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# Cytotoxic T-lymphocyte interaction with fibronectin and vitronectin: activated adhesion and cosignalling

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## SUMMARY

Stimulation of cloned cytotoxic T lymphocytes (CTL) with anti-T-cell receptor (TCR) monoclonal antibody (mAb) in solution resulted in rapid and sustained activation of adhesion to immobilized fibronectin (FN) but did not initiate degranulation. Addition of a second antibody (Ab) to further cross-link the TCR substantially increased the level of adhesion and also activated degranulation, as measured by release of serine esterase, in the presence of immobilized FN but not in its absence. Thus, binding to FN can provide a costimulatory signal to activate degranulation. TCR cross-linking also activated CD8-dependent adhesion to class I, and CD8 provided a costimulatory signal upon binding to class I. However, the requirements for activating adhesion and generating the costimulatory signal differed significantly for FN versus class I ligand, suggesting that these two receptor–ligand systems do not share a common mechanism of action. Co-immobilizing FN and alloantigen resulted in increased serine esterase release in comparison with that stimulated by antigen alone, and required the FN and class I be on the same surface. Peptide and antibody blocking demonstrated that CTL binding to FN, and to vitronectin (VN), was mediated by the $\alpha V\beta_3$ vitronectin receptor (VNR). Thus, VNR is activated by a signal from the TCR to mediate adhesion to FN or VN, and delivers a costimulatory signal for degranulation via a different mechanism than costimulation by CD8 binding to class I.

## INTRODUCTION

Activation of T cells involves not only occupancy of the T-cell receptor (TCR), but also the costimulatory interactions of accessory cell surface receptors and their ligands. Several cell adhesion receptors have been implicated as T-cell costimulatory molecules, including CD8, leucocyte function-associated antigen-1 (LFA-1) and very late antigens (VLA). Previous work has shown that CD8-dependent adhesion of cloned cytotoxic T lymphocytes (CTL) to class I major histocompatibility complex (MHC) is activated via a signal from the TCR, and that the binding of CD8 to class I results in generation of a signal required to activate degranulation and release of serine esterase from the cells,[1,2] one of the mechanisms by which CTL kill target cells. Further, it has been shown that CTL binding to fibronectin (FN) can also generate a costimulatory signal to activate degranulation[3] and induce tyrosine phosphorylation of a 120 000 MW protein,[4] but the receptor mediating the adhesion and signalling was not identified.

Members of the $\beta_1$ integrin family of adhesion receptors bind to various extracellular matrix proteins including FN, a large dimeric protein present in plasma, in tissue and on cell surfaces.[5] VLA-5 mediates attachment to FN via binding to an arginyl-glycyl-aspartyl-serine (RGDS) sequence,[6,7] while VLA-4 interacts with CS1, a sequence found in the alternatively spliced IIIcs region of FN.[8] Resting human T cells express clearly detectable levels of both VLA-4 and VLA-5, but do not adhere to FN unless activated through CD3–TCR or CD2;[9] activation results in a rapid induction of binding with no change in surface expression of the integrins.[9] In addition, several groups have reported that the binding of VLA to FN can provide a costimulatory signal(s) for proliferation of resting T cells in response to anti-CD3 antibody.[10–12] Adhesion to FN can also be mediated by the $\alpha V\beta_3$ vitronectin receptor (VNR), which is present primarily on activated T cells.[13] VNR can function as a costimulatory molecule for T-cell activation[14] but TCR-dependent activation of VNR-mediated adhesion has not previously been reported.

The results described in this report demonstrate that CTL binding to FN is activated via the TCR and delivers a costimulatory signal for degranulation, and that the requirements for these differ from those for CD8 class I adhesion and cosignalling. As predicted by the results using anti-TCR monoclonal antibody (mAb), FN can substantially augment responses to the physiological class I alloantigen stimulus

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when both are present on the same surface. Blocking studies using specific peptides and antibodies demonstrate that the VNR on CTL mediates the adhesion to FN.

## MATERIALS AND METHODS

*Cloned CTL lines and antibodies*

Clones 11, 29, 30 and 35, of (B10.BR × B10.D2)$F_1$ origin, are specific for $K^b$ alloantigen,[2] and clone OE4, of C57BL/6 origin, is specific for H-$2^d$.[15] Clones were maintained by weekly restimulation with irradiated allogeneic spleen cells and 10 U/ml recombinant interleukin-2 (rIL-2).[2] Monoclonal antibody 2C11-145, a hamster antibody (Ab) specific for CD3,[16] was purified from culture supernatant by dielthylaminoethyl (DEAE) chromatography. Monoclonal antibody F23·1, a mouse Ab specific for the TCR V$\beta$8,[17] was purified from ascites fluid by protein A–Sepharose chromatography. Goat anti-mouse IgG (Telios Pharmaceuticals, San Diego, CA) was used to cross-link the F23·1 anti-TCR mAb. Monoclonal antibodies specific for integrin chains were purchased from PharMingen (San Diego, CA) and included 2C9.G2 (anti-$\beta_3$; CD61), 9EG7 (anti-$\beta_1$; CD29), R1-2 (anti-$\alpha_4$; CD49d), 9C10 (anti-$\alpha_4$; CD49d), 5H10-27 (anti-$\alpha_5$; CD49e) and HMa5 (anti-$\alpha_5$; CD49e). Rabbit anti-mouse FN sera (Telios Pharmaceuticals) and purified M1/42 anti-mouse class I mAb[18] were used to quantify immobilized FN and $K^b$ alloantigen, respectively, by enzyme-linked immunosorbent assay (ELISA).

*Ligands and peptides*

H-$2K^b$ and H-$2K^k$ were purified from Triton-X-100 (TX-100) lysates of EL4 ($K^bD^b$) and RDM4 ($K^kD^k$) tumour cells, respectively, by affinity chromatography as described previously,[18,19] and quantified by protein determination and by ELISA relative to standard preparations. Murine FN, RGDS peptide (GRGDSP) and RGES peptide (GRGESP) were purchased from Telios Pharmaceuticals, and murine vitronectin (VN) from Gibco BRL (Gaithersburg, MD). CS1 (DELPQLVTLPHPNLHGPEILDVPST) and RGDS (GRGDSP) were synthesized using a Beckman System 990 peptide synthesizer. For immobilization on plastic, the synthetic peptides were coupled to ovalbumin (OVA) carrier protein.[20]

*Protein immobilization*

Purified H-2 proteins were stored at −20° in 10 mM Tris buffer containing 0·5% deoxycholate and NaCl at concentrations of 20–160 mM, and were diluted into Dulbecco's phosphate-buffered saline (PBS) (pH 7·4) to concentrations ranging from 0·1 to 1 $\mu$g/ml for immobilization. Aliquots, 0·1 ml, of diluted H-2 were added to flat-bottomed microtitre wells (Microtiter III flexible assay plates; Falcon, Oxnard, CA) and incubated for 1·5 hr at 25°. Binding increased linearly with input up to a level of 0·15–0·3 $\mu$g/ml, as determined by ELISA.[21] Following a 1·5-hr incubation, wells were washed three times with 0·1% bovine serum albumin (BSA) in PBS. FN, VN and synthetic peptides coupled to OVA were diluted in PBS, immobilized by addition to wells, and incubated at room temperature for 1·5 hr. The wells then washed and blocked as described above. Co-immobilization of FN and H-2 was accomplished by adding FN to the wells first. After a 1·5-hr incubation, the wells were washed with PBS to remove unbound FN, and H-2 was immobilized as described above.

*Assay of CTL degranulation*

Release of serine esterase activity in response to proteins immobilized in microtitre wells was determined by the addition of $1 \times 10^5$ CTL/well in 0·1 ml of RPMI with 2% fetal calf serum (FCS) and 15 mM HEPES. Plates were centrifuged at 1000 r.p.m. for 1 min and incubated at 37° for times ranging from 30 min to 5 hr. Aliquots, 0·02 ml, of cell-free supernatant were then removed and serine esterase activity determined in a colorimetric assay as described previously,[22] using N-benzyloxycarbonyl-L-lysine thiobenzyl ester as the substrate (Calbiochem, La Jolla, CA) and 5'5-dithiobis-(2-nitrobenzoic acid) (Sigma Chemical Co., St Louis, MO). At the end of the reaction time, the absorbance at 405 nm was determined. CTL incubated in the absence of a stimulus were used to determine the level of spontaneous serine esterase release, and total esterase activity was determined in 0·5% Nonidet P-40 (NP-40) extracts of the cells. Data are expressed as percentage of the total esterase content of the CTL calculated as:

$$\text{percentage specific release} = \frac{(E-S) \times 100}{T-S},$$

where E is the experimental value, S is the esterase activity spontaneously released in the absence of stimulation, and T is the total esterase activity. All conditions were tested in triplicate wells and standard deviations are shown.

*Assay of CTL binding*

CTL were labelled by overnight incubation with 1 mCi [$^3$H]thymidine per $2–5 \times 10^6$ cells or by incubation for 1 hr at 37° with 100 $\mu$Ci of $Na^{51}CrO_4$ per $10^7$ cells. Labelled cells were then washed and resuspended at $10^6$/ml in RPMI with 2% FCS and 15 mM HEPES. F23·1 anti-TCR mAb was added as a stimulus at 0·25 $\mu$g/ml, and goat anti-mouse IgG Ab to further cross-link the TCR was used at 5 $\mu$g/ml. Binding was initiated by adding $10^5$ cells per well in 0·1 ml and centrifuging the microtitre plates at 1000 r.p.m. for 1 min followed by incubation for varying times at 37°. Wells were washed gently with 0·1 ml PBS six times to remove unbound cells, 0·1 ml of 3% sodium dodecyl sulphate (SDS) added to each well, and the plate then incubated at room temperature for 15 min. Well contents were then removed and radioactivity determined. Spontaneous release was determined for cells incubated in parallel under identical conditions. The percentage of bound cells was calculated as:

$$\frac{\text{c.p.m. bound}}{\text{total} - \text{spontaneous c.p.m.}} \times 100.$$

## RESULTS

CTL clones 35 (Fig. 1a), 11 (Fig. 1b) and OE4 (Fig. 1c) were incubated in FN-bearing wells with either no stimulus, anti-TCR mAb, F23·1, or with the combination of F23·1 and goat anti-mouse IgG (anti-Ig), to cross-link the TCR further. Unstimulated CTL showed some detectable binding, particularly at high FN surface densities, but the level of CTL binding was significantly increased upon stimulation with the

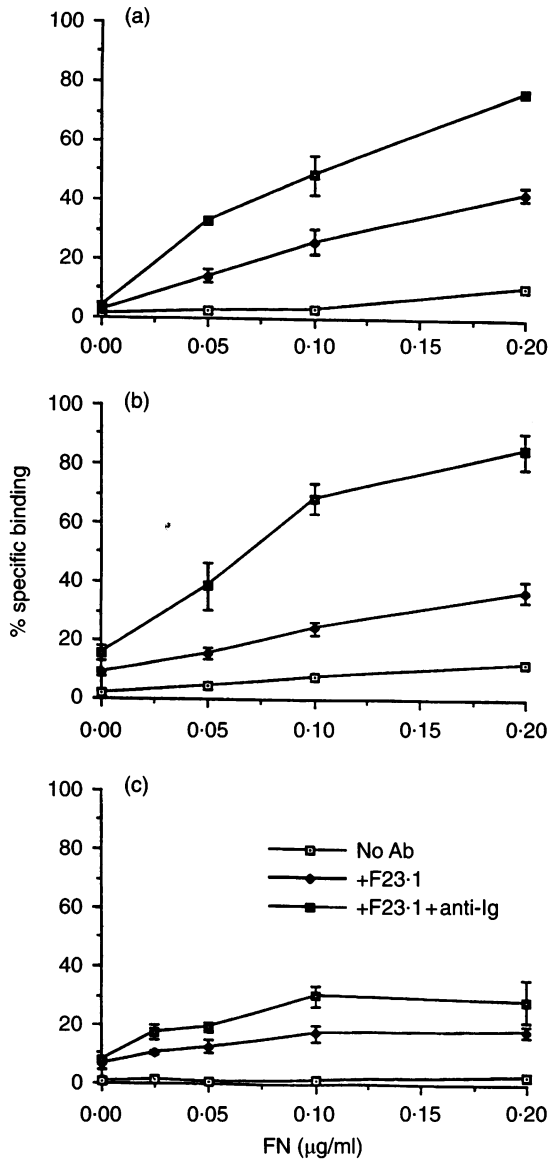


**Figure 1.** Soluble anti-TCR antibody triggers CTL binding to immobilized FN. The indicated concentrations of FN were immobilized in microtitre wells and the wells blocked with 0·1% BSA. Binding of murine FN is linear with input of up to 1·0 μg/well, as determined by ELISA using anti-FN Ab (data not shown). CTL clones 35 (a), 11 (b), and OE4 (c) were stimulated with nothing (open squares), soluble F23·1 alone (closed diamonds) or F3.1 together with goat anti-mouse IgG (anti-IgG; closed squares). After a 2·5-hr incubation at 37°, percentage specific binding was determined as described in the Materials and Methods.

F23·1 mAb. In the presence of the second cross-linking Ab, activated binding further increased to levels approximately double those obtained with F23·1 alone. Binding in the presence of F23·1 mAb alone or with second antibody was detectable within 30 min, reached maximal levels by 1 hr and was sustained for at least several hours (data not shown). Similar results were obtained using 2C11, an anti-CD3 mAb. Levels of adhesion obtained for different clones varied (Fig. 1), but the level of adhesion obtained in these assays for a given CTL clone also varied from experiment to experiment (compare levels in Fig. 1 and subsequent figures). Therefore, it is unclear whether there is any significance in comparisons of binding levels between different clones.

Fluid-phase anti-TCR mAb is not a sufficient stimulus to activate the CTL degranulation response. However, binding of CD8 to non-antigenic class I (i.e. a class I not recognized by the TCR of the CTL being examined) in the presence of fluid-phase anti-TCR results in generation of a costimulatory signal to initiate degranulation.[1] To determine if FN binding could provide a similar costimulatory signal, CTL were incubated in wells bearing either non-antigen class I or FN in the presence of anti-TCR mAb, and the serine esterase release determined. As shown in Table 1, degranulation occurred in response to anti-TCR mAb and class I, as expected. In contrast, no degranulation could be detected from cells incubated with fluid-phase anti-TCR mAb and FN, despite the fact that adhesion to the FN occurred under these conditions (Fig. 1). However, a costimulatory effect was readily apparent when a second Ab was present to cross-link the TCR further (Table 1). Further cross-linking the TCR with a second Ab was not sufficient to trigger degranulation, but serine esterase was released if FN was also present. Addition of a second cross-linking Ab did not increase the response to class I, and in some experiments caused a decrease. Thus, interaction with FN can clearly provide a costimulatory signal, but the requirement for this signal to be effective differs from that for obtaining effective costimulatory signalling as a result of CD8 binding to class I. The level of adhesion obtained for a given clone (Fig. 1) did not correlate directly with the extent of degranulation that occurred upon costimulation with FN (Table 1). This is consistent with previous results showing that CTL clones differ with respect to the maximal level of degranulation that can occur even in response to immobilized anti-TCR mAb.[2]

**Table 1.** Costignalling effects of immobilized class I and FN*

| | | Stimulus | | |
|---|---|---|---|---|
| Clone | Immobilized protein | None | +anti-TCR mAb | +anti-TCR mAb+ anti-Ig Ab |
| 30 | BSA | 0 | 0 | 0 |
| | Class I | 0 | 37±3 | 34±5 |
| | FN | 0 | 0 | 24±1 |
| OE4 | BSA | 0 | 3 | 2 |
| | Class I | 2 | 94±7 | 70±2 |
| | FN | 2 | 5 | 20±6 |
| 11 | BSA | 0 | 2 | 1 |
| | FN | 0 | 1 | 19±1 |

*CTL clones 30, OE4 and 11 were stimulated with either nothing (none), anti-TCR mAb alone, or anti-TCR mAb and anti-Ig Ab to cross-link the TCR further. Cells were then added to wells coated with BSA, non-antigen class I protein (0·15 μg/well) or FN (0·2 μg/well). After 5 h incubation, serine esterase release was determined as described in the Materials and Methods. Results are presented as serine esterase activity released as a percentage of the total serine esterase activity in the cells. Spontaneous release in the absence of any stimulus is subtracted, and was less than 5% in all cases. Standard deviations of triplicate samples are shown.

The above results suggested that interaction with FN might contribute to CTL activation by the physiological TCR stimulus, class I alloantigen. To examine this, FN was immobilized at 0·2 mg/well together with concentrations of $K^b$ alloantigen ranging from 0 to 0·1 mg/well, and adhesion and degranulation were determined in parallel. FN did not stimulate serine esterase release in the absence of alloantigen, but augmented alloantigen-induced release when it was co-immobilized on the same surface (Fig. 2a). At a low alloantigen surface density (0·0125 $\mu$g $K^b$/well), insufficient by itself to stimulate a response, co-immobilization of FN resulted in activation of a detectable response. Co-immobilized FN also caused an increase in the maximal response to a level greater than could be obtained with any amount of alloantigen alone. FN increased the level of adhesion to the FN/alloantigen-coated surface (Fig. 2b). Since binding to FN in the absence of antigen was minimal, this result suggests that TCR and/or CD8 interaction with antigen can provide the signal to activate FN-dependent adhesion. Similar results to those shown in Fig. 2 for clone 35 were obtained in experiments examining clone 30.

To determine if augmented degranulation in response to alloantigen requires FN to be on the same surface, the effects of soluble FN or FN on a different surface were examined.

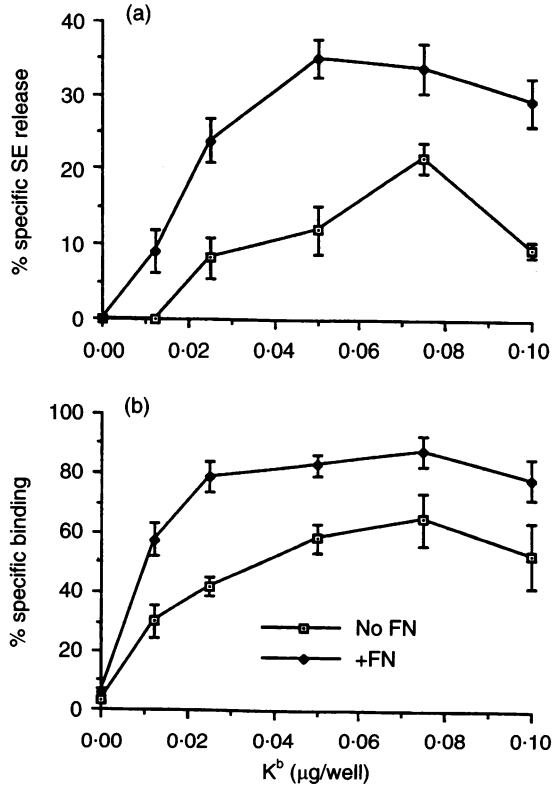


**Figure 2.** Co-immobilized FN enhances CTL response to immobilized alloantigen. CTL clone 35 was cultured in wells coated with the indicated concentrations of $K^b$ alone or in wells coated with 0·2 mg/well FN and the indicated amounts of $K^b$. After a 5-hr incubation, percentage specific serine esterase (SE) release (a) and percentage specific binding (b) were determined as described in the Materials and Methods. Similar results were obtained in experiments examining clone 30.

CTL incubated with $K^b$ immobilized on 5$\mu$ diameter latex beads were stimulated to degranulate (Fig. 3), while no serine esterase release occurred in response to BSA-coated beads (data not shown). Incubation of cells with antigen-coated beads in the presence of FN in solution or in wells bearing immobilized FN resulted in no more degranulation than occurred in the absence of FN (Fig. 3), indicating that FN must be on the same surface as alloantigen to augment the degranulation response.

VLA-5 and VNR bind the RGDS sequence of FN,[6,7,23] while VLA-4 interacts with CS1, a sequence found within the IIIcs region of FN[8,24] Potential contributions of the different receptors binding to FN were examined using peptides having these sequences, and an inactive peptide containing the RGES sequence as a control. CTL were stimulated with anti-TCR mAb and peptides were added immediately before addition to the FN-bearing wells. RGES (Fig. 4a) or CS1 (Fig. 4b) peptides had no effect on adhesion, compared to control CTL with no peptide added (Fig. 5c), while addition of the RGDS peptide at the same concentration almost completely inhibited binding (Fig. 4d). RGDS peptide caused 50% inhibition at approximately 0·013 mg/ml, while the CS1 and RGES peptides did not inhibit at concentrations as high as 0·05 mg/ml, and addition of CS1 and RGDS together resulted in no more inhibition than that obtained with just RGDS (data not shown). Essentially the same results were obtained in examining serine esterase release in response to cross-linked anti-TCR mAb and FN; RGDS inhibited while RGES and CS1 did not (data not shown). Additional experiments confirmed further that the adhesion was dependent on the RGD sequence. The specificity of blocking by RGDS was confirmed in experiments demonstrating that it had no effect on TCR-triggered adhesion to class I protein, and TCR-triggered binding to RGDS, but not RGES or CS1, was found when the peptides were coupled to OVA and immobilized in microtitre wells (data not shown).

The inability of CS1 peptide to block indicated that VLA-4 does not contribute to adhesion of the CTL to FN, and

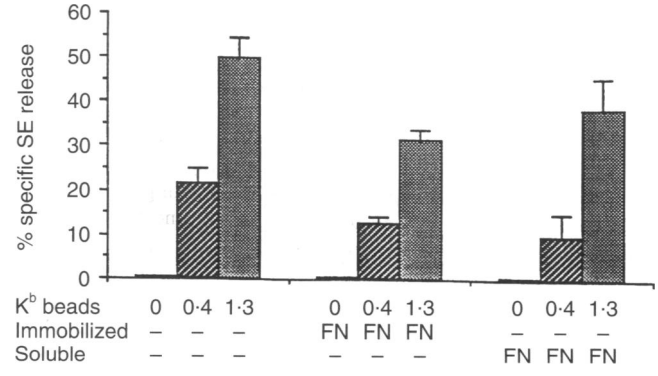


**Figure 3.** FN must be present on the same surface as alloantigen to augment CTL response. CTL clone 35 was cultured in wells coated with either BSA or 0·2 mg/well FN. At the initiation of culture, 5-$\mu$ diameter latex beads coated with $K^b$ as described previously[34] were added at $0.4 \times 10^6$ or $1.3 \times 10^6$beads/well. Soluble FN at 2 $\mu$g/ml was added to CTL and beads in BSA-coated wells where indicated. After a 5-hr incubation at 37°, percentage specific serine esterase (SE) release was determined. Similar results were obtained in experiments examining clone 29.

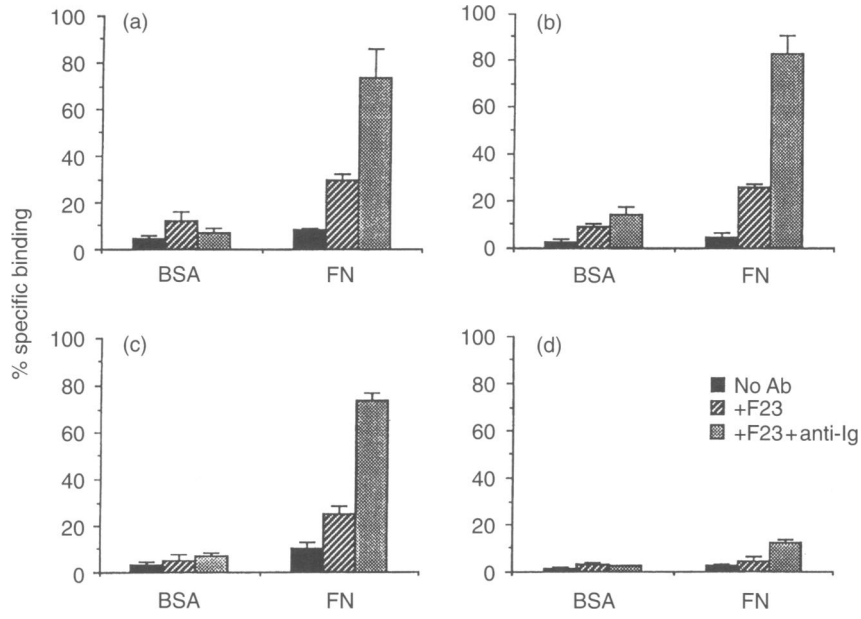


**Figure 4.** RGD-containing peptide block binding of CTL to immobilized FN. CTL clone 11 was activated with soluble F23·1 alone or F23·1 together with goat anti-mouse IgG, and the cells were cultured in wells coated with BSA or 0·1 mg/ml FN. At the initiation of culture, 0·05 mg/ml of RGE (a), CS1 (b), or RGD (d) peptides was added. As a control, no peptide was added (c). After a 2-hr incubation, percentage specific binding was determined. Similar results were obtained in experiments examining clone OE4.

effective blocking by RGDS implicated either VLA-5 or VNR. TCR-triggered adhesion to immobilized murine VN was therefore examined. Adhesion to VN was found to be activated upon stimulation with anti-TCR mAb, and binding increased when a second Ab was added to cross-link the TCR further (data not shown). Adhesion to both FN and VN was effectively inhibited by the 2C9.G2 anti-$\beta_3$ mAb (Fig. 5). In contrast, the 5H10-27 anti-$\alpha_5$ mAb, an Ab that does block $\alpha_5\beta_1$-mediated binding to FN,[25] did not inhibit CTL adhesion to FN or VN (Fig. 5). No reproducible blocking was seen in additional experiments examining the effects of 9EG7 (anti-$\beta_1$), R1–2 (anti-$\alpha_4$), 9C10 (anti-$\alpha_4$), 5H10–27 (anti-$\alpha_5$) or $HMa_5$ (anti-$\alpha_5$) antibodies. Thus, it appears that the binding of the cloned CTL lines examined here is mediated by the VNR receptor, with no detectable contribution from VLA-4 or VLA-5.

## DISCUSSION

Binding of VLA-4 and VLA-5 to FN is induced rapidly upon activation of resting $CD4^+$ T cells with anti-CD3 and CD2 mAb[9] or antigen.[26] The cloned effector CTL examined here can similarly be activated via the TCR to adhere to FN-bearing surfaces (Fig. 1), but in this case adhesion is mediated by the $\alpha V\beta_3$ integrin, VNR (Figs 4 and 5). Thus, like several other members of the integrin family of adhesion receptors, VNR can also be activated via a signal from the TCR to mediate adhesion to its ligands. CTL binding to immobilized FN or VN was sustained for at least several hours, in marked contrast to the transient adhesion to FN reported for resting $CD4^+$ human T cells upon activation with an anti-CD3 mAb.[27] Effector CTL must be able to engage in repeated cycles of adhesion and de-adhesion in order to lyse target cells efficiently, and it is reasonable to expect that interactions between T cells and ligands such as FN might be rapidly reversible. However, the mechanism(s) that regulates these cycles of adhesion and disassociation is not understood, and it is possible that under the experimental conditions used here there is a lack of an 'off signal' needed to terminate binding. Alternatively, sustained binding of the population might reflect an equilibrium established as individual cells cycle between bound and unbound states, as has been demonstrated to be the case for the sustained binding of CTL to immobilized class I alloantigen.[28]

VLA-4 and -5 binding to FN can provide a costimulatory signal for proliferation of resting $CD4^+$ T cells activated with anti-CD3 monoclonal antibody,[10–12] and VNR has been shown to provide costimulation for proliferation of a group of $\gamma\delta$ T cells.[14] VNR can also provide a costimulatory signal to activate the rapid CTL degranulation response (Table 1), one of the mechanisms by which CTL lyse antigen-bearing targets. Interestingly, cosignalling was only evident when both anti-TCR mAb and a second cross-linking Ab were used as the stimulus, despite that fact that anti-TCR mAb alone stimulated significant adhesion to FN (Fig. 1).

The ability of anti-TCR mAb to activate CTL adhesion to FN and provide cosignalling for degranulation suggested that VNR-mediated binding would be activated by the physiological TCR ligand, class I alloantigen, and might enhance response to antigen. These predictions were confirmed in experiments examining the effects of co-immobilizing alloantigen and FN (Fig. 2). Although no binding to FN occurred in the absence of antigen, adhesion to antigen was significantly enhanced when FN was also present, suggesting that TCR engagement by antigen activates VNR binding. FN also strongly enhanced the degranulation response to antigen, allowing a response to occur at a suboptimal antigen density

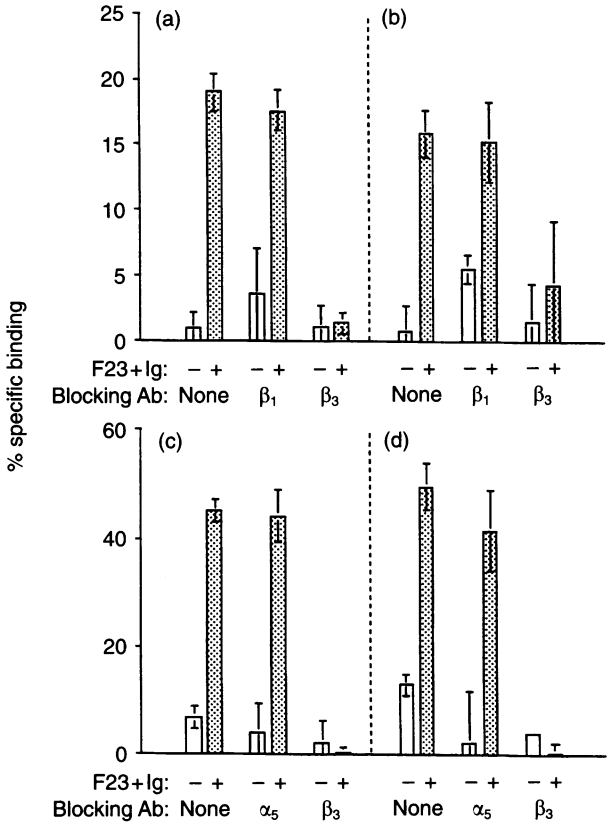


**Figure 5.** Binding to FN and VN is blocked by anti-$\beta_3$ mAb but not by anti-$\beta_1$ or anti-$\alpha_5$ mAb. Adhesion of clone 11 CTL to FN (a and c) or VN (b and d) in the absence (–) or presence (+) of F23·1 mAb (0·5 $\mu$g/ml) and goat anti-mouse IgG (5 $\mu$g/ml) was determined after 2·5-hr incubation. FN and VN were immobilized at 1 $\mu$g/ml. Blocking antibodies included 9EG7 (anti-$\beta_1$), 2C9.G2 (anti-$\beta_3$) and 5H10-27 (anti-$\alpha_5$) and were added at a final concentration of 0·01 mg/ml prior to addition of the cells to the microtitre wells. Data in (a) and (b) are from one experiment, and in (c) and (d) from an independent experiment. Similar results were obtained in experiments examining clone OE4.

and increasing the maximal level of response at optimum antigen density (Fig. 2). Augmentation could result from FN simply increasing the adhesion between the CTL and the antigen-bearing surface to promote a higher level of TCR (and CD8) occupancy. It appears likely, however, that the response is also augmented by VNR-mediated cosignalling. This can clearly occur using cross-linked anti-TCR mAb in solution (Table 1), where increased TCR occupancy due to adhesion cannot contribute. Furthermore, the level of response to alloantigen that is achieved in the presence of FN is greater than that reached at even a very high surface density of alloantigen alone, suggesting that the effect does not simply result from increasing TCR and CD8 occupancy levels. Roles for VNR in increasing TCR and CD8 occupancy levels and providing a costimulatory signal are not mutually exclusive, and it is likely that both contribute to the enhanced response to alloantigen.

Previous work has shown that binding of CTL to FN results in increased production of inositol phosphates,[3] indicating that VNR may deliver a signal to amplify this second messenger pathway. More recently, Brando & Shevach[29] showed that binding of VNR to its ligand induces tyrosine phosphorylation of a 115 000 MW protein in murine T cells that is distinct from the focal adhesion kinase. Similarly, Ostergaard & Ma[4] showed that CTL binding to FN induces phosphorylation of a 120 000 MW protein, and that there is a synergistic increase in phosphorylation of this substrate when FN is co-immobilized with substimulatory amounts of anti-TCR mAb. Although the FN receptor was not identified in this latter study, it is very likely to be the VNR identified here, since some of the cloned CTL lines examined were the same and phosphorylation was inhibited by RGD-containing peptides. Whether the VNR-induced tyrosine phosphorylation of cellular substrates is involved in the amplification of phosphatidy linositol (PI) hydrolysis and costimulation of degranulation remains to be determined.

VNR-mediated augmentation of the response to alloantigen only occurred when FN was co-immobilized on the same surface as the antigen; FN in solution had no effect, nor did FN immobilized in the microtitre well when antigen was presented on latex microspheres (Fig. 3). In contrast, Ostergaard & Ma[4] found that FN on one surface enhanced CTL degranulation in response to sub-optimal anti-TCR mAb immobilized on a different surface. This discrepancy may reflect a difference between the signalling effects of an immobilized anti-TCR Ab versus interaction with the physiological class I antigen, where both TCR and CD8 receptors contribute to the response.

While CD8-dependent adhesion of CTL to non-antigen class I can be activated by fluid-phase anti-TCR mAb, and the binding generates a costimulatory signal to activate degranulation,[1] the results described here demonstrate that CD8 and VNR are distinct with respect to both the requirements for their 'priming' to mediate adhesion and their cosignalling functions. In both cases, adhesion to the respective ligand is activated upon stimulation with fluid-phase anti-TCR mAb and a common mechanism may be involved for both. However, CD8 and VNR differ markedly with respect to the effects of further cross-linking the TCR with a second antibody. This results in a substantial increase in the level of adhesion to FN (Fig. 1) but either no change or a decrease in the level of adhesion to class I.[3] Thus, it appears that there is a second component in the activation of VNR-mediated adhesion that does not occur for CD8, and this is being investigated further. Costimulatory effects also differ, with CD8 initiating degranulation in response to bivalent TCR cross-linking with fluid phase antibody while costimulation by VNR only occurs if the TCR is more extensively crossilinked (Table 1).

*In vivo*, T cells responding to specific antigen are likely to encounter FN in interstitial extracellular matrix, with increased concentrations being found in inflammatory sites.[30] Binding of CTL to FN may allow the cells to adhere to the interstitial matrix, possibly promoting retention of the cells at tissue sites where antigen is present. FN is produced by a variety of cell types including macrophages[31] and T cells,[32] and can be found on the surfaces of various cells including some tumour cells.[33] T cells have a number of co-receptors on their surfaces that appear to promote interactions with antigen-bearing cells, and the particular co-receptors important in a given situation will

depend upon the spectrum of ligands displayed on the cell that bears antigen. The results described here strongly suggest that the VNR may contribute to the efficiency of CTL activation and target lysis in those cases where FN is present on the target cell surface.

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