of the total phenotypic variance). These loci and their effects should be investigated in commercial pig populations as they may have a potential use in marker assisted selection (*16*).

Populations of domesticated animals and their wild ancestors provide a valuable source of genetic diversity that may be exploited to develop animal models for quantitative traits of biological and medical interest. The large effect of the chromosome 4 QTL on fat deposition makes it a potential model for human obesity, an important polygenic syndrome with a strong environmental influence (*17*). Polygenic disease traits are difficult to dissect genetically in studies of humans and a random genome approach, as used in this study, would be arduous. A more rewarding approach may be to study genetic variation at candidate genes identified in an animal model. Comparative gene mapping of porcine chromosome 4 is therefore of interest. Only three type I anchor loci (*18*), suitable for comparative mapping, have yet been mapped to this chromosome: ATP1A1, ATP1B1, and GBA (Fig. 1). The corresponding loci in humans are all found on chromosome 1, which thus may contain one or more of the QTLs detected in this study. In the mouse, several single gene mutations giving an obese phenotype have been described. Two of these, *db* (diabetes) on chromosome 4 and *fat* (fat) on chromosome 8, are closely linked to genes whose homologs are on human chromosome 1 (*19*). The mouse mutants have not yet been cloned, but flanking coding sequences may be mapped in the pig genome to test the possibility of homology to the major locus for fatness described here.

This experiment and the statistical method employed were designed to detect QTLs that were fixed for alternative alleles in the two founder populations. The statistical power is reduced for detecting QTLs where a significant proportion of the total genetic variance is due to segregation within the founder populations. The rather limited size of the F_2 generation implies that only QTLs with fairly large effects were expected to reach statistical significance (*20*). Thus, QTLs with more moderate effects considered nonsignificant in this study may be corroborated by an increase in the family size. The results of the present study provide a strong impetus for more extensive experiments with the aim to further advance our understanding of quantitative trait loci.

REFERENCES AND NOTES

1. A. H. Paterson *et al.*, *Nature* **335**, 721 (1988).
2. H. J. Jacob *et al.*, *Cell* **67**, 213 (1991).
3. C. W. Stuber *et al.*, *Genetics* **132**, 823 (1992).
4. E. S. Lander and D. Botstein, *ibid.* **121**, 185 (1989).
5. C. S. Haley, S. A. Knott, J. M. Elsen, *ibid.*, in press.
6. J. Wiseman, *A History of the British Pig* (Duckworth, London, 1986).
7. M. Johansson *et al.*, *Genomics* **14**, 965 (1992).
8. C. Darwin, *The Variation of Animals and Plants Under Domestication* (John Murray, London, ed. 2, 1882), vol. I, p. 77.
9. H. Petersson, J. Håkansson, S. Eriksson, *Swed. J. Agric. Res.* **9**, 75 (1979).
10. Several of the traits were significantly correlated as estimated on phenotypic data from the F_2 generation. Birth weight showed weak positive correlation to the three growth traits (r = 0.13–0.21; P = 0.07–0.003) and to the length of the small intestine (r = 0.18; P = 0.01). Growth from birth to 70 kg was strongly correlated with growth to 30 kg (r = 0.84; $P < 0.001$) and with growth from 30 to 70 kg (r = 0.62; $P < 0.001$), as expected. The correlation between the two latter growth traits was rather weak (r = 0.19; P = 0.008). The three growth traits showed significant positive correlation with the length of the small intestine (r = 0.20–0.24; P = 0.003–0.0009) and both fat traits (r = 0.28–0.49; $P < 0.001$), except for growth rate from 30 to 70 kg versus abdominal fat (r = 0.14; P = 0.06). The length of the small intestine was negatively correlated with the two fat traits (r = −0.23–0.24; P = 0.001). The two fat traits showed a fairly strong positive correlation (r = 0.58; $P < 0.001$). Pearson correlation coefficients and their significances were estimated with the CORR procedure of SAS [SAS Procedures Guide, release 6.03 edition, SAS Institute Incorporated, Cary, NC (1988)].
11. H. Ellegren *et al.*, *Genetics*, in press.
12. C. S. Haley and S. A. Knott, *Heredity* **69**, 315 (1992).
13. S. S. Shapiro and M. B. Wilk, *Biometrika* **52**, 591 (1965). The test was carried out with the UNIVARIATE procedure of SAS [SAS Procedures Guide, Release 6.03 Edition (1988)].
14. L. Andersson, A. L. Archibald, J. Gellin, L. B. Schook, *Anim. Genet.* **24**, 205 (1993).
15. L. Andersson *et al.*, unpublished results.
16. M. Soller and J. S. Beckmann, *Theor. Appl. Genet.* **67**, 25 (1983).
17. A. J. Stunkard *et al.*, *N. Engl. J. Med.* **322**, 1483 (1990); C. Bouchard and L. Pérusse, *Annu. Rev. Nutr.* **13**, 337 (1993).
18. S. J. O'Brien *et al.*, *Nat. Genet.* **3**, 103 (1993).
19. C. M. Abbott *et al.*, *Mamm. Genome* **4**, S58 (1993); J. D. Ceci, *ibid.*, p. S121.
20. A QTL would have to explain at least 5% of the variance to have a reasonable chance of detection in 200 F_2 individuals from an inbred line cross [van Ooijen, *Theor. Appl. Genet.* **84**, 803 (1992)]. The QTL effect would have to be slightly larger for the same probability of detection in our case, as the markers are not completely informative.
21. Means and standard deviations were estimated without fitting covariates. The absolute sizes of the standard deviations in the Large White population and in the F_2 intercross are difficult to compare because of the different population structure in the two samples. The F_2 intercross comprised only 10 grandparents and large half- and full-sib families, whereas the data on the Large White population included altogether 16 males and 96 females.
22. H. Petersson, thesis, chap. IV, p. 57, Swedish University of Agricultural Sciences, Uppsala, Sweden (1990).

23. We thank G. Bulfield and W. G. Hill for comments on an earlier version of this manuscript and T. Basu, U. Gustafsson, and L. Marklund for technical assistance. Supported by the Swedish Research Council for Forestry and Agriculture, the Nordic Joint Committee for Agricultural Research, the British Agricultural and Food Research Council, and the PiGMaP project of the EC BRIDGE program.

19 October 1993; accepted 15 February 1994

A Subset of CD4$^+$ Thymocytes Selected by MHC Class I Molecules

Albert Bendelac,* Nigel Killeen, Dan R. Littman, Ronald H. Schwartz

To complete their maturation, most immature thymocytes depend on the simultaneous engagement of their antigen receptor [αβ T cell receptor (TCR)] and their CD4 or CD8 coreceptors with major histocompatibility complex class II or I ligands, respectively. However, a normal subset of mature αβ TCR$^+$ thymocytes did not follow these rules. These thymocytes expressed NK1.1 and a restricted set of αβ TCRs that are intrinsically class I–reactive because their positive selection was class I–dependent but CD8-independent. These cells were CD4$^+$ and CD4$^-$8$^-$ but never CD8$^+$, because the presence of CD8 caused negative selection. Thus, neither CD4 nor CD8 contributes signals that direct their maturation into the CD4$^+$ and CD4$^-$8$^-$ lineages.

During the positive selection of CD4$^+$8$^+$ double-positive precursor thymocytes, cells bearing major histocompatibility complex (MHC) class I–specific αβ TCRs are recruited into the CD8 lineage, whereas cells bearing class II–specific TCRs are recruited into the CD4 lineage. The CD4 and CD8 molecules can bind to nonpolymorphic regions of the class II or I molecules (*1*), respectively, and may therefore increase the avidity of TCR-MHC interactions involved in thymic selection. They can also signal through molecules such as lck, a tyrosine kinase associated with both coreceptors (*2*). Although class I and CD8 molecules or class II and CD4 molecules are required for the generation of most CD8$^+$ or CD4$^+$ lymphocytes, respectively (*3–7*), the precise contribution of CD4 and CD8 during

A. Bendelac and R. H. Schwartz, Laboratory of Cellular and Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.
N. Killeen and D. R. Littman, Departments of Microbiology and Immunology and of Biochemistry and Biophysics, Howard Hughes Medical Institute, University of California at San Francisco, San Francisco, CA 94143–0414, USA.

*To whom correspondence should be addressed.

positive selection remains a matter of investigation (8 and 9).

A minor subset of mature CD4$^+$ cells in class II–deficient (class II$^-$) mice provides an apparent exception to the coreceptor-MHC match imposed by positive selection (6). An understanding of the thymic selection requirements of these cells may lead to general insight into the nature of lineage commitment for developing thymocytes. Although such cells might be selected on some cryptic or unusual class II molecule, such as H-2O, they might also be selected on class I molecules or belong to a separate pathway that does not need TCR-MHC interaction for selection. In addition, CD4$^-$8$^-$ cells with helper activity were identified in CD4-deficient (CD4$^-$) mice (*10*), suggesting that CD4 engagement is not required for the positive selection of every helper cell.

We identified in normal mice a subset of thymocytes that expresses CD4 but uses class I for positive selection. Because of its distinctive phenotype, it was possible to investigate its thymic selection requirements and determine the nature of its lineage committment. In adult mice, 10 to 25% of mature [heat-stable antigen (HSA^{low})] thymocytes express features of activation that are more prominent and stable than most thymocytes, including the functional potential to secrete lymphokines such as interleukins-4, -5, and -10 and γ-interferon, as well as several activation-memory surface markers (*11–15*). Among adult C57BL/6 HSA^{low} thymocytes, a homogeneous CD44^{high}NK1.1$^+$ subset can be found that includes mainly CD4$^+$ and CD4$^-$8$^-$ and virtually no CD8$^+$ cells (Fig. 1, A to C, and Table 1). Other activation markers coexpressed on this subset include a Ly6C$^+$3G11^{low}LECAM-1^{low} membrane phenotype, a larger size, and a lower level of surface TCR (*16*). The TCR repertoire was similar for both CD4$^+$ and CD4$^-$8$^-$ cells but different from the mainstream thymocytes. The CD44^{high}NK1.1$^+$ thymocytes used mainly TCR V$_\beta$8 (*14, 15, 17*), as well as V$_\beta$7 and V$_\beta$2, chains, whereas other TCR V$_\beta$ chains were underrepresented (Fig. 1D and Table 1). The V$_\alpha$ chain usage was also restricted and was similar for CD4$^+$ and CD4$^-$8$^-$ cells (*18*), suggesting that their αβ TCR repertoire is largely identical. We refer to this population as mature NK1.1$^+$TCRαβ$^+$ thymocytes, because NK1.1 is the only marker that allows the identification of a set of CD4$^+$ and CD4$^-$8$^-$ T cells in the periphery with a similar TCR repertoire, henceforth called the canonical repertoire (*15, 19–21*).

In β$_2$ microglobulin–deficient mice (β$_2$M$^-$), which do not express surface class I molecules and do not positively select mature CD8$^+$ thymocytes (*7*), the mature NK1.1$^+$TCRαβ$^+$ cells that predominantly express V$_\beta$8, V$_\beta$7, and V$_\beta$2 were absent from the CD4$^-$8$^-$ and CD4$^+$ subsets (Fig. 1E and Table 2). Because it was possible that the CD4$^+$ cells were "normally" selected on some class I– or β$_2$M-derived peptides presented by class II molecules or were first selected on class II molecules and secondarily cross-reacted on class I ligands, we examined class II–deficient mice for these cells, which were found in normal amounts (Fig. 1F and Table 2). It is unlikely, there-

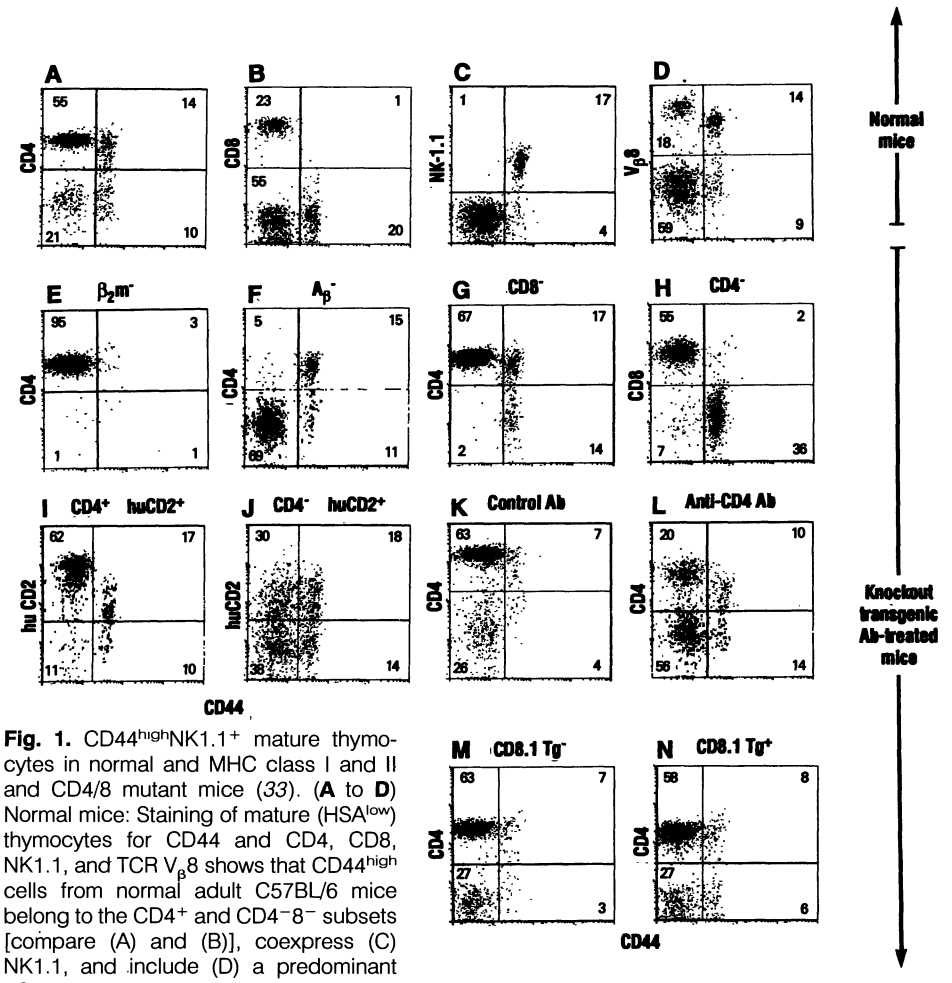

Fig. 1. CD44^{high}NK1.1$^+$ mature thymocytes in normal and MHC class I and II and CD4/8 mutant mice (*33*). (**A** to **D**) Normal mice: Staining of mature (HSA^{low}) thymocytes for CD44 and CD4, CD8, NK1.1, and TCR V$_\beta$8 shows that CD44^{high} cells from normal adult C57BL/6 mice belong to the CD4$^+$ and CD4$^-$8$^-$ subsets [compare (A) and (B)], coexpress (C) NK1.1, and include (D) a predominant TCR V$_\beta$8$^+$ population. (**E** to **N**) Transgenic mice: Staining of mature (HSA^{low}) thymocytes from (E) β$_2$M$^-$, MHC class I$^-$, (F) A$_\beta^-$, MHC class II$^-$, (G) CD8$^-$, (H) CD4$^-$, (I) CD4$^+$huCD2$^+$, expressing the human CD2 gene under the control of the mouse CD4 promoter; (J) CD4$^-$huCD2$^+$, CD4$^-$ expressing the human CD2 gene under the control of the mouse CD4 promoter, (K) control antibody-treated, (L) anti-CD4–treated, (M) CD8.1 Tg$^-$, transgene-negative littermate control, and (N) CD8.1 Tg$^+$. Dot plot displays are pre-gated on αβ TCR$^+$ cells (>95% of the HSA^{low} thymocytes). The lower proportion of CD44^{high} cells in mice from the anti-CD4 (K and L) and the CD8.1 transgenic (M and N) experiments is due to their younger age (5 weeks). Anti-human CD2-PE staining (Amac) correlates with CD4 staining in CD4$^+$huCD2$^+$ mice (*24*). Thymocytes from anti-CD4–treated mice, stained with biotinylated YTA3.1.2 anti-CD4, whose binding is unaffected by the antibody (H1.29) used in vivo, displayed a down-modulated expression of CD4 (three- to fourfold) as a consequence of in vivo antibody binding, which is taken into account in the gate used to score CD4$^+$ cells in (L).

Table 1. Distribution and TCR-V$_\beta$ repertoire analysis of subsets of mature thymocytes in adult C57BL/6 mice (*33*). TCR V$_\beta$ percentages are normalized to the percentage of αβ TCR$^+$ cells in each subset (85% for the CD44^{high}-CD4$^-$8$^-$ cells, >98% for the other subsets). The results are averages of five experiments in which individual thymuses or pools from 4 to 15 mice were analyzed. Standard errors were less than 20% of the mean value for TCR V$_\beta$ determinations.

Thymic subset	HSA^{low} thymocytes (%)	V$_\beta$ (%)			
		V$_\beta$8	V$_\beta$7	V$_\beta$2	V$_\beta$6
	CD44^{low} thymocytes				
CD4$^+$	58	21	2	7	7
CD8$^+$	24	25	6	6	11
	CD44^{high} thymocytes				
CD4$^+$	10	52	14	6	2
CD4$^-$8$^-$	7	69	14	8	1
CD8$^+$	0.7	39	9	8	8

fore, that the activated status of the NK1.1$^+$CD4$^+$ cells is related to some reactivity toward self class II ligands in vivo, as was recently suggested (*22*). The few mature CD4$^+$ cells previously identified in the periphery of class II$^-$ mice are in an activated state (*6*), and their TCR repertoire was also skewed toward the canonical NK1$^+$TCR$\alpha\beta^+$ repertoire, suggesting that they include this population (*16*). Invariant chain-deficient mice (*23*), which represent another model of class II expression deficiency, showed similar results (*16*).

Mice genetically deficient in CD4 (*4*) also generated normal amounts of the NK1.1$^+$TCR$\alpha\beta^+$ cells, which were completely included within the CD4$^-$8$^-$ subset (Fig. 1H) and expressed the canonical TCR repertoire (Table 2). To rule out any involvement of CD4 in the selection of the CD4$^+$ subset, we examined CD4-deficient mice in which a human CD2 transgene (*huCD2*) driven by the CD4 promoter was introduced. Such mice express the human CD2 surface protein in lieu of mouse CD4 (*24*). These mice generated the NK1.1$^+$-TCR$\alpha\beta^+$ population in the huCD2$^+$ (indicating transcriptional activity of the CD4 promoter) and huCD2$^-$ subsets (Fig. 1, I and J). Similarly, mice treated from birth with nondepleting antibodies to CD4 had few mainstream CD4$^+$ cells but had normal NK1.1$^+$TCR$\alpha\beta^+$ cells in both the CD4$^+$ and CD4$^-$ compartments (Fig. 1, K and L). Both huCD2$^+$CD4$^-$ and anti-CD4–treated mice displayed the canonical TCR repertoire in their NK1.1$^+$TCR$\alpha\beta^+$ subset (*16*). These results demonstrate that NK1.1$^+$-TCR$\alpha\beta^+$ CD4$^+$ cells need not use CD4 to be positively selected.

To address the paradox that cells dependent on class I expression are selectively absent from the CD8 lineage, we tested whether CD8 expression results in deletion: Cells that do not appear in a particular lineage may be missing because of negative selection rather than from the absence of positive selection (*25*). Mice expressing CD8.1-α and -β transgenes driven by the CD2 promoter express CD8.1 from the TCR$\alpha\beta^{-/low}$CD8$^+$ immature stage until the mature CD4$^+$ and CD8$^+$ stages (*8*), and the size of their thymus was decreased by 66% on average (Table 2). These mice generated both of the mainstream CD4$^+$ and CD8$^+$ populations as well as the NK1.1$^+$TCR$\alpha\beta^+$ thymocytes in normal relative proportions (Fig. 1, M and N). The NK1.1$^+$TCR$\alpha\beta^+$ population, however, had lost its characteristically skewed TCR repertoire (Table 2), resembling more the rare set of NK1.1$^+$TCR$\alpha\beta^+$ CD8$^+$ thymocytes observed in normal mice (Table 1), whereas the mainstream CD4$^+$ and CD8$^+$ subsets did not change their TCR V_β repertoire (*16*). This result was found for both the CD4$^+$8.2$^-$ and CD4$^-$8.2$^-$ NK1.1$^+$ subsets and was not due to the particular alleles of the CD8-α and -β transgenes because C57BL/6 mice congenic for the alleles of CD8 did not differ (*16*). The net effect of CD8 expression, therefore, was a major and selective depletion of the canonical population of NK1.1$^+$V$_\beta$8$^+$, V$_\beta$7$^+$, and V$_\beta$2$^+$ cells.

We next determined whether CD8 contributed to the positive selection of these class I–reactive CD4$^+$ and CD4$^-$8$^-$ cells by analyzing CD8 deficient mice (*5*) and mice treated from birth with antibodies to CD8. We found that the absolute number of NK1.1$^+$TCR$\alpha\beta^+$ cells and the general skewing of their TCR repertoire were conserved (Fig. 1G and Table 2). However, some subtle changes were observed, such as an increase of V$_\beta$7$^+$ cells from 14% to 19 to 22%. This relative increase, which was found both in CD8-deficient mice and in normal mice treated with anti-CD8 from birth, ranged from 34 to 57% above the cell populations of littermate controls in four separate experiments, including a total of 17 experimental versus 16 control mice, and was observed in both CD4$^+$- and CD4$^-$8$^-$NK1.1$^+$TCR$\alpha\beta^+$ cells. This result shows that, although CD8 is not necessary for the selection of most of the CD4$^+$- and CD4$^-$8$^-$NK1.1$^+$TCR$\alpha\beta^+$ cells, it does influence (by modifying their positive or negative selection) their TCR repertoire. Therefore, it must be expressed in some amount during their thymic selection.

To determine the type of MHC-presenting cells involved in the selection of the NK1.1$^+$TCR$\alpha\beta^+$ thymocytes, we created fetal liver radiation chimeras, in which either the hematopoietic donor cells or the irradiated recipients (and thus the thymic epithelium) were class I–deficient (Fig. 2). Although the development of the mainstream CD8$^+$ thymocytes required class I expression by the recipient (*26*), the NK1.1$^+$TCR$\alpha\beta^+$ thymocytes of either CD4$^+$ and CD4$^-$8$^-$ lineages were selected exclusively when class I molecules were present on donor hematopoietic cells, as was reported for the CD4$^-$8$^-$ subset (*27*). Analysis of these chimeras at different time points after reconstitution confirmed that class I$^+$ into class I$^-$ fetal liver chimeras did not even show a delay in the generation of their NK1.1$^+$TCR$\alpha\beta^+$ population by comparison to the class I$^+$ into class I$^+$ pseudochimeras (*16*). The failure to select NK1.1$^+$TCR$\alpha\beta^+$ cells in the class I$^-$ into class I$^+$ fetal liver radiation chimeras was not due to some intrinsic defect of the class I–deficient thymocytes themselves, because in mixed fetal liver radiation chimeras (class I$^+$ irradiated recipients reconstituted with a mixture of class I$^+$ and class I$^-$ fetal liver cells) class I$^-$ thymocytes did generate the canonical NK1.1$^+$TCR$\alpha\beta^+$ thymocyte subset (*16*). Thus, in contrast with the mainstream thymic population, NK1.1$^+$-

Table 2. TCR V_β repertoire of CD44^{high} thymocytes in mutant mice (*33*). The repertoire is shown for the whole CD44^{high} population (it does not significantly differ between CD4$^+$ and CD4$^-$8$^-$ cells). All thymocytes are CD44^{high}, except where indicated. At least four individual mice were analyzed in each group. Standard errors were less than 20% of the mean value for TCR V_β determinations presented in the table and less than 30% for thymus size. Values significantly different from the normal controls are underlined. ND, not determined.

Mouse	Thymus size (% of control)	V_β (%)			
		V_β8	V_β7	V_β2	V_β6
Normal*	100	22	3	7	8
Normal		59	14	7	2
Aβ$^-$	85	64	12	7	2
CD4$^-$	99	66	13	ND	ND
β_2M$^-$	88	20	2	4	6
CD8$^-$	69	60	19	ND	ND
Anti-CD8	97	58	22	7	3
CD8.1 Tg	34	25	6	4	20

*CD44^{low} thymocytes.

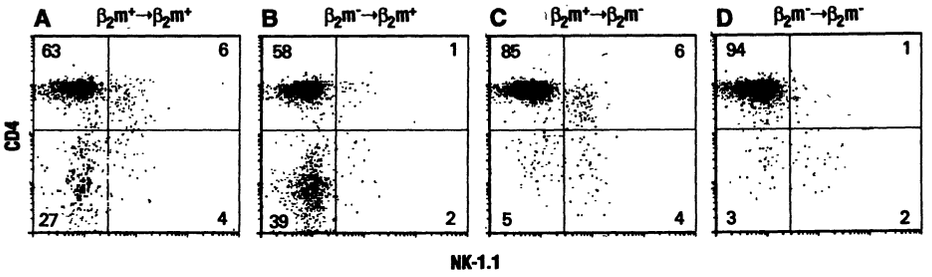

Fig. 2. Fetal liver radiation chimeras (*34*). CD4 and NK1.1 staining of mature (HSA^{low}) thymocyte preparations from the four chimeric combinations of β_2M$^-$ or β_2M$^+$ fetal liver cells and lethally irradiated recipients. Percentages of TCR V$_\beta$8$^+$ cells among TCR$\alpha\beta^+$NK1.1$^-$ and TCR$\alpha\beta^+$NK1.1$^+$ subsets were 23 and 60%, respectively, in (**A**), 23 and 31% in (**B**), 20 and 60% in (**C**), and 26 and 29% in (**D**). More than 95% of mature thymocytes in the chimeras were of donor origin stained by anti–MHC I. Dot plots are from thymocytes analyzed at day 49 after reconstitution, an early time point, which explains the lower proportion of NK1.1$^+$ cells seen here compared to that observed in older mice shown in Fig. 1.

$TCR\alpha\beta^+$ cells are exclusively selected by interaction with MHC on hematopoietic elements.

Finally, we investigated whether the $NK1.1^+TCR\alpha\beta^+$ cells were primarily derived from a bona fide thymic selection process. It could be argued that, because they differ from the mainstream population in that they require the expression of MHC ligands on cells derived from bone marrow and not on thymic epithelial cells for their generation and because they are undetectable in the neonatal thymus and tend to accumulate in the adult thymus (Fig. 3A), they might be generated extrathymically and recirculate back to the thymus (*20*) or even represent thymocytes that do not undergo positive selection and get expanded in response to environmental antigens (*27*). Fetal thymic organ cultures were set up at day 13 or 14 of fetal life, and Fig. 3B shows one of three experiments in which we observed both $CD4^+$ and $CD4^-8^-$ cells bearing the NK1.1 marker as well as TCRs skewed toward $V_\beta7$ and $V_\beta8$ (see legend to Fig. 3). They were detected rather late (day 22 of culture), presumably because their rate of production is low. Thus, the relatively large proportion of these cells among mature thymocytes of adult mice (~10 to 25%) is due to an accumulation related to their slow turnover rate (*21*). In addition, cells recirculating from the periphery back to the thymus in congenically marked thymic chimeras did not contribute to this subset (*28*). Thus, the $NK1.1^+TCR\alpha\beta^+$ population can be generated by selection on purely endogenous self ligands inside the thymus.

We have thus identified a homogeneous set of mature, $\alpha\beta$ TCR^+ thymocytes that displays an unusual phenotype and a coreceptor expression pattern ($CD4^+$ or $CD4^-8^-$, but not $CD8^+$) apparently unmatched with the selecting class I molecule. This subset is the normal counterpart of a significant fraction of the $CD4^+$ cells observed in class II^- mice (*6*) and of some of the $CD4^-8^-$ cells (*10*) previously reported in $CD4^-$ mice. Its positive selection depended on class I presented by thymic cells of hematopoietic origin and was driven by self, not foreign, antigens. By the use of transgenic mice under- or overexpressing CD4-CD8 or MHC molecules, we found that the TCRs expressed by these thymocytes are apparently intrinsically class I–reactive in that they did not require CD8 for their positive selection and were deleted upon the constitutive expression of CD8. The $CD4^+$ cells were selected on class I molecules in the absence of CD4–class II interaction, showing that CD4 is not required to select all $CD4^+$ cells. In this subset, engagement of the class I–specific TCRs at the double-positive stage of thymocyte development might induce stochastic down-modulation of CD4 or CD8, with $CD4^+$ cells being subsequently rescued because of their intrinsic affinity for class I. However, $CD8^+$ cells are deleted because the persistence of CD8, after TCR levels are up-regulated, makes them pass the threshold affinity for negative selection. How then are mature cells with the unusual $CD4^-8^-$ phenotype selected? One model is that TCR engagement during positive selection of double-positive thymocytes may also induce simultaneous down-modulation of both CD4 and CD8, an event that may have been previously unrecognized because few $\alpha\beta$ TCRs have enough intrinsic affinity for MHC ligands, in the absence of CD4 or CD8, to allow the rescue of thymocytes in the double-negative pathway. Alternatively, the $CD4^-8^-$ subset may result from the early engagement of the class I–reactive TCRs at the immature $CD8^+$ (before the $CD4^+8^+$) stage, because this engagement in vitro induces down-modulation of the coreceptors (*29*). Such a rescue from negative selection by coreceptor modulation may also operate in TCR transgenic mice (*30*).

Whether the selection rules of the $NK1.1^+TCR\alpha\beta^+$ thymocytes reflect those of the mainstream $\alpha\beta$ TCR^+ pathway, applied to unusual conditions of class I ligand presentation, or whether they delineate a distinct lineage, which may depend on the expression of molecules of the NK lineage and their interaction with class I molecules (*31*), remains to be determined. The class I ligand recognized by the restricted set of $\alpha\beta$ TCRs that are used by the $NK1.1^+TCR\alpha\beta^+$ thymocytes is likely a nonpolymorphic class I molecule, because of the lack of influence of the MHC haplotype on this TCR repertoire (*18*). The identification of this ligand represents the next step in the dissection of this differentiation pathway, which might be endowed with a specialized function (*32*).

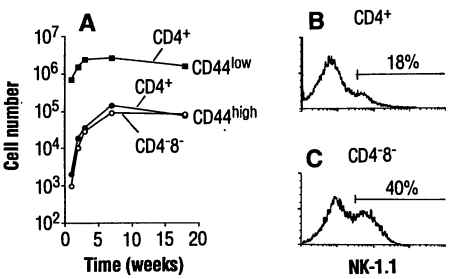

Fig. 3. Intrathymic generation and accumulation of the $NK1.1^+TCR\alpha\beta^+$ population. (**A**) Kinetics of accumulation of mature (HSA^{low}) $CD44^{high}$ and $CD44^{low}$, TCR $V_\beta8^+$ thymocytes in vivo. The number of mature $CD44^{high}$ thymocytes increases 100-fold between the first and the seventh weeks of life, while that of $CD44^{low}$ increases threefold (*35*). (**B** and **C**) Generation of the $NK1.1^+TCR\alpha\beta^+$ subset in fetal thymic organ culture. Mature (HSA^{low}) (B) $CD4^+$ and (C) $CD4^-8^-$ (8 and 5%, respectively, of the HSA^{low} cells at day 22, with the remaining cells being mainly $CD8^+\alpha\beta TCR^+$ and $\gamma\delta TCR^+$ cells) were stained for NK1.1. The percentages of $V_\beta8^+$ and $V_\beta7^+$ cells were 18 and 2%, respectively, in the $CD4^+NK1.1^-$ subset, 46 and 11% in the $CD4^+NK1.1^+$ subset, and 39 and 7% in the $CD4^-8^-$ $NK1.1^+$ subset (*36*).

REFERENCES AND NOTES

J. M. Connolly, T. H. Hansen, A. L. Ingold, T. A. Potter, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2137 (1990); R. D. Salter *et al.*, *Nature* **345**, 41 (1990); G. Cammarota *et al.*, *ibid.* **356**, 799 (1992); R. Konig, L.-Y. Huang, R. N. Germain, *ibid.*, p. 796.

2. A. S. Shaw *et al.*, *Cell* **59**, 627 (1989); R. Zamoyska *et al.*, *Nature* **342**, 278 (1989); J. M. Turner *et al.*, *Cell* **60**, 755 (1990); N. Glaichenhaus, N. Shastri, D. R. Littman, J. M. Turner, *ibid.* **64**, 511 (1991), M. C. Miceli, P. von Hoegen, J. R. Parnes, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2623 (1991).
3. A. Kruisbeek *et al.*, *J. Exp. Med.* **161**, 1029 (1985); J. C. Zuniga-Pflucker *et al.*, *ibid.* **169**, 2085 (1989); S. Marusic-Galesic, D. A. Stephany, D. L. Longo, A. Kruisbeek, *Nature* **333**, 180 (1988); F. Ramsdell and B. J. Fowlkes, *J. Immunol.* **143**, 1467 (1989); M. J. Grusby, R. S. Johnson, V. E. Papaioannou, L. H. Glimcher, *Science* **253**, 1417 (1991).
4. A. Rahemtulla *et al.*, *Nature* **353**, 180 (1991); N. Killeen, S. Sawada, D. R. Littman, *EMBO J.* **12**, 1547 (1993).
5. W. P. Fung-Leung *et al.*, *Cell* **65**, 443 (1991).
6. D. Cosgrove *et al.*, *ibid.* **66**, 1051 (1991).
7. M. Zijlstra *et al.*, *Nature* **344**, 742 (1990); B. H. Koller, P. Marrack, J. W. Kappler, O. Smithies, *Science* **248**, 1227 (1990).
8. E. A. Robey *et al.*, *Cell* **64**, 99 (1991).
9. P. Borgulya, H. Kishi, U. Muller, J. Kirberg, H. von Boehmer, *EMBO J.* **10**, 913 (1991); R. H. Seong, J. W. Chamberlain, J. R. Parnes, *Nature* **356**, 718 (1992); C. B. Davis, N. Killeen, M. E. C. Crooks, D. Raulet, D. R. Littman, *Cell* **73**, 237 (1993); S. H. Chan, D. Cosgrove, C. Waltzinger, C. Benoist, D. Mathis, *ibid.*, p. 225; J. P. M. van Meerwijk and R. N. Germain, *Science* **261**, 911 (1993); N. Killeen and D. R. Littman, *Nature* **364**, 729 (1993); I. T. Chan *et al.*, *Science* **261**, 1581 (1993).
10. R. M. Locksley, S. L. Reiner, F. Hatam, D. R. Littman, N. Killeen, *Science* **261**, 1448 (1993).
11. A. Bendelac and R. H. Schwartz, *Nature* **353**, 68 (1991); A. Bendelac, P. Matzinger, R. A. Seder, W. E. Paul, R. H. Schwartz, *J. Exp. Med.* **175**, 731 (1992).
12. K. Hayakawa, B. T. Lin, R. R. Hardy, *J. Exp. Med.* **176**, 269 (1992).
13. M. Fischer *et al.*, *J. Immunol.* **146**, 3452 (1991).
14. Y. Takahama, S. O. Sharrow, A. Singer, *ibid.* **147**, 2883 (1991).
15. H. I. Levitsky, P. T. Golumbek, D. M. Pardoll, *ibid.* **146**, 1113 (1991).
16. A. Bendelac, unpublished results.
17. B. J. Fowlkes *et al.*, *Nature* **329**, 251 (1987); R. C. Budd *et al.*, *J. Exp. Med.* **166**, 577 (1987).
18. O. Lantz and A. Bendelac, unpublished results.
19. M. Sykes, *J. Immunol.* **145**, 3209 (1990).
20. H. Arase, N. Arase, K. Ogasawara, R. A. Good, K. Onoe, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6506 (1992).
21. A. Bendelac *et al.*, in preparation.
22. I. Kativ, R. R. Hardy, K. Hayakawa, *J. Exp. Med.* **177**, 1429 (1993).
23. S. Viville *et al.*, *Cell* **72**, 635 (1993); E. K. Bikoff *et al.*, *J. Exp. Med.* **177**, 1699 (1993).
24. S. Sawada, N. Killeen, D. R. Littman, unpublished results.
25. P. Matzinger, *Immunol. Rev.* **135**, 81 (1993).
26. M. Bix and D. Raulet, *Nature* **359**, 330 (1992).

27. M. Bix, M. Coles, D. Raulet, *J. Exp. Med.* **178**, 901 (1993).
28. A. Bendelac and P. Matzinger, unpublished results.
29. Y. Takahama and A. Singer, *Science* **258**, 1456 (1992).
30. H. von Boehmer, J. Kirberg, B. Rocha, *J. Exp. Med.* **176**, 699 (1991); N. Auphan *et al.*, *Int. Immunol.* **4**, 1419 (1992).
31. F. M. Karlhofer, R. K. Ribaudo, W. M. Yokoyama, *Nature* **358**, 66 (1992).
32. A. Bendelac, *Semin. Immunol.* **4**, 187 (1992); K. Takeda and G. Dennert, *J. Exp. Med.* **177**, 155 (1993); M. J. Rapoport *et al.*, **178**, 87 (1993); E. Mozes, L. D. Kohn, F. Hakim, D. S. Singer, *Science* **261**, 91 (1993).
33. Mature thymocytes from 8- to 12-week-old normal C57BL/6 or mutant mice were enriched by J11d.2 (anti-HSA) and complement killing, to eliminate $CD4^+CD8^+$ and most immature $CD4^-CD8^-$ thymocytes, with centrifugation on a density gradient (lympholyte M). After Fc receptor blocking with 2.4G2 antibody, the thymocytes were stained with H1.29 anti–CD4-RED-613 or 53.6.7 anti–CD8-RED-613 (Gibco-BRL), IM78 anti–CD44-FITC, and biotinylated PK136 anti-NK1.1 or F23.1 anti-$V_\beta8$ RR4-7 anti-$V_\beta6$, B20.6 anti-$V_\beta2$, TR310 anti-$V_\beta7$ (Pharmingen, San Diego, CA), followed by Streptavidine-PE (Caltag) and analyzed with a FACSCAN flow cytometer. Homozygous A_β^{b-} (Genpharm International), β_2M^-, $CD8^-$, and $CD4^-$ null mutant $H\text{-}2^b$ mice were in their fourth to eighth backcross to C57BL/6. Antibody-treated mice were 5-week-old C57BL/6 mice treated from birth with H1.29 anti-CD4 or 53.6.7 anti-CD8, and CD8.1 transgenic mice were 5 weeks old. Controls included nontransgenic littermates or age- and sex-matched C57BL/6 (for transgenics beyond the sixth backcross to C57BL/6) or littermates injected from birth with phosphate-buffered saline or an isotype-matched control antibody (SFR3-DR5) at the same dose and frequency as those of the experimental littermates (0.5 mg the first week and then 1 mg every 2 days intraperitoneally for 3 weeks). CD8.1 transgenic mice were $H\text{-}2^k$ in their eighth backcross to B10.BR (the B10.BR background does not affect any of the parameters measured on the C57BL/6 background: percent of $V_\beta8$, $V_\beta7$, $V_\beta2$, and $V_\beta6$ were 56, 14, 6, and 4%, respectively, among $CD44^{high}$ thymocytes).
34. Mice 14 to 16 weeks old of the $\beta_2M^-H\text{-}2^b$ strain (backcrossed eight times to C57BL/6), and β_2M^+ mice (C57BL/6) received two intraperitoneal injections of 0.5 mg of PK136 antibody to NK1.1 on day −1 and day 0 of whole body gamma irradiation (1000 rads) and were reconstituted 6 hours after irradiation with 1×10^7 fetal liver cells from 15-day-old $\beta_2M^-H\text{-}2^b$ (backcrossed eight times to C57BL/6) or C57BL/6 fetuses. At days 42, 49, and 74 after reconstitution, thymocytes from individual mice were processed and stained as in Fig. 1. At least five mice were analyzed for each type of chimera.
35. Mature (HSA^{low}) thymocytes were obtained from pools of five to 10 C57BL/6 mice at weeks 1, 2, 3, 7, and 18 of age and stained as described in Fig. 1. The absolute numbers of TCR $V_\beta8^+$ cells per thymus in each subset was computed from the frequency of these cells in the HSA^{low} preparation and the frequency of HSA^{low} cells in the whole thymus, as determined by staining with M1/69-PE anti-HSA (Pharmingen).
36. Thymic organ cultures were set up as described (*11*) from day 14 fetal thymuses. Mature (HSA^{low}) thymocytes were obtained as in Fig. 1, at day 22 of culture, and $NK1.1^+$ cells were first stained with PK136 followed by goat anti-mouse IgG-PE (Southern Biotechnology, Birmingham, AL), then anti–CD4-FITC or a cocktail of FITC-conjugated anti-CD4, anti-CD8, and anti-γδTCR (to gate for $CD4^+$ or $CD4^-8^-\gamma\delta TCR^-$ cells, respectively) and biotinylated antibodies to $V_\beta8$, $V_\beta7$, or panTCR V_β were added, followed by streptavidin–RED-613 (Gibco-BRL).
37. We thank B. J. Fowlkes, O. Lantz, and P. Matzinger for discussions; L. D'Adamio, B. J. Fowlkes, O. Lantz, P. Matzinger, D. Margulies, and W. Paul for reviewing the manuscript; T. Mak, E. Timms, E. Robey, and R. Jaenisch for mice; A. Barnes, S. Brust, and P. Golway for managing the specific pathogen-free mouse colonies; and T. Tran for excellent technical assistance.

20 October 1993; accepted 13 January 1994