MBL Immunology News Jul. 2012

Glycolipid Immunology: NKT cells

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Discovery of NKT cells

The 2011 Nobel Prize in physiology or medicine was awarded to three scientists for their work on the innate immune system, which provides an immediate immune defense against pathogens via utilization of receptors that recognizes structures common to pathogens (i.e., pattern recognition receptors). Once the innate immune system is activated, the adaptive immune system is induced by T as well as B cells bearing receptors that have higher specificity and affinity for antigens. Natural killer T (NKT) cells are known to serve as a link between the innate and adaptive immune systems, and therefore have attracted much attention in the allergy, cancer, and many other fields.

NKT cells that express both the T cell antigen receptor (TCR) and NK receptor were identified in 1990s as a new type of lymphocyte that recognize CD1 molecules.[1] In Japan, basic research on NKT cells has been led by Dr. Masaru Taniguchi (Director, RIKEN Research Center for Allergy and Immunology) and his colleagues. The distribution of NKT cells differs from that of the usual T cells by being largely in the liver and bone marrow with few in lymphatic tissues such as lymph nodes and the spleen. The TCR repertoire of NKT cells is also characteristic. Although they resemble T cells in recognizing antigens via TCRs, their diversity is limited and invariant: only mouse Vα14, Vβ8.2, Vβ7, and Vβ2 in addition to human Vα24 and Vβ11 NKT cells are known (Figure 1). Accordingly, NKT cells are also called invariant NKT cells (hereinafter, referred to as iNKT cells). The antigens captured by TCRs on iNKT cells are also unusual. iNKT cells recognize glycolipids unlike conventional T cells, which recognize antigen polypeptides.[2] Since both human and rodent iNKT cells bind the same glycolipid antigens irrespective of species origin, it is considered that glycolipid recognition by iNKT cells has a fundamental role in bioprotection (glycolipid antigens are described later).

Functions of iNKT cells

It has been indicated that iNKT cells that generate large amounts of interferon (IFN)-γ and interleukin (IL)-4, namely Th1 and Th2 cytokines, are broadly involved in immunity to various diseases such as infections, allergy, and cancer. Recently, novel NKT cells expressing IL-17 receptor B (RB) have been reported to contribute to airway hyperreactivity commonly seen in asthmatic patients.[3] Furthermore, according to the status of CD4 expression, IL-17RB-positive NKT cells are divided into two subsets, CD4-positive and -negative. The former cells produce the Th2 cytokine IL-13, two Th9 cytokines (IL-9 and IL-10), and two Th17 cytokines (IL-17a and IL-22) in response to the stimulus IL-25, whereas the latter cells are retinoid-related orphan receptor (ROR)γt-positive and produce a Th17 cytokine in response to the stimulus IL-23. Moreover, the CD4-positive cells are largely present in the lung where they reportedly play a role in virus-induced bronchitis.[4]

In patients with autoimmune disease (systemic scleroderma, systemic lupus erythematosus [SLE], or rheumatoid arthritis) or atopic dermatitis, the number of iNKT cells in blood is decreased, suggesting some involvement of iNKT cells in the etiology of these diseases.[5–9] It has also been shown that in cancer patients, prognosis is correlated with the number of iNKT cells accumulating in the lesion and the quality of these cells.[10,11]

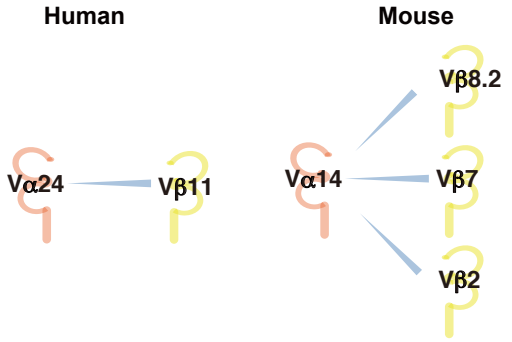

Figure 1 Invariant TCRs expressed on iNKT cells of human and mouse

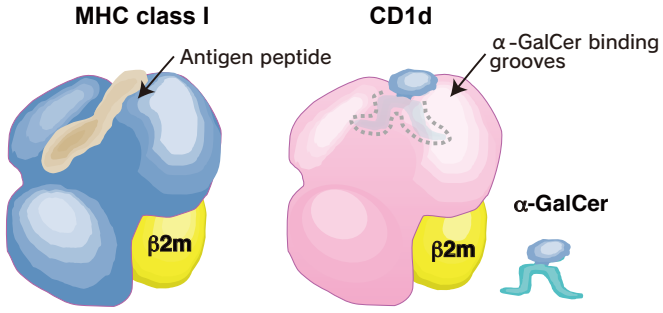

Figure 2 Diagrams of the MHC class I/antigen-peptide complex and the CD1d/α-GalCer complex

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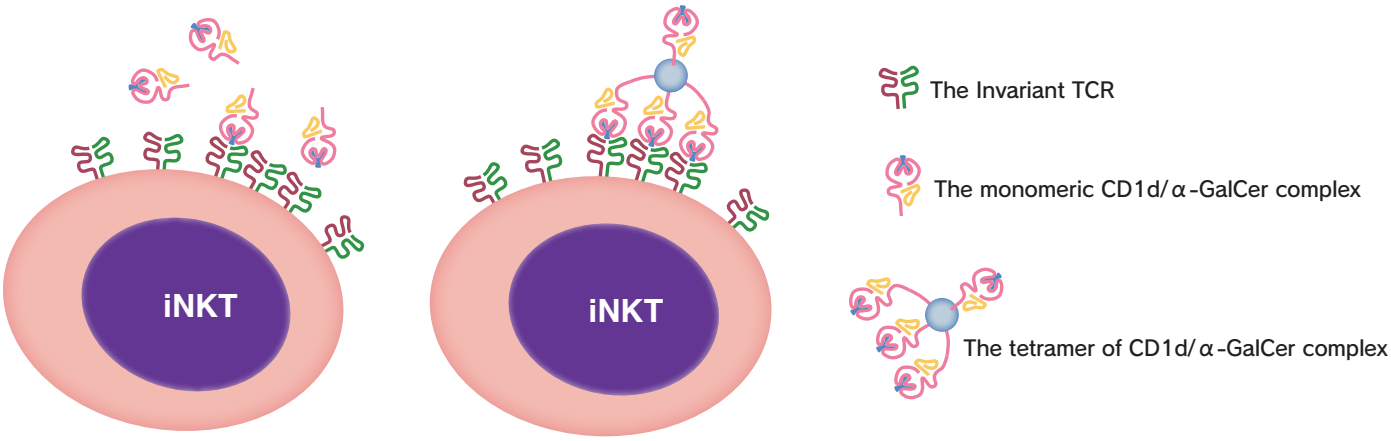

Figure 3 CD1d Tetramer can be used to detect iNKT cells with sustaining the binding to invariant TCR, whereas the monomeric CD1d may be detached from the invariant TCR on iNKT cells due to its low affinity.

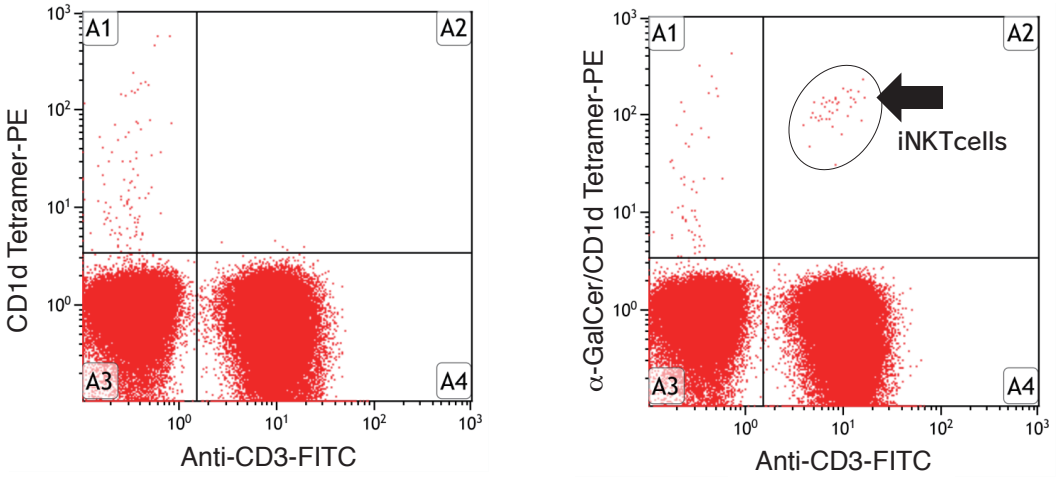

Figure 4 Detection of human iNKT cells by using CD1d Tetramer pre-loaded with α-GalCer.

Antigens for iNKT cells

A glycosphingolipid,α-galactosylceramide (α-GalCer), extracted from sponges is most commonly used as an antigen for stimulating iNKT cells in experiments.[2] As an intrinsic antigen, isoglobotrihexosylceramide (iGb3) (which is a component of lysosome) is considered to be a promising iNKT activator,[12] but iGb3 is absent in humans. As extrinsic antigens, glycolipids derived from particular bacteria (e.g., *Sphingomonas* and *Borrelia*) are reportedly recognized by iNKT cells, [13–15] although the details of the recognition are unclear. However, in 2011, glycolipids derived from *Streptococcus pneumoniae* (a highly lethal Gram-positive bacterium) and Group B *Streptococci* were identified as extrinsic antigens that activate iNKT cells. In addition, not only the sugar but also the fatty acids (vaccenic acid) were shown to be important for iNKT cell activation.[16]

The MHC class I-like glycoprotein CD1

The CD1 molecule is a unique MHC class I-like glycoprotein that can present glycolipids as antigens.[17,18] The CD1 molecule and MHC class I molecule are very similar in protein structure and in binding to β2 microglobulin, however different in the structure of the antigen-binding site. The MHC class I molecule has a hydrophilic groove structure for binding to hydrophilic peptides, whereas the CD1 molecule has two hydrophobic, deep groove structures for binding glycolipids possessing alkyl chains (Figure 2).[19] CD1 molecules can be categorized into group 1 (CD1a, CD1b, and CD1c) and group 2 (CD1d). Humans have CD1 molecules of both groups, whereas mice and rats have only CD1 molecules (CD1d) of group 2. iNKT cells specifically recognize glycolipid-CD1d complexes.

Detection of iNKT cells and CD1d tetramer

The tetramer is, as an example, a complex of four ligand molecules that bind to a cell surface receptor. In immunology, the most well-known tetramer is that of MHC class I molecules. Generally, the affinity of an MHC/peptide complex or CD1d/ligand complex for the TCR is not high enough to maintain binding each other. Thus, engineered tetramers of the MHC-antigen complex were prepared to enhance binding to TCR,

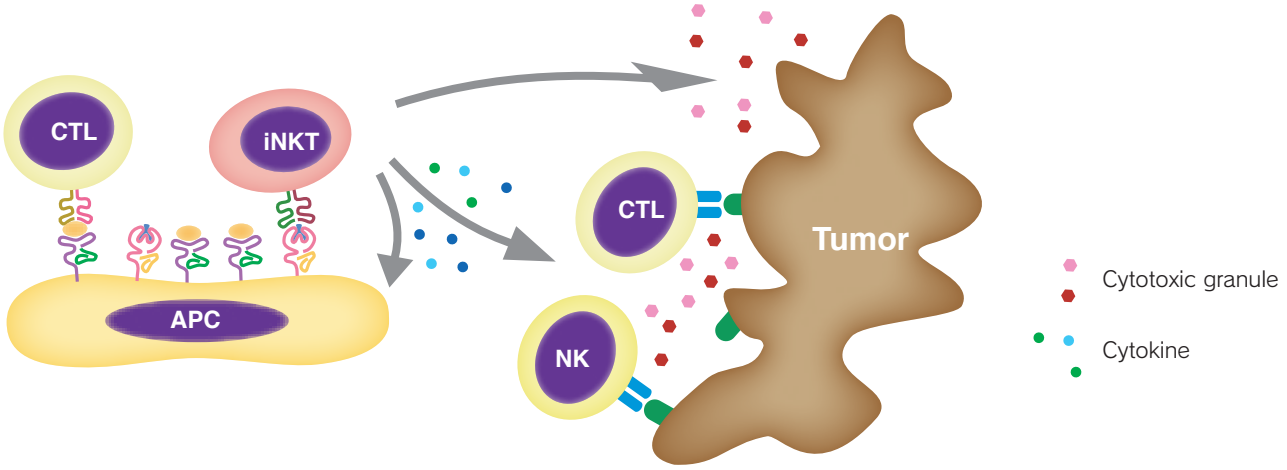

Figure 5 Tumor biology of iNKT cells; Activated iNKT cells can directly attack tumor cells by releasing cytotoxic factors, as well as indirectly by secreting cytokines, which activate tumor specific CTL and NK cells.

facilitating detection of particular TCR-expressing T cells or iNKT cells (Figure 3).

Since the α-GalCer/CD1d tetramer reacts only with iNKT cells, the use of α-GalCer/CD1d tetramer to detect iNKT cells is considered to be the most accurate method of detection (Figure 4). Actually, it has been reported that a comparison of two cell populations allowed to react with antibodies to TCR and the CD1d tetramer revealed different ratios of detected cells.[20)]

Anticancer immunotherapy with iNKT cells

In recent years, iNKT cells have been integrated into anticancer immunotherapy. iNKT cells are easily controlled in activation by incubation with artificially synthesized α-GalCer. More importantly in clinical use, they have promising multiple antitumor effects. Direct anticancer effects include cytotoxicity due to perforin and granzyme release upon iNKT cell activation. Indirect effects include enhancement of NK cell function as well as CD8-positive T cell function via abundant production of cytokines (such as IFN-γ) and production of IL-12 associated with maturation of dendritic cells (Figure 5). In brief, it can be said that advantages of both innate immunity-activating therapy and CTL therapy are realized in anticancer immunotherapy with iNKT cells.

Drs. Shinichiro Motohashi and Toshinori Nakayama *et al*. of Chiba University began two types of clinical trials in patients with lung cancer in 2001.[21)] One was a trial of dendritic cell therapy in which autologous dendritic cells induced from peripheral blood monocytes of the patient are pulsed with α-GalCer and then returned to the patient to activate iNKT cells. Overall survival was prolonged in patients whose response to administration of pulsed dendritic cells and in which numbers of IFN-γ-producing cells are increased as compared with patients without the same response.[22)] This therapy has been approved as a Highly Advanced Medical Therapy by the Japanese Ministry of Health, Labor and Welfare (as of March 2012). The second was a trial of administration of iNKT cells expanded *ex vivo* to enhance their functional recovery. Furthermore, similar clinical trials have been and are being carried out in patients with head and neck cancer in collaboration with Dr. Yoshitaka Okamoto in the Department of Otorhinolaryngology, Chiba University, with favorable results,[23–25)] as seen in the treatment of lung cancer.

Conclusion

When iNKT cells were discovered, their physiological activators were unknown, but these cells were expected to have diverse functions. Attention was first focused on cancer treatment utilizing glycolipid ligand-activated immunity, as evident from the clinical trials in progress. Given that recently found ligands are from infectious pathogens, the application of iNKT therapy may contribute to the treatment of infections with microbial pathogens. Furthermore, since iNKT cells are involved in promoting various inflammatory reactions, methods to control these reactions as well as expansion of iNKT cells should be devised in order to reduce excessive immunoreactions seen in autoimmune diseases, allergies, and other diseases.

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2012.09
144223-12091015N

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