

Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1

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The evolutionary conservation of T lymphocyte subsets bearing T-cell receptors (TCRs) using invariant α -chains is indicative of unique functions. CD1d-restricted natural killer T (NK-T) cells that express an invariant V α 14 TCR α chain have been implicated in microbial and tumour responses, as well as in auto-immunity^{1,2}. Here we show that T cells that express the canonical hV α 7.2-J α 33 or mV α 19-J α 33 TCR rearrangement³ are preferentially located in the gut lamina propria of humans and mice, respectively, and are therefore genuine mucosal-associated invariant T (MAIT) cells. Selection and/or expansion of this population requires B lymphocytes, as MAIT cells are absent in B-cell-deficient patients and mice. In addition, we show that MAIT cells are selected and/or restricted by MR1, a monomorphic major histocompatibility complex class I-related molecule that is markedly conserved in diverse mammalian species⁴. MAIT cells are not present in germ-free mice, indicating that commensal flora is required for their expansion in the gut lamina propria. This indicates that MAIT cells are probably involved in the host response at the site of pathogen entry, and may regulate intestinal B-cell activity.

NK-T and MAIT cells are defined by their use of a restricted TCR repertoire: an invariant TCR α chain—one V α -J α combination—with a CDR3 of constant length paired with a limited number of V β segments¹⁻³. T cells that express an invariant TCR α chain have an activated phenotype (CD44^{hi}), and their high frequency in particular lymphocyte subsets and organs results from oligoclonal expansion. They can be CD4⁺ (CD4 positive), CD4⁻CD8⁻ (double negative or DN) or CD8 α in humans, and do not seem to require CD4 or CD8 accessory molecules for selection. NK-T and MAIT cells are restricted by β 2-microglobulin (β 2m)-dependent major histocompatibility complex (MHC) class Ib molecules in a TAP (transporters associated with antigen processing)-independent manner: CD1d for NK-T cells and a distinct β 2m-dependent molecule for MAIT cells. NK-T cells are abundant in the thymus, liver, spleen and bone marrow; however, the exact tissue distribution of T cells that express hV α 7.2/mV α 19-J α 33 is not known³.

To assess the distribution of MAIT cells, we used fluorescence-activated cell sorting (FACS) to separate TCR $\alpha\beta$ DN T cells from human peripheral blood lymphocytes (PBL) according to the expression level of the gut-specific α 4 β 7 (ref. 5) homing marker, and quantified hV α 7.2-J α 33 transcripts in different fractions. The invariant transcripts were enriched in the TCR $\alpha\beta$ DN α 4 β 7⁺ fraction, as shown by a six-cycle shift to the left of the polymerase chain reaction (PCR) curve, suggesting that hV α 7.2-J α 33⁺ T cells are associated with the gut (Fig. 1a). Enrichment of hV α 7.2-J α 33 transcripts in 11 gut biopsies, as compared with three skin samples, in assays using DN T cells as a positive control and CD4⁺ PBL as a negative control verified the gut location of hV α 7.2-J α 33⁺ T cells (Fig. 1b). Gut-associated T cells comprise intra-epithelial, Peyer's

patch and lamina propria (LPL) lymphocytes⁶. A preliminary analysis of gut biopsies containing variable amounts of intra-epithelial, Peyer's patch and lamina propria tissues suggested that the hV α 7.2-J α 33⁺ T cells were restricted to the lamina propria (data not shown).

To determine whether this gut-specific homing pattern also occurs in mice, we quantified canonical mV α 19-J α 33 expression in gut lymphocytes of B6 mice and in those of TAP- and invariant chain (Ii)-deficient (TAP^{-/-}Ii^{-/-}) mice. Rare in wild-type mice, mV α 19-J α 33⁺ T cells are more abundant in TAP^{-/-}Ii^{-/-} mice, in which the numbers of mainstream T cells are reduced owing to low cell-surface expression of MHC class Ia and II molecules^{3,7}. mV α 19-J α 33 transcripts were tenfold more abundant in LPL than in the mesenteric lymph nodes (MLN) of B6 mice; however, they were absent in LPL of β 2m^{-/-} mice (Figs 1c and 2e). In addition, tenfold more mV α 19-J α 33 transcripts were amplified from TAP^{-/-}Ii^{-/-} LPL than from B6 LPL, confirming that the selection/expansion of MAIT cells is independent of TAP and Ii. mV α 19-J α 33⁺ transcripts were abundant in lamina propria and Peyer's patch samples (albeit less so in Peyer's patch), but not in intra-epithelial lymphocytes (Fig. 1d). Polyclonal sequencing of mV α 19-J α 33 amplicons confirmed the over-representation of the canonical CDR3 specific for this invariant TCR α chain in the gut lamina propria (Fig. 2e). Together, these results show that the gut lamina propria is a favoured location for β 2m-dependent MAIT cells in humans and mice. In both species, preliminary data indicate that hV α 7.2/mV α 19-J α 33⁺ T cells are also present in the lung (data not

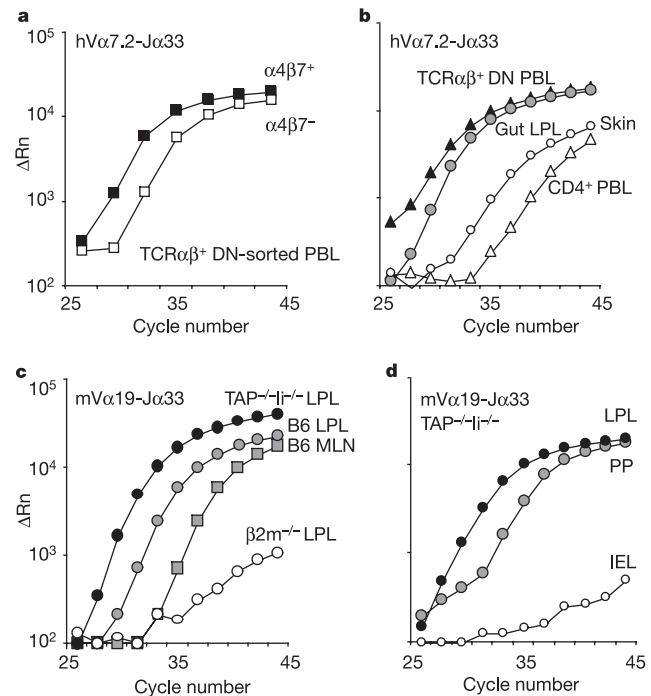


Figure 1 MAIT cells are located in the gut lamina propria of both humans and mice. Lymphocytes were isolated from the indicated organs and RNA was extracted. After cDNA synthesis, kinetic Q-PCR was carried out for C α and the indicated V α -J α combination. Data shown are representative of at least three independent experiments (two for panel a). **a**, hV α 7.2-J α 33⁺ T cells are enriched in the α 4 β 7⁺ fraction of TCR $\alpha\beta$ DN human PBL. α 4 β 7⁺ and α 4 β 7⁻ fractions from CD4⁻CD8 β ⁻TCR β ⁺ PBL were isolated by FACS. **b**, hV α 7.2-J α 33⁺ T cells are present in the human gut. DN and CD4 TCR β ⁺ T cells were isolated from PBL. RNA from skin or gut was isolated from frozen biopsies. Data are representative of 11 (gut) and three (skin) samples. **c**, mV α 19-J α 33⁺ T cells are present in the gut lamina propria and MLN in mice; they are more abundant in TAP^{-/-}Ii^{-/-} mice. **d**, mV α 19-J α 33⁺ T cells are present in the gut Peyer's patch (PP) and in LPL, but not in intra-epithelial lymphocytes (IEL). Δ Rn, change in normalized fluorescence ratio; PBL, peripheral blood lymphocytes.

shown), suggesting that MAIT cells may be present in several mucosal tissues.

Mainstream T cells are selected by classical MHC class I and class II molecules expressed on thymic epithelium⁸, whereas NK-T cells are selected by recognition of CD1d expressed on haematopoietic cells, specifically CD4⁺CD8⁺ (double positive or DP) thymocytes⁹. Urdahl *et al.* recently proposed that most MHC class Ib-restricted T cells may rely on haematopoietic cells for selection¹⁰. As MAIT cells require a $\beta 2m$ -dependent molecule for selection³, we generated fetal liver chimaeras between $\beta 2m$ -deficient and wild-type B6 mice to determine which cell type mediates their selection. As expected, B6 \rightarrow $\beta 2m^{-/-}$ (B6 fetal liver cells transferred into an irradiated $\beta 2m$ -deficient recipient) chimaeras have very few CD8⁺ T cells⁸ (see Supplementary Fig. S1). Conclusively, mV α 19-J α 33 transcripts were present in B6 \rightarrow $\beta 2m^{-/-}$ but not in $\beta 2m^{-/-}$ \rightarrow B6 chimaeras (Fig. 2a). Thus, expression of a $\beta 2m$ -dependent MHC class Ib molecule on haematopoietic cells is necessary and sufficient for the selection/expansion of DN MAIT cells.

To identify the haematopoietic cell type responsible for the selection of MAIT cells, we generated mixed chimaeras in which the bone marrow from $\beta 2m$ -deficient mice (BM-1) was mixed with several sources of bone marrow incapable of generating T cells (BM-2), and then injected into $\beta 2m$ -deficient lymphoid hosts ($\beta 2m^{-/-}$ -RAG2^{-/-}- γ c^{-/-}; that is, hosts lacking $\beta 2m$, recombination-activating gene 2 and the common interleukin receptor γ -chain, respectively). In this setting, T cells repopulating the host thymus are derived from BM-1 and are $\beta 2m$ -deficient, whereas cells originating from BM-2 are the only source of $\beta 2m$. Therefore, when BM-2 is RAG2^{-/-}, only macrophages and dendritic cells express $\beta 2m$ -dependent MHC molecules, whereas when BM-2 is CD3 ϵ ^{-/-}, $\beta 2m$ ⁺ B cells are also present. In all groups, very few CD8^{hi} T cells

were generated (see Supplementary Fig. S2). When only macrophages and dendritic cells were $\beta 2m$ ⁺, no mV α 19-J α 33 transcripts could be detected in LPL. By contrast, the addition of $\beta 2m$ ⁺ B cells was sufficient to give rise to mV α 19-J α 33⁺ LPL (Fig. 2b). These results indicate that B cells mediate MAIT cell selection/expansion, whereas dendritic cells and macrophages do not. The selection of MAIT cells by B cells was confirmed by a significant reduction in mV α 19-J α 33 transcripts in TCR α β ⁺ DN cells from the MLN and LPL of mice negative for the immunoglobulin heavy chain joining region (J_H^{-/-}), mice, which are completely B-cell-deficient¹¹ (Fig. 2c and data not shown). Mice without the transmembrane region of immunoglobulin- μ (IgM; μ MT^{-/-} mice) lack most B cells, but do harbour IgA-producing B cells in their intestine^{12,13}. Normal numbers of MAIT cells were found in LPL from μ MT^{-/-} mice, showing that these residual gut B cells are sufficient for the accumulation of MAIT cells (Fig. 2c). Finally, mV α 19-J α 33 transcripts were amplified equally well from the lamina propria and MLN of xid and control mice (data not shown), indicating that B1 B cells, absent in xid mice¹⁴, are not involved in the selection/expansion of MAIT cells.

To establish whether human MAIT cells are also dependent on B cells for selection/expansion, we quantified hV α 7.2-J α 33 transcripts in TCR α β ⁺ DN human PBL from normal individuals and four patients with mutated Bruton tyrosine kinase¹⁵. hV α 7.2-J α 33 transcripts in DN T cells were extremely rare in two patients, diminished tenfold in another, and unaffected in the fourth, as compared with all normal subjects tested so far (Fig. 2d). Analogous to the situation in μ MT-deficient mice, the normal levels of hV α 7.2-J α 33 transcripts observed in one patient could be related to the presence of some B cells in his gut, despite the absence of B cells (CD19⁺) in his blood (data not shown). These results show that B cells are necessary for the selection/expansion of MAIT cells in both

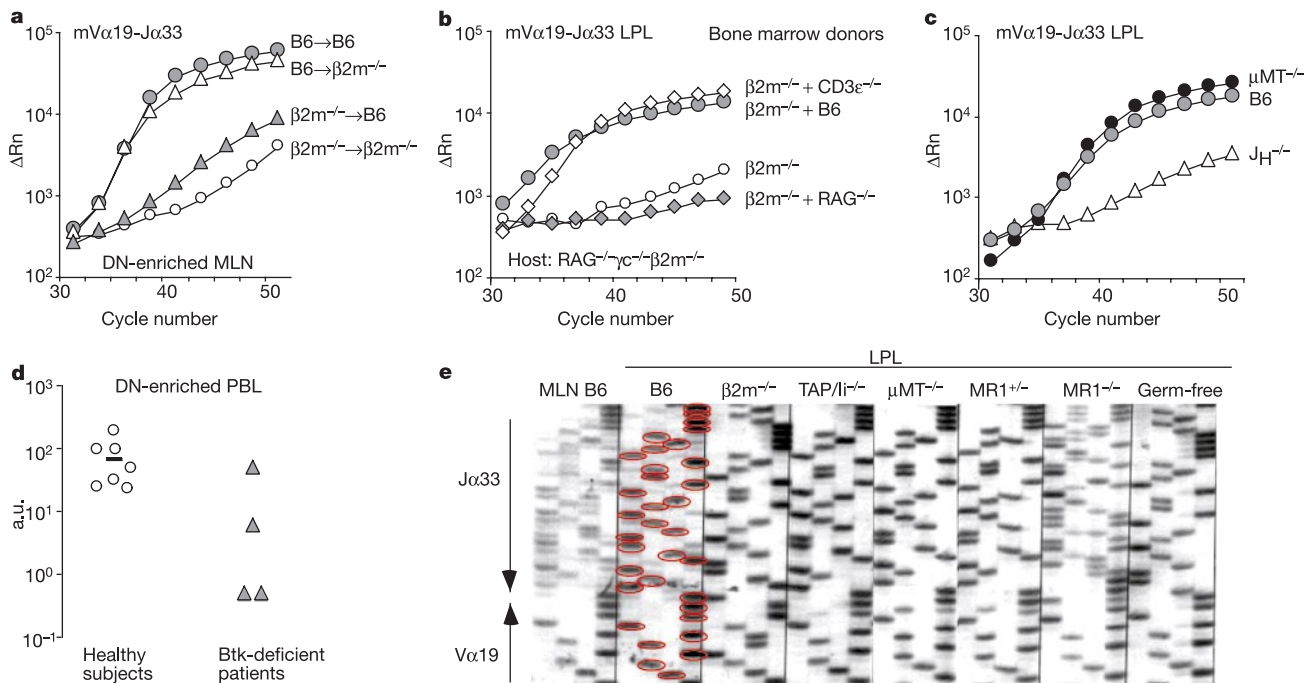


Figure 2 MAIT cells are selected by B cells. **a**, mV α 19-J α 33⁺ T cells are selected by haematopoietic cells. Fetal liver chimaeras with the indicated genotype were prepared as described in the Methods. DN-enriched fractions from MLN were isolated and Q-PCR was carried out. These data are representative of at least three samples per group analysed in two independent experiments. **b**, mV α 19-J α 33⁺ T cells are selected by B cells. 5-Fluorouracil-treated RAG^{-/-}- γ c^{-/-}- $\beta 2m^{-/-}$ recipients were reconstituted with a 1:1 mixture of the indicated bone marrow. After three months, LPL were isolated and Q-PCR was carried out. These data are representative of at least two samples per group. **c**, mV α 19-J α 33⁺ T cells are selected by B cells. LPL were isolated from the indicated

mice and Q-PCR was carried out. Seven mice in each group were analysed; representative data are shown. **d**, hV α 7.2-J α 33⁺ T cells are selected by human B cells. DN-enriched T cells were obtained from the indicated subjects and Q-PCR was performed. Results are normalized to C α and expressed as a percentage relative to one sample, to which we attributed a value of 100 arbitrary units (a.u.). Btk, Bruton's tyrosine kinase. **e**, The mV α 19-J α 33 canonical sequence is not found in MR1-deficient, $\beta 2m$ -deficient or germ-free mice. V α 19-J α 33 amplification was carried out on the indicated samples and polyclonal amplicons sequenced with a V α 19 primer. The canonical sequence is circled in red in the B6 LPL sample.

humans and mice, and suggest that MAIT cells interact with lamina propria B cells.

Thus, B cells express a $\beta 2m$ -dependent, TAP- and Ii-independent MHC class Ib molecule distinct from CD1d that selects MAIT cells. MR1 is by far the most highly conserved MHC class I-related molecule in mammalian species^{16–18}: the 90% sequence identity between mouse and human is unparalleled^{4,19}. Like CD1d, MR1 is encoded on human chromosome 1, but is more closely related to classical class I molecules than are other class Ib family members. These attributes made MR1 a plausible candidate ligand for the MAIT TCR. To demonstrate recognition of MR1 by $mV\alpha 19$ - $J\alpha 33$ ⁺ T cells, we generated several cell lines expressing a full-length murine MR1 complementary DNA fused to enhanced green fluorescent protein (EGFP; see Supplementary Fig. S3), and examined their ability to stimulate a series of $mV\alpha 19$ - $J\alpha 33$ ⁺ T–T hybridomas (ref. 3 and data not shown). Three (18G7, 6C2 and 6H2) out of more than 20 tested hybridomas were specifically stimulated to release interleukin 2 (IL-2) by the MR1 transfectants (Fig. 3a), and anti-TCR antibodies blocked this stimulation (Fig. 3a and

Supplementary Fig. S3c). These results strongly suggest that $mV\alpha 19$ - $J\alpha 33$ ⁺ T cells directly recognize MR1.

To assess the role of MR1 in the selection and expansion of MAIT cells *in vivo*, we analysed mice in which the $\alpha 1$ and $\alpha 2$ domains of MR1 were deleted using gene targeting (see Supplementary Fig. S4). $MR1^{-/-}$ mice contained phenotypically normal T, B and NK cells at the expected number and frequency in the central and peripheral lymphoid organs (thymus, spleen, peripheral lymph nodes (PLN) and MLN) (see Supplementary Fig. S5). $mV\alpha 19$ - $J\alpha 33$ sequences were readily amplified from MLN or lamina propria T cells obtained from littermate controls, but not in those from $MR1^{-/-}$ mice (Fig. 3b and data not shown). These results were confirmed by polyclonal sequencing of $mV\alpha 19$ - $J\alpha 33$ amplicons obtained from lamina propria cells (Fig. 2e). To evaluate the frequency of the canonical sequence in MR1-deficient mice, we sequenced cloned $mV\alpha 19$ - $J\alpha 33$ rearrangements amplified from genomic LN DNA. As shown in Table 1, 80% of the $mV\alpha 19$ - $J\alpha 33$ sequences from $MR1^{+/+}$ mice corresponded to the invariant chain, and only 6% of the sequences were out of frame, demonstrating a very strong selection for this canonical sequence. In sharp contrast to this, only 7% of $mV\alpha 19$ - $J\alpha 33$ junctions sequenced from $MR1^{-/-}$ mice encoded the invariant TCR α chain, and 47% of the sequences obtained were out of frame. The presence of a few canonical sequences in $MR1^{-/-}$ (and $\beta 2m^{-/-}$) mice reflects a strong mechanistic bias for this particular rearrangement, which is germ-line encoded and favoured by homology-directed recombination^{20,21}. $mV\alpha 19$ - $J\alpha 33$ chains with the same CDR3 length as the invariant

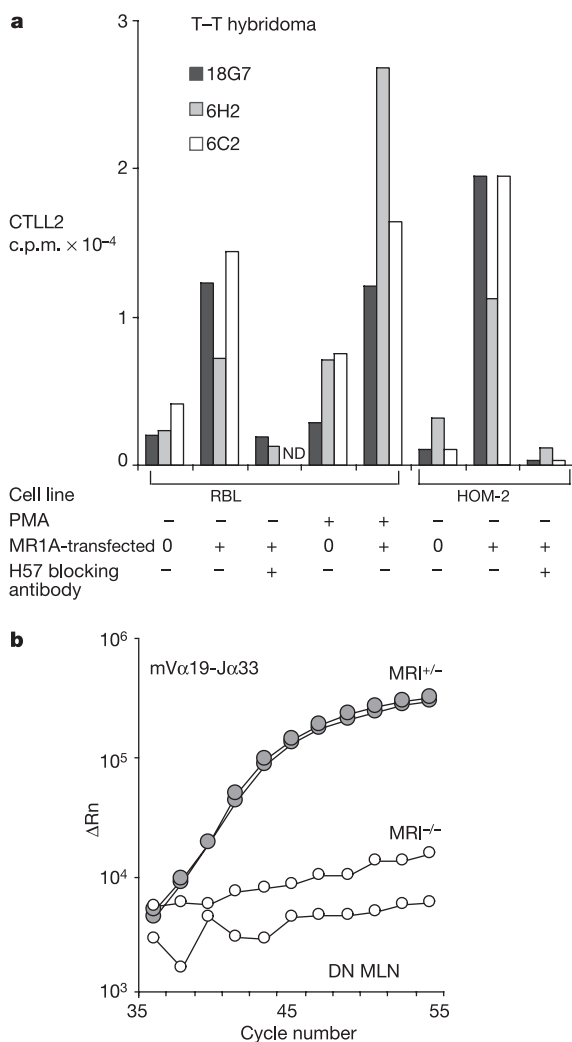


Figure 3 MR1 is the restricting MHC molecule for MAIT cells. **a**, IL-2 production by T–T hybridomas stimulated by MR1 transfectants. A panel of $mV\alpha 19$ ⁺ T–T hybridomas was stimulated with the indicated cell lines with or without anti-TCR β monoclonal antibody ($1 \mu g ml^{-1}$) for 48 h. Supernatants were assayed using the IL-2-dependent cell line CTLL2. PMA, phorbol myristate acetate. **b**, The $mV\alpha 19$ - $J\alpha 33$ junction is not present in DN T cells from $MR1^{-/-}$ MLN. DN TCR $\alpha\beta$ ⁺ T cells were isolated from the MLN of $MR1^{-/-}$ and $MR1^{+/+}$ mice, and Q-PCR performed with C α and $mV\alpha 19$ - $J\alpha 33$ specific primers. ND, not determined.

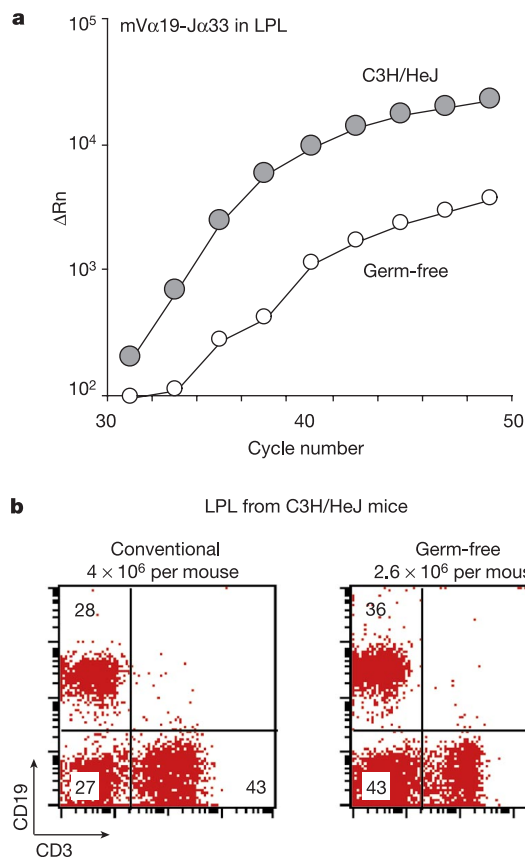


Figure 4 MAIT cells are absent in germ-free mice. **a**, Absence of MAIT cells in germ-free mice. LPL were isolated from germ-free or control C3H mice, and Q-PCR was carried out. Data shown are representative of seven samples per group studied in three independent experiments. **b**, Germ-free mice have significant numbers of CD19⁺ cells. LPL were isolated from the indicated mice, and stained with anti-CD19 and anti-CD3 ϵ monoclonal antibodies. Numbers in each quadrant indicate the percentage of each population.

TCR α chain were also enriched in MR1⁺ mice, some partially encoded by N nucleotides. Thus, mV α 19-J α 33 resembles the canonical mV γ 3-J γ 1 and mV δ 1-D1-D2-J δ 2 chains expressed by murine $\gamma\delta$ dendritic epidermal T cells (DETC), which are mechanistically favoured by homology-directed recombination as well as being selected for at the protein level^{21–23}. Overall, 92% of the MR1⁺ mV α 19-J α 33 sequences were either canonical or of identical CDR3 length, whereas only 12% of the MR1^{-/-} sequences fit these criteria. Together, these results show that MR1 is, indeed, the β 2m-dependent class Ib molecule that is required for the selection/expansion of T cells expressing the invariant mV α 19-J α 33 chain *in vivo*.

MAIT cells may bind MR1 alone or in association with a ligand. NK-T cells recognize as yet unidentified CD1d-bound endogenous glycolipids that are present in fetal thymus organ cultures¹. NK-T cell numbers and phenotype are also unchanged in germ-free mice²⁴. By contrast, we found that mV α 19-J α 33 transcripts were reduced in LPL (Fig. 4a) and in DN MLN T cells obtained from germ-free mice (data not shown). The canonical sequence was absent from LPL, as shown by polyclonal sequencing (Fig. 2e), despite the presence of a significant number of CD19⁺ B cells (Fig. 4b). Thus, the accumulation of MAIT cells is dependent on gut commensal flora and MR1. It is possible that MR1 remains

intracellular in the absence of stress and/or the appropriate ligand, as do MIC (MHC class I chain-related), H2-M3 and HLA-E (human leukocyte antigen E)^{25–27}. In support of this, MR1 molecules expressed by transfection remain largely in the endoplasmic reticulum, associated with molecular chaperones that have been implicated in MHC class Ia assembly. Furthermore, recombinant MR1 heavy chains appear to undergo a folding event similar to class Ia molecules when expressed in highly supplemented medium containing a putative ligand (M. J. Miley *et al.*, unpublished observations). In view of this, it is attractive to speculate that the cell-surface expression of MR1 requires a ligand, which may not be a peptide, derived from or induced by commensal flora. Alternatively, microbial products could facilitate translocation of MR1 to the cell membrane, where it is directly recognized by the hV α 7.2/mV α 19-J α 33 TCR.

NK-T cells and MAIT cells are two phylogenetically conserved MHC class Ib-restricted T-cell subpopulations that share many features: both use a semi-invariant TCR to recognize their restricting elements in a co-receptor-independent manner, and seem to display some level of autoreactivity *in vivo*^{1–3}. Here we show that they share a similar developmental pathway, as MAIT cells also require interaction with bone-marrow-derived cells for selection/expansion. NK-T cells bind CD1d on cortical DP thymocytes⁹,

Table 1 Sequences of V α 19-J α 33 amplicons from MR1-deficient mice and controls

AV19	Canonical junction		CAJ33
TGTGCTGTGAGG GGAT CAGCGC	TGTGCTGTGAGG GGAT AGCAACTATCAGTTGAT		<u>CATGGAT</u> AGCAACTATCAGTTGAT
MR1-deficient	β2m-deficient		MR1-positive littermate controls
B CVV MDGNYQW IWGSG	B CVV MDGNYQW IWGSG	B CVV MDGNYQW IWGSG	B CVV MDGNYQW IWGSG
H CAV KDSNYQL IWGAG	H CAV KDSNYQL IWGAG	H CAV KDSNYQL IWGAG	H CAV KDSNYQL IWGAG
M CAV RDSNYQL IWGSG	M CAV RDSNYQL IWGSG	M CAV RDSNYQL IWGSG	M CAV RDSNYQL IWGSG
Mouse 1 lymph nodes	Lymph nodes	Mouse 1 lymph nodes	
CAV <u>RDSNYQL</u> IWGS (2/11)	CAV <u>RDSNYQL</u> IWGS (3/33)	CAV <u>RDSNYQL</u> IWGS (9/15)	
CAV <u>RRPSNYQL</u> IWGS (1/11)	CAV <u>RLGSNYQL</u> IWGS (6/33)	CAV <u>MDSNYQL</u> IWGS (2/15)	
Out of frame (8/11)	CAV <u>RDRDSNYQL</u> IWGS (15/33)	CAP <u>MDSNYQL</u> IWGS (2/15)	
(7 unique)	CAV <u>RAEGDSNYQL</u> IWGS (3/33)	CAV <u>REGNYQL</u> IWGS (1/15)	
	Out of frame (6/33)	Out of frame (1/15)	
	(3 unique)		
Mouse 2 lymph nodes		Mouse 2 lymph nodes	
CAA <u>HSNYQL</u> IWGS (4/34)		CAV <u>RDSNYQL</u> IWGS (18/26)	
CAV <u>REGYQL</u> IWGS (1/34)		CAV <u>MDSNYQL</u> IWGS (1/26)	
CAV <u>RDSNYQL</u> IWGS (1/34)		CAV <u>LDSNYQL</u> IWGS (2/26)	
CAV <u>MVDSNYQL</u> IWGS (1/34)		CAV <u>RDRNYQL</u> IWGS (1/26)	
CAV <u>RPDSNYQL</u> IWGS (2/34)		CAV <u>RPMSNYQL</u> IWGS (1/26)	
CAV <u>RDRSNYQL</u> IWGS (1/34)		Out of frame (3/26)	
CAV <u>RDRDSNYQL</u> IWGS (15/34)		(3 unique)	
CAV <u>RDGDSNYQL</u> IWGS (4/34)			
Out of frame (5/34)			
(3 unique)			
Mouse 3/4 pooled DN-enriched		Mouse 3/4 pooled DN-enriched	
CAV <u>RDSNYQL</u> IWGS (5/34)		CAV <u>RDSNYQL</u> IWGS (23/26)	
CAV <u>RVDSNYQL</u> IWGS (3/34)		CAV <u>MDSNYQL</u> IWGS (1/26)	
CAV <u>RDRSNYQL</u> IWGS (3/34)		CAV <u>LDSNYQL</u> IWGS (1/26)	
CAV <u>RDPGDSNYQL</u> IWGS (1/34)		Out of frame (1/26)	
Out of frame (22/34)			
(6 unique)			
Mouse 5–7 pooled DN-enriched		Mouse 5–7 pooled DN-enriched	
CAV <u>RDRDYQL</u> IWGS (5/30)		CAV <u>RDSNYQL</u> IWGS (30/34)	
CAV <u>RVDSNYQL</u> IWGS (3/30)		CAV <u>ADSNYQL</u> IWGS (1/34)	
CAV <u>REGSNYQL</u> IWGS (2/30)		CAV <u>RDRMDSNYQL</u> IWGS (1/34)	
CAV <u>RGDSNYQL</u> IWGS (1/30)		CAV <u>RDGRDSNYQL</u> IWGS (1/34)	
CAV <u>RVGGNYQL</u> IWGS (1/30)		Out of frame (1/34)	
CAV <u>RDQGSNYQL</u> IWGS (1/30)			
CAV <u>RDLGSNYQL</u> IWGS (1/30)			
Out of frame (16/30)			
(10 unique)			
Total sequences 109	Total sequences 33	Total sequences 101	
Canonical sequence 8 (7%)	Canonical sequence 3 (9%)	Canonical sequence 80 (80%)	
Same CDR3 length, diff. seq. 5 (4.6%)	Same CDR3 length, diff. seq. 0 (0%)	Same CDR3 length, diff. seq. 12 (12%)	
Different CDR3 length 45 (41%)	Different CDR3 length 24 (73%)	Different CDR3 length 3 (3%)	
Out of frame 51 (47%)	Out of frame 6 (18%)	Out of frame 6 (6%)	

Generation of the canonical junction by the germ-line AV19 and AJ33 segments is shown, with the 4-bp stretch of shared homology in bold and P nucleotides underlined. The amino acid sequences of the bovine (B), human (H) and murine (M) canonical junctions are shown above each data set, and canonical junctions are underlined within the data sets. diff. seq., different sequence.

whereas MAIT cells probably recognize MR1 on intestinal B cells. MAIT cells accumulate in the gut (and perhaps the lung) mucosa, where most microorganisms enter the body. Thus, MAIT cells are probably involved in the host response at the site of pathogen entry. IgA secretion in the gut is dependent on microbe-derived signals and does not always require T cells²⁸. Given the parallel absence of IgA secretion and MAIT cells in germ-free mice²⁹, we propose that these invariant T cells may be involved in a negative feedback loop in which microbial products induce both IgA secretion and the cell-surface expression of MR1/ligand. This would activate MAIT cells, which would in turn secrete inhibitory lymphokines, preventing hyperactivation of the gut immune system. Consistent with this, our preliminary results indicate that the proportion of IgA⁺ cells is increased in MR1^{-/-} mice, which lack MAIT cells. Alternatively, MAIT cells may have a non-immune role, as $\gamma\delta$ DETC do by promoting wound healing in the skin³⁰. Regardless, the strict conservation of both MR1 and MAIT cells among mammalian species implies stringent evolutionary pressure for the function(s) fulfilled by MAIT cells. □

Methods

Mice

$\beta 2m$ -deficient (C57Bl/6 N9, B6N9), CD45.1 congenic B6, Ii-deficient (B6/129) and μ MT-deficient (B6 N10) mice were obtained from the Centre National de la Recherche Scientifique animal facility (Centre de Distribution, de Typages et d'Archivage animal (CDTA)). Germ-free mice (C3H) and matched controls were purchased from the CDTA. TAP-deficient mice were obtained from the Jackson laboratory on a B6/129 background. Ii- and TAP-deficient mice were intercrossed to obtain double-deficient mice. γc -deficient mice were obtained from J. Disanto, and crossed to RAG2^{-/-} and $\beta 2m$ ^{-/-} mice to obtain triple-deficient mice (I. Grandjean *et al.*, manuscript in preparation). *xid* and *J β* -deficient mice were kindly provided by P. A. Cazenave.

Generation of MR1-knockout mice

Made using standard techniques, the targeting construct was designed to cleanly delete a 1.4-kb fragment containing exons 2 and 3, which encode the $\alpha 1$ and $\alpha 2$ domains of MR1. Mice carrying the MR1 $\alpha 1/\alpha 2$ -domain deletion were intercrossed to generate homozygote knockout mice. All the mice studied had a mixed C57Bl/6 \times 129P2/OlaHsd background (see Supplementary Methods).

Cell preparation

Cell suspensions were prepared from thymus, spleen, PLN and MLN as described³. In some experiments, DN (CD4⁻CD8⁻) LN cells were obtained by negative selection using anti-CD4-fluorescein isothiocyanate (FITC) and anti-CD8-FITC monoclonal antibodies and anti-FITC magnetic beads; after separation on MS columns (Miltenyi Biotec), the effluent was collected as the DN fraction. Gut LPL were prepared as described elsewhere⁶ (see Supplementary Methods).

Cytofluorometry

Four-colour cytometry was performed with directly conjugated antibodies (Pharmingen) according to standard techniques, and analysed on a FACScalibur (Becton Dickinson). The following antibodies were used: anti-CD45.2-FITC, anti-CD3 ϵ -FITC, anti-CD4-phycoerythrin (PE) and -allophycocyanin (APC), anti-CD5-APC, anti-CD8-FITC and -APC, anti-V β 6-PE, anti-CD44-PE, anti-CD45.1-PE, anti-TCR β -cychrome, anti-CD19-PE or -FITC, anti-B220-cychrome, anti-IgD-FITC, anti-CD11c-FITC and anti-IgM-PE (clone R6-60.2). Biotinylated anti-CD45.1 antibodies were revealed with streptavidin-Tricolor or PE (Caltag).

Fetal liver chimaeras

Ten-week-old B6 (CD45.1 or CD45.2) or $\beta 2m$ ^{-/-} mice (CD45.2) were injected intraperitoneally at day -1 and day 0 with 0.3 and 0.4 mg of anti-NK1.1 monoclonal antibody (PK136; kindly provided by B. Rocha). Mice were sublethally irradiated (950 cGy), and injected 6 h later with 5×10^6 fetal liver cells prepared from day-14 B6 (CD45.1 or CD45.2) or $\beta 2m$ ^{-/-} (CD45.2) fetuses. Mice were analysed three months later and chimerism determined using the congenic CD45.1/2 markers. Quantitative PCR (Q-PCR) and polyclonal sequencing were carried out using RNA isolated from LN TCR $\alpha\beta$ ⁺ DN-enriched cells. For each group of mice, the frequency of DN among TCR $\alpha\beta$ ⁺ cells was $\geq 80\%$ after enrichment.

Bone marrow chimaeras

Bone marrow cell suspensions from 12-week-old B6 and $\beta 2m$ ^{-/-} mice were depleted of T cells with anti-Thy-1 antibody and complement. Equal numbers (5×10^6) of $\beta 2m$ ^{-/-} and B6, RAG2^{-/-} or CD3 ϵ ^{-/-} BM cells were mixed and injected intravenously into RAG2^{-/-} γc ^{-/-} $\beta 2m$ ^{-/-} hosts that had been treated two days before with 150 mg kg⁻¹ 5-fluorouracil. Mice were analysed three months later.

Preparation of human samples

Frozen biopsies from the skin, gut or lung of patients with non-immune disorders were cut in 50 μ m slices. RNA was extracted with 1 ml of RNABle (Eurobio) as described³.

Cloning and sequencing of V α 19-J α 33 junctions

CD4⁻CD8⁻ LN T cells were prepared with anti-CD4, -CD8 and -MHC class II antibodies and complement, followed by positive selection with anti-TCR β -FITC (Pharmingen) and anti-FITC magnetic beads (Miltenyi Biotec). V α 19-J α 33 junctions were amplified using nested PCR with the following primers: V α 19 external CACTTTCCTGAGCCGCTCGAA, J α 33 external TATAATTAGCTTGGTCCCAGA, V α 19 internal GCTTCTGACAGAGCT CCAG and J α 33 internal CAGAGCCCCAGATCAACTGAT. Fragments were cloned and sequenced using standard techniques.

Mouse MR1A transfectants

Mouse MR1A cDNA was amplified for 35 cycles from B6 splenocytes using the following primers: AAGAAGGAGATCTGTGATGGTGCTCCTGTACCTCTGCTCG and AGAGAAAGAATTGAGAGGGAGAGCTTCCCTCATTCACTTG. The PCR product was cloned into the EGFP-N1 vector (Clontech), sequenced and transfected into RBL (rat mastocytoma) or HOM-2 (human B lymphoblastoid) cell lines. After 10–14 days, growing wells were tested for GFP expression and GFP^{hi} cells were FACS-sorted and plated at one cell per well (see Supplementary Methods).

Screen of haematopoietic cell lines and transfectants

T-T hybridomas were made as described³ from DN mesenteric LN T cells from TAP^{-/-}Ii^{-/-} mice. Cell lines or stable transfectants to be screened were irradiated and distributed at 5×10^4 cells per well in flat-bottomed 96-well plates and 5×10^4 hybridoma were added. After a 48-h incubation, the supernatant was harvested and tested for IL-2 content using the IL-2-dependent CTLL2 cell line. For blocking experiments, serial dilutions of anti-TCR β monoclonal antibody (anti-H57 NA/LE, Pharmingen) or hamster isotype control (clone Ha4/8) were added to the wells (see Supplementary Methods).

Processing of human blood samples, molecular biology methods, RNA extraction, reverse transcription, TCR primers and PCR methods have been described previously³. For clarity, all Q-PCR data have been normalized to C α expression by shifting the V α -J α PCR curves along the x axis.

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Authors' contributions S. Gilfillan and O. Lantz share senior authorship.

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Loss of integrin $\alpha\beta 6$ -mediated TGF- β activation causes Mmp12-dependent emphysema

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Integrins are heterodimeric cell-surface proteins that regulate cell growth, migration and survival. We have shown previously that the epithelial-restricted integrin $\alpha\beta 6$ has another critical function; that is, it binds and activates latent transforming growth factor- β (TGF- β)^{1,2}. Through a global analysis of pulmonary gene expression in the lungs of mice lacking this integrin (*Itgb6* null mice) we have identified a marked induction of macrophage metalloelastase (Mmp12)—a metalloproteinase that preferentially degrades elastin and has been implicated in the chronic lung disease emphysema³. Here we report that *Itgb6*-null mice develop age-related emphysema that is completely abrogated either by transgenic expression of versions of the $\beta 6$ integrin subunit that support TGF- β activation, or by the loss of Mmp12. Furthermore, we show that the effects of *Itgb6* deletion are overcome by simultaneous transgenic expression of

active TGF- $\beta 1$. We have uncovered a pathway in which the loss of integrin-mediated activation of latent TGF- β causes age-dependent pulmonary emphysema through alterations of macrophage Mmp12 expression. Furthermore, we show that a functional alteration in the TGF- β activation pathway affects susceptibility to this disease.

Pulmonary emphysema, which is characterized by simplification of alveolar architecture, loss of lung elasticity, and enlargement of alveolar airspaces, is a worldwide health problem largely attributable to exposure to tobacco smoke. Extracellular proteases, which regulate extracellular matrix homeostasis, have been implicated in tobacco-smoke-induced pulmonary emphysema. Nevertheless, the regulation of these proteases *in vivo* is poorly understood and a regulatory role of lung epithelial cells in this process has yet to be described.

Mice that do not express the $\beta 6$ subunit of the $\alpha\beta 6$ integrin (*Itgb6*⁻) spontaneously develop increased expression of the extracellular macrophage metalloproteinase Mmp12 (macrophage metalloelastase) in their lungs by eight weeks of age. In fact, on the basis of whole-organ gene expression profiling using the

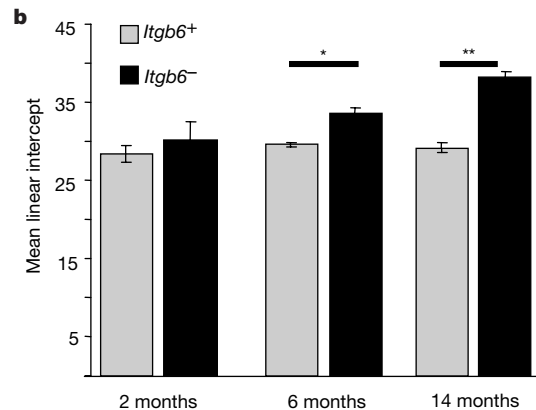
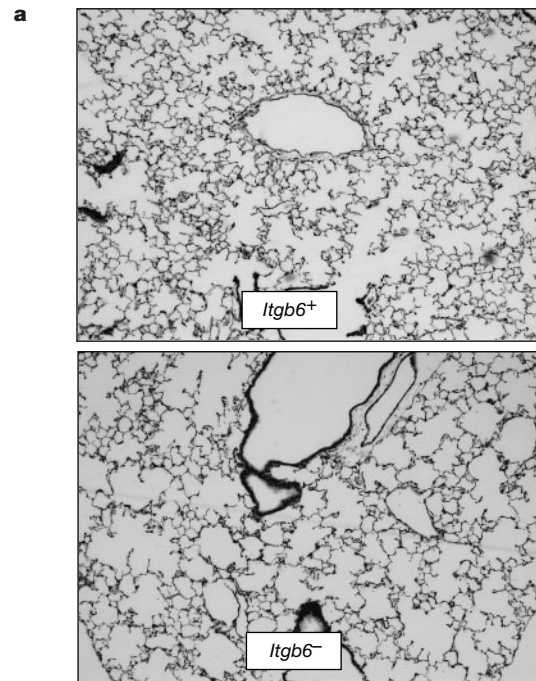


Figure 1 Spontaneous, progressive pulmonary emphysema in *Itgb6*⁻ mice. **a**, Representative histological sections ($\times 10$ objective) of lungs from *Itgb6*⁺ and *Itgb6*⁻ mice at 14 months of age show enlarged alveoli indicative of emphysema in *Itgb6*⁻ mice. **b**, Mean linear intercepts ($\mu\text{m} \pm \text{s.e.m.}$) of alveolar septae measured in the lungs of five *Itgb6*⁺ (wild type) and five *Itgb6*⁻ mice at 2, 6 and 14 months of age. Asterisk, $P = 0.0006$; double asterisk, $P = 0.0002$.

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corrigendum

Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1

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Nature **422** 164–169 (2003).

Owing to mislabelling by the mouse provider, the strain of mice used as a control in the experiment shown in Fig. 4 was not C3H/HeJ but C3H/HeOu. C3H/HeJ have a defect in TLR4-mediated signalling that the other C3H strains do not have. This correction does not affect our conclusions. □

addendum

Non-classical receptive Ca^{2+} mediates switch in a sensory neuron's frequency tuning

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In this Letter, we inadvertently omitted to give the species name, *Apteronotus leptorhynchus* (brown ghost knife-fish), of the weakly electric fish used in our study. □