The Elusive NKT Cell Antigen—Is the Search Over?

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onventional CD4+ and CD8+ T cells of the immune system recognize specific peptide antigens bound to major histocompatibility complex (MHC) class II or MHC class I molecules, respectively. In contrast, a specialized subpopulation of T cells called NKT cells recognizes glycolipid antigens presented by the MHC class I–like molecule, CD1d (1). NKT cells express both a conserved αβ T cell receptor (TCR) and natural killer (NK) cell receptors. These cells are important for suppressing autoimmunity and graft rejection, enabling resistance to infection, and promoting tumor immunity (2, 3). Yet surprisingly little is known about the specific endogenous antigens that NKT cells recognize. This is set to change with the report by Zhou et al. (4) on page 1786 of this issue. Through a combination of deduction and experimentation that unfolds like a detective story, these investigators identify the glycosphingolipid, isoglobotrihexosylceramide (iGb3), as a key endogenous NKT cell antigen.

During development in the thymus, NKT cells branch from the mainstream T cell precursor pool when they randomly generate a TCR that interacts with CD1d. CD1d presents endogenous glycolipid antigens that have been processed in lysosomes to NKT cells, and this presentation is necessary for efficient NKT cell development (see the figure) (5). Mature NKT cells display a perpetually activated/memory phenotype and low-level autoreactivity, which suggests the presence of endogenous CD1d-restricted antigens on the surface of antigen-presenting cells in the periphery (see the figure) (3, 5). In most studies of NKT cell function, these cells are stimulated with a synthetic glycosphingolipid called α-galactosylceramide, originally derived from a marine sponge (6). This molecule potently stimulates NKT cells in both mice and humans in a CD1d-dependent manner. In addition to providing valuable insights into the possible function of NKT cells, α-galactosylceramide is currently being tested in cancer patients (2, 3). However, because α-galactosylceramide is not a normal product of mammalian cells, a key question is whether equivalent mammalian glycolipid antigens exist, and if they do, whether they are involved in NKT cell development and activation.

In their study, Zhou and colleagues (4) demonstrate that mice deficient in the enzymes β-hexosaminidase A and B, which degrade glycosphingolipids in lysosomes, exhibit defective NKT cell development. Subsequent experiments narrowed down the possible causative abnormalities in these mice to an apparent deficiency in the production of lysosomal iGb3. Their data show that iGb3 is a broadly reactive agonist ligand for mature NKT cells that induces robust stimulation of these cells that is comparable to stimulation by α-galactosylceramide. Although these data suggest that iGb3 is a primary ligand for NKT cells, the authors do not exclude the possibility that other CD1d-restricted antigens (possibly mammalian, tumor, or microbial-derived) also activate NKT cells in the periphery (5).

Indeed, partial diversity in the β chain of the TCR of NKT cells implies that peripheral NKT cells may have multiple antigen specificities. This possibility is supported by the clonal expansion of an NKT cell subset in response to the disialoganglioside GD3 (7).

The Zhou et al. (4) provides multiple lines of evidence to suggest that iGb3 is an (possibly the) endogenous ligand for NKT cells. Yet the presence of this glycolipid in the thymus and peripheral lymphoid organs of mice and humans remains to be formally demonstrated. Probably the most contentious issue is whether iGb3 is an endogenous ligand for human NKT cells. This molecule contains a Galα1,3Gal carbohydrate linkage considered foreign to human immune cells as hu-
mans lack a functional α1,3Galactosyltransferase enzyme (8). Indeed, ~1% of human immunoglobulin G (IgG) reacts with Galα1,3Gal moieties, providing a major barrier to xenotransplantation (9, 10). However, the observation that human IgG does not react with iGb3 (4) suggests that this, or a closely related, glycolipid may not be considered foreign by human immune cells. Theoretically, this could result in the selective clonal deletion of human B cells with specificity for the Galα1,3Gal moiety in the context of iGb3. In further support of this possibility, formation of the Galα1,3Gal linkage in iGb3 is specifically controlled by the enzyme iGb3 synthase rather than by α1,3Galactosyltransferase (see the figure) (11). Furthermore, NKT cell autoreactivity against human dendritic cells can be blocked with the Galα1,3Gal-specific lectin, isolectin-B4 (4). These observations are at least consistent with the possibility that iGb3 is an endogenous ligand for NKT cells in humans as well as mice.

If iGb3 is an endogenous ligand for NKT cells, important questions and exciting possibilities emerge. From the standpoint of developmental biology: How do NKT cells undergo positive selection in the thymus in response to a ligand that activates them in the periphery? It is possible that iGb3 levels vary among tissues or among different cell types, or that costimulatory factors like interleukin-12 determine the extent to which NKT cells respond to this self antigen (5). Are the residual NKT cells in β-hexosaminidase-deficient mice selected by different glycolipid ligands, or might a few iGb3 molecules be loaded into CD1d independently of the lysosomal degradation pathway? An intriguing possibility is that variable levels of iGb3 may be responsible for determining the wide range in numbers of NKT cells observed between humans and distinct mouse strains (2, 3). In this context, it will be interesting to discover whether patients with Sandhoff disease, who lack β-hexosaminidase A and B (12), are deficient in NKT cells.

Regarding the development of potential therapeutics, it will be important to know whether iGb3 is involved in NKT cell–mediated immune suppression of autoimmune disease and tissue grafts, and whether this molecule, or related agonist compounds, could be used to enhance immunological tolerance. Conversely, if iGb3 contributes to destructive NKT cell activities such as the promotion of atherosclerosis or airway hypersensitivity (2), it may be possible to ameliorate these diseases by specifically blocking this ligand. It also will be important to discern whether levels of iGb3 in tumor cells correlate with NKT cell–dependent tumor rejection, and whether transplanting tumor cells with the gene encoding iGb3 synthase would generate more effective tumor vaccines.

As more is learned about the factors that determine NKT cell development and activity, we will improve our ability to manipulate these cells therapeutically. The identification of iGb3 as a mammalian NKT cell ligand is an important step in the right direction. Whether iGb3 is unique or just one of many other ligands that activate NKT cells is the next burning question to be answered.

References and Notes
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PLANEY SCIENCE

Proof for Water, Hints of Life?

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On 25 January of this year, NASA’s Opportunity rover landed on Mars’ Meridiani Planum, a smooth, flat plain unlike any feature studied by earlier martian landers. Eleven papers in this issue characterize Opportunity’s landing site in detail (1–11). The analyzed rocks mainly consist of iron oxides and hydrated magnesium, calcium, and iron sulfates; they were deposited in or altered by salty, acidic water, perhaps a sea (1, 2). Together with orbital observations (12), the reports for the first time document the geology and geochemistry of a martian hydrological event. The results indicate aqueous sedimentation or aqueous alteration and are consistent with models of a warmer, wetter martian past (12–15).

Opportunity was the last of a recent international armada of space probes to reach Mars. Just a few weeks before it touched down, its twin rover, Spirit, landed in Gusev crater, halfway around the planet. But previous landers, including Spirit, found only volcanic rock rubble and inorganic soils. Opportunity was the first to sample bedrock (see the figure). Sediments appear to have accumulated layer-by-layer and experienced episodic drying (2). The regular fine lamination (see figure, panels C, D, and G), bundled sets of laminae (panel G), thicker bedding on the meter scale (panels B and F), and much thicker layers (panel A) indicate several frequencies of cyclic deposition. If the fine-scale rhythm (panels C and D) is annual, and if it constitutes much of the 600-m-thick sedimentary rock record in Meridiani Planum, then this sequence could have formed in about 250,000 Mars years. Annual laminae would imply a seasonal response of the water mass, thin or no ice cover, and a much warmer climate than today’s or else ultracold concentrated acid solutions. Polygonal cracks (panels D, E, H, and I) suggest that hydrous deposition or alteration also played a role (2). It remains unclear whether the polygons formed in drying, salty mud or whether they resulted from subsequent salt dehydration or from repetitive freezing and thawing of ice.

The strata contain large fractions of magnesium, calcium, and iron sulfates, traces of chlorine, bromine, and phosphorus, and insoluble or weakly soluble iron oxides and aluminosilicate impurities (3–7). The salty outcrops are generally much softer than volcanic rocks examined by the Spirit rover in Gusev crater (8). They are softer than many terrestrial sedimentary rocks and are similar to highly hydrated salts in Earth’s ephemeral desert lakes. The outcrops have a spongy texture like that caused by dissolution or dehydration. The mineral jarosite detected at Meridiani Planum (6) requires highly acidic conditions (16–21). Other minerals that have been observed or modeled at the site are consistent with acid brine, as is the absence of calcium carbonate (which reacts to gypsum in acid sulfate solution). High sulfur-to-chlorine ratios and high iron contents of the nine salty rocks analyzed by Opportunity (5) suggest a relatively warm (~265 K) acid sulfate solution (18). The mineral assemblage and chemistry is typical of acid mine drainage systems affected by sulfide oxidation (19–21). To explain the huge amounts of sulfur, sulfate salts had to be in-

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