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Structure and Biology of Self Lipid Antigens

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Abstract Self lipid antigens induce selection and expansion of autoreactive T cells which have a role in immunoregulation and disease pathogenesis. Here we review the important biological rules which determine lipid immunogenicity. The impact of lipid structure, synthesis, traffic, membrane distribution and CD1 loading are discussed.

1 Introduction

T cells recognize glycolipids associated with CD1 antigen-presenting molecules. The CD1–glycolipid complexes are structurally related to MHC–peptide complexes and this may explain the capacity of the TCR αβ to interact with both types of complexes. Although important immunological properties of lipid-specific T cells remain to be disclosed, it is clear that certain aspects of their biology resemble those of peptide-specific T cells. This is the case for thymic selection, upregulation of chemokine receptors necessary for thymus

exit, acquisition of phenotype typical of naïve cells in the periphery and of memory or effector cells after antigen encounter and challenge.

T cells recognizing self glycolipids can be operationally divided into two groups. T cells restricted by CD1d which express a semi-invariant TCR, composed of the invariant Vα24-Jα18 and variable Vβ11 chains in humans (Vα14-Jα18 paired with Vβ8.2, Vβ7 or Vβ2 chains in mice) are included in the first group. Because of the conserved TCR structure, these cells are known as invariant NK T cells. NKT cells are activated by the neutral self glycosphingolipid isogloboside 3 (iGb3) (Zhou et al. 2004b) and by a variety of bacterial glycolipids such as α-glucuronosylceramide and α-galacturonosylceramide (Kinjo et al. 2005; Mattner et al. 2005). Both bacterial glycolipids are produced by *Sphingomonas*, bacteria which do not synthesize lipopolysaccharide (Kawahara et al. 1999) and which rarely cause disease in humans. NKT cells are also stimulated during infection with *Ehrlichia muris* (Mattner et al. 2005), another Gram-negative bacterium which does not produce LPS. It has been suggested that the immune system has evolved this type of recognition to promptly react to LPS-deficient bacteria. NKT cells also react to the sponge-derived glycolipid, α-galactosylceramide (α-GalCer), which behaves as a superagonist for this cell population. α-GalCer has been instrumental in studying responsiveness of NKT cells and their involvement in immunoregulation, as well as protection from infections and pathogenesis of autoimmune diseases (Godfrey and Kronenberg 2004).

T cells belonging to the second group express a variety of TCR heterodimers, apparently without bias for unique V or J genes, and they are restricted by CD1a, CD1b, CD1c and CD1d molecules. Among these diverse CD1-restricted T cells, evidence to date has not yet revealed a strong bias for CD4 and CD8 co-receptors in identifying subpopulations preferentially restricted to CD1 molecules, in contrast with T cells that recognize MHC molecules. Human CD1-restricted T cells may express CD4 or CD8 and, in some cases, are CD4 and CD8 double-negative.

A major question is whether lipid-specific T cells are a minor population of T cells or whether instead they present at high frequencies such that they could represent an effector arm of the immune response. A limited number of studies have addressed this important issue. In an initial report, it was found that human T cells reacting to self-glycosphingolipids are present in the circulating blood at the same frequency as classically MHC-restricted and peptide-specific T cells (Shamshiev et al. 1999). These initial studies have been confirmed by ongoing studies showing that T cells specific for sulfatides are present at frequencies of 1 in 2,000–10,000 T cells. These frequencies are very similar to those detected for myelin protein-specific and MHC class II-restricted T cells. Other studies have investigated the frequency of T cells rec-

ognizing microbial lipid antigens (Kawashima et al. 2003; Ulrichs et al. 2003) and have also shown that T cells with these specificities can be easily detected and isolated. Thus, lipid-specific T cells do not represent a unique and rare population of lymphocytes and are activated in immune responses against infectious microorganisms, tumour cells and are also involved in autoimmunity.

2 Nature of Self Lipids Stimulating T Cells

The two most prominent families of self lipids which stimulate T cells are sphingolipids and phospholipids. The structure of some immunogenic self lipids are shown in Figs. 1, 2 and 3. The sphingolipids gangliosides, sphingomyelin and sulfatides (Shamshiev et al. 1999, 2000) and the phospholipids phosphatidylcholine (PC), phosphytidylethanolamine (PE) and phosphatidylglycerol (PG) have been found to stimulate specific human T cells (Agea et al. 2005). Also, the ganglioside GD3, which is enriched in human tumours of neuroectodermal origin, stimulates specific T cells after immunization (Wu et al. 2003). Mouse T cells reacting to phosphatidylinositol (PI),

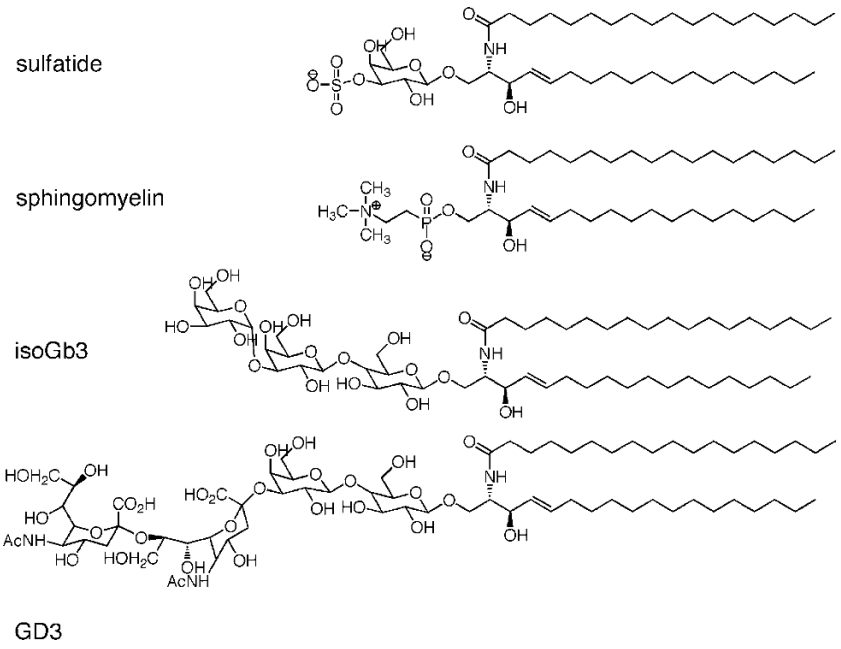

Fig. 1 Structures of immunogenic sphingolipids

PE and PG have also been described (Gumperz et al. 2000). NKT cells are reactive against the self iGb3 (Zhou et al. 2004b), which is generated during the lysosomal degradation of isogloboside 4. Importantly, the structure of the sphingoid base and of the associated fatty acids determine the half-life of the lipid, its distribution in the membranes and association with membrane proteins (Pomorski et al. 2001), thus also influencing the immunogenicity of the lipid (see below).

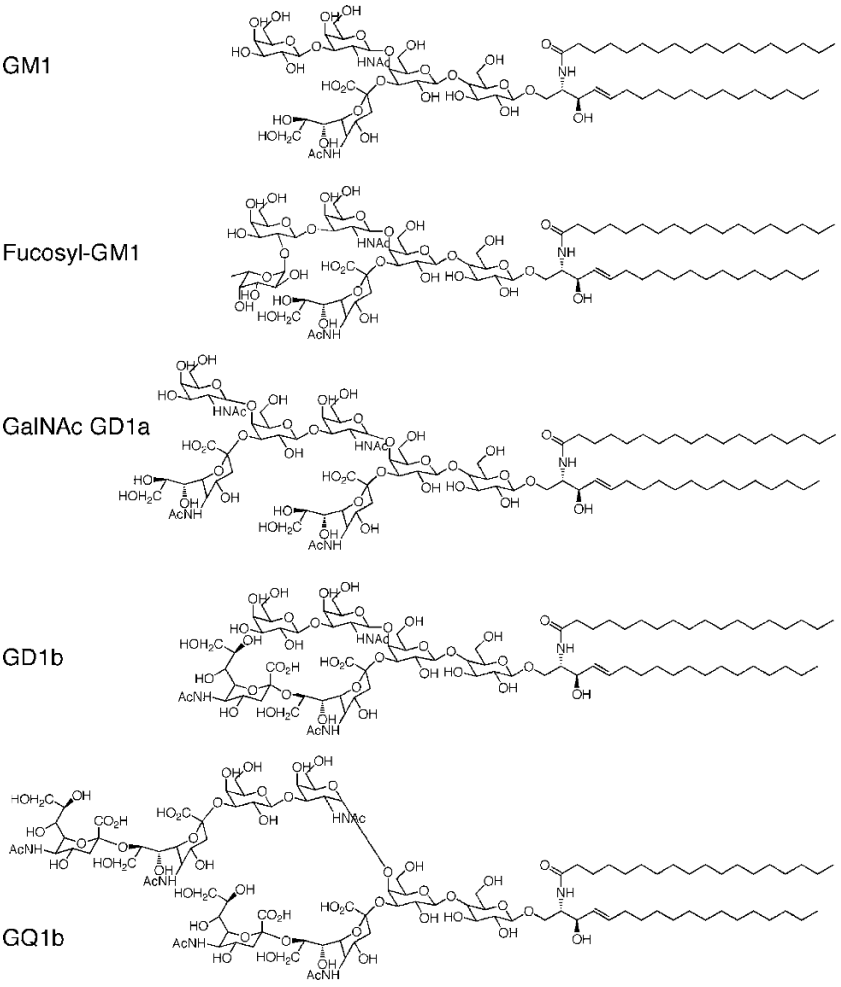

Fig. 2 Structures of immunogenic gangliosides

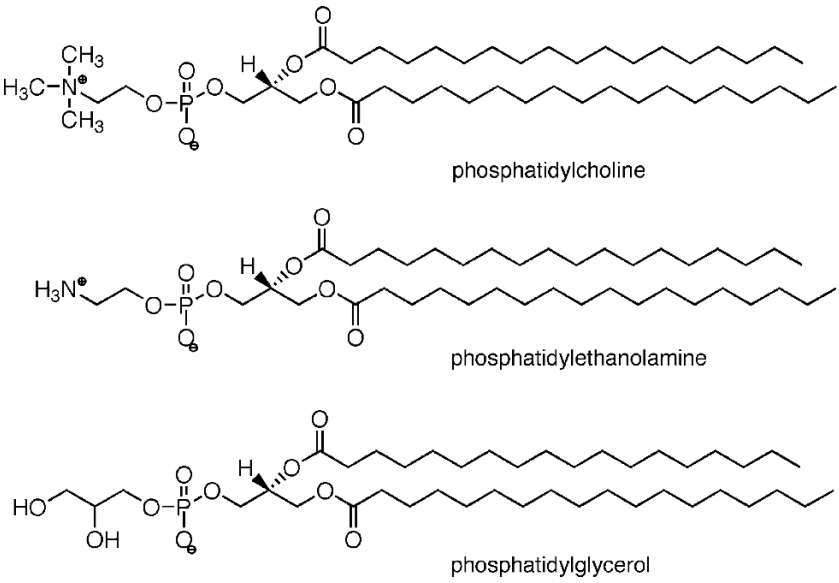

Fig. 3 Structures of immunogenic phospholipids

2.1 Structure of Self Lipids Stimulating T Cells

Sphingolipids have a backbone made of the basic alcohol sphingosine or a related long-chain base, which usually contains between 14 and 24 carbon atoms. Bases with up to two double bonds as well as one additional hydroxyl group may also be present. Sphingosine combines in amide linkage with a fatty acid to form a ceramide, which contains a free hydroxyl group combining with another component. When phosphate is added to ceramide, followed by choline, sphingomyelin is formed. This lipid is very abundant in all cell membranes. When glucose or galactose are added, glycosylceramides are generated. Synthases of mammalian cells link glucose or galactose monosaccharides to ceramide with a beta glycosidic bond, thus conferring a definable orientation to the polar head of the glycosphingolipid (GSL). This has important consequences with respect to antigen recognition by the TCR.

2.2 How the Structure of the Lipid Moiety Influences TCR Recognition

The hydrophobic part of GSL directly participates in the immunogenicity of GSLs through several discernible mechanisms. The importance of the acyl

chain structure was described in the model of ganglioside GM1 recognition (Shamshiev et al. 2000). When a series of GM1 analogues were tested, it was clear that the structure of the ceramide tail contributes to the efficacy of the lipid when measured in vitro. The lyso form of GM1, which lacks the acyl chain and bears only the sphingosine base, as well as the sugar component devoid of ceramide, are not recognized by GM1-specific T cells, probably because these compounds do not bind to CD1. The length of the acyl chain is also important because GM1 containing a C18 acyl chain is more stimulatory than the analogue bearing a C24 acyl chain. The fact that GSLs with longer acyl chains are less immunogenic is not a general rule, because in another model of self lipid recognition, sulfatide molecules bearing a C24 acyl chain are more immunogenic than the analogue bearing a C18 acyl chain. As both GM1 and sulfatide are presented by CD1b, this difference is not ascribed to the presentation molecule, but more likely to the specificity of individual TCR. The importance of the acyl chain structure has also been outlined in another model antigen, i.e. PE recognition by a CD1d-restricted mouse T cell hybridoma (Rauch et al. 2003). These T cells are activated by PE containing at least one unsaturated acyl chain, with the cis configuration of double bonds being mandatory. These structural requirements were associated with a more efficient binding to CD1d. Similar constraints apply to the response of human T cells which specifically recognize phosphatidylcholine from pollens and discriminate the length and saturation of acyl chains (Agea et al. 2005).

In the GM1 recognition model, it was also found that modifications to the sphingosine base, namely the presence of a sphinganine, which unlike sphingosine does not bear unsaturated bonds, also decreases GM1 immunogenicity. This finding suggests that the rigidity of the base is also important, although the mechanism by which these lipid alterations influence T cell activation is not yet fully clear. Sphinganine is less polar than sphingosine and thus might also modify the critical micelle concentration (CMC), so that the capacity of GM1 to be solubilized and therefore to be delivered to CD1 might be affected. The CMC is an important biophysical property of lipids, which has two consequences of immunological relevance. If lipids are aggregated in a form that prevents their transfer to CD1 as monomers, this phenomenon may lead to overestimation of the lipid concentration which activates T cells. Also, differing solubilities and CMCs may render the comparison of immunogenicity between lipid analogues difficult.

The presence of a less rigid lipid base may also affect the orientation inside CD1 and the stability of binding. This latter mechanism has been found to play a role when an analogue of α-GalCer with a truncated sphingosine, called OCH, was tested (Miyamoto et al. 2001; Oki et al. 2004). This ligand induces a strong IL-4 release by NKT cells and a weak IFN-ɣ response. This effect likely

depends on the fast off rate of OCH from CD1d and a reduced interaction with the TCR. As a consequence, activated T cells do not upregulate CD40 ligand, which is important for efficient IFN-γ secretion (Oki et al. 2005). Recent studies suggest that substitution of phytosphingosine with sphinganine in OCH also generates a compound which preferentially induces IL-4 secretion (Ndonye et al. 2005).

The importance of the lipid tail may have great relevance during immune responses against self lipids in vivo. Different tissues synthesize complex GSL using the most abundant fatty acids available inside their constitutive cells. This leads to the accumulation of GSL, which differ in their fatty acid composition and, therefore, may have immunologically different behaviours. Furthermore, the type of fatty acid used may change during ontogenesis and modifications of lipid metabolism occur after oncogenesis, viral transformation, cell activation and growth (Hakomori 1981). It is tempting to speculate that in these instances the immune system may be confronted with unusual GSL to which it is not tolerant and thus specific immunity is readily initiated.

The mechanisms of thymic selection and central tolerance of T cells recognizing self lipids have not yet been investigated. Mouse and human NKT cells are positively selected on double-positive thymocytes which express CD1d. Positive selection of NKT cells requires co-stimulation by a still unidentified member of the protein family called signalling lymphocytic activation molecule (SLAM). SLAM molecules are expressed by immature NKT cells and make homotypic interactions with other unidentified SLAM molecules expressed by CD4 and CD8 double-positive thymocytes. Upon this interaction, the SLAM protein expressed by NKT cells signals through a SLAM-associated protein (SAP) and contributes to further maturation of NKT cells (MacDonald and Schumann 2005). SAP is a key molecule, which probably facilitates recruitment of Fyn to the TCR. Indeed, in SAP-deficient patients and mice, either NKT cells or other non-MHC-restricted T cells do not develop (Borowski and Bendelac 2005). It is not clear whether T cells bearing a receptor interacting with self GSL undergo the same type of negative selection as peptide-specific T cells. Furthermore, it is not known how broad the repertoire of GSL in the thymus is, thus permitting induction of central tolerance to this type of antigenic lipids.

2.3 How the Structure of the Polar Part Influences TCR Recognition

The polar head of lipid antigens has two main functions. First, it makes direct interactions with residues of the alpha helices of CD1, thus assisting in forming stable CD1–lipid complexes, facilitating TCR interaction. Secondly,

it participates directly in the cognate interaction with the TCR. The specificity of recognition is therefore dependent on the presence of the antigen residues making both types of interactions. The positioning of the polar residues is also dictated by the structure of the lipid moiety, as outlined in the section above, and thus the final shape of the lipid–CD1 complex is influenced by both the polar and apolar parts of the antigen.

Some complex glycolipid antigens require trimming of their terminal sugars, in order to generate immunogenic molecules. This has been shown with a synthetic di-galactosylceramide molecule, which stimulates NKT cells only after the terminal galactose is cleaved, thus generating the stimulatory α-GalCer (Prigozy et al. 2001). A second example is the presentation of the mycobacterial hexamannosylated phosphatidyl-myo-inositol (PIM_6) antigens (de la Salle et al. 2005). PIM_6s are characterized by the presence of six mannoses, which are trimmed into the dimannosylated molecules (PIM_2) by the lysosomal α-mannosidase. PIM_2s are then presented by CD1b to specific T cells. Recently, the important role of CD1e has been identified in processing of PIM_6. CD1e is able to bind PIM_6 and facilitates its processing to PIM_2 by the enzyme acidic α-mannosidase present in lysosomes (de la Salle et al. 2005).

T cells recognizing processed self GSL have not been isolated so far. In the GM1 model, this antigen, which contains an large glycan, is recognized as an intact molecule and does not require any processing, as shown by the ability of recombinant CD1 proteins to present the antigen in the absence of APCs. Also, gangliosides which lack individual sugars present in GM1 are not recognized. Furthermore, more complex gangliosides, such as GD1a, GD1b, GT1b, and GQ1b are also recognized by the same T cells without processing (Shamshiev et al. 2000). These more complex gangliosides have additional sialic acid units, which make them more negatively charged as compared to GM1. The TCR of these specific T cells makes cognate interaction with the α-helices of CD1b (Melian et al. 2000) and hence it is unlikely that it binds to the sugars only. Furthermore, the sequence of the TCR complementarity determining region 3 (CDR3) has a length comparable to that of MHC-restricted TCR and, therefore, it is also unlikely that this CDR3 region forms a cavity accommodating the additional sialic acid sugars. Another possibility is that more distal sialic acid residues assume a position that is lateral to the CD1–TCR interface so that they do not sterically hinder the contacts between CD1, TCR and the sugars common to GM1. This model resembles recognition of long peptides associated with MHC class II molecules (Stern et al. 1994). This latter possibility is suggested by the fact that fucosyl-GM1 is not recognized by these T cells (Fig. 4). In this ganglioside, the terminal fucose is linked with an alpha 1–2 glycosidic bond, thus assuming a disposition very different

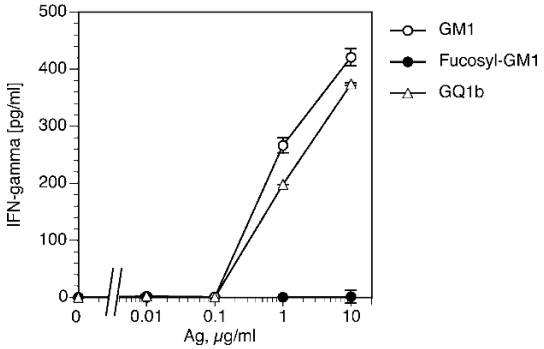

Fig. 4 Comparison of immunogenicity of gangliosides

from that of the sialic acid residues present in the other tested gangliosides. These results confirm the fine antigen specificity of the TCR and show that, like peptide-specific T cells, lipid-specific T cells also show a certain degree of cross-reactivity between different lipid antigens. Whether this cross-reactivity may lead to autoreactive responses remains to be investigated.

3 Where Glycolipids and Phospholipids Are Synthesized

The cellular localization of the enzymes which synthesize glycolipids and phospholipids influences the capacity of these lipids to bind to CD1 molecules.

The enzymes responsible for ceramide synthesis are located on the cytosolic membrane leaflet of the endoplasmic reticulum (ER) (Mandon et al. 1992). Then ceramide is transported to the trans-Golgi cisternae by a dedicated lipid transfer protein called CERT (Hanada et al. 2003) or is translocated inside the luminal membrane of ER in which it can be utilized to generate galactosylceramide in some cell types (van Meer and Holthuis 2000). Therefore, if ceramide binds to nascent CD1 molecules, a limiting step might be its translocation into the ER lumen. Ceramide is the common precursor of sphingomyelin and GSL. Sphingomyelin is predominantly synthesized in the luminal part of Golgi apparatus vesicles (Futerman et al. 1990) and is formed by the transfer of phosphorylcholine on the 1-hydroxyl group of ceramide. This requires the translocation of ceramide to the luminal leaflet of Golgi. After its synthesis, sphingomyelin traffics to the plasma membrane following the secretory pathway.

The Golgi apparatus is also the place where sugars are added to ceramide and GSL are synthesized. Glucosylceramide (GlcCer) is synthesized on the

cytosolic leaflet of the Golgi apparatus (Jeckel et al. 1992). A major fraction of newly synthesized GlcCer is rapidly transported to the plasma membrane by a non-Golgi pathway and then rapidly degraded (Warnock et al. 1994). Alternatively, GlcCer is translocated in the luminal leaflet of Golgi vesicles and further modified by addition of other sugars.

Lactosylceramide (LacCer) is the GSL synthesized after GlcCer by the addition of a galactose moiety and is the common precursor for the GSL series. LacCer synthesis, as well as synthesis of more complex glycosylated lipids which contain sialic acid, occurs on the luminal leaflet of Golgi membranes (Lannert et al. 1998). GM3 and GD3 synthesis occurs in early Golgi compartments, whereas complex gangliosides are predominantly synthesized in the *trans* Golgi (Allende et al. 2000; Giraudo et al. 1999). These GSL then reach the plasma membrane following the secretory pathway. Because GSL and sphingomyelin are synthesized in the Golgi lumen and are not substrates for lipid translocators, they are localized exclusively in the noncytosolic leaflet of membranes.

Phospholipids are synthesized in the endoplasmic reticulum. The enzymes involved in phospholipid synthesis have their active sites facing the cytosol, in which the required metabolites are present. After their synthesis, phospholipids move to the luminal leaflet of ER. This movement is very fast and is facilitated by scramblases, which thus equilibrate the distribution of phospholipids across the ER membrane. The luminal localization of phospholipids may explain their association with nascent CD1d (Giabbai et al. 2005; Zajonc et al. 2005) and CD1b (Garcia-Alles et al. 2006) molecules. In the plasma membrane, phospholipid flippases specifically remove phospholipids containing free aminogroups (PS and PE) from the extracellular leaflet, generating an asymmetric phospholipids composition (Holthuis et al. 2003). How phospholipids are loaded on CD1 molecules in lysosomes is not clear.

4 Regulation of Antigenic Self Lipid Synthesis

Several mechanisms appear to participate in the regulation of antigenic self lipid synthesis. Ganglioside generation is influenced by intrinsic properties of the sugar transferases such as their enzymatic kinetics, localization within the Golgi compartments, and the contiguous presence of other transferases. For example, N-acetyl-galactosyl-transferase and galactosyltransferase II form a complex, which accepts GM3 and generates GM1, without releasing GM2 (Giraudo et al. 2001). This may explain why GM2, which is an intermediate of GM1 synthesis, is poorly represented in membranes.

Another mechanism of glycolipid regulation is based on the relative abundance and activity of glycosyltransferases which have common substrates (Ruan and Lloyd 1992; Yamashiro et al. 1993). The presence of increased amounts of GM3 synthase leads to accumulation of GSL of the ganglioside series (Dumonceaux and Carlsen 2001; Prinetti et al. 2003). This may result in important changes in cell behaviour, including the capacity to adhere to other cells in vitro, formation of metastasis in vivo and resistance to fenretinide.

The physicochemical characteristics of each GSL, the trafficking capacity through membranes of different organelles and the availability of required sugar substrates, may also contribute to their relative abundance.

Finally, gene regulation of sugar transferases influences GSL synthesis. Indeed, in transformed cells and during ontogenesis transcription of transferase genes undergo profound changes, thus influencing the type of accumulating GSL. Also, feedback regulation on gene transcription exerted by accumulated GSL or phosphorylation of glycosyl transferases and the pH of their environment may affect GSL synthesis.

5
Lipid Traffic in the Cell

The ER and Golgi are the two organelles in which immunogenic self lipids are synthesized. Therefore, the mechanisms regulating lipid traffic from these cellular compartments to the membranes of other compartments are important because they regulate membrane lipid composition as well as the possible loading of CD1 molecules.

The ER is a highly dynamic center of lipid distribution. The lipids synthesized in the ER or moving to the ER may be efficiently transferred to other organelles through the combined action of different lipid transfer proteins (LTP) (Holthuis and Levine 2005). These proteins are characterized by the capacity to bind and transport lipid monomers across aqueous phases. Most LTP bind lipid with some degree of specificity, with a 1:1 stoichiometry, and have the capacity to extract lipids from membranes. Several LTPs are composed of domains conferring the lipid-binding capacity and of domains, which may exert functions such as recognition of proteins associated with other organelles, gene regulation or GTPase regulation.

The specific transfer between two compartments is assured by the presence of additional LTP protein domains, some of which are specific for the donor membrane and others for the acceptor membrane. This unique structure allows precise transport and also explains why transfer of bound lipid is rapid. The presence of two domains increases the affinity of binding to the

membranes and allows the formation of a bridge, thus avoiding random navigation in the cytoplasmic space. The effects of cytoplasmic LTP on lipid presentation to T cells have not been investigated and it is possible that LTP involved in trafficking of relevant self lipids may have important roles.

Another mechanism controlling lipid distribution in the membranes is provided by the biophysical characteristics of each lipid. For example, sphingolipids synthesized in the Golgi do not traffic to the ER and move only to the plasma membrane through anterograde transport vesicles. The reason why they are not incorporated in retrograde transport vesicles seems to be related to their capacity to form large numbers of hydrogen bonds, leading to formation of rigid lipid domains in the membrane of Golgi. Similar mechanisms may also occur in sorting lipids in the endosomal compartment. In early endosomes, lipids in more fluid microdomains more easily recycle to plasma membrane, whereas lipids in less fluid ones traffic to late endosomes. This may also explain why glycolipids with similar structures but differing in the length of the lipid tail traffic with different preferences: the ones with short lipid preferentially recycling in the early endosomal compartment, the ones with long lipids reaching the late endosomal compartment. An elegant example has been provided with analogues of the mycobacterial antigen glucose monomycolate which bears acyl chains of different length (Moody et al. 2002). Lipid differences in recycling may have important consequences for the loading of CD1 molecules. Indeed, phospholipids commonly recycling in early endosomes are efficiently presented by CD1a (Agea et al. 2005), which also recycles in this compartment (Moody and Porcelli 2003), whereas more complex GSL, such as gangliosides, reach late endosomes and are presented by CD1b (Shamshiev et al. 1999), recycling in this compartment.

Another important feature of late endosomes is the capacity to sort proteins and lipids into multivesicular bodies (MVB), small vesicles which bud towards the same endosomal lumen. Lipid composition of MVB is different from that of endosomal membranes, as they are enriched in cholesterol and the negatively charged lipids bis-(monoacylglycero)-phosphate and phosphatidylinositol-3-phosphate. Complex glycolipids which are degraded in lysosomes are sorted in the MVB membrane leaflet facing the endosomal lumen. This location allows their processing and might also aid in transfer to CD1 molecules, which recycle in the same compartment. Furthermore, in late endosomes LTP such as saposins and GM2-activator protein are present, which behave as liftases and facilitate GSL attack by hexohydrolases and GSL loading on CD1 molecules (Zhou et al. 2004a).

6
Where Self Lipids Are Loaded on CD1

The ER is the first compartment in which endogenous lipids may associate with CD1 molecules. During CD1d assembly, the microsomal triglyceride transfer protein (MTP) plays an important role in its stabilization (Brozovic et al. 2004), likely by promoting binding of phospholipids (Dougan et al. 2005) such as PC, which is associated with nascent CD1d (Giabbai et al. 2005). Whether MTP is also important in stabilization of other CD1 molecules is not clear.

During assembly, a subpopulation of CD1d molecules form complexes with the invariant chain (Ii) and MHC class II molecules (Jayawardena-Wolf et al. 2001; Kang and Cresswell 2002). This association drives this population of CD1d molecules to late endosomes and might have relevance during inflammation in which the cellular levels of Ii also change.

Recent data show that newly synthesized CD1d (Zajonc et al. 2005) and CD1b molecules (Garcia-Alles et al., submitted) may associate with spacer molecules, during their assembly inside ER. These spacers stabilize CD1d and CD1b during their traffic to the cell membrane and likely also prevent binding of lipids with long acyl chains. Therefore, it is unlikely that complex GSL, which are synthesized inside the luminal part of Golgi, associate with nascent CD1d and CD1b molecules. Upon reaching the cell surface, CD1 molecules recycle into late endosomal compartments in which the low pH facilitates their partial denaturation (Ernst et al. 1998) and loading with other lipid antigens.

An exception to this scenario is CD1a, which recycles in early endosomes. In this compartment, CD1a is proximal to lipid molecules that do not traffic further in deeper compartments because of the lack of tyrosine containing cytoplasmic tail motifs, which mediated internalization through binding to adaptor protein complexes. How self lipids might be extracted from early endosomal membranes is unclear, since no LTP such as saposins and GM2 activator are present in these organelles. One possibility is that lipoproteins present in serum, and which upon internalization are sorted in the recycling endosomes, may provide this function. This is supported by the finding that serum lipids control the maintenance of CD1a molecules with appropriate conformation on the cell surface (Manolova et al. 2006). In the absence of serum, CD1a molecules maintain their plasma membrane location, but are altered and lose the capacity to present lipid antigens. This behaviour does not depend on the recycling properties, since hybrid CD1a molecules expressing the CD1b cytoplasmic tail and recycling in late endosomes behave as wild type CD1a. Most likely the unique structure of CD1a accounts for

this dependence on exogenous lipid and lipoproteins. Indeed, the CD1a F′ pocket, which represents the portal through which lipids enter the groove, is partially open towards the upper part of the molecule facing the extracellular space (Zajonc et al. 2003). This may facilitate ready exchange of lipids when appropriate acceptors such as membranes or lipoproteins come in contact with the lipid-binding part of CD1a.

Complex glycolipids traffic in late endosomes where they are loaded on CD1 molecules present in the same compartment. This is facilitated by saposins and GM2 activator, which participate in glycolipid degradation as well as their loading on CD1 molecules. The importance of saposins in CD1 loading is outlined by a series of findings with both mouse and human CD1-restricted T cells. Mice lacking functional prosaposin gene do not develop normal numbers of NKT cells (Zhou et al. 2004a). Furthermore, presentation of α-GalCer is partially impaired when saposin-deficient APCs are used (Kang and Cresswell 2004; Zhou et al. 2004a). When saposins were tested in a CD1d loading assay *i*n vitro with sulfatide, saposins A and C were more efficient than saposin B, whereas saposin D was inactive (Zhou et al. 2004a). In a third study, it was found that CD1b-restricted presentation by human saposin-deficient dendritic cells of mycobacterial lipoarabinomannan, glucosylmonomycolate and mycolic acid was inefficient. Loading of these antigens required the presence of saposin C and not of saposin B (Winau et al. 2004). These studies suggest that each saposin may preferentially interact with individual CD1 proteins. However, this conclusion is not supported by the published data, and another possibility can be considered. Saposins and GM2 activator preferentially bind different types of lipids and therefore it is the type of lipid antigen to be loaded which selects the LTP involved in CD1 loading. For example, when mouse CD1d is loaded with sulfatide, saposins A and C are very efficient, whereas they are inactive in loading α-GalCer. Instead, GM2 activator, which binds α-GalCer, is very efficient in assisting the formation of the CD1d-α–GalCer complex (Zhou et al. 2004a).

7
Role of Self Glycolipids in Diseases

The identification of the possible role of self lipid-specific T cells in human diseases is a difficult task. First, there is a limited number of studies conducted in human diseases and therefore it is still premature to make final conclusions. Secondly, to investigate the role of these cells in vivo, it is important to investigate disease models in animals which can be manipulated. As small rodents do not express group I CD1 molecules, they have not been useful

for this purpose. However, mice have been instrumental in investigations into the role of NKT cells, which also recognize self lipids, and these studies have provided direct evidence of the important regulatory function of these cells. The function of NKT cells has already been appropriately reviewed (Bendelac et al. 1997; Godfrey and Kronenberg 2004; Kronenberg and Gapin 2002; Taniguchi et al. 2003; Van Kaer 2005) and this discussion focuses on how self lipid-reactive T cells restricted by group I CD1 molecules may have a role in human diseases.

7.1 Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease characterized by areas in the brain becoming demyelinated as result of autoimmune attack. Activated T cells accumulate at the borders of the lesions in the brain and in the perivascular spaces and are likely to contribute to persistence of inflammation. These T cells are mostly specific for myelin components such as myelin proteins and myelin lipids. In the circulating blood of MS patients, there is a high frequency of T cells recognizing gangliosides, sulfatide and sphingomyelin (Shamshiev et al. 1999). These self glycosphingolipids can be presented by all CD1 molecules expressed on the cell surface without apparent bias for any particular isoform (Shamshiev et al. 2002). The T cell response against self lipids appears to be more pronounced in patients with the primary progressive form of MS (Pender et al. 2003), which is also the more malignant form of this disease. Patients with primary progressive MS also develop high titers of anti-glycolipid antibodies in the CSF and serum (Acarin et al. 1996; Sadatipour et al. 1998). These findings support the hypothesis that myelin lipids are highly immunogenic in patients, and it is likely that the specific T and B cell immune responses are correlated with the progression of MS.

Another important finding is that mice with experimental allergic encephalomyelitis (EAE), a model of autoimmune disease which leads to brain lesions and paralysis and resembles MS in some respects, show increased numbers of CD1d-restricted and sulfatide-specific T cells. Interestingly, these T cells accumulate in the brain at the time of the disease peak, whereas in disease-free animals they are mostly present in the spleen (Jahng et al. 2004). If mice are immunized with sulfatide before EAE induction, a milder form of disease develops.

Additional data supporting the importance of self lipid-specific immunity is provided by the EAE model in guinea pigs, which, in contrast to mice, express group I CD1 molecules. EAE in guinea pigs is exacerbated (Kusunoki et al. 1988; Moore et al. 1984) or inhibited (Mullin et al. 1986) by injecting

gangliosides present in myelin. Self lipids also induce generation of specific antibodies in MS patients (Arnon et al. 1980; Endo et al. 1984; Kanter et al. 2006), in mice (Kanter et al. 2006) and in guinea pigs (Schwerer et al. 1984) with EAE.

An open question remains: How are self lipid-reactive T cells activated? One possibility is that some T cells cross-react with microbial lipoglycans. However, this remains a hypothesis, as there is no experimental evidence for this possibility. An alternative mechanism is that during infection there is a modulation of self lipid metabolism, which facilitates their synthesis and presentation by CD1 molecules. Indeed, when dendritic or monocytic cells are infected with different types of bacteria or stimulated with different bacterial products, they increase the de novo synthesis of glycosphingolipids and acquire the capacity to stimulate CD1-restricted and glycolipid-specific T cells (De Libero et al. 2005). Thus, infection promotes recognition of induced self lipids, which might result in disease exacerbation. This mechanism is observed independently of the bacteria used for infection and is in accordance with the findings that MS attacks are more frequent after infection, although there is no evidence of association with a unique infectious agent.

7.2 Guillaume Barré Syndrome

Guillaume Barré syndrome (GBS) is a postinfectious autoimmune neuropathy caused by the presence of autoantibodies cross-reacting with gangliosides present in myelin and in the lipopolysaccharides (LPS) of some *Campylobacter jejuni* strains (Willison and Yuki 2002), which usually only causes enteritis. Chemical analyses of the core oligosaccharides of neuropathy-associated *C. jejuni* strains have revealed structural homology with human gangliosides (Moran and Prendergast 2001). Serum antibodies against gangliosides are found in one-third of GBS patients but are generally absent in enteritis cases. It is assumed that the antibodies are induced by antecedent infection with *C. jejuni*, and subsequently react with nerve tissue, causing damage. Although there is still no evidence for a direct role of self lipid-specific T cells in this disease, the observation that most GBS patients produce IgG antibodies specific for lipid structures supports the possibility that these T cells might be present and help glycolipid-specific B cells to switch to Ig isotypes. That these patients might also have a genetic predisposition to the development of this type of autoimmune response remains an open possibility.

8 Conclusions

The capacity of T cells to react against self lipids shows the high plasticity of T cell recognition and has important consequences for immune response. The apparent lack of functional polymorphism in CD1 molecules raises questions concerning the evolutionary mechanism that forced this type of antigen recognition. The hydrophobic structures of the self glycolipids responsible for anchoring to CD1 molecules may have provided an important evolutionary constraint. However, it not clear why CD1 molecules have not acquired polymorphic residues on the two alpha helices which make cognate interactions with the TCR. Lack of polymorphism might simplify the design of novel types of vaccines inducing the proliferation of self lipid-specific T cells with immunoregulatory or protective immune functions.

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