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Immunology

Fibroblasts can induce thymocyte positive selection *in vivo*

(differentiation)

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ABSTRACT **During development in the thymus, thymocytes bearing $\alpha\beta$ T-cell receptors are selected to mature if the receptors they bear are able to interact in some way with major histocompatibility complex (MHC) proteins expressed on thymic stromal cells. It has been shown that thymus cortical epithelial cells are usually the cells presenting the MHC molecules involved in this process of so-called positive selection. Here we tested the ability of fibroblasts to mediate positive selection *in vivo*. Fibroblasts transfected with the genes for the MHC I-A^b proteins were injected intrathymically into irradiated H-2^k animals reconstituted with H-2^{bxk} F_1 fetal liver cells. Eight weeks later, the recipient mice were immunized and shown to contain peptide-specific I-A^b-restricted T cells. This demonstrates the ability of I-A^b-transfected fibroblasts to participate in positive selection. Thus a cell type that is not specialized to process and present antigens in the context of MHC class II molecules can mediate positive selection when transfected with an appropriate MHC molecule. The data also support the idea that the ability to mediate positive selection may not be limited to thymic cortical epithelium.**

In order to mature into functional T cells, thymocytes undergo positive selection, a process that selects for maturation those cells that will be able to recognize foreign antigens associated with self major histocompatibility complex (MHC) proteins (1, 2). Positive selection is usually thought to occur during interaction between the $\alpha\beta$ receptors [T-cell receptors (TcRs)] on thymocytes and MHC on thymic stromal cells (3–5). Thymocytes that have been positively selected because of their affinity for self could in principle all be eliminated in the thymus, through clonal deletion, because they represent potential autoreactive clones. This is obviously not the case. To reconcile this paradox two main hypotheses have been suggested. The affinity model (6) proposes that thymocytes with low but perceptible affinity for self-MHC are positively selected in the thymus. Conversely, cells with an affinity reaching levels that would potentially make them autoreactive are deleted. Prompted by reports that cortical epithelial cells are crucial to positive selection (7–11), it was suggested that this thymic compartment may bear specialized proteins that participate in the adherence of the developing thymocytes (12). Alternatively, or in addition, the cortical epithelial cells may bear MHC proteins to which a special set of self-peptides might be bound. Thus according to the latter model, referred to as the altered self-peptide model (12), developing thymocytes could be positively selected by interaction of their TcR with these MHC/self-peptide complexes without danger of recognition of the same complexes outside the thymus, and consequent autoreactivity. The altered self-peptide hypothesis, therefore, relies on the fact that the ability to induce positive selection is restricted to the cortical epithelial cells.

Recently, however, Bix and Raulet (13) have reported that MHC class I molecules on hematopoietic cells used to reconstitute MHC class I-deficient mice could participate, although inefficiently, in positive selection of MHC class I-restricted $CD4^-CD8^+$ T cells. This suggests that special properties on the part of the MHC-bearing cells are not required for the process. On the other hand, many studies (reviewed in ref. 14) including the corresponding experiment using MHC class II-deficient mice showed that MHC class II molecules on bone marrow-derived cells could not participate in positive selection of MHC class II-restricted $CD4^+CD8^-$ T cells (15). These reports are not necessarily contradictory since most of the bone marrow-derived cells in the thymus cortex where positive selection is thought to occur (7–11) lack MHC class II molecules but bear MHC class I proteins (16).

Recently we (17) and others (18) have developed an assay that allows us to assess the ability of various cell lines, injected into the thymus *in vivo*, to participate in positive selection. Using this assay we have previously shown that a thymic epithelial cell line, 2E4, which bears the MHC class II protein I-A^b, can select thymocytes for recognition of foreign antigen associated with I-A^b molecules (17). In this paper, we examine the ability of a fibroblast cell line, transfected with I-A^b genes, to perform the same function. Although fibroblasts are not normally thought to be involved in positive selection, and although these cells do not normally bear MHC class II molecules, the data presented here show that these cells could indeed select thymocytes for recognition of foreign antigen associated with I-A^b molecules.

MATERIALS AND METHODS

Mice. B10.BR (H-2^k) and C57BL/10 (H-2^b) mice were purchased from The Jackson Laboratory and bred in our animal facility under pathogen-free conditions. Day 15–16 embryos were harvested from pregnant females, day 0 corresponding to the day a vaginal plug was found.

Chimeras and Intrathymic Injections. Chimeric animals were prepared as described (5, 17). Briefly, recipient mice received 9.5 Gy of γ-irradiation and were reconstituted 2 hr later with an intravenous injection of 10^7 fetal liver cells from (C57BL/10 × B10.BR)F_1 (H-$2^{b/k}$) day 15–16 donor embryos. Three weeks later, chimeric animals received an intrathymic injection of 10–20 μl of BSS per lobe with or without 1–5 × 10^6 cells.

Cell Lines. The 2E4 thymic epithelial cell line was described previously (17). The LTk fibroblast line was derived from C3H H-2^k mice (19); FT2.3.H4 fibroblasts (a gift from R. Germain, National Institute of Allergy and Infectious Diseases) were obtained by transfecting LTk cells with the genes for the α and β chains of I-A^b (20). We also used the

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Abbreviations: IFN-γ, interferon γ; MHC, major histocompatibility complex; TcR, $\alpha\beta$ T-cell receptor; (T,G)-A- -L, (Tyr,Glu)-poly(DL-Ala)- -poly(Lys).

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B-cell lymphoma CH12.1 (21), the T-cell hybrid BO-97.11 (E. Kushnir, J.W.K., and P.M., unpublished data), the thymic macrophage cell line 1G.18LA (a gift from A. Zlotnik) (22), and a thymic epithelial cell line, HKM5.1, established in this laboratory and derived from B10.M (H-2^f) mice (P.H., J.W.K., and P.M., unpublished data).

Immunization and Proliferative Assay. Mice were immunized in the base of the tail with 50 μg of (Tyr,Glu)-poly(DL-Ala)- -poly(Lys) [(T,G)-A- -L] (a gift from M. Sela) in complete Freund's adjuvant (Sigma). Seven days later, draining lymph nodes were collected from three or four mice per group and pooled, and nylon wool-enriched T cells were cultured (2.5×10^5 per well) for 4 days in Click's medium with mitomycin C-treated H-2^b splenocytes (5×10^5 per well) in the absence or presence of 100 μg of (T,G)-A- -L per ml. Cultures were pulsed with 1 μCi of [^{3}H]thymidine per well (Amersham; 5 Ci/mmol; 1 Ci = 37 GBq) for the last 9 hr of incubation. The cells were then harvested and [^{3}H]thymidine incorporation was counted and expressed as mean cpm.

Flow Cytometric Analysis. The method used was described elsewhere (23) with slight modifications. Briefly, adherent cells were detached by mild treatment with 0.05% trypsin/0.53 mM EDTA (GIBCO) and then washed in 20% fetal bovine serum (FBS) to inhibit trypsin. One million cells were suspended in 50 μl of phosphate-buffered saline (PBS) with 1% FBS/0.1% sodium azide and added to microculture wells with 10 μl of human immunoglobulin (10 μg/ml) and stained with 30 μl of primary antibodies at appropriate dilutions. The following antibodies were used: anti-CD44 (Pgp-1, clone IM7.8.1, American Type Culture Collection; TIB235), fluorescein-conjugated anti-CD54 (ICAM-1, PharMingen), anti-CD11a/18 (LFA-1, clone I21/7.7) (24), anti-CD11b/18 (MAC-1, clone MI/70.15.11, American Type Culture Collection; TIB128), anti-CD31 (clone MEC1.3.3, PharMingen), anti-I-A^b (clone 28-16-8S, American Type Culture Collection; HB35), anti-pan-specific H-2K (clone MI/42.3.9.8, American Type Culture Collection; TIB126), anti-VCAM-1 and anti-ICAM-2 (clones 429 and 3C4.3, respectively, generously given by T. Springer), anti-B7/BB1 [clone 1G10, a gift from N. Nabavi and D. Godfrey (25)], and anti-Lgp55 (clone PA3-795.4.16) (26). After a 25-min incubation at 4°C, cells were washed and incubated with fluorescein isothiocyanate-conjugated sheep anti-mouse immunoglobulin (Silenus), sheep anti-rat immunoglobulin (Silenus), or monoclonal mouse anti-rat κ chain (RG7/9.1). Cells were then analyzed using an Epics C (Coulter Electronics) and data were plotted using the MKFLOW program (written by J.W.K.). As negative controls, cells were stained with the second step reagent only.

RESULTS

Fibroblasts do not express MHC class II molecules (16) and therefore cannot be involved in positive selection of MHC class II-restricted T cells. To determine whether fibroblasts could participate in this process we used fibroblasts transfected with the genes for I-A^b. B10.BR (H-2^k) mice were lethally irradiated and reconstituted with (C57BL/10 × B10.BR)F_1 H-2^{bxk} (F_1) fetal liver cells. Three weeks later, I-A^b-expressing FT2.3.H4 fibroblasts were injected into the thymic lobes of reconstituted mice. Other mice were injected with the I-A^b-expressing thymic epithelial cell line 2E4. This cell line has been shown previously to induce positive selection in this system (17). Negative control animals were injected with the MHC class II^- parental cells of FT2.3.H4, LTk cells. As positive controls, lethally irradiated F_1 mice were reconstituted with F_1 fetal liver cells and mock injected in the thymus. Eight weeks after intrathymic injection mice were immunized with the (T,G)-A- -L synthetic copolymer, an antigen recognized well by T cells in association with I-A^b but poorly in association with I-A^k molecules (17, 27). One week later lymph node T cells were recovered and assessed for their response to (T,G)-A- -L presented by H-2^b splenocytes.

A good response to (T,G)-A- -L was seen with T cells from mock injected $F_1 \rightarrow F_1$ chimeras (Fig. 1). This was expected since the T cells in these animals were positively selected on I-A^b-bearing thymic epithelium. As previously reported, a weak background response to (T,G)-A- -L was obtained with T cells from $F_1 \rightarrow$ B10.BR chimeras given LTk cells that lack I-A^b. This weak response might indicate the presence of some I-A^b-restricted T cells positively selected on I-A^b-bearing,

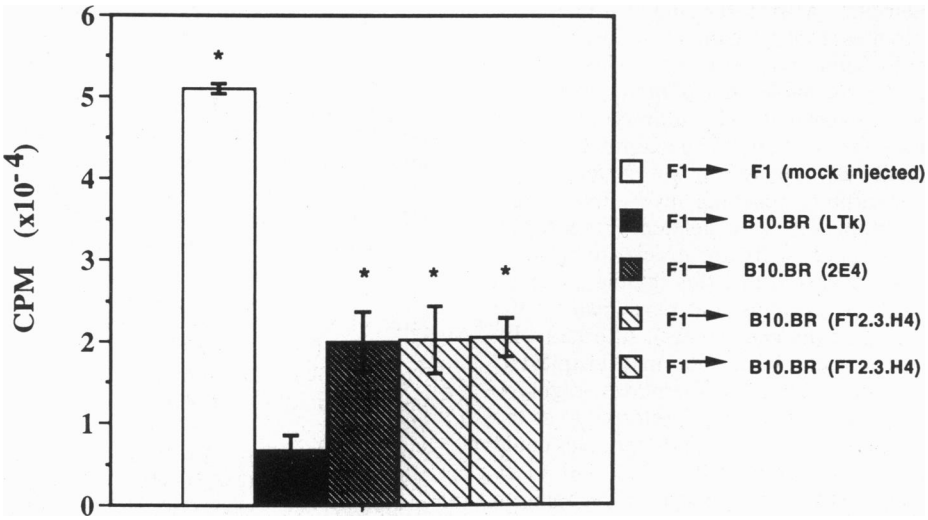

FIG. 1. Capacity of fibroblasts to mediate positive selection. Response to (T,G)-A- -L was measured in $F_1 \rightarrow$ B10.BR chimeras injected intrathymically with I-A^b fibroblasts (FT2.3.H4) and compared to that of $F_1 \rightarrow$ B10.BR chimeric animals injected with I-A^b 2E4 thymic epithelial cells or I-A^- fibroblasts (LTk) (negative control). The response was also measured in mock injected $F_1 \rightarrow F_1$ chimeras (positive control). Results are expressed as the mean ± SE of four to eight replicates with antigen minus background response in absence of antigen. All responses were significantly greater than that of the negative control ($F_1 \rightarrow$ B10.BR chimeras injected with LTk cells); *, $P \leq 0.05$ (Student's t test). Another group of mock injected $F_1 \rightarrow F_1$ chimeras gave equivalent results (data not shown). Two independent groups of $F_1 \rightarrow$ B10.BR chimeras injected intrathymically with I-A^b FT2.3.H4 are presented. Similar increases of (T,G)-A- -L response were obtained from $F_1 \rightarrow$ B10.BR chimeras injected with FT2.3.H4 fibroblasts in two other independent experiments including three groups of mice (not shown).

fetal liver-derived, hematopoietic cells in these animals. If so, such selection was quite inefficient, as previously reported (13). T cells from $F_1 \rightarrow$ B10.BR chimeric mice injected with I-A^b-bearing cell lines, either FT2.3.H4 or 2E4, gave intermediate responses to (T,G)-A- -L. This suggested that I-A^b-restricted T lymphocytes could be found in these chimeric animals.

To demonstrate that the (T,G)-A- -L responses in $F_1 \rightarrow$ B10.BR chimeras given I-A^b-bearing stromal cells were indeed I-A^b-restricted, we examined the effect of an anti-I-A^b antibody on the *in vitro* response to (T,G)-A- -L. This treatment inhibited by >98% the response of T cells from $F_1 \rightarrow F_1$ chimeras (Table 1) expected to contain such I-A^b-restricted T cells. Similarly, the responses of T cells from $F_1 \rightarrow$ B10.BR chimeras injected with FT2.3.H4 or 2E4 cells were suppressed by 75–99%. Thus, the T cells in these mice were responding to (T,G)-A- -L presented by I-A^b. This indicates that intrathymically injected I-A^b-bearing fibroblasts, like thymic epithelial cells, increase the number of I-A^b-restricted T cells in recipient animals. Thus the result shows that fibroblasts can participate in positive selection of MHC class II-restricted T cells.

It is conceivable that some adhesion molecules are essential to the process of positive selection. These might act to increase the overall avidity of interaction between lymphocytes and the selecting cells. We therefore examined the expression of such molecules on the FT2.3.H4 fibroblasts and 2E4 epithelial cell line described above. Both cell types expressed CD44 and surface expression was increased by incubation with interferon γ (IFN-γ) (Fig. 2), a process that probably mimics the effect of intrathymic incubation (17, 28). The role of CD44 on stromal cells and its role in complex formation with thymocytes through one of its ligands, hyaluronate (29), have not been demonstrated yet. Lgp55, for which CD11a/18 might be a counterreceptor (26), was also found on the FT2.3.H4 and 2E4 cells. Lgp55 was initially thought to be ICAM-2 (26) but this has recently been contested (30). CD54 (ICAM-1; counterreceptor, CD11a/18), a molecule thought to be involved in thymocyte differentiation (31, 32), was not expressed on fibroblasts. The low constitutive levels of CD54 on 2E4 cells were elevated by IFN-γ. VCAM-1 [counterreceptor, CDw49d/29 (VLA-4)] was also expressed constitutively on 2E4 but not FT2.3.H4 cells. On the other hand, very low levels of B7/BB1 (counterreceptor, CD28) and ICAM-2 (counterreceptor, CD11a/18) were seen on FT2.3.H4 but not on 2E4 cells. Other adhesion molecules, CD11a/18 (LFA-1; counterreceptor, CD54), CD11b/18 (MAC-1; counterreceptor, CD54), and CD31, were not expressed by either FT2.3.H4 or 2E4 cells. This study revealed that two molecules, CD44 and Lgp55, are expressed on both of the cell types identified as able to induce positive selection. It is therefore possible that either or both of these molecules contributed to the event.

Table 1. Effect of anti-I-A^b antibody on (T,G)-A- -L response of T cells from chimeric animals given FT2.3.H4 fibroblasts or 2E4 cells

Host	Donor	Treatment	Response to (T,G)-A- -L: Control	Response to (T,G)-A- -L: + anti-I-A^b antibody
F_1	F_1	Mock injected	62,801	898
B10.BR	F_1	LTK (I-A^{b-})	1,535	ND
B10.BR	F_1	FT2.3.H4 (I-A^{b+})	25,596	<100
B10.BR	F_1	FT2.3.H4 (I-A^{b+})	4,338	<100
B10.BR	F_1	2E4 (I-A^{b+})	3,070	<100

T cells were harvested from chimeric mice and immunized as described in the legend to Fig. 1 except that immunization with (T,G)-A- -L was performed 5 weeks following intrathymic injection of stromal cells. Nylon wool-enriched T cells (5×10^5) were incubated in microculture wells with or without antigen and H-2^b splenocytes. To inhibit the response, protein A-purified mouse IgG2a anti-I-A^b antibody (75 μg/ml) (clone 25-9-17S, American Type Culture Collection; HB26) was added for the duration of the culture. The addition of 75 μg of mouse IgG2a anti-I-A^k antibody per ml (clone 10.3.6.2, American Type Culture Collection; TIB95) to the T-cell stimulation assay from mock injected $F_1 \rightarrow F_1$ chimeras resulted in <20% inhibition, whereas the anti-I-A^b antibody did not block an I-E$\alpha^d\beta^b$-restricted response in a separate assay (data not shown). ND, not determined.

DISCUSSION

During intrathymic development thymocytes go through several selective events. One of these is positive selection, which picks out cells to mature that will be able to recognize foreign peptides bound to self-MHC molecules. Another event is that of negative selection. Developing thymocytes die if they engage self-peptides bound to self-MHC molecules in the thymus. Both of these events involve interaction between the receptors of developing thymocytes and MHC molecules expressed on thymus stromal cells.

A number of ideas that reconcile these two apparently contradictory events have been proposed. The altered self-peptide hypothesis, for example, suggests that thymic cortical epithelial cells bear a special set of peptides bound to their MHC molecules (12). Positive selection would therefore occur following interaction between TcR and self-peptide/MHC complexes found only on the cortical epithelium, whereas negative selection would involve recognition of MHC molecules associated with self-peptides representative of those found in the periphery, on thymus bone marrow-derived cells. Since the positively selecting peptide/MHC complexes are suggested to be unique to the thymic cortex, this hypothesis would explain the observation that positively selected mature T cells are not activated by self-peptide/MHC complexes found in the periphery.

Since the altered self-peptide hypothesis requires a special property of the selecting cell, its ability to place a special set of self-peptides in its MHC molecules, the hypothesis could be tested by examining the ability of nonspecialized cells to participate in the process. In this study fibroblast cells, transfected with genes for I-A^b, were used as examples of such cells. Intrathymic injection of these cells did increase the ability of T cells that have matured in their presence to respond to peptide antigens associated with I-A^b molecules. Indeed, the T cells in animals injected intrathymically with I-A^b-bearing fibroblasts responded to I-A^b-associated peptides as powerfully as did the T cells from mice injected with an I-A^b-bearing thymic epithelial cell line. However, the variables of the system, such as cell survival after intrathymic injection and the amplification that takes place *in vivo* after injection of antigen (33), do not allow direct comparison of the ability of these two cell types to participate in positive selection. The results also do not demonstrate that fibroblasts can mediate all steps of positive selection and that the process did not rely on the presence of endogenous thymic cortical epithelium. Nevertheless, these results show that a cell type that is not specialized for participation in positive selection, and that does not even normally express MHC class II molecules (16), can present the MHC molecules that are involved in the process. Almost certainly, therefore, the ability to introduce a special set of self-peptides into MHC class II molecules is not a prerequisite for the selecting cells. The altered self-peptide hypothesis is thus probably not correct.

Positive and negative selection can also be reconciled by the affinity hypothesis (6). This idea argues that positive selection occurs during low-affinity reactions between TcR on developing thymocytes and self-peptide/MHC complexes

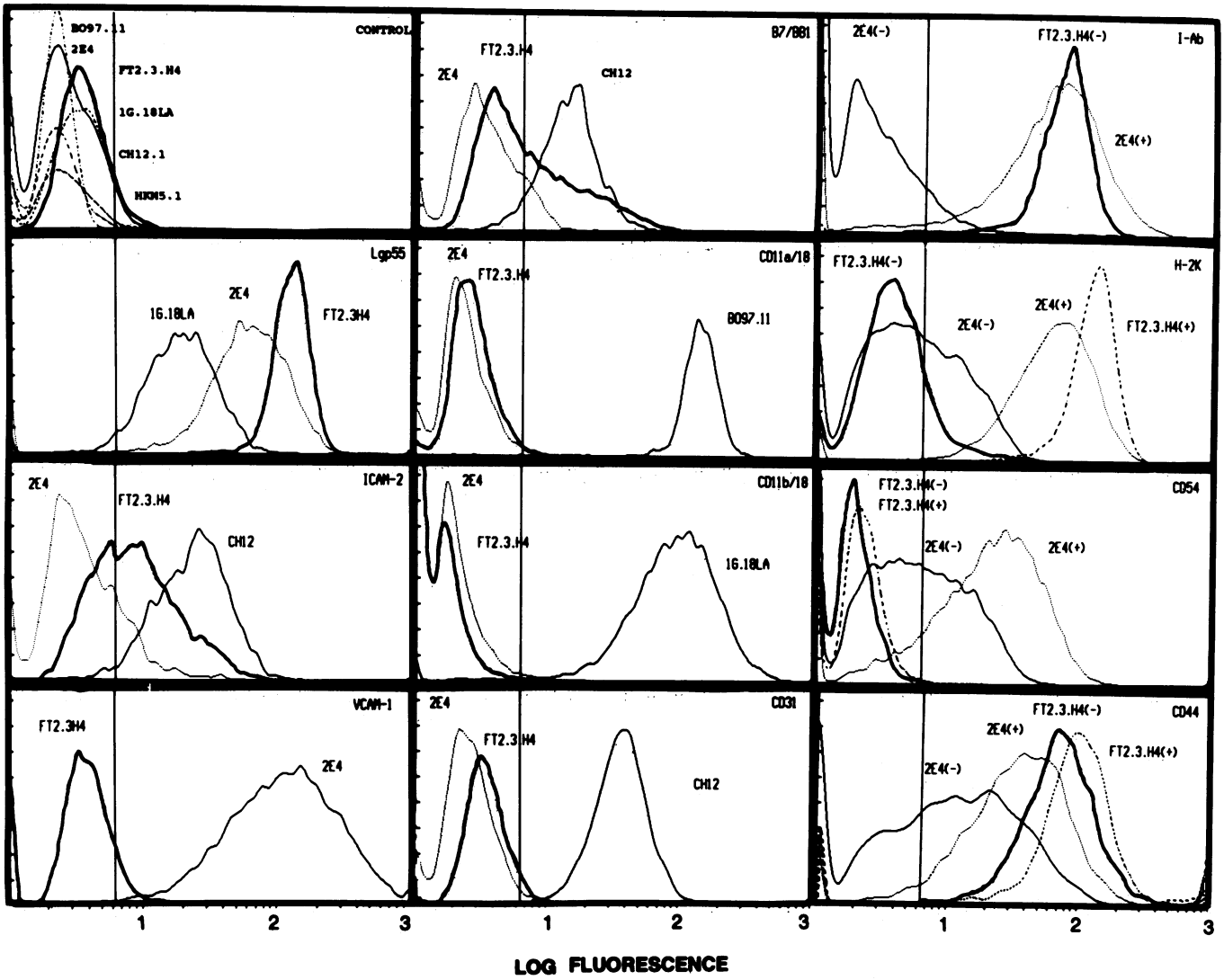

FIG. 2. Expression of selected adhesion molecules by L-cell fibroblasts and 2E4 thymic epithelial cells. Expression of MHC molecules and adhesion molecules on cultured 2E4 cells or I-A^b transfected FT2.3.H4 fibroblasts was determined by flow cytometry. Expression of I-A^b, H-2K^b, CD54, and CD44 was determined on cells cultured 72 hr in absence (−) or presence (+) of 400 units of IFN-γ per ml. As negative control, cells were stained with the second step reagent only. The B-lymphoma CH12.1, T-cell hybrid BO.97-11, thymic macrophage cell line 1G.18LA, and a thymic epithelial cell line, HKM5.1, were treated with trypsin/EDTA and used as positive controls for markers not expressed by 2E4 or FT2.3.H4 cells.

in the thymus. The affinity of these reactions is suggested to be too low to cause subsequent negative selection or activation of the cells once they have become mature T cells and migrated to the periphery. Some versions of the affinity hypothesis suggest that the reaction between thymocytes and stromal cells might be bolstered by special adhesion molecules on the selecting stromal cells and their counterreceptors on thymocytes. If this is true, the fibroblasts and cortical epithelial cells used in this study must express and perhaps share such adhesion molecules. We have found that both fibroblasts and 2E4 thymic epithelial cells express CD44 and Lgp55. Unfortunately, expression of the corresponding counterreceptors for the molecules on thymocytes has not yet been confirmed and their relevance, at least in the process of positive selection, is dubious. Nevertheless, they represent likely candidates for adhesion molecules essential for positive selection. The absence of other molecules, such as CD54, ICAM-2, and VCAM-1, either on fibroblasts or on 2E4 cells indicates that their presence is not essential for positive selection. However, other adhesion molecules such as CD40, CD58 (LFA-3), and ICAM-3 for which counterreceptors are present on thymocytes (gp39, CD2, CD11a/18, respectively) were not examined in this study, since suitable mouse reagents are not yet available. Moreover, it is possible that the thymic milieu provides signals, other than IFN-γ, that induce special adhesion molecules on the selecting cells.

Taken together, our results suggest that fibroblasts can induce positive selection *in vivo*. This implies that no "special properties" are required of the selecting cell itself and therefore that the altered self-peptide hypothesis is not correct. However, since positive selection occurs most efficiently in the thymic cortex, this tissue almost certainly provides ancillary signals that contribute to the event.

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1. Bevan, M. (1977) *Nature (London)* **269**, 417–418.
2. Zinkernagel, R. M., Callahan, G. N., Cooper, S., Klein, P. A. & Klein, J. (1978) *J. Exp. Med.* **147**, 882–896.
3. Benoist, C. & Mathis, D. (1989) *Semin. Immunol.* **1**, 117–124.

4. Pardoll, D. & Carrera, A. (1992) *Curr. Opin. Immunol.* **4**, 162–165.
5. Hugo, P., Kappler, J. W. & Marrack, P. (1993) *Immunol. Rev.* **135**, in press.
6. Lo, D., Ron, Y. & Sprent, J. (1986) *Immunol. Res.* **5**, 221–232.
7. van Ewijk, W., Ron, Y., Monaco, J., Kappler, J., Marrack, P., Le Meur, M., Gerlinger, P., Durand, B., Benoist, C. & Mathis, D. (1988) *Cell* **53**, 357–370.
8. Benoist, C. & Mathis, D. (1989) *Cell* **58**, 1027–1033.
9. Berg, L. J., Pullen, A. M., Fazekas de St. Groth, B., Mathis, D., Benoist, C. & Davis, M. (1989) *Cell* **58**, 1035–1046.
10. Bill, J. & Palmer, E. (1989) *Nature (London)* **341**, 649–651.
11. Cosgrove, D., Chan, S. H., Waltzinger, C., Benoist, C. & Mathis, D. (1992) *Int. Immunol.* **4**, 707–710.
12. Marrack, P. & Kappler, J. (1987) *Science* **238**, 1073–1079.
13. Bix, M. & Raulet, D. H. (1992) *Nature (London)* **359**, 330–333.
14. Sprent, J., Lo, D., Gao, E. K. & Ron, Y. (1988) *Immunol. Rev.* **101**, 173–190.
15. Markowitz, J. S., Auchincloss, H., Grusby, M. J. & Glimcher, L. H. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2779–2783.
16. van Ewijk, W. (1991) *Annu. Rev. Immunol.* **9**, 591–615.
17. Hugo, P., Kappler, J. W., Godfrey, D. I. & Marrack, P. (1992) *Nature (London)* **360**, 679–682.
18. Vukmanovic, S., Grandea, A. G., Faas, S. J., Knowles, B. B. & Bevan, M. J. (1992) *Nature (London)* **359**, 729–732.
19. Kit, S., Dubbs, D. R., Piekarski, L. J. & Hsu, T. C. (1963) *Exp. Cell Res.* **31**, 297–312.
20. Braunstein, N. S. & Germain, R. N. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2921–2925.
21. Arnold, L. W., LoCascio, N. J., Lutz, P., Pennell, C. A., Klapper, D. & Haughton, G. (1983) *J. Immunol.* **131**, 2064–2068.
22. Ransom, J., Wu, R., Fischer, M. & Zlotnik, A. (1991) *Cell. Immunol.* **134**, 180–190.
23. Hugo, P., Waanders, G. A., Scollay, R., Shortman, K. & Boyd, R. L. (1990) *Int. Immunol.* **2**, 209–218.
24. Trowbridge, I. S. & Bishr Omary, M. (1981) *J. Exp. Med.* **154**, 1517–1524.
25. Nabavi, N., Freeman, G. J., Gault, A., Godfrey, D., Nadler, L. M. & Glimcher, L. H. (1992) *Nature (London)* **360**, 266–268.
26. Golde, W. T., McDuffie, M., Kappler, J. & Marrack, P. (1990) *J. Immunol.* **144**, 804–810.
27. McDevitt, H. O., Deak, B. D., Shreffler, D. C., Klein, J., Stimpeling, J. H. & Snell, G. D. (1972) *J. Exp. Med.* **135**, 1259–1278.
28. Lo, D. & Sprent, J. (1986) *J. Immunol.* **137**, 1772–1775.
29. Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C. B. & Seed, B. (1990) *Cell* **61**, 1303–1313.
30. Xu, H., Tong, I. L., de Fougerolles, A. R. & Singer, T. A. (1992) *J. Immunol.* **149**, 650–655.
31. Kaye, J. & Ellenberger, D. L. (1992) *Cell* **71**, 423–435.
32. Fine, J. S. & Kruisbeek, A. M. (1991) *J. Immunol.* **147**, 2852–2859.
33. MacDonald, H. R., Casanova, J. L., Maryanski, J. L. & Cerottini, J. C. (1993) *J. Exp. Med.* **177**, 1487–1492.