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Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We thank K. J. L. Hammond for critical reading of the manuscript, and S. Sidobre, L. Sidobre, K. J. L. Hammond and A. Khurana for mCD1d protein. This work was supported by grants from the National Institutes of Health (to M.K., to C-H.W. and to M.T.). Y.K. was supported in part by the Yamada Science Foundation.

Competing interests statement The authors declare that they have no competing financial interests.

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Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections

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CD1d-restricted natural killer T (NKT) cells are innate-like lymphocytes that express a conserved T-cell receptor and contribute to host defence against various microbial pathogens[1,2]. However, their target lipid antigens have remained elusive. Here we report evidence for microbial, antigen-specific activation of NKT cells against Gram-negative, lipopolysaccharide (LPS)-negative alpha-Proteobacteria such as *Ehrlichia muris* and *Sphingomonas capsulata*. We have identified glycosylceramides from the cell wall of *Sphingomonas* that serve as direct targets for mouse and human NKT cells, controlling both septic shock reaction and bacterial clearance in infected mice. In contrast, Gram-negative, LPS-positive *Salmonella typhimurium* activates NKT cells through the recognition of an endogenous lysosomal glycosphingolipid, iGb3, presented by LPS-activated dendritic cells. These findings identify two novel antigenic targets of NKT cells in antimicrobial defence, and show that glycosylceramides are an alternative to LPS for innate recognition of the Gram-negative, LPS-negative bacterial cell wall.

CD1 is a family of β2-microglobulin-associated, major histocompatibility complex-like molecules that evolved to capture lipid antigens in different cellular compartments for display at the surface of antigen-presenting cells[1,2]. A variety of self and microbial lipid antigens can be presented by CD1a, b and c for specific recognition by αβ T cells expressing diverse T-cell receptors (TCRs). In contrast, CD1d is associated with an innate-like population of memory/effector, NK receptor-expressing NKT cells that predominantly use a conserved, semi-invariant mouse Vα14-Jα18/Vβ8 or human Vα24-Jα18/Vβ11 TCR, suggesting different modalities of activation. Like NK cells, NKT cells constitutively express messenger RNA but not protein for interferon-γ (IFN-γ), a hallmark of their poised effector stage[3]. NKT-deficient mice have impaired antimicrobial defence due in part to defective early IFN-γ secretion[4–6], but the mechanism of NKT cell activation during infection is only partially understood. *In vitro*, dendritic cells (DCs) pre-treated with *Salmonella typhimurium* extract induce the production of IFN-γ by NKT cells in a CD1d-dependent manner, indicating a requirement for CD1d-mediated presentation of antigen. DCs and high doses of semi-purified LPS or interleukin-12 (IL-12) also induced the production of IFN-γ by NKT cells in a CD1d-dependent manner, suggesting the unusual possibility that self-antigens might in fact serve as targets of NKT cells in some conditions. However, because studies so far have failed to identify the putative lipid targets of NKT cells, with the exception of phosphatidylinositolmannosides expressed by mycobacteria[7], the mechanisms of their *in vivo* recruitment during infection remain a matter of speculation.

In a co-culture system combining fresh, bone marrow-derived DCs (BMDCs) and CD1d tetramer-sorted NKT cells, the addition of various heat-killed bacteria induced substantial IFN-γ secretion by mouse NKT cells in a CD1d-dependent manner (Fig. 1a, left). Similar results were found with a human NKT cell line co-cultured with DCs derived from peripheral blood mononuclear cells (PBMCs), including the requirement for CD1d expression shown by antibody blocking (Fig. 1a, right). Whole spleen cell suspensions cultured in the presence of these heat-killed bacteria for six days showed a marked expansion and proliferation of NKT cells, only slightly inferior to that induced by pure α-galactosylceramide (αGalCer) KRN7000, a pharmacological NKT cell ligand (Fig. 1b and Supplementary Fig. S1). These bacteria included Gram-negative, LPS-positive *Salmonella typhimurium* and also, surprisingly, the Gram-negative *Ehrlichia muris*, a lethal pathogen which, unlike *Salmonella*, does not express LPS[8], as well as *Sphingomonas capsulata*, another Gram-negative LPS-negative member of the same class of alpha-Proteobacteria whose cell wall lipids have been extensively characterized[9] (Fig. 1). We tested the involvement of Toll-like receptors (TLRs) in these anti-microbial responses by examining *MyD88*$^{-/-}$, *Trif*$^{lps2/lps2}$ and *MyD88*$^{-/-}$*Trif*$^{lps2/lps2}$ spleen cells lacking one or both of the adaptors, MyD88 and Trif (also known as Ticam-1), necessary for TLR signalling[10]. In the whole spleen cell culture assay, *Salmonella*-induced IFN-γ was drastically reduced to 2–15% of control, on average, in the absence of either one or both TLR adaptors (Supplementary Fig. S2). In sharp contrast, the splenic IFN-γ response to LPS-negative *Ehrlichia* and *Sphingomonas* was largely independent of MyD88 and Trif. *CD1d*$^{-/-}$ spleen cells lacking NKT cells failed to respond to

Sphingomonas and *Ehrlichia*, whereas the response to *Salmonella* was only marginally reduced. Similarly, wild-type NKT cells co-cultured with MyD88-deficient DCs responded to *Sphingomonas* and *Ehrlichia* but not *Salmonella* (Fig. 2a). These results suggest that in total spleens exposed to heat-killed *Salmonella*, IFN-γ production is initiated after TLR signalling of antigen-presenting cells and subsequent recruitment of NKT cells as well as other cell types such as NK cells. In contrast, IFN-γ stimulation by *Ehrlichia* and *Sphingomonas* was primarily dependent on NKT cells and CD1d, with minimal contribution from TLRs.

We have recently identified the glycosphingolipid isoglobotrihexosylceramide (iGb3) as a major species of endogenous glycolipid antigens recognized by NKT cells in healthy, non-infected cells[11]. *Hexb* $^{-/-}$ DCs fail to generate iGb3 in the lysosome because they lack the β-hexosaminidase required to remove the terminal GalNAc of iGb4, the precursor to iGb3 (ref. 12). *Hexb* $^{-/-}$ DCs pulsed with heat-killed *Ehrlichia* or *Sphingomonas* were able to stimulate NKT cells as well as wild-type DCs. In contrast, *Salmonella*-pulsed *Hexb* $^{-/-}$ DCs did not stimulate NKT cells (Fig. 2b). The terminal Gal(α1–3)Gal disaccharide of iGb3 is specifically recognized by the lectin IB4[13]. This lectin also blocked the recognition of iGb3 but not αGalCer presented by CD1d to NKT cells (Fig. 2c), thus serving as a probe for endogenous ligand recognition by NKT cells. The lectin did not impair the stimulation of NKT cells by DCs pulsed with heat-killed *Ehrlichia or Sphingomonas*, consistent with direct recognition of a distinct microbial antigen. However, the lectin readily blocked stimulation by *Salmonella* (Fig. 2c). Together, the role of β-hexosaminidase and the blockade by Gal(α1–3)Gal-specific lectins define a sequence of three carbohydrates, β-hexosamine/Gal(α1–3)/Gal, which is highly characteristic of the isoglobo-series of endogenous glycolipids. Combined with the report that IL-12 or LPS can replace *Salmonella* in the DC-NKT co-culture system[6], our results identify the endogenous ligand iGb3 rather than a microbial antigen as the target of NKT cells in their response to *Salmonella* infection.

Ehrlichia and *Sphingomonas* are examples of Gram-negative bacteria that do not express LPS[8,9,14], and belong to the same class of alpha-Proteobacteria. Recent analysis of the *Sphingomonas* cell wall revealed the presence of a family of monoglycosylceramides that are substitutes for LPS[9]. Because of their structural analogies with KRN7000, a pharmacological ligand of NKT cells, we synthesized several of the *Sphingomonas* cell wall glycosylceramides and tested their immunological properties. Both α-glucuronosylceramide (PBS 30) and to a lesser degree α-galacturonosylceramide (PBS 59) (Fig. 3a) strongly activated mouse and human NKT cell proliferation as well as IFN-γ secretion, whereas control β-glucuronosylceramide (PBS 50) did not (Fig. 3b and data not shown). Tetramers of CD1d–α-glucuronosylceramide stained all human NKT cells and ~25% of mouse NKT cells (Fig. 3c). In clonal assays, however, 16/16 different mouse Vα14 hybridomas responded to both α-glucuronosylceramide and α-galacturonosylceramide, suggesting that tetramer staining underestimated the

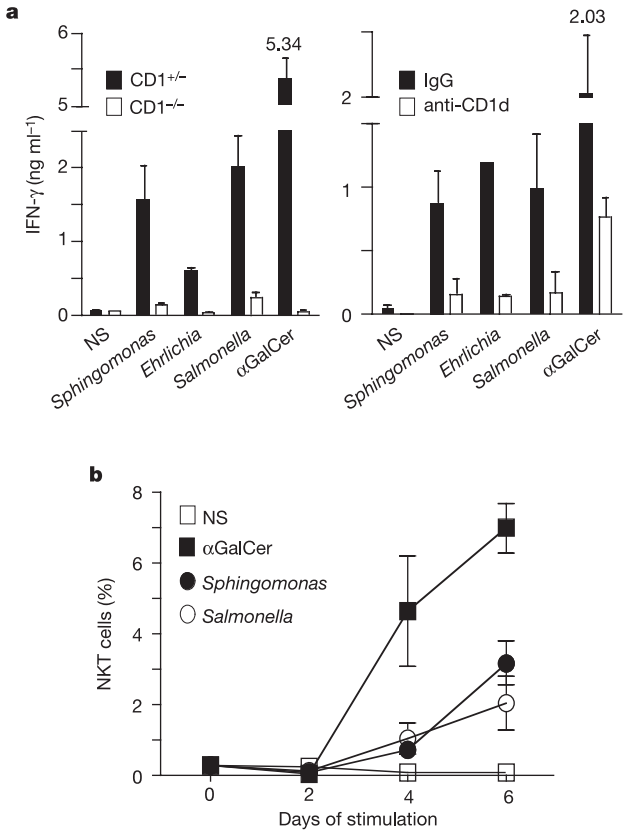

Figure 1 CD1d-restricted antimicrobial NKT cell responses. **a**, Left, purified mouse Vα14 NKT cells and *CD1* $^{+/-}$ or *CD1* $^{-/-}$ BMDCs were co-cultured in the presence of 5×10^6 heat-killed bacteria or 100 ng ml^{-1} αGalCer as indicated (NS, no bacteria), and IFN-γ release was measured after 48 h. Right, similar experiment with a human Vα24 NKT cell line and PBMC-derived DCs, in the presence of 1 μg ml^{-1} anti-CD1d or control IgG1. **b**, Whole spleen cells from B6 mice were stimulated for 6 days with 5×10^6 heat-killed bacteria or 100 ng ml^{-1} αGalCer, and the frequency of CD1d-αGalCer$^+$ NKT cells measured at indicated time points. Absolute numbers of lymphocytes were comparable for each stimulus and time point analysed. Data in **a** and **b** show means ± s.d. from three separate experiments.

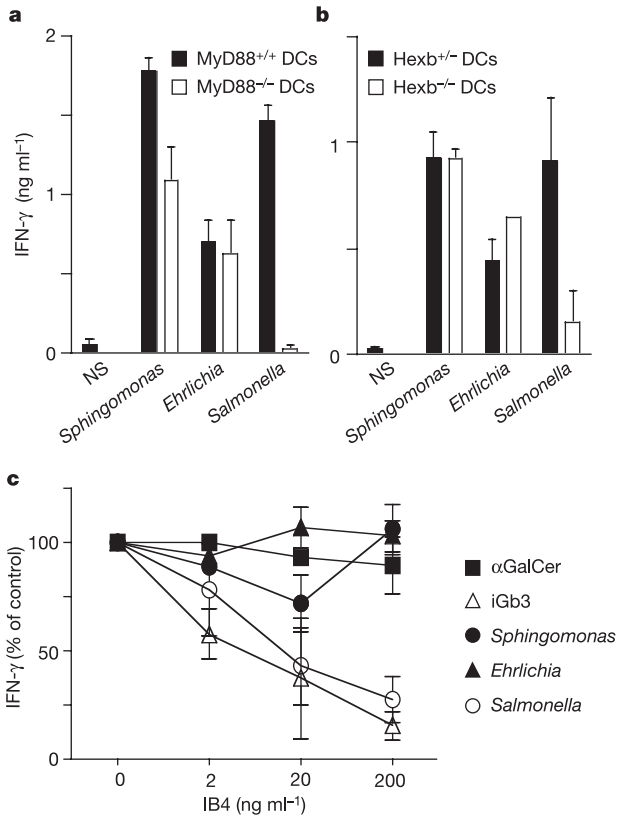

Figure 2 Differential requirements for the IFN-γ response to *Sphingomonas* and *Ehrlichia* versus *Salmonella*. **a**, **b**, IFN-γ released by purified mouse NKT cells cultured with *MyD88* $^{+/+}$ or *MyD88* $^{-/-}$ (**a**), *Hexb* $^{+/-}$ or *Hexb* $^{-/-}$ DCs (**b**) and heat-killed bacteria. Data show means ± s.d. from two or three separate experiments. **c**, Blockade of human NKT cell responses to DCs + antigen by the lectin IB4, which is specific for the [Gal(α1–3)Gal] epitope of iGb3 bound to CD1d. Points show means ± s.d. from two experiments.

frequency of responders (not shown). These findings reveal that the lipids replacing LPS in the cell wall of at least some Gram-negative bacteria have themselves become targets of innate immunity, as they are directly recognized by the conserved TCR of innate-like NKT cells.

We conclude therefore that the Gram-negative, LPS-positive bacterium *Salmonella* acts primarily through TLR activation of antigen-presenting cells, subsequently inducing NKT cells to produce IFN-γ through the combined stimulation by their endogenous iGb3 antigen and IL-12. NKT cells were just one of several IFN-γ-producing cell-types recruited by *Salmonella*, explaining why NKT-deficient mice do not appear to be particularly susceptible to *Salmonella* (data not shown). In contrast, Gram-negative, LPS-negative *Ehrlichia* and *Sphingomonas* activate NKT cells primarily through direct recognition of microbial lipids, which include but are not necessarily restricted to cell-wall glycosylceramides (in the case of *Sphingomonas*). These microbial

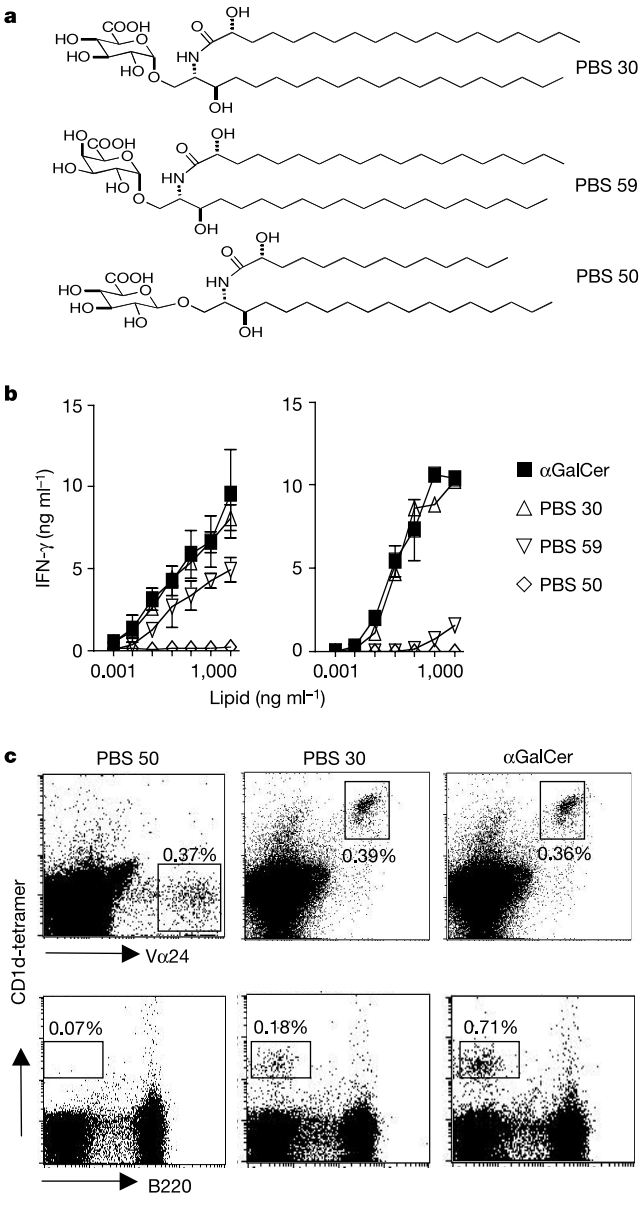

Figure 3 Different lipid antigens stimulate NKT cells during microbial infections. **a**, Structures of synthetic *Sphingomonas* cell wall antigens PBS 30 and PBS 59. PBS 50 is a control β-glucuronosylceramide. **b**, IFN-γ response of a human Vα24-Jα18 NKT line (left) and fresh purified mouse NKT cells (right) stimulated by the indicated lipid antigens and DCs. Points shown means ± s.d. from two experiments. **c**, CD1d-tetramer staining of fresh human NKT cells (upper row) and fresh mouse spleen cells (lower row) with indicated lipid antigens. NKT cell gate and percentage as indicated. Similar staining results were obtained for fresh or cultured NKT cells from three individuals.

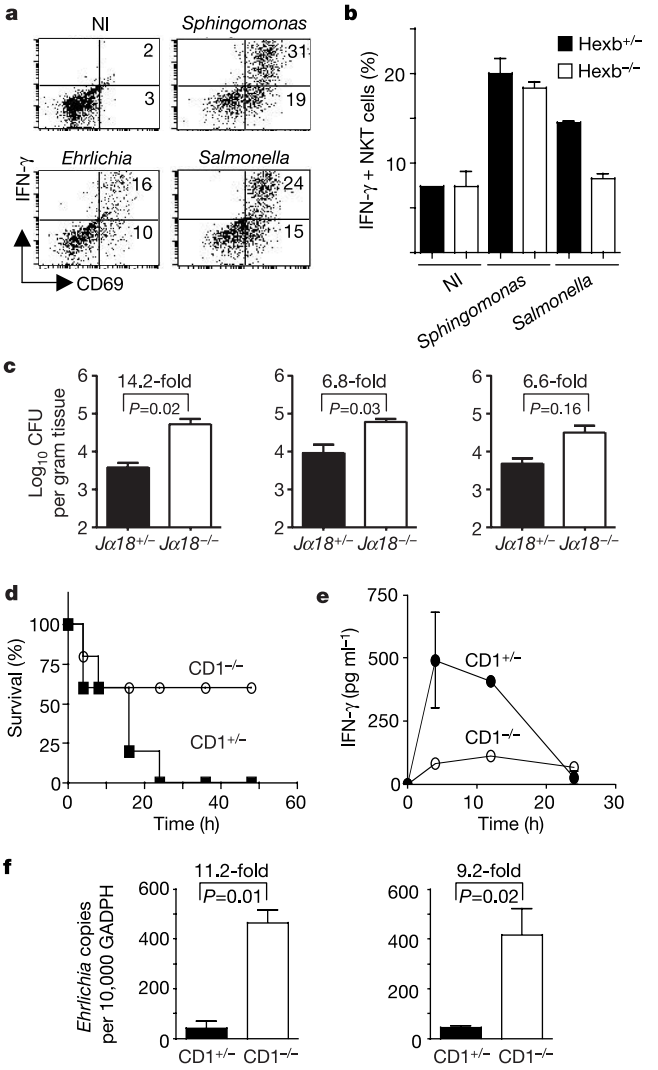

Figure 4 *In vivo* role of NKT cells during microbial infection. **a**, *In vivo* activation of NKT cells 24 h after intravenous infection with *Sphingomonas* (1×10^7), *Ehrlichia* (1×10^8) and *Salmonella* (1×10^6). NI, not infected. NKT cells gated as tetramer$^+$ B220$^-$ cells were analysed for surface CD69 and intracellular IFN-γ. Similar results were obtained in two experiments. **b**, IFN-γ production by NKT cells in response to *Salmonella* requires a *Hexb*-sufficient host. CFSE-labelled Vα14 transgenic thymocytes were injected intrasplenically 2 h after intraperitoneal challenge with 5×10^6 *Sphingomonas* or *Salmonella*. Intracellular staining for IFN-γ was performed one day after infection and results are shown as the percentage of IFN-γ$^+$ cells among NKT cells (mean ± s.d.). The difference between *Hexb* $^{+/+}$ and *Hexb* $^{-/-}$ was significant for *Salmonella* ($P = 0.001$). Three mice per group were analysed, and similar results obtained in two independent experiments. **c**, Bacterial burden in the lungs of *Jα18* $^{+/-}$ and *Jα18* $^{-/-}$ mice on days 1 (left), 3 (middle) and 5 (right) post-infection with 5×10^6 CFU of *Sphingomonas* (each bar represents mean ± s.d. of four to five mice). Fold increase and *P* values are indicated. One experiment representative of three is shown. **d**, Acute lethality after inoculation of a high dose of 5×10^8 *Sphingomonas capsulata* to *CD1d* $^{+/-}$ versus *CD1d* $^{-/-}$ mice ($n = 24$ each, $P < 0.0001$). **e**, Acute serum release of IFN-γ after infection with 1×10^7 *Sphingomonas capsulata* in *CD1d* $^{+/-}$ and *CD1d* $^{-/-}$ mutant mice. Similar results were obtained in two independent experiments. **f**, *Ehrlichia* PCR counts in spleens of *CD1d* $^{+/-}$ and *CD1d* $^{-/-}$ mice at 2 days (left) and 7 days (right) post-infection. Data represent mean ± s.d. from three mice; one experiment representative of two is shown. Fold increase and *P* values are indicated.

lipids subsequently induce DC activation, probably through CD40L/CD40 interaction, as reported for αGalCer[15]. We confirmed the early activation of NKT cells and their secretion of IFN-γ within 24 h of infection by each of these bacteria *in vivo* (Fig. 4a). Further, we examined the response to *Salmonella* and *Sphingomonas* by NKT cells transferred into *Hexb*$^{-/-}$ mice lacking lysosomal iGb3, and found that the response to *Salmonella* was selectively abrogated (Fig. 4b). These results are in direct support of the proposed model of NKT cell activation by iGb3 in the case of *Salmonella* infection.

To test the central role that NKT cells might play *in vivo* against some Gram-negative, LPS-negative bacteria, we intravenously infected NKT-deficient mice and their littermate controls with *Sphingomonas*, for which the NKT cell antigens are well defined. After infection with 1×10^6 or 5×10^6 bacteria, both *CD1d*$^{-/-}$ and *Jα18*$^{-/-}$ mice had delayed bacterial clearance compared with heterozygous littermate controls, with up to 12–14-fold higher bacterial load in the lung at early time points; this demonstrates the antimicrobial function of NKT cells (Fig. 4c and Supplementary Fig. S3). Infection with a high dose of 5×10^8 *Sphingomonas* colony-forming units (CFU) was rapidly lethal in all the wild-type mice, but in contrast, a majority of NKT-deficient mice survived (Fig. 4d and Supplementary Fig. S4). The lethal outcome in wild-type mice was associated with the explosive release of IFN-γ and IL-12 in the serum, whereas NKT deficient mice released significantly less of the two cytokines (Fig. 4e and Supplementary Fig. S5). These results demonstrate that NKT cells mediate septic shock in response to high doses of *Sphingomonas*. Similarly, NKT-deficient mice are unable to clear *Ehrlichia* (Fig. 4f and Supplementary Fig. S6).

This study reveals two very different strategies of microbial recognition by NKT cells. For the Gram-negative, LPS-positive bacterium *Salmonella*, microbial invasion was detected by TLRs expressed on DCs, and NKT cells functioned downstream of this primary event, side by side with NK cells, through the recognition of their endogenous (self) ligand iGb3. For the Gram-negative, LPS-negative *Sphingomonas*, microbial invasion was directly detected by NKT cells through specific recognition of cell-wall glycosylceramides in the place of LPS. Although *Sphingomonas* is capable of inducing septic shock in immunocompromised humans, it is reported to be an infrequent pathogen[16]; however, recent findings in primary biliary cirrhosis have suggested that chronic infection might underlie severe liver inflammation associated with NKT cell redistribution[17,18]. Our findings suggest that similar mechanisms of direct, acute NKT cell activation by microbial cell-wall lipids may operate for other alpha-Proteobacteria, including *Ehrlichia*, a severe natural pathogen in mice and humans[19]. Other *Rickettsiales*, which belong to the same class of alpha-Proteobacteria, also lack peptidoglycan and LPS[8,9,14] and appear to be controlled by TLR2-, TLR4- and MyD88-independent mechanisms[20]. Thus, it is tempting to speculate that NKT cells might be specifically involved in immunity against some microbial pathogens lacking cell-wall ligands for TLRs. These novel mechanisms of NKT cell activation highlight their dual antimicrobial functions, monitoring both endogenous and exogenous glycosphingolipids in various infectious settings. □

Methods

Mice

CD1d$^{-/-}$ and *Trif*$^{lps2/lps2}$ mice were generated in our laboratories, *MyD88*$^{-/-}$ were from S. Akira, *Jα18*$^{-/-}$ were obtained from M. Taniguchi and *Hexb*$^{-/-}$ were from R. Proia. All mice were in the C57Bl/6 background. In all cases, littermates obtained from heterozygous matings were genotyped by polymerase chain reaction (PCR) and used for comparative analysis. All mice were raised in a specific pathogen-free environment at the University of Chicago, according to the Institutional Animal Care and Use Committee guidelines.

CD1d-restricted T cell responses

Lymphocyte preparations, cell staining and sorting with CD1d–glycolipid tetramers were performed as described[21,22]. *Griffonia simplicifolia* isolectin B4 (IB4) was from Vector Laboratories, and anti-human CD1d monoclonal antibody 51 was obtained from S. Porcelli. The human Vα24 NKT cell lines were derived from peripheral blood lymphocytes (PBLs) stimulated with αGalCer. Stimulation assays were performed with whole spleen cells (5×10^5 per 200 μl well) or with purified T cells and antigen-presenting cells. T cells were either sorted CD1d-αGalCer$^+$ mouse spleen cells (5×10^4 per 200 μl well), human PBLs (5×10^5 per 200 μl well, obtained after Ficoll centrifugation of heparinized blood) or human NKT cell lines (2.5×10^5 per 200 μl well). Antigen-presenting cells for mouse assays were bone marrow-derived DCs (2.5×10^5 per 200 μl well) cultured with granulocyte–macrophage colony-stimulating factor (GMCSF) and IL-4 (100 ng ml^{-1} each, R&D Systems); for human assays, human irradiated allogeneic PBMCs (2×10^5 per 200 μl well) were used, either fresh or cultured for 5 days with GMCSF and IL-4 (referred to as DCs) (Vα24 NKT lines do not respond to allogeneic MHC antigens). Cells were washed twice and starved for 6 h in medium alone before addition to the stimulation experiments. T cells were stimulated for 48 h with the indicated lipid concentrations in 96-well round-bottom plates containing RPMI 1640 (Biofluids) supplemented with glutamine, antibiotics, 5×10^{-5} M 2-mercaptoethanol and 10% FCS (mouse studies) or 5% human AB serum (human studies). Concentrations of mouse and human IFN-γ in the supernatant were measured using the respective enzyme-linked immunosorbent assay (ELISA) kits (BD Bioscience, lower detection limit of 12.5 pg ml^{-1}).

Bacterial strains

Sphingomonas capsulata (American Type Culture Collection 14666) and *Salmonella typhimurium* R71 were grown in Mueller-Hinton agar. *Ehrlichia muris* were prepared as described[23]. Bacteria were heat-killed by 2-h exposure to 74 °C, and 2.5–5 × 10^6 CFU-equivalents per well were used for *in vitro* stimulation.

Live infection experiments

Sphingomonas and *Salmonella* were grown overnight in Mueller-Hinton broth, diluted in fresh medium, grown for 8 h at 37 °C to an absorbance of 0.5 at 600 nm, washed and diluted in PBS buffer. 5–7-week-old *CD1*$^{+/-}$ and *CD1*$^{-/-}$, or *Jα18*$^{+/-}$ and *Jα18*$^{-/-}$, or *Hexb*$^{+/-}$ and *Hexb*$^{-/-}$ littermates were intravenously inoculated with 100 μl of bacterial suspension. For survival experiments, dead/moribund (euthanized) mice were recorded every 2–4 h. At indicated time points after infection, bacterial counts were performed after tissue homogenization in 0.5% Triton X-100 and cultured for colony formation. For *Ehrlichia* infection experiments, mice were infected intraperitoneally with 500 μl of a 10^{-1} dilution of the *Ehrlichia muris* stock as described[23].

Determination of *Ehrlichia* cell numbers

The *Ehrlichia* load in tissues was determined by real-time PCR of the *Ehrlichia dsb* gene as described previously[23]. Results were normalized to GAPDH levels in the same sample and expressed as numbers of *Ehrlichia* copies per 10^4 GAPDH copies.

NKT cell transfers

5×10^6 Vα14 TCR transgenic thymocytes[24] were labelled with CFSE (carboxyfluorescein diacetate succinimidyl ester) and transferred in a volume of 50 μl directly into the spleen of *Hexb*$^{+/-}$ and *Hexb*$^{-/-}$ littermates.

Lipid antigens

The synthesis of *Sphingomonas* lipids (PBS 30 and PBS 59) and control PBS 50 will be described elsewhere. Synthetic compounds were characterized via ^{1}H- and ^{13}C-NMR spectroscopy and mass spectroscopy; results were consistent with the published structures of the *Sphingomonas* glycolipids. Purity was assayed using thin-layer chromatography and ^{1}H-NMR spectroscopy.

Flow cytometry

CD1d–lipid tetramers were generated as described[21]. CFSE labelling and intracellular cytokine staining were performed according to manufacturer's instructions (Molecular Probes and BD Pharmingen). Anti-B220 and anti-CD69 (mouse) antibodies were from BD Pharmingen and anti-Vα24 (human) antibodies were from Beckman Coulter. Cells were analysed using a FACSCalibur (BD Biosciences) machine with CellQuest software.

Received 13 December 2004; accepted 24 January 2005; doi:10.1038/nature03408.

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Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We thank D. Wei for reading the manuscript, K. Thompson for help with biochemical characterization of *Sphingomonas* and growth of bacteria, S. Porcelli for the gift of anti-human CD1d, R. Duggan, J. Marvin and B. Eisfelder for cell sorting, L. Taylor for managing the mouse colonies, and the University of Chicago Digestive Disease Research center for equipment. Supported by NIH grants to A.B., P.S.B. and L.T., an NIH award to K.L.D. and O.S., an NIH grant to B.B., a Cancer Research Institute fellowship to J.M. and D.Z., and a fellowship from the Fondation pour la Recherche Medicale to P.S.-M.

Authors' contributions P.B.S. and A.B. are co-senior authors.

Competing interests statement The authors declare that they have no competing financial interests.

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Fission yeast Mes1p ensures the onset of meiosis II by blocking degradation of cyclin Cdc13p

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Meiosis is a special form of nuclear division to generate eggs, sperm and spores in eukaryotes. Meiosis consists of the first (MI) and the second (MII) meiotic divisions, which occur consecutively. MI is reductional, in which homologous chromosomes derived from parents segregate. MI is supported by an elaborate mechanism involving meiosis-specific cohesin and its protector[1]. MII is equational, in which replicated sister-chromatids separate as in mitosis. MII is generally considered to mimic mitosis in mechanism. However, fission yeast Mes1p is essential for MII but dispensable for mitosis. The *mes1-B44* mutant arrests before MII[2]. Transcription of *mes1* is low in vegetative cells and boosted in a narrow window between late MI and late MII[3]. The *mes1* mRNA undergoes meiosis-specific splicing[4]. Here we show that Mes1p is a factor that suppresses the degradation of cyclin Cdc13p at anaphase I. Mes1p binds to Slp1p, an activator of APC/C (anaphase promoting complex/cyclosome), and counteracts its function to engage Cdc13p in proteolysis. Inhibition of APC/C-dependent degradation of Cdc13p by Mes1p was reproduced in a *Xenopus* egg extract. We therefore propose that Mes1p has a key function in saving a sufficient level of MPF (M-phase-promoting factor) activity required for the execution of MII.

MPF is a complex of Cdc2 kinase and M-phase cyclin Cdc13p. To investigate MPF dynamics during meiosis in wild-type and *mes1Δ* cells, we analysed zygotes (h^{90}/h^{90}) expressing Cdc13p tagged with green fluorescent protein (GFP) at various stages of meiosis (Fig. 1a–c). Figure 1a shows a profile of Cdc13p-GFP in wild-type zygotes. Each cell was assigned to a specific meiotic phase according to the number of nuclei and the length of the spindle(s) revealed by CFP-tubulin. Typical examples for metaphase I, anaphase I, metaphase II and anaphase II are displayed. At metaphase I, Cdc13p-GFP was detected in the nucleus, especially on the spindle. It remained in the nucleus until anaphase I. This contrasted with mitosis, during which Cdc13p disappears at anaphase[5]. At metaphase II, Cdc13p-GFP was again detected on the spindle. It disappeared completely from the nucleus at anaphase II. These observations confirmed a previous report that MPF activity is maintained from the initiation of MI to the end of MII in fission yeast[6]. *mes1Δ* zygotes showed the same Cdc13p-GFP localization until metaphase I, but all Cdc13p-GFP disappeared from the nucleus at anaphase I (Fig. 1b). Quantitative analysis of Cdc13p-GFP fluorescence per cell indicated that wild-type cells kept half of Cdc13p remaining at anaphase I, whereas *mes1Δ* cells lost most of it (Fig. 1c).

We then measured Cdc2 kinase activity during synchronous meiosis induced by thermal inactivation of Pat1 kinase in pre-starved homozygous (h^-/h^-) diploid cells, using histone H1 as a substrate. We monitored the progression of meiosis by counting the number of nuclei in a cell (Fig. 1d, e). In wild-type cells, the activity increased at the onset of MI (3.75 h) and remained high until early MII (4.5 h) (Fig. 1d, f), as reported[6]. In contrast, the activity seemed to decrease after MI in *mes1Δ* cells (Fig. 1e, f). In immunoblotting, Cdc13p disappeared about 20 min earlier in *mes1Δ* than in wild-type cells (Fig. 1d, e). Mes1p tagged with three copies of a haemagglutinin epitope (3HA), expressed from the authentic *mes1* promoter, reached its peak in about late MI/early MII (Fig. 1d). Cdc2p was equally abundant throughout meiosis in *mes1Δ* and wild-type cells, and its phosphorylation at tyrosine 15, a measure of the kinase activity[7], was indistinguishable between them (Fig. 1d,e).

We attempted to raise the level of Cdc13p in *mes1Δ* cells by using a plasmid pP*mes-cdc13*, which carried the *cdc13* open reading frame under the control of the *mes1* promoter. *mes1Δ* cells transformed with pP*mes-cdc13* produced four-spored asci efficiently, whereas control cells carrying the vector pP*mes* were arrested at MII (Fig. 2a). We found that pP*mes-cig2*, carrying the *cig2* open reading frame[8], could also suppress *mes1Δ* (Fig. 2a). The *cig2* gene, encoding a G1/S cyclin, is known to be expressed at about MII, although it is not strictly essential for MII[6]. Immunoblot analysis indicated that Cig2p was slightly less abundant and disappeared earlier in *mes1Δ* cells (Fig. 1d, e). Efficient suppression of *mes1Δ* by the supply of either Cdc13p or Cig2p suggested that the central role of Mes1p might be to maintain cyclin between MI and MII.

CORRIGENDUM

doi:10.1038/nature04475

Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections

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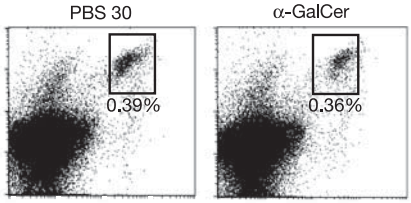
Nature 434, 525–529 (2005)

Figure 3 of this Letter contains an inadvertently duplicated panel: the PBS 30 panel is identical to the αGalCer panel (top right). The corrected panels are shown here. Our results and conclusions are unaffected by this oversight.



CORRIGENDUM

doi:10.1038/nature04484

Genome sequencing in microfabricated high-density picolitre reactors

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Nature 437, 376–380 (2005)

The following were omitted from the original author listing: Alex de Winter, James Drake, Robin Forte, Steve Hutchinson, William L. Lee, Michael Reifler and David A. Willoughby. These names are included in the revised authorship shown here and either were or are at 454 Life Sciences Corporation, Branford, Connecticut 06405, USA.

CORRIGENDUM

doi:10.1038/nature04572

Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*

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Nature 438, 1151–1156 (2005)

There are two errors in the author listings for this Letter: the surname of Anne Lafon was misspelt as 'Lafton' and the affiliation of Hiroyuki Horiuchi should have been number 16 (and not 15, as was published).

ERRATUM

doi:10.1038/nature04476

Regulated cell-to-cell variation in a cell-fate decision system

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Nature 437, 699–706 (2005)

In Fig. 1b of this Article, the *x* axis of the right-hand plot should be labelled 'α-Factor system output in each cell (CFP F.U. × 10^6)' and not 'ACT1 system output in each cell (CFP F.U. × 10^5)'. In addition, Supplementary Fig. S6 was incorrect as originally published and was replaced on 26 January 2006.