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Harnessing CD1d-restricted T cells towards anti-tumor immunity in humans

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Abstract

Natural killer T (NKT) cells are a distinct subset of T cells that recognize lipid antigens in the context of CD1d molecules. There is considerable body of evidence implicating a role for NKT cells in regulating anti-tumor immunity in mice. α-galactosylceramide (α-GalCer) is a potent agonist ligand for type I NKT cells. We and others have shown that injection of α-GalCer loaded DCs leads to clear expansion of NKT cells in vivo in cancer patients. Preclinical studies suggest the capacity of thalidomide analogues to enhance ligand dependent NKT activation, and provide the rationale for combination approaches that are now being designed. Recently, we have demonstrated the presence of CD1d restricted T cells specific for an inflammation associated lipid, lysophosphatidylcholine in patients with advanced myeloma. These studies suggest that type II NKT cells may play a role in sensing and regulating inflammation. Harnessing CD1d restricted T cells in cancer may depend on regulating the balance between type I and II NKT cells, and holds promise as a broad strategy for immune therapy of several cancers.

Keywords

Natural killer T cells; lipid antigens; myeloma; CD1d; cancer immunotherapy

Diversity of CD1d-restricted T cells

NKT cells are innate lymphocytes that are implicated in the resistance to tumors and pathogens. [1] [2] Over the past two decades, several lines of evidence led to the concept of a specialized subset of TCRαβ+ T cells that recognized antigens presented by the class I-like MHC molecule CD1d, expressed NK1.1 and a semi-invariant TCR (Vα14Jα18 in mice, Va24 in humans). The discovery of α-Galactosylceramide (α-GalCer), derived from a marine sponge (or microorganisms symbiotic with the sponge) as a potent agonist for these cells spurred considerable research on their properties. Although this T-cell population was originally discovered as a T-cell subset expressing both T and NK markers,[3] there is now consensus that these cells are part of a broader repertoire of lipid specific T cells.[4] At least 3 distinct subsets of CD1d restricted T cells are recognized. The best studied of the subsets are those expressing the invariant T cell receptor (Vα14Jα18 TCR in the mouse or Vα24Jα18 in the human), called type I NKT or invariant NKT cells. Type I NKT cells can be further divided into CD4+ and CD4−CD8− DN subsets, and a small subset of human NKT cells may even express CD8αα.[5] Type I NKT cells have also been distinguished by their tissue localization. In the mouse, these cells have their highest prevalence in the liver, where they may represent up to 30% of CD3+ T cells in the mouse.[6] The liver-resident type I NKT cells are not only more prevalent, but also show different functional characteristics, in that they have been shown

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to be more protective against tumors than NKT cells from the spleen or thymus.[7] Invariant or type I natural killer T cells have a phenotype of activated T cells, and can be rapidly activated by a synthetic glycolipid ligand, α-GalCer. NKT activation by GalCer leads to rapid downstream activation of NK cells and induction of adaptive immunity via activation of dendritic cells and T cells.[8,9] Invariant NKT cells are evolutionarily conserved, thus suggesting an important role in the immune system.

Another subset of NKT cells that are also CD1d-restricted but express more diverse TCRs were termed type II NKT.[10–12] These are likely to be a heterogenous cell population. Compared to the knowledge about type I NKT cells, that of type II NKT cells is very limited. Finally, there is another heterogeneous subset of T cells with diverse TCRs that express NK markers, but are not CD1d restricted or glycolipid-reactive, and have been termed type III NKT cells. [13]

Studies have shown that type I NKT cells can recognize some microbial glycolipids (such as those from sphingomonas and Borrelia) and self-antigens, such as isoglobotrihexosylceramide (iGb3).[14] The nature of antigens specifically recognized by type II NKT cells is less clear and limited to sulfatide and nonlipidic small molecules.[15,16] In some settings, GD3 and tumor-derived glycosphingolipids have been implicated as ligands for iNKT cells.[17,18] However, the nature of the specific endogenous ligands recognized by either type I or II NKT cells in inflammation or cancer in humans remains obscure. Our group recently identified a distinct population of human CD1d-restricted T cells specific for inflammation-associated lysolipids. This evidence suggests a novel mechanism for inflammation mediated immune regulation in human cancer.[19]

In summary, CD1d-restricted T cells fill a unique niche in providing the immune system a cellular arm to recognize lipid antigens. The ability of NKT cells to recognize self lipids may be one reason they can have profound impact on autoimmune disease. On the other hand, their ability to recognize bacterial lipids, gives the adaptive T-cell immune system another handle on invading microbes, by detecting their lipid content as well as their proteins.[6] Thus, NKT cells serve as regulatory cells and potentially effector cells in responses ranging from autoimmune disease and allergy to infectious diseases and cancer.

Role of CD1d restricted T cells in tumor immunity

In tumor immunity, type I NKT cells have been postulated to play primarily a protective role dependent on their ability to make interferon γ. The importance of the CD1d-invariant NKT cell axis in immune regulation has been clearly demonstrated in many murine models of anti-tumor responses.[17,20,21] Several studies have confirmed the anti-tumor activity of NKT cells stimulated by α-GalCer in vivo. An important role NKT cells play in immunosurveillance of tumors was shown based on the use of knockout mice deficient in NKT cells.[22] Mice deficient in NKT cells have increased propensity for cancer in both spontaneous as well as carcinogen induced models. The antitumor effects of NKT cells are due to several mechanisms, including enhancement of immune effectors, particularly IFN-γ mediated activation of NK cells and antiangiogenesis.[23–28] Syngeneic DCs pulsed with α-GalCer were able to treat established liver metastases of the B16 melanoma.[23] More recent studies show that a C-glycoside analogue of α-GalCer, skewed more toward an interferon-γ response, was even more effective against melanoma metastases.[29] In addition to the role of IFN-γ in recruiting NK cells, NKT cells were shown to activate DCs to make IL-12, and this mechanism also played an important role in anti-tumor activity.[30] Human Vα24+ NKT cells can mediate antitumor effects in vitro; a deficiency of NKT cells, or defects in their function, have been described in cancer patients.[31–34] Studies in some mouse models suggested that NKT cells could also suppress tumor immunosurveillance based on their production of the Th2 cytokine IL-13.

[35,36] However it is now clear that this is best explained by differing properties of type I versus II NKT cells.

NKT cells in human multiple myeloma

Multiple myeloma (MM) is a hematologic malignancy characterized by the accumulation of transformed plasma cells in the bone marrow. Myeloma is an interesting target for NKT mediated anti-tumor immunity due to several considerations. In prior studies, we have shown that progression to clinical myeloma is associated with loss of interferon-γ secreting function of NKT cells.[34] We studied the function of natural killer T cells from the blood and tumor bed in patients with premalignant gammopathy, nonprogressive myeloma, or progressive multiple myeloma. NKT cells from patients with progressive disease, but not nonprogressive myeloma or premalignant gammopathy, had a marked deficiency of ligand-dependent interferon- γ production. This functional defect could be overcome in vitro using dendritic cells pulsed with the NKT ligand, α-GalCer. These data suggested that clinical progression in patients with monoclonal gammopathies is associated with an acquired but potentially reversible defect in NKT cell function and support the possibility that these innate lymphocytes play a role in controlling the malignant growth of this incurable tumor. Thus, measurement of NKT cell function may be a useful predictor of clinical outcome in these patients. These data provided impetus for further clinical studies to boost or restore NKT cell function in malignancy.

It is worth noting that in myeloma, tumor cells grow predominantly in the bone marrow and that a significant proportion of T cells in the human marrow are CD1d restricted.[11] Moreover, we have previously shown that myeloma cells express CD1d, are sensitive to lysis by NKT cells, and therefore are particularly interesting target for iNKT directed therapies. Our group and others have observed that CD1d is highly expressed in tumor cells in pre-malignant and early myeloma. Expression of CD1d is reduced with disease progression and eventually lost altogether in most myeloma cell lines, suggesting that CD1d impacts negatively on myeloma cell survival. Consistent with this, engagement of CD1d by anti-CD1d mAbs induces cell death of myeloma cell lines with restored CD1d expression and primary myeloma cells.[37] As discussed above, NKT activation may also mediate anti-myeloma effects by anti-angiogenesis, as well by activation of NK cells, and tumor specific T cells.

Dendritic Cell mediated activation of human NKT cells in cancer

Several studies have reported a deficiency in iNKT numbers and/or function in the blood of patients with various advanced malignancies, such as in monoclonal gammopathies, myelodysplastic syndromes, prostate cancer and head and neck cancers. This loss of NKT function appears to correlate with clinical stage of tumors, and may be reversible, at least in part. Therefore, the ability to manipulate NKT cells in humans has a valuable therapeutic potential. The discovery and availability of α-GalCer as a clinical grade reagent has allowed studies employing this ligand to enhance NKT cells in vivo. Despite encouraging preclinical data using α-GalCer, injection of the ligand alone in humans with cancer led to only modest and transient effects without substantial NKT expansion.[38] In contrast, injection of glycolipid loaded DCs led to prolonged NKT activation. These results have led the investigators to test alternative approaches utilizing targeted antigen presenting cells to enhance NKT cell activation.

To understand the impact of different APCs on the activation of human NKT cells we conducted study on freshly isolated human NKT cells and quantified them by an enzyme-linked immunospot (ELISPOT) assay.[39] We observed that human NKT cells have a Th1 profile after stimulation with a-GalCer loaded DCs. We evaluated APC requirements for human NKT cell activation in fresh blood and showed that monocyte derived DCs are more effective than

monocytes/macrophages for detecting and activating NKT cells in fresh blood, with mature α-GalCer pulsed DCs being optimal. In this study, DCs were also efficient APCs for expanding NKT cells in culture and generating NKT cell lines. NKT cells expanded with DCs were functional, secreting both IFN-γ and IL-4, and killing NKT-sensitive targets. Optimal activation of these lines was seen using mature DCs loaded with α-GalCer. DCs matured with several different stimuli were effective. These data helped to establish the conditions for loading DCs with α-GalCer for immune therapeutic targeting of NKT cells, and provided a new simple assay to monitor NKT function in humans.

Based on these data we carried out a phase I clinical trial to test the safety and tolerability of α-GalCer loaded mature DCs in patients with advanced cancer.[40] Monocyte-derived mature DCs that were loaded with α-GalCer were intravenously injected in patients with advanced cancer. Injection of α-GalCer-pulsed DCs led to greater than 100-fold expansion of several subsets of NKT cells in all patients. Interestingly, these expansions could be detected for up to six months after vaccination. NKT activation was associated with an increase in serum levels of IL-12 and IFN-γ-inducible protein-10. In addition, we observed enhancement of antigen specific T cell responses. There was also an increase in cytomegalovirus specific memory $CD8^+$ T cells after injection of GalCer loaded DCs. These data demonstrated the feasibility of sustained expansion of NKT cells in vivo in patients with advanced cancer, and suggested that NKT activation might be a first step toward harnessing these innate effectors for therapeutic benefit.

Immuno-modulatory drugs as adjuvants for NKT activation

Given the complex interaction between malignant cells, immune effectors and microenvironment of the tumor bed, simply activation of NKT cells in vivo may not be sufficient for significant anti-tumor effects. A more effective strategy might be to use combined modalities to attain maximal anti-tumor effects of immunotherapy. Thalidomide and its derivative lenalidomide can enhance costimulation of human T cells.[41] These agents have anti-inflammatory, antiangiogenic, and immunomodulatory properties, and may target tumor cells by direct cytotoxicity and indirectly by interfering with several components of the bone marrow microenvironment. Lenalidomide (LEN) retains antitumor activity equal to or greater than the parent compound, but carries less toxicity. Importantly, LEN demonstrates greater potency at T cell costimulation. LEN has been shown to have significant clinical activity in multiple myeloma and LEN based regimens have become a common frontline approach in myeloma. Clinical response to these drugs is associated with increase in immune cytokines, however the nature of specific immune targets remains unclear.

To elucidate immune targets of lenalidomide and study its influence on NKT cell function we conducted a trial in healthy volunteers and patients with myeloma.[42] We showed that LEN enhances antigen-specific expansion of NKT cells in response to α-GalCer in both healthy donors and patients with myeloma. NKT cells activated in the presence of LEN had greater ability to secrete interferon-γ. Antigen-dependent activation of NKT cells was greater in the presence of dexamethasone (DEX) plus LEN than with DEX alone. Therapy with LEN/Thal also led to an increase in NKT cells in vivo in patients with myeloma and del5q myelodysplastic syndrome. Together these data demonstrate that LEN and its analogues enhance CD1d-mediated presentation of glycolipid antigens and support combining these agents with other NKT targeted approaches. Another group recently explored the effects of lenalidomide on iNKT cells from newly diagnosed and advanced multiple myeloma patients, and confirmed the capacity of LEN to enhance Th1 polarization of iNKT cells.[43] These results provide additional preclinical evidence for the iNKT cell-mediated immunotherapy and a rationale for its use in combination with lenalidomide in multiple myeloma treatment. The above studies provide foundation for an upcoming clinical trial that will utilize α-GalCer-loaded DCs in

combination with an immunomodulatory agent lenalidomide in order to exploit their adjuvant properties to expand NKT cells, and boost both innate and adaptive immunity to tumors.

Ligands for type II NKT cells in humans

Recent studies have shown that type I NKT cells can recognize some microbial glycolipids and self-antigens, such as isoglobotrihexosylceramide (iGb3). However, the nature of antigens specifically recognized by type II NKT cells is less clear. Binding of CD1d molecules to phospholipids has also been demonstrated. However, whether these molecules are commonly recognized by populations of human T cells is not known.

To identify the nature of CD1d-binding ligands in human myeloma, we took a biochemical approach to directly isolate and characterize the CD1d-binding lipids from the plasma of these patients.[19] Characterization of these ligands revealed several lysophosphatidylcholine (LPC) species. Several species of LPC exist in vivo depending on the properties of the acyl chain. We focused on 2 of the species (C16:0 and C18:1) identified in our experiments. CD1d dimers loaded with LPC showed clear staining for human T cells. Human LPC-CD1d dimmer binding cells were T-cell receptor αβ T cells, but predominantly Vα24-Vβ11-. In contrast to CD1d-LPC, CD1d dimer loaded with α-GalCer clearly stained V α 24^{+} T cells in these cultures. Cytokine secretion by LPC-specific T cells was skewed toward IL-13 secretion, and the frequencies of these cells were increased in myeloma patients relative to healthy donors. These data identified a distinct population of human CD1d-restricted T cells specific for inflammation-associated lysolipids and suggested a novel mechanism for inflammation mediated immune regulation in human cancer.

Possible roles of LPC specific T cells in cancer and inflammation

LPC is generated by the action of phospholipase A2 on phosphatidylcholine (PC). It is a major constituent of oxidized low density lipoprotein in atherosclerotic plaques, and elevated levels of lysophospholipids have been observed in allergic and autoimmune inflammation, asthma, and human cancer, including in multiple myeloma. Therefore, the findings made in the above study may apply to the putative role for CD1d-restricted T cells in diverse inflammatory states. Prior studies have suggested that PC may play chaperone-like roles in intracellular assembly of CD1 molecules. In our studies, PC competed with loading of LPC on CD1d, and subsequent LPC dependent binding of T cells. Our data are consistent with a model where the recognition of CD1d by these T cells may depend on the balance between PC and LPC, suggested to be a sensitive indicator of inflammation. Studies support the hypothesis that some CD1d-restricted cells may serve as a cellular sensor system for the detection of inflammation in tissues.

Inflammation is a common feature of many human cancers and can lead to high levels of several species of LPC or related ligands in the malignant tissue. Therefore, the generation of LPC may be a mechanism by which inflammation might paralyze innate immunity against cancer. The preferential production of IL-13 by LPC-specific T cells is also of interest because these cytokines have been implicated in promoting tumor growth, fibrosis, and angiogenesis in malignant tissues and inflammation. Specific targeting of these LPC-specific T cells may therefore provide novel approaches to regulate inflammation and innate immunity in clinical setting.

Conclusions

Several subsets of CD1d restricted T cells are strongly implicated in tumor immunity. Recent clinical studies demonstrate the feasibility of harnessing type I NKT cells in vivo in patients with malignancy. NKT activation might be a first step toward utilizing these innate effectors for therapeutic benefit in tumor immunotherapy. Despite expanding knowledge, much remains

to be learned about the characteristics and properties of NKT cell subsets and their ligands in vivo. Systematic studies in defined clinical settings are needed to further advance our knowledge of biologic effects of these cells and translating it to clinical benefit.

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