International Immunology, Vol. 5, No. 1, pp. 11 – 17

Functional expression of a human TCRβ gene in transgenic mice

Joachim Rothe, Stefan Ryser, Urs Mueller[1], Michael Steinmetz[2], and Horst Bluethmann

Biology Department, Pharmaceutical Research New Technologies, F.Hoffmann-La Roche Ltd, 4002 Basel, Switzerland
[1]Basel Institute of Immunology, 4002 Basel, Switzerland

[2]Present address: Department of Biotechnology Research, F. Hoffmann-La Roche Ltd, Nutley, NJ 07110, USA

Key words: allelic exclusion, human TCRβ gene, thymocyte development, transgenic mice

Abstract

A functionally rearranged TCRβ (*Tcrb*) gene was isolated from a cloned human T helper cell recognizing the CS.T3 epitope of *Plasmodium falciparum* with HLA-DR2. Transgenic mice were generated by co-injection of the human gene together with the mouse *Tcrb* enhancer. Analysis of transgenic mice shows that the functional *Tcrb* gene of xenogenic, i.e. human, origin exerts allelic exclusion of endogenous *Tcrb* genes. Cytofluorometric analysis revealed expression of the human TCRβ chain on virtually all thymocytes and peripheral T cells together with endogenous TCRβ chains and CD3 components. No surface expression of mouse TCRβ chain or rearrangement of endogenous *Tcr* genes was detectable. Expression of the hybrid receptor causes a reduction in the number of thymocytes and a bias for $CD4^+CD8^-$ T cells in the thymus as compared with non-transgenic littermates. Peripheral transgenic T cells mount a normal proliferative response against allogeneic targets in mixed lymphocyte reactions. These results show that a hybrid mouse/human TCR is able to pass positive and negative selection in the thymus, and is functional in transgenic mice

Introduction

T cells recognize foreign antigens presented by self MHC class I or class II molecules with the help of highly diverse, but clonally distributed TCRs (for review see 1). TCRs are composed of variable α and β chains associated with non-variant polypeptides of the CD3 complex. The diversity of the TCRα and β chains is generated somatically in T cells by rearrangement of distinct germ line gene segments. During maturation of thymocytes the T cell repertoire is subjected to positive and negative selection in the thymus to render it functional and self-tolerant (2,3).

Transfection experiments have previously demonstrated that a TCRαβ – CD3 complex containing xenogeneic polypeptide chains can be expressed on the surface of human cells and mediate T cell activation (4,5). In particular, human Jurkat cell variants with inactive *Tcra* or *b* genes were transfected with functionally rearranged mouse *Tcra* or *b* genes to replace the inactivated subunit and shown to express hybrid mouse α – human β and human α – mouse β heterodimers associated with the human CD3 complex on the surface. Following ligand binding, the hybrid receptors could mediate transmembrane signals as measured by lymphokine secretion. More recently in transgenic mice, a hybrid TCR – CD3 complex containing a human CD3ε chains was shown to be expressed on mouse T cells and to be able to transduce activation signals (6).

Transgenic mice with functionally rearranged *Tcr* genes have proven to be an excellent model to study T cell maturation and repertoire selection (7,8). Introduction of functionally rearranged mouse *Tcrb* genes into the germline of mice was shown to lead to suppression of endogenous variable (V_β) gene segment rearrangement (9 – 12). Whereas allelic exclusion was found to strictly apply to the *Tcrb* locus, it seems that rearrangement of the *Tcra* locus is controlled in a different way. Several T cell clones have been described which contain two productively rearranged *Tcra* alleles (13 – 17).

Here we demonstrate that a functionally rearranged human *Tcrb* gene can be expressed on T cells of transgenic mice and is able to suppress endogenous V_β gene segment rearrangement. T cells bearing hybrid TCRs, consisting of the transgenic human β chain associated with mouse α chains and the CD3

Correspondence to: H.Bluethmann

Transmitting editor: M. Reth

Received 16 July 1992, *accepted* 21 September 1992

components, can mature in transgenic mice and form a T cell repertoire with an apparently normal frequency of alloreactive cells.

Methods

Generation of transgenic mice

Constructs were injected into zygotes of (C57BL/6 × DBA/2)F_1 mice as described (18,19). Founders were backcrossed with strain C57BL/6J to establish transgenic lines B6-Tg58 and B6-Tg62. Screening for transmission of the transgene in the offspring was performed either by Southern blot analysis using a ^{32}P-labelled fragment of the human V_β5.1 segment as a probe or by the polymerase chain reaction (PCR), essentially as described elsewhere (20). The 5′ primer used to detect the transgene was specific for the 3′ end of the human V_β5.1 segment (CCAGTAAAGGCTGGAGTCAC) and the 3′ primer was specific for the human J_β1.2 region (TACAACGGTTAACCT-GGTCC).

PCR

Oligonucleotides specific for sequences of the murine V_β segments 2, 6, 8.3 and 14 were used as 5′ primers together with an oligonucleotide recognizing a sequence 3′ of J_β2.6 as a 3′ primer at final concentrations of 1 mM each (21). Depending on the primers used, the annealing temperature varied from 50 to 65°C and the magnesium concentration from 1 to 2 mM. PCR was routinely performed with 25 cycles using 2 U Taq-Polymerase (Cetus) and 100 ng genomic DNA isolated from relevant organs or cells before separating the amplified fragments on agarose gels and transferring them to a nylon membrane (Zeta-Probe, Bio-Rad Laboratories, Richmond, CA) by vacuum blotting.

DNA probes

For detection of the transgene we used a 300 bp *Eco*RV – *Hin*cII fragment of the human V_β5.1 gene segment. A 1.4 kb *Pst*I fragment specific for mouse D_β1 was used to analyze $D_\beta - J_\beta$ rearrangements, while a 1.2 kb *Eco*RI – *Cla*I fragment covering the mouse J_β2 cluster was applied to detect $V_\beta - D_\beta J_\beta$ rearrangements after PCR amplification (22). All probes were labelled to high specific activity with [^{32}P]dCTP by random primer extension.

Cytofluorometric analysis

Preparation of T cells and analysis by flow cytometry was essentially as described (19). Briefly, thymus and lymph nodes were homogenized, and the cells recovered and washed twice in PBS/5% FCS/0.1% NaN_3. Samples of 10^6 cells were stained in the same buffer at 4°C with optimal concentrations of antibodies. After two further washes, 10^5 cells were analyzed using a FACScan cytometer (Becton-Dickinson, Mountain View, CA). Data were processed with the FACScan research software. In order to make surface antigens accessible to the antibody βF1, cells were fixed prior to staining with 10 volumes of a freshly prepared 1% solution of formaldehyde in PBS at room temperature. After 15 min incubation, cells were washed twice with PBS.

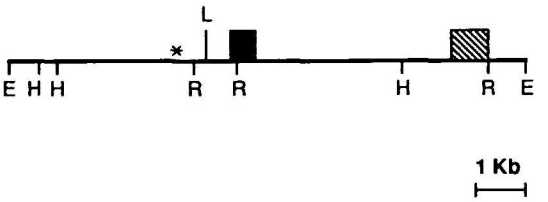

Fig. 1. Functionally rearranged *Tcrb* gene from the T helper cell clone MG-30. Schematic representation of the *Tcrb* construct used for the generation of transgenic mouse lines Tg58 and Tg62. The black box indicates the human V_β5.1 segment functionally rearranged to D_β1 and J_β1.2 gene segments. The striped box represents the human C_β1 segment. L: leader; *location of a non-functional leader sequence. H: *Hin*dIII; R: *Eco*RV; E: *Eco*RI.

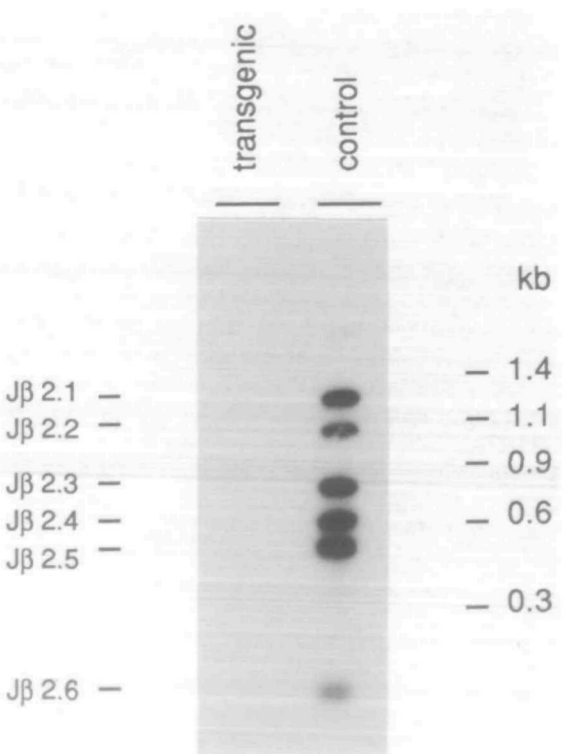

Fig. 2. Suppression of rearrangements of endogenous V_β8.3 gene segments in transgenic mice. Southern hybridization of PCR amplified fragments using DNA from transgenic and non-transgenic thymocytes from control littermates. The 5′ primer was specific for V_β8.3 and the 3′ primer specific for a sequence 3′ of J_β2. The probe for hybridization was specific for J_β2. There is no detectable signal with DNA from transgenic thymocytes, while V_β8.3 rearrangements to all six J_β2 segments are clearly visible in the non-transgenic control.

Antibodies

Cells were stained with the following antibodies: anti-human pan-TCRβ (βF1, T-Cell sciences, Cambridge, MA), anti-mouse CD4 – phycoerythrin (PE; L3T4, Becton-Dickinson), anti-mouse CD8 – FITC (Lyt-2, Becton-Dickinson), anti-mouse CD3ε-biotinylated (145-2C11, (23)), anti-mouse pan-TCRβ (H 57-597, (24)), anti-mouse V_β2 (B20.6.5, (25)), anti-mouse V_β3 (KJ 25, (26)), anti-mouse V_β6 (44-22-1, (27)), and anti-mouse V_β8 (F23.1,

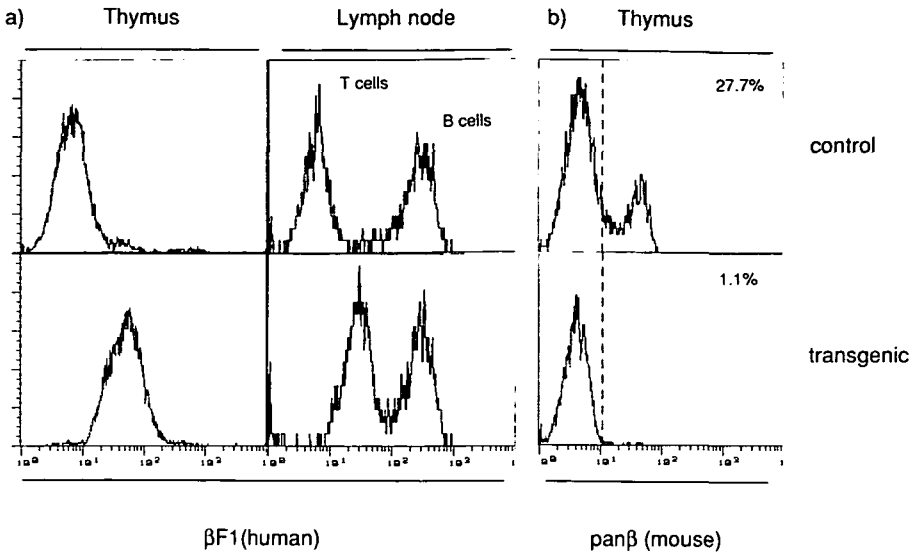

Fig. 3. (a) Expression of the human TCRβ chain on thymocytes and lymph node T cells. Cytofluorometric analysis of fixed thymocytes (left) and lymph node cells (right) from transgenic and non-transgenic mice. All thymocytes and peripheral T cells from transgenic mice were stained with the pan-human TCRβ-specific antibody βF1. The brightly stained population in the right panels are B cells which were directly stained by the second reagent (anti-Ig – FITC). (b) Expression of endogenous TCRβ chains on thymocytes. Staining of transgenic and non-transgenic thymocytes with the pan-mouse TCRβ chain-specific antibody H57-597. No mouse TCRβ chains are expressed on thymocytes transgenic for the human TCRβ chain.

(28)). Secondary reagents were either FITC-labeled anti-mouse Ig (Silenus Laboratories, Victoria, Australia) or PE-labeled streptavidin (Becton-Dickinson).

Mixed lymphocyte reaction (MLR)

Purified spleen and lymph node cells (2.5×10^5/well) from transgenic B6-Tg58 or B6-Tg62 animals and non-transgenic controls were co-cultured in triplicate with 10^6 irradiated syngeneic or allogeneic spleen cells as stimulators for 24, 48, or 72 h. Addition of 1 μCi of [^{3}H]thymidine (Amersham, Arlington Heights, IL) to each well was 20 h before harvesting and measuring the [^{3}H]thymidine uptake by liquid scintillation counting.

Results

Isolation of a functionally rearranged human Tcrb gene

We used the cloned human T helper cell MG-30, recognizing the CS.T3 epitope (amino acids 378 – 398) of the circumsporozoite protein of *Plasmodium falciparum* in association with the HLA class II molecule DR2, to isolate a human *Tcrb* gene. Screening of EMBL3 genomic libraries made from this clone using C_β probes yielded full length clones containing the functionally rearranged *Tcrb* gene. A 10.5 kb *Eco*RI genomic fragment which contains a functionally rearranged $V_\beta 5.1$ gene segment joined to $D_\beta 1$ and $J_\beta 1.2$ gene segments was subcloned into pUC 19 (Fig. 1). About 500 bp upstream of the $V_\beta 5.1$ leader exon, a second leader sequence was identified, which is non-functional due to a mutation of the splice donor site.

Generation of transgenic mice

Transgenic mice were generated by microinjection of the 10.5 kb genomic *Eco*RI fragment containing the functionally rearranged human *Tcrb* gene together with a 5.5 kb fragment carrying the mouse *Tcrb* enhancer (29). The mouse enhancer was co-injected to ensure expression of the human transgene in T cells at a high level. Both fragments were injected in equimolar amounts into zygotes from (C57BL/6 × DBA/2)F_1 mice. Of the four founder mice obtained, two did not transmit the transgenes to their offspring. Of the others, transgenic lines B6-Tg58 and B6-Tg62 were established by backcrossing with C57BL/6 mice. Southern blot analysis revealed that the transgenes had integrated in 1 – 2 and ~ 10 copies respectively. The human *Tcrb* gene was found to co-segregate with the enhancer transgene, meaning that both had integrated into the same chromosomal site.

The human Tcrb transgene exerts allelic exclusion of endogenous mouse Tcrb genes

Endogenous $D_\beta - J_\beta$ rearrangements were assayed in thymocytes of transgenic mice by Southern blot analysis. Similarly to a functional murine *Tcrb* transgene (9), the human *Tcrb* transgene did not inhibit endogenous $D_\beta - J_\beta$ rearrangements (data not shown).

Endogenous $V_\beta - D_\beta J_\beta$ rearrangements were analyzed by PCR with primers specific for different V_β gene segments (see Methods). As shown in Fig. 2, six fragments were amplified when DNA from non-transgenic thymocytes and a primer pair specific for $V_\beta 8.3$ and 3′ $J_\beta 2$ sequences were used. These six fragments correspond to rearrangements of $V_\beta 8.3$ gene segments of one of each of the six $J_\beta 2$ segments. No $V_\beta 8.3$ rearrangements, however, were detectable in thymocytes from transgenic mice. Analogous results were obtained with primers specific for $V_\beta 2$, $V_\beta 6$, and $V_\beta 14$ regions (not shown). This shows that the human transgene is able to completely suppress endogenous $V_\beta - D_\beta J_\beta$ rearrangements.

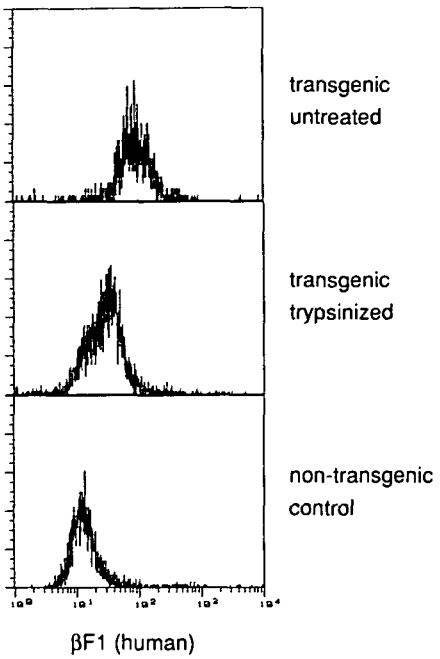

Fig. 4. Surface expression of the human TCRβ chain. Thymocytes from transgenic mice were fixed and stained with the antibody βF1 before or after treatment with trypsin for 15 min. Thymocytes from non-transgenic mice were used as control. The decrease in signal intensity after trypsin treatment is clearly visible and even more pronounced after longer incubation times.

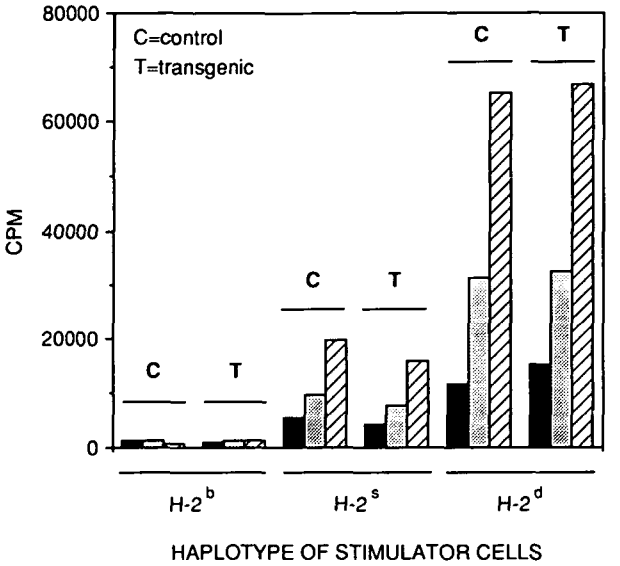

Fig. 5. Kinetics of MLR. Transgenic (T) and control (C) responder cells were co-cultured with irradiated syngeneic (H-2^b) and allogeneic (H-2^s and H-2^d) spleen cells. [^{3}H]thymidine incorporation was measured after 24 (black), 48 (grey) and 72 (striped) h.

The human Tcrb transgene is transcribed in a cell-type specific way

Cell-type specific transcription of the human transgene was analyzed by Northern blot hybridization (data not shown). In both transgenic mouse lines, mature transcripts of 1.3 kb in size were detected in transgenic thymus and to a lesser extent also in spleen, but not in brain, heart, lung, kidney or bone marrow. A low level of unspecific transcription was found in testes.

The human TCRβ chain is expressed on the surface of transgenic T cells

Attempts to immunoprecipitate the human β chain from the surface of transgenic T cells using the antibody βF1, specific for a common determinant on human TCRβ chains, were unsuccessful. Cytofluorometric analysis, however, demonstrated that the human β chain is expressed on virtually all T cells in transgenic mice.

T cells were fixed with formaldehyde to allow the βF1 antibody to recognize its determinant on the human β chain (30). As shown in Fig. 3(A), essentially all thymocytes and peripheral T cells from transgenic mice were stained with βF1 demonstrating expression of the human β chain. To ensure that the determinant seen by βF1 is indeed located on the cell surface and not in the cytoplasm exclusively, cells were gently trypsinized for different time intervals prior to formaldehyde fixation and staining. Prolonged trypsinization led to decreasing signals as revealed by βF1 and reached control levels after 30 min of trypsinization, proving that the human β chain is indeed expressed on the cell surface (Fig. 4).

Thymocytes from transgenic mice were also analyzed for the expression of endogenous mouse TCRβ chains with the antibody H57-597 which detects a common determinant on mouse β chains. Whereas in normal mice ~30% of thymocytes were stained with this antibody, no staining above background levels were obtained for transgenic thymocytes (Fig. 3B). In addition, antibodies specific for different mouse V_β regions (V_β2, V_β3, V_β6, and V_β8)—covering together more than one third of the normal mouse V_β repertoire—did not stain transgenic thymocytes (not shown).

Transgenic thymocytes and peripheral T cells were also stained with the antibody 145-2C11 specific for the ε subunit of the murine CD3 complex. No difference with respect to the number of $CD3^+$ T cells as well as the level of CD3 surface expression was found in transgenic mice when compared with normal littermates (not shown). Taken together, these results demonstrate that the human TCRβ chain is expressed on transgenic T cells in the complete absence of mouse β chains.

The human transgenic β chain participates in the formation of a functional TCR

Since the human β transgene exerts allelic exclusion of endogenous mouse β genes and leads to the expression of a human β chain on the surface of transgenic T cells in the absence of detectable mouse β chains, it is reasonable to assume that the human β chain is associated with mouse TCRα chains and CD3 polypeptides. Are these hybrid receptors able to recognize foreign antigens and to trigger T cell responses?

The function of transgenic T cells was assayed by their ability to respond to allogeneic targets in mixed lymphocyte reactions (MLRs). Splenocytes from SJL (H-2^s, *Mls-1*^{b}, *Mls-2*^{a}) and DBA/2 (H-2^d, *Mls-1*^{a}, *Mls-2*^{a}) mice were used as stimulators. As seen in Fig. 5, T cells from transgenic as well as control littermates (H-2^b, *Mls-1*^{b}, *Mls-2*^{b}) were indistinguishable in their ability to recognize allogeneic target cells and to respond by proliferation.

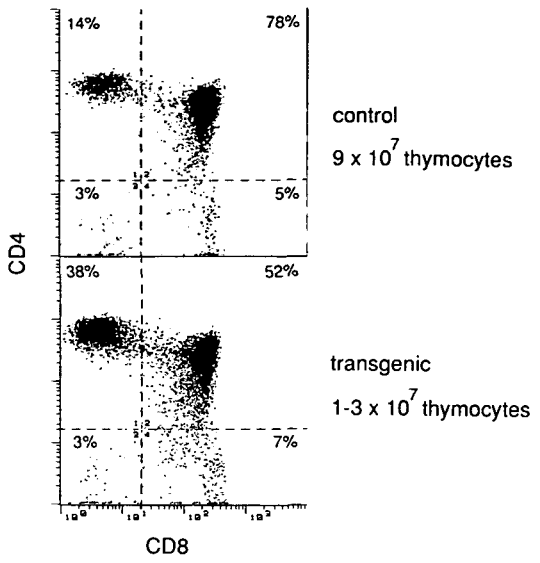

Fig. 6. CD4/CD8 subpopulations of thymocytes. Two-color cytofluorometric analysis of thymocytes for non-transgenic (upper panel) and transgenic mice (lower panel). Numbers of quadrants give the relative percentage of thymocyte subpopulations.

These results reveal that the frequency of alloreactive T cells is as high in transgenic as in control animals. Thus the invariant human TCRβ chain together with a variable mouse TCRα chain and murine CD3 molecules can form functional TCR in transgenic mice.

Expression of the human transgene in H-2^b mice affects thymocyte differentiation

The accumulation of functional T cells in the periphery of transgenic mice, expressing a mouse – human hybrid TCR receptor, indicates that these receptors can pass positive and negative selection in the thymus. The expression of the human TCRβ chain in transgenic mice, however, has a strong impact on the number and phenotype of T cells in the thymus (Fig. 6). Whereas normal littermates contain $\sim 9 \times 10^7$ thymocytes, this number is reduced to $\sim 1-3 \times 10^7$ in transgenic mice. Furthermore, a relative increase of CD4$^+$ single-positive and a decrease of CD4$^+$CD8$^+$ double-positive T cells were found. The relative size of the CD4$^-$CD8$^-$ and CD4$^-$CD8$^+$ subpopulations remained unchanged.

In contrast to what was observed in the thymus, the periphery of transgenic mice contained normal numbers of T cells and CD4$^+$ or CD8$^+$ subpopulations. Also the number of B cells is as in control littermates.

Discussion

Experiments using *Tcrb* transgenes in mice have shown that allelic exclusion is a regulated rather than a stochastic event (9 – 12). Here we demonstrate that a functional *Tcrb* transgene of human origin is able to completely suppress $V_\beta - D_\beta J_\beta$ rearrangements of the endogenous *Tcrb* loci in the mouse. Thus, the mechanisms regulating allelic exclusion seem to be conserved between mouse and man. Experiments with a mutant *Tcrb* transgene showed that expression of the C region domain is sufficient to induce allelic exclusion in transgenic mice (31). The transcript by itself, however, had no effect. Analyses of mice double transgenic for either a *Tcrb* minilocus and a functional *Tcrb* gene (21), or two functional *Tcrb* transgenes (32), revealed that allelic exclusion may operate at a level after gene rearrangement.

Surface expression of the human TCRβ chain was detected with the pan-specific mAb βF1 after fixation of T cells, which is necessary to render the determinant accessible to the antibody. The gradual elimination of the determinant by prolonged digestion with trypsin clearly demonstrates that the human TCRβ chain is expressed on the cell surface. No antibody specific for the variable region of the human transgene was available. The antibody 42 1C1 (33), raised against the human $V_\beta 5.3$ domain, did not detect the $V_\beta 5.1$ region encoded by our transgene.

By immunoprecipitation we could not give direct evidence for a hybrid formation of the human TCRβ chain with various mouse TCRα chains, because the pan-human TCRβ antibody βF1 detects a hidden determinant of the TCR – CD3 complex on the cell surface. This determinant is accessible to the antibody only after fixation of the cells, which bears the risk of exposing intracellular components. However, several observations argue for the expression of a hybrid mouse α – human β TCR in our transgenic mice. (i) A prerequisite for T cell maturation in the thymus is a complete TCRαβ receptor. Although it has been shown in *Tcrb* transgenic *scid/scid* mice (8,34) that TCRβ chains can appear on thymocytes at early stages of differentiation together with an as yet non-characterized invariant molecule, such a receptor cannot pass positive selection in the thymus, and, in addition, would not explain the alloreactive responses observed with peripheral T cells in our transgenic mice. (ii) All mature T cells in the periphery express CD3, which is constitutively co-expressed with the TCRαβ receptor. (iii) In the complete absence of endogenous TCRβ chains, only the transgenic human β chain can pair with endogenous α chains and associate with mouse CD3 polypeptides on the surface of T cells. With human T cell lines, the formation and functionality of such a human TCR – CD3 complex, where the TCRα or β chain is replaced by the corresponding mouse TCR chain, was demonstrated (4,5).

Although the requirements for the formation of a hybrid mouse – human receptor seem to be met, this process might be of lower efficiency, permitting less thymocytes to proliferate as compared with normal mice. An alternative explanation for the reduced number of thymocytes, however, could be an enhanced negative selection of the transgenic T cells expressing a monomorphic β chain. No change, however, was detected in the number of T cells in lymph nodes. It appears that both pool sizes are regulated independently.

The hybrid mouse – human receptor is functional in thymocyte development. It appears to be able to interact with mouse MHC molecules and transduce signals necessary for further maturation to single-positive thymocytes. The relative enrichment of CD4$^+$ single-positive thymocytes might reflect an intrinsic affinity of the human TCRβ chain—which was derived from a class II restricted cell—for mouse MHC class II molecules. A preferential development of CD4$^+$ T cells in transgenic mice expressing MHC class II-restricted TCRs was reported by Berg (35) and Kay (36); however, not for a TCRβ chain alone. When we made mice

double transgenic for the TCRα and β chain of the T helper cell MG-30, we found the same bias for $CD4^+$ thymocytes as in the single transgenic ones (unpublished results).

The invariant human TCRβ chain reduces the TCR repertoire in our transgenic mice by a factor of ~10,000. Since the frequency of alloreactive cells in the transgenic mice is still comparable with control littermates, it seems that the antigenic determinants on allogeneic cells are diverse enough to trigger a restricted T cell repertoire to the same extent as a fully developed one.

Acknowledgements

We thank Beatrice Schmutz and Monika Seiler for expert help in the maintenance and analysis of transgenic mice and Dr Joost van Meerwijk for the PCR primers used to analyze rearrangements of the *Tcrb* locus.

Abbreviations

MLR	mixed lymphocyte reaction
PCR	polymerase chain reaction
PE	phycoerythrin

References

1 Blackman, M., Kappler, J., and Marrack, P. 1990. The role of the T cell receptor in positive and negative selection of developing T cells. *Science* 248:1335.
2 Teh, H. S., Kisielow, P., Scott, B., Kishi, H., Uematsu, Y., Bluethmann, H., and von Boehmer, H. 1988. Thymic major histocompatibility complex antigens and the αβ T cell receptor determine the CD4/CD8 phenotype of T cells. *Nature* 335:229.
3 Kisielow, P., Bluethmann, H., Staerz, U. D., Steinmetz, M., and von Boehmer, H. 1988. Tolerance of T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. *Nature* 333:742.
4 Saito, T., Weiss, A., Miller, J., Norcross, M. A., and Germain, R. N. 1987. Specific antigen-Ia activation of transfected human T-cells expressing murine Ti αβ-human T3 receptor complexes. *Nature* 325:125.
5 Saito, T., Weiss, A., Gunter, K. C., Shevach, E. M., and Germain, R. N. 1987. Cell surface T3 expression requires the presence of both α- and β-chains of the T cell receptor. *J. Immunol.* 139:625.
6 de la Hera, A., Muller, U., Olsson, C., Isaaz, S., and Tunnacliffe, A. 1991. Structure of the T cell antigen receptor (TCR): two CD3 epsilon subunits in a functional TCR/CD3 complex. *J. Exp. Med.* 173:7.
7 von Boehmer, H. 1990. Developmental biology of T cells in T cell-receptor transgenic mice. *Annu. Rev. Immunol.* 8:531.
8 Bluethmann, H. 1991. Analysis of the immune system with transgenic mice: T cell development. *Experientia* 47:884.
9 Uematsu, Y., Ryser, S., Dembic, Z., Borgulya, P., Krimpenfort, P., Berns, A., von Boehmer, H., and Steinmetz, M. 1988. In transgenic mice the introduced functional T cell receptor β gene prevents expression of endogenous β genes. *Cell* 52:831.
10 Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., Russell, J. H., and Loh, D. Y. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature* 336:73.
11 Pircher, H., Ohashi, P., Miescher, G., Lang, R., Zikopoulos, A., Buerki, K., Mak, T. W., MacDonald, H. R., and Hengartner, H. 1990. T cell receptor (TcR) β chain transgenic mice: studies on allelic exclusion and on the TcR^+ γδ population. *Eur. J. Immunol.* 20:417.
12 Iglesias, A., Hansen-Hagge, T., von Bonin, A., and Weltzien, H. U. (1992) Increased frequency of TNP-specific, H-2^b-restricted CTL precursors in transgenic mice expressing a TCR-β chain gene from a H-2^b-restricted, TNP-specific cytolytic T cell clone. *Eur. J. Immunol.* 22:335.
13 Fondell, J. D., Marolleau, J. P., Primi, D., and Marcu, K. B. 1990. On the mechanism of non-allelically excluded Vα – Jα cell receptor secondary rearrangements in a murine T cells lymphoma. *J. Immunol.* 144:1094.
14 Furutani, M., Yanagi, Y., Fujisawa, I., Nakayama, T., Kishimoto, H., Kuida, K., Asano, Y., and Tada, T. 1989. Post-transcriptional allelic exclusion of two functionally rearranged T cell receptor alpha genes. *Int. Immunol.* 1:281.
15 Malissen, M., Trucy, J., Letourneur, F., Rebai, N., Dunn, D. E., Fitch, F. W., Hood, L., and Malissen, B. 1988. A T cell clone expresses two T cell receptor α genes but uses one αβ heterodimer for allorecognition and self MHC-restricted antigen recognition. *Cell* 55:49.
16 Marolleau, J. P., Fondell, J. D., Malissen, M., Trucy, J., Barbier, E., Marcu, K. B., Cazenave, P. A., and Primi, D. 1988. The joining of germ-line Vα to Jα genes replaces the preexisting Vα – Jα complexes in a T cell receptor αβ positive T cell line. *Cell* 55:291.
17 Schittek, B., Unkelbach, E., and Rajewsky, K. 1989. Violation of allelic exclusion of the T cell receptor beta genes in a helper T cell clone. *Int. Immunol.* 1:273.
18 Hogen, B., Costantini, F., and Lacy, E. 1986. *Manipulating the Mouse Embryo: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
19 Bluethmann, H. and Steinmetz, M. 1990. Trangenic mice for analysis of T cell development. In Lefkovits, I. and Pernis, B., eds, *Immunological Methods,* vol. IV, p. 311. Academic Press, Orlando.
20 Maniatis, T., Fritsch, E. F., and Sambrook, J. 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
21 van Meerwijk, J. P., Iglesias, A., Hansen-Hagge, T., Bluethmann, H. and Steinmetz, M. 1991. Allelic exclusion of a T cell receptor-beta minilocus. *J. Immunol.* 147:3224.
22 Malissen, M., Minard, K., Mjollsness, S., Kronenberg, M., Goverman, J., Hunkapiller, T., Prystowsky, M. B., Yoshikai, Y., Fitch, F., Mak, T. W., and Hood, L. 1984. Mouse T-cell antigen receptor: structure and organization of constant and joining gene segments encoding the β polypeptide. *Cell* 37:1101.
23 Leo, O., Foo, M., Sachs, D. H., Samelson, L. E., and Bluestone, J. A. 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl Acad. Sci. USA* 84:1374.
24 Kubo, R. T., Born, W., Kappler, J. W., Marrack, P., and Pigeon, M. 1989. Characterization of a monoclonal antibody which detects all murine αβ T cell receptors. *J. Immunol.* 142:2736.
25 Gregoire, C., Rebai, N., Schweisguth, F., Necker, A., Mazza, G., Auphan, N., Millward, A., Schmitt-Verhulst, A. M., and Malissen, B. 1991. Engineered secreted T-cell receptor alpha beta heterodimers. *Proc. Natl Acad. Sci. USA* 88:8077.
26 Pullen, A. M., Marrack, P., and Kappler, J. W. 1988. The T cell repertoire is heavily influenced by tolerance to polymorphic self antigens. *Nature* 335:796.
27 Payne, J., Huber, T. H., Cannon, N. A., Schneider, R., Schilam, M. W., Acha-Orbea, H., MacDonald, H. R., and Hengartner, H. 1988. Two monoclonal rat antibodies with specificity for the β-chain variable region of the murine T-cell receptor. *Proc. Natl Acad. Sci. USA* 85:7695.
28 Staerz, U., Rammensee, H., Benedetto, J., and Bevan, M. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994.
29 Krimpenfort, P., de Jong, R., Uematsu, Y., Dembic, Z., Ryser, S., von Boehmer, H., Steinmetz, M., and Berns, A. 1988. Transcription of T-cell receptor β-chain genes is controlled by a downstream regulatory element. *EMBO J.* 7:745.
30 Brenner, M. B., Mclean, J., Scheft, H., Warneke, R. A., Jones, N., and Strominger, J. L. 1987. Characterization and expression of the human αβ T cell receptor by using a framework monoclonal antibody. *J. Immunol.* 138:1502.
31 Krimpenfort, P., Ossendorp, F., Borst, J., Melief, C., and Berns, A. 1989. T cell depletion in transgenic mice carrying a mutant gene for TCRβ. *Nature* 341:742.
32 van Meerwijk, J. P., Romagnoli, P., Iglesias, A., Bluethmann, H., and Steinmetz, M. 1991. Allelic exclusion at DNA rearrangement level is required to prevent coexpression of two distinct T cell receptor β genes. *J. Exp. Med.* 174:815.
33 Boylston, A. W. 1989. Current status review: antibodies to the T cell antigen receptor. *Br. J. Exp. Pathol.* 70:489.
34 von Boehmer, H. Kishi, H., Borgulya, P., Scott, B., van Ewijk, W.,

Teh, H. S., and Kisielow, P. 1989 Control of T-cell development by the TCR$\alpha\beta$ for antigen. *Cold Spring Harbor Symp. Quant. Biol.* 54:111.

35 Berg, L. J., Pullen, A. M., Fazekas de St Groth, B., Mathis, D., Benoist, C., and Davis, M. M. 1989. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. *Cell* 58:1035.

36 Kaye, J., Hsu, M. L., Sauron, M. E., Jameson, S. C., Gascoigne, N. R. J., and Hedrick, S. M. 1989. Selective development of $CD4^+$ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. *Nature* 341:746.