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5. Mixed forebrain cultures from embryonic rats were prepared as described [S. A. Goldman, W. A. Pulsinelli, W. Y. Clarke, R. P. Kraig, F. Plum, *J. Cereb. Blood Flow Metab.* **9**, 471 (1989)]. Cultures were grown 1 to 6 weeks in vitro before use. The cells were loaded with 10 μM fluo-3 acetomethoxyester (fluo-3 AM; Molecular Probes) for 1 hour at 37°C. A Bio-Rad confocal scanning microscope MCR600 attached to an inverted microscope (Olympus IMT-2) was used for imaging of the fluo-3 signal. Excitation was provided by the 488-nm line of a 25-mW argon laser filtered to less than 1% by neutral density filters. Emission was long pass–filtered (515 nm) and detected with the confocal aperture set to its maximal opening (7 mm). Images were acquired every 0.5 to 3 s and recorded on a Panasonic TQ-2028F optical memory disk recorder. Relative changes in fluorescence were calculated and normalized against the base line fluorescence ($F$) by $\Delta F/F$. Background counts were subtracted from all measurements. Experiments were carried out at room temperature in Hanks balanced salt solution (HBSS; Gibco). For experiments performed with no extracellular $Ca^{2+}$, HBSS was exchanged for a similar solution containing no $Ca^{2+}$ and 0.5 mM EGTA.
6. Focally applied electric fields were produced as described [N. B. Patel and M.-M. Poo, *J. Neurosci.* **4**, 2939 (1984); R. W. Davenport and S. B. Kater, *Neuron* **9**, 405 (1992)]. Constant current (dc) of 2 to 28 μA was passed for 0.5 s between the micropipette and an Ag-AgCl ground electrode and monitored throughout the experiment. The current density, $J$, at a distance $r$ from the electrode tip was calculated by

$$J = I/2\pi r^2$$

where $I$ is the total current flow. Although in some experiments astrocytic $Ca^{2+}$ waves were also elicited by either mechanical stimulation (briefly deforming the plasma membrane with the electrode tip) or by laser excitation, in several experiments laser stimulation caused an uncontrollable degree of fluo-3 bleaching, and mechanical stimulation often induced irreversible damage of the target cell. Therefore, in routine experiments astrocytic $Ca^{2+}$ waves were elicited by electric field stimulation.
7. GFAP (G 9269; Sigma) and MAP-2 (clone AP-20; Sigma) staining were performed according to standard procedures [M. Nedergaard, S. A. Goldman, S. Desai, W. A. Pulsinelli, *J. Neurosci.* **11**, 2489 (1991)]. After staining, each culture was viewed by epifluorescence.
8. In routine experiments, a perfusion rate of 0.1 mm/s was used. In selected experiments, this perfusion rate was increased to 1 to 2 mm/s without affecting the intercellular $Ca^{2+}$ wave.
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12. Cultures were incubated with 2 μM dicarboxydichlorofluorescein diacetate (CDCF) for 5 min and incubated in the absence of CDCF for another 30 min [M. Nedergaard, S. Desai, W. Pulsinelli, *Anal. Biochem.* **187**, 109 (1990)]. After a base line fluorescence image of the culture was obtained, the area of laser scanning was reduced to include only one target cell. Complete or almost complete photobleaching occurred after 10 to 20 scans, each lasting 1 s at full laser power. Subsequently, the microscope settings were returned to recording configuration, and the refill was monitored for 20 min [M. H. Wade, J. E. Trosko, M. Schindler, *Science* **232**, 525 (1986); S. M. Finkbeiner, *Neuron* **8**, 1101 (1992); J. Mantz, J. Cordier, C. Giaume, *Anesthesiology* **78**, 892 (1993)].
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# Genetic Mapping of Quantitative Trait Loci for Growth and Fatness in Pigs

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**The European wild boar was crossed with the domesticated Large White pig to genetically dissect phenotypic differences between these populations for growth and fat deposition. The most important effects were clustered on chromosome 4, with a single region accounting for a large part of the breed difference in growth rate, fatness, and length of the small intestine. The study is an advance in genome analyses and documents the usefulness of crosses between divergent outbred populations for the detection and characterization of quantitative trait loci. The genetic mapping of a major locus for fat deposition in the pig could have implications for understanding human obesity.**

Quantitative genetic variation is the major determinant of intra- and interpopulation differences for many traits of biological, medical, and agricultural significance. Quantitative (or polygenic) inheritance implies that variation in the trait is determined by the action of alleles at several loci together with environmental factors. We have, however, only vague ideas about the number, location, and action of loci controlling quantitative variation. Recently, the ability to dissect genetically quantitative traits has been improved by the development of detailed linkage maps based on DNA markers. With these the segregation of individual chromosome segments can be traced in appropriate pedigrees. Thus, quantitative trait loci (QTLs) segregating in crosses between inbred lines of tomato (*1*), rat (*2*), and maize (*3*) have been mapped. The statistical method used in these studies, maximum likelihood interval mapping (*4*), cannot be used in studies of most domesticated animal species because of the lack of inbred lines. However, an analytical method based on least squares for the identification of QTLs segregating in crosses between divergent outbred lines has been described (*5*). The present study is an experimental application of the method.

European domesticated pigs are thought to have been derived largely from the European wild boar, with some crossbreeding with Chinese domesticated breeds in the 18th to 19th centuries (*6*). Despite striking phenotypic differences between the wild boar and domesticated pigs, they are sufficiently closely related to interbreed easily. We generated a three-generation pedigree by crossing two European

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wild boars with eight Large White sows (*7*). Four $F_1$ males and 22 $F_1$ females were intercrossed to generate 200 $F_2$ offspring. Growth and fat deposition traits were recorded because there has been an intensive artificial selection for high growth and leanness in domesticated pigs. The length of the small intestine was also measured as it has long been recognized to increase in length in response to domestication (*8*), and this trait shows a strong positive correlation with growth (*9*). As expected, the $F_2$ animals grew more slowly, had a shorter small intestine, and deposited more fat than purebred Large White animals (Table 1) (*10*).

A linkage map covering an estimated 75% of the porcine genome has been developed by analysis of 117 genetic markers in the pedigree (*11*). The QTL analysis in the present study was based on 105 genetic markers assigned to linkage groups on 15 of the 18 autosomes (Table 2). The QTLs that differentiate the Large White and wild boar will segregate in the $F_2$ population. In the analysis, the marker data were used to calculate the probabilities of each $F_2$ individual inheriting none, one, or two alleles from each breed for an unobserved QTL at a given position in the genome. For an inbred line, in which markers will be completely informative, these genotypic probabilities can be calculated with only the two markers flanking a given position (*4, 12*). Because the pig breeds used were outbred, markers may be uninformative in particular individuals; thus it was necessary for us to use all the markers in a linkage group to calculate the genotypic probabilities as accurately as possible (*5*).

Coefficients of additive and dominance effects of a QTL were calculated from the genotypic probabilities on the assumption that the two breeds were fixed for alternative QTL alleles. The additive and dominance effects of a QTL at a given position were defined as the deviation of animals homozygous for the wild boar allele or heterozygous, respectively, from the mean of the two homozygotes. (Negative values of the additive and dominance effects would hence indicate that animals homozygous for the wild boar allele or heterozygous, respectively, have trait values lower than the mean of the two homozygotes.) The trait data were regressed onto the coefficients of additive and dominance effects for each individual, and if a QTL was present at the position under study, a regression *F* ratio statistic greater than one was expected. By repeating this procedure at 1-cM intervals, one can plot a curve of *F* ratio values, analogous to a likelihood curve (*4*), along the chromosome, with the highest point representing the most likely position of a QTL (*5, 12*). In this procedure multiple tests at 1-cM intervals are performed, so the value of the *F* ratio at which significance is declared cannot be easily derived theoretically. Thus, using the pedigree and marker genotypes in the actual data, we simulated and analyzed 5000 replicates of trait data in which no QTL was segregating. From each replicate the highest *F* ratio in the whole genome was selected. From these the *F* ratio thresholds at which there was a 5, 1, or 0.1% probability of detecting a QTL somewhere in the entire genome when none were present were calculated as 7.65, 9.73, and 12.66, respectively.

The data analyzed were from 193 animals for birth weight and from 191 animals for the other traits. The use of the regression method of analysis (*5*) allows environmental effects to be estimated simultaneously with the QTL effects. This removes environmental "noise" and thus increases the chance of detecting QTLs and reduces the risk of biases in the analysis. Analyses for all traits included the effect of sex, family, and parity of the animal. The effect of a feeding treatment was included in the statistical model for all traits except birth weight and growth to 30 kg. The following covariates were also included in the analyses: size of litter of birth for birth weight, exact weight at start of period for growth rate from 30 to 70 kg, exact weight at end of period for growth rates to 30 and 70 kg, and weight at slaughter for abdominal fat percentage, average back fat depth, and small intestine length. After fitting the covariates, the distribution of the residuals was tested for normality with the Shapiro-Wilk test (*13*). None of the traits deviated significantly from the normal distribution.

We found evidence for QTLs on chromosome 4 with large effects on growth, length of the small intestine, and fat deposition (Table 3). Wild boar alleles were associated with reduced growth, a shorter small intestine, and a higher fat content consistent with the differences between the founder populations (compare Tables 1 and 3). There were also indications of a QTL on chromosome 13 affecting early growth (Table 3). The chromosome 13 effect on daily gain to 30 kg of weight was significant at approximately the 5% level, but there was also a relatively large effect on birth weight (significant at the 15% level) mapping to the same region. The gene action at those loci on chromosomes 4 and 13 which were significant at the 5% level appeared to be largely additive, that is, the phenotypic value of the heterozygotes was intermediate to the two homozygotes, except that there was an indication of dominance for increased fat depth (Table 3). Further analyses revealed no significant interactions between the effects of the detected QTLs and sex or the feeding treatment. No QTL effects associated with the remaining 13 chromosomes reached statistical significance.

The double peak obtained for both fat deposit traits (Fig. 1A) and the flat curves for growth rate (Fig. 1B) suggested the presence

**Table 1.** Growth and fat deposition traits in purebred Large White pigs and $F_2$ animals from a wild boar intercross. Means and the within-population standard deviation (in parentheses) are given (*21*). The animals were weighed at birth, at 3, 6, and 9 weeks of age, and every second week during the growth period. Growth rates were calculated from the recorded weights nearest to either 30 or 70 kg and the corresponding ages. Animals were also weighed before slaughter, and the length of the small intestine and fat deposit traits were measured after slaughter.

| Trait | Population | |
|---|---|---|
| | Large White* | $F_2$ intercross† |
| Birth weight (kg) | 1.53 (0.29) | 1.38 (0.24) |
| Growth rate from birth to 30 kg (g/day) | 323 (32) | 236 (38) |
| Growth rate from birth to 70 kg (g/day) | 524 (40) | 367 (49) |
| Growth rate from 30 to 70 kg (g/day) | 909 (90) | 624 (107) |
| Abdominal fat (%)‡ | 1.79 (0.44) | 2.40 (0.69) |
| Average depth of back fat (mm)§ | — | 26.4 (4.9) |
| Length of small intestine (m) | 21.2 (1.8) | 17.5 (1.9) |

*Data on birth weight and growth were from 200 Large White animals representing the same population as used in the wild boar intercross. A random sample of gilts and castrates, raised at the same time as the $F_2$ generation, was used for this purpose. The data on abdominal fat were based on 32 animals. Data on the length of small intestine were from the literature (*22*). †Data on birth weight were from 193 animals and from 191 animals for the other traits. ‡Represents the weight of fat tissue in the abdominal cavity as a percentage of the total carcass weight. §Represents the average of five measurements along the dorsal midline at shoulder, last rib, and loin; data for this trait are missing for the purebred Large White animals as it is not measured at a comparable weight.

**Table 2.** Summary of linkage map data.

| Chromosome* | Number of markers | Average heterozygosity† | Map length (cM) |
|---|---|---|---|
| 1 | 4 | 0.60 | 43.3 |
| 2 | 5 | 0.78 | 44.3 |
| 3 | 9 | 0.52 | 86.3 |
| 4 | 10 | 0.85 | 102.0 |
| 5 | 6 | 0.75 | 81.4 |
| 6 | 9 | 0.57 | 79.2 |
| 7 | 10 | 0.67 | 99.3 |
| 8 | 8 | 0.55 | 47.8 |
| 9 | 11 | 0.48 | 75.8 |
| 11 | 4 | 0.31 | 9.5 |
| 12 | 5 | 0.67 | 72.8 |
| 13 | 6 | 0.66 | 102.6 |
| 14 | 8 | 0.47 | 67.0 |
| 15 | 4 | 0.67 | 35.9 |
| 16 | 6 | 0.60 | 65.0 |

*No markers were available on chromosomes 10, 17, 18, and X. †Represents marker heterozygosity observed among the 26 $F_1$ animals.

of two or more linked QTLs for these traits. To test for the presence of a second QTL in a single chromosome, we compared the best model with two QTLs with the best model with a single QTL (*12*) using the significance threshold appropriate for that chromosome derived by simulation. Models with two QTLs on chromosome 4 for the fatness and growth traits gave a better fit than a one-locus model, but the support for the two-locus models was not considered statistically significant (*F* ratio = 3.9–5.0). Because QTLs are mapped with relatively low precision, we cannot exclude the possibility that there is one QTL, located in the proximal part of chromosome 4, with pleiotropic effects on fat deposition, intestinal length, and growth. Moreover, the QTLs identified here may constitute a single gene with a large effect, or a cluster of linked genes each with a smaller effect. One can address this by following the segregation of a QTL in subsequent generations because a cluster may be dissociated by recombination. By utilizing recombinants in the particular region, one should also be able to determine the location of the QTL more precisely as well as test whether the same locus has pleiotropic effects on growth and fatness.

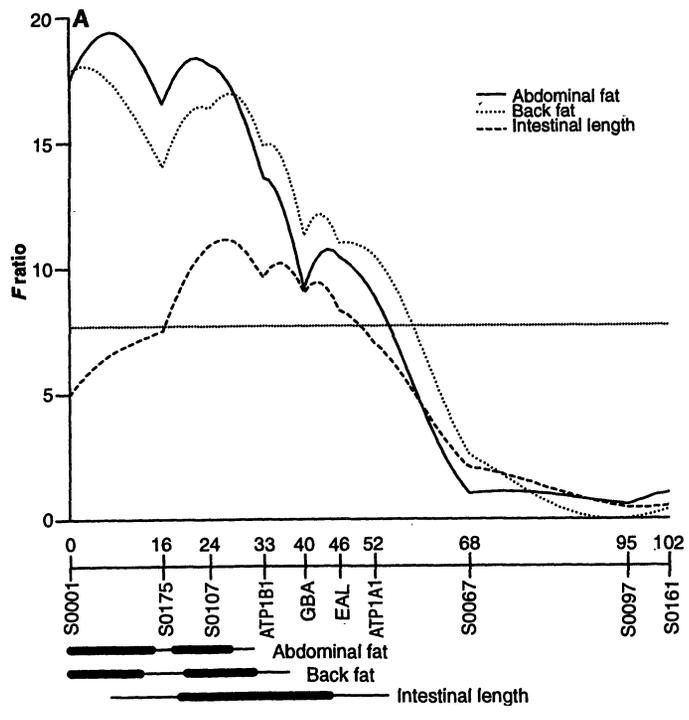
This study illustrates the power of genome analysis in finding the chromosomal location of genes with phenotypic effects. An alternative strategy would be to study candidate genes such as those involved in the growth hormone axis. The segregation at three such candidate loci could be followed with the markers employed in this study, for example, the growth hormone locus on chromosome 12, the growth hormone receptor locus on chromosome 16, and the insulin-like growth factor 1 locus on chromosome 5 [see (*11*) and (*14*) for map data]. However, none of the effects estimated for these loci reached the significance threshold used in this study (*15*).

The QTLs detected on chromosome 4 must have been important in the response to selection for high growth and leanness during the evolution of European domesticated pigs. The QTL on chromosome 4 had an estimated additive effect on growth rate from birth to 70 kg of 24 g per day, explaining ~12% of the total phenotypic variance in the $F_2$ population (and thus an even larger proportion of the genetic variance). Animals homozygous for the wild boar "allele" had a daily gain of almost 50 g less than those homozygous for the Large White "allele," leading to a difference between the two homozygotes of about 10 kg in weight when the pig is 6 months of age. Similarly, the QTL effect on fat deposition explains a significant proportion of the genetic difference in this trait between the wild boar and domesticated animals (about 5 mm in average back fat depth between homozygotes, with the locus accounting for 18%

**Table 3.** Summary of significant QTL effects for growth and fat deposit traits. The test statistics represent the highest *F* ratio found for a given chromosome.

| Trait | *F* ratio | $df_1$, $df_2$ | Map position† | Additive effect‡ | Dominance effect‡ | Percent of $F_2$ variance§ |
|---|---|---|---|---|---|---|
| | | | *Chromosome 4* | | | |
| Growth from birth to 70 kg (g/day) | 11.8** | 2, 158 | 58 | −23.5 ± 4.9 | 0.7 ± 7.3 | 11.9 |
| Length of small intestine (m) | 11.1** | 2, 158 | 27 | −0.87 ± 0.18 | 0.03 ± 0.27 | 11.3 |
| Average back fat depth (mm) | 18.0*** | 2, 158 | 3 | 2.30 ± 0.42 | 1.47 ± 0.65 | 17.6 |
| Abdominal fat percentage (%) | 19.4*** | 2, 158 | 7 | 0.38 ± 0.06 | 0.15 ± 0.10 | 18:7 |
| | | | *Chromosome 13* | | | |
| Growth from birth to 30 kg (g/day) | 7.6* | 2, 160 | 53 | −13.5 ± 3.6 | 6.0 ± 5.2 | 7.5 |

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. †The map position is the one giving the highest *F* ratio on that chromosome estimated in centimorgans from the proximal end as defined (*11*). ‡Estimates are given as mean ± SE. §The reduction in the residual variance of the $F_2$ population effected by inclusion of a QTL at the given position.



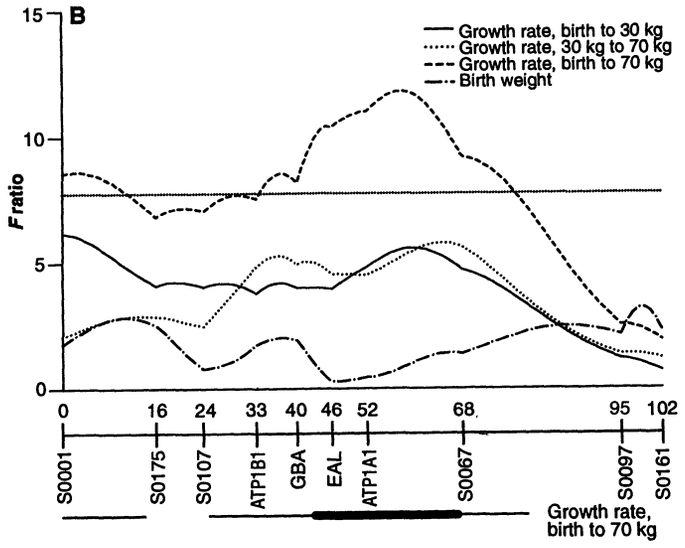




**Fig. 1.** Test statistic curves for chromosome 4. (**A**) Abdominal fat percentage, average back fat depth, and length of the small intestine. (**B**) Birth weight, growth rate from birth to 30 kg, growth rate from 30 to 70 kg, and growth rate from birth to 70 kg. The graphs plot the *F* ratio testing the hypothesis of a single QTL in a given position on the chromosome. The dotted horizontal line represents the 5% significance threshold. The map of chromosome 4 is shown with the distances between markers in centimorgans (*11*). Support intervals for the location of the significant QTLs, approximately equivalent to one lod (thick bar) (logarithm of the likelihood ratio for linkage) and two lod (thin bar) support, are shown beneath the graph.

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.

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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)].
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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.
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# A Subset of CD4$^+$ Thymocytes Selected by MHC Class I Molecules


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**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


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