Review

Cancer Research

Targeting γδ T Lymphocytes for Cancer Immunotherapy: From Novel Mechanistic Insight to Clinical Application

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Abstract

Abundant interferon-γ secretion, potent cytotoxicity, and major histocompatibility complex–independent targeting of a large spectrum of tumors make γδ T cells attractive mediators of cancer immunotherapy. However, a better understanding of the molecular mechanisms involved in tumor cell recognition and γδ T-cell activation is required to improve the limited success of γδ T-cell–mediated treatments. Here, we review key advances in basic knowledge made over the past 3 years, and summarize the results of γδ T-cell–based clinical trials concluded to date. We also highlight new research directions on the basis of the modulation of receptors that control the function of γδ T cells. *Cancer Res; 70(24); 10024–7. ©2010 AACR.*

Introduction

Cellular immunotherapy of solid and hematopoietic malignancies is regarded as a promising approach to deal with the common relapse or resistance to conventional treatments. Among the most potent antitumor cytolytic mediators are γδ T cells, innate-like lymphocytes that recognize their targets independently of major histocompatibility complex (MHC)–mediated antigen presentation (1, 2). Human γδ T cells kill a vast repertoire of tumor cell lines and primary samples *in vitro*, including leukemia and lymphoma, melanoma, neuroblastoma, and multiple types of carcinoma (3–7). More recently, *in vitro*–activated γδ T cells have also been shown to target a small population of colon cancer stem cells, responsible for tumor resistance to conventional therapies (7), and to kill chemotherapy (imatinib)–resistant chronic myelogenous leukemia lines (4). Moreover, γδ T cells have been frequently isolated from tumor-infiltrating lymphocytes and shown to react *in vitro* to tumors but not to healthy cells (reviewed in ref. 1). On the other hand, activated human γδ T cells produce large amounts of interferon-γ (8), a central cytokine in antitumor immune responses. There is, therefore, great interest in targeting γδ T cells for cancer immunotherapy.

Here, we review key developments over the last 3 years in the basic mechanisms of tumor cell recognition and activation of human γδ T cells, and summarize the results of clinical studies concluded to date.

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T-Cell Receptor Agonists for Activation of Vγ9Vδ2 T Cells

Most (60 to 95%) human γδ peripheral blood lymphocytes (γδ-PBL) express a Vγ9Vδ2 T-cell receptor (TCR) that enables them to uniquely respond to nonpeptidic prenyl pyrophosphate metabolites, generically known as phosphoantigens (2). Among these, (E)-4-hydroxy-3-methyl-but-enyl pyrophosphate (HMBPP), which is synthesized in *Eubacteria* and *Protozoa* organisms via the nonmevalonate pathway of isoprenoid biosynthesis, has been shown to trigger bona fide TCR signaling and to activate the antitumor functions of Vγ9Vδ2 T cells even below nanomolar concentrations (8). Other phosphoantigens, such as isopentenyl pyrophosphate (IPP), which are produced in eukaryotic cells through the mevalonate pathway, can also activate Vγ9Vδ2 T cells, but at much higher concentrations, which may only be found in some tumor cells but not in healthy tissues (2). In fact, it was shown that mRNA knockdown of the IPP-consuming enzyme, farnesyl pyrophosphate synthase (FPPS), induced a Vγ9Vδ2 T-cell stimulatory activity in otherwise nonstimulatory tumor cells (9).

Although putative interactions between phosphoantigens and the Vγ9Vδ2 TCR have yet to be characterized, Mookerjee-Basu and colleagues suggested that cell surface–bound F1.ATPase can act as a phosphoantigen-presenting molecule (10). F1.ATPase-coated beads stably bound to an adenylated derivative of IPP, and promoted TCR aggregation, cytokine secretion, and cytotoxic activity (10). These studies collectively support the concept that activation of Vγ9Vδ2 T cells requires a certain density of membrane-bound phosphoantigens to induce efficient TCR signaling, which is necessary for the activation of their antitumor cytotoxic properties (4, 5, 7).

Tumor Cell Recognition Via NKG2D Ligands

Although the TCR plays a central role in the activation of γδ T cells, their cytolytic response to tumors clearly involves

Table 1. Summary of efficacy data from γδ T-cell–based cancer immunotherapy clinical trials

Cancer Immunotherapy	Reference No.	Cancer Type	No. of Patients*	Percent PD	Percent SD	Percent PR
Autologous γδ T-cell infusions	15	Renal cell carcinoma (metastatic)	10	40	60	0
	16	Non–small cell lung cancer	8	63	37	0
Aminobisphosphonates *in vivo*	17	Non-Hodgkin lymphoma (relapsed and/or refractory) or multiple myeloma	A = 9	89	11	0
			B = 9	45	22	33
	18	Metastatic prostate cancer (hormone-refractory)	A = 9	78	11	11
			B = 9	33	45	22
	19	Breast cancer (advanced)	10	70	20	10

Abbreviations: PD, progressive disease; SD, stable disease; PR, partial remission. No complete remissions were observed in any trial.
*Number of patients evaluated at study endpoint. A and B refer to separate cohorts that received distinct regimens.
NOTE: Regimen details and reported side effects are listed in Supplementary Table S1.

other receptors, particularly natural killer (NK) receptors. For example, DNAX accessory molecule-1 (DNAM-1) was shown to regulate γδ T-cell cytotoxicity upon interaction with Nectin-like-5 expressed in carcinoma cells (11). Even more critical for tumor cell recognition is NKG2D, which is expressed on essentially all Vγ9Vδ2 T cells and provides activation signals upon binding to nonclassical MHC proteins of the MIC and ULBP families. Two NKG2D ligands, ULBP1 and ULBP4, have recently been proposed as determining Vγ9Vδ2 T-cell recognition of leukemias and/or lymphomas (12) and ovarian and/or colon carcinomas (13), respectively. Although the role of ULBP4 was inferred from ectopic expression studies (13), the specific mRNA knockdown of ULBP1 in leukemia cell lines showed the physiologic importance of this NKG2D ligand for Vγ9Vδ2 T-cell recognition (12). These data are consistent with a two-step model in which phosphoantigen (TCR)-activated Vγ9Vδ2 T cells discriminate tumor from healthy cells through engagement of NKG2D. In this context, reduced NKG2D-ligand expression is likely to constitute a key immune evasion mechanism. This may explain why various tumors, particularly primary samples, are resistant to Vγ9Vδ2 T-cell cytotoxicity *in vitro* (3), and why ULBP1 expression segregates with leukemia and/or lymphoma susceptibility to γδ T-cell targeting (12).

A controversial aspect about NKG2D ligands is their ability to bind to TCRγδ. Thus, Kong and colleagues showed binding between ULBP4 and a soluble Vγ9Vδ2 TCR chimeric protein (13), whereas MICA tetramers had been previously reported to bind the Vδ1 TCR characteristic of tissue-homing and/or intraepithelial γδ T cells (reviewed in ref. 1). Although this issue deserves further investigation, the strong evidence for tumor cell recognition based on NKG2D-ligand expression, by both $V\delta2^+$ (12, 13) and $V\delta1^+$ (1) T cells, and for costimulation properties of NKG2D (14), should be promptly transferred to the clinic.

Targeting γδ T Cells in the Clinic

Several clinical trials involving patients with advanced disease, refractory to conventional treatments, have been designed to test the safety and efficacy of γδ T-cell–based immunotherapy. Trials that have evaluated clinical efficacy are listed in Table 1 (other studies were not reviewed because of space limitations). Some regimens consisted of the activation and expansion of autologous Vγ9Vδ2 T cells *ex vivo* and their infusion into the patients (15, 16). Either phosphoantigens, like bromohydrin pyrophosphate (BrHPP, phosphostim), or aminobisphosphonates, such as zoledronate or pamidronate, were used for *in vitro* stimulation (Supplementary Table S1). Zoledronate or pamidronate have also been used for *in vivo* activation of Vγ9Vδ2 T cells in a distinct set of clinical trials (Supplementary Table S1; Table 1; refs. 17–19). These drugs, which are therapeutically used in bone cancer, are thought to stimulate Vγ9Vδ2 T cells through inhibition of FPPS and consequent accumulation of intracellular IPP (1, 2). It is of note that partial tumor remissions have only been reported in the *in vivo* drug administration trials (Table 1). Recently, in the context of their studies on metastatic renal cell carcinoma, Kobayashi and colleagues have reported a complete remission in a patient who underwent 6 monthly cycles of autologous γδ-PBLs, activated and/or expanded *in vitro* with HMBPP plus interleukin (IL)-2, combined with the infusion of zoledronate (4 mg) plus low-dose IL-2 (1.4×10^6 IU; ref. 20). This response was associated with a sharp increase in interferon-γ–producing Vγ9Vδ2 T cells following adoptive transfer, and the patient has been disease free for 2 years without any additional treatment (20).

A very recent phase I-II trial involving 21 cancer patients has evaluated the efficacy of zoledronate (4 mg intravenously at day 1) combined with low doses of IL-2 (2×10^6 IU/m^2 subcutaneously at days 1 to 6) on both hematologic and solid

malignancies. Partial tumor remission was observed in 2 out of 8 (25%) of acute myelogenous leukemia patients, whereas none of 13 patients with solid tumors (metastatic renal cell carcinoma or metastatic melanoma) showed an objective response (V. Kunzmann and M. Wilhelm, personal communication). Although a similar regimen has resulted in partial remissions in one patient with prostate carcinoma (18) and one patient with breast cancer (Table 1; ref. 19), the available data (see also ref. 17) could suggest hematologic tumors are more susceptible to current protocols activating Vγ9Vδ2 T cells *in vivo*.

In general, the clinical trials completed to date, particularly those stimulating γδ T cells *in vivo*, have shown objective responses in the range of 10 to 33% (Table 1). If, in some cases, the lack of response to therapy could be attributed to deficient expansion of effector Vγ9Vδ2 T cells (17–19), many patients exhibiting significant and sustained *in vivo* activation and proliferation of Vγ9Vδ2 T cells also failed to respond to treatment (ref. 18; and V. Kunzmann and M. Wilhelm, personal communication). Thus, current γδ T-cell–based treatments, although feasible and safe (Supplementary Table S1), have obvious limitations. New clinical studies, incorporating the latest advances in our understanding on how γδ T cells work, are necessary to improve their therapeutic efficacy.

Future Directions

One critical aspect to explore toward clinical application is the simultaneous manipulation of TCRγδ and NKG2D signals. On the TCR side, it will be important to evaluate the clinical effect of synthetic TCR agonists such as phosphostim (BrHPP) or picostim (analogous to HMBPP), which are much more potent (*in vitro*) than aminobisphosphonates. Another interesting prospect for the use of phosphoantigens is the combination with therapeutic antibodies, as suggested by the improved leukemia and/or lymphoma *in vitro* killing upon coadministration of BrHPP and rituximab (6). As for NKG2D, activating antibodies could overcome potential immune evasion mechanisms that downregulate the expression of its ligands in tumors. However, it would be important to assess the impact on the discrimination between transformed and healthy tissues by NKG2D-activated lymphocytes.

Besides TCRγδ and NKG2D, another receptor whose manipulation may provide immunotherapeutic benefit is CD27, which is expressed by approximately 80% of human γδ-PBL, particularly those with naïve or central memory phenotypes. We have recently looked at CD27 function in γδ-PBL, using either blocking antibodies or agonist ligands (soluble CD70). We observed that CD70-CD27 interactions promote the survival and proliferation of γδ-PBL that produce high levels of interferon-γ (21). The synergy between TCRγδ and CD27 signals should, thus, be explored for clinical expansion of γδ-PBL. These cells include the dominant $V\delta2^+$ PBL subset, but also $V\delta1^+$ cells that express very high levels of CD27 (21). In fact, future research should explore the potential of manipulating $V\delta1^+$ T cells either *in vivo* or upon adoptive cell therapy.

On the tumor side, it is worth exploring the adjuvant effect of Toll-like receptor (TLR) stimulation, because *in vitro* treatment of tumor cells with TLR3 and TLR7 agonists resulted in enhanced cytotoxicity of γδ T cells isolated from cancer patients (22).

Finally, as with all therapies, patient selection is of vital importance; this requires the identification of biomarkers that may predict clinical outcome. For example, a recent study has identified a panel of 10 genes encoding cell surface proteins that segregated "γδ-susceptible" from "γδ-resistant" hematologic tumors (3). In this era of genome-wide studies, equivalent markers could be promptly characterized in multiple cancer types, and their predictive value should then be evaluated in γδ T-cell–based clinical trials. We believe the combination of "susceptible" tumor profiles with improved strategies for γδ T-cell activation *in vivo* may be *the* way forward for γδ T-cell–based cancer immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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