Targeting gamma delta T cells for cancer immunotherapy: bench to bedside

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γδ T lymphocytes represent a minor subset of peripheral blood in humans (<10%). γδ T cells expressing Vγ9Vδ2 T cell receptor recognise the endogenous pool of isopentenyl pyrophosphate (IPP) that is overproduced in cancer cells as a result of dysregulated mevalonate pathway. Aminobisphosphonates increase the endogenous pool of IPP in cells by blocking the enzyme farnesyl pyrophosphate synthase (FPPS) of the mevalonate pathway. Activated γδ T cells release copious amounts of interferon (IFN)-γ and tumour necrosis factor (TNF)-α and exhibit potent anti-tumour activity. Combination of γδ T cells with therapeutic monoclonal antibodies can efficiently mediate antibody dependent cellular cytotoxicity against tumours. These features makes γδ T cells attractive mediator of cancer immunotherapy. We review here, the basic properties and importance of γδ T cells in tumour immunity, and highlight the key advances in anti-tumour effector functions of γδ T cells achieved over the last few years and also summarize the results of the clinical trials that have been done till date. Future immunotherapeutic approach utilizing γδ T cells holds considerable promise for treatment of different types of cancer.

Key words Aminobisphosphonates - anti-tumor cytotoxicity - clinical trials - immunotherapy - γδ T cells - phosphoantigens

Introduction

The immune system has evolved to protect the host from infections and cancer. Typically, the immune system is divided into two categories- innate immunity and adaptive immunity. The innate immune system comes into play immediately after the appearance of antigen whereas the adaptive immune system provides antigen-specific response. In addition to these defense mechanisms, there are unconventional T cells like the gamma delta (γδ) T lymphocytes and natural killer T (NKT) cells that functionally and phenotypically belong to both the innate and the adaptive immune system and are able to bridge the two1-3. In the peripheral circulation of humans, γδ T cells comprise about 1-10 per cent of the circulating T cells, though this percentage can rise to as high as 50 per cent at some mucosal sites4. γδ T cells are involved in combating infectious diseases and have non-redundant capacities in the inhibition of tumour development and progression5,6.

Antigen recognition and activation of γδ T lymphocytes

Unlike αβ T cells, γδ T cells do not require the help of conventional major histocompatibility complex
(MHC) class I and class II molecules for recognizing the antigens. Antigen recognition by γδ T cells is dependent upon the particular variable (V) region of the T cell receptor (TCR) as opposed to the entire rearranged TCR required by αβ T cells. γδ T cells expressing Vδ1 are abundantly found at mucosal sites and these respond to the expression of non-classical MHC molecules on the surface of virally-infected or tumour cells. Vδ2+ (Vγ9Vδ2) cells are predominantly present in the peripheral circulation and respond to non-peptide phosphoantigens. Vγ9Vδ2 T cells recognize self and microbial phosphorylated metabolites generated in the eukaryotic mevalonate pathway and in the microbial 2-C-methyl-D-erythritol 4-phosphate (MEP) or non-mevalonate pathway. It was observed that during bacterial and protozoan infections, Vγ9Vδ2 T cells expand to high levels which in some individuals represented the majority of circulating T cells. The first chemically defined antigens for Vγ9Vδ2 were found to be alkyl phosphates. One natural antigen from mycobacteria was isolated and identified as isopentenyl pyrophosphate (IPP). Subsequent characterization of the microbial antigens recognized by human γδ T cells revealed that these are non-proteinaceous in nature and have critical phosphate residues. The Vγ9Vδ2 crystal structure confirmed the presence of a basic, positively charged region in the binding groove that could directly interact with the negatively charged pyrophosphate moiety of the antigen. These phosphoantigens are generated during the non-mevalonate and mevalonate pathways utilized by prokaryotic and eukaryotic cells, respectively. Various compounds like steroid hormones, cholesterol, many types of vitamins, rubber, etc. are derived from this pathway. There are now many synthetic phosphorylated compounds that are capable of stimulating γδ T cells like bromohydrin pyrophosphate (BrHPP), 4-hydroxy-3-methyl-buty-2-enyl pyrophosphate (HMBPP) and mono-ethyl pyrophosphate (BrHPP) and mono-ethyl pyrophosphate (MEP) or non-mevalonate pathway. It was observed that during bacterial and protozoan infections, Vγ9Vδ2 T cells expand to high levels which in some individuals represented the majority of circulating T cells. The first chemically defined antigens for Vγ9Vδ2 were found to be alkyl phosphates. One natural antigen from mycobacteria was isolated and identified as isopentenyl pyrophosphate (IPP). Subsequent characterization of the microbial antigens recognized by human γδ T cells revealed that these are non-proteinaceous in nature and have critical phosphate residues. 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The influx of TILs (tumour infiltrating lymphocytes) to the tumour site enhances the potential for anti-tumour immune responses. The numbers and types of lymphocytes present in the infiltrate are related to the chemokines produced by both the tumour cells and tissue stromal cells located at the tumour site. The infiltration of circulating lymphocytes to the tumour is facilitated by these chemokines. For example, breast, cervix and pancreatic tumours as well as ovarian tumour produce CC and CXC chemokines that are important mediators of macrophage and lymphocyte infiltration in those tumours. Interestingly, both Vδ1 and Vγ9Vδ2 T cells display distinct chemokine receptors that bestow these cells the property to migrate to the tumour site. Vδ1 express CCR5 and Vγ9δ2 express both CCR5 and CXCR3. In addition, Vγ9δ2 T cells express NK receptor P1A (NKR-P1A) platelet endothelial cell-adhesion molecules (PECAM) while Vδ1 use NK receptor P1A NKR-P1A for transendothelial migration. Vδ1 T cell subsets from the peripheral blood utilize a larger array of adhesion molecules, namely LFA-1, VLA-α4, VLA-α5, L-selectin and αEβ7, to bind to squamous cell carcinoma cells compared to the restricted usage of LFA-1, L-selectin and CD44V6 by the Vδ2 T cells. The mutually exclusive pattern of chemokine receptor expression in both the subsets of γδ T cells indicates independent mechanism of homing to tumour site that might have an important aspect in cancer immunotherapy.

Anti-tumour activity of γδ T lymphocytes

Ability of γδ T lymphocytes to produce abundant proinflammatory cytokines like IFN-γ, potent cytotoxic effector function and MHC-independent recognition of antigens makes it an important player of cancer immunotherapy. γδ T cells kill many different types of cells.
of tumour cell lines and tumours in vitro, including leukemia, neuroblastoma and various carcinomas.

Accumulation of mevalonate metabolites in tumour cells is a powerful danger signal that activates the γδ T cells. In normal cells, IPP produced by mevalonate pathway are at a concentration that is insufficient to trigger γδ T cells response. However, dysregulation of mevalonate pathway in certain tumours leads to production of higher concentrations of IPP, which is sensed by γδ TCR as a tumour antigen. It was also shown that mRNA knockdown of IPP-consuming enzyme, FPPS, induced Vγ9Vδ2 T cell stimulation in otherwise non-stimulatory tumour cells. γδ T cells are able to recognize and kill many different differentiated tumours cells, either spontaneously or after treatment with different bisphosphonates, including zoledronate. It has been shown that human tumour cells can efficiently present aminobisphosphonate and pyrophosphomonoester compounds to γδ T cells, inducing its proliferation and IFN-γ production.

Combination treatment utilizing Vγ9Vδ2 T cells along with chemotherapeutic agents and zoledronate has been shown to induce an increase in the cytotoxic function of γδ T cells against solid tumour. The ability of γδ T cells to efficiently kill bisphosphonates treated colon cancer stem cells and ovarian cancer stem-like cells has also been reported.

In addition to phosphoantigens, γδ T lymphocytes can also be activated by mitochondrial F1-ATPase-related structure expressed together with apolipoprotein A-I, which are expressed on the surface of some tumour cells. ATP F1 synthase is an intracellular protein complex involved in ATP generation. F1-ATPase displays characteristic of antigen presentation molecule by binding to the adenylated derivative of IPP and promoting TCR aggregation, cytokine secretion and cytotoxic activity.

**NK receptors and anti-tumour activity of γδ T cells**

Natural killer (NK) receptors expressed on γδ T cells play a crucial role in mediating the anti-tumour response of γδ T cells. Natural killer group 2, member D protein (NKG2D) expressed on Vγ9Vδ2 T cells is critical for tumour recognition and provides activation signals upon binding to non-classical MHC molecules of the MHC class I chain-related molecules (MIC) and UL-16 binding protein (ULBP) families expressed on tumour cells. This ligand binding to NKG2D can affect the release of TNF-α, interleukin (IL)-2 α receptor (CD25) upregulation and increase cytolytic potential of γδ T cells. ULBP molecules are involved in Vγ9Vδ2 T cells recognition of leukemias and lymphomas and also ovarian and colon carcinomas. γδ T cells utilizing the Vδ1 chain isolated from tumour-infiltrating lymphocytes can also kill cancer cells. Vδ1 γδ T lymphocytes have been shown to mediate cytolytic activity by recognizing MIC, MICB or ULBP expressed on cancer cells.

γδ T cells resemble NK cells as these also express CD16 (FcγRIII) receptor. Upon recognition of phosphoantigens, a subset of Vγ9Vδ2 T cells upregulates CD16. It has been reported that CD16 represents activation/memory status of γδ T cells and these CD16-high cells have specific phenotypic features that distinguish these from the CD16-low subset. These constitutively express several natural killer receptors (NKG2A/CD94) and high amounts of perforin, but express low levels of chemokine receptors (CXCR3, CCR6) and IFN-γ. CD16/FcγRIII receptor binds to Fc portion of immunoglobulin G (IgG) and engagement of CD16 by γδ T cells leads to antibody-dependent cellular cytotoxicity (ADCC). ADCC is a process in which CD16+ effector cells actively lyse tumour cells that have been bound by specific antibodies. Several reports have proven that in vitro γδ T cells respond to activation via CD16 and mediate ADCC against tumour with therapeutic anti-tumour monoclonal antibodies (mAbs) like rituximab, trastuzumab, ofatumumab and alemtuzumab. It has also been shown that stimulated γδ T cells increase the efficacy of trastuzumab in vivo in Her2+ breast cancer patients.

**Application of γδ T cell immunotherapy in clinics**

Given the potent antitumour effector function of γδ T cells and broad reactivity to many different types of tumours has raised a great interest to explore their therapeutic potential. An important feature of γδ T cells is that these favourably kill cancer cells and show low (if any) reactivity towards non-transformed cells which makes these very good candidates for cancer immunotherapy. The safety and efficacy of γδ T cell-based immunotherapy have been evaluated in several clinical trials. Presently, two strategies for γδ T cells in tumour immunotherapy have been applied. These are 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 recombinant IL2 (rIL2).

Studies carried out in nude mice demonstrated that repeated infusion of γδ T cells leads to tumour growth arrest. Another study carried out in SCID mice showed...
the anti-tumour effector functions of NK cells and γδ T lymphocytes against autologous melanoma cells. In one pilot study, patients with B-cell malignancies that failed conventional therapy were treated with intravenous administration of pamidronate and rIL2 to stimulate Vγ9Vδ2 T cells in vivo. It was observed that in vivo Vγ9Vδ2 T cells were expanded in five out of nine patients; three out of these five responding patients had partial remissions and one had stable disease. Other trials with adoptive transfer of γδ T cells include patients with advanced cancer like metastatic renal cell carcinoma and non-small cell lung carcinoma where stable disease was found in 60 and 37 per cent patients, respectively. In these cases, the regimen consisted of ex vivo activation and expansion of autologous Vγ9Vδ2 T cells with either phosphoantigens, such as BrHPP or aminobisphosphonates, like zoledronate or pamidronate or their infusion into the patients. Aminobisphosphonates have also been used in clinical trials to treat metastatic prostate cancer and advanced breast cancer where partial remissions have been reported. Complete remission of lung metastasis in a patient with renal cell carcinoma has also been reported after adoptive transfer of γδ T cells. It was shown that the patient was disease free for two years without any additional treatment following in vitro activation and expansion of autologous γδ T cells with HMBPP plus rIL2, combined with the infusion of zoledronate and rIL2. There is also increasing evidence that stimulating γδ effector T cells can enhance monoclonal antibody-induced cytotoxicity and thereby improve the anticancer effects of mAbs. It was found that repeated infusions of phosphoantigens stimulated γδ T cells and trastuzumab increased the efficacy of γδ T cells against HER-2+ breast carcinoma cell lines in vivo. In addition, a survival advantage to patients with an increased γδ T cells following allogeneic stem cell transplantation (ASCT) has been reported. A long-term survival advantage in a group of high-risk acute leukemia patients who recovered with increased number of circulating γδ T cells following partially mismatched related hematopoietic stem cell transplantation was reported.

Conclusions

The unique features of human γδ T cells related to antigen recognition, tissue tropism, lack of antigen processing requirement and cytotoxic function make these ideal candidates for cancer immunotherapy. γδ T cells recognize increased pool of endogenous IPP (a consequence of dysregulated mevalonate pathway) in cancer cells, release IFN-γ/TNF-α and mediate cytolytic effector functions. Expression of NKG2D receptors provides a selective advantage to γδ T cells to recognize tumours that express stress induced molecules like MICA/B. This property of γδ T cells can be exploited for immunotherapy as tumours downregulate MHC molecules to evade immune recognition (Fig.). Human γδ T cells show potent cytotoxic effector functions...
against various types of tumours. One way to exploit γδ T cells for cancer immunotherapy is the use of synthetic phosphoantigens like BrHPP or HMBPP which can act as γδ TCR agonists. Future trials should harness bisphosphonate activated γδ T cells in combination with chemotherapy or monoclonal antibodies for treatment of solid tumours and haematologic malignancies.

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