Antagonist Peptide Selects Thymocytes Expressing a Class II Major Histocompatibility Complex-restricted T Cell Receptor into the CD8 Lineage

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Summary

CD4/CD8 lineage decision is an important event during T cell maturation in the thymus. CD8 T cell differentiation usually requires corecognition of major histocompatibility complex (MHC) class I by the T cell receptor (TCR) and CD8, whereas CD4 T cells differentiate as a consequence of MHC class II recognition by the TCR and CD4. The involvement of specific peptides in the selection of T cells expressing a particular TCR could be demonstrated so far for the CD8 lineage only. We used mice transgenic for an MHC class II-restricted TCR to investigate the role of antagonistic peptides in CD4 T cell differentiation. Interestingly, antagonists blocked the development of CD4$^+$ cells that normally differentiate in thymus organ culture from those mice, and they induced the generation of CD8$^+$ cells in thymus organ culture from mice impaired in CD4$^+$ cell development (invariant chain-deficient mice). These results are in line with recent observations that antagonistic signals direct differentiation into the CD8 lineage, regardless of MHC specificity.

Key words: antagonist • major histocompatibility complex • thymocyte differentiation • thymic selection • T cell receptor

The T cell repertoire is shaped in the thymus by positive and negative selection, such that the mature T cell population recognizes foreign antigens and tolerates self-peptides. The involvement of self-peptide in both selection processes has been explained by the avidity model, according to which high avidity interactions between thymocytes and antigen presenting cells lead to deletion of T cells, whereas low avidity interactions induce positive selection (for reviews see references 1, 2).

The selection of CD8$^+$ cells expressing MHC class I-restricted TCRs appears to follow this model. Positive selection of T cells expressing a class I-restricted transgenic TCR was induced in fetal thymic organ culture with low concentrations of antigenic peptide, whereas high concentrations of the same peptide induced negative selection (3, 4). More efficiently, analogues of the antigenic peptide could be used for the induction of positive selection (4, 5). Such analogues are frequently antagonists of mature T cells and were shown to provide a decreased affinity interaction with the TCR (6). The fact that analogues of the antigenic peptide, but rarely unrelated peptides (7–9), caused positive selection of CD8$^+$ cells indicated a high degree of peptide specificity for selection whenever single peptides were used.

Surprisingly, nobody has reported positive selection of transgenic class II-restricted cells using similar assays. On the other hand, mice expressing class II molecules loaded with a single peptide were used to identify T cells selected by this single ligand. In H2-M-deficient mice that almost exclusively display class II-associated invariant chain peptides on their class II molecules (10–12), as well as in mice expressing an Eα peptide in the context with H2-A$^b$ in the absence of endogenous class II molecules (13), a diverse repertoire was selected, although with less efficiency than in wild-type mice. This indicated a less stringent peptide specificity for positive selection of class II-restricted TCRs.

Also, introduction of single peptides into mice by means of intrathymic injection of an adenovirus vector expressing such peptides provided evidence that in addition to antigenic peptide and its analogues, some unrelated peptides could induce positive selection (14).

A disadvantage of these polyclonal, nontransgenic systems is that they do not give information about particular TCR Vα/Vβ sequences selected. The fact that none of several different transgenic TCRs was positively selected in H2-M-deficient and Eα/H2-A$^b$ expressing mice (15–17) shows that unrelated single peptides are less adequate for positive selection than one might conclude from a poly-
clonal repertoire. The only report in which a transgenic class II-restricted TCR system with known TCR V, Vβ sequences was used to investigate positive selection mediated by antigen-related peptides was by Spain et al. (18). They showed that the addition of antagonist peptide to fetal thymic organ culture of class II-restricted thymocytes inhibits the development of CD4+ cells. However, this study was performed on a normally selecting background in which endogenous peptides are presented. Therefore, the inhibition of CD4+ cell development might have been due to interference of the antagonist peptide with the physiological positively selecting peptide.

To circumvent this potential problem, we used invariant chain deficient mice (Ii−/−) that express reduced levels of surface MHC class II molecules with greatly increased ability to present exogenously added class II binding peptides (19, 20). These mice were crossed to the MHC class II-restricted TCR transgenic mouse line A18 to enable analyses of selection requirements for a particular TCR. This TCR is specific for a peptide derived from the fifth component of complement (21). Neonatal thymic lobes (NTOC) from these mice did not generate mature CD4+ cells, due to an altered repertoire of self-peptides bound to class II that is lacking the endogenous peptide responsible for positive selection. When antagonist peptide was loaded exogenously onto class II molecules, CD4+ cell development could not be rescued. Instead, this treatment resulted in the generation of CD8+ cells. These data address important questions about the interaction of the TCR and its ligand, leading to CD4 versus CD8 lineage commitment.

Materials and Methods

Mice. The C5 TCR transgenic mouse line A18 on the Rag1−/− background (21) was crossed to invariant chain deficient mice (20) to generate Rag−/−, Ii−/− A18 transgenic mice. Genotyping was performed by PCR analysis.

Peptides. The agonist peptide for the A18 T cell receptor is peptide 106-121 from mouse C5. Two antagonists were generated by replacement of lysine residue 113 with either isoleucine for antagonist 113I (K→I) or with valine for antagonist 113V (K→V). Antagonist 113V was used for the organ cultures.

Antagonist Assay. Dendritic cells derived from cultures of bone marrow cells with GM-CSF as described (22) were pulsed with 10 nM A18 agonist peptide for 2 h. They were then washed and cultured with different doses of antagonist or control peptide in the presence of spleen T cells from A18 Rag1−/− TCR transgenic mice. After 48 h of culture, supernatants were transferred to fresh wells with 5,000/well IL-2–dependent CTLL indicator cells. IL-2 response of A18 TCR transgenic spleen cells in response to 10 nM A18 agonist peptide, and then cultured with bone marrow dendritic cells (2 × 105/well) in the presence of 1 μM C5 peptide 106-121 for 72 h. Supernatant was tested for the presence of IL-2 by its ability to support growth of IL-2-dependent CTLL indicator cells.

Results

Identification of Peptide Antagonists for the Transgenic TCR A18. Exchange of lysine residue 113 in the C5 peptide recognized by the A18 TCR results in loss of stimulatory activity for C5-specific A18 T cells. Two such altered peptides with a change of K→I (peptide 113I) or K→V (peptide 113V) displayed antagonist activity as judged by their ability to inhibit activation of A18 T cells to the agonist A18 peptide. Both peptides are nonstimulatory for A18 T cells in a range of concentrations from 1 nM to 10 μM.
(data not shown). Fig. 1 shows that both 113I and 113V blocked the IL-2 response of A18 T cells to dendritic cells prepulsed with agonist peptide, whereas an unrelated H2-E\textsuperscript{k} binding peptide from hen egg lysozyme (HEL 1-18, 2G7; reference 26) did not influence the response.

TCR Antagonists Efficiently Induce the Differentiation of CD8\textsuperscript{+} Cells in Thymus Organ culture from A18, Invariant Chain-deficient Mice. To decrease presentation of endogenous peptides and enable preferential loading of exogenously added peptides onto MHC class II molecules, we crossed class II-restricted TCR-transgenic A18 mice on the RAG-1\textsuperscript{-/-} background to invariant chain-deficient (Ii\textsuperscript{-/-}) mice. MHC class II expression in Ii\textsuperscript{-/-} mice has been reported to be reduced but not abolished and the absence of invariant chain interferes with CD4 T cell development of many, but not all, specificities (20, 27). Neonatal thymus organ culture of A18 Ii\textsuperscript{-/-} mice showed no differentiation of mature CD4\textsuperscript{+} cells (Fig. 2 A, medium), placing A18 in the former category. The few CD4\textsuperscript{+} cells that were found in NTOC displayed an immature phenotype (TCR\textsuperscript{lo-int} and HSA\textsuperscript{hi}). Thymus cortical epithelial cells, which are responsible for the induction of positive selection of T cells (28), show a twofold reduction in MHC class II levels (data not shown). Since we know that the A18 TCR is positively selected in F1 mice with the nonselecting H2-q background (Barthlott, T., R. J. Wright, and B. Stockinger, manuscript submitted for publication), we can exclude that this degree of reduction in MHC class II expression is the reason for the lack of mature CD4\textsuperscript{+}HSA\textsuperscript{lo} cells in A18 Ii\textsuperscript{-/-} lobes. An alternative and more likely explanation for the defect in positive selection of A18 cells is an impairment in the processing of the normally positively selecting peptide or its proper assembly with class II, due to the absence of Ii. The greatly increased peptide binding capacity of Ii\textsuperscript{-/-} antigen-presenting cells allowed us to test the effect of exogenously added peptides on positive selection of A18 T cells.

Antagonist peptides have been shown to be successful in generating class I-restricted CD8\textsuperscript{+} T cells. We asked whether modified A18 peptides with antagonist activity for the nominal C5 peptide would rescue the development of CD4 T cells in NTOC of invariant chain-negative A18 thymocytes. However, thymus organ culture in the presence of antagonist peptide did not result in the generation of mature CD4\textsuperscript{+} cells at any concentration tested. Fig. 2 shows antagonist peptide 113V as an example; antagonist 113I behaved in the same way (data not shown). Instead of expected CD4\textsuperscript{+} cells, CD8\textsuperscript{+} cells with the characteristics of mature T cells (TCR\textsuperscript{hi}HSA\textsuperscript{lo}) were generated (Fig. 2 A, 1 \textmu M antagonist and 10 \textmu M antagonist). These cells express CD8\alpha\beta heterodimers on the surface (Fig. 3). The increased percentage of CD8\textsuperscript{+} cells was reproducibly reflected in a 5–10-fold increase in absolute

Figure 2. Generation of MHC class II-restricted CD8\textsuperscript{+} cells in NTOC in the presence of antagonist peptide. Thymocytes from A18 Rag-1\textsuperscript{-/-} newborns on the Ii\textsuperscript{-/-} background (A) or Ii\textsuperscript{+/+} background (B) were analyzed for their expression of CD4, CD8, TCR, and HSA 7 d after organ culture in the absence of peptide (medium) or in the presence of 1 or 10 \textmu M antagonist peptide 113V. Percentages of CD4 and CD8 single positive cells are given in each dot plot. Expression of V\beta 8.3 and HSA is shown on gated CD4 single positive cells and gated CD8 single positives, as indicated. Gray-filled curves in each histogram represent expression on thymocytes from NTOC in medium alone for comparison.
cell numbers as shown in Fig. 4. These CD8+ cells could not have been derived by endogenous receptor rearrangements leading to expression of a class I-restricted TCR because of the absence of RAG-1 protein in those mice. Therefore, it clearly demonstrated that the antagonist peptide caused T cells with the class II-restricted A18 TCR to differentiate into the CD8 lineage. The antagonist peptide is presented on MHC class II since anti–class II antibodies block the generation of CD8 cells in NTOC (Fig. 3). The antigenic C5 peptide was also tested for its influence on positive selection in NTOC. However, it mostly led to deletion of A18 thymocytes. Very inefficient selection of CD8 and CD4 cells occurred in some but not all experiments with C5 peptide at picomolar concentrations (data not shown).

Figure 3. Thymuses from A18 Rag1−/− newborns on the II−/− background were cultured with 10 μM antagonist peptide 113V in the presence of 50 μg/ml anti-class II antibody 14.4.4 (right) or mouse IgG as control (left). Percentages of CD4 and CD8α are given in each dot plot. The histogram below shows expression of CD8β on cells gated for CD8α expression to illustrate coexpression of both CD8 chains.

Figure 4. Increased absolute numbers of CD8 single positive cells after NTOC in the presence of antagonist peptide. Thymic lobes from A18 Rag-1−/− II−/− newborn mice were cultured in medium alone or in medium containing 1 or 10 μM antagonist peptide 113V for 7 d. Thymocytes were counted, stained for the expression of CD4 and CD8, and analyzed by flow cytometry. Absolute numbers of CD8 single positive cells per lobe of five lobes from four independent experiments are shown.

Discussion

We have demonstrated that antagonist peptide can alter lineage decision from CD4+ to CD8+ cells. Previously, Spain et al. (18) did not report an enhanced differentiation into the CD8 lineage, presumably because their mice were not deficient for RAG and therefore contained significant numbers of CD8+ cells in the absence of antagonist peptide. CD8+ cells derived in the presence of antagonist A are functionally mature. The CD8+ cells generated with antagonist peptide in NTOC from A18 II−/− mice displayed a mature phenotype according to their high expression of the TCR and downregulation of expression as shown in Fig. 2.

To investigate the functional ability of these cells, we cultured them with dendritic cells presenting the C5 agonist peptide and assayed for IL-2 production. As expected, given that they contained no mature single positive cells, thymocytes recovered from NTOC from A18 II−/− mice did not react to C5 peptide (Fig. 5, medium). In contrast, lobes cultured in the presence of antagonist peptide gave rise to thymocytes responding to C5 peptide (Fig. 5, 1 μM antagonist and 10 μM antagonist). This response was mediated by the CD8+ population, as depletion of CD8+ cells before the functional assay ablated C5 reactivity. In contrast, depletion of CD4+ cells did not affect C5 reactivity. These results confirm the maturity of the CD8+ cells and also their TCR specificity for C5 peptide. C5 peptide/class II complex recognition by the A18 TCR in the absence of CD4 was observed previously in CD4-negative T cell hybrids (data not shown).
positive selection of CD8+ cells was achieved with peptide analogues of the antigenic peptide or low concentrations of the nominal antigen presented by the selecting MHC class I molecule (3–5). In contrast, positive selection of CD4+ cells could be induced through coengagement of the TCR/CD3 complex with a variety of thymocyte surface molecules such as CD2, CD4, CD5, CD8, CD24, CD28, CD49d, CD81, or TSA-1 (29–33). In addition, cross-linking of TCRβ or CD3γε with mAb (34), as well as targeting thymocytes to thymic cortical epithelium via anti–TCR/CDR-1 hybrid antibodies (31), resulted in the exclusive generation of CD4+ cells even in the absence of MHC molecules. Thus, the signals for CD4 differentiation seemed to be promiscuous in comparison to signals for CD8 differentiation and it was suggested that development into the CD8+ lineage follows a “default” pathway (32). However, we show here that CD8+ rather than CD4+ cells developed, even without the involvement of either CD8 or class I-specific signals. Instead of a default model for either lineage, the involvement of distinct signals seems to be more likely.

In terms of the effect of antagonist peptides, it has been shown that binding of the TCR to MHC molecules occupied by antagonist peptide results in a higher off rate (35, 36). A shorter interaction time between the TCR and its ligand might not allow sufficient time for coreceptor binding and consequently for the recruitment of the tyrosine kinase p56lck (37). Lack of lck recruitment is presumably more debilitating for CD4 lineage cells since a much larger fraction of CD4 than CD8 molecules is associated with lck (38, 39), implying a more prominent role for lck in CD4+ cell development. Therefore, the generation of class II-restricted CD8+ cells in NTOC might be the consequence of insufficient lck recruitment in the presence of antagonist peptide (40). In support of this, class II-restricted T cells choose the CD8 pathway in mice lacking the CD4 molecule (41). Recruitment of lck to the TCR complex was first implied as a major player in CD4 lineage decision by Itano et al. (42). By introducing a hybrid protein consisting of the extracellular and transmembrane domain of CD8 and the cytoplasmic part of CD4, they could generate a large number of MHC class I-restricted CD8+ T cells in transgenic mice. The only known difference to CD8 transgenic mice was more efficient lck recruitment by the CD4 cytoplasmic domain. Basson et al. (43) directed differentiation into the CD8 lineage through TCR engagement independently of MHC specificity by using CD3ε-specific F(ab')2 antibodies. The CD3-F(ab')2 reagent was unable to activate mature T cells and instead resembled an antagonist peptide in terms of downstream signaling and inhibitory effect on agonist peptide responses. Based on this, they hypothesized that CD8+ cell development is favored by antagonist-like signals, which have limited participation of lck signals. On the other hand, CD4 differentiation would require a stronger lck signal in relation to the TCR signal. Our results are consistent with these models and demonstrate for the first time the ability of a single peptide to convert CD4/CD8 lineage decision.

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