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Immunology

Adhesion of immature thymocytes to thymic stromal cells through fibronectin molecules and its significance for the induction of thymocyte differentiation

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ABSTRACT **Only 10–15% of unseparated thymocytes adhered to culture plates precoated with fibronectin (FN), but 60–70% of the CD4$^-$8$^-$ (double-negative) thymocyte population did. This population bound to FN but not to collagen, laminin, or vitronectin. Its binding to FN was inhibited by anti-FN antibody or a mixture of synthetic peptides corresponding to two different sites of FN, termed the V10 sequence and the RGDS (Arg-Gly-Asp-Ser) sequence, which interact, respectively, with the VLA-4 and VLA-5 FN receptors expressed on T-lineage cells. CD4$^-$8$^-$ thymocytes also adhered to a monolayer of a thymic stromal cell clone, MRL104.8a, that induces growth-maintenance and differentiation of such thymocytes. The involvement of FN–FN receptor interaction in this adhesion was demonstrated by the following lines of evidence: (*i*) the MRL104.8a cells expressed FN molecules on their surface and (*ii*) the adhesion of CD4$^-$8$^-$ thymocytes to MRL104.8a monolayers was almost completely inhibited by simultaneous addition of anti-FN antibody and a mixture of peptides (V10 plus RGDS) capable of binding to anti-FN receptors (VLA-4 and -5). Most important, blocking the adhesion of CD4$^-$8$^-$ thymocytes to the thymic stromal cell monolayer resulted in potent inhibition of the differentiation of these thymocytes, which was otherwise induced toward the expression of CD4 and/or CD8 molecules. These results indicate that immature CD4$^-$8$^-$ thymocytes adhere to thymic stromal cells preferentially through FN–FN receptor interaction and that such adhesion has a critical role in inducing and/or supporting the differentiation of these thymocytes.**

Lymphocytes express a number of surface molecules that mediate the adhesion of cells to one another or to extracellular matrix (ECM) components (1–4). For example, cell–cell interaction molecules such as CD2 and LFA-3 are used to augment specific adhesion of T cells to antigen-presenting cells (5), and other cell–cell interaction molecules function as homing receptors (6, 7). Thus, these cell surface molecules have been recognized as regulating the migration of lymphocytes as well as the interactions of activated lymphocytes during immune responses.

Another type of cell adhesion mechanism involves the interaction of cells with ECM (2, 4). While the importance of such cell–cell interactions in developmental biology has been documented (8–12), few studies have investigated the role of cell–ECM adhesion mechanisms in lymphocyte development in lymphopoietic microenvironments such as the bone marrow and thymus. This may be ascribed to the lack of *in vitro* systems for such analyses and to the complexity arising from the expression of various forms of ECM molecules regulated in a tissue- and cell-specific fashion, as exemplified by fibronectin (FN) molecules (13–15). In this context, several lines of marrow and thymic stromal cells have been isolated to provide tools for analyzing the interaction between developing lymphocytes and stromal cells (16). We have also established a thymic stromal cell clone that is capable of providing an *in vitro* model for intrathymic T-cell development (17–19).

The present study investigates the role of FN molecules expressed on thymic stromal cells in thymocyte–stromal cell adhesion and thymocyte differentiation. The results demonstrate that CD4$^-$8$^-$ ("double-negative") thymocytes adhere to thymic stromal cells through FN molecules on the stromal cells. Molecular analyses revealed that two adhesion sites, the classic RGDS (Arg-Gly-Asp-Ser) sequence and the RGDS-independent V10 sequence in the V region, on FN molecules were involved, although the adhesion through the latter was more potent. Most important, blocking of the adhesion of CD4$^-$8$^-$ thymocytes to stromal cells resulted in potent inhibition of differentiation of CD4$^-$8$^-$ thymocytes on the thymic stromal cell monolayer, indicating the critical role of FN–FN receptor interaction in intrathymic T-cell development.

MATERIALS AND METHODS

Thymic Stroma-Derived Cell Clone. The clone MRL104.8a was established from long-term liquid-phase cultures of thymic stromal cells from MRL-*lpr/lpr* mice (17).

Reagents. Bovine serum albumin and mouse FN were from Chemicon; porcine collagen, mouse laminin, and human vitronectin from the Iwaki Glass Works (Tokyo); peptides GRGDSP and GRGESP from Bachem; rabbit anti-mouse FN antibody from Biomedical Technologies (Stoughton, MA); and normal (control) rabbit IgG from Cappel Laboratories. Peptides V10 (GPEILDVPST) and V10-s (VIPDLTESPG; the scrambled version of the V10 peptide containing the same amino acids but in a different order; ref. 15) were synthesized on a peptide synthesizer (Applied Biosystems) and purified on an ODS column (RQ-2, Wako Pure Chemical, Osaka) at the Shionogi Research Laboratories.

Preparation of CD4$^-$8$^-$ Thymocyte Population. Thymocytes from young adult (3- to 5-week-old) C3H/He mice were treated with antibodies to CD8 (3.155) and CD4 (RL172.4) in the presence of complement as described (19). Only the CD4$^-$8$^-$ cell preparations that were >95% and >98% pure, respectively, were used in the thymocyte-binding assays and differentiation cultures. Approximately 30% of the CD4$^-$8$^-$ cells were CD3$^+$ (expressing either the $\alpha\beta$ or the $\gamma\delta$ T-cell antigen receptor).

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Abbreviations: ECM, extracellular matrix; FN, fibronectin.

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Culture Medium. For short-term thymocyte binding assays, Eagle's minimum essential medium (MEM) was used. ASF301 culture medium (Ajinomoto, Kawasaki, Japan) was used without supplementation with serum for 24-hr cocultures of thymic stromal cells and thymocytes to examine thymocyte differentiation.

Thymocyte Binding Assays. Twenty-four-well culture plates (Corning) were coated with various proteins by overnight exposure to 10 μg/ml solutions of each protein. Monolayers of the MRL104.8a thymic stromal cell clone were prepared in 24-well Corning culture plates (18). Thymocytes were added to the protein-coated plates or plates of the MRL104.8a monolayers and incubated for 30 min at room temperature in the absence or presence of various blocking reagents. Unattached cells were removed by gentle pipetting, and wells of the plates were washed three times with MEM. Attached cells were recovered with phosphate-buffered saline (PBS) containing EDTA. The unattached and attached cells were counted, and percent binding was calculated as follows: % binding = (no. of attached cells/no. of unattached and attached cells) × 100. Relative percent binding of thymocytes in the presence of various blockers was calculated as follows: relative % binding = (% binding in the presence of blocker/% binding in the absence of blocker) × 100.

Immunofluorescence Staining and Flow Microfluorometry. Thymocytes were doubly stained with fluorescein-conjugated anti-L3T4 (GK1.5) antibody and with biotinylated anti-Lyt-2 (3.155) antibody followed by phycoerythrin-conjugated streptavidin (19). Thymic stromal cells were stained with rabbit anti-FN antibody followed by fluorescein-conjugated goat anti-rabbit immunoglobulin antibody.

Northern Blot Analysis. Isolation of mRNA and Northern blot analysis were done as described (20). Poly(A)$^+$ RNA (5 μg per lane) was electrophoresed in a 2.2 M formaldehyde/1% agarose gel and transferred onto a nylon membrane. The hybridization probes were the 135-base-pair *Sac* II–*Pst* I genomic DNA fragment of pFC40R3K, for chicken FN exon 2 (21), and a 30-mer oligodeoxynucleotide, for the V10 segment of the V region of rat FN (22).

RESULTS

Selective Adhesion of CD4$^-$8$^-$ Thymocytes to FN-Coated Plates. CD4$^-$8$^-$ thymocytes were isolated from normal C3H/He thymuses after four killing cycles using anti-CD4 and anti-CD8 antibodies in the presence of complement (19). The whole or CD4$^-$8$^-$ fraction of thymocytes was allowed to attach to culture plates precoated with various proteins such as bovine serum albumin (control), FN, collagen, laminin, and vitronectin. After a 30-min incubation, unattached cells were removed by gentle pipetting, and the plates were washed three times with MEM. Attached cells were recovered with PBS containing EDTA. The unattached and attached cells were counted, and the percent binding of the thymocytes was calculated. Only 10–15% or 5–15% of the whole fraction of thymocytes bound to FN or vitronectin, respectively. In contrast, 60–70% of CD4$^-$8$^-$ thymocytes adhered selectively to FN-precoated plates without exhibiting adhesion to plates precoated with other ECM proteins (Table 1). While a typical CD4$^-$8$^-$ population contained about 30% CD3$^+$ cells, the ratio of CD3$^-$ cells to CD3$^+$ cells was changed only marginally in CD4$^-$8$^-$ thymocytes adhering to FN-coated plates.

Adhesion Sites on FN for CD4$^-$8$^-$ Cells. We tried to determine the sites of FN molecules to which CD4$^-$8$^-$ thymocytes adhere. Two kinds of peptides, a hexapeptide containing the RGDS sequence and the V10 decapeptide, each of which is reactive to VLA-5 (1, 2, 14) or VLA-4 (15) protein on lymphoid cells, were used as competitive blockers for the adhesion of CD4$^-$8$^-$ thymocytes to FN-coated plates. Table 2 shows that addition of the RGDS peptide to culture plates resulted in no (Exp. 1, 0.5 mg/ml) or ≈35% (Exp. 2, 1 mg/ml) reduction of relative percent binding of CD4$^-$8$^-$ thymocytes to FN molecules, whereas the V10 peptide induced as much as 50% inhibition. More potent (Exp. 1) or almost complete (Exp. 2) inhibition of the binding was obtained when both types of peptides were simultaneously included or when rabbit anti-mouse FN antibody was used as a blocker instead of peptide samples. Neither the mixture of control RGES and V10-s peptides (the scrambled version of the V10 peptide containing the same amino acids but in a different order) nor normal (control) rabbit IgG inhibited the binding. The binding of CD4$^-$8$^-$ thymocytes to FN-coated plates and its inhibition by various blockers are also illustrated by phase-contrast micrographs (Fig. 1). These results indicate that CD4$^-$8$^-$ thymocytes adhere to FN at two sites, corresponding to the RGDS adhesion sequence or the RGDS-unrelated V10 segment in the V region. Binding is more potent at the V10 site than at the RGDS site.

Table 1. Binding of whole or CD4$^-$8$^-$ thymocyte population to various adhesion molecules

Exp.	Thymocyte fraction	% binding to coated plates				
		BSA	FN	Coll	Lam	VN
1	Whole	1.4	15.3	2.3	0.6	10.4
	CD4$^-$8$^-$	6.0	70.5	4.1	1.2	2.3
2	Whole	0.3	12.6	ND	ND	ND
	CD4$^-$8$^-$	3.4	60.2	3.2	1.3	0.0

Plastic tissue culture plates were coated with the indicated proteins by overnight exposure to 10 μg/ml solutions of each protein. The whole and CD4$^-$8$^-$ fractions of thymocytes (2 × 10^6 cells per well) were placed onto the protein-coated plates and incubated for 30 min at room temperature. Unattached cells were removed and attached cells were recovered with PBS containing EDTA. Percent binding was calculated according to the formula in *Materials and Methods* and expressed as the mean of duplicate wells. Standard errors, which were <15% in each group, were excluded for simplicity. BSA, bovine serum albumin; Coll, collagen; Lam, laminin; VN, vitronectin; ND, not done.

Binding of CD4$^-$8$^-$ Thymocytes to MRL104.8a Monolayers and Its Inhibition by Various Blockers. We next asked whether CD4$^-$8$^-$ thymocytes adhere to thymic stromal cells and, if they do, whether FN–FN receptor interaction is involved in the event. Monolayers of the MRL104.8a thymic stromal cell clone were prepared in 24-well culture plates coated with collagen. The plates were precoated with collagen to strengthen the binding of stromal cells to them. CD4$^-$8$^-$ thymocytes were added onto the MRL104.8a monolayers in the absence or presence of various blocking reagents. Strong binding of CD4$^-$8$^-$ thymocytes to the MRL104.8a monolayer was seen in the absence of blockers (Fig. 2*A*). Percent binding was in the range 60–80% in five consecutive experiments (data not listed). Binding was appreciably blocked by either the RGDS/V10 peptide mixture or anti-FN antibody and more efficiently blocked by the combination of peptides plus anti-FN antibody (Table 3; Fig.

Table 2. Blocking of CD4$^-$8$^-$ cell–FN interaction by synthetic peptides reactive with FN receptors or by anti-FN antibody (α-FN Ab)

Exp.	Relative % binding to FN						
	No blocker	RGDS	V10	RGDS/ V10	RGES/ V10-s	Control IgG	α-FN Ab
1	100	117.6	57.2	40.6	105.4	124.2	10.7
2	100	65.4	49.9	6.0	110.3	105.4	10.6

CD4$^-$8$^-$ thymocytes (2 × 10^6 cells per well) were added to FN-coated plates in the absence or presence of the indicated blocking reagents at 0.5 mg/ml (Exp. 1) or 1 mg/ml (Exp. 2). Relative percent binding was calculated as described in *Materials and Methods*.

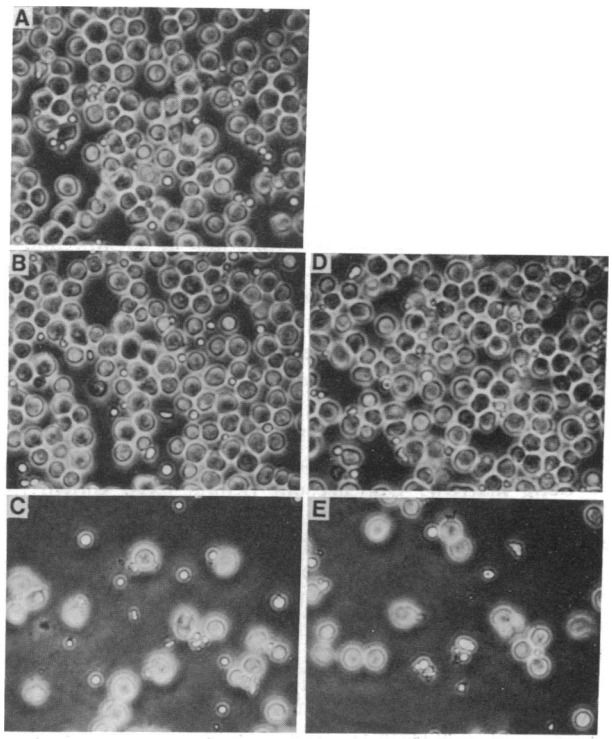

FIG. 1. Inhibition of $CD4^-8^-$ thymocyte–FN interaction by various blockers. $CD4^-8^-$ thymocytes (2×10^6 cells per well) were cultured on FN-precoated plates in the absence of inhibitor (*A*) or in the presence of the RGES/V10-s control peptide mixture (*B*), the RGDS/V10 peptide mixture (*C*), control IgG (*D*), or anti-FN antibody (*E*). Each blocker was present at 1 mg/ml. Pictures were taken after removal of unattached thymocytes. (×100.)

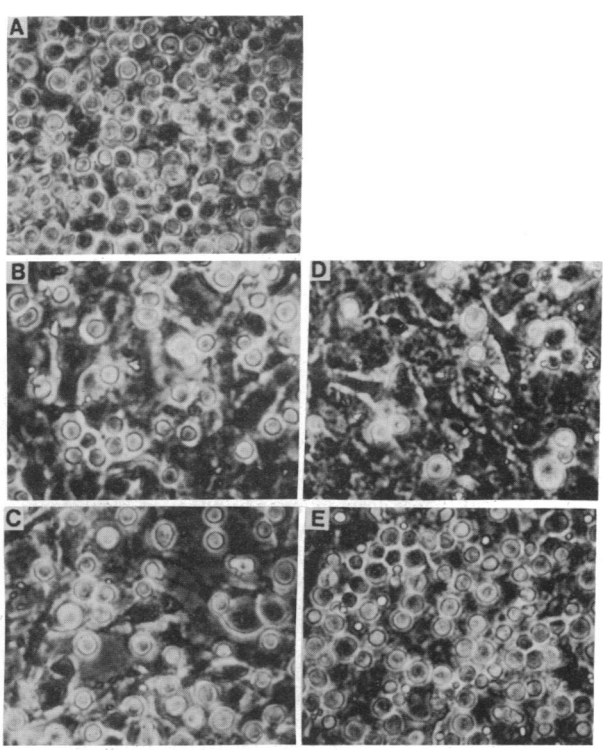

FIG. 2. Inhibition of $CD4^-8^-$ thymocyte–MRL104.8a interaction by various blockers. $CD4^-8^-$ thymocytes (2×10^6 cells per well) were cultured on a monolayer of the MRL104.8a clone in the absence of blocker (*A*) or presence of RGDS plus V10 (*B*), anti-FN antibody (*C*), RGDS plus V10 plus anti-FN antibody (*D*), or RGES plus V10-s plus control IgG (*E*). Concentration of each blocker was 1 mg/ml. (×100.)

Table 3. Inhibition of $CD4^-8^-$ thymocyte binding to MRL104.8a monolayer by anti-FN antibody (α-FN Ab) and/or synthetic peptides reactive with FN receptors

	Relative % binding to MRL104.8a monolayer						
Exp.	No blocker	RGDS	V10	RGDS/ V10	α-FN Ab	RGDS/ V10/ α-FN Ab	RGES/ V10-s/ control IgG
1	100	97.7	87.8	65.9	67.8	33.2	101.3
2	100	99.9	55.6	54.9	59.9	18.1	104.8

Monolayers of the MRL104.8a thymic stromal cell clone were prepared in 24-well culture plates that had been coated with collagen. $CD4^-8^-$ thymocytes (2×10^6 cells per well) were added to the MRL104.8a monolayers in the absence or presence of various blockers at 0.5 mg/ml (Exp. 1) or 1 mg/ml (Exp. 2).

2 *B–E*). These results indicate that the FN–FN receptor interaction is deeply involved in the adhesion of $CD4^-8^-$ thymocytes to MRL104.8a thymic stromal cells.

Expression of FN Molecules Including the V10 Segment by MRL104.8a Thymic Stromal Cells. The preceding results suggested the expression of FN molecules on MRL104.8a cells. To demonstrate this directly, we first examined the expression of FN mRNA by MRL104.8a cells. mRNA was isolated from MRL104.8a cells and analyzed by Northern blotting using a FN DNA probe as well as a synthetic oligonucleotide probe for the V10 segment. MRL104.8a cells expressed high levels of ≈9-kilobase FN mRNA (Fig. 3*A*, lane b) whereas $CD4^-8^-$ thymocytes did not express detectable FN mRNA (lane a). FN mRNA from MRL104.8a cells also hybridized with the oligonucleotide probe corresponding to the V10 segment (lane c), indicating the presence of FN mRNA bearing the nucleotide sequence for the V10 segment in the total FN mRNA.

As shown in a representative flow microfluorometric analysis (Fig. 3*B*), MRL104.8a cells expressed FN on the cell surface, whereas $CD4^-8^-$ thymocytes did not.

Differentiation of $CD4^-8^-$ Thymocytes on an MRL104.8a Monolayer Is Inhibited by Reagents Able to Block Cell–Cell

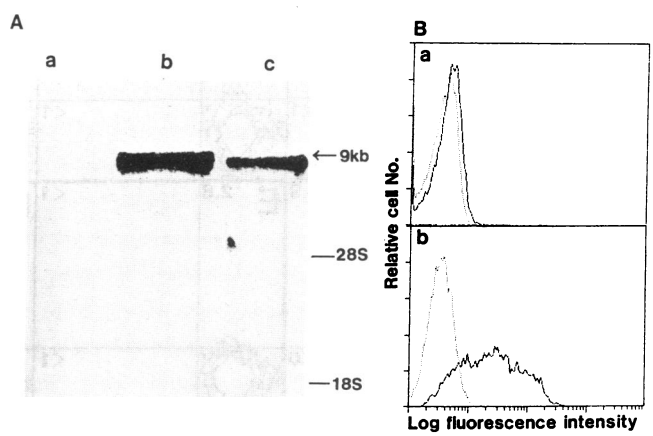

FIG. 3. Expression of FN molecules by MRL104.8a thymic stromal cells. (*A*) Northern blot analysis. Five micrograms of poly(A)$^+$ RNA from $CD4^-8^-$ thymocytes (lane a) or MRL104.8a cells (lanes b and c) was electrophoresed and transferred to a filter. The filter was hybridized with FN DNA (lanes a and b) or V10-specific oligonucleotide (lane c) as a probe. The probes were then removed and the filter was rehybridized with a 1.1-kilobase (kb) fragment of mouse β-actin cDNA to ensure that equivalent amounts of RNA were loaded and transferred (data not shown). Positions of the 9-kb FN mRNA and 28S and 18S rRNAs are indicated. (*B*) Flow microfluorometry. $CD4^-8^-$ thymocytes (*a*) or MRL104.8a cells (*b*) were stained with normal rabbit IgG (----) or rabbit anti-mouse FN antibody (—) followed by fluorescein-conjugated goat anti-rabbit immunoglobulin.

Adhesion. We investigated whether the adhesion between stromal cells and $CD4^-8^-$ thymocytes that is mediated by FN–FN receptor interaction has a biological significance for thymocyte differentiation. The $CD4^-8^-$ thymocyte population (>98% purity) was cultured alone or on the MRL104.8a monolayer in ASF301 culture medium (serum-free) in the absence or presence of various adhesion-blocking reagents. After 1 day in culture, cells were recovered and stained for CD8 and CD4 antigens. The recovery of viable cells was 10–20% of input and <3.6% of the cells expressed CD4 and/or CD8 when the cells were cultured alone (Fig. 4*A*). In contrast, 30–40% of input cells were recovered after culture for 1 day on the MRL104.8a monolayer and an appreciable percentage of these cells expressed CD8 (CD8 single-positive; an intermediate stage of cells) or CD8 plus CD4 (double-positive) (Fig. 4*B*, Exps. 1 and 2), which is essentially concordant with previous results obtained by using culture medium supplemented with fetal bovine serum (19). Such differentiation was not influenced by addition of the RGDS/V10 peptide mixture to the cultures (Fig. 4*D*). However, the addition of anti-FN antibody alone (Fig. 4*C*) or anti-FN antibody plus the peptide mixture (Fig. 4*E*, Exps. 1 and 2) to cultures resulted in inhibition of differentiation of these thymocytes. The combination of control blockers did not affect the differentiation (Fig. 4*F*, Exps. 1 and 2). The viable cell recovery was not significantly affected by any of the blockers; but the recovery was 30–40% in five consecutive experiments, including Exps. 1 and 2. Thus, these observations indicate that the adhesion of $CD4^-8^-$ thymocytes to thymic stromal cells is critical for their differentiation toward the expression of CD4 and/or CD8.

DISCUSSION

The major findings of this study are that (*i*) $CD4^-8^-$ thymocytes adhere to thymic stromal cells mainly through FN (on stromal cells)–FN receptor (on thymocytes) interactions, (*ii*) two different sites on FN molecules (RGDS sequence and RGDS-independent V10 sequence in the V region) are involved in this interaction, and (*iii*) the FN–FN receptor interaction has a critical role in inducing the differentiation of $CD4^-8^-$ thymocytes toward the expression of CD4 and/or CD8 antigen.

These findings should be discussed individually. (*i*) Few studies have investigated the function of ECMs and/or integrins involved in lymphocyte development. It has been found that immature lymphoid cells such as pre-B cells in bone marrow (23) and $CD4^-8^-$ thymocytes in the thymus (24) bind to FN by using FN receptors that they preferentially express. The immature lymphoid cells became bound to FN-coated plates, but those studies did not investigate whether they interact with stromal cells that represent a hematopoietic inductive microenvironment in the thymus and bone marrow. Our present results have shown that $CD4^-8^-$ thymocytes interact strongly with thymic stromal cells expressing a large amount of FN.

(*ii*) For several of the adhesion molecules, a single gene may produce a variety of different transcripts through alternative splicing of the relevant RNA, and these transcripts generate different protein products (14, 25–30). Humphries *et al.* (31–33) have reported that one of the alternatively spliced segments (V region) of FN contains a cell adhesion-related portion. Guan and Hynes (15) have demonstrated that the V25 segment, which can be selectively spliced out independently of the rest of the V region, represents a cell adhesion site different from the classic adhesion site, the RGDS sequence. Their results also indicated that the adhesion site is limited to the V10 sequence of the V25 segment and that the V10 segment acts as the ligand for the VLA-4 protein expressed on lymphoid cells (15). Although VLA-4 has been identified as the receptor for the VCAM-1 adhesion molecule expressed on endothelial cells

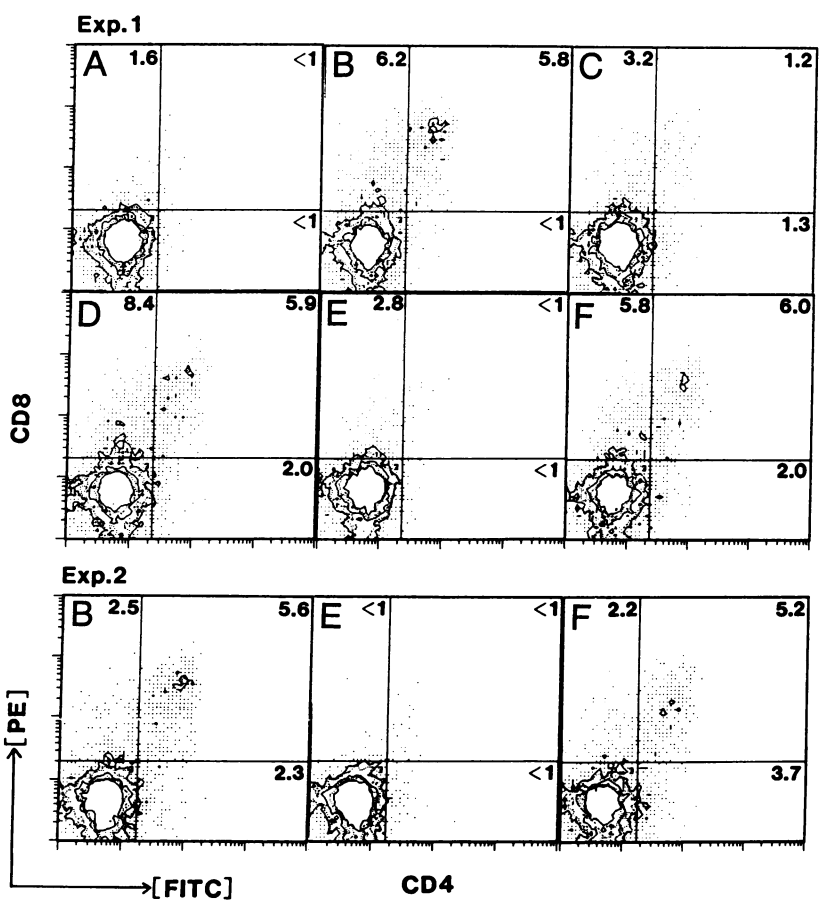

FIG. 4. Inhibition of $CD4^-8^-$ thymocyte differentiation by adhesion-blocking reagents. $CD4^-8^-$ thymocytes (10^6 per well) were cultured alone (*A*) or on the MRL104.8a monolayer in the absence (*B*) or presence of anti-FN antibody (*C*), RGDS plus V10 peptides (*D*), anti-FN antibody plus RGDS plus V10 (*E*), or control IgG plus RGES and V10-s peptides (*F*) in 24-well culture plates for 1 day. Concentration of each blocker was 0.5 mg/ml. Cells recovered 1 day after culturing were stained doubly for CD8 and CD4 antigens and analyzed for two-color fluorescence (PE, phycoerythrin; FITC, fluorescein isothiocyanate). Numbers in the $CD4^-8^+$, $CD4^+8^+$, and $CD4^+8^-$ quadrants are the percentages of cells stained by the reagents.

(34, 35), it appears that the V10 segment of FN is also a ligand for VLA-4 (5, 35). The amino acid sequence of the V25 segment is well conserved in various species (36, 37), suggesting an important role for this segment.

The rat V10 peptide and corresponding oligonucleotide were prepared and used in the present mouse system. The results indicated the efficient blocking capacity of the rat V10 peptide in the binding assay and hybridization of the rat V10 oligonucleotide probe in Northern blot analysis. It appears that this segment on FN represents the primary cell adhesion site for $CD4^-8^-$ thymocytes (Table 2, Exp. 1). It is also likely that the RGDS sequence is another cell adhesion site on $CD4^-8^-$ thymocytes, because the FN binding of these double-negative thymocytes is more efficiently blocked by the mixture of V10 and RGDS peptides (Table 2, Exp. 2). Thus, $CD4^-8^-$ thymocytes adhere to FN at its V10 and RGDS sites, which interact with the VLA-4 and VLA-5 integrins, respectively.

(*iii*) What is the biological significance of $CD4^-8^-$ thymocyte–stromal cell adhesion through FN–FN receptor (VLA-4 and -5) interactions? The blocking of such adhesion with anti-FN antibody plus the V10/RGDS peptide mixture resulted in almost complete inhibition of differentiation of $CD4^-8^-$ thymocytes (Fig. 4). While the mixture of V10 and RGDS peptides functioned as an efficient blocker in the short-term cell adhesion assays, this combination of reagents failed to inhibit the differentiation of $CD4^-8^-$ thymocytes in 1-day cultures. In contrast, differentiation was strongly inhibited by an anti-FN antibody that blocked the adhesion of $CD4^-8^-$ thymocytes to the MRL104.8a monolayers no more effectively than the peptide mixture. This could be explained by postulating differential turnovers of peptides and antibody during 1-day cultures.

In a previous study (19), the differentiation of $CD4^-8^-$ thymocytes into a $CD4^-8^+$ intermediate but not into a $CD4^+8^+$ stage was induced in the absence of the MRL104.8a monolayers by addition of partially purified samples of stroma-derived growth and differentiation factors (19). However this was observed in culture medium supplemented with fetal bovine serum, which contains serum FN capable of coating the culture plates, whereas the present study used serum-free culture medium. It remains to be determined whether FN coated on culture plates produces some contribution to $CD4^-8^-$ thymocyte differentiation. However, when $CD4^-8^-$ thymocytes were cultured without the stromal cell monolayer in medium free of fetal bovine serum (serum FN), they failed to differentiate into even a $CD4^-8^+$ intermediate stage (Fig. 4*A*). Thus, the interaction of $CD4^-8^-$ thymocytes (FN receptors) with thymic stromal cells (FN molecules) is likely to be critical for differentiation of $CD4^-8^-$ thymocytes on stromal cells.

These observations raise the question of whether the signal(s) from FN receptors (VLA-4 and VLA-5) on $CD4^-8^-$ thymocytes are involved in inducing their differentiation. It is evident that VLA proteins (2, 38–40) as well as adhesion receptors such as LFA-1 (41–43) induce signal transduction for cellular proliferation when they are stimulated by the relevant ligands. However, it is unclear whether these adhesion-related receptors function to transduce differentiation signals in immature cells of the T lineage. Thus, the present approach could provide a model system for investigating the role and molecular mechanism of FN receptors in T-cell differentiation in the thymus.

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