

T CELL ANTIGEN RECEPTOR EXPRESSION IN ATHYMIC (*nu/nu*) MICE

Evidence for an Oligoclonal β Chain Repertoire

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The thymus is well established as the major site of T cell lymphopoiesis in mammals (reviewed in 1 and 2). As such, diversification and selection of the T cell antigen specificity repertoire would be expected to occur in that organ. With the recent availability of cloned genes corresponding to the α and β chains of the T cell receptor for antigen (TCR)¹ (3–5), more direct approaches to understanding the role of the thymic microenvironment in TCR expression have become feasible. Thus, several laboratories have demonstrated that rearrangement and expression of TCR β genes precedes expression of TCR α genes during thymus ontogeny (6–10). Furthermore, recent *in vitro* experiments with fetal thymus organ cultures have provided strong presumptive evidence that the thymic microenvironment is sufficient for TCR expression (11, 12).

Despite the overwhelming evidence in favor of thymic control of TCR expression, several models of extrathymic T cell development exist. Foremost among these is the congenitally athymic (*nu/nu*) mouse. Although nude mice were originally considered devoid of T lymphocytes, evidence from many laboratories has established that cells sharing phenotypic and functional properties of T cells do develop as a function of age in these animals (reviewed in 13 and 14). In addition, limiting-dilution studies have shown that antigen-specific precursors of both cytolytic (15) and helper (16) T cells exist in nude mice, albeit at a significantly (5–10-fold) lower frequency than in their euthymic (*nu/+*) littermates.

In view of the presence of (operationally defined) antigen-specific T cells in athymic mice, it is of interest to establish whether nude T cells rearrange and express conventional (i.e., α/β) TCR genes, or alternatively, make use of some other surface structures to recognize antigen. In the present report, we demonstrate the expression of conventional TCR in nude T lymphocytes by both RNA and protein measurements. In addition, we provide evidence at both the protein and DNA level suggesting that the T cell repertoire in individual nude mice is frequently oligoclonal.

¹ *Abbreviation used in this paper:* TCR, T cell receptor.

Materials and Methods

Mice. Congenitally athymic (*nu/nu*) mice on a N:NIH(s)II background and their heterozygous (*nu/+*) littermates were bred under pathogen-protected conditions in the animal facilities maintained by the Ludwig Institute for Cancer Research, Epalinges, Switzerland. Breeding pairs were kindly provided by Dr. C. Hansen, National Institutes of Health, Bethesda, MD. As described elsewhere (17), these animals are doubly congenic in that they carry the X-linked B cell immunodeficiency inherited from CBA/N mice, as well as the nude mutation. Nude mice used in this study were 4–5 mo of age.

Monoclonal Antibodies. mAbs directed against Lyt-2 (18) and L3T4 (19) have been described. mAbs KJ16-133 (20) and F23.1 (21) are directed against partially overlapping idiotypic determinants present on ~20–25% of TCR β chains in most mouse strains. A rat mAb to Thy-1 (III5) was generated as described previously (22).

Culture Conditions. Nylon wool-purified or unseparated T cells (5×10^5) from nude or *nu/+* lymph nodes were cultured in flat-bottomed culture wells (16-mm-diam; Costar, Cambridge, MA) in 1 ml Dulbecco's modified Eagle's medium supplemented with 5% FCS, 5×10^{-5} M 2-ME, and 5% (vol/vol) supernatant from PMA-stimulated EL4 thymoma cells as a source of IL-2 (23). Phorbol-12-myristate acetate (PMA) was added at a final concentration of 3 ng/ml and ionomycin at a final concentration of 0.25 μ g/ml. In some experiments, Con A was added at 2.5 μ g/ml. Cultures were harvested after 6–10 d for phenotypic analysis and/or extraction of RNA and DNA as described below.

Flow Microfluorometry. Single-parameter (24) and two-parameter (25) flow microfluorometric analyses were performed as previously described. For the present two-parameter studies, cells were stained sequentially at 4°C in 100 μ l with purified F23.1 IgG (10 μ g) FITC-conjugated goat anti-mouse Ig (Tago Inc., Burlingame, CA), biotinylated Lyt-2 or L3T4 IgG, and avidin-phycoerythrin. Samples were passed on a FACS II flow cytometer modified for four-parameter analysis (26). Cytograms representing 10^5 viable cells were accumulated on a logarithmic scale and displayed on an Ortho 2150 computer (Ortho Diagnostics Systems, Raritan, NJ). Appropriate fluorescence compensation was achieved by passing an artificial mixture of FITC- and phycoerythrin-stained samples.

Cell Surface Labeling, Immunoprecipitation, and Polyacrylamide Gel Electrophoresis. The procedures have been described in detail previously (27). Briefly, 2.5 – 5.0×10^7 cells were labeled by lactoperoxidase-catalyzed iodination and lysed in a buffer containing 0.5% NP-40 and 0.5% deoxycholate. Lysates were precleared twice with normal human Ig coupled to Sepharose 4 B (Pharmacia Fine Chemicals, Uppsala, Sweden). The cleared lysates were filtered and then immunoprecipitated with F23.1 coupled directly to Sepharose 4 B (5 mg IgG per milliliter of packed beads). Immunoprecipitates were run on a 12.5% SDS-PAGE gel under reducing conditions, and autoradiographed.

Preparation of RNA and DNA. Total cellular RNA was extracted from fresh lymph node tissue using the guanidinium/cesium chloride method (28), whereas cytoplasmic RNA was extracted from cultured cells using NP-40 and phenol. Isolation of genomic DNA followed the procedure of Wigler et al. (29).

Filter Hybridizations. Glyoxylated RNA and restriction enzyme-digested genomic DNA were electrophoresed on agarose gels, transferred to Hybond-N nylon membrane (Amersham Corp., Amersham, United Kingdom), and hybridized as described previously (30, 31). cDNA were subcloned into SP6 plasmids and were used to transcribe in vitro 32 P-labeled RNA probes complementary to mRNA. The recombinant plasmids were characterized by restriction enzyme mapping, detection of the correct germline bands and/or detection of the correct size of the message on Northern blots. cDNA were kindly provided by Dr. T. Mak (TCR- α , VJC, and 3' untranslated sequences), Dr. E. Palmer (TCR- β , J $C_{\beta 2}$, and 3' untranslated sequences), Dr. B. Malissen (TCR-V $\beta_{8.1}$ TB $_{12}$ sequence 5' of Pvu II site [32]) and Dr. S. Hedrick (Thy-1.1 [TM8], [4]). The 217 bp Bal I fragment of the TCR- β constant domain was also subcloned into SP6 plasmids and used to transcribe probes giving equal hybridization to both C_{β} loci on genomic blots.

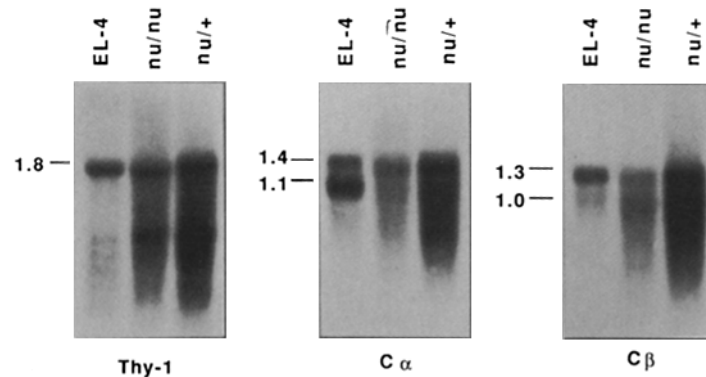


FIGURE 1. TCR α and β gene expression by freshly isolated nude T lymphocytes. Total cellular RNA from *nu/nu* (24 μ g) or *nu/+* (12 μ g) lymphoid cells was run on an agarose gel, transferred to nitrocellulose and hybridized with 32 P-labeled antisense RNA probes corresponding to Thy-1, TCR α , and β genes. RNA from EL-4 thymoma cells (12 μ g) was included for comparison (scale in kb). No hybridization of any of the probes was observed with control RNA from the B lymphoma A20/2J (data not shown).

Results

Expression of TCR Genes by Freshly Isolated Nude T Lymphocytes. In previous studies (24), we determined that the development of T lymphocytes in lymphoid organs of athymic mice was both age and strain dependent. Among the strains tested, the highest proportion of T cells was found in aged nude mice on a N:NIH II genetic background (14). These mice were therefore selected for further analysis of TCR expression. To this end, lymph node and spleen cells from nude or control (*nu/+*) N:NIH II mice were pooled. Total cellular RNA was extracted from these cells, run on agarose gels, transferred to nitrocellulose, and hybridized with cDNA probes specific for TCR- α and - β chains. A control Thy-1 cDNA probe was used in this and subsequent studies to ensure that comparable amounts of T cell-specific mRNA were present in all samples tested. An additional RNA sample from EL4 thymoma cells was included as a positive control.

As shown in Fig. 1, both nude and control (*nu/+*) RNA preparations hybridized to the Thy-1 probe, revealing the expected 1.8 kb message. As predicted from the relative proportions of Thy-1⁺ cells in these populations (Fig. 2), the level of Thy-1 message was two- to three-fold lower in nudes than control *nu/+*. Messages of 1.4 kb and 1.3 kb, corresponding to full-length transcripts of the TCR- α and - β chains, were also found in both *nu/nu* and *nu/+* populations. Truncated (1.1 and 1.0 kb) transcripts of both chains were also observed, although apparently to a lesser extent for the α chain in nudes. By densitometric analysis, the amount of full-length TCR- α and - β message was significantly (two- to three-fold) lower in the nude samples, even when normalized on the basis of expression of Thy-1.

Expression of TCR Proteins on Fresh and Activated Nude T Lymphocytes. Although the presence of full-length TCR- α and - β gene transcripts is a necessary prerequisite for expression of TCR protein, other factors (such as TCR assembly with the CD3 complex) could theoretically limit cell surface expression of these molecules. To study cell surface TCR expression, we therefore made

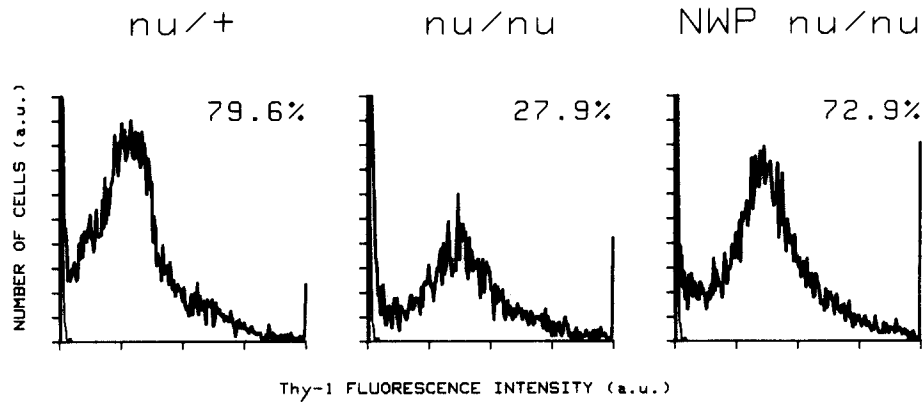


FIGURE 2. T cell content of unseparated and nylon wool-purified lymph node cells from nude mice. Cells were stained with anti-Thy-1 mAb followed by fluoresceinated goat anti-rat Ig and run on a FACS II flow cytometer gated to exclude nonviable cells. Unseparated *nu/+* lymph node cells are included for comparison. The percentage of positive cells (after subtraction of cells staining with the fluorescent conjugate alone) is indicated.

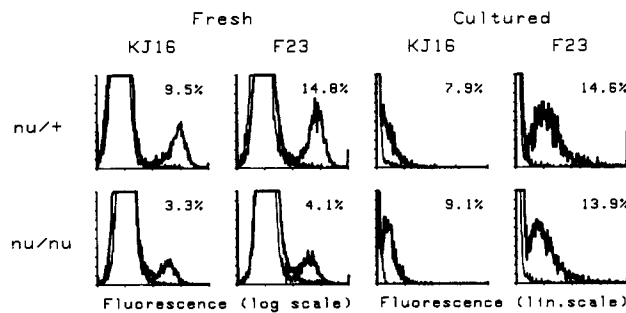


FIGURE 3. Flow microfluorometric analysis of TCR expression by T cells from nude and control (*nu/+*) mice. Freshly isolated or PMA/ionomycin-cultured *nu/nu* and *nu/+* lymph node cells were stained by indirect immunofluorescence with the TCR-specific mAbs KJ16-133 and F23.1 as indicated. Histograms of stained cells (*heavy lines*) are compared to control samples stained with the fluorescent conjugate alone (*thin lines*). The percentage of positive cells is indicated. Note that fluorescence intensity of fresh or cultured cells is expressed on a logarithmic or linear scale, respectively.

use of mAbs KJ16-133 (20) and F23.1 (21), which react with protein products of a family of TCR- β chain variable region genes (termed $V_{\beta 8}$; see references 33 and 34), apparently in association with any α gene product. For these experiments, nude lymph node cells were purified on nylon wool columns to give a three- to four-fold enrichment in Thy-1⁺ cells, thus making this population equivalent in T cell content ($\sim 70\%$) to the nonpurified *nu/+* control lymph node (Fig. 2). As shown in Fig. 3, ~ 10 and 15% of *nu/+* lymph node cells stained with KJ16-133 and F23.1 mAbs, respectively, while a lower proportion ($\sim 5\%$) of NWP nude T cells stained with these reagents. Staining of freshly isolated nude T cells was clearly biphasic on a logarithmic scale, although the absolute intensity was twofold lower than for the *nu/+* control (Fig. 3).

Upregulation of expression of TCR proteins following *in vitro* activation by lectins, phorbol esters, and/or calcium ionophores has been observed for certain populations of phenotypically immature mouse T cells (35) and human leukemia

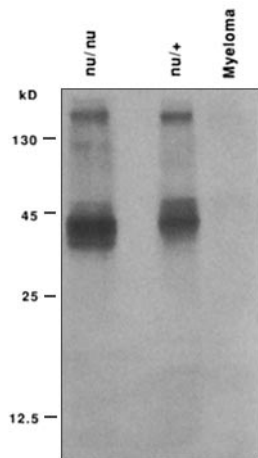


FIGURE 4. Immunoprecipitation analysis of TCR proteins expressed by *nu/nu* and *nu/+* T cells. PMA/ionomycin-stimulated T cells from nude or control (*nu/+*) mice were surface iodinated. Detergent-soluble lysates were then immunoprecipitated by F23.1-conjugated Sepharose beads. Immunoprecipitates were run on a 12.5% SDS-polyacrylamide gel under reducing conditions and autoradiographed for 24 h. Lysates from labeled myeloma cells were included as a negative control. Molecular mass standards are indicated.

cells.² We therefore investigated expression of KJ16-133 and F23.1 antigens on nude T cells that had been cultured for 6 d with a combination of PMA, ionomycin, and IL-2, a protocol that has been found to induce optimal proliferation of both Lyt-2⁺ and L3T4⁺ mature T cells (36) as well as immature (Lyt-2⁻, L3T4⁻) thymocytes (37). As shown in Fig. 3, a similar proportion (~10%) of PMA/ionomycin-cultured nude lymphoid cells expressed KJ16 antigen at about the same level as control PMA/ionomycin-stimulated *nu/+* lymphoid cells. ~15% of cells in both cultured populations also reacted with F23.1 (Fig. 3). The higher proportion of F23.1⁺ (as opposed to KJ16⁺) T cells is in accord with earlier studies (20, 21) and presumably reflects the fact that F23.1 reacts with all three members of the $V_{\beta 8}$ family, whereas KJ16 reacts with only two (38). Stimulation of nude or *nu/+* lymphoid cells with Con A plus IL-2 resulted in comparable levels of KJ16 and F23 expression as PMA/ionomycin stimulation (data not shown).

To exclude the unlikely possibility that F23.1/KJ16-133 expression on nude T cells resulted from crossreactivity of the antibodies with a cell surface structure unrelated to the TCR, cultured nude (or control *nu/+*) cells were surface iodinated and the detergent-soluble lysates were immunoprecipitated with purified F23.1 antibodies conjugated to Sepharose beads. Similarly labeled species of 38–43 kD were specifically immunoprecipitated from both nude and *nu/+* preparations under reducing conditions, while control lysates from myeloma cells were negative (Fig. 4). Thus F23.1 reacted with a molecular complex on nude T cells with the expected physical properties of the α/β dimer.

TCR Expression in Individual Nude Mice. Previous reports from our laboratory (16) and others (39, 40) have demonstrated a considerable degree of variability in the functional antigen-specific immune responsiveness of individual nude mice. Because the preceding experiments were carried out with pooled lymphoid cells from several animals, a corresponding potential variability in TCR expres-

² Carrel, S., J.-P. Mach, G. Miescher, S. Salvi, L. Giuffrè, M. Schreyer, and P. Isler. 1987. Phorbol-12-myristate-13-acetate (PMA) induces surface expression of T3 on human immature T cell lines with and without concomitant expression of the T cell antigen receptor complex. Manuscript submitted for publication.

TABLE I
Surface Phenotype of Cultured T Cells from Individual nu/+ and nu/nu Mice

Mouse	Percentage of cells staining positively for:				
	Thy-1	F23	KJ16	Lyt-2	L3T4
<i>nu/+</i>					
1	72.0	16.5	7.9	ND	ND
2	79.3	13.8	8.6	46.6	42.2
3	88.7	23.8	ND	47.4	40.2
4	86.0	15.3	13.6	27.1	35.9
5	72.3	15.5	ND	35.0	17.9
<i>nu/nu</i>					
1	86.2	1.6	ND	23.3	30.9
2	82.1	0	ND	11.6	42.6
3	95.7	5.6	ND	46.9	4.4
4	96.2	14.4	7.2	32.3	9.8
5	85.1	1.7	0	3.2	15.6
6	88.0	17.6	15.5	29.6	1.3
7	92.7	7.6	3.3	38.7	0.5
8	82.0	27.1	ND	41.7	16.1
9	67.5	19.2	ND	31.9	16.8
10	86.6	43.2	ND	29.6	35.2
11	96.6	46.8	ND	46.4	37.8

Total lymph node cells from 6–10-wk-old *nu/+* mice or nylon wool-purified lymph node cells from 5–6-mo-old *nu/nu* mice were cultured for 5–7 d with 3 ng/ml PMA, 250 ng/ml Ionomycin, and a source of IL-2. Recovered cells were stained with the indicated mAbs followed by fluorescently labelled anti-Ig and passed on a FACS II flow cytometer. Data are presented as the percent positive cells compared with samples stained with the fluorescent conjugate alone (cf. Fig. 2).

sion would tend to be masked. We therefore decided to investigate TCR expression in individual animals. As shown in Table I, there was a remarkable variability in F23.1 and KJ16-133 expression in PMA/ionomycin-stimulated lymph node preparations from a series of 11 individual N:NIH II nude mice, with the proportion of F23⁺ cells ranging from undetectable to 47%. In contrast, the proportion of F23⁺ cells in control (PMA/ionomycin-stimulated) *nu/+* lymph node cells was much more constant in individual mice (ranging from 14 to 24% in five determinations). Analysis of V_{β8}-specific message (using a V_{β8.1} probe that hybridizes with all three family members) further indicated a correlation with F23/KJ16 expression in individual nude mice (Fig. 5).

Several trivial explanations for the highly variable expression of F23/KJ16 antigens on cultured nude lymphoid cells were considered. First, the possibility that high F23 expression in certain mice reflected a selection for Thy-1⁺ cells in culture could be excluded, because almost all cultured populations contained >80% Thy-1⁺ cells (Table I). An alternative explanation, namely that V_{β8} (F23/KJ16)-positive cells were somehow selected for by the culture conditions, also seems highly improbable in view of the fact that full-length TCR-α and -β transcripts were found in PMA/ionomycin-stimulated cells from individual nude mice irrespective of the level of F23/KJ16 (or V_{β8}) expression (Fig. 5). Thus, it

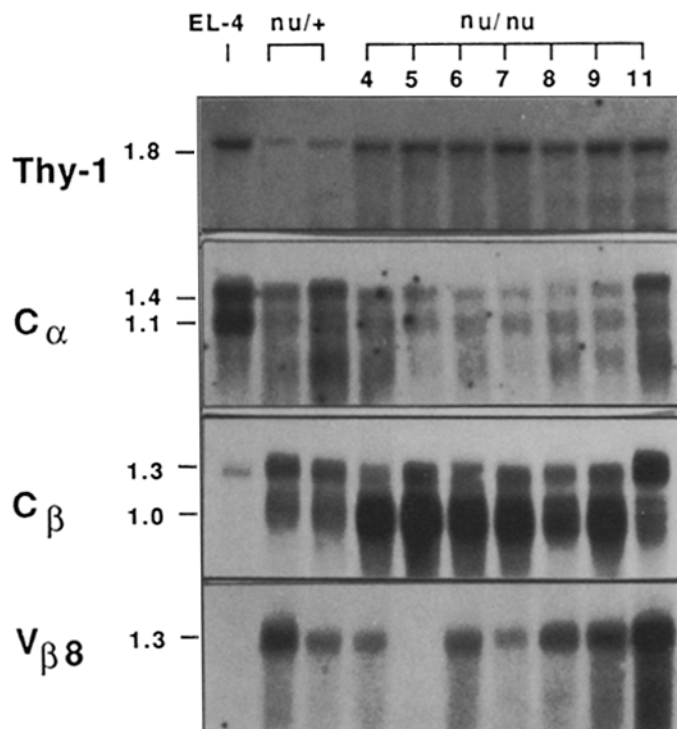


FIGURE 5. Expression of TCR α , β , and $V_{\beta 8}$ genes by cultured nude or $nu/+$ T cells. RNA extracted from PMA/ionomycin-stimulated T cells from individual $nu/+$ (5 μ g/lane) or nu/nu (10 μ g/lane) mice was run on replicate agarose gels, transferred to nitrocellulose, and hybridized with 32 P-labeled antisense RNA probes corresponding to Thy-1, C_{α} , C_{β} and $V_{\beta 8.1}$.

seems likely that the variable F23/KJ16 expression in individual nude mice reflects a true nonrandom usage of TCR- β chain variable gene segments.

TCR Expression in T Cell Subsets from Nude Mice. We have recently reported that T cell subsets are abnormally distributed in athymic mice (25). Thus while virtually all normal peripheral T cells express either L3T4 or Lyt-2 (in a roughly equal ratio), individual nude mice frequently express disproportionate levels of these differentiation markers as exemplified in Table I. In view of the marked variability of F23/KJ16 expression in individual nude mice, we therefore investigated whether TCR expression was linked to a particular subset of T cells. For these experiments, a two-color immunofluorescence technique was used in which F23 was revealed by a green (FITC-conjugated) anti-Ig antibody and biotinylated Lyt-2 or L3T4 was revealed by red (phycoerythrin-conjugated) avidin. Results from several representative PMA/ionomycin-stimulated nude lymphoid populations, as well as cultured control ($nu/+$) cells, are shown in Fig. 6 and a detailed summary of all data is given in Table II. Several important points emerge from this analysis. First, as is the case in normal mice (41), F23 expression is not restricted to either the Lyt-2 $^{+}$ or L3T4 $^{+}$ subset of nude T cells, since clear examples of F23 expression by both subsets are evident. However, in contrast to normal mice, where a similar proportion of Lyt-2 $^{+}$ or L3T4 $^{+}$ cells express F23

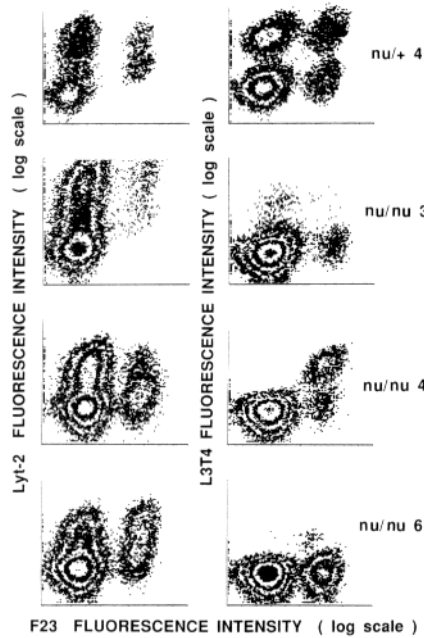


FIGURE 6. Expression of F23.1 by T cell subsets in individual *nu/+* or *nu/nu* mice. PMA/ionomycin-stimulated cells were stained sequentially with F23.1 mAb, fluoresceinated goat anti-mouse Ig, biotinylated anti-Lyt-2 or anti-L3T4 mAb and avidin-phycoerythrin. Samples were passed on a FACS II flow cytometer. Cytograms (representing 10^5 viable cells) are displayed on a logarithmic scale. The proportion of F23⁺, Lyt-2⁺ and F23⁺, L3T4⁺ cells in these and other similarly processed samples are summarized in Table II.

TABLE II
Expression of F23.1 in Lyt-2⁺ and L3T4⁺ T Cell Subsets of Individual *nu/+* and *nu/nu* Mice

Mouse	F23 ⁺ , Lyt-2 ⁺ cells per total Lyt-2 ⁺	F23 ⁺ , L3T4 ⁺ cells per total L3T4 ⁺
	%	
<i>nu/+</i>		
2	16.2	15.7
3	23.8	37.1
4	18.8	38.1
5	29.7	40.9
Mean ± SD	22.1 ± 5.9	32.9 ± 11.6
<i>nu/nu</i>		
3	13.6	29.5
4	9.3	91.8
6	27.3	—*
7	10.8	—*
8	29.2	92.5
9	34.7	32.1
10	27.0	92.8
11	17.6	97.8
Mean ± SD	21.2 ± 9.5	72.7 ± 32.6

Cultured T cells from individual mice were double stained with F23.1 mAb and either Lyt-2 or L3T4 mAbs as described in Fig. 6. The proportion of F23⁺ cells in each subset is indicated. Populations containing <2% F23⁺ cells were excluded from the analysis.

* <2% L3T4⁺ cells.

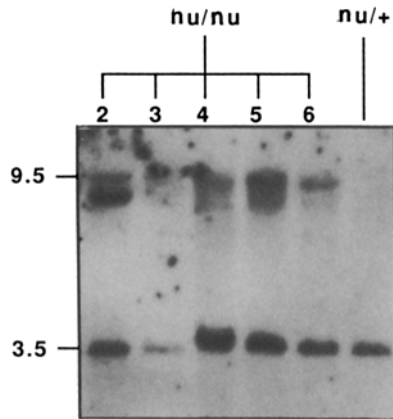


FIGURE 7. Analysis of TCR β chain rearrangements in individual *nu/+* or *nu/nu* T cells. 10 μ g DNA extracted from PMA/ionomycin-stimulated T cells was digested with Hind III, run on an agarose gel, transferred to nitrocellulose, and hybridized with 32 P-labeled C_{β} probe. The positions of the two germline (liver) restriction fragments are indicated.

(41) (Table II), there is a striking tendency for F23⁺ cells to be present predominantly in only one subset (i.e., Lyt-2⁺ or L3T4⁺) of nude T cells. Even more surprisingly, F23⁺ cells can account for virtually all the T cells in the L3T4⁺ subset of certain individual nude mice. Because F23⁺ cells account for only ~15% of normal T cells on this genetic background, such a result implies either a strong selection for certain TCR- β chain variable region genes during extrathymic differentiation, or the selective expansion of very small numbers of preexisting T cells expressing these genes.

TCR- β chain Rearrangements in Nude T Cells. The results detailed above raised the possibility that T cell populations in nude mice might be oligoclonal in origin. To test this hypothesis directly at the molecular level, DNA extracted from PMA/ionomycin-stimulated T cells from individual nude mice was digested with appropriate restriction enzymes, run on an agarose gel and transferred to nitrocellulose. When probed with a β -chain constant region probe, a Hind III digest of control germline (liver) DNA revealed the expected bands of 9.5 kb and 3.5 kb, corresponding to fragments containing the $C_{\beta 1}$ and $C_{\beta 2}$ loci, respectively (42). In DNA from cultured *nu/+* T cells, no 9.5 kb band was detectable (Fig. 7), indicating that virtually all cells in this normal population had rearrangements or deletions at the $C_{\beta 1}$ locus. No new bands could be seen, presumably because the rearrangements were polyclonal (and hence random). When DNA from several cultured nude T cell populations was analyzed in the same fashion, a different pattern was observed (Fig. 7). First, a small but detectable amount of unrearranged (9.5 kb) DNA was present. In addition, a series of new bands (or smears) were apparent below the 9.5 kb germline band in all nude mice, suggesting nonrandom rearrangements to the $C_{\beta 1}$ locus. In one particular case (nude mouse 2), a strong rearranged band corresponding to 80–90% germline intensity was observed, presumably indicating the presence of a high proportion of clonally derived T cells in this individual. The apparently clonal nature of most T cells in nude mouse 2 was confirmed by digesting the same DNA preparation with Hpa II and probing with C_{β} (data not shown).

Discussion

The data presented in this report demonstrate formally that expression of TCR α/β heterodimers can occur extrathymically. Thus, freshly isolated T cells from aged nude mice expressed full-length transcripts for both the TCR α and β genes and low (but detectable) levels of TCR protein at the cell surface. Immunoprecipitation analysis confirmed that the surface structure recognized by anti-TCR mAb F23 on activated nude and normal T cells was similar, and that it corresponded to the expected properties of the α/β heterodimer. Our data are in apparent contradiction with two recent reports indicating that TCR α and β chain transcripts were either absent (11) or truncated (43) in freshly isolated cells from nude mice. However, these studies did not confirm the presence of other T cell-specific messages in the RNA preparations used. Furthermore, the source of nude T cells used (2–3-mo-old BALB/c mice) would be expected on the basis of earlier studies (24) to be particularly low in mature T cell content.

A striking finding in the present study was the variability of expression of F23/KJ16 determinants on T cells from individual nude mice. F23.1 and KJ16-133 antibodies are known to react with TCR heterodimers containing the product of a particular family of β chain variable region genes (known as $V_{\beta 8}$) (33, 34), and the reduced proportion of KJ16⁺ (as opposed to F23⁺) cells in polyclonal T cell populations reflects the fact that KJ16 recognizes only two of the three members of the $V_{\beta 8}$ family (38). Thus, the widely variable F23/KJ16 expression in T cells of individual nude mice could be most readily interpreted to mean that there is a strong but random selection for V_{β} gene usage in these animals. An alternative possibility, namely that V_{β} selection is nonrandom and biased towards $V_{\beta 8}$ in nude mice seems highly unlikely in view of the fact that T cells from individual nude mice expressed detectable amounts of full-length TCR α and β transcripts irrespective of whether or not they expressed F23/KJ16.

Several other observations are relevant in the context of a model in which particular V_{β} genes are selected for in individual nude mice. Thus, analysis of F23 expression in Lyt-2⁺ or L3T4⁺ subsets of nude T cells revealed that, in contrast to normal mice (41), F23 expression was frequently associated predominantly with either Lyt-2⁺ or L3T4⁺ cells. Even more strikingly, several independent examples were seen of L3T4⁺ subsets of nude T cells that were virtually 100% F23⁺, and there was a strong tendency toward overexpression of F23 in general among L3T4⁺ nude T cells (73 vs. 33% in control *nu/+*). The mechanism underlying this apparent selection for the $V_{\beta 8}$ gene family among L3T4⁺ nude T cells is unknown, but could be speculated to be the result of a developmental regulation of expression of V_{β} gene segments. In this context, we have recently observed that $V_{\beta 8}$ genes are overexpressed in a subset of immature (Lyt-2⁻, L3T4⁻) normal thymocytes (44). Because rearrangement and expression of TCR genes in athymic mice appears to be severely retarded, preferential expression of developmentally immature V_{β} segments might be expected. In such a case, the fact that F23 expression was normal among Lyt-2⁺ nude T cells (21%) would imply either a distinct developmental sequence of V_{β} gene expression for L3T4⁺ and Lyt-2⁺ T cells (and hence independent lineages), or alternatively, a more

efficient differentiation of the Lyt-2⁺ lineage leading to a greater degree of receptor polyclonality.

We have previously observed that the specificity pattern of alloreactive T cells in individual nude mice is more restricted than that of their euthymic littermates (16). On the basis of these and other similar findings (39, 40), we (16) and others (13) proposed that the repertoire of antigen-specific T cells in nude mice may be oligoclonal. Direct evidence in favor of this hypothesis is provided in the present study by the observation of more restricted (and in some cases unique) rearrangements at the TCR β locus in cultured T cells from certain individual nude mice. No such presumably monoclonal rearrangements were seen in control (*nu/+*) T cells in this or (to our knowledge) any other similar study. In evaluating the apparent frequency of these rearrangements, it should be noted that (for practical reasons) we were unable to evaluate TCR β chain rearrangements in isolated Lyt-2⁺ or L3T4⁺ subsets from nude mice. Thus the failure to detect unique rearranged bands in most animals does not preclude the possibility that additional monoclonal rearrangements may exist at the subset level.

The presence of oligoclonal β chain rearrangement in athymic mice raises the question of whether α chain rearrangements are likewise restricted. Unfortunately, the large size of the α locus (45–47) makes direct analysis of α rearrangements difficult without specialized probes and gel technology. Because α rearrangements occur later in normal T cell development than β rearrangements (6–10), it might nevertheless be anticipated that whatever mechanism leads to restricted expression of β chains in athymic mice may also influence α chain expression. If so, and assuming that association of α and β chains is random, a practical consequence of such a putative oligoclonal α chain repertoire would be to further restrict the number of possible α/β protein heterodimers expressed in nude T cells. At present, we have no data bearing directly on this issue; however, the more discrete pattern of TCR proteins precipitated by F23.1 from nude (as opposed to *nu/+*) T cells would be consistent with such an interpretation.

The mechanism responsible for the observed upregulation of expression of TCR protein in nude T cells following exposure to PMA/ionomycin or Con A requires further study. Other examples of increased surface expression of TCR protein upon *in vitro* stimulation have been seen in a subset of phenotypically immature (Lyt-2⁻, L3T4⁻) T cells in autoimmune *lpr* mice (35) and in certain human T cell leukemias with immature phenotype.² Whether such upregulation reflects changes in the transcription rate of TCR genes themselves or in other molecules (such as the CD3 complex) that may limit their assembly and surface expression remains to be established.

Finally, it should be noted that the present demonstration of α/β heterodimers on at least some nude T cells does not exclude the possibility that other CD3-associated structures may also be expressed in athymic mice. In this regard, recent studies of phenotypically immature T cells in both human (48, 49) and murine (50, 51) systems have clearly established the existence of CD3-associated molecular complexes containing the protein product of the rearranging T cell-specific γ gene previously shown to be structurally homologous to α and β (52). Because the level of expression of this gamma gene is elevated in freshly isolated (43) and PMA/ionomycin-stimulated nude T cells (G. Meischer, R. K. Lees, and

H. R. MacDonald, unpublished data), it remains possible that some nude T cells express the gamma protein in association with CD3. Such a finding would be consistent with the presence of increased numbers of immature (Lyt-2⁻, L3T4⁻) T cells in athymic mice (25), since cells of this phenotype have been shown elsewhere to express the gamma protein (49–52).

Summary

The expression of T cell antigen receptors (TCR) in congenitally athymic (nude) mice has been investigated. Lymph node T cells from 4–5-mo-old athymic mice expressed full-length transcripts for the TCR α and β chains at a level two- to three-fold lower than normal littermate (*nu/+*) controls. Low levels of expression of TCR protein at the surface of a proportion of nude T cells was demonstrated by staining with monoclonal antibodies KJ16-133 and F23.1 (directed against protein products of a family of TCR β chain variable region genes known as $V_{\beta 8}$). Immunoprecipitation studies confirmed that F23.1 reacted with a similar molecular species on nude and *nu/+* T cells.

Studies with individual nude mice revealed a striking heterogeneity in the proportion of T cells expressing KJ16/F23.1 that was not seen in normal animals. This heterogeneity correlated with the expression of mRNA specific for $V_{\beta 8}$ but not with total expression of full-length β chain transcripts. Analysis of Lyt-2⁺ and L3T4⁺ T cell subsets in individual nude mice further demonstrated that F23.1 expression was frequently associated with only one subset, and several cases were seen in which all L3T4⁺ cells expressed F23.1. In contrast, a similar (and constant) proportion of Lyt-2⁺ or L3T4⁺ T cells expressed F23.1 in control mice. Southern blotting of Hind III-digested DNA from nude T cells with a C_{β} probe revealed a more restricted pattern of TCR β chain rearrangements than was seen for normal T cells. Taken together, these data provide compelling evidence that TCR gene rearrangement and expression can occur extrathymically. Furthermore, they suggest a model according to which the restricted functional repertoire of T cells previously observed in individual nude mice results from an oligoclonal expansion of T cells that have randomly rearranged and expressed TCR β chain genes.

We thank P. Zaech and E. Santoli for FACS analysis and P. Brunet for secretarial assistance.

Received for publication 29 January 1987 and in revised form 26 March 1987.

References

1. Miller, J. F. A. P., and D. Osoba. 1967. Current concepts of the immunological function of the thymus. *Physiol. Rev.* 47:437.
2. Moore, M. A. S., and J. J. T. Owen. 1967. Experimental studies on the development of the thymus. *J. Exp. Med.* 126:715.
3. Yanagi, Y., Y. Yoshikai, K. Leggett, S. P. Clark, I. Aleksander, and T. W. Mak. 1984. A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature (Lond.)* 308:145.
4. Hedrick, S. M., D. L. Cohen, E. A. Nielsen, and M. M. Davis. 1984. Isolation of

- cDNA clones encoding T cell-specific membrane-associated proteins. *Nature (Lond.)*. 308:149.
5. Saito, H., D. Kranz, Y. Takagaki, A. Hayday, H. Eisen, and S. Tonegawa. 1984. A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. *Nature (Lond.)*. 312:36.
 6. Raulet, D. H., R. D. Garman, H. Saito, and S. Tonegawa. 1985. Developmental regulation of T-cell receptor gene expression. *Nature (Lond.)*. 314:103.
 7. Snodgrass, H. R., Z. Dembic, M. Steinmetz, and H. von Boehmer. 1985. Expression of T-cell antigen receptor genes during fetal development in the thymus. *Nature (Lond.)*. 315:232.
 8. Born, W., J. Yague, E. Palmer, J. Kappler, and P. Murrack. 1985. Rearrangement of T-cell receptor β -chain genes during T-cell development. *Proc. Natl. Acad. Sci. USA*. 892:2925.
 9. Samelson, L. F., T. Lindsten, B. J. Fowlkes, P. van den Elsen, C. Terhorst, M. M. Davis, R. N. Germain, and R. H. Schwartz. 1985. Expression of genes of the T-cell antigen receptor complex in precursor thymocytes. *Nature (Lond.)*. 315:765.
 10. Haars, R., M. Kronenberg, W. M. Gallatin, I. L. Weissman, F. L. Owen, and L. Hood. 1986. Rearrangement and expression of T cell antigen receptor and γ genes during thymic development. *J. Exp. Med.* 164:1.
 11. Owen, M. J., E. J. Jenkinson, G. T. Williams, R. Kingston, and J. J. T. Owen. 1986. An investigation of T cell receptor gene rearrangement and expression in organ cultures of normal embryonic thymus and Thy-1⁺ cells of nude mice. *Eur. J. Immunol.* 16:875.
 12. Born, W., M. McDuffie, N. Roehm, E. Kushnir, J. White, D. Thorpe, J. Stefano, J. Kappler, and P. Murrack. 1987. Expression and role of the T cell receptor in early thymocyte differentiation in vitro. *J. Immunol.* 138:999.
 13. Hunig, T. 1983. T-cell function and specificity in athymic mice. *Immunol. Today*. 4:84.
 14. MacDonald, H. R. 1984. Phenotypic and functional characteristics of "T-like" cells in nude mice. *Exp. Cell. Biol.* 52:2.
 15. Maryanski, J. L., H. R. MacDonald, B. Sordat, and J.-C. Cerottini. 1981. Cytolytic T lymphocyte precursor cells in congenitally athymic C57BL/6 nu/nu mice: Quantitation, enrichment and specificity. *J. Immunol.* 126:871.
 16. MacDonald, H. R., and R. K. Lees. 1984. Frequency and specificity of interleukin 2-producing cells in nude mice. *J. Immunol.* 132:605.
 17. Azar, H. A., C. T. Hansen, and J. Costa. 1980. N:NIH(s)II-nu/nu mice with combined immunodeficiency: a new model for human tumor hetero-transplantation. *J. Natl. Cancer Inst.* 65:421.
 18. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47:63.
 19. Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch. 1983. Characterization of the murine T cell surface molecule designated L3T4 identified by monoclonal antibody GK 1.5: Similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol.* 131:2445.
 20. Haskins, K., C. Hannum, J. White, N. Roehm, R. Kubo, J. Kappler, and P. Murrack. 1984. The major histocompatibility complex-restricted antigen receptor on T cells. VI. An antibody to a receptor allotype. *J. Exp. Med.* 160:452.
 21. Staerz, U. D., H.-G. Rammensee, J. D. Benedetto, and M. J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on the T cell antigen receptor. *J. Immunol.* 134:3994.
 22. MacDonald, H. R., C. Bron, M. Rousseaux, C. Horvath, and J.-C. Cerottini. 1985.

- Production and characterization of monoclonal anti-Thy-1 antibodies that stimulate lymphokine production by cytolytic T cell clones. *Eur. J. Immunol.* 15:495.
23. Farrar, J. J., J. Fuller-Farrar, P. L. Simon, M. L. Hilfiker, B. M. Stadler, and W. L. Farrar. 1980. Thymoma production of T cell growth factor (interleukin-2). *J. Immunol.* 125:2555.
 24. MacDonald, H. R., R. K. Lees, B. Sordat, P. Zaech, J. L. Maryanski, and C. Bron. 1981. Age-associated increase in expression of the T cell surface markers Thy-1, Lyl-1 and Lyl-2 in congenitally athymic (nu/nu) mice: analysis by flow microfluorometry. *J. Immunol.* 126:865.
 25. MacDonald, H. R., C. Blanc, R. K. Lees, and B. Sordat. 1986. Abnormal distribution of T cell subsets in athymic mice. *J. Immunol.* 136:4337.
 26. MacDonald, H. R., and P. Zaech. 1982. Light scatter analysis and sorting of cells activated in mixed leukocyte culture. *Cytometry.* 3:55.
 27. Luescher, B., and C. Bron. 1985. Biosynthesis of mouse Thy-1 antigen. *J. Immunol.* 134:1084.
 28. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18(24):5294.
 29. Wigler, M., R. Sweet, G. K. Sim, B. Wold, A. Pellicer, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel. 1979. Transformation of mammalian cells with genes from procaryotes and eucaryotes. *Cell.* 16:777.
 30. Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12(18):7035.
 31. Church, G. M., and W. Gilbert. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA.* 81:1991.
 32. Barth, R. K., B. S. Kim, N. C. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershenfeld, C. Okada, D. Hansburg, I. L. Weissman, and L. Hood. 1985. The murine T-cell receptor uses a limited repertoire of expressed V_{β} gene segments. *Nature (Lond.).* 316:517.
 33. Sim, G. K., and A. A. Augustin. 1985. V_{β} gene polymorphism and a major polyclonal T cell receptor idotype. *Cell.* 42:89.
 34. Behlke, M. A., H. S. Chou, K. Huppi, and D. Y. Loh. 1986. Murine T-cell receptor mutants with deletions of β -chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 83:767.
 35. Budd, R. C., H. R. MacDonald, J. W. Lowenthal, J.-L. Davignon, S. Izui, and J.-C. Cerottini. 1985. Growth and differentiation in vitro of the accumulating Lyl-2⁻/L3T4⁻ subset in *lpr* mice. *J. Immunol.* 135:3704.
 36. Erard, F., M. Nabholz, A. Dupuy-d'Angeac, and H. R. MacDonald. 1985. Differential requirements for the induction of interleukin-2 responsiveness in L3T4⁺ and Lyl-2⁺ T cell subsets. *J. Exp. Med.* 162:1738.
 37. Palacios, R., and H. von Boehmer. 1986. Requirements for growth of immature thymocytes from fetal and adult mice in vitro. *Eur. J. Immunol.* 16:12.
 38. Behlke, M. A., T. J. Henkel, S. J. Anderson, N. C. Lan, L. Hood, V. L. Braciale, T. J. Braciale, and D. Y. Loh. 1987. Expression of a murine polyclonal T cell receptor marker correlates with the use of specific members of the $V_{\beta 8}$ gene segment subfamily. *J. Exp. Med.* 165:257.
 39. Hunig, T., and M. J. Bevan. 1984. Ability of nude mice to generate alloreactive, xenoreactive and H-2 restricted cytotoxic T-lymphocyte responses. *Exp. Cell. Biol.* 52:7.

40. Miller, R. G., H. R. Derry, and B. J. Sarjeant. 1983. The extent of self-MHC restriction of cytotoxic T cells in nude mice varies from mouse to mouse. *J. Immunol.* 130:63.
41. Roehm, N., L. Herron, J. Cambier, D. DiGuisto, K. Haskins, J. Kappler, and P. Marrack. 1984. The major histocompatibility complex-restricted antigen receptor on T cells: Distribution on thymus and peripheral T cells. *Cell.* 38:577.
42. Malissen, M., K. Minard, S. Mjolsness, M. Kronenberg, J. Goverman, T. Hunkapiller, M. B. Prystowsky, Y. Yoshikai, F. Fitch, T. W. Mak, and L. Hood. 1984. Mouse T cell antigen receptor: structure and organization of constant and joining gene segments encoding the β polypeptide. *Cell.* 37:1101.
43. Yoshikai, Y., M. D. Reis, and T. W. Mak. 1986. Athymic mice express a high level of functional γ -chain but greatly reduced levels of α - and β -chain T-cell receptor messages. *Nature (Lond.)*. 324:482.
44. Budd, R. C., G. C. Miescher, R. C. Howe, R. K. Lees, C. Bron, and H. R. MacDonald. 1987. Developmentally regulated expression of T cell receptor β chain variable domains in immature thymocytes. *J. Exp. Med.* In press.
45. Hayday, A. C., D. J. Diamond, G. Tanigawa, J. S. Heilig, V. Folsom, H. Saito, and S. Tonegawa. 1985. Unusual organization and diversity of T-cell receptor α -chain genes. *Nature (Lond.)*. 316:828.
46. Winoto, A., S. Mjolsness, and L. Hood. 1985. Genomic organization of the genes encoding mouse T-cell receptor α -chain. *Nature (Lond.)*. 316:832.
47. Yoshikai, Y., S. P. Clark, S. Taylor, U. Sohn, B. I. Wilson, M. D. Minden, and T. W. Mak. 1985. Organization and sequences of the variable, joining and constant region genes of the human T-cell receptor α -chain. *Nature (Lond.)*. 316:837.
48. Brenner, M. B., J. McLean, D. P. Dialynas, J. L. Strominger, J. A. Smith, F. L. Owen, J. G. Seidman, S. Ip, F. Rosen, and M. S. Krangel. 1986. Identification of a putative second T cell receptor. *Nature (Lond.)*. 322:145.
49. Bank, I., R. A. DePinho, M. B. Brenner, J. Cassimeris, F. W. Alt, and L. Chess. 1986. A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. *Nature (Lond.)*. 322:179.
50. Lew, A. M., D. M. Pardoll, W. L. Maloy, B. J. Fowlkes, A. Kruisbeck, S.-F. Cheng, R. N. Germain, J. A. Bluestone, R. H. Schwartz, and J. E. Coligan. 1986. Characterization of T-cell receptor gamma chain expression in a subset of murine thymocytes. *Science (Wash. DC)*. 234:1401.
51. Nakanishi, N., K. Maeda, K. Ito, M. Heller, and S. Tonegawa. 1987. T γ protein is expressed on murine fetal thymocytes as a disulfide-linked heterodimer. *Nature (Lond.)*. 325:720.
52. Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa. 1984. Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. *Nature (Lond.)*. 309:757.