

660 Human Protein Biomarkers  
200 Mouse Protein Biomarkers  
67 Rat Protein Biomarkers



See What You Have Been  
Missing!

The Largest Multiplex Protein  
Detection Panels Available



*The Journal of  
Immunology*

## Donor Unrestricted T Cells: A Shared Human T Cell Response

Ildiko Van Rhijn and D. Branch Moody

This information is current as  
of February 9, 2016.

*J Immunol* 2015; 195:1927-1932; ;  
doi: 10.4049/jimmunol.1500943  
<http://www.jimmunol.org/content/195/5/1927>

---

### References

This article **cites 68 articles**, 41 of which you can access for free at:  
<http://www.jimmunol.org/content/195/5/1927.full#ref-list-1>

### Subscriptions

Information about subscribing to *The Journal of Immunology* is online at:  
<http://jimmunol.org/subscriptions>

### Permissions

Submit copyright permission requests at:  
<http://www.aai.org/ji/copyright.html>

### Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:  
<http://jimmunol.org/cgi/alerts/etoc>



## Donor Unrestricted T Cells: A Shared Human T Cell Response

Ildiko Van Rhijn<sup>\*,†</sup> and D. Branch Moody<sup>\*</sup>

The now-famous term “restriction” derived from experiments in which T cells from Donor A failed to recognize Ags presented by cells from Donor B. Restriction results from interdonor variation in MHC genes. Donor restriction dominates immunologists’ thinking about the T cell response because it governs organ transplantation and hinders the discovery of disease-associated Ags. However, other T cells can be considered “donor unrestricted” because their targets, CD1a, CD1b, CD1c, CD1d, or MR1, are expressed in a similar form among all humans. A striking feature of donor unrestricted T cells is the expression of invariant TCRs with nearly species-wide distribution. In this article, we review new evidence that donor unrestricted T cells are common in humans. NKT cells, mucosa-associated invariant T cells, and germline-encoded mycolyl-reactive T cells operate outside of the familiar principles of the MHC system, providing a broader picture of T cell function and new opportunities for therapy. *The Journal of Immunology*, 2015, 195: 1927–1932.

In 1974, Zinkernagel and Doherty (1) reported that T cells from Donor A cannot recognize viral Ags presented by cells from Donor B and so are “restricted” to Donor A. Restriction was explained through discovery of MHC genes, which vary in sequence from donor to donor. The medical implications of interdonor MHC protein variation are vast. MHC polymorphism is the central explanation for rejection of organs from unrelated donors (2). Based on the complex patterns of MHC protein expression in human populations, genetic restriction of MHC-reactive T cells creates a situation in which the immunodominant T cell Ags for any infection or autoimmune disease cannot be described in a general way for every patient, which limits Ag-based immunodiagnosis and therapy.

Although immunologists rarely use the term, “donor unrestricted” T cells also exist. That is, certain  $\alpha\beta$  T cells recognize Ags presented by proteins that are not donor specific in the sense that they are not dependent on the genotype of the

donor. CD1a, CD1b, CD1c, CD1d, MR1, and HLA-E genes are expressed as protein heterodimers on the surface of APCs in a similar or identical form among nearly all humans. Single-nucleotide polymorphisms in CD1a and CD1d were described, but the affected amino acids are not located in or near the Ag-binding groove or the TCR-binding surface (3), which justifies the common description of CD1 genes as nonpolymorphic. Unlike Zinkernagel and Doherty’s experiments, CD1-reactive T cells from Donor A will respond to Ag presented by APCs from any human, with the only known exception being that CD1a expression levels differ among donors (4).

Among all donor unrestricted  $\alpha\beta$  T cells, two widely known T cell types recognize MR1 or CD1d. As the names mucosa-associated invariant T (MAIT) cells and NKT cells imply, these T cell types have secondary properties related to organ localization or expression of NK markers, such as CD161. However, in the modern usage of these terms, the essential defining feature of NKT cells and MAIT cells is the expression of invariant TCRs with two key properties: expansion of T cells with many similar, but nonidentical, TCR sequences (intradonor conservation) and recapitulation of these patterns among nearly all humans (interdonor conservation). Moving beyond CD1d and MR1, we review new evidence that CD1a, CD1b, and CD1c proteins also generate inter- and intradonor conserved T cell responses in humans, including newly discovered T cell types defined by invariant TCRs. Because humans express four types of CD1 proteins, and each of these binds to many lipids, we speculate that structurally diverse CD1–lipid complexes could support networks of genetically unrestricted T cells.

### Human $\alpha\beta$ TCR diversity

The basic mechanisms that generate  $\alpha\beta$  TCR diversity are understood in detail and were reviewed previously (5). In maturing T cells, variable (V), diversity (D), and joining (J) gene segments recombine in a stochastic manner. At the joints, nucleotides may be deleted and nontemplated (N) nucleotides may be added to create diverse sequences at the TCR  $\alpha$  and  $\beta$  loci. These rearrangements occur independently in each maturing T cell, generating diverse repertoires of paired  $\alpha$ - and

<sup>\*</sup>Division of Rheumatology, Immunology, and Allergy, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115; and <sup>†</sup>Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, 3584CL Utrecht, the Netherlands

Received for publication April 28, 2015. Accepted for publication June 11, 2015.

This work was supported by the National Institute of Allergy and Infectious Diseases (Grants AI049313, AR048632, and AI111224 to D.B.M.) and by a Bill and Melinda Gates Foundation Vaccine Accelerator Award.

Address correspondence and reprint requests to Dr. Ildiko Van Rhijn, Division of Rheumatology, Immunology, and Allergy, Brigham and Women’s Hospital, One Jimmy Fund Way, Room 514, Boston, MA 02115. E-mail address: i.vanrhijn@uuh.nl

Abbreviations used in this article: GEM, germline-encoded mycolyl reactive; GMM, glucose monomycolate; MAIT, mucosal-associated invariant T; N, nontemplated.

Copyright © 2015 by The American Association of Immunologists, Inc. 0022-1767/15/\$25.00

$\beta$ -chains in every person. Positive and negative selection of T cells is mediated by Ags and Ag-presenting molecules expressed in the thymus. An individual's TCR repertoire size can be compared with the larger number of the theoretically possible  $\alpha\beta$  TCRs or to all  $\alpha\beta$  TCRs actually expressed by humankind. Each human has  $\sim 10^{12}$  T cells (5, 6), but the number of unique TCRs is lower because clonal expansion creates subpopulations with identical TCRs. Because of technical limitations, most experiments that measure human TCR repertoire size focus on the  $\beta$ -chain, and results range from  $0.5$  to  $5 \times 10^6$  unique sequences/person (6–10). Several groups estimate the actual number of TCR  $\alpha\beta$ -chain combinations at  $\sim 2 \times 10^7$ /human (6). The theoretical maximum for TCR  $\beta$ -chain amino acid sequences was estimated to be  $5 \times 10^{11}$  (8) or  $3.5 \times 10^{23}$  (10), whereas for TCR  $\alpha\beta$ -chain combinations it was estimated to be  $10^{15}$  (11).

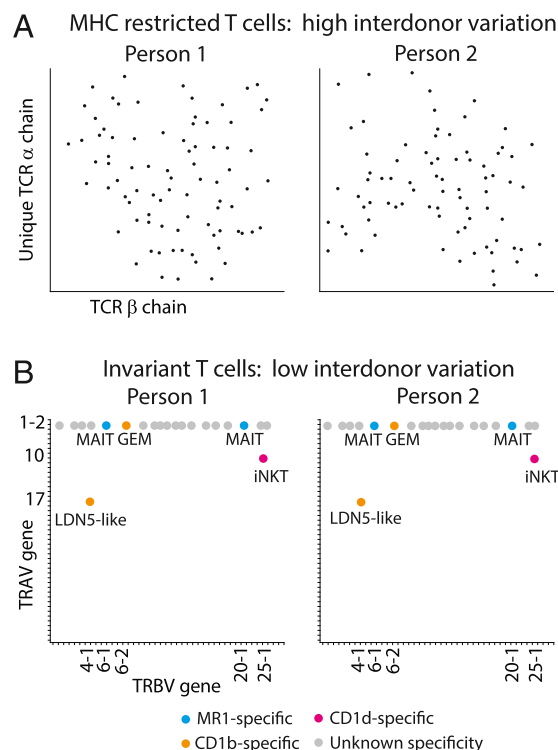
#### Private TCRs in the MHC system

These estimates indicate that the TCR repertoire in one person represents a tiny fraction of all possible TCRs, so most of the potential TCR space, considered as a two-dimensional plot of TCR  $\alpha$  and  $\beta$  sequences, is not covered by all of the  $\alpha\beta$  TCRs in any person (Fig. 1A). Therefore, it follows, and was experimentally observed (8, 12), that most TCRs present in an individual are found in few or no other individuals and so are known as “private” TCRs. Given these facts, two questions arise. How could highly similar TCRs that define invariant NKT cells or MAIT cells come to exist as many clonotypes in one person (intradonor conservation)? And why does the process leading to intradonor conservation repeat itself independently in most or all humans (interdonor conservation)? Some kind of mechanism for directed recombination might be responsible, but studies of mouse NKT cells point away from this explanation (13). Instead, emerging data suggest that this process is related to mechanisms that also underlie public TCRs in the MHC system, which, when applied to nonpolymorphic Ag-presenting molecules, creates a special kind of public TCR that occurs in most or all people, known as invariant TCRs.

#### Public TCRs in the MHC system

Public TCRs are a meaningful exception to the otherwise unmitigated intradonor and interdonor TCR diversity in the MHC system. Public TCRs have highly similar TCR  $\alpha$ - and  $\beta$ -chain sequences and arise in unrelated humans as a result from common HLA alleles, such as HLA-A2, presenting immunodominant Ags from chronic or common viral infections. Fig. 2 summarizes known public TCRs and certain viruses that generate them, including CMV, EBV, and HIV (Fig. 2). Even if genetic polymorphism and Ag exposure are removed as a basis for generating diverse TCRs by HLA matching donors with the same viral infection, it is not clear that public TCRs should come to exist. As discussed above and illustrated in Fig. 1A, TCRs are randomly generated and the number of TCRs in one person is much smaller than all possible TCRs by several orders of magnitude, which suggests that even MHC-matched donors with the same infection would use mostly private TCRs.

Venturi et al. (14) proposed the convergent recombination hypothesis to explain public TCRs in the MHC system. The core idea is that some TCRs have an increased probability of being generated if their sequences are formed by simple and common rearrangements of germline sequences. Extensive,



**FIGURE 1.** Polymorphic and nonpolymorphic Ag-presenting molecules stimulate different kinds of TCR repertoires. **(A)** For two genetically unrelated persons, the number of  $\alpha\beta$  TCR pairs defined as unique amino acid sequences (dots) represent a small fraction of all possible TCRs (6, 8, 10, 11). In the MHC system, interdonor TCR diversity is high, and two unrelated donors have mostly private TCRs that differ in TCR  $\alpha$ - and  $\beta$ -chain pairing (8, 12). **(B)** Invariant TCRs on invariant NKT (iNKT) cells, MAIT cells, GEM T cells, and LDN5-like T cells are genetically unrestricted T cell types that show low interdonor diversity. In addition, 16 TCRs of unknown Ag specificity are conserved among  $>50\%$  of human donors, representing candidate invariant TCRs (51) (gray dots).

nonrepetitive N-nucleotide additions decrease the likelihood of a TCR chain being generated repeatedly. Indeed, public TCRs show hallmarks of simple rearrangements. Also, interdonor-conserved TCR chains often have one or two variable amino acid positions that do not influence the Ag specificity of the TCR chain, which greatly increases the number of ways to create the chain.

Public TCRs are identifiable in highly selected populations that are matched for MHC and viral infection, but they do not extend to the majority of humans or the whole of humanity. The reasons are straightforward: no MHC protein and few Ag exposures occur broadly in outbred human populations. Even the most common MHC class I allele, HLA-A\*0201, is expressed at a rate of 18–48% in three major ethnic populations in the United States (Fig. 2). Most other alleles range from 0.01 to 30%. To our knowledge, no MHC-restricted public TCR has been demonstrated in unselected populations, and the rates of MHC expression in populations predict that none will be discovered in the future (Fig. 2). Thus, public TCRs are not so penetrant in human populations as to form meaningful TCR-based T cell subclasses, and they have not been used widely in immunodiagnosis.

#### Invariant TCRs

Returning to the question of how NKT cells and MAIT cells arise in nearly everyone, this observation might be explained by

	Expression in population				T cell receptor			Specificity	
	Presenting molecule	African American	White	Chinese	Name	TRAV	TRBV	**Antigen	Pathogen
Public TCRs	A*0201	23%	48%	18%	AS01 RA14 JM22	TRAV5 TRAV24 TRAV27	TRBV20-1 TRBV6-5 TRBV19	BMLF1 (280-288) pp65 (495-503) MP (58-66)	EBV CMV Influenza
	B*3501	13%	11%	4.4%	ELS4	TRAV1-2	TRBV10-3	BZLF1 (54-64)	EBV
	B*3508	0%	0.86%	0.10%	D1 cl.10	TRAV8-6	TRBV28	pp65 (103-114)	CMV
	B*5701	1.4%	7.2%	1.0%	LON005	TRAV5	TRBV19	Gag (62-172)	HIV
	B*0702	14%	24%	1.6%	D5 cl.4 1B1	TRAV23 TRAV19	TRBV4-3 TRBV12-4	pp65 (265-275) VP22 (49-57)	CMV HSV
	B*0801	7.5%	22%	0.92%	IM6	TRAV26-2	TRBV7-6	EBNA3 (339-347)	EBV
Invariant TCRs	CD1b	100%*	100%*	100%*	GEM LDN5-like	TRAV1-2 TRAV17	TRBV6-2 TRBV4-1	Glucose- monomycolate	Mycobacterial species
	MR1	100%*	100%*	100%*	MAIT	TRAV1-2	TRBV20-1 TRBV6-1	Vitamin B metabolites	many bacteria
	CD1d	100%*	100%*	100%*	iNKT	TRAV10	TRBV25-1	$\alpha$ -Galactosylceramide	

**FIGURE 2.** The distribution of MHC-restricted public TCRs is limited by the allele frequency of the Ag-presenting molecule. Expression of allelic variants of MHC-encoded Ag-presenting molecules is limited to small human subpopulations, and so are the public T cells that recognize these molecules (60–67). CD1 and MR1 genes are expressed similarly in nearly 100% of humans, so T cells that recognize CD1 or MR1 can be considered donor unrestricted (17, 43, 44, 68). Allele frequency datasets were obtained from <http://www.allelefrequencies.net>. African American (USA NMDP African American),  $n = 416,581$ ; White (USA NMDP European Caucasian),  $n = 1,242,890$ ; Chinese (USA NMDP Chinese),  $n = 99,672$ . \*Single nucleotide polymorphisms are known (3), but functionally distinct alleles of CD1 and MR1 molecules have not been discovered. Individuals with low or high expression levels of CD1a on their dendritic cells were described (4). \*\*For each viral protein Ag that is recognized by public TCRs, the location of the peptide epitope is shown in parentheses.

convergent recombination generating T cells that recognize Ag-presenting molecules expressed in ~100% of the human population (Fig. 2, lower half). Such randomly, but commonly, formed TCRs can be positively selected in every human being if the selecting Ag is also ubiquitously expressed. TCRs that are generated multiple times in one person also would be expected to occur in nearly everyone (15). In this formulation, intradonor and interdonor conservation arise from the same mechanisms and are linked phenomena. Last, this hypothesis predicts that invariant TCRs are largely germline encoded, with few N-region additions. Next, we consider how well these predictions play out in experimental observations of both widely known and newly discovered invariant T cell populations.

*CD1d and MR1*

NKT cells (16–18) and MAIT cells (16, 19) were discovered with unbiased sequencing methods that identified unexpectedly conserved TCRs among the expected pattern of highly diverse TCRs for the MHC system. The overall patterns showed linked intradonor and interdonor conservation, and chains encoded by germline V and J genes with few N-region additions, fulfilling the predictions outlined above. Specifically, NKT cells showed nearly invariant TCR  $\alpha$ -chains of TRAV10 joined to TRAJ18, which is sometimes referred to as a type 3 bias (20), in which a complete TCR chain is conserved. The NKT  $\beta$ -chain almost always uses TRBV25-1 (16, 21), but without a preferred J gene or a fixed length, which is considered a type 1 TCR bias (20). MR1 activates TCRs with  $\alpha$ -chains that use TRAV1-2 joined to TRAJ33 that paired with TRBV20-1 or TRBV6-1 (16, 19) (Fig. 2). These observations are consistent with the interpretation that TCRs on NKT cells and MAIT cells are like public TCRs in the MHC system but are much more penetrant in human populations. The fact that MAIT and NKT cells are activated or expanded in all humans, even at a very young age (22–24), suggests that selecting Ags are widespread and most likely are not limited to one pathogen. For NKT cells, self (25, 26) or microbiota-derived  $\alpha$ -linked sphingolipids are implicated (27, 28), but self-ligands for MR1 are unknown.

*A broader view of CD1*

With >20,000 publications on NKT cells and MAIT cells, it might seem that the overall repertoires of cells recognizing CD1 and MR1 are completely or at least broadly characterized. Therefore, we emphasize in this article that the current understanding of the human CD1-reactive T cell repertoire is highly incomplete. Considering CD1d, Cardell and colleagues (29, 30) described CD1d and sulfatide-reactive TCRs that are outside the sequence motifs for invariant NKT cells. Other T cell clones recognize CD1d-presented Ags that are structurally unrelated to  $\alpha$ -linked ceramides or sulfatides, such as phenyl pentamethyldihydrobenzofuran sulfonate (31), phospholipids (32), lysophospholipids (33), and ether-linked lipids (34). The extent to which these clones represent invariant TCR patterns in the repertoire has not been extensively evaluated. Likewise, new evidence suggests that certain MR1-reactive T cells lack the canonical MAIT cell TCRs (35, 36). The recent validation of MR1 tetramers loaded with vitamin B metabolites now allows investigation of the MR1-reactive repertoire that may include T cells outside the TCR motifs described by Porcelli and Lantz (16, 19). Further, the human CD1 system is not represented by CD1d only, but includes three structurally distinct Ag-presenting molecules, CD1a, CD1b, and CD1c, which are known as the group 1 CD1 proteins. All four human CD1 Ag-presenting molecules present self and foreign lipid Ags to human  $\alpha\beta$  T cells (37), but the responding TCRs are only beginning to be systematically analyzed.

*Earliest analyses of the group 1 CD1 repertoire*

Initial analyses of group 1 CD1-reactive TCRs failed to find evidence for conserved TCR gene usage (38, 39). These initial surveys led to the description of the group 1 CD1-reactive TCR repertoire as diverse, which contrasts with the invariant nature of the CD1d-reactive TCR repertoire. The notion of a diverse group 1 CD1-reactive TCR repertoire invited comparisons to diverse MHC-reactive T cells. However, early analyses were small in scale and compared panels of TCRs that recognize different human CD1 isoforms presenting various



Ag. TCRs recognizing differently shaped CD1–lipid complexes are expected to differ in structure. The concepts outlined above invite a different question about TCR diversity measured across the human species. Do T cells from genetically unrelated donors that respond to one lipid Ag presented by the same CD1 isoform show conserved or diverse TCRs? The recent validation of human CD1a, CD1b, and CD1c tetramers (40–42) now offers a method to answer this question, providing the first glimpses into the polyclonal TCR repertoire recognizing group 1 CD1 proteins.

#### *Invariant TCRs recognizing CD1b*

Human CD1b tetramers loaded with an immunodominant mycobacterial ligand, glucose monomycolate (GMM), were used to interrogate TCRs in patients with tuberculosis. This approach enabled rapid generation of panels of T cell clones from unrelated donors (43, 44). When the question of interdonor diversity was framed as measuring the types of TCRs recognizing one immunodominant glycolipid (GMM) presented by one CD1 protein (CD1b), evidence for two conserved TCRs emerged. The first invariant TCR showed the kind of pattern also seen in invariant NKT cells and MAIT cells: stringent conservation of the TCR  $\alpha$ -chains that are encoded by germline V and J segments, and a biased use of TCR  $\beta$ -chains with a certain V gene (Fig. 1B) (43). Based on the origin of the TCR and Ag, these CD1b-reactive invariant T cells were named germline-encoded mycolyl-reactive (GEM) T cells. Both GEM T cells and MAIT cells express TRAV1-2, but they use different joining genes and different TCR  $\beta$ -chains to recognize their Ags without cross-reactivity (Fig. 1B).

A second pattern of TCR conservation was identified among genetically unrelated tuberculosis patients: a biased use of TRBV4-1 in the TCR  $\beta$ -chain and TRAV17 in the TCR  $\alpha$ -chain (44). These T cells were designated LDN5-like T cells because their TCRs strongly resembled those from a T cell clone (LDN5) previously isolated from a skin lesion infected with *Mycobacterium leprae* (45). This name emphasizes that the LDN5 clone was first thought to be a unique reagent that expresses a private TCR, but instead appears to be a polyclonal pool that occurs among genetically unrelated donors. The most characteristic aspect of the pattern is type 1 bias (shared V gene usage in the absence of simple recombination events or J gene sharing) in both TCR chains. LDN5-like TCRs have only been measured in small patient groups, so the extent of their interdonor conservation is not known. LDN5-like TCRs consistently show lower affinity for CD1b-GMM than GEM TCRs. LDN5-like TCRs show less stereotyped TCR patterns, much like the relationship of diverse NKT TCRs to invariant NKT TCRs. Overall, these data suggest that one Ag in the CD1b system generates at least two interdonor conserved TCRs that differ in affinity and the patterns of TCR conservation. The CD1a- and CD1c-reactive repertoires have not been extensively probed. However, a recent study of CD1c-phosphomycoketide-reactive TCRs showed overrepresentation of TRBV7-8 and TRBV7-9, which might represent an example of TCR conservation in the CD1c repertoire (46).

#### *The broader CD1 repertoire*

Based on TCR patterns observed for a small number of Ags in the CD1d and CD1b systems, questions that guide research

into the overall CD1-reactive TCR repertoire can now be meaningfully framed. First, we predict that other invariant TCRs exist in humans but have not been discovered. It is notable that two types of GMM-reactive TCRs were discovered using the first Ag studied in this way, and CD1b is known to present many Ags, including free mycolate (47), glycerol monomycolate (48), sulfolipid (49), and others. The overall breadth of Ags presented by the four types of CD1 proteins remains unknown, but one study detected hundreds of lipids eluting from each of the four human CD1 proteins produced in cells (50). Although most research focuses on NKT cells, the cellular system for generating CD1–lipid complexes has combinatorial diversity, and the many types of CD1–lipid complexes formed in cells could support correspondingly high numbers of Ag-specific TCRs. Thus, it is plausible to suggest that a network of donor unrestricted T cells exists and that it functions in parallel to the MHC-restricted system.

#### *High-throughput TCR sequencing*

Such a system could be discovered through high-throughput TCR sequencing of human T cells. Most existing datasets focus on the TCR  $\beta$ -chain only, and many studies of this type used populations that share MHC alleles to search for MHC-restricted public TCRs. As the discussion above highlights, invariant TCRs are distinguished by high penetrance in populations and recognition of nonpolymorphic Ag-presenting molecules. Therefore, approaches that would distinguish invariant TCRs from both private and public MHC-restricted TCRs would start with unselected human populations, use stringent penetrance criteria (>50% of donors), and identify the Ag-presenting molecule. To our knowledge, this has not been accomplished, but a recent study of TCR  $\alpha$ -chains using TRAV1-2 identified 16 invariant TCRs (Fig. 1B, gray dots) (51). The Ag-presentation molecule(s), if it exists, remains unknown. However, these data suggest that broadly conserved TCR chains exist in the human repertoire and are not limited to sequences that are known from MAIT cells, NKT cells, and GEM T cells. NKT cells, MAIT cells, and GEM T cells show higher conservation in the TCR  $\alpha$ -chain, but LDN5-like T cells show bias in V $\alpha$  and V $\beta$  gene usage. Therefore, the ideal approach is to measure paired  $\alpha$ - and  $\beta$ -chain sequences, a technique that is becoming feasible (52).

#### *Private TCRs in the CD1 system*

A second prediction is that private TCRs recognizing CD1–lipid complexes also exist. Broadly shared Ag-presenting molecules are necessary for the development of invariant T cell populations. However, there is no reason to assume that they do not also stimulate T cells expressing private TCRs. Recombination with diverse N-region additions represents a powerful diversity-generating mechanism. Studies comparing TCR profiles in unrelated donors, HLA-matched siblings, and identical twins found similar rates of shared TCR sequences, making it clear that even shared Ag-presenting molecules most often generate private, rather than public, TCRs (8, 9, 12). Therefore, noninvariant CD1–lipid complex-specific T cells might be derived from this private pool of TCRs. Some individual clones recognizing CD1a, CD1b, and CD1c contain extensive N-nucleotide regions (38, 44, 46), which are not likely to exist as species-wide conserved

TCRs. Also, NKT cells and MAIT cells were recognized and defined based on conserved TCR motifs, but the recent validation of Ag-loaded tetramers allows the broader survey of CD1d- and MR1-reactive T cells, providing a means to broadly survey TCRs with these specificities.

## Conclusions

The central idea discussed in this article is that the widely accepted principles of donor-specific genetic restriction are important, but they should not be assumed to apply to all  $\alpha\beta$  T cells. Recent evidence indicates that donor unrestricted T cells are more common than previously thought. CD1a-autoreactive TCRs are particularly abundant in blood and skin, with frequencies ranging from 0.1 to 1% (53) or from 1 to 10% (54). MAIT cells (1–20%) (55) and NKT cells (0.1–5%) (56) circulate in large numbers in human blood and tissue, and recent studies showed that  $\gamma\delta$  or  $\delta/\alpha\beta$  T cells also recognize CD1 (57, 58). Collectively, these various types of donor unrestricted T cells make up a substantial fraction of the overall human T cell repertoire (58). Thus, for the basic immunologist, studying donor unrestricted T cells offers a broad way to test the relationship between Ag-presenting molecule polymorphism and the diversity of T cell responses. For example, the considerations outlined above make experimentally verifiable predictions: interdonor and intradonor TCR conservation are linked, and additional invariant TCRs that respond to nonpolymorphic Ag-presenting molecules in the human system exist, but remain undiscovered.

Further, practical application of knowledge of TCR diversity in the CD1 system can be considered a basis for diagnosis and therapy. For T cells restricted to genetic elements that are rare in the population, defining immunodominant Ags that act broadly across human populations has been difficult. Therefore, peptides are rarely used for T cell modulation. Interdonor variation in TCR response prevents practical use of TCR sequences for immunodiagnosis. Similarly, it is difficult to use peptides as recall Ags in immunodiagnosis, notwithstanding successes that came about through complex discovery and engineering promiscuous peptides for tuberculosis testing (59). For donor unrestricted T cells, these roadblocks are absent. The nonpolymorphic nature of CD1 and MR1 proteins creates a situation in which species-wide Ag specific responses exist and, in some cases, correspond to species-wide invariant TCRs. Uniform responses across populations could make T cell Ags tractable for diagnosis and therapy. We know that most humans respond to orally delivered  $\alpha$ -galactosylceramide. MAIT cells account for up to 20% of all T cells in some tissues (55), so ribityl lumazine Ags might be used to modulate responses in vivo. Two conserved T cell types were identified using only one of the many Ags in the CD1b system. Therefore, dissection of the human  $\alpha\beta$  TCR pool with CD1a, CD1b, CD1c, and MR1 tetramers may show that NKT cells, MAIT cells, and GEM T cells are not lonely islands of TCR conservation in a sea of diverse MHC-restricted T cells, but instead are harbingers of larger networks of species-wide Ag responses.

## Acknowledgments

We thank Vanessa Venturi for thoughtful comments.

## Disclosures

The authors have no financial conflicts of interest.

## References

1. Zinkernagel, R. M., and P. C. Doherty. 1974. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semi-allogeneic system. *Nature* 248: 701–702.
2. Zinkernagel, R. M. 1997. The Nobel Lectures in Immunology. The Nobel Prize for Physiology or Medicine, 1996. Cellular immune recognition and the biological role of major transplantation antigens. *Scand. J. Immunol.* 46: 421–436.
3. Han, M., L. I. Hannick, M. DiBrino, and M. A. Robinson. 1999. Polymorphism of human CD1 genes. *Tissue Antigens* 54: 122–127.
4. Seshadri, C., M. Shenoy, R. D. Wells, T. Hensley-McBain, E. Andersen-Nissen, M. J. McElrath, T. Y. Cheng, D. B. Moody, and T. R. Hawn. 2013. Human CD1a deficiency is common and genetically regulated. *J. Immunol.* 191: 1586–1593.
5. Nikolich-Zugich, J., M. K. Slika, and I. Messaoudi. 2004. The many important facets of T-cell repertoire diversity. *Nat. Rev. Immunol.* 4: 123–132.
6. Arstila, T. P., A. Casrouge, V. Baron, J. Even, J. Kanellopoulos, and P. Kourilsky. 1999. A direct estimate of the human alpha beta T cell receptor diversity. *Science* 286: 958–961.
7. Robins, H. S., P. V. Campregher, S. K. Srivastava, A. Wachter, C. J. Turtle, O. Khsai, S. R. Riddell, E. H. Warren, and C. S. Carlson. 2009. Comprehensive assessment of T-cell receptor beta-chain diversity in alpha beta T cells. *Blood* 114: 4099–4107.
8. Robins, H. S., S. K. Srivastava, P. V. Campregher, C. J. Turtle, J. Andriesen, S. R. Riddell, C. S. Carlson, and E. H. Warren. 2010. Overlap and effective size of the human CD8+ T cell receptor repertoire. *Sci. Transl. Med.* 2: 47ra64.
9. Warren, R. L., J. D. Freeman, T. Zeng, G. Choe, S. Munro, R. Moore, J. R. Webb, and R. A. Holt. 2011. Exhaustive T-cell repertoire sequencing of human peripheral blood samples reveals signatures of antigen selection and a directly measured repertoire size of at least 1 million clonotypes. *Genome Res.* 21: 790–797.
10. Wang, C., C. M. Sanders, Q. Yang, H. W. Schroeder, Jr., E. Wang, F. Babrzadeh, B. Gharizadeh, R. M. Myers, J. R. Hudson, Jr., R. W. Davis, and J. Han. 2010. High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets. *Proc. Natl. Acad. Sci. USA* 107: 1518–1523.
11. Davis, M. M., and P. J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature* 334: 395–402.
12. Zvyagin, I. V., M. V. Pogorelyy, M. E. Ivanova, E. A. Komech, M. Shugay, D. A. Bolotin, A. A. Shelenkov, A. A. Kurnosov, D. B. Staroverov, D. M. Chudakov, et al. 2014. Distinctive properties of identical twins' TCR repertoires revealed by high-throughput sequencing. *Proc. Natl. Acad. Sci. USA* 111: 5980–5985.
13. Shimamura, M., T. Ohteki, U. Beutner, and H. R. MacDonald. 1997. Lack of directed V alpha 14-J alpha 281 rearrangements in NK1+ T cells. *Eur. J. Immunol.* 27: 1576–1579.
14. Venturi, V., D. A. Price, D. C. Douek, and M. P. Davenport. 2008. The molecular basis for public T-cell responses? *Nat. Rev. Immunol.* 8: 231–238.
15. Greenaway, H. Y., B. Ng, D. A. Price, D. C. Douek, M. P. Davenport, and V. Venturi. 2013. NKT and MAIT invariant TCR $\alpha$  sequences can be produced efficiently by VJ gene recombination. *Immunobiology* 218: 213–224.
16. Porcelli, S., C. E. Yockey, M. B. Brenner, and S. P. Balk. 1993. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4–8– alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. *J. Exp. Med.* 178: 1–16.
17. Fowlkes, B. J., A. M. Kruisbeek, H. Ton-That, M. A. Weston, J. E. Coligan, R. H. Schwartz, and D. M. Pardoll. 1987. A novel population of T-cell receptor alpha beta-bearing thymocytes which predominantly expresses a single V beta gene family. *Nature* 329: 251–254.
18. Budd, R. C., G. C. Miescher, R. C. Howe, R. K. Lees, C. Bron, and H. R. MacDonald. 1987. Developmentally regulated expression of T cell receptor beta chain variable domains in immature thymocytes. *J. Exp. Med.* 166: 577–582.
19. Tilloy, F., E. Treiner, S. H. Park, C. Garcia, F. Lemonnier, H. de la Salle, A. Bendelac, M. Bonneville, and O. Lantz. 1999. An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. *J. Exp. Med.* 189: 1907–1921.
20. Turner, S. J., P. C. Doherty, J. McCluskey, and J. Rossjohn. 2006. Structural determinants of T-cell receptor bias in immunity. *Nat. Rev. Immunol.* 6: 883–894.
21. Lantz, O., and A. Bendelac. 1994. An invariant T cell receptor alpha chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4–8– T cells in mice and humans. *J. Exp. Med.* 180: 1097–1106.
22. van Der Vliet, H. J., N. Nishi, T. D. de Gruij, B. M. von Blomberg, A. J. van den Eertwegh, H. M. Pinedo, G. Giaccone, and R. J. Scheper. 2000. Human natural killer T cells acquire a memory-activated phenotype before birth. *Blood* 95: 2440–2442.
23. Martin, E., E. Treiner, L. Duban, L. Guerri, H. Laude, C. Toly, V. Premel, A. Devys, I. C. Moura, F. Tilloy, et al. 2009. Stepwise development of MAIT cells in mouse and human. *PLoS Biol.* 7: e54.
24. Gold, M. C., T. Eid, S. Smyk-Pearson, Y. Eberling, G. M. Swarbrick, S. M. Langley, P. R. Streeter, D. A. Lewinsohn, and D. M. Lewinsohn. 2013. Human thymic MR1-restricted MAIT cells are innate pathogen-reactive effectors that adapt following thymic egress. *Mucosal Immunol.* 6: 35–44.
25. Kain, L., B. Webb, B. L. Anderson, S. Deng, M. Holt, A. Costanzo, M. Zhao, K. Self, A. Teyton, C. Everett, et al. 2014. The identification of the endogenous ligands of natural killer T cells reveals the presence of mammalian  $\alpha$ -linked glycosylceramides. [Published erratum appears in 2014 *Immunity* 41: 867.] *Immunity* 41: 543–554.
26. Brennan, P. J., R. V. Tatituri, C. Heiss, G. F. Watts, F. F. Hsu, N. Veerapen, L. R. Cox, P. Azadi, G. S. Besra, and M. B. Brenner. 2014. Activation of iNKT cells by a distinct constituent of the endogenous glucosylceramide fraction. *Proc. Natl. Acad. Sci. USA* 111: 13433–13438.

27. Kawano, T., J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, et al. 1997. CD1d-restricted and TCR-mediated activation of  $\alpha$ 14 NKT cells by glycosylceramides. *Science* 278: 1626–1629.
28. Wieland Brown, L. C., C. Penaranda, P. C. Kashyap, B. B. Williams, J. Clardy, M. Kronenberg, J. L. Sonnenburg, L. E. Comstock, J. A. Bluestone, and M. A. Fischbach. 2013. Production of  $\alpha$ -galactosylceramide by a prominent member of the human gut microbiota. *PLoS Biol.* 11: e1001610.
29. Cardell, S., S. Tangri, S. Chan, M. Kronenberg, C. Benoist, and D. Mathis. 1995. CD1-restricted CD4<sup>+</sup> T cells in major histocompatibility complex class II-deficient mice. *J. Exp. Med.* 182: 993–1004.
30. Jahng, A., I. Maricic, C. Aguilera, S. Cardell, R. C. Halder, and V. Kumar. 2004. Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide. *J. Exp. Med.* 199: 947–957.
31. Van Rhijn, I., D. C. Young, J. S. Im, S. B. Levery, P. A. Illarionov, G. S. Besra, S. A. Porcelli, J. Gumperz, T. Y. Cheng, and D. B. Moody. 2004. CD1d-restricted T cell activation by nonlipidic small molecules. *Proc. Natl. Acad. Sci. USA* 101: 13578–13583.
32. Tatituri, R. V., G. F. Watts, V. Bhowruth, N. Barton, A. Rothchild, F. F. Hsu, C. F. Almeida, L. R. Cox, L. Eggeling, S. Cardell, et al. 2013. Recognition of microbial and mammalian phospholipid antigens by NKT cells with diverse TCRs. *Proc. Natl. Acad. Sci. USA* 110: 1827–1832.
33. Fox, L. M., D. G. Cox, J. L. Lockridge, X. Wang, X. Chen, L. Scharf, D. L. Trott, R. M. Ndonye, N. Veerapen, G. S. Besra, et al. 2009. Recognition of lyso-phospholipids by human natural killer T lymphocytes. *PLoS Biol.* 7: e1000228.
34. Facciotti, F., G. S. Ramanjaneyulu, M. Lepore, S. Sansano, M. Cavallari, M. Kistowska, S. Forss-Petter, G. Ni, A. Colone, A. Singhal, et al. 2012. Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus. *Nat. Immunol.* 13: 474–480.
35. Reantragoon, R., A. J. Corbett, I. G. Sakala, N. A. Gherardin, J. B. Furness, Z. Chen, S. B. Eckle, A. P. Uldrich, R. W. Birkinshaw, O. Patel, et al. 2013. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J. Exp. Med.* 210: 2305–2320.
36. Gold, M. C., J. E. McLaren, J. A. Reistetter, S. Smyk-Pearson, K. Ladell, G. M. Swarbrick, Y. Y. Yu, T. H. Hansen, O. Lund, M. Nielsen, et al. 2014. MR1-restricted MAIT cells display ligand discrimination and pathogen selectivity through distinct T cell receptor usage. *J. Exp. Med.* 211: 1601–1610.
37. Layre, E., A. de Jong, and D. B. Moody. 2014. Human T cells use CD1 and MR1 to recognize lipids and small molecules. *Curr. Opin. Chem. Biol.* 23: 31–38.
38. Grant, E. P., M. Degano, J. P. Rosat, S. Stenger, R. L. Modlin, I. A. Wilson, S. A. Porcelli, and M. B. Brenner. 1999. Molecular recognition of lipid antigens by T cell receptors. *J. Exp. Med.* 189: 195–205.
39. Cohen, N. R., S. Garg, and M. B. Brenner. 2009. Antigen Presentation by CD1 Lipids, T Cells, and NKT Cells in Microbial Immunity. *Adv. Immunol.* 102: 1–94.
40. Kasmar, A. G., I. van Rhijn, T. Y. Cheng, M. Turner, C. Seshadri, A. Schiefner, R. C. Kalathur, J. W. Annand, A. de Jong, J. Shires, et al. 2011. CD1b tetramers bind  $\alpha\beta$  T cell receptors to identify a mycobacterial glycolipid-reactive T cell repertoire in humans. *J. Exp. Med.* 208: 1741–1747.
41. Kasmar, A. G., I. Van Rhijn, K. G. Magalhaes, D. C. Young, T. Y. Cheng, M. T. Turner, A. Schiefner, R. C. Kalathur, I. A. Wilson, M. Bhati, et al. 2013. Cutting Edge: CD1a tetramers and dextramers identify human lipopeptide-specific T cells ex vivo. *J. Immunol.* 191: 4499–4503.
42. Ly, D., A. G. Kasmar, T. Y. Cheng, A. de Jong, S. Huang, S. Roy, A. Bhatt, R. P. van Summeren, J. D. Altman, W. R. Jacobs, Jr., et al. 2013. CD1c tetramers detect ex vivo T cell responses to processed phosphomycolate antigens. *J. Exp. Med.* 210: 729–741.
43. Van Rhijn, I., A. Kasmar, A. de Jong, S. Gras, M. Bhati, M. E. Doorenspleet, N. de Vries, D. I. Godfrey, J. D. Altman, W. de Jager, et al. 2013. A conserved human T cell population targets mycobacterial antigens presented by CD1b. *Nat. Immunol.* 14: 706–713.
44. Van Rhijn, I., N. A. Gherardin, A. Kasmar, W. de Jager, D. G. Pellicci, L. Kostenko, L. L. Tan, M. Bhati, S. Gras, D. I. Godfrey, et al. 2014. TCR bias and affinity define two compartments of the CD1b-glycolipid-specific T cell repertoire. *J. Immunol.* 192: 4054–4060.
45. Moody, D. B., B. B. Reinhold, M. R. Guy, E. M. Beckman, D. E. Frederique, S. T. Furlong, S. Ye, V. N. Reinhold, P. A. Sieling, R. L. Modlin, et al. 1997. Structural requirements for glycolipid antigen recognition by CD1b-restricted T cells. *Science* 278: 283–286.
46. Roy, S., D. Ly, N. S. Li, J. D. Altman, J. A. Piccirilli, D. B. Moody, and E. J. Adams. 2014. Molecular basis of mycobacterial lipid antigen presentation by CD1c and its recognition by  $\alpha\beta$  T cells. *Proc. Natl. Acad. Sci. USA* 111: E4648–E4657.
47. Beckman, E. M., S. A. Porcelli, C. T. Morita, S. M. Behar, S. T. Furlong, and M. B. Brenner. 1994. Recognition of a lipid antigen by CD1-restricted  $\alpha$   $\beta$  T cells. *Nature* 372: 691–694.
48. Layre, E., A. Collmann, M. Bastian, S. Mariotti, J. Czaplicki, J. Prandi, L. Mori, S. Stenger, G. De Libero, G. Puzo, and M. Gilleron. 2009. Mycolic acids constitute a scaffold for mycobacterial lipid antigens stimulating CD1-restricted T cells. *Chem. Biol.* 16: 82–92.
49. Gilleron, M., S. Stenger, Z. Mazorra, F. Wittke, S. Mariotti, G. Böhmer, J. Prandi, L. Mori, G. Puzo, and G. De Libero. 2004. Diacylated sulfolipids are novel mycobacterial antigens stimulating CD1-restricted T cells during infection with *Mycobacterium tuberculosis*. *J. Exp. Med.* 199: 649–659.
50. Huang, S., T. Y. Cheng, D. C. Young, E. Layre, C. A. Madigan, J. Shires, V. Cerundolo, J. D. Altman, and D. B. Moody. 2011. Discovery of deoxyceramides and diacylglycerols as CD1b scaffold lipids among diverse groove-blocking lipids of the human CD1 system. *Proc. Natl. Acad. Sci. USA* 108: 19335–19340.
51. van Schaik, B., P. Klarenbeek, M. Doorenspleet, A. van Kampen, D. B. Moody, N. de Vries, and I. Van Rhijn. 2014. Discovery of invariant T cells by next-generation sequencing of the human TCR  $\alpha$ -chain repertoire. *J. Immunol.* 193: 5338–5344.
52. Turchaninova, M. A., O. V. Britanova, D. A. Bolotin, M. Shugay, E. V. Putintseva, D. B. Staroverov, G. Sharonov, D. Shcherbo, I. V. Zvyagin, I. Z. Mamedov, et al. 2013. Pairing of T-cell receptor chains via emulsion PCR. *Eur. J. Immunol.* 43: 2507–2515.
53. de Jong, A., V. Peña-Cruz, T. Y. Cheng, R. A. Clark, I. Van Rhijn, and D. B. Moody. 2010. CD1a-autoreactive T cells are a normal component of the human  $\alpha\beta$  T cell repertoire. *Nat. Immunol.* 11: 1102–1109.
54. de Lalla, C., M. Lepore, F. M. Piccolo, A. Rinaldi, A. Scelfo, C. Garavaglia, L. Mori, G. De Libero, P. Dellabona, and G. Casorati. 2011. High-frequency and adaptive-like dynamics of human CD1 self-reactive T cells. *Eur. J. Immunol.* 41: 602–610.
55. Dusseaux, M., E. Martin, N. Serriari, I. Péguillet, V. Premel, D. Louis, M. Milder, L. Le Bourhis, C. Soudais, E. Treiner, and O. Lantz. 2011. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood* 117: 1250–1259.
56. Gumperz, J. E., S. Miyake, T. Yamamura, and M. B. Brenner. 2002. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. *J. Exp. Med.* 195: 625–636.
57. Bai, L., D. Picard, B. Anderson, V. Chaudhary, A. Luoma, B. Jabri, E. J. Adams, P. B. Savage, and A. Bendelac. 2012. The majority of CD1d-sulfatide-specific T cells in human blood use a semiinvariant V $\delta$ 1 TCR. *Eur. J. Immunol.* 42: 2505–2510.
58. Pellicci, D. G., A. P. Uldrich, J. Le Nours, F. Ross, E. Chabrol, S. B. Eckle, R. de Boer, R. T. Lim, K. McPherson, G. Besra, et al. 2014. The molecular bases of  $\delta/\alpha\beta$  T cell-mediated antigen recognition. *J. Exp. Med.* 211: 2599–2615.
59. Lalyani, A., and M. Pareek. 2010. A 100 year update on diagnosis of tuberculosis infection. *Br. Med. Bull.* 93: 69–84.
60. Miles, J. J., A. M. Bulek, D. K. Cole, E. Gostick, A. J. Schauben, G. Dolton, V. Venturi, M. P. Davenport, M. P. Tan, S. R. Burrows, et al. 2010. Genetic and structural basis for selection of a ubiquitous T cell receptor deployed in Epstein-Barr virus infection. *PLoS Pathog.* 6: e1001198.
61. Trautmann, L., M. Rimbart, K. Echasserieau, X. Saulquin, B. Neveu, J. Dechanet, V. Cerundolo, and M. Bonneville. 2005. Selection of T cell clones expressing high-affinity public TCRs within human cytomegalovirus-specific CD8 T cell responses. *J. Immunol.* 175: 6123–6132.
62. Moss, P. A., R. J. Moors, W. M. Rosenberg, S. J. Rowland-Jones, H. C. Bodmer, A. J. McMichael, and J. I. Bell. 1991. Extensive conservation of alpha and beta chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide. *Proc. Natl. Acad. Sci. USA* 88: 8987–8990.
63. Wynn, K. K., Z. Fulton, L. Cooper, S. L. Silins, S. Gras, J. K. Archbold, F. E. Tynan, J. J. Miles, J. McCluskey, S. R. Burrows, et al. 2008. Impact of clonal competition for peptide-MHC complexes on the CD8<sup>+</sup> T-cell repertoire selection in a persistent viral infection. *Blood* 111: 4283–4292.
64. Gillespie, G. M., G. Stewart-Jones, J. Rengaraj, T. Beattie, J. J. Bwayo, F. A. Plummer, R. Kaul, A. J. McMichael, P. Easterbrook, T. Dong, et al. 2006. Strong TCR conservation and altered T cell cross-reactivity characterize a B\*57-restricted immune response in HIV-1 infection. *J. Immunol.* 177: 3893–3902.
65. Brennan, R. M., J. Petersen, M. A. Neller, J. J. Miles, J. M. Burrows, C. Smith, J. McCluskey, R. Khanna, J. Rossjohn, and S. R. Burrows. 2012. The impact of a large and frequent deletion in the human TCR  $\beta$  locus on antiviral immunity. *J. Immunol.* 188: 2742–2748.
66. Dong, L., P. Li, T. Oenema, C. L. McClurkin, and D. M. Koelle. 2010. Public TCR use by herpes simplex virus-2-specific human CD8 CTLs. *J. Immunol.* 184: 3063–3071.
67. Argat, V. P., C. W. Schmidt, S. R. Burrows, S. L. Silins, M. G. Kurilla, D. L. Doolan, A. Suhrbier, D. J. Moss, E. Kieff, T. B. Sculley, and I. S. Misko. 1994. Dominant selection of an invariant T cell antigen receptor in response to persistent infection by Epstein-Barr virus. *J. Exp. Med.* 180: 2335–2340.
68. Treiner, E., L. Duban, S. Bahram, M. Radosavljevic, V. Wanner, F. Tilloy, P. Affaticati, S. Gilfillan, and O. Lantz. 2003. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* 422: 164–169.