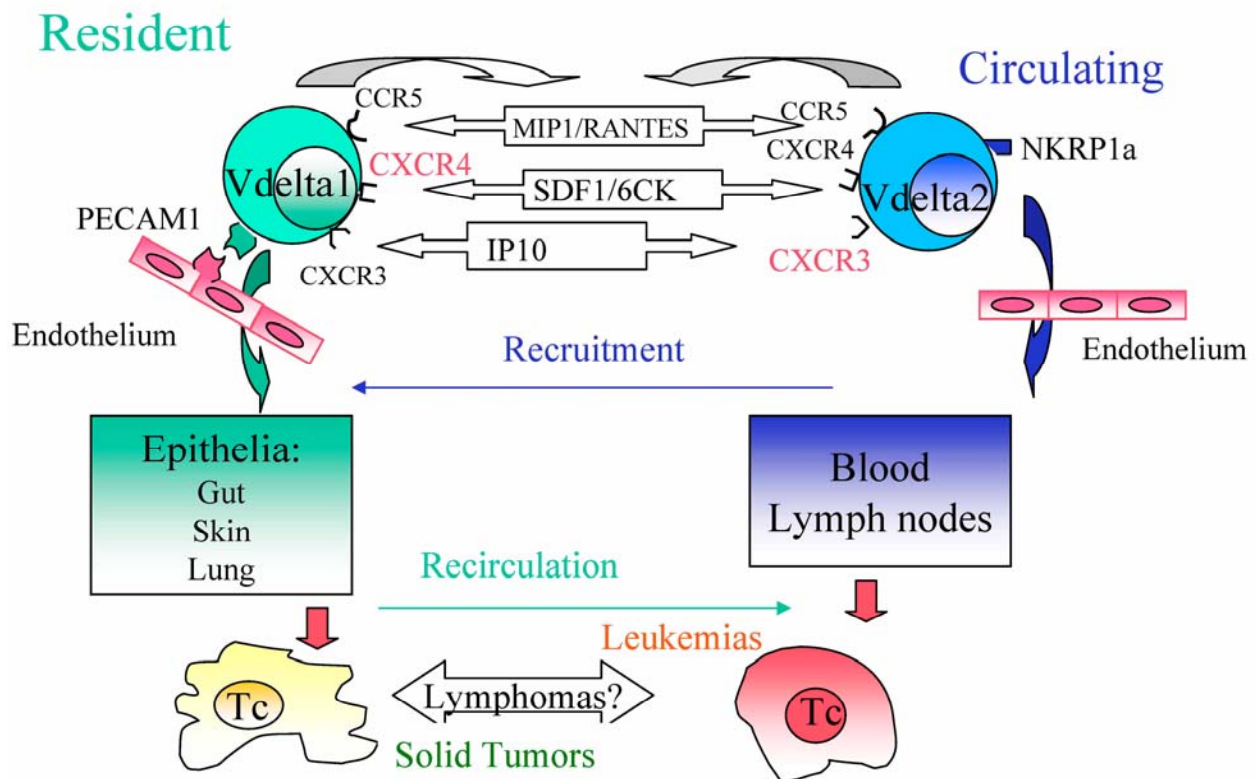# Resident and circulating gammadelta T cell subsets

Figure 1. Subsets of resident and circulating gammadelta T lymphocytes. Vdelta1 T lymphocytes (green) are mainly localized in the epithelia, while Vdelta2 T cells (blue) are found in peripheral blood and lymph nodes, the former population being involved in the response against solid tumors (yellow, Tc), the latter against hematological malignancies (red). Both populations might contribute to the immune response against lymphomas, that share some characteristics of solid cancers and leukemias; each gammadelta subset is possibly recruited to the tumor site, or recirculate, in response to selected chemokines, which can be produced by gammadelta T cells themselves. Vdelta1 or Vdelta2 subsets are more responsive to constitutive (such as SDF1 or 6Ckine) or inflammatory (IP-10) chemokines respectively, according to the intensity of expression of their specific receptors (CXCR4 vs CXCR3). Moreover, the Vdelta1 population is PECAM1^{+} and use this molecule to undergo haptotactic migration, whereas Vdelta2 T cells express NKRP1a and use it to transmigrate across endothelium.

tract (5, 6). The immunologic function of gammadelta T cells in humans is gradually unraveling: there is increasing consensus on the hypothesis that this subtype of T lymphocytes serves as first line of defense in the recognition of microbial antigens that do not undergo the conventional major histocompatibility complex (MHC)-driven antigen presentation (5, 6). A large body of evidence on the role played by these T lymphocytes comes from the observation that they are expanded in peripheral blood in malaria, in the intestine and in the lung during tuberculosis infection, and in the skin granulomatous lesions in leprosy and leishmaniasis, thus playing a role against intracellular pathogens (4-8).

The reported preferential expansion of gammadelta T cells in certain malignancies supports the notion that they are also important in the immunological surveillance against cancer. Indeed, besides alphabeta T cells (9), which have been extensively studied in human

neoplasias, T lymphocytes bearing the gammadelta TCR can infiltrate solid cancers, especially those localized in the gut, lung and skin, and exhibit a selective lytic activity against a variety of tumors (10-13). In addition, gammadelta T lymphocytes are able to produce and release a number of cytokines, including tumor necrosis factor (TNF)-alpha, and interferon (IFN)-gamma, known to contribute to anti-cancer immunity. Two main subsets of gammadelta T cells have been described: one, expressing the TCR variable regions Vgamma9 and Vdelta2, represents the majority of peripheral blood gammadelta lymphocytes (figure 1) (1-3). Gammadelta T cells of this subset are mainly responsible for the response against intracellular pathogens and are also involved in the defense against some hematological malignancies (3, 5, 10, 14); indeed, activated Vgamma9/Vdelta2 lymphocytes are capable of *in vitro* killing of myeloma and lymphoma cell lines (10, 14, 15), and there is increasing evidence that they are also involved in the response against some human
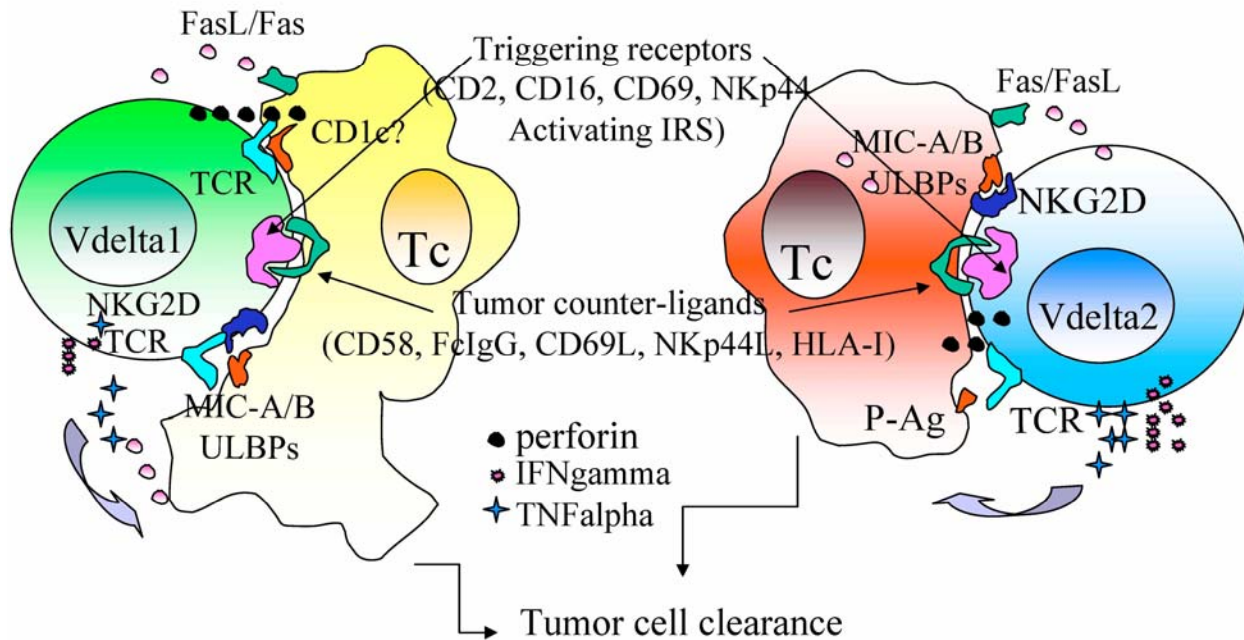
Figure 2. Role of gammadelta T lymphocyte subsets in anti-cancer surveillance. The resident Vdelta1 T cell population (green) interacts with MIC-A/B or ULBPs expressed by solid tumor cells (yellow, Tc) through the NKG2D receptor and/or TCR or recognize CD1-restricted molecules, whereas the circulating Vdelta2 subset (blue) displays TCR specificity to phosphate antigens (P-Ag) mainly expressed by haematological malignancies (red). Upon this interaction gammadelta T cells receive an activating signal leading to tumor cell lysis and/or to secretion of IFN-gamma and/or TNF-alpha. In the figure, activating receptors other than NKG2D and TCR, contributing to the trigger of anti-tumor activity, and their counter ligands possibly expressed on Tc are depicted.

leukemias. Vdelta2 T cells have been found with high frequency in disease-free survivors of acute leukemia after bone-marrow transplantation (16), possibly due to the potential role played by these cells in graft-versus-leukemia effect. By contrast, Vdelta1 T cells are resident mainly within epithelial tissues, where they might provide a first line of defence against infections or malignancies of the gastrointestinal and respiratory tract (4-8, 17) (figure 1).

Along this line, we and others have reported that gammadelta tumor infiltrating lymphocytes (TILs) from epithelial tumors belong to the resident population, exert an *in vitro* anti-tumor killing and recognize antigens over-expressed at the tumor site (13, 17-19). In particular, a selective anti-neoplastic activity has been reported for gammadelta TIL infiltrating colo-rectal cancers, lung adenocarcinomas, esophageal tumors and, recently, also pancreatic cancers (20-23). Interestingly, mice lacking gammadelta T cells are highly susceptible to multiple regimens of cutaneous carcinogenesis; in these mice, skin-associated gammadelta T lymphocytes killed skin carcinoma cells with high efficiency and specificity, supporting a role for these lymphocytes also in spontaneous or chemically-induced cutaneous tumors (13, 14). In keeping with this, regional lymph nodes of patients with squamous cell carcinomas of the head and neck have been reported to be infiltrated by gammadelta T lymphocytes

(25). Finally, human gammadelta T cells exert natural, beside interleukin (IL)-2-induced, cytotoxicity to neuroblastoma tumor cells and can be activated *in vitro* by IL-12 against autologous tumor cells in patients with glioblastoma (26, 27).

3. ANTIGEN RECOGNIZED BY GAMMADELTA T LYMPHOCYTES ON TUMOR CELLS

Gammadelta T lymphocytes display a unique repertoire of antigen specificity, which is not confined to proteins. In particular, human Vdelta2 T lymphocytes recognize cells that have been exposed to low molecular weight phosphate-containing nonpeptide molecules (28-30). These include natural ligands, such as phosphorylated uridine and thymidine containing compounds, isopentenyl or prenyl pyrophosphate derivatives and certain bacteria-specific intermediates of the isoprenoid biosynthesis pathway, as well as synthetic phosphate analogs (31-35). This recognition is TCR-mediated and does not require antigen processing and presentation by conventional MHC molecules (3, 29, 35-37) (figure 2). Notably, nonpeptidic antigens recognized by Vdelta2 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 (31). A similar link in the recognition of microbial

pathogens and hematopoietic tumors by Vdelta2 T lymphocytes, might be provided by prenyl pyrophosphate intermediates, which are also present in mammalian cells (32, 35). Along this line, certain aminobiphosphonates expand populations of Vgamma9/Vdelta2 T lymphocytes exhibiting cytotoxic activity against myeloma cells, both *in vitro* and *in vivo* (14).

In mice, gammadelta T lymphocytes have been shown to recognize protein antigens, including MHC class II and non classical MHC class I molecules and a herpes simplex virus (HSV) glycoprotein (38). At variance with alphabeta T cells, all these proteins are recognized directly with no requirement for antigen processing (38, 39), suggesting an Ig-like recognition by gammadelta T lymphocytes (38). The specificity of recognition is influenced deeply by post-translational modifications, such as N-linked glycosylation, of the protein (40); interestingly, glycosylation patterns are altered substantially by malignant trasformation or infection, further supporting the concept that gammadelta T cells recognize "stress signals". A similar pattern of protein recognition has also been proposed in humans: Vdelta2 lymphocytes 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 (41). Moreover, earlier studies suggested that Vgamma9Vdelta2 lymphocytes recognize proteins belonging to the heat shock protein family (HSPs), which might be over-expressed by "stressed" or transformed cells (3, 5).

3.1. Antigens expressed by solid tumors

We have reported an association between the expression of HSP72 by lung tumor cells and the presence of gammadelta TILs belonging to the Vdelta1 subset (18, 42). A direct recognition of these molecules by either Vdelta2 or Vdelta1 T lymphocytes has never been demonstrated convincingly; however, evidence for a recognition by gammadelta T cells of HSPs or other "stress-inducible" molecules has been reported in epithelial tumors of the gastrointestinal tract, including coloncarcinomas and esophageal or oral cancers (19, 43-46).

Recent studies provide evidence that Vdelta1 T lymphocytes recognize the human MHC-class-I-related molecules MIC-A and MIC-B (19, 44) (figure 2). These are stress-induced antigens, expressed under the control of the heat-shock-responsive promoters by intestinal epithelia and by epithelial tumors, including carcinomas of the lung, kidney and colon (19, 43-45). Stress-induced MHC class-I related molecules have been also described in human melanomas and hepatocellular carcinomas (47, 48). Notably, the same tumors were infiltrated by Vdelta1 T lymphocytes (19) and further evidence has proven that gammadelta T cells can indeed be stimulated by MIC-A-expressing cells (44, 49-51). Although the killing of MIC-A/B-expressing cancers by Vdelta1 TILs was TCR-mediated, a direct interaction between MIC-A/B and gammadelta TCR has not been demonstrated, leaving the possibility that the latter recognizes as yet unknown molecules on tumor cells. The natural killer cell receptor

NKG2D, identified as the ligand for MIC-A/B (52, 53), has also been found, in addition to NK cells, on gammadelta T cells (see below) and has been shown to modulate both their anti-viral and anti-tumor activity (49, 53-55). Therefore, it is conceivable that activation of Vdelta1 TILs by MIC-A/B expressing tumors might be achieved through two different pathways. Indeed, enhancement of TCR-mediated effector functions upon MIC-A-NKG2D interaction has been demonstrated recently for Vdelta2 T lymphocytes (49) (figure 2). Recently, a nonredundant role for resident skin-associated gammadelta T cells in the immune response against cutaneous malignancies has been substantiated in experimental models using TCRdelta^{-/-} mice, which were more susceptible to chemically induced or transplanted tumors than wild type congenics (13). In these models, gammadelta T cells were shown to kill tumor cells via the interaction between NKG2D and the murine equivalent of human MIC-A/MIC-B molecule, i.e.RAE-1 (13, 53).

Other MHC class I-related molecules, such as CD1c, are recognized specifically by Vdelta1 T lymphocytes and have been proposed to present self-derived lipid antigens (figure 2) (56). However, there is no direct evidence, so far, for a role played by CD1-molecules in antigen presentation; likewise, apparently there is no requirement for MHC-like restriction in antigen recognition by gammadelta T cells. Accordingly, we could not find evidence for an involvement of CD1 or MHC molecules in the recognition of lung tumor cell by Vdelta1 TILs (20). This is in keeping with the observation that CD1c is expressed mainly by professional antigen presenting cells, rather than normal or neoplastic epithelial cells, where MIC-A/B molecules are conceivably the surface structures responsible for TCR and/or NKG2D engagement on Vdelta1 TILs.

Consistent with their preferential distribution in epithelial tissues, Vdelta1 T cells can interact with molecules expressed or produced by epithelial cells. We reported that gammadelta TILs infiltrating lung cancers can recognize tumor cells expressing the monomeric laminin receptor (MLR) (18). This receptor, usually localized at the baso-lateral side of normal epithelia, is distributed in cancer cells along the whole cell membrane and is involved in tumor invasion and metastasis (18). In our study, we found that only Vdelta1 TILs were capable of selective lysis of MLR[+] autologous tumor cells; this cytotoxicity could be inhibited by masking the MLR but not the TCR (18). These data further confirm that multiple pathways are used by gammadelta T lymphocytes, in particular those localized in epithelial tissues, to recognize and clear tumor cells.

3.2. Antigens expressed by hematological tumors

Similar to the MLR, the oncofetal-antigen-immature laminin-receptor protein (OFA-iLRP) has been recently described as a potential tumor associated antigen capable of inducing specific cytotoxic T lymphocytes (57). OFA-iLRP is a highly conserved protein, preferentially expressed in fetal tissues and in many types of cancers, including hematopoietic malignancies,

but not in differentiated adult cells. Several lymphoma and leukemia cell lines, as well as cells from patients with acute myeloid leukemia and chronic lymphatic leukemia, express this onco-fetal protein and can stimulate *in vitro* OFA-iLRP-specific cytotoxic T cells (57).

Nevertheless, the major non-peptide family of antigens recognized by gammadelta T cells on hematological tumors, seem to be represented by non-peptide phosphate-containing molecules. Recognition of these antigens has been originally described for Vgamma9/Vdelta2 T cells activated by Mycobacterium tuberculosis (58); both the phosphate and the amine antigens identified as targets for gammadelta T cells are small molecules consisting of short straight or branched aliphatic chains and either a phospate or an amine moiety. The mechanism by which these molecules are presented is not known, but it does not involve MHC class-I or class-II peptide antigen-presentig molecules and it has been proposed that they may be recognized as haptens by TCR in a Ig-like fashion (55). Indeed their recognition is critically dependent on the CDR3 sequence of the gammadelta TCR (59).

Notably, the recognition of these small aliphatic moieties has been referred, so far, exclusively to the gammadelta T cells of the Vdelta2 subset, and it is claimed that this is the molecular basis of the cytotoxic effect exerted by Vdelta2 T cells against Daudi lymphoma tumor cells (60). A large body of evidence is emerging from the literature pointing to the ability of different phosphate-containing molecules and their derivatives to stimulate proliferation, cytolytic activity and cytokine production by Vdelta2 T lymphocytes (61-65), ultimately leading to an anti-tumor effect directed against different types of hematopoietic tumors, including non-Hodgkin lymphomas (66-68). More importantly, induction of gammadelta T cell functions has been observed *in vivo*, after administration of biphosphonates in patients affected by different cancers, including hematological neoplasias (14, 15, 68). In these patients, stimulation of gammadelta T cells with alendronate, pamidronate and risendronate induced the activation and IL-2-dependent proliferation of Vgamma9/Vdelta2 T cells with anti-plasmacell activity *in vitro* and an *in vivo* cytoreductive effect was also observed in some patients (14).

Recently, it has become evident that aminobiphospshonates do not represent Vgamma9/Vdelta2 ligands themselves, but rather induce accumulation of mevalonate metabolites which, in turn, are recognized by TCR gammadelta. Indeed, treatment of Daudi cells and other tumor cell lines with inhibitors of the mevalonate pathway, after exposure to aminobiphosphonates, impaired the activation of gammadelta T cells, while reconstitution of this pathway rescued the gammadelta T cell response (65). As the mevalonate pathway is essential for cell survival and growth, it is conceivable that tumor cells overproduce phosphorylated metabolites which may allow the immune system to identify cells with significant metabolic abnormalities; accumulation of these metabolites

in excess might represent the alarm signal to activate Vgamma9/Vdelta2 lymphocytes.

Other possible targets for gammadelta T cells in hematological neoplasias is represented by the recently described UL16-binding proteins (ULBPs), glucosylphosphatidyl inositol (GPI)-linked non-conventional-MHC proteins, belonging to the "stress-inducible" protein family and distantly related to MIC-A and MIC-B (50, 53, 69) (figure 2). The human cytomegalovirus glycoprotein UL16 binds to members of this ULBPs family, which shares with MIC-A and MIC-B the property of interacting with the NKG2D receptor (69-71). The four identified members of ULBPs are expressed on numerous tumor cell lines in humans (72) and in the mouse, a family of proteins structurally related to ULBPs, the RAE molecules not only functions as ligands for NKG2D, but are induced on tumor cells by carcinogens and stimulate anti-tumor activity of gammadelta T lymphocytes (13, 53, 70, 71). Furthermore, RAE-1-transduced cell lines can be eliminated *in vivo* by NKG2D-expressing NK and T cells (73).

Different from MIC-A and MIC-B, which are preferentially distributed on epithelial tumors, ULBPs are expressed on various cancers of hematopoietic origin, including acute myeloid and lymphoblastic leukemias (74), thus possibly representing a good antigen for gammadelta T lymphocytes, in particular those belonging to the Vdelta1 cell subset. This would further support the role of gammadelta T cells in host defence against hematological malignancies (16, 75).

4. GAMMADELTA T LYMPHOCYTES AS ANTI-TUMOR EFFECTORS

Recent advances in our understanding of the anti-tumor effect exerted by the immune system include the characterization of the biochemical events occurring both upon direct contact of cytotoxic lymphocytes and tumor cells and as a consequence of such interaction in terms of soluble factors secretion (figure 2). The former mechanism is based on the delivery of lytic enzymes directly through the membrane of tumor target cells; the latter includes the release of proteins, such as Fas ligand (FasL) or cytokines able to induce tumor cell apoptosis, as an early or late event following tumor cell encountering. Granule exocytosis is the main pathway for tumor cell elimination by cytotoxic T lymphocytes and NK cells (76-78). After target cell recognition, the release of cytotoxic granule content into the immunological synapse formed between the killer cell and its target induces tumor cell lysis; in turn, Fas/FasL interaction leads to tumor cell apoptosis, that is the programmed cell death which avoids the release of pro-inflammatyory factors into the extracellular milieu (76-78). Among the anti-tumor cytokines, TNF-alpha and the proteins related to this superfamily, by interacting with their counterreceptors induce apoptosis via a mechanism superimposable to that mediated by FasL/Fas interaction (79, 80). Another cytokine with outstanding anti-tumor effects is IFN-gamma, produced and secreted by T lymphocytes, including gammadelta T cells, and NK cells

upon contact with infected or transformed cells (81). It is now generally accepted that perforins contained in cytolytic granules on one side and secreted interferon-gamma on the other side are key effector molecules in the immune response against cancer (24).

4.1. Mechanisms of tumor cell death

Apoptosis is generally initiated by two principal pathways: one emerging from mitochondria, the other activated via the ligation of the so-called death receptors (78). The prototypical death receptor is Fas, which upon engagement by FasL triggers the caspase cascade through its cytoplasmic domain and ultimately leads to DNA fragmentation, nuclear blebbing and cell shrinking (78, 80). In turn, the granules of cytotoxic lymphocytes contain two membrane-perturbing proteins: perforin and granulysin, and a family of serine-proteases known as granzymes, all able to activate a cascade of events leading to caspase-independent cell death (76, 77). Perforin-mediated cytotoxicity of tumor targets has been reported for Vdelta2 and Vdelta1 lymphocytes (82, 83); moreover, intraepithelial gammadelta T cells are equipped with granzyme B and granzyme M, whose expression seems to be confined to this T lymphocyte population, beside NK cells, suggesting that this enzyme is peculiar of innate immune responses (83, 84).

On the other hand, the two gammadelta T cell subsets up-regulate the expression of FasL following TCR engagement (85), and have, therefore, the potential to kill Fas-positive tumor cells (82) (figure 2). In addition, it has been determined by immunomorphometry that a large proportion of intraepithelial lymphocytes, including gammadelta T cells, contain FasL and use it to kill Fas-expressing tumor cell lines in a TCR-independent mechanism (82, 86). However, Fas-FasL interaction is implicated not only in killing tumor targets, but also in the activation-induced cell death (AICD) of effector cells (82, 83), that might represent a down-regulation of local immune response exploited by cancers to escape the immune system surveillance. Long-term activated, Fas-sensitive Vgamma9/Vdelta2 cells undergo Fas-mediated apoptosis upon TCR-mediated recognition of Daudi lymphoma cells; interestingly, this phenomenon can be prevented partially by the use of specific caspase inhibitors, thus rescuing the gammadelta-mediated anti-cancer immune response (87). Nevertheless, it is conceivable that, upon chronic recognition of persistent antigens, gammadelta TILs are deleted as well, resulting in an additional mechanism of tumor immune escape.

4.2. Production of anti-tumor cytokines

Both Vdelta1 and Vdelta2 lymphocytes secrete cytokines, in particular IFN-gamma, to eliminate tumors (81) (figure 2). In agreement with a proposed role for Vdelta1 TILs in the immune response against solid cancers, Vdelta1 cell clones from ovarian tumors released IFN-gamma upon challenge with MIC-A$^+$ tumor cells (17, 19). We also found evidence for IFN-gamma production by Vdelta1 TILs from lung adenocarcinoma (M. Ferrarini *et al.*, unpublished). In pancreatic tumors, intracellular

staining revealed IFN-gamma positive gammadelta T lymphocytes with anti-tumor cytotoxic activity (23). Of note, the amount of IFN-gamma produced by Vdelta2 T cell subset is increased when anti-tumor activity is elicited upon stimulation with non-peptide antigens (88); this is also the case of anti-myeloma specific gammadelta T lymphocytes that produce IFN-gamma when triggered with aminobiphosphonates (14). Thus, both in the case of Vdelta1 and Vdelta2 T cells, an antigen-specific TCR mediated stimulation seems to be needed; given that both TCR and NKG2D can be triggered by MIC-A/B expressing tumors, it is tempting to speculate that the predominant engagement of either pathway is able to shape the effector function of gammadelta anti-tumor T cells in terms of cytotoxic activity or cytokine release. In addition, the engagement of NKG2D may provide a co-stimulatory signal to Vdelta1 TILs enhancing their antigen-dependent effector function, similarly to that described for Vdelta2 T lymphocytes (49). Among the other cytokines with potential anti-tumor activity, production of TNF-alpha by human Vgamma9/Vdelta2 T cells has been demonstrated in response to bacterial products and non-peptidic antigens (89, 90), thus providing innate immunity with another weapon against cancer cells (figure 2).

Finally, gammadelta T cells might orchestrate the development of an immune response through the secretion of chemokines (91) (figure 1). Indeed, both circulating and resident gammadelta lymphocytes secrete chemokines, including MIP-1alpha and -beta, RANTES and IL-8, well-known chemoattractants for activated lymphocytes, professional antigen presenting cells and neutrophils (92, 93). Further, chemokine production by endothelial cells and/or stromal cells might be induced by cytokines released by activated gammadelta cells, thus providing an amplification loop in leukocyte recruitment during inflammation (93). The evaluation of chemokine secretion by human gammadelta TILs in epithelial malignancies would help to clarify whether these cells can also contribute, through the release of soluble factors, to tumor-associated inflammation and recruitment of effector leukocytes.

5. ACTIVATION AND INHIBITION OF GAMMADELTA T CELL FUNCTION

To accomplish their anti-tumor function, both in terms of cytotoxicity and cytokine production, effector cells, including gammadelta T lymphocytes, must receive an activating signal that triggers the intracellular biochemical pathways needed for eliminating tumor cells. Recently, it has become evident that both activating and inhibiting molecules are present at the surface of the same effector lymphocyte; this co-expression is conceivably aimed to finely tune the immune response and avoid the uncontrolled release of potentially dangerous soluble factors. A number of inhibiting receptors, belonging to the "inhibitory-receptor superfamily" (IRS), has been described in natural killer (NK) and gammadelta T cells (12). These molecules are counteracted by activating receptors that contribute, together with NKG2D and TCR, to the

complete activation of the effectors of natural immunity directed to the control of tumor cell growth.

5.1. Activating receptors

Triggering of cytolysis and cytokine production can be achieved in gammadelta T lymphocytes via receptors whose engagement by the corresponding ligand expressed by tumor cells delivers an activating signal. Besides CD3/TCR complex and CD2 receptor, which are functional also in alphabeta T lymphocytes, gammadelta T cells acquire the CD69 antigen upon IL2-activation (94). Engagement of CD69 with specific monoclonal antibodies, triggers cytolysis in gammadelta T cells like in NK cells. In addition, our recent findings (Poggi *et al.*, unpublished) indicate that the large majority of both Vdelta1 and Vdelta2 T cell clones, displaying a strong cytolytic activity against cultured leukemic or solid tumor cell lines, express the previously reported NK specific cell markers NKp44 (95). Furthermore, the engagement of NKp44 triggers gammadelta T cells to kill Fcgamma receptor positive target cells, suggesting that this receptor is functional in this T cell subset, as reported in NK cells (90).

In addition, it has been shown that gammadelta T cells can bear, at the cell surface, the FCgammaRIIIa (CD16) whose engagement leads to TNF-alfa production (90) (figure 2). This effect is evident in Vdelta2 T cell subset where CD16 is upregulated upon stimulation of the TCR with phosphoantigens. Altogether, these findings suggest that gammadelta T cells represent a unique T cell population resembling phenotypic and functional activities characteristic of both NK cells and T lymphocytes. Indeed, gammadelta and NK cells bear the NKG2D receptor, which, according to several reports, represent a co-receptor of both TCR and CD16 (95). It is tempting to speculate that gammadelta T cells exert a powerful anti-tumor function using activating mechanisms of both innate and adaptive immune response.

5.2. Inhibiting receptors

Gammadelta T cells resemble NK cells not only for the common expression of several activating receptors but also for the expression of a series of inhibiting receptors, represented by members of the IRS family, that interact with discrete HLA-I allele (12, 96). Indeed, IRS can be subdivided into two structural types of molecules: i) one consists of Immunoglobulin (Ig)-superfamily receptors (ISIR) and ii) the C-type lectin inhibitory receptors (CLIR). The nature of the inhibitory signal delivered by inhibitory receptors has been elucidated mainly in NK cells. In fact, the engagement of IRS members counteracts the kinase activity triggered by activating receptors by recruiting tyrosine phosphatase (usually the SHP-1) leading to the block of early signals in the activation cascade (96).

Gammadelta T lymphocytes can also express ISIR (96), including Killer Ig-Like receptors (KIR) as well as Leukocyte-Associated Ig-like Receptor (LAIR-1) (97) and/or CLIR, like NKG2/CD94 complex (98). Indeed, it has been shown that the majority of CD8 positive gammadelta T cells, in particular the Vgamma9/Vdelta2 subset, in peripheral blood and in the gut express CLIR and

KIR or both. The ligation of these receptors together with Vdelta2 TCR on gammadelta T cells leads to a detectable inhibition of the activating signal delivered via Vdelta2 TCR alone. Similarly, co-engagement of Vdelta2 TCR and CLIR strongly decreased cytolytic activity and cytokine production mediated through Vdelta2 TCR (98). Interestingly, KIR expressed by Vgamma9/Vdelta2 T lymphocytes have been reported to down-regulate TCR-mediated signal in response to HLA-I expressing B cell lymphomas (99). Likewise, ligation of KIR3DL1 by HLA-Bw4 allotypes resulted in inhibition of cytotoxicity against HLA-B*4403 transfected melanomas, as well as against melanomas endogenously expressing HLABw4 allotypes; similarly, interaction of KIR2DL2 or KIR2DL3 with HLA-Cw3-related allotypes on melanomas resulted in decreased tumor cell lysis (100). Recently, Vgamma9/Vdelta1 T lymphocytes expressing the KIR p58.2 (CD158b) specific for group2 HLA-C, isolated from the peripheral blood of an acute myeloid leukemia (AML) patient, have been shown to lyse autologous leukemic cells, which express very low levels of HLA, but not HLA-matched non malignant hematopoietic cells. Transfection of HLA-Cw*0304 into the HLA negative 721.221 cell line inhibited lysis, conceivably through the engagement of KIR p58.2 (101). Thus, anti-tumor cytolytic activity of Vdelta2 T lymphocytes is apparently KIR-regulated.

Data regarding the role of IRS members in Vdelta1 gammadelta T cells are less frequent in the literature (96) but it is conceivable that also in this T cell subset their engagement reduce TCR-mediated activation. The physiological significance of the negative regulation exerted by IRS on activation of gammadelta T cells is still to be defined. It is possible that IRS function is the protection of self from the lytic effect of gammadelta T cells acting as a safeguard against autoreactivity.

5.3. Activating isoforms of inhibiting receptors (IRS)

Most subfamilies of receptors within IRS include members with activating rather than inhibiting function (96). These receptors can be associated with the adaptor protein DAP12, a disulphide-bonded homodimer containing an immunoreceptor tyrosine-based activation motif in its cytoplasmic domain, which can transduce an activating signal (96). The physiological role of activating isoforms of IRS is still to be determined either in NK or T cell subset. Scattered and few data regarding expression and function of activating IRS in gammadelta cells are present in the literature (96). In our experience, gammadelta T cells, either Vdelta1 or Vdelta2 clones, can express these receptors and their ligation with specific monoclonal antibodies leads to the activation of cytolysis and secretion of anti-tumor cytokines as IFN-gamma and TNF-alfa (Poggi *et al.*, unpublished; figure 2). In addition, gammadelta T cell clones bearing the activating IRS can lyse *in vitro* target cells bearing the corresponding HLA-I counter receptor, while target cells lacking the right HLA-I are not killed. Altogether, these findings would suggest that also activating IRS can trigger gammadelta T cells to kill tumor cells and thus these receptors may have a synergic effect with TCR-mediated signaling in eliminating tumor cells.
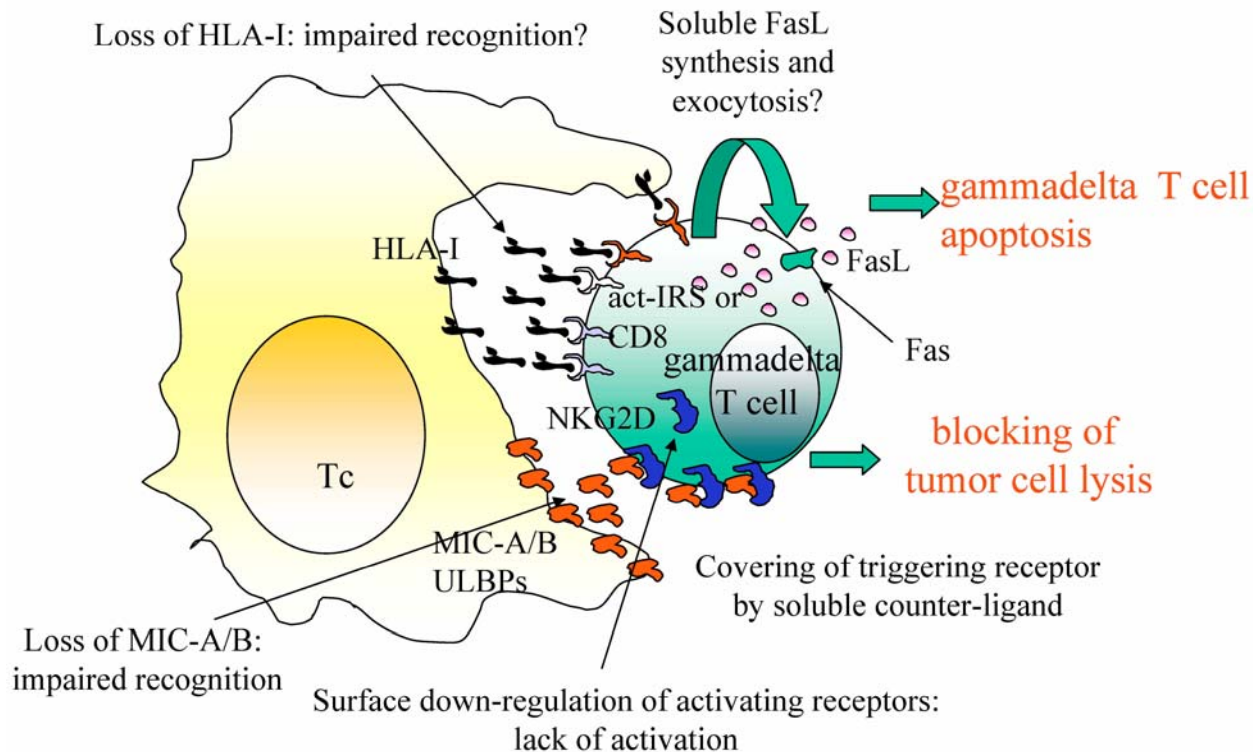
Figure 3. Mechanisms of tumor escape from gammadelta T cell surveillance. Tumor cells (yellow, Tc) can escape gammadelta T cell (green) surveillance by multiple mechanisms: 1) release of HLA-I molecules which, interacting with either CD8 or activating IRS (act-IRS), induce synthesis and secretion of FasL that binds to Fas receptor on effector gammadelta T cells leading to their apoptosis; 2) release of MHC-related antigens, such as MIC-A/B, which induce down-regulation of NKG2D, thus blocking the trigger of cytolysis; 3) loss of HLA-I and/or MIC-A/B causes the lack of recognition of Tc by gammadelta T lymphocytes; 4) covering of NKG2D by soluble MIC-A/B might impair the interaction between NKG2D and surface MIC-A/B occurring upon gammadelta T lymphocyte/Tc binding.

6. MECHANISMS OF TUMOR ESCAPE

It is evident that many tumors must escape the host immune response as they can grow and kill the host. Indeed, tumors are composed of autologous cells and thus they have a low immunogenic potential. In some instances, tumor cells loose co-stimulatory molecules which are essential in inducing a powerful immune response (figure 3). Further, tumor cells can produce cytokines such as transforming growth factor (TGF)-beta, which are strongly immunosuppressive (102). In addition, tumor cells can loose one or more HLA-I allele, thus impairing the presentation of tumor-derived antigenic peptides. However, the loss of HLA-I molecules could promote the innate immune response, recruiting NK and gammadelta T cells; indeed, these lymphocyte subsets expressing inhibitory isoforms of IRS, in absence of the corresponding ligand are able to kill tumor cells. Anyway, since tumor cells very often overcome these control mechanisms, additional escaping means should play a role.

6.1. Soluble molecules

Soluble counter receptors of activating surface structures expressed by effector cells may represent a useful tool for tumor cells to avoid recognition by the immune system. Indeed, it has been shown that soluble MIC-A (sMIC-A) molecules are actually released by cancer cells and are present in the sera of patients affected by gastrointestinal tumors (103). Thus, it is conceivable that the NKG2D activating receptor on effector gammadelta T cells can bind to sMIC-A, instead of MIC-A expressed by tumor cells. In addition, it has been reported that soluble MIC ligands impair NKG2D expression and consequent NKG2D-mediated cell activation (104). These two mechanisms may allow tumor cells to evade immune system-mediated control. If this is true, it would be essential to analyse the mechanisms by which tumor cells can shed MIC-A, in order to prevent or block this loss. Thus, by preserving MIC-A at the tumor cell surface, effector cells could deliver the lethal hit and eliminate tumor cells. Like MIC-A, tumor cells can also loss HLA-I antigens. Although it is not clear how much it is relevant HLA-I for antigen recognition of gammadelta T cells, the lack of HLA-I can reduce antigen specific recognition (figure 3). Finally, other MHC-related molecules, such as ULBPs, which are found in the sera of leukemic patients

(74), might be released by tumor cells and contribute to tumor escape (figure 3).

6.2. Effector cell apoptosis

Programmed cell death (PCD), also called apoptosis, represents a cellular mechanism playing a role in regulating the integrity of the immune system during the lifetime. Indeed, there is a network functions to make certain the activation and expansion of cells during immune response and the elimination of lymphocytes that are no longer needed at the end of this response (105). PCD is a controlled process that utilizes regulating signalling cascades and leads to the organized breakdown of cellular structures (105). It is conceivable that any time an immune response is triggered highly regulated negative feed-back mechanisms should exist to switch down this response (105). This would occur also during anti-tumor immune response. Indeed, it has been reported that gammadelta T cells can undergo apoptosis upon the interaction with Daudi lymphoma target cells (87). This phenomenon is called activation induced cell death (AICD) and it should occur to avoid, after the elimination of tumor cells, an undesired reaction against autologous normal tissue. However, AICD could favour at the same time, the survival of unrecognized tumor cells and facilitate tumor escape. Importantly, AICD can be downregulated by the treatment of gammadelta T lymphocytes with caspase-3 inhibitors (87) thus allowing these cells to maintain their anti-tumor activity for a longer time. This indicates that the block of the executioners of cell death, like caspases, may reinforce the immune response to cancer cells.

Apoptosis of T cells may be induced not only after the interaction with target cells but also by soluble molecules. In fact, lymphocytes express at the cell surface the death receptor Fas and undergo PCD in the presence of Fas ligand (FasL) (105). Thus, FasL may represent not only a mean to kill tumor cells bearing Fas (figure 2) but also a molecule which induces lymphocyte suicide (figure 3). In fact, FasL is present in effector cells in vesicles which are secreted upon activation (106) and thus it is possible that the engagement of activating receptors leads, at the same time, to the delivery of the lethal hit and to effector cell death.

In addition, the synthesis and release of FasL can occur upon ligation of surface receptors which are not directly involved in the activation of cytolytic machinery. With this regard, recently, we have shown that sHLA-I can induce apoptosis of either alphabeta or NK cells by interacting with CD8 (107, 108). Indeed, sHLA-I, upon CD8 ligation, leads to FasL mRNA upregulation, synthesis and release of soluble FasL; then, FasL induces the death of effector cells. Importantly, CD8 engagement do not trigger cytolysis of T or NK cells suggesting that cytolysis and apoptosis may utilize different biochemical mechanisms (107, 109). This is confirmed by the finding that cyclosporin A inhibits only sHLA-I-induced apoptosis but not effector cell-mediated tumor cell lysis. We have demonstrated that the sHLA-I leads to apoptosis of gammadelta T cells upon ligation of CD8 on that this effect occurs with a kinetics slower than that triggered by the

engagement of CD3/TCR complex (Poggi *et al.*, unpublished). Furthermore, we observed that the ligation of a given activating IRS with the right sHLA-I allele on the gammadelta T cell surface leads to PCD via FasL/Fas interaction (109). This finding indicates that common mechanisms exist for regulating cytolytic effector cells. In addition, the pharmacological down-regulation of these mechanisms may enhance the anti-tumor immune response.

7. LOCALIZATION AT THE TUMOR SITE

In mice, gammadelta T cells recirculate preferentially through non lymphoid tissues and show a particular tropism for skin and gut (110). The precise mechanism for this preferential migration is unclear; in general, lymphocyte extravasation is initiated by rolling on vascular endothelium, sustained through the engagement of specific glycoproteins, including the P-selectin ligand PSGL-1, by selectins, followed by integrin-induced adhesion to endothelial cells and leukocyte arrest (111). Then, lymphocytes must move to intercellular junctions between endothelial cells, where transmigration occurs (112, 113); however, little is known about the ability of the two subsets of gammadelta lymphocytes to undergo successful transendothelial migration and tissue localization and to recirculate. In this regard, it has been reported that in some haematological malignancies circulating gammadelta T cells are increased: in particular an expansion of Vdelta1 T cells has been reported with high frequency in disease-free patients with acute leukemia after bone marrow transplantation, while an increase of Vdelta2 T lymphocytes has been described in multiple myeloma (14, 16). A remarkable increase in the number of Vdelta2 or Vdelta1 T cells has been also found in the spleen or in the peripheral blood of patients with acute or chronic B cell leukemia (16, 75, 114). This suggests that circulating gammadelta T lymphocytes might be susceptible *in vivo* to chemotactic or haptotactic gradients, thus, being able to be targeted to damaged tissues.

Migration of lymphocytes, including gammadelta T cells, depends not only on the combined actions of various adhesion molecules (112, 113) but it is also controlled by the interactions between chemokines and their receptors (115, 116). In human CXC chemokines, two of four conserved cysteines are separated by an aminoacid X, while CC chemokines display two cysteines side by side (92). Based on their physiological features, one can distinguish between "inflammatory" (or inducible) chemokines, which include I-309 (CCL1) and interferon-inducible protein 10 (IP-10, CXCL10), and "homeostatic" (or constitutive) chemokines, such as stromal cell-derived factor-1 (SDF-1, CXCL12) and 6Ckine/SLC (CCL21) (92, 115, 116). Functional studies indicate that circulating gammadelta T cells migrate in response to RANTES, MIP-1alpha, MIP-1beta and IP-10 (93, 116, 117), while in terms of chemokine receptors, Vdelta2 T lymphocytes express CCR5 (receptor for RANTES, MIP-1alpha, MIP-1beta), and CXCR3 (whose ligands are represented by IP-10/CXCL10 and 6Ckine/SLC/CCL21

(116-118) which in turn is not always found on the Vdelta1 T cell surface (119).

7.1. Adhesion molecules used for transendothelial migration

To extravasate, leukocytes use a number of adhesion molecules, which interact with endothelial counter-receptors and/or with sub-endothelial matrix components. In both cases, cells must modify their shape and polarize towards the endothelial cell junction to complete transmigration. Among the adhesion molecules known to participate in extravasation, the Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1/CD31), which is expressed both at the endothelial cell borders and at the surface of leukocytes, creates a haptotactic gradient able to recruit circulating cells and to drive transmigration (113).

Interestingly, we have shown that Vdelta1 T lymphocytes preferentially express PECAM-1, which drives transendothelial migration of this cell subset, at variance with Vdelta2 T cells that bear the NKRP1a type II membrane glycoprotein, containing a C-type lectin domain, and use this receptor to transmigrate across endothelial cells in the absence of chemotactic stimuli (120). In turn, cells of the Vdelta1 cell subset lack NKRP1a, while Vdelta2 T cells are mostly PECAM-1 negative (120). Both PECAM-1 and the NKRP1a molecule might be important in driving the localization of circulating gammadelta T lymphocytes, with potential anti-cancer activity, to the tumor site. Because the expression of NKRP1A is regulated by interleukin-12 (IL-12), potential release of IL-12 at the level of the damaged tissue might modulate the recirculation of NKRP1a$^+$ gammadelta T cells.

Moreover, we have reported that gammadelta T lymphocytes 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 sub-endothelial matrix (121). These Vdelta1 TILs can use N-CAM for invasion of heparan sulfate matrices and reverse (i.e. in the basal-apical direction) transendothelial migration (A. Poggi *et al.*, unpublished). Thus, N-CAM/CD56 might contribute to the regulation of both extravasation of circulating gammadelta lymphocytes and recirculation of resident gammadelta TILs. Interestingly, leukocyte function-associated antigen (LFA)-1, a beta2 integrin involved in the adhesion of leukocyte to endothelial cells, which is the first step of extravasation, has a low-grade expression on intraepithelial gammadelta T lymphocytes, and is variably expressed by gammadelta TILs (A. Poggi *et al.*, unpublished). Therefore, it is conceivable that N-CAM/CD56 can substitute for LFA-1 in transendothelial migration of Vdelta1 TILs weakly expressing this integrin, as we reported for T lymphocytes from a patient with leukocyte adhesion deficiency syndrome, a genetic disease resulting from mutations in the beta2 subunit of LFA-1 (122).

7.2. Kinases activated for transmigration

We have reported that PECAM-1/CD31 is associated to the phosphoinositide-3 kinase (PI-3K), an enzyme that phosphorylates the D-3' position of the inositol ring and produces novel inosities, in human neutrophils (123, 124). PI-3K, which can also be activated via LFA-1, in the earliest steps of adhesion, induces phosphorylation and activation of the serine-threonine kinase Akt/PKB and this pathway is essential for the control of actin organization and cell migration (123, 124). On the other hand, in neutrophils, another kinase, the calcium calmodulin kinase II (CAMKII) has been shown to be crucial in mediating cytoskeletal rearrangement and cell polarization during locomotion (125).

Noteworthy, PECAM-1 expressed by the Vdelta1 subset and NKRP1a on Vdelta2 T cells activate different signal transduction pathways (126). Indeed, transendothelial migration of Vdelta2 T lymphocytes is inhibited by blockers of CAMKII, whereas the Vdelta 1 subset is blocked by PI-3K inhibitors. In turn, the engagement of NKRP1a on Vdelta2 lymphocytes leads to CAMKII activation, while PECAM-1 oligomerization on Vdelta1 T cells triggers the phosphorylation of the PI-3K-dependent Akt/PKB. Thus, the two subsets use different adhesion molecules and signaling pathways to transmigrate, suggesting that resident and circulating gammadelta T lymphocytes are equipped with distinct biochemical and molecular mechanisms to regulate their selective tissue localization.

It is of interest that PI-3K is also coupled to chemokine receptor signal transduction: recently, it has been reported that chemokine-induced high-affinity state of the beta2 integrin LFA-1 is controlled by a PI-3K-dependent signalling pathway (127). This might account, at least in part, for the faster kinetics of transendothelial migration displayed by Vdelta2 T cells, that we showed to highly express LFA-1. Indeed, a rapid integrin-driven lymphocyte arrest conceivably facilitates the interaction between other adhesion molecules and their endothelial ligand(s) involved in the progression of transmigration. Likewise, the presence of PSGL-1 on this subset might contribute to start transendothelial motility, in keeping with other data (111).

The preferential usage of a PI-3K-dependent pathway by the resident subset of gammadelta T lymphocytes might also imply that they have been recruited to the tissues by soluble factors (e.g. chemokines) produced by T lymphocytes or other cell types, especially during inflammation (128-130). Finally, the identification of signaling pathways which differently regulate the homing of the two gammadelta T cell subsets might be of interest, as they may be crucial to direct one or the other subset to the tumor site.

7.3. Response to chemokines

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 (92, 118). With regard to alphabeta TILs, we have shown that they are responsive to chemotactic factors produced by autologous tumor cells

in lung cancers (131). *In vitro*, migration of human gammadelta T cells in response to inflammatory chemokines, in particular MIP-1alpha and -beta, and RANTES, has been reported (93, 117). Vgamma9/Vdelta2 T lymphocytes express a wide range of CC-chemokine receptors, including CCR1 and CCR5 (119), which represent the receptors for the afore-mentioned cytokines.

Our preliminary observations indicate that Vdelta1 T lymphocytes infiltrating lung cancers express CXCR3 and CCR5 (M. Ferrarini *et al.* unpublished). Expression of CXCR3 has been demonstrated recently in intrathymic gammadelta lymphocytes and shown to mediate cell migration *in vitro* to IP-10 specifically. Vdelta1 also express high levels of CXCR4, whereas in Vdelta2 T cells the expression of CXCR4 is low. Conversely, we could not detect CCR3, the receptor for monocyte chemotactic protein (MCP)-2, -3 and -4 and eotaxin, which is present on eosinophils, basophils and T helper 2 (Th2) lymphocytes (93, 119).

According to their expression of CXCR3 or CXCR4, Vdelta1 and Vdelta2 T cell clones from healthy donors, transmigrate across endothelial monolayers in response to the specific chemokines, i.e. IP-10/CXCL10 for CXCR3$^+$ cells and SDF-1/CXCL12 for the CXCR4$^+$ clones (132). Of note, the homeostatic chemokine 6Ckine/SLC/CCL21 is more effective than IP-10/CXCL10 in driving transendothelial migration of the Vdelta1 clones expressing CXCR3, suggesting that the resident Vdelta1 T cell population preferentially respond to homeostatic, constitutive chemokines. In this regard, Vdelta1 T cells also bear CCR7, which is reported to be expressed on bovine gammadelta T lymphocytes and drive tissue localization of these cells in response to 6Ckine/SLC/CCL21 (118). On the other hand, Vdelta2 CXCR3$^+$ cells are driven more efficiently by IP-10/CXCL10, supporting that circulating gammadelta T cells are more sensitive to inflammatory chemokines. In addition, we showed that CXCR3 can activate CAMKII, which is needed for transendothelial migration mediated by IP-10/CXCL10; conversely, CAMKII is not involved in SDF-1/SLC/CXCL12-driven transmigration. We also found that transmigration of the two gammadelta T cell subsets induced by IP-10/CXCL10 or 6Ckine/SLC/CCL21 or SDF-1/CXCL12 was dependent on PI-3K activation (132), in agreement with the reported finding that CXCR3 and CXCR4 receptors are coupled to this kinase (133, 134).

Both circulating and resident gammadelta lymphocytes seem, therefore, to express a distinct functional pattern of chemokine receptors, which, potentially, allow them to extravasate and migrate throughout the extracellular matrix and reach the tumor site in response to specific stimuli. However, it is conceivable that redistribution of Vdelta1 and Vdelta2 cell subsets during tumor onset and progression is also regulated by cytokines, such as TNFalpha, which increase adhesiveness to vascular endothelium thus influencing migratory capabilities of lymphocytes. Of note, intraepithelial gammadelta T cells can themselves produce a number of chemokines (90), thereby possibly contributing to the recruitment of circulating lymphocytes, including other gammadelta lymphocytes.

8. PERSPECTIVES

Recent advances in the characterization of the functional capabilities of resident Vdelta1 and circulating Vdelta2 T cells indicate that they display unique features in terms of antigen specificities, requirements for antigen recognition and tissue distribution, which make them suitable candidates as anti-tumor effectors (figure 1 and 2). In particular, given the lack of antigen-processing requirements and the capability of recognizing non conventional MHC-like molecules, gammadelta T lymphocytes might complement the MHC-restricted tumor-specific immune response mediated by alphabeta T lymphocytes. Moreover, the expression of NK-like receptors, such as NKG2D and activating isoforms of killer inhibitory receptors (12, 45, 52, 54), provides gammadelta T cells with additional effector mechanisms in the defence against tumors that bear "stress-induced" molecules or which have down-regulated expression of MHC-class I molecules, a frequent occurrence during cancer progression.

These characteristics can be exploited further to enhance anti-tumor immunity. First, fusion proteins and/or humanized monoclonal antibodies might be designed to trigger gammadelta T cells through a TCR independent pathway, such as that mediated by NKG2D; in addition, drugs, such as retinoic acid, increasing the expression of NKG2D ligands might potentiate this pathway of activation. Second, vaccines may be designed based on the use of herpes simplex virus-derived oncolytic viruses, able to enhance the innate response mediated by gammadelta T cells, creating a link to adaptive immunity (135, 136). Moreover, manipulation of the cytokine and/or chemokine milieu might be envisaged, to enhance the anti-neoplastic function(s) of gammadelta TILs and promote the recruitment of circulating gammadelta T lymphocytes to the tumor site. Third, phosphate antigens might be considered as potential drugs to activate the responding Vdelta2 gammadeltaT-cell population in certain hematological malignancies (14, 68). Indeed, they are commonly used in the prevention of osteoporosis and of osteolytic lesions in multiple myeloma; interestingly, results of a recent randomized, double-blind, placebo-controlled study, showed significant improvement of the survival rates in patients assuming biphosphonates (14, 15); furthermore, a pilot study of a combination therapy with low-dose IL-2 and pamidronate proved to be useful also in patients with relapsed/refractory low-grade non-Hodgkin lymphoma (15, 67). Finally, the identification of specific signaling pathways that selectively inhibit AICD in gammadelta T cells, retaining MHC-unrestricted (innate) anti-tumor activity (87), will allow the development of future immunotherapy strategies.

9. ACKNOWLEDGMENTS

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Send correspondence to: Dr Maria Raffaella Zocchi, Laboratory of Tumour Immunology and Department of Internal Medicine, H San Raffaele Scientific Institute, Via Olgettina 60, I-20132 Milano, Italy, Tel: 39-02-26432612, Fax: 39-02-26432611, E-mail: zocchi.maria@hsr.it