Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes

Pawel Kisielow*†, Horst Blüthmann‡, Uwe D. Staerz*, Michael Steinmetz‡ & Harald von Boehmer*

* Basel Institute for Immunology, CH-4005 Basel, Switzerland
‡ Central Research Units, F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basel, Switzerland

The mechanism of self-tolerance is studied in T-cell-receptor transgenic mice expressing a receptor in many of their T cells for the male (H-Y) antigen in the context of class I H-2D^b MHC antigens. Autospecific T cells are deleted in male mice. The deletion affects only transgene-expressing cells with a relatively high surface-density of CD8 molecules, including nonmature $CD4^+CD8^+$ thymocytes, and is not caused by anti-idiotype cells.

T LYMPHOCYTES recognize antigens on the surface of other cells in the context of molecules encoded by the major histocompatibility complex (MHC)[1] by virtue of the heterodimeric T cell receptor (TRC) which is composed of α and β polypeptide chains[2,3]. In binding to its ligand, the $\alpha\beta$TCR is assisted by CD8 or CD4 accessory molecules[4,5], which presumably interact with nonpolymorphic portions of class I or class II MHC molecules respectively[6–10]. Mature T lymphocytes usually do not respond to self-MHC molecules presenting self-antigens. The question of whether the mechanism of immunological tolerance involved deletion of autospecific lymphocytes has concerned immunologists over decades[11], but no direct evidence for such a mechanism has been obtained, because the great diversity of receptors generated during lymphocyte development had made it impossible to follow individual clones of cells expressing receptors specific for self-antigens.

Recently, two groups of investigators obtained monoclonal antibodies (mAb) against the products of certain Vβ genes that are expressed with unusually high frequency on T cells specific for certain class II MHC-associated alloantigens[12–14]. Using these antibodies, Kappler *et al.* and MacDonald *et al.* were able to show that in mice expressing the relevant class II MHC-associated antigens, cells expressing the particular Vβ gene products were absent from the pool of peripheral T cells and medullary thymocytes[12–14], but were present among cortical $CD4^+8^+$ thymocytes[12,13]. These results can be explained by deletion of autospecific cells, but the alternative possibility that their absence is the result of a change of their phenotype caused by modulation or masking of surface molecules has not been excluded.

The development of transgenic mice offers another approach to analyse the mechanism of self-tolerance. To this end we have constructed transgenic mice expressing in a large fraction of their T cells an $\alpha\beta$TCR specific for a minor histocombatibility antigen (H-Y) present on male, but not female, cells. Fertilized eggs obtained from a cross of C57BL/6J × DBA/2J mice were injected with genomic DNA harbouring the productively rearranged TCR α and β genes isolated from the B6.2.16 cytolytic T-cell clone[15]. This clone is specific for H-Y antigen in the context of class I (H-2D^b) MHC antigen and expresses a TCR β-chain coded in part by the Vβ8.2 gene segment which can be identified by the F23.1 antibody[16].

The transgenic founder mouse 71 contained four copies of the α and two copies of the β transgenes integrated on the same chromosome[17]. It was crossed with C57L mice expressing H-2^b MHC antigens, but lacking the Vβ8 gene family. Here we show that cells with the phenotype of the B6.2.16 clone that responded to H-Y antigen were frequent in female but not in male transgenic offspring, despite the fact that peripheral T cells in animals

† Permanent address: The Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.

Table 1 Frequency of male (H-Y) antigen-specific precursors of proliferating T cells (PT-P) among $CD4^+8^-$ and $CD4^-8^+$ T cells from normal and $\alpha\beta$TCR transgenic mice

Stimulation: spleen cells (3000R) + IL-2	CD4/CD8 phenotype	Donor of responding T cells: C57L female			Donor of responding T cells: $\alpha\beta$TCR transgenic female			Donor of responding T cells: $\alpha\beta$TCR transgenic male		
		1/frequency		p*	1/frequency		p	1/frequency		p
C57BL/6 female	$CD4^-8^+$	>25,000			>25,000			>25,000		
C57BL/6 female + Con A	$CD4^-8^+$	2.3	(1.6–3.3)	0.66	1.8	(1.2–2.6)	0.87	2.3	(1.3–4.0)	0.18
	$CD4^+8^-$	NT†			6.4	(4.5–9.1)	0.60	NT†		
C57BL/6 male	$CD4^-8^+$	15,985	(5,029–50,802)	0.61	6.6	(4.8–9.0)	0.67	>25,000		
	$CD4^+8^-$	NT†			>25,000			NT†		

Lymph node cells were stained with a mixture of anti CD4-PE and anti-CD8-FITC mabs (see Fig. 1). $CD4^+8^-$ and $CD4^-CD8^+$ T cells were separated on fluorescein activated cell sorter (FACS440, Becton Dickinson). Limiting numbers of $CD4^+8^-$ $CD4^-8^+$ T cells (24 wells per group) were cultured for 8 days together with irradiated (3,000R) spleen cells (5×10^5 cells per cell) and interleukin-w (5% v/v) of partially purified supernatant from Con A-stimulated rat spleen cells[24] without or with Con A (2.5 $\mu g\,ml^{-1}$). Cells were collected after addition of [^{3}H]thymidine for the last 12 h of culture and incorporated radioactivity was measured by liquid scintillation counting. Negative control cultures contained no responder cells. Frequencies were calculated as described elsewhere[25].

* Probability, p, attached to the computed χ^2 (ref. 25). † NT, not tested.

of both sexes expressed both transgenes[17]. T cells in male (but not female) mice had an abnormal CD4/CD8 phenotype: over 90% of T cells in male transgenic mice were $CD4^-8^-$, or expressed only low levels of CD8 molecules, and the numbers of $CD4^+8^-$T cells were very small. The cellular composition of male thymuses revealed that this unusual phenotype of peripheral T cells was the consequence of deletion of autospecific thymocytes expressing high levels of CD8 molecules, predominantly cortical $CD4^+8^+$ thymocytes. The deletion process spared cells expressing low levels of CD8 molecules, but affected the precursors of single positive $CD4^+8^-$ cells that were not male-specific. This latter observation provides strong evidence that double-positive $CD4^+8^+$ thymocytes contain precursors of single positive $CD4^+8^-$ and $CD4^-8^+$ T cells.

T cells in females

Lymph nodes of female transgenic mice contained normal proportions of $CD4^+8^-$ and $CD4^-8^+$ T ($Thyl^+$) cells which had normal levels of CD4 as well as CD8 accessory molecules (Fig. 1*a*, *b*, *d* and *e*). But these differed in two respects from T cells in normal mice. Firstly, as previously described for β transgenic mice[15], most of them expressed the transgenic β chain on their surface (Fig. 1*b*). Secondly, as shown by limiting dilution analysis of $CD4^-8^+$ T cells, one in six proliferated specifically in response to C57BL/6 male stimulator cells, as compared with one in 16,000 in normal C57L female mice (see Table 1). As only every second plated T cell responded to concanavalin A (Con A), we conclude that at least 30% of $CD4^-8^+$ T cells in transgenic females have a phenotype similar to that of the B6.2.16 clone. $CD4^+8^-$ T cells from transgenic mice did not show any male-specific proliferation, but did respond to Con A (Table 1).

T cells in males

As in transgenic females, lymph nodes of transgenic males contained normal proportions of $Thy1^+$ cells, and most of them expressed the transgenic β chain on their surface (Fig. 1*c*). Northern blot analysis of the α transgene revealed comparable levels of expression in T cells from female and male mice[17]. However, the CD4/CD8 phenotype of T cells in male mice was very different from that of females: 58% of $Thyl^+$ cells were $CD4^-8^-$, 35% were $CD4^-8^+$ but expressed low levels of CD8, and 7% were $CD4^+8^-$ and expressed normal amounts of CD4 (Fig. 1*f*). Limiting dilution analysis showed that one in two $CD4^-8^+$ T cells could be induced to grow by Con A. There was, however, no detectable response to male C57BL/6 stimulator cells (Table 1). Likewise, $CD4^-8^-$ and $CD4^+8^-$ T cells were unresponsive to H-Y antigen (data not shown). These results indicate that male-specific T cells with the phenotype of the B6.2.16 clone are absent from male transgenic mice and that the lack of or low level of CD8 on transgene-expressing cells precluded male-specific responses. This conclusion is supported by the observation that cytolytic activity of the B6.2.16 clone can easily be inhibited by anti-CD8 antibodies (not shown), and by CD8 gene transfection experiments which show that CD8 molecules strongly assist antigen recognition by T cells[4,5]. As shown elsewhere[17], a high proportion of $CD4^-8^-$T cells in male mice expressed both transgenes, but had their endogenous α and β genes in germline configuration. This result, and the fact that only a few T cells expressed normal amounts of CD8 in male transgenic mice, is consistent with the notion that most T cells in male mice and $CD8^+$ T cells in female mice carry transgenic $\alpha\beta$TCR on their surface. But, owing to reduced density of CD8 molecules in male mice, they are not autoreactive.

Thymocytes

The number of thymocytes was drastically lower in male ($0.5–1.6\times10^7$ per thymus) than in female ($1.0–1.6\times10^8$ per thymus) transgenic mice.

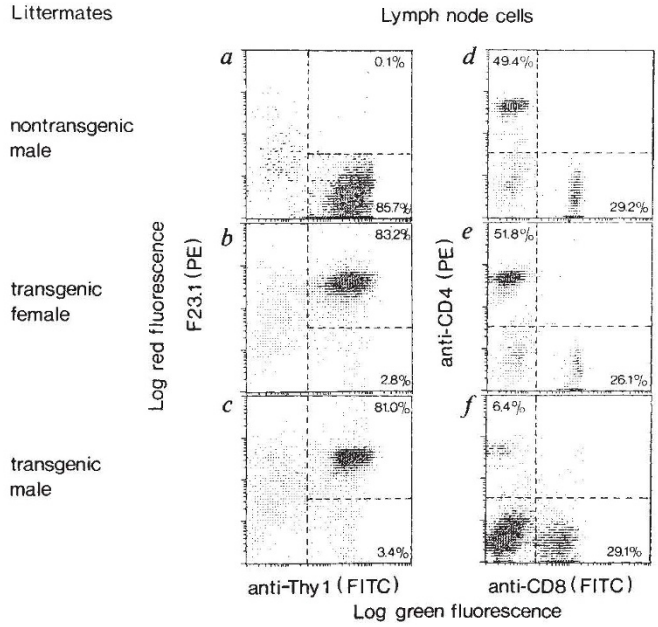

Fig. 1 Comparison of cell surface expression of $F23.1^+$ TCR β chain, CD4 and CD8 molecules on lymph node T ($Thyl^+$) cells from female and male $\alpha\beta$TCR transgenic mice and their nontransgenic male littermate as analysed by two-colour flow cytometry. Lymph node cells were stained with biotinylated F23.1 monoclonal antibody (mAb) followed by a mixture of fluorescein (FITC)-labelled anti-Thyl mab with phycoerythrin-streptavidin (PEA) (*a*, *b* and *c*) or with PE-conjugated anti-CD4 mAb followed by FITC conjugated anti-CD8 mab (*d*, *e* and *f*). In panels *a*, *b* and *c* some $Thyl^-$ cells (B cells) stain nonspecifically with F23.1 mAb due to the binding by Fc receptor. The presented data were obtained with one pair of 7-week-old $\alpha\beta$TCR transgenic female and male littermates. The same results were obtained with 3 other pairs of transgenic mice.

Methods. Single cell suspensions were prepared from lymph nodes (mesenteric, axillary, inguinal) and washed twice in RPM1-1640 and once in PBS with 5% FCS. For staining the following mAbs were used: FITC-conjugated anti-Thyl (ref. 21), biotin-conjugated F23.1 (ref. 16), PE-conjugated anti-CD4 (anti-mouse L3T4, Becton Dickinson) FITC conjugated anti-CD8 (anti-mouse Lyt2, Becton Dickinson). Biotin or FITC conjugation of mAbs was performed by standard procedures. Optimal concentrations of staining reagents were determined in preliminary experiments. All incubations and washings were done at 4 °C. Cells ($0.5–1\times10^6$) were incubated with either biotinylated F23.1 mAb (*a*, *b* and *c*) or anti-CD4-PE mAb (*d*, *e* and *f*). After 20 min, cells were washed twice and incubated again for 20 min with anti-Thyl-FITC mAb plus PEA (Becton Dickinson) (*a*, *b* and *c*) or with anti-CD8-FITC mAb (d, e and *f*). Finally, cells were washed three times in PBS 5% FCS and analysed for two-colour fluorescence on FACScan (Becton Dickinson) flow cytometer with a single Argon laser and logarithmic intensity scales using FACScan research software program (FRSP). Ten thousand viable cells were analysed in each sample. Dead cells were excluded from analysis using a combination of low-angle and sideways light scatter. The results are presented as 'density' plots, generated by analysis of processed data reduced to a 64×64 matrix with 16 levels. Percentages of stained and non-stained cells were calculated using FRSP. Markers were set against the 'density' plots of control samples which involved substitution of diluent alone for either one or both antibodies.

As shown in Fig. 2*a*, double-staining with F23.1 and CD3 antibodies demonstrated that most (>95%) thymocytes from transgenic females and males expressed the β transgene, and that the amount of TCR expression corresponded to the higher values of the normal spectrum of TCR densities observed in C57BL/6 mice.

In the thymus of transgenic females, two populations expressing different levels of TCR could be distinguished (Fig. 2*a*, middle panel). The one with relatively low TCR density included

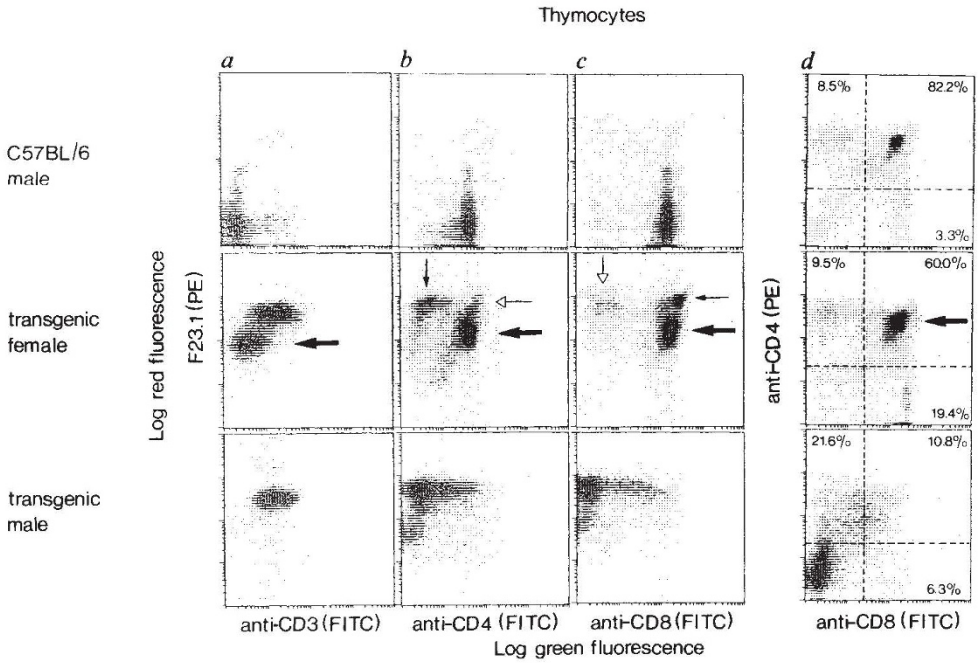

Fig. 2 Expression of F23.1$^+$ TCR β chain, CD3, CD4, and CD8 molecules on thymocyte subpopulations from normal C57BL/6 and from $\alpha\beta$TCR transgenic female and male mice. In panel *a*, cells were incubated consecutively with anti-CD3 mAb, FITC-conjugated goat anti hamster immunoglobulin, mouse immunoglobulin, biotinylated F23.1 mAb and PEA. In panels *b* and *c*, cells were stained bith biotinylated F23.1 mAb, followed by a mixture of anti-CD4-FITC (*b*) or anti-CD8-FITC (*c*) mAbs with PEA. In panel *d*, cells were stained with anti-CD4-PE followed by anti-CD8-FITC mabs. The number of cells per thymus in C57BL/6 male, $\alpha\beta$TCR transgenic female and $\alpha\beta$TCR transgenic male were: 100×10^6, 105×10^6 and 13×10^6 respectively. Thick arrows indicate the population of CD4$^+$8$^+$ thymocytes expressing a lower level of TCR, which is mostly depleted in male thymus. Open-head arrows indicate the population of CD4$^+$8$^-$, and thin arrows of CD4$^-$8$^+$ female thymocytes that express higher levels of TCR. Populations indicated by open-head thin arrows and in the upper left quadrant of middle panels *b* and *c* also contain CD4$^-$CD8$^-$ thymocytes, as indicated by virtual absence of cells in the lower left quadrant of middle panel *c*. Presence of cells in lower left quadrant of middle panel *b* is due to imperfect staining of this particular sample in this experiment. In other experiments, no F23.1$^-$CD4$^-$ cells could be seen under the same conditions.

Methods. Single thymocyte suspensions were prepared by squeezing the whole thymus through a nylon mesh into medium RPM1-1640 with 5% FCS. After washing, cells were resuspended in PBS with 5% FCS, counted and stained as indicated above with extensive washings between each step (see Fig. 1). For staining with anti-CD3, unconjugated mAb 145.2c11 (ref. 22) was used. To saturate free binding-sites of second-step reagent, cells were incubated with mouse immunoglobulin (Sigma, 1 mg ml^{-1}) for 15 min. FITC-conjugated anti-CD4 (GK1.5, ref. 23) mAb was prepared by standard procedures. Control samples were stained with each reagent alone, or in combinations omitting each single reagent. Ten thousand viable cells were analysed in each sample by FACScan flow cytometry. For details see Fig. 1.

CD4$^+$8$^+$ cells, whereas the other, expressing about tenfold more TCR, contained CD4$^+$8$^-$, CD4$^-$8$^+$ and CD4$^-$8$^-$ cells (Fig. 2*b* and *c*, middle panels).

Double-staining with CD4 and CD8 antibodies revealed significant differences between transgenic and normal C57BL/6 females with regard to the size of CD4$^-$8$^+$, CD4$^+$8$^+$ and CD4$^-$8$^-$ thymocyte subpopulations (Fig. 2*d*, upper and middle panels). The proportion of CD4$^+$8$^-$ thymocytes in transgenic females was normal, but the proportion of CD4$^-$8$^+$ thymocytes was enlarged, resulting in a reversed ratio of CD4$^+$8$^-$ to CD4$^-$8$^+$ cells as compared with normal nontransgenic mice. The proportion of CD4$^-$8$^-$ thymocytes was also noticeably higher in transgenic females than in normal mice. The increase in proportion of CD4$^-$8$^+$ and CD4$^-$8$^-$ cells was matched by a corresponding decrease in the size of the CD4$^+$8$^+$ population.

In contrast to the females, the thymus of transgenic males was severely depleted of CD4$^+$8$^+$ cells with decreased expression TCR, but contained about the same total number of CD4$^-$8$^-$ cells, which constituted the bulk of the population of male thymocytes (Fig. 2*d*, middle and lower panels). Most CD4$^-$8$^+$ cells showed low expression of CD8. Thus, the male thymus was depleted of transgene-expressing cells with relatively high levels of CD4/CD8 accessory molecules and the nonmature CD4$^+$8$^+$ thymocytes expressing decreased amounts of TCR were the main target of depletion.

Because CD4$^+$8$^+$ thymocytes are extremely steroid-sensitive, it was important to find out whether the deletion of these cells in transgenic males was a result of stress rather than of an antigen-specific deletion process. Stress in the male mice could possibly be caused by autoimmunity not detectable by *in vitro* assay. We addressed this question in reconstitution experiments using haemopoietic stem cells from transgenic (F23.1$^+$) and nontransgenic C57L (F23.1$^-$) mice (Fig. 3). T-cell-depleted bone marrow cells (BMC) from transgenic females were transferred either along or together with BMC from normal C57L females into lethally X-irradiated female and male C57L recipients. Five weeks after the transfer of the transgenic BMC, the cellular composition of the thymus in male recipients was very much like that in male transgenic mice (Fig. 3*a*, lower panel). But in the thymus of male recipients which had received a mixture of BMC from transgenic and C57L females, CD4$^+$8$^+$ thymocytes derived from F23.1$^-$ C57L donors developed normally and outgrew the transgenic F23.1$^+$ cells, which were mostly deleted (Fig. 3*b* and *c*, lower panel). On the other hand, in the female recipient, CD4$^+$8$^+$ thymocytes developed from both F23.1$^+$ and F23.1$^-$ donors (Fig. 3*b* and *c*, upper panel). Thus, because the deletion selectively affected transgene-expressing F23.1$^+$ CD4$^+$8$^+$ thymocytes in the male recipients, this experiment indicates that the deletion is a result of the interaction of autospecific thymocytes with radioresistant male cells in the thymus, and not of stress and steroid release. If the latter possibility were true, the F23.1$^-$ CD4$^+$8$^+$ thymocytes derived from C57L donors of BMC should also have been affected.

Discussion

Our study with $\alpha\beta$TCR transgenic mice provides new observations relevant to the understanding of the mechanism of self-tolerance and relevant to the clarification of the function of cortical CD4$^+$8$^+$ thymocytes in T-cell development. The drastically decreased number of thymocytes in male but not female mice is direct evidence for a deletion of autospecific cells at the level of CD4$^+$8$^+$ nonmature thymocytes. Furthermore, our results indicate that CD4 and CD8 accessory molecules are involved in the deletion process of autospecific cells.

Two questions relating to the function of double-positive

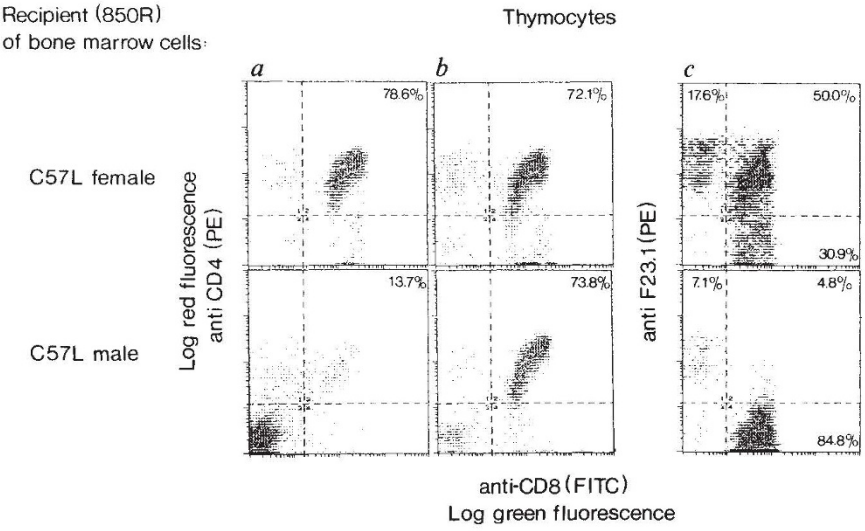

Fig. 3 Surface phenotype of thymocytes from C57L male and female irradiation chimeras reconstituted with bone marrow of $\alpha\beta$TCR transgenic female, either alone (*a*) or with normal bone marrow from C57L female (*b*, *c*). Thymocytes were stained with anti-CD4-PE (*a*, *b*) or biotinylated F23.1 (*c*) mAbs, followed by anti-CD8-FITC mAb (*a*, *b*) or a mixture of CD8-FITC mAb with PEA (*c*). In (*a*), 80% of thymocytes in the female recipient and 67% in the male recipient were stained with F23.1 mAb (data not shown).

Methods. Bone-marrow cells from transgenic or normal C57L dnor were treated with cytotoxic anti Thyl mAb (T24, ref. 21) plus rabbit complement (Cedar Lane, Ontario, Canada) for 45 min at 37 °C. After washing, 5×10^6 viable cells from the transgenic donor were injected intravenously (i.v.) into lethally irradiated (850R) eight-week-old C57L females and males, either alone or together with 0.5×10^6 viable bone marrow cells from normal female C57L. Five weeks later the mice were killed, their thymuses removed and single-cell suspensions prepared, counted and stained with anti-CD4, -CD8 and -F23.1 mAb, and analysed as described in Figs 1 and 2.

$CD4^+8^+$ thymocytes are why so many of these cells should die within the thymus[18] and whether or not they contain precursors of single positive $CD4^+8^-$ and $CD4^-8^+$ cells[19]. Our results show that the death of at least some cortical thymocytes can result from antigen-specific elimination of autoreactive cells. The deletion of nonfunctional, antigen-specific $CD4^+8^+$ thymocytes would make sense if $CD4^+8^+$ thymocytes contained precursors of functional $CD4^+8^-$ and $CD4^-8^+$ cells. Consistent with this view is our observation that $CD4^+8^-$ cells were severely depleted in male transgenic mice, despite the fact that such cells from transgenic female mice cannot be activated by male cells. An analogous finding has been reported by MacDonald *et al.*[14], who observed that $CD4^+8^-$ and $CD4^-8^+$ T cells staining with V_{β^6} antibodies were reduced to the same extent in animals positive for the *Mls*^{a}-allele of the minor lymphocyte stimulating locus (*Mls*), even though $CD4^-8^+$ from *Mls*^{a}-negative animals lacked specificity for *Mls*^{a}.

We thus favour the view that at least some double-positive $CD4^+8^+$ thymocytes act as precursors for functional single positive cells[20], even though further investigation is needed. Although we have shown that the deletion predominantly affects $CD4^+8^+$ thymocytes, we could argue that it might occur independently of accessory molecules at any stage of T-cell development. But this view is not compatible with our observation that the deletion process spares T cells that lack accessory molecules, or even T cells having low expression of CD8. Thus our experiments provide the first direct evidence that these molecules play a crucial role in the induction of tolerance. Taken together, the three observations made in male transgenic mice, namely the drastically reduced number of $CD4^+8^+$ thymocytes, the reduction of $CD4^+8^-$ cells and the occurrence of transgene-expressing cells with virtually no CD8, argue that the deletion of autospecific cells is dependent on CD4 and CD8 accessory molecules.

The results of our experiments with transgenic mice differ in at least two important aspects from others recently reported[12–14] for normal mice. Firstly, in the experiments of Kappler *et al.*[12,13], the depletion of autospecific T cells did not appear to affect $CD4^+8^+$ thymocytes. One possible reason for the difference is that different antigens are under investigation: we are looking at an antigen in the context of class I MHC antigens found throughout the cortex whereas Kappler *et al.*[12,13] are looking at an entity[21] related to class II MHC antigens which are usually not detected in the outer cortex. Thus in the latter case $CD4^+8^+$ cells can meet antigen only when reaching the cortico-medullary junction. Consequently only a minor subset of $CD4^+8^+$ cells would be deleted in the experiments of Kappler *et al.*, and this would be difficult to detect. Another possible reason for the different findings is the fact that in our transgenic mice the expression of TCR proteins is skewed towards higher levels of the range observed in $CD4^+8^+$ from normal mice. This phenomenon, as well as the increased proportion of $CD4^-8^+$ thymocytes in transgenic females, could reflect a positive selection of thymocytes by H-2^b antigens, or alternatively may be a direct consequence of expression of transgenes. We could argue therefore that the deletion of $CD4^+8^+$ thymocytes was easily detected because the majority of $CD4^+8^+$ thymocytes in transgenic mice represent a minor and more mature population of $CD4^+8^+$ thymocytes that may escape detection in normal mice, especially when representing only a fraction of cells expressing a certain idiotype. Whatever the reason for the apparent discrepancy in the results, our data indicate that the nonmature $CD4^+8^+$ populaton can be a target of deletion, whereas it is not clear whether $CD4^+8^-$ or $CD4^-8^+$ cells are susceptible to the same deletion process.

The second difference between our results and those of Kappler *et al.*[12,13] and MacDonald *et al.*[14] is that these authors did not report the presence of cells with few or no accessory molecules, spared by the deletion. Again in this case, such cells would constitute a very minor population in their experimental system, because the pool of T cells in normal mice can be easily replenished by T cells expressing different TRCs, which is not the case in transgenic mice.

As we observed normal numbers of T cells in the periphery, but not in the thymus, of transgenic males, we propose that the number of peripheral T cells can be adjusted independently of the export of newly formed cells from the thymus. This would allow the accumulation of cells with rare phenotypes in the periphery of male mice, as shown here and in the accompanying paper[17]. The fact that transgene-expressing cells with few or no accessory molecules accumulate in male mice, tends to rule out a role of anti-idiotypic cells in the deletion process; such a mechanism should eliminate transgene-expressing cells, rather than cells expressing high levels of accessory molecules.

We would like to thank Katrin Hafen, Abigail Garner and Verena Stauffer for technical assistance, Drs Werner Haas and Hung Sia Teh for critical reading and S. Kirschbaum for typing the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffman-LaRoche & Co. Ltd, Basel, Switzerland.

Received 7 April; accepted 6 May 1988.

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LETTERS TO NATURE

Spin-down of the X-ray pulsar GX1+4 during an extended low state

K. Makishima*, T. Ohashi*, T. Sakao*, T. Dotani†, H. Inoue†, K. Koyama†, F. Makino†, K. Mitsuda†, F. Nagase†, H. D. Thomas‡, M. J. L. Turner‡, T. Kii§ & Y. Tawara§

* Department of Physics, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
† Institute of Space and Astronautical Science, 3-1-1 Yoshinodai, Sagamihara, Kanagawa, Japan
‡ X-ray Astronomy Group, Department of Physics, University of Leicester, Leicester LE1 7RH, UK
§Department of Astrophysics, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan

X-ray pulsars[1,2] are magnetized, spinning neutron stars accreting matter from their binary companions. Their pulse periods P, ranging over four orders of magnitude, increase and decrease in complex ways[1,3,4]. The more luminous ones tend to show faster spin-up[1,5]. A puzzle is that the spin-up timescales of many X-ray pulsars are much shorter than their binary-evolution timescales, thus apparently violating the steady-state condition. It has therefore been suspected[6] that there exist many 'turned-off' X-ray pulsars currently spinning down undetected. An excellent test for this hypothesis became available using the X-ray pulsar GX1+4, which used to show the fastest spin-up over a decade[1,7–10] and then faded away[11]. Using the X-ray satellite Ginga[12], we detected GX1+4 at ~1/40 the previous intensity, and found that it now has an average spin-down trend. This discovery apparently supports the above hypothesis.

GX1+4 (4U1728−24) is a luminous binary X-ray pulsar near the Galactic centre[13], with a ~2 min (or possibly twice this) pulse period and probably a >100-day binary period[7,13]. From its discovery in the early 1970s until 1980, it was spinning up rapidly, with $\dot{P} = -7.5 \times 10^{-8}$ s s^{-1} ($\dot{P}/P = -0.02$ yr^{-1}) (refs 7–10), the fastest among binary X-ray pulsars[1]. The optical companion is an M6III symbiotic star[13]; it is thus a rather rare system composed of an evolved late-type primary star and a strongly magnetized neutron star. Although in the 1970s GX1+4 had an X-ray intensity of 50–200 mCrab (10^{37-38} erg s^{-1}; ref. 7), in 1983 it was found by Exosat to be <4 mCrab (ref. 11). This and subsequent Exosat observations (K. Mukai, personal communication) showed that GX1+4 had entered an extended low state (<0.5 mCrab), although the hard X-ray flux[14] might have remained relatively unchanged[15] and the optical emission showed a complex time variation[16,17].

The present observation was carried out with the large area counter (LAC; M.J.L.T. *et al.*, manuscript in preparation) on board Ginga launched on 5 February 1987[12]. The LAC experiment, a UK–Japan collaboration, uses eight identical proportional counters, achieving 4,000 cm^2 total effective area. The LAC was pointed to GX1+4 from 07:24 to 09:08 UT on 28 March, and 16:52 on 30 March to 03:02 on 31 March 1987. The available data consisted of 10 separate segments of ~20 min each. The data covered an energy range of 1–36 keV with 48 spectral channels and 0.5-s time resolution. Figure 1 shows a portion of the raw X-ray count-rate record; the source was unambiguously detected at about 33 c s^{-1} (1–30 keV), or 3 mCrab, and showed significant flickering. The pulse-phase averaged X-ray spectrum is slightly softer than those observed previously[2,8,18]. At a 10 kpc distance, the observed 2–20 keV luminosity becomes 1.5×10^{36} ergs s^{-1}.

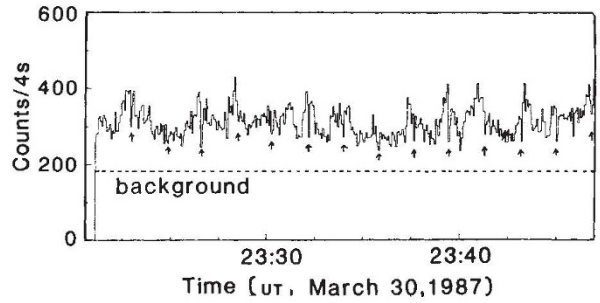

Fig. 1 A part of the 1–20 keV count rate record during the present GX1+4 observation, binned at 4.0 s. The arrows indicate the expected dip recurrence at 110.2 s. The background level, determined using the standard Ginga background estimation procedure (M.J.L.T. *et al.* 1988, manuscript in preparation), should be accurate to ~2 c s^{-1}.

A standard Fourier analysis revealed a highly significant period at ~110 s, together with several higher harmonics, but no other periodicity. Through a folding analysis, we refined the heliocentric period to $P = 110.233 \pm 0.003$ s (ref. 19) with a reduced chi-squared of 15.4 for 99 degrees of freedom. As plotted in Fig. 2, this value for P is longer than the value 109.668 ± 0.002 s last obtained in 1980[8]. Therefore the average behaviour of GX1+4 between 1980 and 1987 proves to be spin-down at a rate $\dot{P} = 2.6 \times 10^{-9}$ s s^{-1}, in contrast to the previous rapid spin-up. A joint Australia–Germany–Italy balloon experiment in November 1986 obtained a similar value for P (A. B. Giles, J. Greenhill and D. P. Sharma, personal communication).

The folded pulse profile (Fig. 3) turned out to be extremely peculiar: the pulse peak involves a sharp dip, which is resolved to two roughly symmetric minima separated by a local narrow maximum. On closer inspection, we can identify individual dips in the raw data of Fig. 1. Such a peculiar feature has never been