LETTERS

Apolipoprotein-mediated pathways of lipid antigen presentation

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Peptide antigens are presented to T cells by major histocompatibility complex (MHC) molecules, with endogenous peptides presented by MHC class I and exogenous peptides presented by MHC class II. In contrast to the MHC system, CD1 molecules bind lipid antigens that are presented at the antigen-presenting cell (APC) surface to lipid antigen-reactive T cells[1]. Because CD1 molecules survey endocytic compartments[2], it is self-evident that they encounter antigens from extracellular sources. However, the mechanisms of exogenous lipid antigen delivery to CD1-antigen-loading compartments are not known. Serum apolipoproteins are mediators of extracellular lipid transport for metabolic needs[3]. Here we define the pathways mediating markedly efficient exogenous lipid antigen delivery by apolipoproteins to achieve T-cell activation. Apolipoprotein E binds lipid antigens and delivers them by receptor-mediated uptake into endosomal compartments containing CD1 in APCs. Apolipoprotein E mediates the presentation of serum-borne lipid antigens and can be secreted by APCs as a mechanism to survey the local environment to capture antigens or to transfer microbial lipids from infected cells to bystander APCs. Thus, the immune system has co-opted a component of lipid metabolism to develop immunological responses to lipid antigens.

Serum, lymph and interstitial fluid are the physiological contexts in which extracellular lipid antigens are encountered by CD1-bearing antigen presenting cells *in vivo*. Lipid-transport particles termed lipoproteins are a major constituent of these extracellular compartments[3], yet their role in CD1 antigen presentation has not been investigated. To assess their contribution to the immune recognition of lipids, we first used an *in vitro* system in which human dendritic cells (DCs) were cultured with CD1-presented lipid antigens in human serum to measure their uptake and stimulatory capacity for T cells. We used a model CD1d-presented glycolipid, galactosyl(α1-2)galactosyl ceramide (GGC) because this antigen strictly requires uptake and delivery to lysosomes where it is converted to the active antigenic form, α-galactosyl ceramide (α-GC)[4]. GGC was allowed to distribute into human serum, after which we separated the various serum fractions to resolve the major lipoprotein components of serum, namely very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The fractions were then assayed for their ability to stimulate α-GC-reactive, CD1d-dependent natural killer (NK) T cells. The antigenic activity of GGC was distributed almost entirely in the VLDL fraction of human serum (Fig. 1a). VLDL is a complex of lipids and apolipoproteins, including apolipoprotein B (apoB) and apolipoprotein E (apoE), which independently participate in receptor-mediated uptake into cells[5,6]. Only antibodies against apoE effectively blocked the

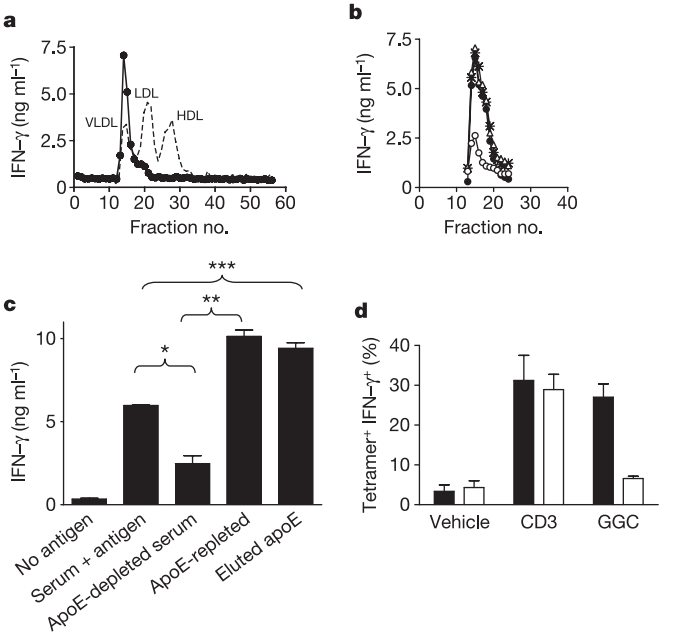

Figure 1 | Distribution and presentation of lipid antigens in serum, and dependence on apoE. a, VLDL localization of CD1-restricted T cell activity. GGC was incubated in human serum and separated by FPLC, and fractions were analysed for NK T-cell reactivity (solid line) and for cholesterol and triglycerides (dashed line). Data are representative of six separate experiments. IFN-γ, interferon-γ. **b**, Dependence of antigen reactivity on apoE. As in **a**, T-cell activity was measured in the absence (filled circles) or presence (open circles) of polyclonal blocking antibodies against apolipoprotein E versus isotype-matched antibodies against apolipoprotein B (triangles) and apolipoprotein A-II (asterisks). See also Supplementary Fig. 1. **c**, Depletion of apoE from serum diminishes GGC reactivity. T-cell reactivity to GGC was compared in 5% normal human serum, the same serum depleted of apoE, the apoE-depleted fraction plus added apoE ($2.5\,\mu g\,ml^{-1}$) and the apoE-positive fraction eluted from the column. Data are representative of four independent experiments. Asterisk, $P < 0.001$; two asterisks, $P < 0.001$; three asterisks, $P < 0.01$. **d**, Diminished CD1-restricted T-cell activity in apoE$^{-/-}$ mice. Mice were injected intravenously with 200 ng of GGC, 1.5 μg of anti-CD3, or vehicle alone. CD1d-tetramer-positive cells were analysed for intracellular interferon-γ production by flow cytometry. Data are representative of three separate experiments with 15 apoE$^{-/-}$ mice (open bars) and 15 controls (filled bars). Error bars represent s.e.m.

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VLDL-mediated T-cell activity (70–80% inhibition; see Supplementary Information), in comparison with isotype-matched antibodies against apoB or apoA-II (Fig. 1b). This blockade was specific for VLDL-bound GGC and did not affect the presentation of free GGC or phytohaemagglutinin (PHA) controls (Supplementary Fig. 1). Thus, the exogenously supplied lipid antigen GGC binds in the VLDL fraction of serum, and is acquired by DCs in an apoE-dependent manner, processed, and then presented to T cells.

To show the importance of apoE in serum in the uptake and presentation of this lipid antigen, we depleted apoE from human serum with an immunoaffinity column[7]. ApoE-depleted serum incubated with GGC had a significantly lower capacity to stimulate T cells than normal serum, and the presenting capacity was recovered by adding back purified apoE (Fig. 1c). Furthermore, the apoE-positive fraction of serum had a greater stimulatory capacity than serum alone. These results indicate that apoE might be the major factor in human serum mediating the presentation of GGC to CD1d-reactive T cells.

To assess the importance of apoE in the presentation of lipid antigens *in vivo*, we analysed the CD1d-restricted response to GGC in apoE-deficient mice. Although there were comparable numbers of α-GC-reactive NK T cells in apoE-deficient and wild-type mice, the response to intravenously administered GGC was drastically reduced in the apoE-deficient mice (Fig. 1d). This was not due a non-specific deficit of T cell reactivity, because intravenous administration of anti-CD3 antibodies activated CD1d–α-GC tetramer-labelled T cells similarly in both apoE-deficient mice and controls. Thus, apoE has a crucial function in the presentation of an exogenous lipid antigen *in vivo*. However, it has been shown that much higher doses of α-GC can still activate NK T cells in apoE-deficient mice[8,9], indicating that less efficient alternative pathways of exogenous lipid presentation also exist.

To examine directly the binding of apoE to lipid antigens such as GGC, we incubated antigens with apoE and then separated free from bound lipid with a heparin affinity column. Whereas free lipid readily passed through the column (Fig. 2a, upper panel), apoE bound with high affinity. After a 10-min incubation of apoE with GGC at room temperature, all detectable lipid antigen remained bound to apoE, was retained by the column and could be eluted with NaCl (Fig. 2a, lower panel). Even in the presence of a 500-fold excess of albumin, which itself can non-specifically bind lipids[10], more than 95% of the GGC still bound to apoE. ApoE therefore binds efficiently to a model CD1-presented lipid antigen.

Next we determined whether delivery of lipid antigens bound to apoE enhances CD1-restricted T-cell responses in comparison with free lipid antigen. The antigenic potency of GGC incubated with VLDL-derived apoE or recombinant apoE was increased almost 50-fold (Supplementary Information) compared with freshly sonicated free GGC or GGC delivered with other lipoprotein or serum fractions such as HDL (not shown), LDL or albumin (Fig. 2b). This effect was specific for lipid-dependent T-cell responses because there was no effect of apoE on an MHC class II restricted response to tetanus toxoid, an exogenous protein antigen requiring endocytic processing (Fig. 2c) or on control T-cell stimulators such as CD3, PHA or phorbol 12-*myristate*-13-acetate (PMA) plus ionomycin (Supplementary Fig. 3). Given the known affinity of apoE for a wide variety of lipid classes[3], we proposed that the binding and delivery of lipid antigens might apply to many CD1-presented lipid antigens. T-cell responses to naturally occurring microbial lipid antigens were also enhanced in the presence of apoE for the presentation of glucose monomycolate (GMM), mycolic acid (CD1b-presented) and manosyl-phosphomycoketide (CD1c-presented)[11] (Fig. 2d and data not shown). Taken together, these results establish that apoE specifically enhances T-cell responses to a variety of CD1-presented lipid antigens for multiple CD1 isoforms.

The efficient uptake of soluble antigens by professional APCs such

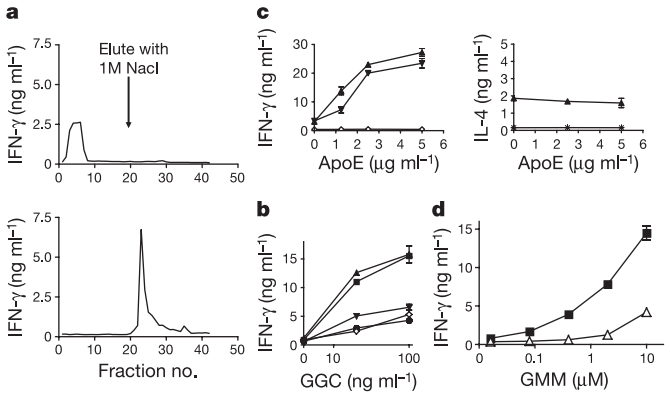

Figure 2 | ApoE binds lipid antigens and specifically enhances CD1-dependent T-cell responses. **a**, ApoE–GGC binding. Freshly sonicated GGC (top) or apoE-bound GGC (bottom) was loaded on a heparin affinity column and fractions were assayed for antigenic activity. ApoE was eluted with 1 M NaCl. Data are representative of at least six separate experiments. **b**, ApoE enhancement of GGC reactivity. GGC was incubated with apoE3 (squares), VLDL–apoE (upright triangles), LDL (inverted triangles) or BSA (circles), or freshly sonicated (diamonds), before being assayed for NK T-cell reactivity. **c**, ApoE specifically enhances CD1-dependent T-cell responses (left) but not MHC-dependent T-cell responses (right). α-GC (100 ng ml^{-1}; upright triangles in left panel), GGC (100 ng ml^{-1}; inverted triangles) or tetanus toxoid (TT, 10 μg ml^{-1}; upright triangles in right panel) was incubated with various concentrations of apoE3 before being assayed for T-cell activity (left, α-GC-reactive clone; right, TT-reactive clone). IL, interleukin. **d**, Enhancement of a CD1b-dependent T-cell response against foreign antigens. GMM was incubated with or without apoE and assayed for antigenic activity with the GMM-reactive clone LDN5. All error bars represent s.e.m.

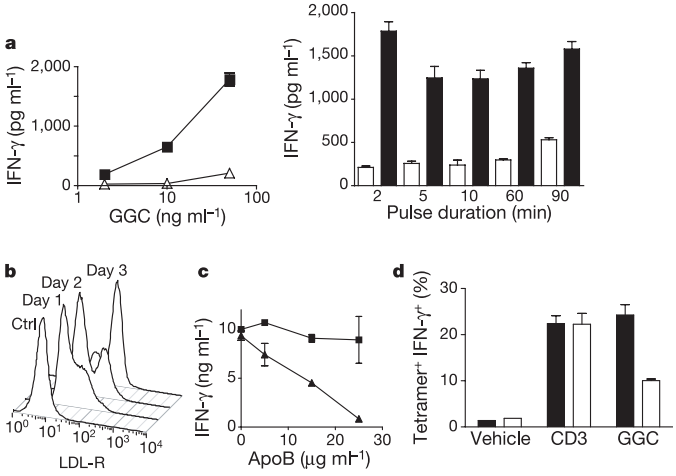

Figure 3 | Receptor-mediated uptake of apoE-bound lipid antigens. **a**, ApoE enhances the efficiency of uptake of GGC. ApoE-loaded GGC (filled squares (left) and filled bars (right)) or sonicated free GGC (open triangles (left) and open bars (right)) was used to pulse DCs for the indicated durations before being washed and tested for T-cell activity. Left: dose–response curve of GGC for a 2-min pulse. Right: various pulse durations are shown for 50 ng ml^{-1} GGC. **b**, The LDL-R is upregulated as monocytes differentiate into DCs; monocyte-derived DCs were harvested on the indicated days and LDL-R expression was determined by flow cytometry compared with the control (Ctrl). **c**, Free GGC (squares) or apoE–GGC (triangles) was pulsed onto DCs in the presence of increasing concentrations of apoB, and NK T-cell activity was measured. Data are representative of at least three experiments. **d**, LDLR$^{-/-}$ (open columns) and matched wild-type controls (filled columns) were challenged intravenously with 200 ng of GGC, 1.5 μg of anti-CD3 or vehicle. Data are representative of two separate experiments with 10 LDLR$^{-/-}$ and 10 wild-type mice.

as DCs is accomplished by robust macropinocytosis. In contrast, how APCs sample their local environment for lipid antigens is not known. ApoE is known to be secreted locally in tissues by macrophages and can account for 10% of all newly synthesized protein[3]. We also noted that apoE was coordinately upregulated with CD1 molecules in microarray experiments in human DCs[12,13], further indicating that apoE might be involved in lipid antigen presentation. We confirmed that apoE is abundantly produced by both DCs and macrophages, because apoE accumulated in supernatants at concentrations up to $10\,\mu g\,ml^{-1}$ in culture (Supplementary Fig. 2). Thus, by locally producing apoE in their environment, DCs or macrophages can be able to promote the capture and presentation of lipid antigens to T cells.

ApoE is known to be taken up into the endocytic system of cells by receptor-mediated endocytosis[14]. Receptor-mediated uptake is much more efficient than macropinocytosis[15] and may be of particular relevance for lipids. To determine the rapidity and efficiency of lipid uptake into DCs, we used free lipid antigen or apoE-bound lipid antigen to pulse DCs for varying durations before washing the cells and assaying their subsequent ability to present antigen to T cells. ApoE-delivered GGC achieved maximal stimulation of T cells after a 2-min exposure to DCs, whereas free lipid in medium alone required 4–6 h to achieve its maximal stimulation, which still reached only 48 ± 11% (mean ± s.e.m.) of that achieved in 2 min with apoE (Fig. 3a, and data not shown). This markedly enhanced rapidity and efficiency of uptake of the antigen is consistent with the difference between receptor-mediated endocytosis (apoE-delivered lipid) and macropinocytosis.

ApoE is known to be taken up by several cell-surface receptors, the most prominent being the LDL-R and the LDLR-like protein (LRP or CD91) (ref. 16). LRP is expressed on DCs[17], but to our knowledge LDL-R has not been documented on DCs. Flow cytometric analysis showed surface expression of both LRP and LDL-R on DCs, the latter being markedly upregulated during the differentiation of monocytes to DCs (Fig. 3b). To determine the roles of these cell surface receptors in the uptake of apoE-bound lipid, we took complementary approaches. First we compared the ability of apoE3 (the most common human variant) with that of apoE2 (a variant with a single amino-acid change that diminishes binding to the LDL-R[18]) to mediate lipid antigen uptake in DCs. In comparison with apoE3, apoE2 had only 40–47% the capacity to enhance the CD1d-dependent response (data not shown), indicating that the LDL-R

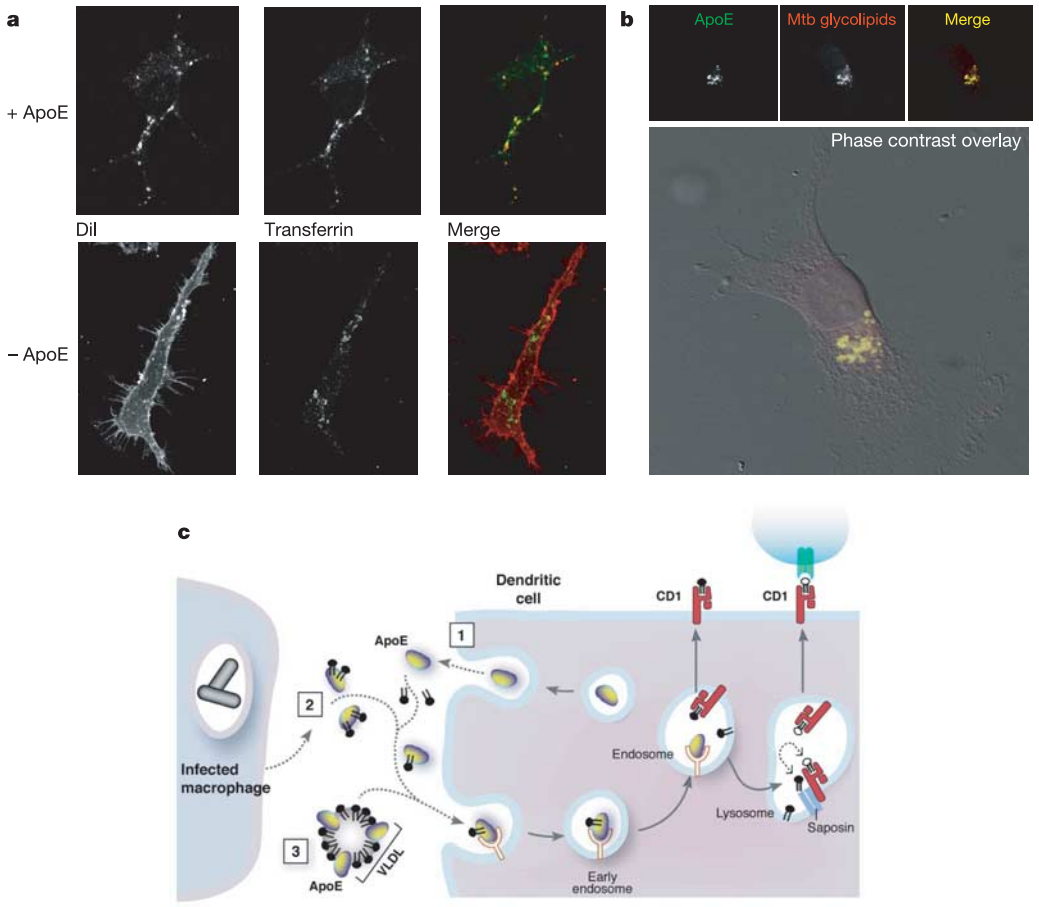

Figure 4 | Directed delivery of lipids to endosomal compartments by apoE. **a**, Dendritic cells were pulsed with DiI (red) with apoE (+apoE) or freshly sonicated (−apoE). Alexa-488 transferrin (green) was simultaneously pulsed with the lipid to reveal the early endosomes. After 5 min, cells were fixed and observed by confocal microscopy. Results are representative of multiple images in four separate experiments. **b**, ApoE transfers mycobacterial glycolipid antigens to uninfected DCs. *M. tuberculosis* (Mtb) was labelled with Alexa-555 and used to infect human macrophages. ApoE was isolated from culture supernatants by immunoaffinity purification and used to pulse DCs before observation by confocal microscopy. As controls, transfer of uninfected supernatants showed apoE staining with no labelled glycolipid, and the infected apoE-negative fraction showed labelled glycolipid with no associated apoE (data not shown). **c**, Exogenous pathways for lipid antigen presentation mediated by apoE. Delivery of lipids to the endosomal system can occur by three possible mechanisms indicated by the numbers in squares: 1, secretion-capture, in which secreted (or recycled) apoE can capture lipid antigens; 2, bystander acquisition, in which infected cells such as macrophages may shed lipid antigens associated with apoE that are delivered to bystander (uninfected) DCs; and 3, serum lipoproteins (VLDL in the case of GGC) serve as a depot for lipid antigens and may stimulate a response far from the source of the antigen.

might have a significant function in the uptake of apoE–antigen complexes. Second, we took advantage of the fact that apoB, the primary component of LDL, also binds to the LDL-R and can serve as a competitive ligand but does not bind LRP[19]. DCs pulsed with apoE–GGC in the presence of increasing amounts of apoB exhibited a dose-dependent diminution of antigen presentation compared with controls (Fig. 3c and Supplementary Fig. 4) further implicating the LDL-R in uptake. Last, we analysed the response to GGC in LDL-R deficient mice. In comparison with wild-type controls, LDL-R knockouts had a greatly diminished response to antigen while responding similarly to non-specific stimulation by anti-CD3 (Fig. 3d). We therefore conclude that the LDL-R has a significant function in the uptake of apoE–lipid-antigen complexes and presentation by CD1.

Next, we wished to address the mechanism through which receptor-mediated uptake of apoE-bound lipids enhances presentation by CD1. We proposed that the apoE-mediated delivery of lipids resulted in more effective lipid antigen delivery to CD1 antigen loading compartments. To reveal the delivery of free lipids versus apoE-bound lipids directly, we used a fluorescent lipid probe, 1,1′-dioctadecyl-3,3,3′,3′- tetramethylindocarbocyanine perchlorate (DiI). This molecule resembles CD1-presented lipid antigens in having two 18-carbon fatty-acyl chains and a distinct headgroup, in this case giving it fluorescent properties permitting detection within cells[20]. The intracellular fate of DiI delivered as apoE-bound DiI was compared with that of free DiI by confocal microscopy in dendritic cells (Fig. 4a). ApoE-delivered DiI resulted in rapid uptake in a punctate endosomal pattern, co-localizing with the early endosomal marker transferrin (Fig. 4a, top panels). Freshly sonicated free DiI also rapidly entered dendritic cells but distributed in an apparent plasma membrane distribution with very little endosomal localization (Fig. 4a, bottom panels). Thus, receptor-mediated endocytosis of apoE concentrates lipid molecules rapidly in the endosomal system, allowing enhanced access to the trafficking pathways followed by CD1 (refs 1 and 2).

Last, we sought to reveal the apoE-mediated transfer of exogenous lipid antigens in the setting of an infection. Macrophages are the host cells during infection by *Mycobacterium tuberculosis*, harbouring the bacterium while being incapable of presenting lipid antigens by means of CD1a, CD1b or CD1c (owing to lack of expression). We therefore proposed that lipid antigens might be transferred to uninfected CD1a,b,c-positive DCs to enable antigen presentation. Because macrophages produce apoE abundantly, we infected these cells in culture with live *M. tuberculosis* labelled with a fluorescent dye to tag mycobacterial surface glycolipids[21]. The infected human macrophages were washed and cultured for two to three days, after which we isolated secreted apoE with an immunoaffinity column. The apoE fraction contained abundant fluorescently labelled bacterial glycolipid that was rapidly (in 15 min) taken up into DCs, accumulating in a punctuate endosomal pattern completely co-localizing with apoE (Fig. 4b) and partly coinciding with CD1b (data not shown). ApoE produced by infected macrophages therefore transfers glycolipid antigens to uninfected 'bystander' DCs. It remains to be determined what mechanism transfers the lipid cargo from apoE to CD1 molecules.

These experiments outline an exogenous pathway for lipid antigen presentation mediated by apoE through receptor-mediated endocytosis. *In vivo*, the ability of bystander APCs to pick up microbial antigens from the extracellular fluid may be essential for effective immunity when infected cells cannot themselves present antigen. We propose that apoE readily binds exogenous lipid antigens and efficiently targets them for receptor-mediated uptake by DCs either in the infected tissue or in distant lymphoid organs to stimulate systemic immune responses. This may occur by the secretion and capture of apoE–lipid-antigen complexes in the local milieu or by apoE–VLDL–lipid-antigen complexes acquired from the circulation or from tissue fluid (Fig. 4c). We have not ruled out the role of other pathways in CD1 antigen presentation, such as apoB/LDL-R[29] or oxidized LDL/scavenger receptors[22], and consider it likely that structurally distinct lipid antigens may distribute differently in lipoprotein compartments. In addition to foreign lipids, apolipoproteins might also be important in providing a pathway for the delivery of self-lipid antigens and contribute to inflammatory diseases such as multiple sclerosis or atherosclerosis, in which both apoE and CD1 have been independently implicated[9,16,23,24]. The exogenous lipid antigen delivery pathway mediated by apolipoproteins therefore has important implications for microbial immunity, autoimmunity and atherosclerosis.

METHODS

Cells and reagents. Immature monocyte-derived DCs were prepared from fresh leukapheresis mononuclear cells or peripheral blood mononuclear cells as described previously[25]. Macrophages were generated by culturing leukocytes in IMDM medium for 7 days with 10% human serum and recombinant human macrophage colony-stimulating factor. The following CD1-restricted T cells and respective antigens were used as described previously: CD1d-restricted α-GC-reactive clones[25], CD1b-restricted T cell lines DN1 (mycolic acid-reactive)[26], and LDN5 (GMM-reactive)[27] and the CD1c-restricted mannosyl-phosphomycoketide-reactive T-cell line CD8-1 (ref. 11), tetanus-toxoid-specific MHC II-restricted clones[25].

Mice and analysis of NK T cell activation. ApoE-deficient mice and matched C57BL/6 controls were from Taconic. LDL-R-deficient mice and matched C57BL/6 controls were obtained from Jackson Laboratories. GGC was sonicated at 1 μg ml^{-1} in saline plus 0.045% Tween 20, and 200 μl was injected intravenously; alternatively, as controls, anti-CD3 (1.5 μg per 200 μl) (BD Pharmingen) or vehicle alone was used. Spleens and livers were harvested after 2 h (for GGC) and 30 min (for anti-CD3) and analysed for the specific activation of NK T cells with the use of FITC-labelled α-GC-loaded CD1d tetramers, (TcRβ-positive, CD19-negative) and intracellular staining for interferon-γ as described previously[25]. Isotype-matched antibodies and unloaded CD1d tetramers were used as controls.

T-cell assays. Unless specified otherwise, DCs were pulsed with antigen (either apoE-loaded or freshly sonicated) in serum-free medium (SFM), consisting of RPMI supplemented with L-glutamine and penicillin/streptomycin and 4 mg ml^{-1} BSA (used as a non-specific carrier) or serum-free medium from Gibco. CD1-restricted T cells were then added directly (or following a wash step as in pulse–wash experiments; Fig. 3a) in the same SFM supplemented with human serum (final concentration 2%). Interferon-γ production by T cells was measured after an overnight (16–24 h) culture at 37 °C. PHA (Sigma-Aldrich), anti-CD3 stimulation (antibody OKT3 at 100 ng ml^{-1}) and PMA/ionomycin were used as positive controls.

Lipid loading of apoE or other serum components. Lipid antigens were suspended in SFM by sonication in a water bath for 2 min. Recombinant human apoE3 and apoE2 (Invitrogen), VLDL-derived apoE and LDL-derived apolipoprotein B (Biodesign), HDL and LDL (Intracel) were then incubated at 5 μg ml^{-1} with lipid unless otherwise indicated.

FPLC separation of serum fractions by gel filtration. GGC was sonicated in DMSO at 100 μg ml^{-1}, then added to 1 ml of human serum (filtered in a 5-μm filter (Pall Corporation)), incubated for 1–6 h at 37 °C and loaded on a Superose-6 column (Amersham). Fractions (600 μl) were collected and assayed for T-cell reactivity as described above and for cholesterol and triglyceride with standard colorimetric assays (Thermo Electron Corporation) as described previously[28]. Blocking antibodies were from Biodesign.

Depletion and isolation of apoE. Human AB serum (Gem Cell) was depleted of apoE as described previously[7] with an immunoaffinity column. In brief, Econopac columns were packed with 2.5 ml of resin complex prepared from apoE polyclonal goat antibodies (Academy Biomedical) bound to Sepharose at 5.5 mg ml^{-1}. Depletion was confirmed by ELISA to be at least 95%. The apoE-positive fraction was eluted from the column with sodium thiocyanate, and both apoE-negative and positive fractions were washed with PBS and concentrated back to the original sample volume with 20-ml VivaSpin Concentrators (VivaScience).

Isolation of apoE-bound lipid by heparin column chromatography. Recombinant apoE (5 μg ml^{-1}) was incubated with lipid (100 ng ml^{-1} GGC or 5 μg ml^{-1} DiI) in loading buffer (10 mM $NaHPO_4$ pH 7). The sample was passed over 1 ml Hi-Trap Heparin columns (Amersham) and 250-μl fractions were collected in a 96-well plate (Costar). After being washed, the apoE was eluted with 1 M NaCl pH 7 and fractions were assayed for T-cell activity as described above.

Flow cytometry. Monocyte-derived DCs were prepared as described above and stained for flow cytometry with FITC-conjugated anti-LRP (CD91) antibodies (BD Pharmingen) and biotinylated rabbit anti-LDL-R (Research Diagnostics, Inc.). Streptavidin-Alexa-488 (Molecular Probes) was used as a secondary reagent (1:1000 dilution). Isotype-matched antibodies (BD Pharmingen) and secondary reagents were used as controls.

Confocal microscopy. DiI (5 μg ml^{-1}; Molecular Probes) was incubated for 1 h with apoE (5 μg ml^{-1}), and apoE-bound lipid was isolated as described above, with a heparin column. The isolated apoE-DiI complex was used to pulse live human dendritic cells adhered to fibronectin-coated coverslips. Free DiI (5 μg ml^{-1}) was freshly sonicated in SFM before pulsing DCs. Transferrin was conjugated to Alexa-488 (Molecular Probes) and used to pulse cells simultaneously at 5 μg ml^{-1}. After a 5-min pulse, cells were fixed for 30 min with 2% paraformaldehyde and viewed by confocal microscopy on a Nikon TE2000 with EZ-C1 V.2.20 software. Images were generated with Adobe Photoshop.

Infection of macrophages with *M. tuberculosis*. *M. tuberculosis* (strain H37-Rv) was cultured in 7H9 medium with OADC supplements (Gibco) and 0.05% Tween 80. Mid-exponential phase cultures were labelled with Alexa-555-hydrazide (Molecular Probes) as described previously[21] and used to infect human macrophages at a multiplicity of infection of 10. After a 1-h infection, macrophages were washed extensively to remove extracellular mycobacteria and cultured for three days in serum-free medium (Ex-Vivo medium; Gibco); supernatants were collected and passed through a 0.45-μm syringe filter to remove any whole bacteria or bacterial debris. apoE production was determined by ELISA, and apoE–lipid-antigen complexes were isolated with an immunoaffinity column as described above. The apoE-positive fraction (or, as controls, the apoE-negative or uninfected fractions) was used to pulse dendritic cells for 15 min and viewed by confocal microscopy as described above.

Statistical analysis. Unless otherwise stated, error bars and error values represent s.e.m.

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