Perspectives of γδ T Cells in Tumor Immunology

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Abstract

Subsets of human γδ T cells recognize tumor cell–expressed ligands that are not seen by the T-cell receptor of conventional αβ T cells. Vδ1 T cells recognize MHC class I chain–related molecules A and B and UL-16–binding proteins expressed at variable levels on epithelial tumor cells and some leukemias and lymphomas. In addition, therapeutically used aminobisphosphonates and synthetic phosphoantigens activate Vδ2 T cells, the dominant subset of γδ T cells in human peripheral blood that display strong cytotoxicity towards various epithelial tumors. Intentional activation of γδ T cells *in vivo* and/or adoptive cell therapy with *in vitro* expanded γδ T cells holds considerable promise as a novel immunotherapy in certain types of cancer. [Cancer Res 2007;67(1):5–8]

Introduction

T cells carrying the γδ T-cell receptor (TCR) account for 2% to 5% of $CD3^+$ T cells in the peripheral blood but constitute a major T-cell subset in other anatomic localizations, such as the intestine or the skin (here, however, only in the murine but not in human skin; ref. 1). In the blood of most healthy individuals, T cells expressing the *Vδ2* gene paired with one particular Vγ chain (Vγ9) account for 50% to >90% of the γδ T-cell population. In contrast, intestinal intraepithelial γδ T cells frequently express the *Vδ1* gene, which can associate with different Vγ elements (1).

An obvious question is why the immune system has afforded to maintain two different types of TCR throughout evolution. There are no major differences between αβ and γδ T cells with regard to effector functions. Thus, activated γδ T cells have strong cytotoxic effector activity (using both death receptor/death ligand and cytolytic granule pathways) and produce various cytokines (frequently including tumor necrosis factor-α and IFN-γ; ref. 1). It seems, however, that the γδ TCR recognizes novel ligands that are not seen by αβ T cells, thereby providing an essential additional pathway of local immunosurveillance with immediate relevance for tumor defense (1, 2). In addition, increasing evidence indicates that γδ T cells also play a nonredundant role in the antimicrobial immune defense through the selective recognition of bacterial metabolites and some viral antigens (1, 3). Most γδ T cells lack cell surface expression of CD4 or CD8, in agreement with their MHC-nonrestricted recognition of such unconventional antigens.

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doi:10.1158/0008-5472.CAN-06-3069

Tumor-Expressed Antigens Recognized by the TCR of Human γδ T Cells

Vδ1 T cells recognize MHC class I chain–related molecules A and B and UL-16–binding proteins. Vδ1 γδ T cells isolated from tumor-infiltrating lymphocytes (TIL) of patients with colorectal cancer have been shown to lyse not only autologous but also various allogeneic epithelial tumor cells (4). The MHC class I chain–related molecules A and B (MICA and MICB) and the UL-16 binding proteins 1 to 3 (ULBP1–3) can be induced on epithelial cells by heat shock or oxidative stress and are constitutively expressed to variable levels on many epithelial tumor cells and also on some leukemias and lymphomas. Such MICA/MICB or ULBP-expressing tumor and lymphoma cells are recognized and killed by Vδ1 γδ T cells (5, 6). It has been debated whether MICA/MICB and ULBPs are directly recognized by the Vδ1 TCR or, alternatively, activate Vδ1 T cells through alternative receptors, such as stimulatory natural killer (NK) receptors (see below). Although this issue is unresolved for the ULBPs, two lines of evidence suggest direct recognition of MICA by the Vδ1 TCR (Fig. 1*A*). First, MICA tetramers bind to TCR transfectants expressing the relevant Vδ1 TCR but lacking NKGD2, the activating NK receptor for MICA (7). Second, we have recently generated soluble recombinant Vδ1 TCR protein from MICA-reactive Vδ1 γδ T cells. The soluble Vδ1 TCR, but not similarly constructed control Vδ2 or Vδ3 TCR proteins, specifically bound to immobilized recombinant MICA protein as shown by surface plasmon resonance (8). Hence, it seems that tumor-expressed MICA/MICB antigens can be specifically recognized by the TCR of human Vδ1 γδ T cells. It is quite likely, however, that the Vδ1 TCR recognizes additional as yet undefined tumor cell ligands (Fig. 1*A*).

Vδ2 T cells recognize phosphoantigens and ectopically expressed mitochondrial ATPase. Early studies have shown that the major population of Vδ2Vγ9 peripheral blood γδ T cells is strongly activated by a variety of bacteria and parasites (reviewed in ref. 1). The relevant antigens have been identified as nonpeptidic phosphorylated intermediates of the nonmevalonate pathway of bacterial isoprenoid biosynthesis ("phosphoantigens"), and analogues with potent and selective stimulatory capacity for Vδ2Vγ9 T cells, such as bromohydrin pyrophosphate (Phosphostim), have been synthesized (9). In contrast to many bacteria, eukaryotic cells use the mevalonate pathway for isoprenoid biosynthesis. This generates isopentenyl pyrophosphate (IPP), which also activates Vδ2Vγ9 T cells but only at concentrations that are not achieved physiologically in nontransformed cells. Interestingly, however, certain tumors produce higher concentrations of IPP, which then can be sensed by the TCR of Vδ2Vγ9 T cells as a tumor antigen (3, 10). The intracellular levels of IPP can be manipulated by therapeutically administered drugs. Aminobisphosphonates, which are in clinical use for the treatment of osteoporosis and bone metastasis, are potent inhibitors of the IPP-processing enzyme farnesyl pyrophosphate synthase, thereby leading to the intracellular accumulation of IPP and in consequence to potent activation

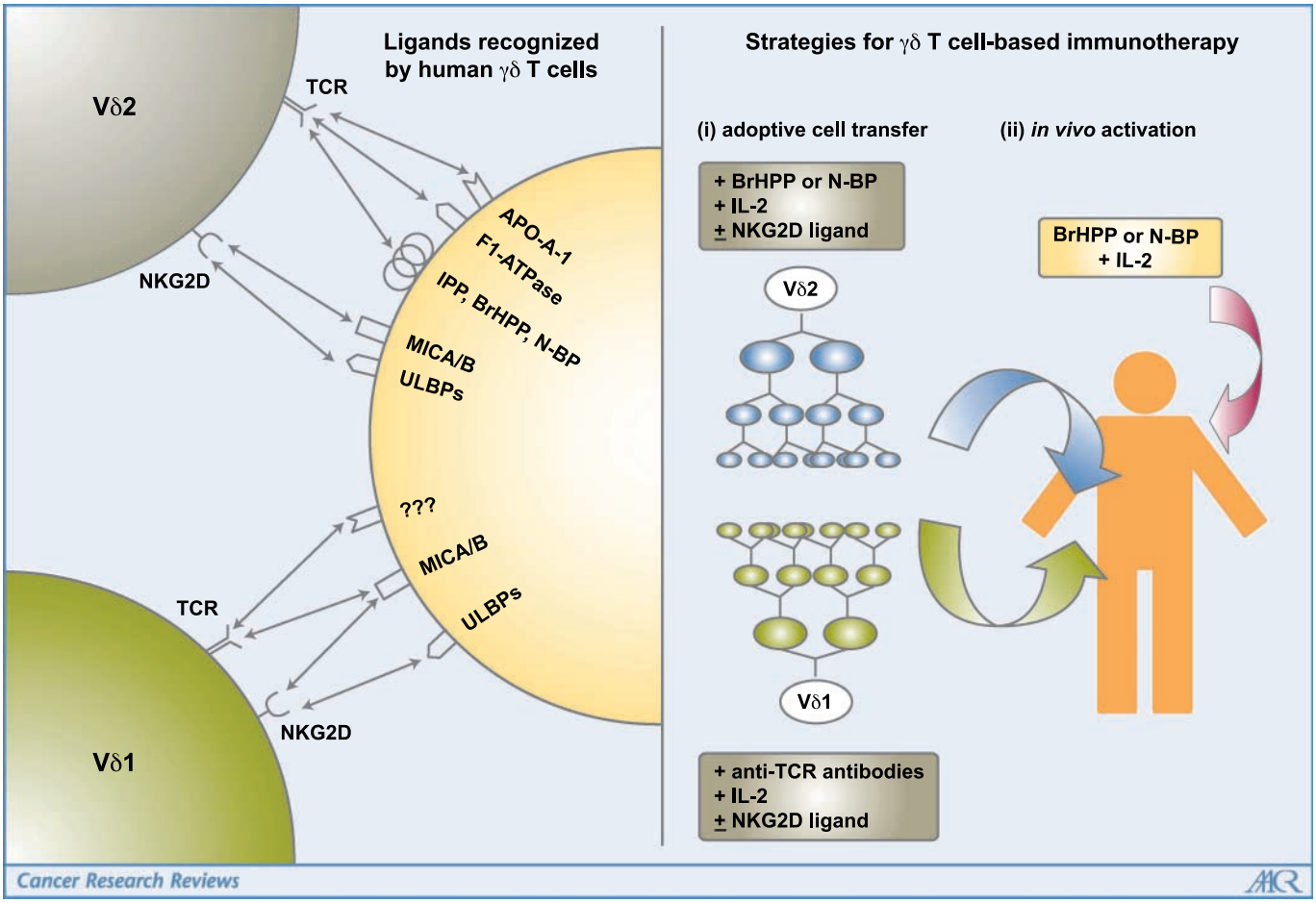

Figure 1. Tumor cell ligands recognized by human γδ T cells and strategies for γδ T cell–based immunotherapy. *Left panel,* the dominant population of Vδ2Vγ9 T cells recognizes via its TCR nonpeptidic phosphoantigens, such as the naturally occurring isoprenoid metabolite IPP, the synthetic bromohydrin pyrophosphate (*BrHPP*; Phosphostim), aminobisphosphonates (*N-BP*), the ectopically expressed F1-ATPase, and apolipoprotein A-I. Ligands recognized by the Vδ1 TCR have been less well defined but include MICA. In addition, MICA/MICB and ULBPs frequently expressed on tumor cells are ligands for the activating NK receptor NKG2D, which is present on Vδ1 and Vδ2 γδ T cells. *Right panel,* strategies for γδ T cell–based immunotherapy include (*i*) the adoptive cell transfer of *in vitro* expanded γδ T cells and (*ii*) the *in vivo* activation of Vδ2Vγ9 T cells by phosphoantigens (e.g., bromohydrin pyrophosphate/Phosphostim) or aminobisphosphonates and low-dose IL-2. Vδ2Vγ9 T cells can be readily activated and expanded *in vitro* by phosphoantigens (bromohydrin pyrophosphate) or aminobisphosphonates in the presence of IL-2. Possible beneficial effects of additional NKG2D stimulation by activating antibodies or naturally occurring NKG2D ligands require further investigation. In the absence of defined ligands (except for MICA) that are recognized by the Vδ1 TCR, tumor-reactive Vδ1 γδ T cells can be activated and expanded *in vitro* by stimulation with mitogenic anti-TCR antibodies and IL-2 in the absence or presence of additional NKG2D ligation.

of Vδ2Vγ9 T cells (3, 10, 11). In view of the strong reactivity of Vδ2Vγ9 T cells towards different tumors (9, 12), the potential application of synthetic phosphoantigens (e.g., Phosphostim) and aminobisphosphonates for the activation and expansion of tumor-reactive Vδ2Vγ9 T cells is under investigation.

In addition to the phosphoantigens, the very same Vδ2Vγ9 TCR also recognizes a mitochondrial F1-ATPase–related structure and delipidated apolipoprotein A-I, which are expressed on the surface of tumor cells (13). Thus, it is striking to note that one single molecularly defined TCR (i.e., Vδ2Vγ9) recognizes structurally completely unrelated moieties, such as isoprenoid metabolites, F1-ATPase, and apolipoprotein A-I, all of which play a role as tumor antigens and contribute to the antitumor reactivity of human γδ T cells (see Fig. 1*A*). To identify possible additional tumor cell ligands for Vδ2 γδ T cells, we have synthesized peptides corresponding to the Vδ2 CDR3 region of ovarian epithelial carcinoma–infiltrating γδ T cells. Such Vδ2 CDR3 peptides and recombinant Vδ2 CDR3-grafted antibodies bind to certain epithelial tumor cells and tumor cell lysates, indicating the direct involvement of the TCR Vδ2 region in tumor cell recognition (14). In ongoing investigations, we use the CDR3δ peptides and the CDR3δ-grafted recombinant antibodies in an attempt to identify the bound tumor cell ligands.

Modulation of γδ T-Cell Activation by NK Receptors

T-cell responses are modulated by activating and inhibitory NK receptors, which have been first identified on NK cells but are also present on T-cell subsets, notably $CD8^+$ αβ T cells and γδ T cells. Many NK receptors recognize MHC class I molecules as ligands and signal through their immunoreceptor tyrosine–based activation motifs (activating receptors) or immunoreceptor tyrosine–based inhibitory motifs (inhibitory receptors). Of special relevance for γδ T cells is the activating NKG2D receptor as it is expressed on many γδ T cells. NKG2D interacts with MICA/MICB and ULBPs frequently expressed on tumor cells and thus can interfere with TCR-dependent signaling events in tumor-reactive γδ T cells. As discussed above, there is good evidence that the Vδ1 subset recognizes MICA/MICB (but not ULBPs) directly via the TCR.

Therefore, NKG2D-positive Vδ1 γδ T cells might recognize MICA/MICB–expressing tumor cells simultaneously through the TCR and NKG2D, and both pathways likely contribute to cellular activation and tumor cell lysis (Fig. 1*A*). NKG2D, however, is also expressed on Vδ2 γδ T cells. In these instances, the TCR-dependent recognition of unusual ligands described above and the subsequent activation of Vδ2 γδ T cells can be enhanced by MICA engagement of NKG2D (15). Importantly, however, NKG2D-positive Vδ2 T cells can also be directly activated by MICA in the absence of TCR-dependent ligand recognition (16). Irrespective of the additional complexity due to the effect of MHC class I–specific inhibitory NK receptors, these results point to possible strategies of exploiting the NKG2D receptor as a target for activation and expansion of tumor-reactive Vδ2 T cells.

Perspectives of γδ T Cells for Immunotherapy of Cancer

Tumor-infiltrating γδ T cells are tumor reactive. γδ T cells have been consistently identified and isolated from TIL in various types of cancer, including colorectal, breast, prostate, ovarian, and renal cell carcinoma (4, 5, 9, 12, 14). γδ T cell lines and clones established from TIL recognize and kill not only the autologous tumor but generally also a broad range of related tumors, presumably due to the recognition of shared ligands as described above. Importantly, the γδ T cells preferentially kill tumor cells and show low (if any) reactivity towards nontransformed cells, a feature that has raised great interest to explore their therapeutic potential (9, 12).

Tumor-reactive γδ T cells are stimulated by phosphoantigens and aminobisphosphonates. Synthetic phosphoantigens, such as bromohydrin pyrophosphate (Phosphostim) and aminobisphosphonates, are potent activators of Vδ2Vγ9 T cells. In the presence of interleukin-2 (IL-2), these ligands induce a rapid and exponential expansion of γδ T cells to large cell numbers for potential application in adoptive cell transfer (9). *In vitro* expanded Vδ2Vγ9 T cells maintain their antitumor activity *in vivo* upon adoptive transfer into immunodeficient mice transplanted with human tumor cells, indicating the feasibility of this approach in a preclinical model (17). Therefore, two strategies for the potential usage of γδ T cells in tumor immunotherapy are presently being envisaged i.e., the adoptive cell transfer of *in vitro* expanded γδ T cells and the *in vivo* therapeutic application of γδ-stimulating phosphoantigens or aminobisphosphonates together with low-dose IL-2 (Fig. 1*B*). Ongoing clinical phase I trials evaluate Phosphostim together with low-dose IL-2 in patients with renal cell carcinoma (18), and other types or cancer, including pancreatic adenocarcinoma and colon carcinoma, are under consideration. Trials with aminobisphosphonates plus IL-2 are also done in multiple myeloma (11).

Potential Obstacles of γδ T Cell–Based Immunotherapy

As discussed above, tumor-expressed MICA/MICB and ULBPs trigger γδ T-cell activation via the TCR and/or NKG2D. These NKG2D ligands can be proteolytically shed from the surface of tumor cells, and the soluble NKG2D ligands have been shown to inhibit NK cell and CD8 T-cell function through the down-modulation of NKG2D. Soluble MICA present in the serum of tumor patients has recently been found to down-regulate also the cytotoxic activity of γδ T cells (19). Moreover, sustained activation of NKG2D by MICA is known to inhibit NKG2D-expressing cytotoxic cells. Therefore, therapeutic approaches have to consider that tumor-derived soluble NKG2D ligands might interfere with γδ T-cell immunotherapy in tumor patients.

Moreover, it is obvious that γδ T cells are part of the multicellular immune system that is tightly regulated by multiple pathways, including regulatory T cells (Treg). Therefore, γδ T cell–based immunotherapies (like any other cellular therapy, including dendritic cell vaccination) should also consider the possible negative effect of Treg on the generation of effective antitumor responses (20). Presently, it is unknown to what extent the activation of γδ T cells is controlled by naturally occurring Treg. Our own preliminary results suggest that the *in vitro* activation of human γδ T cells in response to phosphoantigens is enhanced in the absence of $CD4^+CD25^{high}$ Treg, supporting the idea that dampening of Treg activity might be beneficial also in γδ T cell–based immunotherapy of cancers (20).

Conclusion and Future Directions

The identification of unusual tumor-expressed ligands that are recognized by γδ but not by conventional αβ T cells together with their potent cytotoxic antitumor effector activity have recently stimulated great interest in the development of γδ T cell–based immunotherapies in certain types of cancer, such as renal cell carcinoma, colon carcinoma, pancreatic adenocarcinoma, multiple myeloma, and certain leukemias. In contrast to other potential effector cells, it is possible to envisage combined *in vivo* activation and adoptive cell therapy with *ex vivo* expanded γδ T cells because aminobisphosphonates and IL-2 are licensed for clinical application (and Phosphostim is in clinical trials). Although γδ T cells are thus an attractive target for cellular immunotherapy, protocols for their therapeutic use need to be optimized. In this regard, it could be explored whether activating anti-NKG2D antibodies might help to costimulate γδ T-cell expansion *in vitro* to obtain the large cell numbers required for adoptive cell transfer (Fig. 1*B*). Future studies should also address the possible advantage of combining γδ T cell–based immunotherapy with conventional chemotherapy or other therapeutic approaches, such as antiangiogenic drugs.

Acknowledgments

Received 8/18/2006; revised 10/6/2006; accepted 11/7/2006.

Grant support: Deutsche Forschungsgemeinschaft grant Ka 502/8-3 (D. Kabelitz); National Program for Key Basic Research Projects, Ministry of Science and Technology of P.R. China grants 2001CB510009 and 2004CB518706; and National Natural Science Foundation of P.R. China grant 30490244 (W. He).

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Cancer Res 2007;67:5-8.