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*J Immunol* 1999; 163:6598-6605; [http://www.jimmunol.org/content/163/12/6598](http://www.jimmunol.org/content/163/12/6598)

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Commitment to the CD4 Lineage Mediated by Extracellular Signal-Related Kinase Mitogen-Activated Protein Kinase and Lck Signaling

Leslie L. Sharp and Stephen M. Hedrick

The development of T cells results in a concordance between the specificity of the TCR for MHC class I and class II molecules and the expression of CD8 and CD4 coreceptors. Based on analogy to simple metazoan models of organ development and lineage commitment, we sought to determine whether extracellular signal-related kinase (Erk) mitogen-activated protein (MAP) kinase pathway signaling acts as an inductive signal for the CD4 lineage. Here, we show that, by altering the intracellular signaling involving the Erk/MAP kinase pathway, T cells with specificity for MHC class I can be diverted to express CD4, and, conversely, T cells with specificity for MHC class II can be diverted to express CD8. Furthermore, we find that activation of the src-family tyrosine kinase, p56Lck, is an upstream mediator of lineage commitment. These results suggest a simple mechanism for lineage commitment in T cell development. The Journal of Immunology, 1999, 163: 6598-6605.

Two different models have been suggested to explain the process of T cell lineage commitment, including the association of TCR specificity and the appropriate expression of the CD4 and CD8 coreceptors (1). The major tenet of the stochastic/selective model is that there is no specificity to the signal received as a result of MHC class I recognition vs MHC class II recognition. The proposal is that, upon TCR/MHC interaction, one coreceptor randomly becomes down-regulated and further survival depends upon continuous MHC interactions with both the TCR and the CD4 or CD8 coreceptor. Cells expressing the correct coreceptor survive, whereas cells that express a coreceptor not in concert with the specificity of the TCR do not. Alternatively, mechanisms that include an MHC-specific signal to guide lineage commitment in accordance with TCR specificity fall under the rubric of instructional models.

Oddly, there is evidence in support of both mechanisms. An analysis of MHC-deficient (class I- class II-) mice showed that, by the criterion of CD4+CD8- expression levels, no differentiation past the CD4+CD8- double positive (DP) step occurs. Notably, in MHC class I- class II+ mice, a population of immature CD4 thymocytes (CD4+CD8low) arose. On the assumption that these cells were precursors to mature CD4 single positive T cells, the idea was put forth that MHC class I or II molecules can give an initial nonspecific signal to begin the process of positive selection (2). Similarly, in the absence of MHC class I, but the presence of MHC class II, there was evidence for an immature CD8 population (2, 3). Further analysis indicated that the above assumption may be too simplistic (4, 5). A second line of experimentation showed that, in some cases, constitutive, transgenic expression of a coreceptor could permit the appearance of T cells with a specificity not matched to coreceptor expression (6-8). In other words, a transgenic coreceptor could "rescue" T cells that expressed a coreceptor not in concert with the TCR specificity. In other experiments along this line, there was no rescue seen (1), but, in any event, the rescued T cells were always few in number. These results may be consistent with a mechanism whereby there is an initial stimulation that causes cells to initiate differentiation, but this differentiation is not strictly random and is influenced by the signaling resulting from MHC class I or class II recognition (7).

Indeed, other experiments support the notion that there are signals that direct the differentiation of uncommitted thymocytes along one or another pathway. The use of Abs directed to the TCR in organ cultures causes a bias toward the CD4 lineage, except in one case where it causes a bias toward the CD8 lineage (9-11). There are no reports of balanced CD4 and CD8 maturation that should come from a mechanism based strictly on stochastic differentiation and selection. Other work showed that the signals required for CD4 and CD8 commitment appear to be asymmetric (12). Recent data looking at the signaling molecules that drive differentiation are also consistent with the notion that the nuances of signal transduction influence lineage commitment. We have shown that a hypersensitive form of extracellular signal-related kinase (Erk)-2 biases differentiation toward the CD4 lineage, and inhibition of the Erk-2 activation pathway using a pharmacological inhibitor biases differentiation toward the CD8 pathway. The deletion of Csk, a src family regulator, causes thymocytes to differentiate toward the CD4 lineage exclusively and in the complete absence of TCR expression (13). In addition, a deficiency in c-Cbl, a regulator of T cell signaling, promotes CD4 and not CD8 maturation (14). Finally, either activated Notch or Bcl-2 overexpression appears to promote differentiation to the CD8 lineage (15-17).

A simplistic view of thymocyte development is that TCR and coreceptor interactions with MHC class I or class II molecules signal cells to differentiate. The quality of the signal as well as other receptor-counter receptor interactions influence whether the...
incipient T cell differentiates toward the CD4 or CD8 lineage (or dies as a manifestation of negative selection). The quality of the signal appears to depend on the strength of signaling through src family kinases and ultimately the activation of mitogen-activated protein (MAP) kinases such as Erk-2. Nonetheless, the initial signal may provide only an inductive bias, and it is possible that further signaling could be required to provide proofreading as means to substantially increase the accuracy of a lineage decision. This could be described as an instructional-selective model. It is consistent with much of the available evidence, and it also conforms to general schemes of lineage commitment worked out for organ development in simple metazoans (18). In this report, we provide evidence that CD4 and CD8 lineage commitment can be subverted by altering the strength of MAP kinase pathway signaling. Furthermore, we provide evidence that this signaling can originate from p56	extsuperscript{lck} activation.

Materials and Methods

Mice

Mice were bred and maintained at the University of California at San Diego animal facilities. Timed matings were set up by placing 2–3 females with one male for 16 h. The day the male was removed was considered day 0 of gestation. Erk2	extsuperscript{−/−} mice are transgenic for Erk2D319N (19). AND mice are transgenic for Vα11 and Vβ3, which confers specificity for pigeon cytochrome c (PCC) (20, 21). OT-1 mice are transgenic for Vα2 and Vβ5, conferring specificity for the OVA peptide SIINFEKL and were a gift from Michael Bevan (22). p56	extsuperscript{lck}−/−F505 mice were a gift from Roger Pernmüttner (23).

Fetal thymus organ culture

Thymus lobes were harvested on E16 and placed on Transwell filters (Costar, Cambridge, MA). Thymuses were cultured in IMDM (supplemented with 10% FCS, glutamine, sodium pyruvate, nonessential amino acids, 2-ME, and antibiotics) in the presence or absence of indicated concentrations of PMA, ionomycin, and PD98059 (New England Biolabs, Beverly, MA) at 37°C, 5% CO2, for 5 days unless otherwise indicated. Medium was replaced every 48 h. Lobes were strained through nylon mesh to release thymocytes. Viable cells were counted using the flow cytometer.

Flow cytometry and Abs

Thymocytes were washed in PBS containing 2% FCS and 0.1% sodium azide. Cells were surface stained using combinations of anti-CD4 PE, anti-CD8 Tricolor (Caltag, South San Francisco, CA), anti-Vα11-FTIC (PharMingen, San Diego, CA), anti-Vα2-FTIC (PharMingen), and anti-HSA-biotin (PharMingen) + Streptavidin-APC (PharMingen). BrdU analysis was performed by adding 25 μM BrdU (Sigma, St. Louis, MO) to the culture medium for 16 h before harvest. Staining was performed following the protocol of Carayon and Bord (24). Anti-BrdU-FTIC was obtained from Becton Dickinson (Mountain View, CA). Events were collected before analysis on a FACSscan (Becton Dickinson) or FACScalibur (Becton Dickinson). Collection and analysis were performed using CellQuest software (Becton Dickinson). New analysis gates were generated with each experiment to compensate for changes in forward scatter or side scatter as well as Ab-staining intensity.

Results and Discussion

Erk Pathway inhibition increases CD8SP in AND TCR transgenic thymocytes

We have shown that fetal thymocytes from normal mice cultured in the presence of PD98059, a specific inhibitor of MAP/Erk kinase (MEK), have a marked decrease in CD4SPs, but no substantial decrease in CD8SPs (19). To show the time course of the effects of MEK inhibition, we added PD98059 to fetal thymic organ cultures (FTOCs) at different stages of development (Fig. 1). The percentage of recovered CD4SP-TCRβ-chain high thymocytes was plotted vs the day of fetal life at the initiation of a 5-day culture. As shown, the percentage of CD4s always exceeded that of CD8s in FTOCs treated with vehicle alone whereas, in the presence of 30 μM PD98059, the percentage of CD8SPs exceeded that of CD4SPs at every point. This experiment confirms our previous results (19) and demonstrates that the increased CD8SPs are αβ T cells. It conflicts to some extent with the results of one subsequent report (25), but, as discussed below, it is consistent with a very recent report (26).

In cultures initiated before day E15, the recovery of thymocytes in the presence of PD98059 is diminished to between 20–30% of controls. This is because the inhibition of MEK is very effective in eliminating the proliferation and differentiation that gives rise to DPs (19); for this reason, a comparison of the actual number of recovered cells is difficult. As the day of culture initiation progresses, a substantial number of thymocytes have made the transition to the DP stage, and the recoveries are less affected to the point where recoveries are actually increased in day 16 cultures. In the series of d15 experiments presented in Table I, the number of CD4SPs recovered was greater than the number of CD8SPs in

<table>
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<tr>
<th>Initiation Day of 5 Day FTOC</th>
<th>DMSO</th>
<th>PD98059</th>
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<tr>
<td>E13</td>
<td>6.2</td>
<td>22.9</td>
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<tr>
<td>E14</td>
<td>3.5</td>
<td>6.9</td>
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<td>E15</td>
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CD4 and CD8 subsets present after MEK inhibition

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<thead>
<tr>
<th></th>
<th>DMSO Control</th>
<th>30 μM PD98059</th>
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<tr>
<td>Total recovery</td>
<td>865 ± 160</td>
<td>506 ± 140</td>
</tr>
<tr>
<td>CD4 &quot;CD8+&quot;</td>
<td>666 ± 130</td>
<td>333 ± 130</td>
</tr>
<tr>
<td>CD4SP-TCRβhigh</td>
<td>57.5 ± 15.1</td>
<td>6.9 ± 3.5</td>
</tr>
<tr>
<td>CD8SP-TCRβhigh</td>
<td>18.9 ± 6.2</td>
<td>22.9 ± 2.0</td>
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*Total number of recovered live cells (× 10^3) from individual organ cultures of E15 thymuses cultured for 5 days (n = 4 thymuses per group).
control cultures, whereas the reverse was true of cultures treated with PD98059. Although the mean number of CD8SPs found with the addition of PD98059 was greater than that of controls, the variation precluded achieving statistical significance. At least under these conditions, we found that CD4SP differentiation was preferentially or even exclusively inhibited, but from this experiment we cannot tell whether class II-specific cells that failed to mature into CD4SPs changed their fate to become CD8SPs.

To determine whether MHC class II-specific precursors can be redirected to differentiate into CD8SPs, we analyzed the development of T cell precursors expressing a receptor known to be specific for MHC class II molecules. AND transgenic mice express TCRα- and β-chain genes that encode a TCR specific for pigeon cytochrome c and H-2E<sup>k,b</sup> molecules. In the presence of H-2<sup>b</sup> or H-2<sup>k</sup>, there is a very large population of CD4SPs and virtually no CD8SPs that express V<sub>a11</sub> at high levels (20, 21). However, we note that, in FTOCs from AND-H-2<sup>b</sup> mice, there is an unusual population of CD8s with highly variable levels of CD8 (Fig. 2A). We have seen this with other MHC class II-specific TCR transgenic mice as well. Nonetheless, thymuses from AND-H-2<sup>k</sup> mice, there is a very large population of CD4SPs and virtually no CD8SPs that express V<sub>a11</sub> at high levels (20, 21). However, in FTOCs from AND-H-2<sup>k</sup> mice, there is an unusual population of CD8s with highly variable levels of CD8 (Fig. 2A).

We have seen this with other MHC class II-specific TCR transgenic mice as well. Nonetheless, thymuses from AND TCR transgenic mice were cultured in the presence or absence of 30 μM PD98059 and analyzed for the expression of CD4, CD8, and V<sub>a11</sub>. A. A flow cytometric profile of a typical experiment using 30 μM PD98059. B. Histograms representing the percentage of V<sub>a11</sub>CD4SP cells (left) and V<sub>a11</sub>CD8SP cells (right).

FIGURE 2. MEK inhibition increases CD8SP in MHC class II-specific thymuses. E16 thymuses from AND TCR transgenic mice were cultured for 5 days in the presence of the indicated concentration of PD98059. Thymocytes were stained for expression of CD4, CD8, and V<sub>a11</sub>. A. A flow cytometric profile of a typical experiment using 30 μM PD98059. B. Histograms representing the percentage of V<sub>a11</sub>CD4SP cells (left) and V<sub>a11</sub>CD8SP cells (right).

FIGURE 3. PMA and ionomycin can induce the development of CD4SP in MHC class I-restricted thymocytes. E16 thymuses from OT-1 mice were cultured for 5 days in the presence of the indicated concentrations of PMA and ionomycin. A. Thymocytes were stained with anti-CD4-PE, anti-CD8-Tricolor, and anti-V<sub>a2</sub>-FITC. Numbers represent the percentage of live cells within the indicated quadrant. B. Thymuses were cultured in the presence of BrdU during the final 16 h before harvest and stained with anti-CD4-PE, anti-CD8-Tricolor, anti-V<sub>a2</sub>-biotin + streptavidin-APC and anti-BrdU-FITC. Numbers represent the percentage of BrdU-positive cells within the CD4SP or CD8SP population.

Erk pathway signaling generates CD4SP in class I-restricted thymocytes

To determine whether increased Erk signaling could induce thymocytes that would normally become CD8SP to become CD4SP, we used an FTOC method described by Takahama (27) in which we did not see a more dramatic increase in the absolute number of CD8SPs in C57BL/6 cultures because the population is heterogeneous. These results are consistent with those of Bommhardt et al. (26). In this recent study, PD98059 was shown to enhance CD8 maturation in neonatal thymus organ cultures from either MHC class I- and MHC class II-specific TCR transgenic mice. In addition, they showed that an Ab stimulus that normally gives rise to CD4SPs would promote the appearance of CD8SPs in the presence of PD98059. All of these studies support our original contention that the Erk-signaling pathway is preferentially required for CD4 differentiation, but, in addition, it is now clear that the inhibition of Erk can convert differentiation to the CD8 lineage. Two questions have yet to be addressed: one, can the stimulation of the Erk pathway convert MHC class I-specific cells to the CD4 lineage; and two, how does MHC class II specificity promote Erk activation?
thymocytes are incubated in the presence of PMA and ionomycin. PMA is known to activate the Erk/MAP kinase pathway through protein kinase C-mediated stimulation of Raf-1 (28). We examined whether PMA-mediated increases in Erk/MAP kinase signaling could alter the fate of MHC class I-restricted OT-1 thymocytes. The OT-1 TCR is formed by the combination of Vα2 and Vβ5 and is specific for the OVA peptide SIINFEKL presented by H-2Kb (22). OT-1 thymuses were cultured in the presence of PMA and ionomycin. This is consistent with results obtained by Takahama (27), and with our observation that PMA plus ionomycin does not increase the proportion of CD4SPs and CD8SPs in cultures from C57BL/6 mice (data not shown).

To further analyze these newly formed CD4SPs for maturity, we compared the level of α-chain expression and the maturation marker heat stable Ag (HSA). As shown, CDSP induced by PMA plus ionomycin has the same level of Vα2 expression as CD8SP thymocytes (Fig. 4A). In some experiments we analyzed the expression of Vβ5 and found, as expected, that it was uniformly high and consistent with exclusion of endogenous receptor rearrangements (data not shown). As such, there is no significant contribution of endogenous TCR chains to the receptor expressed by the CD4SPs, and thus we deduce that development was mediated by an MHC class I-specific TCR. Additionally, PMA plus ionomycin induced a population of Vα2+CD4SP cells expressing low levels of HSA that virtually were absent in controls (Fig. 4B). This indicates that cells with an MHC class I-specific receptor could be diverted to the CD4SP phenotype by signaling, which included the mobilization of calcium and signaling through the MAP kinase pathway. Finally, to show that the increase in CD4SPs was MEK dependent, thymuses were cultured in the presence of PMA and ionomycin with and without 30 μM PD98059. As shown in Fig. 5, inhibition of MEK using PD98059 inhibits the formation of Vα2+CD4SP, suggesting that MHC class I-specific thymocytes can be induced to become CD4SPs in a MEK-dependent manner.

This induction of CD4SP, although small, is consistent with the findings of Takahama et al. (27), in which TCR α-chain-deficient thymocytes cultured in the presence of PMA and ionomycin allowed the development of some CD4SP thymocytes. It would appear to us that the CD4SP are converted from the CD8 lineage since both the proportion and number of CD4SPs that bear high levels of the Vα2 chain. C57BL/6 E16 thymuses cultured with PMA and ionomycin showed no increase in the percentage of CD4SP (data not shown). We also found that addition of PMA increased the DN population. This was noted by Takahama et al. and shown to be an increase in immature cells (27). One possibility is that the increase in CD4SPs is due to proliferation of the preexisting small population caused by PMA and ionomycin. To test this, cultures were set up and labeled with BrdU for 16 h before harvesting. As shown in Fig. 3B, the proportion of labeled CD4SPs or CD8SPs did not increase with the addition of PMA and ionomycin. This is consistent with results obtained by Takahama (27), and with our observation that PMA plus ionomycin does not increase the proportion of CD4SPs and CD8SPs in cultures from C57BL/6 mice (data not shown).
elegans vulval development, since overexpression of lin-12 imparts the 2° fate to all vulval precursor cells (30).

Erk sem does not cause a general increase in positive selection

We have previously shown that a hypersensitive form of Erk-2 (Erk2sem) expressed in transgenic mice causes an increase in the percentage of CD4SPs in normal mice and also in AND, MHC class II-specific TCR transgenic mice (19). To determine whether Erk2sem can promote maturation to the CD8 lineage in TCR transgenic mice, we crossed the Erk2 sem mice with the MHC class I-restricted TCR transgenic line OT-1 and compared OT-1 mice with OT-1:Erk2sem mice. For illustration purposes, an analysis of AND and AND: Erk2 sem mice is shown. There was no increase in CD8SPs in OT-1:Erk2 sem mice whereas, as previously published, there was a substantial increase in the CD4SPs in AND:Erk2sem mice (Fig. 6). Curiously, in the OT-1:Erk2sem mice there was also no consistent increase in the CD4SPs that appear. Given the results presented above, we propose two explanations. One is that the Erk2sem is a hypersensitive, but not a constitutively active, allele and as such does not provide enough activity in this setting to alter the fate of OT-1 T cells. A second possibility is that an increase in Erk activity alone is not sufficient to cause a deviation in lineage commitment. As shown above, PMA alone does not cause the appearance of CD4SPs in OT-1 mice but requires the addition of ionomycin.

The role of p56 lck in lineage commitment

The data presented are consistent with a mechanism of lineage commitment that favors differentiation to the CD4 lineage as a consequence of strong signaling mediated through the Erk/MAP kinase pathway. How then does MHC class II recognition lead to a stronger inductive signal? One possibility that has been suggested lies with the MHC-specific coreceptors themselves. CD4 is known to bind to p56lck with a higher stoichiometry than CD8 (31). Thus, signaling through the TCR and CD4 provides for a greater number of activated p56lck molecules when compared with signaling through the TCR and CD8 (11). This, in turn, could lead to a more highly activated MAP kinase cascade. Indeed, the cytoplasmic tail of CD4 and CD8 have both been implicated in lineage commitment (32–35), and the absence of CD4 allows class II-specific T cell precursors to differentiate into CD8SPs (36).

To directly address the role of p56lck in lineage commitment, we introduced an activated form of p56lck (p56lck-F505) into various TCR transgenic mice. As previously published (37), p56lck-F505 caused a potent down-regulation of the TCR in AND TCR transgenic mice (data not shown). However, when combined with the OT-1 TCR transgenes, we found that there was no down-regulation of expression of Vα2 (Fig. 7A) or Vβ5 (data not shown), nor were there any changes in cellularity. As an aside, we noticed that OT-1 mice possessed a synthetic α-chain gene driven by an H-2Kk promoter and IgH enhancer (22). Another TCR transgenic line, AD10 (21), using an identical α-chain construct, did not correspond with the results obtained with OT-1; it too showed a strong down-modulation of TCR expression in the presence of p56lck-F505 (data not shown). At this point, we do not understand the fortuitous lack of TCR modulation in OT-1:p56lck-F505 mice.
To determine whether p56<sup>lck</sup> activation can preferentially give rise to CD4SP differentiation, the thymocyte subset proportions were determined for groups of OT-1:p56<sup>lck</sup>-F505 and OT-1 mice. As shown in Fig. 7B, there was a statistically significant rise in the number of V<sub>a</sub>high CD4SPs but not V<sub>a</sub>highCD8SPs. The percentage of V<sub>a</sub>highCD4SP was increased from 18.7 ± 5.7 in OT-1 mice to 28.7 ± 7.6 in OT-1:p56<sup>lck</sup>-F505 mice (p = 0.007, Student's t test). The number of CD4SPs was also increased from an average of 9.03 ± 5.54 million in OT-1 mice to 20.29 ± 13.33 million in OT-1:p56<sup>lck</sup>-F505 mice. This effect is consistent with the predicted concept of the CD4 and p56<sup>lck</sup> complex as a mediator of the lineage decision.

Mechanistically, p56<sup>lck</sup> appears to play a direct role in activating the MAP kinase pathway. Stimulation of p56<sup>lck</sup>-deficient J.CaM1 cells with anti-CD3 fails to activate Raf-1 and MEK-1 (38). Conversely, thymoblasts containing constitutively active p56<sup>lck</sup> show constitutive activation Ras, Raf-1, and Erk-1 and -2 (39). Recent reports have shown that the Src homology (SH) 3 domain of Lck is important for activating the Erk pathway and that this Erk activation is independent of CD3 and ZAP-70 phosphorylation (40). This is also consistent with a report showing that p56<sup>lck</sup> has downstream signaling effects independent of TCR-proximal ZAP-70 activation (41). Furthermore, HIV-induced CD4 aggregation was shown to cause p56<sup>lck</sup> aggregation and activation, and Raf-1 was shown to be associated with active p56<sup>lck</sup> in this situation (42). Finally, p56<sup>lck</sup> was found to be associated with active Erk after stimulation through CD3 and during IL-2-mediated proliferation (43–45).

A basis for lineage commitment

To further examine the role of the Erk/MAP kinase pathway in lineage commitment, we attempted to alter the fate of TCR transgenic thymocytes by varying the levels of Erk/MAP kinase signals. We were able to see an increase in CD4 T cells with specificity for MHC class I by increasing MAP kinase signaling and, in accord with Bommahardt et al. (26), an increase in MHC class II-specific CD8 T cells by decreasing MAP kinase signaling. A mechanism by which differential signaling can occur is suggested by the increase in CD4SPs promoted by an activated form of p56<sup>lck</sup>. By virtue of its higher stoichiometric association with CD4 vs CD8, MHC class II recognition would result in increased p56<sup>lck</sup> activation, as compared with MHC class I recognition. In turn, this may result in the activation and possibly the intracellular translocation of Erk-1 and -2 (Fig. 8A). These results could be almost perfectly scripted from basic organogenesis defined for R7 differentiation in the Drosophila retina and especially vulva differentiation in Cae norhabditis. An inductive pathway originating from a receptor tyrosine kinase, which activates Erk, causes cells to assume a primary phenotype, whereas a weaker inductive stimulus causes cells to assume a secondary phenotype. If we assume CD4 T helper cells to be the primary phenotype, or as Singer et al. described it, the “default pathway,” then T cell lineage commitment fits easily into this now canonical scheme (46). This analogy to development in early metazoans was first suggested by experiments that addressed the role of Notch in T cell lineage commitment (16), and though there is still some controversy concerning the interpretation of these results (17), Notch will almost certainly be involved at some level (47, 48).

As described in the introduction, we propose that the observations to date can be best understood in the framework of an instructive-selective model. No single signaling event may be accurate enough to promote differentiation with the fidelity of the T cell lineage commitment, so that the previously proposed “quantitative model” describes only part of the process. We can imagine that a robust and accurate process would incorporate a degree of fuzziness at any one point in time, but, in a process of serial selection events that continue as T cell differentiate, the accuracy of the lineage selection would be sharpened to a very high degree. This is illustrated by an imaginary progression of MHC class II-specific
Although we find it interesting to consider the conservation of differentiation mechanisms over a vast evolutionary time frame, we caution that most studies of differentiation in simple metazoans focus on spatial differentiation, and not on cyto differentiation (18). The concept of spatial differentiation is that cells acquire a phenotype based, in part, on their relative position within the developing organism. Arising partly from the circuitry of Notch signaling, as an example, bristle formation is pattern dependent. This is true of most studies, perhaps for the prosaic reason that morphologic differences are easiest to score in a genetic screen. On the other hand, lymphoid development is not an example of spatial differentiation. Although there is lymphoid organ anatomy, cells may not acquire their phenotype on the basis of position. Rather, they differentiate and then migrate to specific areas of primary and secondary lymphoid organs. As such, the signaling circuitry that drives lymphoid differentiation is likely to have many conceptually unique aspects not anticipated by a study of vulva development. The results described in this report, following on the work of many investigators, establishes that there exists a signaling pathway capable of guiding lineage commitment in T cell development. Without question, this is but the smallest glimpse into the intricate mechanisms governing lymphocyte development.

Acknowledgments

We thank Dawn Page for advice on fetal thymic organ cultures and for critical reading of the manuscript.

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