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Transgenic mice with a diverse human T cell antigen receptor repertoire

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Because of tolerance mechanisms, it has been hard to identify the T cell receptors (TCRs) of high-avidity T cells against self (for example, tumor) antigens. TCRs that are specific for foreign human antigens from the nontolerant T cell repertoire can be identified in mice. Moreover, if mice are constructed to express the human TCR repertoire, they can be used to analyze the unskewed repertoire against human self antigens. Here we generated transgenic mice with the entire human TCRαβ gene loci (1.1 and 0.7 Mb), whose T cells express a diverse human TCR repertoire that compensates for mouse TCR deficiency. A human major histocompatibility class I transgene increases the generation of CD8$^+$ T cells with human compared to mouse TCRs. Functional CD8$^+$ T cells against several human tumor antigens were induced, and those against the Melan-A melanoma antigen used similar TCRs to those that have been detected in T cell clones from individuals with autoimmune vitiligo or melanoma. These mice will allow researchers to identify pathogenic and therapeutic human TCRs.

Each T cell clone expresses a unique TCR of either the TCRαβ or the TCRγδ type. Clonal diversity is created by stochastic rearrangement of V(D)J (variable, diversity, joining) gene segments that span large genomic regions[1–3]. The human TCR α gene locus (TRA) is spread over 1.1 Mb on chromosome 14; the TCRβ gene locus (TRB) is located on a 0.7-Mb fragment on chromosome 7. Together, they contain around 170 functional TCR gene segments. For positive selection in the thymus[4–6], survival in the periphery[7] and their final purpose, the specific response to pathogens, T cells require the TCRαβ for binding to major histocompatibility complex (MHC) molecules that present self or foreign peptides[8,9]. It has been shown that the mouse TCRαβ evolved for binding to MHC proteins[10–13], but similar analysis of the human TCR has not been possible. Even though it is known that TCRαβ and TCRγδ T cells develop in the thymus from a common precursor, and either T cell subset can drive differentiation of the other lineage, the intricate relationship that controls lineage choice is incompletely understood[14].

Mice with a humanized T cell recognition system provide a powerful tool for addressing questions related to tolerance mechanisms in humans. Tolerance to self antigens, such as tumor-associated antigens (TAAs), is generated and maintained by central (deletional) and peripheral tolerance. The relative contributions of both mechanisms might depend on the respective (peptide) antigen. CD8$^+$ T cell responses against some TAAs, such as Melan-A/MART-1, can occur at high frequency in individuals with melanoma or autoimmune vitiligo[15–19]. In contrast, high-avidity T cells specific for TAAs are usually clonally deleted[20]. However, the TCRs used by such T cells might be the most effective for use in TCR gene therapy to treat cancer[21]. Mice with a human TCR repertoire might allow the identification of high-affinity TCRs from the nontolerant repertoire against those human peptide antigens that differ between mice and humans, with the additional advantage that the human TCRs are not immunogenic in humans. The comparison between TCRs used by T cells from mice and humans might also reveal whether and against which antigens similar TCRs are used and, thus, whether self-reactive T cells survived central tolerance mechanisms. Here we have constructed mice with a diverse human TCR repertoire.

RESULTS

Generation of mice transgenic for human TCR gene loci

The available yeast artificial chromosomes (YACs) with TCRα and TCRβ gene loci lacked either many 5′ V genes or 3′ (D) J and constant (C) genes. We chose YACs y852C12 (~0.82 Mb of the TCR genomic region) and y27BE8 (0.9 Mb) for TCRα gene locus construction because these YACs covered the whole chromosomal locus on overlapping DNA fragments (**Fig. 1a**). Similarly, YACs yWSS4181 (0.62 Mb) and yWSS335 (0.24 Mb) together covered the whole TCRβ gene locus and overlapped (**Fig. 1b**). We introduced yeast selectable markers into the YACs to allow selection of YACs that recombined by homologous recombination (**Supplementary Figs. 1** and **2**). PCR analysis of recombined YACs, termed yHuTRA and yHuTRB, indicated successful recombination. Apart from a small deletion of Vβ5.1 and Vβ6.1 in yHuTRB, both yHuTRA and yHuTRB were positive for all tested V, (D), J and C genes (**Supplementary Figs. 3** and **4**). We fused YAC-containing yeast cells with embryonic stem (ES) cells and selected ES cells for the neomycin resistance marker introduced into the YACs. Then, we injected ES cells containing either yHuTRA or

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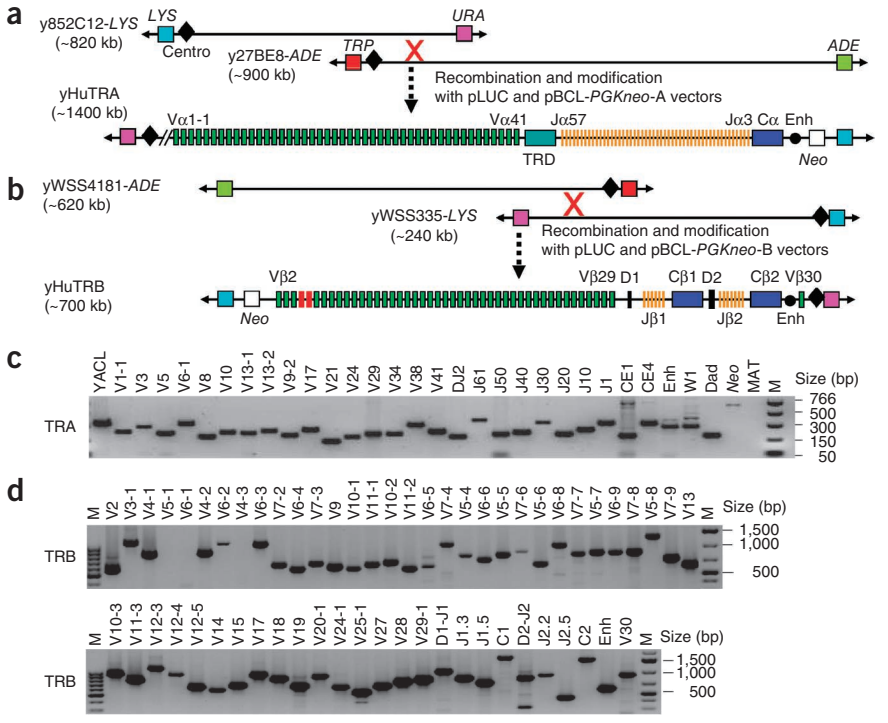

Figure 1 Generation of mice transgenic for the human TCRα and TCRβ gene loci. (**a**,**b**) Schematic diagram of construction of YACs containing the genomic region of the TCRα (yHuTRA) (**a**) and TCRβ (yHuTRB) (**b**) loci by homologous recombination of YACs containing partial gene loci on overlapping fragments in yeast cells. The TCR regions contained in the YACs and estimated size of inserts (not drawn to scale) are indicated. Note the further modification after recombination of YACs with pBII-LysUraCen (pLUC) and pBCL-*PGKneo*-A/B vectors. A detailed depiction of YAC construction is in **Supplementary Figures 1**–**4**. (**c**,**d**) PCR analysis of yHuTRA- (**c**) and yHuTRB- (**d**) transgenic mice with a set of TCR-specific primers. Uracil (*URA*), lysine (*LYS*), adenosine (*ADE*) and tryptophan (*TRP*) genes are yeast-selectable markers. Centro, yeast centromere; V, variable gene; D, diversity gene; J, joining gene; C, constant gene; CE, constant exon; Enh, TCR enhancer; TRD, TCRδ locus; YACL, YAC left arm sequence; Neo, neomycin gene; W1 and Dad, sequences 3′ of the TRA enhancer; M, DNA size marker. The red boxes in yHuTRB indicate deletion of Vβ5.1 and Vβ6.1 during homologous recombination of YACs. Details of gene segments in the transgenic mice can be found in **Supplementary Table 1**.

yHuTRB into blastocysts to produce chimeric mice. The offspring of the chimeric mice were positive for all V, (D), J and C gene sequences that were present in the YACs, which indicated that germline transmission by the fused ES cells was successful (**Fig. 1c**,**d**).

We crossed human TCRα locus–transgenic (hTRA-Tg) mice to mouse TCRα–deficient (*Tcra*$^{-/-}$) mice and also crossed human TCRβ–transgenic (hTRB-Tg) mice to mouse TCRβ–deficient (*Tcrb*$^{-/-}$) mice. We analyzed both mouse lines for CD3$^+$ T cells in the blood. In contrast to *Tcra*$^{-/-}$ and *Tcrb*$^{-/-}$ mice (data not shown), hTRA-Tg *Tcra*$^{-/-}$ and hTRB-Tg *Tcrb*$^{-/-}$ mice had substantial numbers of CD3$^+$ T cells in the blood, albeit slightly fewer than in wild-type mice (**Supplementary Fig. 5a**). These data show that in these mice human TCR gene segments are functionally rearranged and that mouse and human TCRα and TCRβ chains can pair with each other. We crossed hTRA-Tg *Tcra*$^{-/-}$ to hTRB-Tg *Tcrb*$^{-/-}$ mice to obtain hTRA-Tg, hTRB-Tg *Tcra*$^{-/-}$;*Tcrb*$^{-/-}$ (termed AB*ab*) mice that are deficient for mouse TCRα and TCRβ expression and transgenic for the human TCRα and TCRβ gene loci. AB*ab* mice contained CD3$^+$, CD4$^+$ and CD8$^+$ T cells in the thymus (**Fig. 2a**) and CD3$^+$ T cells in the blood (**Fig. 2b**), showing that human TCRαβ chains can compensate for mouse TCRαβ deficiency. In addition, T cells with human TCRs were positively selected by mouse MHC molecules. AB*ab* mice were crossed to HHDII mice, which carry *HLA-A*0201* as a transgene (as an *HLA-A*0201–H2-D*^{b} fusion gene to allow binding to mouse CD8 molecules) fused to the human β2-microglobulin (*B2M*) gene and are deficient for the mouse *B2m* and *H2-D*^{b} genes[22]. We called these mice AB*ab*DII (for AB*ab* HHDII). Thus, we could compare AB*ab* (human TCRs and mouse MHC I), HHDII (mouse TCRs and a single human MHC I) and AB*ab*DII (human TCRs and a single human MHC I gene) mice with regard to development of a polyclonal T cell pool. All mice expressed mouse MHC II molecules.

T cell development in mice transgenic for human TCR gene loci

The absolute number of thymocytes and spleen cells in the three mouse lines was slightly lower than in wild-type mice (**Supplementary Fig. 6a**). We observed an approximate 50% decrease in total CD3$^+$ cell numbers was detected in spleens of AB*ab* compared to WT mice and an approximate decrease of 30% in AB*ab*DII compared to HHDII mice (**Supplementary Fig. 6b**). AB*ab* mice contained approximately 20% as many CD3$^+$ thymocytes compared to WT mice and AB*ab*DII approximately 50% as many CD3$^+$ thymocytes compared to HHDII mice (**Supplementary Fig. 6b**). All mice transgenic for human TCR gene loci that were analyzed in this study were heterozygous, which might reduce the chance of successful TCR recombination. Analysis of the ratio of CD4$^+$ and CD8$^+$ single-positive and CD4$^+$CD8$^+$ double-positive thymocytes showed a higher frequency of CD4$^+$CD8$^+$ cells in AB*ab* (89.3%) and AB*ab*DII (89.3%) compared to wild-type (77.3%) and HHDII mice (77.7%) but similar frequencies of double-negative cells (**Fig. 2a**). We found more CD4$^+$CD8$^+$ thymocytes in hTRA-Tg *Tcra*$^{-/-}$ mice but not in hTRB-Tg *Tcrb*$^{-/-}$ mice than in wild-type mice, suggesting that the TCRα rearrangement, which, in contrast to TCRβ rearrangement, is not necessary for progression to the CD4$^+$CD8$^+$ stage of T cell maturation[23], was less efficient or delayed (**Supplementary Fig. 5b**). The reduced progression from CD4$^+$CD8$^+$ to CD8$^+$ and CD4$^+$ single-positive cells in AB*ab* and AB*ab*DII mice might explain the high frequency of double-negative thymocytes in the CD3$^+$ population (**Fig. 2a**). These cells were TCRγδ$^+$ T cells (data not shown), which constituted 10.6% and 15.4% of peripheral CD3$^+$ cells in AB*ab* and AB*ab*DII mice compared to 2.0% and 2.9% in wild-type and HHDII mice (**Supplementary Fig. 7**). In wild-type mice, TCRγδ rearrangement is terminated at the double-positive stage, and neither TCRα- nor TCRβ-deficient mice have substantially altered numbers of TCRγδ$^+$ T cells[23]. Therefore, the increased frequency of TCRγδ$^+$ T cells in mice with human TCRαβ gene loci supports a model of competitive TCRαβ$^+$ and TCRγδ$^+$ T cell development, in which T cells with human TCRs have a prolonged window of development through which TCRβ selection and TCRγδ lineage commitment occur simultaneously[24–28]. Notably, AB*ab* and AB*ab*DII mice carried a single (human) functional TCRα gene locus and three copies

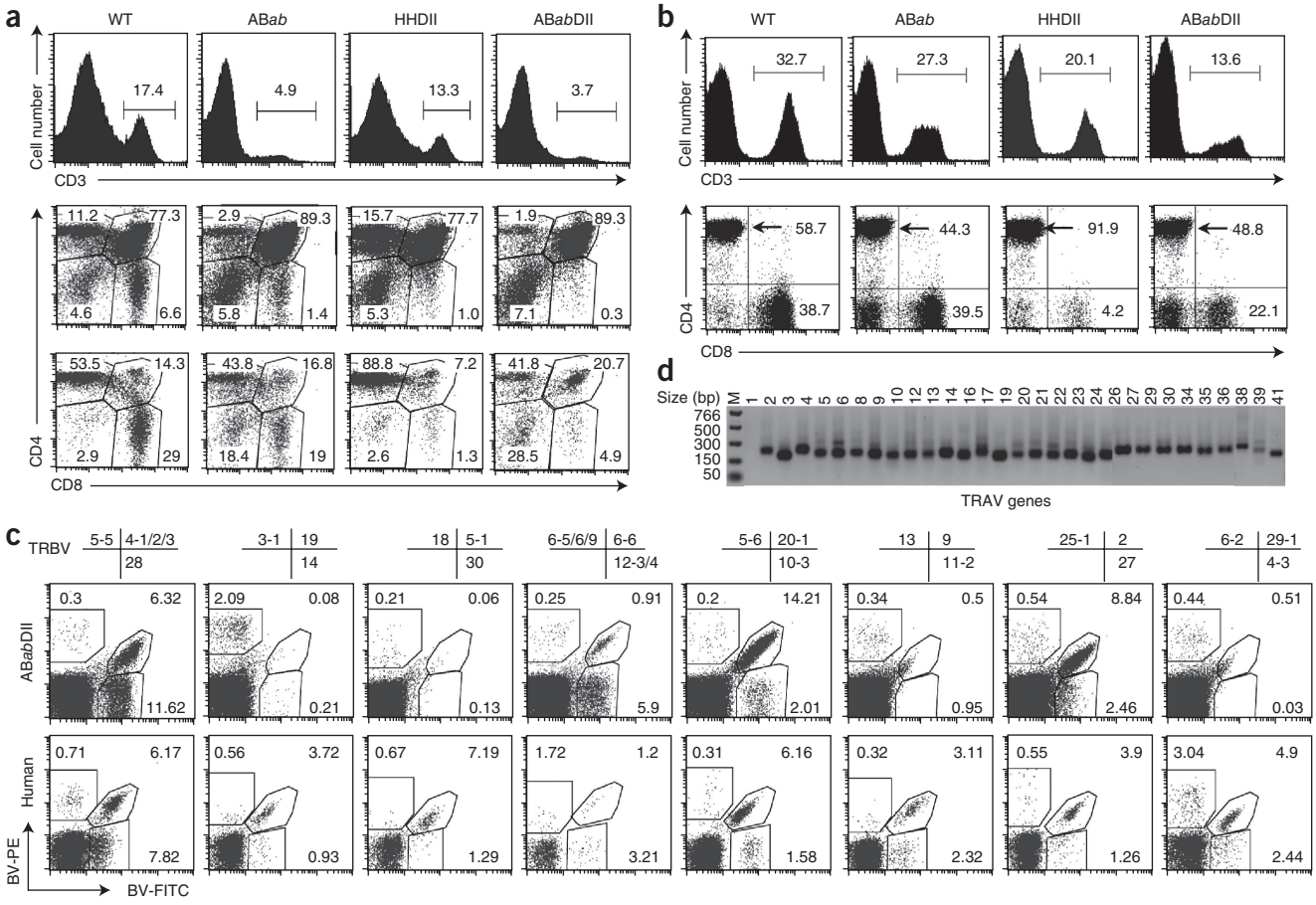

Figure 2 T cell development in human TCRαβ gene loci transgenic mice with a diverse TCR repertoire. (**a**,**b**) Thymocytes (**a**) and blood cells (**b**) from the indicated mice at 1–2 months of age were stained with antibodies specific for CD3, CD4 and CD8 and analyzed by flow cytometry. (**a**) Top, CD3$^+$ cells in the living thymocyte population; middle, CD4 and CD8 single-positive and double-positive cells gated on the thymocyte population; bottom, CD4 and CD8 single-positive and double-positive cells gated on the CD3$^+$ cell population. Percentage of positive cells is indicated. Data are representative of six analyzed mice per group. (**b**) Top, CD3$^+$ cells in the living lymphocyte population; bottom, CD4$^+$ and CD8$^+$ cells gated on the living CD3$^+$ cell population. (**c**) Spleen cells of an AB*ab*DII mouse and human peripheral blood lymphocytes were analyzed for Vβ expression on CD4$^+$ and CD8$^+$ T cells. Each staining contained three different antibodies specific for individual Vβ chains, as indicated above the diagrams. Shown are the Vβ$^+$ cells within the CD4$^+$ and CD8$^+$ cell population. Percentages of Vβ$^+$ cells are indicated on the diagrams. PE, phycoerythrin. (**d**) RT-PCR analysis of spleen cells of an AB*ab*DII mouse with 5′ Vα primers (specific for TRAV genes as indicated) and a 3′ C-region primer. Data in **c** and **d** are representative of two experiments.

(two mouse and one human) of the TCRδ gene locus that is embedded within the TCRα gene locus. Human TCRδ genes contributed to the development of TCRγδ$^+$ cells (data not shown).

Intrinsic affinity of human TCRs for human MHC I

In HHDII mice, 88.8% of the CD3$^+$ thymocytes were CD4$^+$ and 1.3% were CD8$^+$ (**Fig. 2a**). In AB*ab*DII mice, the ratio was 41.8% CD4$^+$ and 4.9% CD8$^+$ (**Fig. 2a**). As HHDII and AB*ab*DII mice differ by the use of mouse versus human TCRs, the data can best be explained by a higher intrinsic affinity of human compared to mouse TCRs for HLA-A2 molecules. The shift in the ratio of CD4$^+$ and CD8$^+$ T cells between HHDII (91.9 versus 4.2%) and AB*ab*DII mice (48.8 versus 22.1%) was even more obvious in the blood (**Fig. 2b**). In absolute numbers, the spleens of AB*ab*DII mice contained fewer CD4$^+$ and more CD8$^+$ T cells than those of HHDII mice (**Supplementary Fig. 8a**). However, there were more CD8$^+$ T cells in AB*ab* mice than in AB*ab*DII mice, suggesting that multiple mouse MHC I alleles that are expressed at physiological levels, contrasting with a single human MHC I allele expressed at a low level[22], overrides the intrinsic affinity between human TCRs and human MHC I. The lower number of CD8$^+$ T cells in the spleen of AB*ab*, HHDII and AB*ab*DII mice compared with wild-type mice was accompanied by higher frequencies of cells with an activated or memory phenotype (CD44^{hi}; **Supplementary Fig. 8b**). The higher frequency of CD44^{hi} T cells in the periphery indicated that thymic output was reduced[29]. Because the total number of T cells and expression of CD44 were normal in hTRB-Tg *Tcrb*$^{-/-}$ mice, the genetic background and presence of the human TCRβ gene locus do not account for the differences.

Diverse T cell repertoire in mice transgenic for human TCR loci

We analyzed whether T cells in AB*ab*DII mice use a diverse human TCR repertoire. We found that 21 out of 24 Vβ-specific antibodies stained a fraction of CD3$^+$ spleen cells in AB*ab*DII mice and human peripheral blood lymphocytes (**Fig. 2c**) but not in wild-type mice (data not shown). We analyzed gene expression by RT-PCR and found that 29 out of the 30 analyzed Vα genes were expressed in AB*ab*DII mice (**Fig. 2d**). We sequenced a limited number of randomly chosen cDNA clones and found 91 unique out of 99 analyzed TCRα sequences and 68 unique out of 71 analyzed TCRβ sequences. A complete list of V and J segments so far found to be rearranged in peripheral

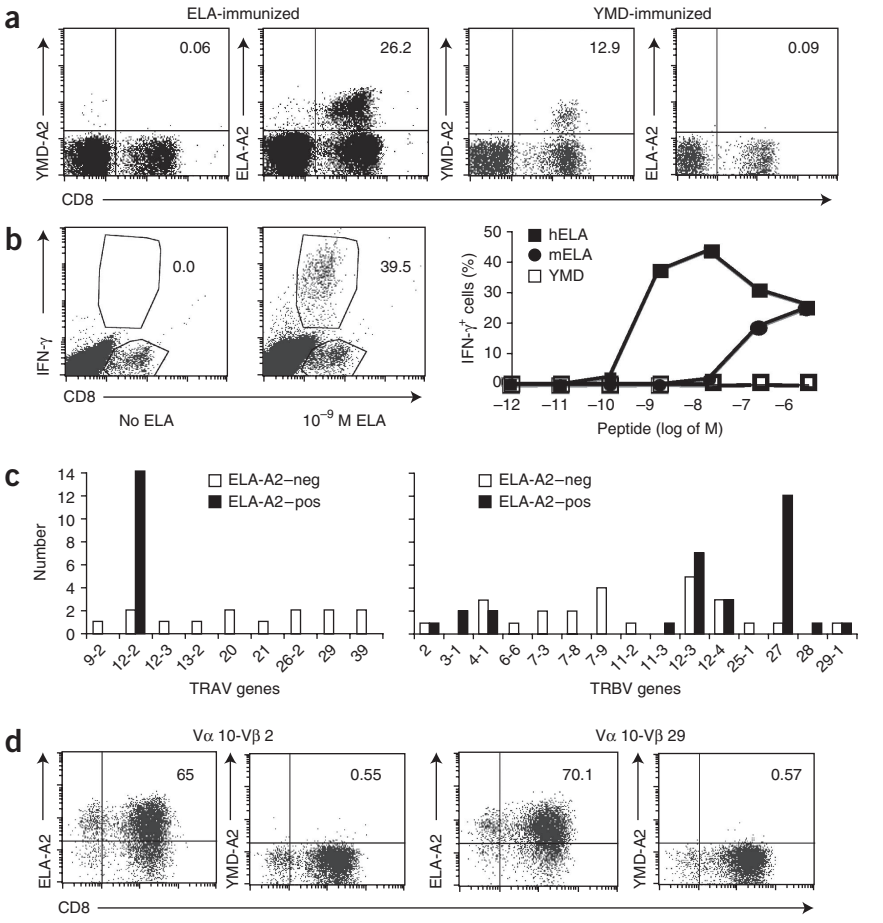

Figure 3 CD8+ T cells in AB*ab*DII mice are functional and use similar TCRs as human CD8+ T cells against an immunogenic antigen. (**a**) Flow cytometric analysis of peripheral blood cells of mice immunized with ELA or YMD peptides 7–9 d earlier and stained with ELA-A2 or YMD-A2 tetramer molecules together with CD3- and CD8-specific antibodies. Percentage of peptide-tetramer+ cells of CD8+ cells is indicated. Data are representative of three (YMD) and ten (ELA) experiments. (**b**) Analysis of CD8 and intracellular IFN-γ expression in spleen cells of ELA-immunized mice stimulated *in vitro* with various concentrations of human ELA (hELA), mouse ELA (mELA) and YMD peptides. Left, ELA-specific IFN-γ expression in response to 10^{-9} M peptide; right, percentages of IFN-γ+ cells of the CD8+ cells. One out of three experiments with similar results is shown. (**c**) The rearranged TCRα (ELA-A2 negative, $n = 14$; ELA-A2 positive, $n = 14$) and TCRβ (ELA-A2 negative, $n = 27$; ELA-A2 positive $n = 30$) sequences in spleen cells of ELA-immunized mice after sorting into the ELA-A2 tetramer–positive (95%) and ELA-A2 tetramer–negative fractions. Shown is the number of isolates of the indicated genes. Sequences from the ELA-A2 tetramer+ fraction are shown in **Supplementary Table 2**. (**d**) FACS analysis of binding of ELA-A2 tetramers or control tetramers (YMD-A2) on CD8+ TCRαβ-deficient Jurkat cells retrovirally transduced with TCRα 10 and TCRβ 2 or 29 cDNA clones. Shown are cells gated on CD3. Percentage of positive cells of the CD8+ cells is indicated.

T cells is shown in **Supplementary Table 1**. The Jβ2–Cβ2 gene cluster was preferentially used. Analysis of the complementarity-determining region-3 (CDR3) of Vα genes showed length variability for 29 of 30 of the analyzed genes and inclusion of non–template-encoded nucleotides (**Supplementary Figs. 9** and **10**). Thus, the major mechanisms that create TCR diversity, such as random V(D)J gene rearrangement and the generation of CDR3 diversity, function in the T cells of AB*ab*DII mice.

CD8+ T cell responses to human tumor antigens

We analyzed whether AB*ab*DII mice could mount an antigen-specific CD8+ T-cell response and whether the TCR usage in these mice was similar to that in humans. We chose the HLA-A2–presented Melan-A–derived peptide 26–35 (ELA) because it is immunogenic in humans and because the TCRs are known for a large number of ELA-specific T cell clones, which preferentially use the TCRα variable 12-2 (TRAV12-2) gene with several TCRβ variable (TRVβ) genes[15–19]. We immunized AB*ab*DII mice with ELA or, as a second antigen, the tyrosinase 369–378 peptide (YMD), and 7–9 d later we stained CD8+ T cells with peptide-specific HLA-A2 tetramers (ELA-A2 and YMD-A2). In ELA-immunized mice, 26.2% of the CD8+ T cells stained positive with ELA-A2 tetramers compared to 0.06% in YMD-immunized mice (**Fig. 3a**). Conversely, in YMD-immunized mice, 12.9% of the CD8+ T cells stained positive with YMD-A2 tetramers compared to 0.09% in ELA-immunized mice, showing specific induction of CD8+ T cells (**Fig. 3a**). CD8+ T cells from ELA-immunized mice produced interferon-γ (IFN-γ) after specific peptide stimulation *in vitro*, showing that CD8+ T cells from AB*ab*DII mice were functional (**Fig. 3b**). The CD8+ T cells responded to 1% the amount of human (10^{-9} M) compared to mouse (10^{-7} M) ELA peptide; the two peptides differ by one amino acid. We sorted ELA-A2 tetramer+ cells and determined their TCR usage. Whereas the negative fraction showed random TCRαβ usage, the positive fraction revealed unique usage of AV12-2 and limited Vβ gene usage (**Fig. 3c** and **Supplementary Table 2**). The Vα CDR3 regions were strikingly similar to those previously isolated from Melan-A–specific CD8+ T cells from individuals with autoimmune vitiligo or melanoma, and one clone was identical (**Table 1**). The CDR3 regions of several of the Vβ clones were also very similar to those isolated from individuals with vitiligo or melanoma (**Table 1**). Jurkat cells transduced to express two TCRαβ combinations bound the ELA-A2 tetramers (**Fig. 3d**). Thus, the CD8+ T cells in AB*ab*DII mice and humans use very similar and, in part, identical TCRs against an antigen that is immunogenic in humans.

To ask how reproducible CD8+ T cell responses can be induced in AB*ab*DII mice, we immunized them with peptides derived from a panel of human TAAs whose sequences are foreign to mice. For six out of six analyzed antigens (α-fetoprotein, gp100, melanoma-associated antigen-A1 (MAGE-A1), MAGE-A10, cancer-testis antigen (NY-ESO-1) and six-transmembrane epithelial antigen of the prostate (STEAP)), a considerable fraction of the CD8+ T cells produced IFN-γ upon *in vitro* stimulation with the specific (between 0.9% and 11%) but not unspecific (between 0.1% and 1.5%) peptide (**Fig. 4**).

DISCUSSION

Previously, transgenic mice containing human immunoglobulin heavy and light chain gene loci proved that human immunoglobulin

Table 1 Similar Melan-A–specific TCR usage in AB*ab*DII mice and T cell clones from individuals with vitiligo and melanoma

Source of TCR	AV		CDR3		AJ
AB*ab*DII 10	12-2	CAV	NIGFGNVL	HCG	35
Melanoma	12-2	CAV	NIGFGNVL	HCG	35
Melanoma	12-2	CAV	SIGFGNVL	HCG	35
Vitiligo	12-2	CAV	TIGFGNVL	HCG	35
Vitiligo	12-2	CAV	SRGFGNVL	HCG	35
AB*ab*DII 21	12-2	CAV	NDAGKS	TFG	27
Vitiligo	12-2	CAV	GAGKS	TFG	27
AB*ab*DII 26	12-2	CAV	NDSGAGSYQL	TFG	28
Melanoma	12-2	CAV	PDQGAGSYQL	TFG	28
Source of TCR	**BV**		**CDR3**		**BJ**
AB*ab*DII 2	27	CASS	FLGDTQ	YFG	2-3
Melanoma	27	CASS	SLGDTQ	YFG	2-3
Melanoma	27	CAS	SLGNEQ	FFG	2-1
Melanoma	27	CAS	SLGVATGEL	FFG	2-2
AB*ab*DII 29	3-1	CASS	P LAGYTGEL	FFG	2-2
AB*ab*DII 22	28	CASSQ	PGLAGYEQ	YFG	2-7
Vitiligo	3-1	CASS	PGLAYYEQ	YFG	2-7
Vitiligo	15	CATSR	APGLAVTDTQ	YFG	2-3
Melanoma	4-2	CASSQ	EGLAGASQ	YFG	2-7
AB*ab*DII 37	3-1	CASSQ	GTSGVNEQL	FFG	2-1
Melanoma	27	CASS	MTSY NEQ	FFG	2-1

CDR3 amino acid alignment of TRAV and TRBV genes isolated from the ELA-A2 tetramer+ fraction (all clones are shown in **Supplementary Table 2**) and ELA-specific human T cell clones from individuals with vitiligo or melanoma[14–18]. AV, TCRα variable gene; BV, TCRβ variable gene; AJ, TCRα joining gene; BJ, TCRβ joining gene.

gene segments could be functionally rearranged by mouse recombination enzymes[30]. Immunoglobulin and TCR rearrangements are executed by the same mechanism. In analogy, mice humanized for the TCRαβ gene loci (and MHC I) are a useful tool to identify high-affinity TCRs against human antigens from the nontolerant repertoire and to address whether high-avidity T cells against self antigens—for example, TAAs, which are clonally deleted in humans—can be detected in mice. Human TCRs were rearranged in mouse thymocytes and generated a diverse T cell pool. T cells with human TCRs were positively selected by mouse MHC molecules, even though TCR-MHC interactions involve germline-encoded CDR1/2 regions[12,13] and mouse and human TCR and MHC genes differ by 30–40%. However, human TCRs only partially replaced mouse TCRs if restricted to mouse MHC I molecules. The increased thymic selection of cells using human rather than mouse TCRs by human MHC I molecules and the preferential choice for human MHC I rather than mouse MHC II molecules, when AB*ab*DII and HHDII mice were compared, provide evidence for evolutionarily conserved binding affinity of human TCRs to human MHC I molecules and a lower recognition of mouse MHC I molecules by human TCRs. These data are consistent with the intrinsic affinity of mouse TCRs for mouse MHC II molecules[13]. Although there is obviously substantial flexibility because cells using human TCRs were selected by mouse MHC molecules, an evolutionary pressure for a certain TCR-MHC binding affinity, probably imposed by pathogens selecting for efficient T cell responses, may exist in humans as well. Nonetheless, AB*ab* mice contained more total peripheral CD8+ T cell numbers than AB*ab*DII mice. AB*ab* mice, which are on a mixed H-2^b × H-2^d genetic background, contain several MHC I alleles that may favor positive selection. The single human *HLA-A2* allele is expressed at low levels that might decrease positive selection. Thus, multiple physiologically expressed mouse MHC I molecules successfully compete with a single low-expressed human MHC I restriction element for selection of T cells with human TCRs. The interpretation of an extended period of TCRαβ and TCRγδ lineage choice, caused for unknown reasons by the human TCRα gene locus, remains preliminary because other factors such as increased TCRγδ+ T-cell proliferation cannot be excluded. However, our interpretation is compatible with the observation that

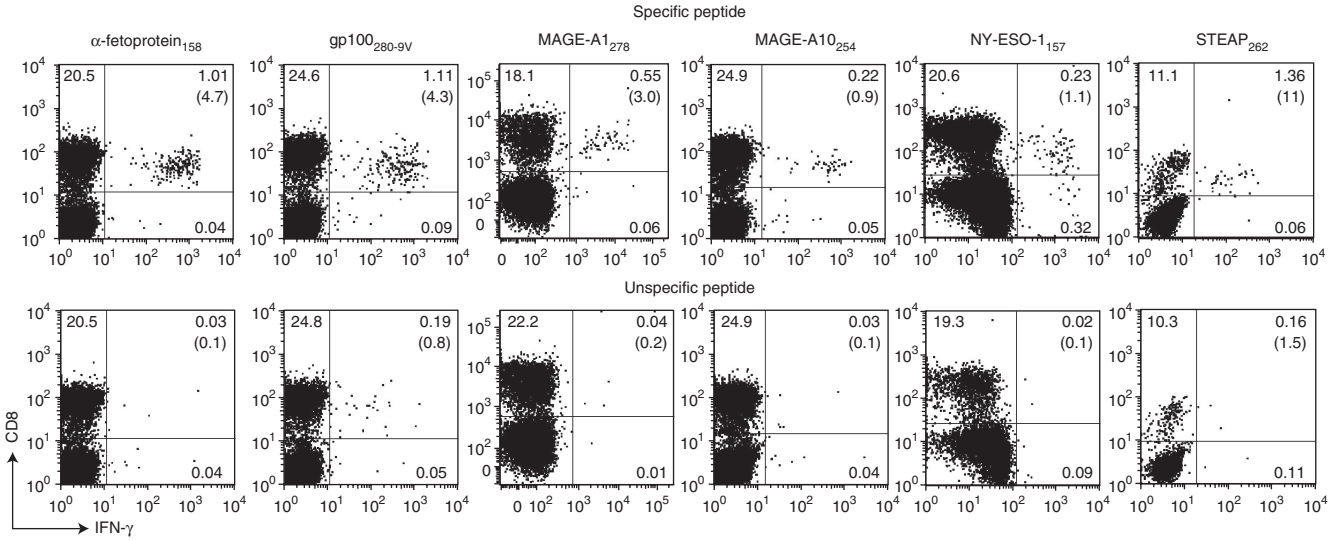

Figure 4 Specific CD8+ T cell responses against a panel of human TAAs in AB*ab*DII mice. Mice were immunized with the indicated human TAAs. Seven to eleven days later, pooled spleen and lymph node cells were stimulated *in vitro* with specific (top; the position of the first amino acid in the peptide is indicated by the subscript) or nonspecific (bottom) peptides and analyzed for expression of CD3, CD8 and intracellular IFN-γ. Shown are CD8+ and IFN-γ+ cells within the CD3+ cell population (percentages indicated by numbers). In parentheses, the percentage of CD8+ and IFN-γ+ T cells within the CD8+ T cell population is given. Shown is one representative out of at least three experiments per experimental group. Unspecific peptides were NY-BR-1 960-968, STEAP 262-270. YMD 369-378 and Melan-A 26-35 (ELA). These peptides used for *in vitro* stimulation were always different from the peptide used for immunization. 9V in the gp100 peptide indicates an amino acid exchange.

human double-positive thymocytes can give rise to a substantial proportion of TCRγδ$^+$ T cells in fetal thymic organ cultures[26].

The main purpose of having constructed these mice is to identify TCRs that are involved in human health and disease. Even though we do not yet know to what extent the T cell repertoire in the mice represents that of humans (for example, the number of different T cell clones in the human blood has been estimated as 2.5×10^7 and as 2×10^6 in the mouse spleen[31,32]), AB*ab*DII mice generated efficient primary CD8$^+$ T cell responses to eight of eight human TAAs. Mouse and human CD8$^+$ T cells respond to similar epitopes derived from human viruses such as HIV, Epstein-Barr virus and hepatitis B virus[33]. Thus, it might be possible to identify the protective TCRs against human pathogens. Conversely, pathogenic TCRs causing autoimmune disease can be identified, as suggested by our finding that TCR usage in ELA-immunized AB*ab*DII mice is very similar to that found in individuals with autoimmune vitiligo. Finally, because the mice are not tolerant for most human TAAs, they might allow the identification of high affinity TCRs, which are deleted in humans, for TCR gene transfer into patients' T cells and adoptive T cell therapy[21].

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

L.-P.L. designed experimental strategies and contributed to writing of the manuscript. He contributed **Figures 1c**, **2a–c** and **3**, **Supplementary Figures 3**, **5–7** and **9**, **Table 1** and **Supplementary Tables 1–3**. J.C.L. contributed to experimental design and to **Figures 1d** and **2c**, **Supplementary Figure 4** and **Supplementary Tables 1** and **3**. X.C. contributed to **Figures 2d**, **3d** and **4**, **Supplementary Figure 10** and **Supplementary Table 1**. C.L. contributed to **Figure 4** and **Supplementary Figure 8**. J.P. contributed to **Figure 4**. W.M. was responsible for microinjection of ES cells to obtain chimeric mice. T.B. proposed and supervised the project, interpreted data and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

YAC construction. YACs y852C12 and y27BE8 were used for TRA construction; yWSS4181 and yWSS335 for TRB construction. Each of the two YACs contained partial TCR regions that overlapped and were determined to be in the same orientation. Homologous recombination of the two TRA-YACs and two TRB-YACs was achieved by mating and subsequent meiotic recombination and selection of recombinant clones by marker genes previously introduced into the YACs by standard methods[34]. Then, a gene encoding phosphoglycerate kinase promoter–neomycin was introduced into the YACs by standard methods. Integrity of the YACs was confirmed during all steps by PCR with a series of gene loci–specific primers covering the entire gene loci (**Supplementary Table 3**).

Mice. Yeast spheroplasts that contained the human TCR-YACs were fused with 129Sv-derived 14.1 ES cells by 50% polyethyleneglycol 1500 with 5–10 mM $CaCl_2$, 5% DMSO and 50 μM 2-mercaptoethanol, and G418-resistant clones were isolated. ES cells were injected into blastocysts, and chimeric mice were obtained by standard protocols. Chimeric mice were crossed with C57BL/6 or BALB/c mice to obtain hTRA-Tg and hTRB-Tg mice, which were crossed to *Tcra*$^{-/-}$ and *Tcrb*$^{-/-}$ mice, respectively, and then the two lines were intercrossed to obtain AB*ab* mice. These mice were crossed to HHDII mice that are *B2m*$^{-/-}$ and *H2-D*$^{b-/-}$ and transgenic for the HLA-A*0201–*H2-D*^{b} fusion, which is fused to the human *B2M* gene[22]. Experiments were approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit, Berlin.

T cell receptor analysis and cloning. CDR3 length diversity was determined by standard methods[31]. V(D)J rearrangement was detected by RT-PCR (rapid amplification of cDNA ends (RACE) PCR with C gene–specific primers or with V and C gene-specific primers). PCR products were cloned and sequenced. Rearranged *TCRA* and *TCRB* genes were introduced into the retroviral plasmid MP71, and retroviruses were generated to transduce TCRαβ-deficient Jurkat cells as described[35].

T cell analysis. Thymus, spleen or peripheral blood cells were incubated with antibodies specific for CD3 (clone 145-2C11), CD4 (clone RM4-5), CD8a (clone 53-6.7), CD44 (clone IM7), γδ (clone GL3) (all BD Biosciences), intracellular IFN-γ (clone AN18.17.24, Miltenyi Biotec), various Vβ (IOTest Beta Mark Kit, IM3497), HLA-A*0201–peptide tetramers (Melan$A_{26–35}$, ELAGIGILTV; tyrosinase$_{157–165}$, YMDGTMSQV) (T20132; T20143, Beckman Coulter) and analyzed by flow cytometry. Mice were immunized subcutaneously with 50–150 μg peptide and 50 μg CpG oligonucleotides 1826, emulsified in incomplete Freund's adjuvant. Between 7 and 11 d after immunization or boosting, T cells were analyzed. The following peptides were used for immunization: Melan-A 26–35 (ELA), tyrosinase 369–378 (YMD), NY-ESO-1 157–165, MAGE-A1 278–286, STEAP 262–270, MAGE-A10 254–262, α-fetoprotein 158–166 and gp100 280–288 (Genscript).

Additional methods. Detailed methodology is described in the **Supplementary Methods**.

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