centrations of IL-2 receptors, we were surprised to find that the cells responded only weakly to purified recombinant IL-2. Interestingly, a vigorous proliferative response of immature thymocytes to IL-2 was revealed by co-stimulation with the T-cell mitogen Con A, whereas the cells responded only weakly to Con A alone; this was true of both adult and fetal L3T4$^-$, Lyt-2$^-$ thymocytes. However, in two experiments the response of the fetal thymocytes to IL-2 titrated more steeply than that of the young adult L3T4$^-$, Lyt-2$^-$ thymocytes. Whether this reflects differences in receptor affinity or some other difference in these cell populations is unknown. We note that inclusion of irradiated T-cell-depleted syngeneic splenic accessory cells did not augment the response to Con A plus IL-2, nor did the accessory cells substitute for either Con A or IL-2 in stimulating the proliferative response (data not shown).

The expression of apparently functional IL-2 receptors by immature thymocytes suggests that IL-2 plays a role in thymus cell ontogeny. Of obvious interest in this context is the ontogeny of IL-2-producing cells in the thymus. IL-2-producing thymocytes are not detected before day 19 of fetal ontogeny and seem to correlate with the L3T4$^+$, Lyt-2$^-$ subpopulation[7], but it is not clear whether the methods of that study used appropriate conditions to detect IL-2 production by immature thymocytes. We are now assessing other protocols for stimulating IL-2 production by immature thymocytes.

A possible role of IL-2 in the positive selection of thymocytes is suggested by the requirement of Con A for potent proliferative responses to IL-2 despite the fact that the cells express IL-2 receptors without *in vitro* induction. In the case of mature resting T cells, which do not constitutively express IL-2 receptors, mitogenic or antigenic stimulation leads to expression of IL-2 receptors and subsequent IL-2-dependent proliferation. Although expression of IL-2 receptors above a threshold level may be a sufficient condition for IL-2-dependent proliferation of mature T cells, our data suggest that additional requirements, provided by mitogenic stimulation, are operative, at least in the case of immature thymocytes. Con A may act by stimulating cell surface structures on immature thymocytes involved in recognition of MHC antigens and/or nominal antigens. *In vivo*, stimulation of the receptor might aid positive selection of thymocytes during the 'education' of thymocytes.

Finally, the present results do not imply that all thymocyte proliferation is IL-2 dependent, as thymic blasts with phenotypes distinct from the L3T4$^-$, Lyt-2$^-$ subpopulation have been reported[7,11].

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Table 1 Proliferative responses of Lyt-2$^-$, L3T4$^-$ thymocytes

Stimulant in culture	Lyt-2$^-$, L3T4$^-$ thymocytes	
	Adult	Day 16 fetal
None	91 ± 17	52 ± 4
IL-2*, 5 U ml^{-1}	4,518 ± 701	1,220 ± 201
IL-2, 1 U ml^{-1}	2,477 ± 84	294 ± 61
IL-2, 0.2 U ml^{-1}	1,131 ± 96	246 ± 187
Con A†	253 ± 26	924 ± 85.7
Con A + IL-2, 5 U ml^{-1}	28,282 ± 1,128	18,020 ± 1,610
Con A + IL-2, 1 U ml^{-1}	18,855 ± 1,114	1,464 ± 401
Con A + IL-2, 0.2 U ml^{-1}	6,073 ± 634	215 ± 45

Thymocytes were treated once (fetal thymocytes) or twice consecutively (young adult thymocytes) with anti-Lyt-2 and anti-L3T4 monoclonal antibodies plus complement (see Fig. 2) and viable cells purified on gradients of Ficoll-Isopaque. Recoveries from complement lysis were 1.3% and 68% for the young adult and fetal cells respectively. In tests of stimulant activity, triplicate cultures of cells in round-bottom microtitre wells (Costar 3799 plates) contained 1×10^5 cells in 0.2 ml RPMI 1640 medium, 5% fetal calf serum, 50 μM 2-mercaptoethanol, 0.02% glutamine, antibiotics and the additions indicated. After culture for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air, 0.5 μCi ^{3}H-thymidine (6.7 Ci mmol^{-1}) was added to each well and the cultures continued for 4 h, at which time the cells were collected and lysed on glass fibre filters and counted by liquid scintillation spectrometry.

* Purified human recombinant IL-2 (AmGen Biologicals) was titred in our laboratory and compared with a standard sample of IL-2 provided by Dr Richard Robb (Dupont, Nemours and Co.) which is referenced against the unit defined by Gillis and Smith[20]. The specific activity of IL-2 we calculate to be 2×10^5 U per mg protein.

† Con A (Pharmacia) was added to a final concentration of 2 μg ml^{-1}.

Developmental regulation of T-cell receptor gene expression

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In contrast to B cells or their antibody products, T lymphocytes have a dual specificity, for both the eliciting foreign antigen and for polymorphic determinants on cell surface glycoproteins encoded in the major histocompatibility complex (MHC restriction)[1–4]. The recent identification of T-cell receptor glycoproteins[5–7] as well as the genes encoding T-cell receptor subunits will help to elucidate whether MHC proteins and foreign antigens are recognized by two T-cell receptors or by a single receptor. An important feature of MHC restriction is that it appears to be largely acquired by a differentiating T-cell population under the influence of MHC antigens expressed in the thymus[8–10], suggesting that precursor T cells are selected on the basis of their reactivity with MHC determinants expressed in the host thymus[9–11]. To understand this process of 'thymus education', knowledge of the developmental regulation of T-cell receptor gene expression is necessary. Here we report that whereas messenger RNAs encoding the β-and γ-subunits are relatively abundant in immature thymocytes, α mRNA levels are very low. Interestingly, whereas α mRNA levels increase during further development and β mRNA levels stay roughly constant, γ mRNA falls to very low levels in mature T cells, suggesting a role for the γ gene in T-cell differentiation.

cDNA clones encoding the α- and β-subunits of the T-cell receptor have been isolated[12–17] and identified by comparison with partial protein sequences[18–20]. As reported elsewhere, the predicted primary structures of the β- and α-subunits and, in the case of the β-subunit, the genomic organization of the corresponding genetic elements show striking similarities to their

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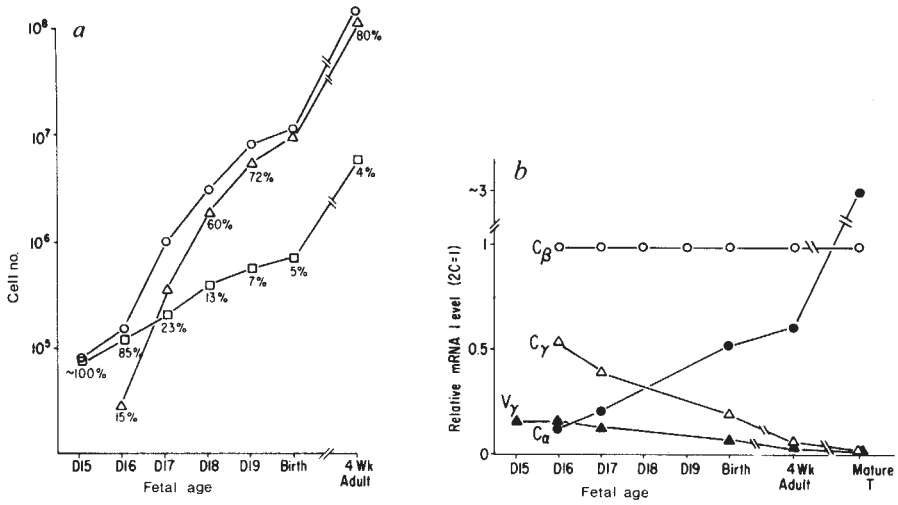

Fig. 1 *a*, Contribution of different subpopulations to thymocyte differentiation. Circles represent the approximate total cellularity of the thymus at different stages; triangles and squares represent the approximate contribution, at each stage, of 'double-positive' and 'double-negative' thymocytes, respectively. The numbers beneath the symbols represent percentages of the total. Data from Ceredig *et al.*[27]. *b*, Approximate relative levels of α, β and γ mRNAs in thymocytes and T cells as a function of fetal and adult age. Data are from the present study. The levels are relative to RNA from the 2C line, and are determined primarily from the Northern data (Figs 3, 4) with reference to the ribonuclease assay data (Fig. 2).

immunoglobulin counterparts[12-17,21-24]. A third gene, γ, has striking similarities to α and β, and may encode an additional T-cell receptor subunit[15,25,26].

Figure 1 represents our depiction of the results of Ceredig *et al.*[27] on the representation of different thymocyte subpopulations during murine development. Thymocytes with the Lyt-2$^-$, L3T4$^-$, Thy-1$^+$ cell surface phenotype (hereafter referred to as 'double-negative' thymocytes) represent the large majority of thymocytes before day 17 of fetal development, and decrease in proportional representation during development until, in the young adult (4-week-old) thymus, they represent only ~4% of the total. After day 16 of fetal development, most thymocytes express the Lyt-2$^+$, L3T4$^+$ ('double-positive') phenotype. Thymocytes with the phenotypes characteristic of mature T cells (Lyt-2$^+$, L3T4$^-$ and Lyt-2$^-$, L3T4$^+$) are detectable shortly after this time and increase in numbers during development to represent ~15% of young adult thymocytes.

The exact lineage interrelations of these subpopulations and the identity of the direct precursor of mature peripheral T cells remain controversial issues, but recent evidence shows that double-negative thymocytes are precursors of the other thymocyte subpopulations[28], and presumably of peripheral T cells. In the analyses reported here, we examine RNA from thymocytes of embryos of days 15, 16, 17 and 20 (newborn) of fetal development, and of 4-week old mice. We also examine RNA from purified double-negative thymocytes from 4-week-old mice and mature lymph-node T-cell RNAs.

T-cell receptor mRNAs were detected by Northern blot analysis and with a recently developed ribonuclease protection assay. We used RNA from the cloned alloreactive cytotoxic T-lymphocyte (CTL) line 2C (ref. 29; see also refs 15, 16) as a standard in each experiment. Titrations of 2C RNA were included so that the relative amounts of each mRNA could be estimated. As a basis for comparison, the frequencies of β and γ cDNA clones in an unselected 2C cDNA library were estimated at 0.1% and 0.02% respectively (H.S., unpublished). The library has not been screened with an α probe, but the frequency of α clones in a subtracted 2C cDNA library is one-half that of β clones[14].

The β probe corresponded to the last third of the β constant region and the 3′-untranslated region of the 3′-most of the two C_β genes ($C_{\beta'}$) (Fig. 2*a*; refs 15, 30, 31); as these are the regions of least homology between the two genes (69%), our assays probably selectively detect transcripts of the $C_{\beta'}$ gene. Analysis of β-chain RNA in developing thymocytes, using the ribonuclease protection assay, showed that β-chain constant-region transcripts are already abundant at day 15 of fetal development (Fig. 2*a*), corresponding to about three times the level found in the reference 2C CTL line. The level of C_β-RNA increases somewhat on day 17 of fetal development and remains approximately constant throughout further thymocyte development. Northern blot analysis showed that approximately two-thirds of the β transcripts in RNA from day 16 fetal thymocytes are 1.0 kilobases (kb) long, and about one-third of the transcripts are 1.3 kb long (Fig. 3*a*). Studies in the mouse[24] and human[32,33] systems suggest that the functional V-D-J-C transcripts of β are 1.3 kb long whereas the 1.0-kb mRNA corresponds to transcripts from incompletely rearranged loci. We estimate the level of 1.3-kb β mRNA in day 16 fetal thymocytes to be about equal to the level in RNA from the 2C cell line. The level of 1.3-kb β mRNA is roughly constant throughout further thymocyte development, and is similar in peripheral resting T cells, in T cells activated for 2 days with concanavalin A (Con A) (Figs 2*a*, 4*b*), and in the immature double-negative thymocytes from 4-week-old adult mice (Figs 2*a*, 4*b*). The fact that our β probe preferentially detects $C_{\beta'}$ mRNA may explain why we fail to observe higher levels of β mRNA in thymocytes than in mature T cells, as has been reported by others[12,24]; recent studies suggest that 97% of β transcripts in murine thymocytes use C_β, although $C_{\beta'}$ is used in most functional T cells thus far examined[30].

Genomic analysis suggests that there is only a single C_α gene (A. Hayday, G. Tanigawa, D. Diamond, H.S. and S.T., unpublished). α mRNA is barely detectable at day 16 of development and increases in level thereafter, although even in adult thymocytes it is lower than in 2C cells (Figs 2*b*, 3*b*). The late appearance of α mRNA may explain recent data suggesting that before day 17 of fetal development, thymocytes do not express the α/β heterodimer on the cell surface[34]. The level of α mRNA in resting or activated mature T cells is approximately three times that in 2C cells. The level of α mRNA in immature double-negative adult thymocytes is ~2-3-fold lower than that in 2C cells, or 6-9-fold lower than in mature peripheral T cells (Figs 2*b*, 4*a*).

Sequences coresponding to the constant region of the γ gene (C_γ) are relatively abundant in day 16 fetal thymocytes (approximately half the level in 2C by Northern analysis) and decrease in level thereafter (Figs 2*c*, 3*c*). C_γ mRNA is nearly 10-fold less abundant in young adult thymocytes than in 2C cells. The level of C_γ mRNA in adult double-negative thymocytes is the highest of any cells we have examined, corresponding to about twice the level in 2C RNA (Figs 2*c*, 4*c*). In striking contrast, the level of C_γ mRNA in mature T cells is extremely low (Figs 2*c*, 4*c*). A longer exposure of the gel shown in Fig. 2*c* was required to reveal a weak band corresponding to C_γ mRNA in mature T cells (not shown); we estimate the level of C_γ mRNA in these T cells to be more than 20-fold lower than that in 2C cells. The level of C_γ mRNA in T cells activated for 2 days with Con A is also very low (Figs 2*c*, 4*c*). C_γ mRNA levels were also very low in BALB.B lymph node T cells (not shown).

In contrast to the α and β constant-region gene segments, which appear to use a larger number of different variable-region gene segments, a single γ-chain variable-region gene segment is transcribed in each CTL clone thus far examined[15,21]. Therefore, we have been able to analyse the regulation of V_γ mRNA expression in developing thymocytes. V_γ-containing mRNA was detected in day 15 fetal thymocyte RNA using the ribonuclease

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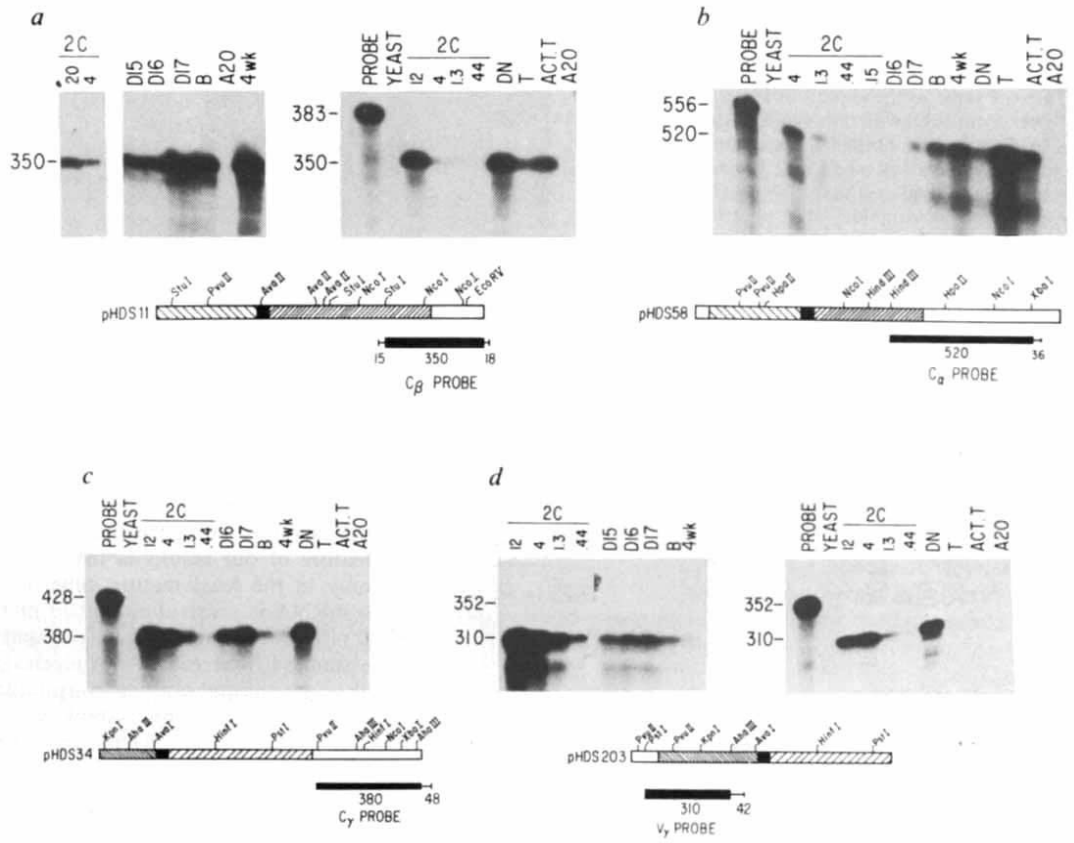

Fig. 2 Analysis of β (*a*), α (*b*), C_γ (*c*) and V_γ (*d*) mRNA levels in BALB/c AnN thymocyte and T-cell RNAs, using the ribonuclease protection assay[40]. 4 μg of total cellular RNA, or the indicated amounts of a standard preparation of total cellular RNA from the 2C cell line, were analysed. Samples containing <4 μg of 2C RNA were supplemented with yeast RNA. D15, D16 and D17 refer to RNAs prepared from thymocyte suspensions of fetuses killed 15, 16 or 17 days after detection of the vaginal plug (day 0). B, Newborn thymocytes (day 20 after detection of the vaginal plug); 4 wk, thymoctes from 4-week old mice; T, RNA from nylon-wool column-purified lymph-node T cells from 8-week-old mice, and ACT.T, RNA from the same cells activated for 42 h with Con A in the presence of equal numbers of irradiated splenic accessory cells; DN, RNA from 'double-negative' thymocytes purified from thymocyte suspensions from 4-week-old mice by two rounds of lysis with anti-L3T4 (GK1.5; ref. 41) and anti-Lyt-2 (AD4(15); ref. 42) monoclonal antibodies plus complement, and Ficoll–Isopaque purification of viable cells. Such cells are estimated to >90% pure double-negative thymocytes by cell sorter analysis (not shown). A20, RNA from the A20 B-lymphoma cell line. A20 RNA and yeast soluble RNA (4 μg) served as negative controls.

Methods. The probes were derived by subcloning in reverse orientation the indicated restriction fragment from pHDS 11 (β; ref. 15), pHDS 58 (α; ref. 17), pHDS 203 (V_γ; ref. 15) and pHDS 34 (C_γ) downstream from the SP6 promoter in the SP6 plasmids[40]. (pHDS 34 is a cDNA clone containing the 3′ portion of the γ gene; ref. 25.) ^{32}P-labelled single-stranded (SS) RNA probes complementary to T-cell receptor mRNAs were transcribed *in vitro* from the linearized SP6 plasmids, using the SP6 polymerase, in the presence of 10 μM ^{32}P-UTP (600 Ci mmol^{-1}) and excess cold ATP, GTP and CTP[40]. The lengths of the probes derived from the β, α and γ cDNAs are indicated in the diagrams (blacked in), as well as the length of the SP6 plasmid sequences also included in the transcripts (flanking lines). The ^{32}P-labelled probes were gel-purified and hybridized for 12 h at 40 °C with total cellular RNA, isolated by the guanidinium isothiocyanate/CsCl technique[43] from different cell types. The hybridization mixture was then treated with ribonuclease at 22 °C to digest single-stranded RNA. The ribonuclease was destroyed by incubation with proteinase K followed by phenol–chloroform extraction, and the RNA was ethanol-precipitated. The samples were electrophoresed on denaturing 7M urea/6% polyacrylamide gels, and the gels were subjected to autoradiography. Undigested probe was run on each gel; digestion of the SP6 plasmid-derived sequences from the probe in the experimental samples confirms the specificity of the assay.

protection assay, at ~10–15% the level found in clone 2C RNA (Fig. 2*d*). As the level of 1.5-kb C_γ mRNA in fetal thymocyte RNA is ~50% the level in 2C cells (see above), fetal thymocytes apparently contain 1.5-kb transcripts with C_γ sequences that do not hybridize with our V_γ probe. These transcripts may represent so-called 'sterile transcripts' of the γ gene, although sterile transcripts are usually shorter than their complete counterparts[24,32,33]. Alternatively, high-level transcription of C_γ rearranged to a distinct V_γ gene segment which does not hybridize with our V_γ probe would also account for the observed discrepancy.

V_γ mRNA declines to very low levels in adult thymocytes. Northern blot analysis confirmed that V_γ mRNA levels are low early in development compared with 2C and fall further after day 17 of fetal development (Fig. 3*d*). However, the level of V_γ mRNA in the adult double-negative thymocytes is much higher (approximately sevenfold) than that in fetal thymocytes (Fig. 2*d*). As is the case for C_γ-containing mRNAs, the level of V_γ mRNA in mature resting or activated T cells is extremely low, requiring for detection a longer exposure of the gel shown in Fig. 2*d*.

As the γ locus appears to undergo similar gene rearrangements on most rearranged chromosomes[15,25], Southern blot analysis of DNA from a T-cell population allows one to make a positive assessment of the extent of gene rearrangement. Rearrangement of C_γ gene segments is already detectable in DNA from fetal thymocytes at day 15, as evidenced by the appearance of a faint band of high relative molecular mass, and the diminution in intensity of the 13.4-kb germline band (Fig. 4*d*). The frequency of rearranged alleles increases during development, but ~50% of the alleles are still unrearranged even in adult thymocytes[25] (Fig. 4*d*). Our data suggest that the level of V_γ mRNA per rearranged γ allele prior to day 17 of fetal development is roughly comparable to the level in the 2C CTL line.

Approximately 50% of the γ alleles have undergone rearrangement in double-negative adult thymocytes, paralleling the increase in V_γ mRNA in these cells compared with fetal thymocytes (which also express the double-negative phenotype). These results suggest that the double-negative subpopulation has undergone maturation between the fetal and young adult stages with respect to rearrangement and expression of the γ genes. In contrast, mature T cells have a large proportion (~70%) of rearranged γ alleles[21] (Fig. 4*d*), yet have very low levels of γ mRNA.

Figure 1*b* shows the levels of α, β and γ mRNAs in thymocytes as a function of fetal age; the values given are rough estimates of the levels of 1.7-, 1.3- and 1.5-kb α, β and γ mRNAs respectively, relative to RNA from the 2C cell line, determined

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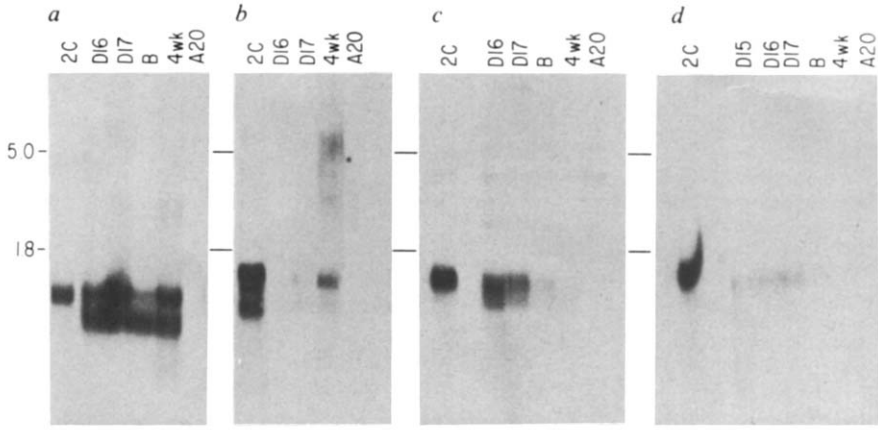

Fig. 3 Northern blot analysis of RNA samples from developing thymocytes. The probes are: *a*, C_β; *b*, C_α; *c*, C_γ; *d*, V_γ. The RNA samples are the same as those used in Fig. 2. The blots were hybridized with the same SP6-derived ^{32}P-labelled single-stranded probes as used in the ribonuclease assay (see Fig. 2 legend).
Methods. Total cellular RNA (5 μg) was denatured in formaldehyde, subjected to electrophoresis in 1.1% agarose/formaldehyde gels[43], then transferred to nitrocellulose filters, and hybridized with 3×10^7 d.p.m. of ^{32}P-labelled ssRNA probes(see Fig. 2 legend and probe diagrams) essentially as described by Melton *et al.*[40], except that the hybridized filters were washed at 70 °C rather than 65 °C in 0.1 × SSC, 0.1% SDS before autoradiography.

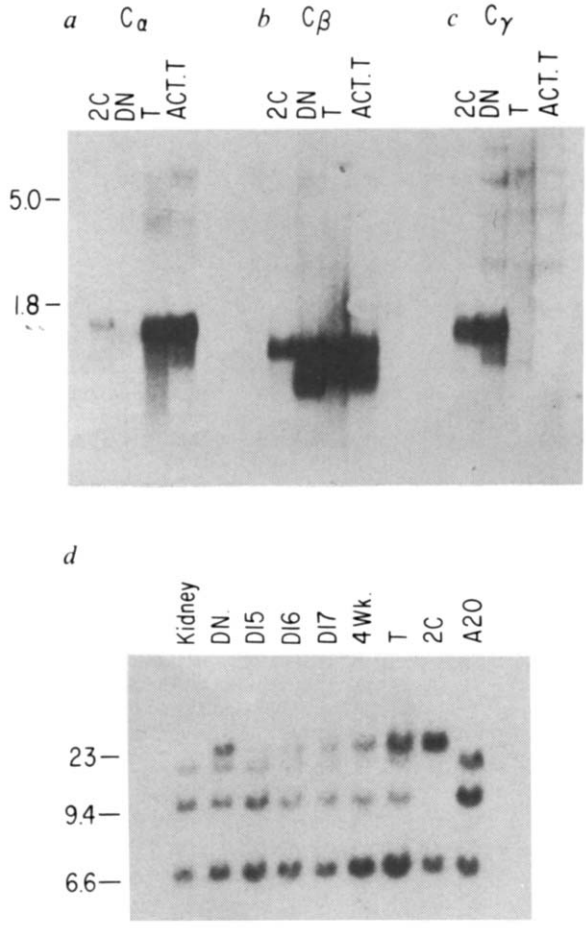

Fig. 4 *a–c*, Northern blot analysis of 5 μg of total cellular RNA from 'double-negative' adult thymocytes (DN), lymph-node T cells (T), activated T cells (ACT.T), and 2C CTLs (2C). The probes are *a*, C_α; *b*, C_β; *c*, C_γ. *d*, Rearrangements of the γ gene in thymocytes and T cells. Southern blot analysis[44] of *Eco*RI-digested genomic DNA from BALB/c kidneys, fetal thymocytes (D15, D16 and D17), 4-week-old thymocytes (4 wk) and purified lymph-node T cells from 8-week-old mice (T). DNAs from the A20 B-cell lymphoma (BALB/c-derived) and the 2C line (BALB.B-derived) were also included in the analysis. BALB/c and BALB.B embryo patterns are indistinguishable[21].
Methods. *a–c*, Northern blot analysis was performed as described in Fig. 3 legend. The preparation of the cells and RNAs were as described in Fig. 2 legend. *d*, Samples (5 μg) of each DNA preparation were digested with *Eco*RI, electrophoresed through 0.8% agarose gels, and transferred to nitrocellulose filters. The filters were hybridized with a ^{32}P-labelled, nick-translated probe corresponding to the constant region of the pHDS 34 γ cDNA clone. The probe extended from the *Ava*I site near the *J* region in pHDS 34 to the 3′ end (see Fig. 2*c*).

primarily from the Northern data (Figs 3, 4) with reference to the ribonuclease protection data (Fig. 2). The most striking feature of our results is that γ mRNA is relatively abundant only in the least mature cells of the T-cell lineage, whereas α mRNA is more abundant in mature than immature T cells. β mRNA is roughly equally abundant in each cell population examined. Whereas mRNA levels do not necessarily predict the absolute amount of the corresponding protein in a cell, the data presented here, which show shifts in the relative levels of mRNAs, probably reflect developmental regulation of the corresponding proteins.

What role might the γ gene play in T-cell recognition? Both class II-restricted helper T cells and class I-restricted CTL use a heterodimer of the α and β chains as at least part of their antigen/MHC receptor[6,7]. Although T-cell recognition is apparently not mediated by the independent recognition of MHC and antigen by independent receptors[35–37], other variations of the two-receptor model are still viable, particularly those which emphasize the constraints on physical interactions between H-2 molecules and antigens. Thus, the γ gene may encode part of a second T-cell receptor. However, based on recent evidence[38], it is unlikely that the specificity of either of the two putative receptors can be neatly assigned to either antigen or MHC determinants.

An alternative hypothesis is suggested by our data: perhaps the γ gene functions primarily in early T-cell differentiation. γ mRNA levels appear to be inversely related to α mRNA levels in thymocyte populations. Perhaps early in development a heterodimer of the γ and β chains is present on the surface of thymocytes prior to expression of the α gene. The putative γ/β heterodimer may serve as a receptor on immature thymocytes, allowing selection or suppression of clones expressing particular β chains. Later in thymocyte development, accompanying high-level expression of the α gene and a decrease in γ mRNA, the α-subunit may replace the γ subunit to form the receptor on mature T cells. It should be emphasized that the role of a γ-subunit in such a process need not be passive: for example, the γ chain may focus the γ/β-receptor complex onto MHC glycoproteins.

The finding that 2C and other CTL clones express relatively abundant levels of γ mRNA compared with normal T cells may result from the non-physiological growth conditions of the cells or, alternatively, induction of the γ gene in highly activated CTLs for purposes related to CTL function. However, the levels of γ mRNA are very low in T cells activated for 2 days with Con A (Figs 2, 4), which demonstrate significant CTL activity[39] (data not shown).

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Identification of antigen receptor-associated structures on murine T cells

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The specific antigen receptor on human and murine T lymphocytes is a heterodimer of relative molecular mass (M_r) 80,000–90,000 (80–90K) composed of two 40–50K disulphide-linked glycoprotein subunits[1–8]. Peptide map analysis of the α- and β-chains of receptor isolated from distinct tumour cell lines suggests the presence of both constant and variable regions[2,9–12]. Unlike the antigen receptor on B lymphocytes (that is, surface immunoglobulin), the human T-cell antigen receptor seems to be non-covalently associated with another invariant structure recognized by monoclonal antibodies to the cell-surface antigens T3 and Leu 4 (refs 4, 5, 9, 12). Meuer *et al.*[5] have demonstrated co-modulation of the T3 structure and T-cell antigen receptor using anti-clonotypic and anti-T3 monoclonal antibodies. Furthermore, immunoprecipitation with anti-T3 weakly co-precipitates a small amount of the 80–90K heterodimer in certain conditions[4,9,12]. The murine homologue of the Leu 4/T3 structure has not been identified, although Gunter *et al.* have suggested that Thy-1 may be the counterpart of Leu 4/T3 (ref. 13). Here we describe a Leu 4/T3-like structure, distinct from Thy-1, associated with the T-cell receptor of a murine T-lymphoma cell line.

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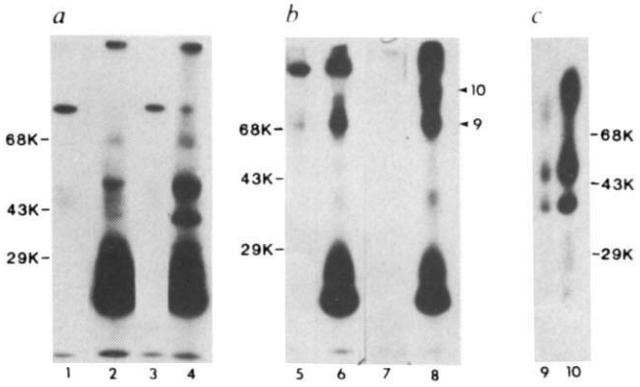

Fig. 1 SDS-PAGE analysis of human Leu 4 and associated structures. *a*, Reduced samples; *b*, non-reduced samples, run on 10% gels. Lanes 1, 2, 5, 6, sham-treated cells; lanes 3, 4, 7, 8, DTBP crosslinked cells; lanes 1, 3, 5, 7, P3 IgG1; lanes 2, 4, 6, 8, anti-Leu 4. *c*, Bands at arrows in *b* were cut out of dried gels, rehydrated in SDS sample buffer containing 50 mM dithiothreitol for 1 h, then loaded into sample wells and resolved on 12.5% gels.
Methods. Human HPB-ALL T-leukaemia cells (provided by Dr Jun Minowada) were labelled with ^{125}I (Amersham) using lactoperoxidase (Sigma)-catalysed radioiodination[14]. Half of the labelled cells were incubated for 30 min at room temperature (25 °C) in phosphate-buffered saline (PBS, 0.1 M phosphate, *p*H 7.5) containing 10 mM DTBP (Pierce Chemicals) as described by Wang and Richards[15], with minor modifications. Control cells were incubated in PBS without DTBP. After washing in 5 mM ammonium acetate/0.1 M PBS(*p*H 7.3) to inhibit the reaction, cells were placed in lysate buffer (0.05 M Tris-HCl, *p*H 8.0) containing 0.15 M NaCl, 1 mM EDTA (Sigma), 0.02% NaN, 0.5% Nonidet P-40 (Sigma), 20 KIU ml^{-1} aprotinin (Sigma) and 50 mM *N*-ethylmaleimide (Sigma) for 20 min at 4 °C. Lysates were centrifuged at 13,000*g* for 15 min, pre-cleared for 10 min with formalin-fixed *S. aureus* (Enzyme Center), then incubated at 4 °C with anti-Leu 4 antibody (Becton Dickinson Monoclonal Center) or P3X IgG1 myeloma protein. Immune complexes were isolated from lysates by incubation with rabbit anti-mouse IgG-coated formalin-fixed *S. aureus*. After washing, bound material was eluted by boiling in sample buffer containing 2.3% SDS (Bio-Rad) with or without 5% 2-mercaptoethanol (Sigma). Samples were analysed by SDS-PAGE as described by Laemmli[24]. Protein standards (Sigma) were included for M_r determination. Gels were dried and autoradiographed at −70 °C on Kodak XR film using intensifying screens (Cronex Lightning Plus).

The human T-cell antigen-specific receptor co-modulates on the cell surface with a 20–25K glycoprotein structure identified by the anti-T3 monoclonal antibody[5]. Immunoprecipitation of the T3 antigen from ^{125}I-labelled T-cell lines also weakly co-precipitates a small amount of the disulphide-linked 80–90K heterodimer under certain non-stringent conditions[4,9,12]. Here, we use chemical crosslinkers to perform a nearest-neighbour analysis of structures associated with human and murine T-cell receptor. We labelled the cell surface proteins of murine or human T cells with ^{125}I using lactoperoxidase-catalysed radiodination[14], then crosslinked them using the cleavable reagent dimethyl dithiobispropionimidate (DTBP)[15]. The cells were solubilized with detergent and immunoprecipitates obtained with monoclonal antibodies and analysed by SDS-PAGE. Figure 1 shows such an analysis of human T-leukaemia HPB-ALL cells immunoprecipitated with an anti-Leu 4 antibody. Under reducing conditions (*a*), immunoprecipitates obtained from crosslinked cells contained significant amounts of two additional proteins of 37 and 45K in addition to the major Leu 4 bands at 22 and 27K. Lesser amounts of the 37 and 45K components were observed in the sham-treated controls. The properties of these proteins are consistent with the clonotypic heterodimer subunits described by Meuer *et al.*[4,5]. Immunoprecipitations of crosslinked and sham-treated samples with monoclonal antibodies to Leu 1 (T1), Leu 2 (TB), Leu 3 (T4) and Leu 5 (T11) failed to co-precipitate the 37 and 45K components, indicating

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