The Vitronectin Receptor Serves as an Accessory Molecule for the Activation of a Subset of γ/δ T Cells

By Kevan Roberts, Wayne M. Yokoyama, Patricia J. Kehn, and Ethan M. Shevach

From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Summary

Constitutive production of cytokines was observed in 3 of 12 γ/δ T cell lines derived from murine epidermis and correlated with the expression of the Cγ4, Vδ6 T cell receptor (TCR). After adaptation of one of the lines (T195/BW) to serum-free culture conditions, cessation of the "spontaneous" production of interleukin 4 (IL-4) was observed and IL-4 production could then be induced by the addition of RGD-containing extracellular matrix (ECM) proteins to the culture. The response to the ECM proteins could be completely inhibited by a mAb to the murine vitronectin receptor (VNR). However, the induction of IL-4 production could also be inhibited by anti-CD3 and by an anti-clonotypic mAb to the TCR-γ/δ of T195/BW. As TCR-γ/δ loss mutants of T195/BW also failed to respond to ECM proteins, these data demonstrate that engagement of the VNR by its ligand is necessary, but not sufficient, for the induction of IL-4 production. Furthermore, the VNR is expressed by many other T cell clones (both γ/δ and α/β), none of which produce lymphokines constitutively. Taken together, these observations strongly favor the view that not only is coexpression of the VNR and TCR required for the induction of IL-4 production, but that the TCR must also be engaged by its ligand, most likely a cell surface antigen expressed by the hybridoma itself.

T lymphocytes can be divided into two major lineages based on the molecular characteristics of their receptors. The major subpopulation of peripheral T lymphocytes express α/β chains and recognize peptide antigens in association with either MHC class I or class II molecules. Although numerous recent studies have documented the existence of a second subpopulation of lymphocytes that express TCR γ/δ chains, a consensus view does not exist either concerning the nature of the antigen recognized by the TCR-γ/δ or the nature of the restriction element, if any, utilized by this population of cells (1). A number of laboratories have reported the characteristics of γ/δ clones/lines that recognize allo- and self-MHC including both class I (2, 3), class II (4), and nonclassical MHC antigens (5–7). Certain γ/δ T cell clones have been generated that recognize conventional protein antigens (8, 9) and studies from several laboratories have suggested that γ/δ T cells may play a unique role in host defense against certain bacterial infections because their receptors are skewed to recognize a set of bacterial antigens (10–14), particularly mycobacterial heat shock proteins (15–17). Although it has been reported that protein antigen specific γ/δ T cell clones may use conventional MHC class II antigens (8) or nonclassical MHC antigens (9) as restriction elements, many antigen-specific γ/δ T cells demonstrate no specific MHC restriction (14) or no requirement for the addition of non-T APCs (15). These latter results do not rule out the possibility that the clones present antigen to themselves and use an uncharacterized cell surface antigen as a restriction element that may exhibit limited polymorphism.

Because γ/δ T cells are predominantly $CD4^-$, $CD8^-$, an alternative approach to the analysis of γ/δ T cell function has been to define unique cell surface antigens, in addition to the TCR, which are expressed on this population of cells and might play a role in their function, possibly as accessory molecules for the recognition of antigen (18, 19). During the course of such studies, we identified (20) two mAbs to a novel integrin on murine lymphoid cells that was expressed on many γ/δ T cell lines and hybridomas as well as on α/β T cell lines and chronically activated α/β T cells in vitro. The antigen family of cell surface heterodimers consists of at least three groups of cell surface antigens which are identified by their usage of a common β which is associated with one of several α chains (21). The VLA antigens use the β1 chain, the leukocyte adhesion proteins the β2 chain, and the cytoadhesins the β3 chain. Recent studies have documented the existence of other β chains (22–24). The integrin identified on murine cells by our mAbs mediated cell binding to the extracellular matrix (ECM)[1] proteins, fibronectin, fibrinogen,

[1] *Abbreviations used in this paper:* DETC, dendritic epidermal T cells; ECM, extracellular matrix; VNR, vitronectin receptor.

and vitronectin and adhesion could be completely inhibited by the tetrapeptide RGDS. Although the precise molecular relationship of this integrin to the proposed classification system of human integrins has not been determined, we have recently shown (24a) that this murine integrin is likely to be a member of the $\beta 3$ family, most likely the vitronectin receptor (VNR).

In this report, we characterize several γ/δ cell lines derived from murine dendritic epidermal T cells (DETC) that produce substantial amounts of cytokines in culture in the apparent absence of stimulation by exogenous agents. We demonstrate that this "spontaneous" production of cytokines requires the expression of the VNR and the interaction of the VNR with RGD-containing ECM proteins. Furthermore, this phenomenon is associated with the usage of the Cγ4, Vδ6 TCR and is absolutely dependent on expression of this TCR. Collectively, these data strongly suggest that the VNR plays a critical role as an accessary molecule in the stimulation of the Cγ4, Vδ6 subset of cells.

Materials and Methods

Cell Lines. The DETC lines Y93A, Y245, and the T195/BW hybridoma have been previously described (25–28) and are grown in RPMI 1640 supplemented with 10% FCS, 5 mM Hepes, 1 mM sodium pyruvate, nonessential amino acids, 2 mM glutamine, penicillin/streptomycin, and 5 μM 2-ME (CM-RPMI). The T195 cell line was grown in CM-RPMI supplemented with 20% rat Con A supernatant. Each adherant line was removed from plastic by treatment with PBS and 0.02% EDTA before resuspension in CM-RPMI. DETC were also grown in AIM V media (Gibco Laboratories, Grand Island, NY) without serum, but otherwise supplemented as described for RPMI-CM. CTLL cells were maintained as previously described (29). CT.4S was grown in CM supplemented with 500 U/ml rIL-4 and the DA-1 line was grown in 5% WEHI-3 supernatant.

Reagents. Vitronectin, fibronectin, and PepTite 2000 were obtained from Telios Pharmaceuticals, Inc. (San Diego, CA). Rat collagen IV, BSA, PMA, and ionomycin were purchased from Sigma Chemical Co., St. Louis, MO. Peptides (RGDS, GRGDS, RGES) used in inhibition experiments were purchased from Peninsula Laboratories, Inc. (Belmont, CA). Proteolytic fragments of human fibronectin were kindly provided by Joyce Czop (Harvard Medical School, Boston, MA) and have previously been described (30).

Production of Lymphokine-containing Supernatants. DETC were incubated at a concentration of 2.5×10^5 in 1.0 ml of CM-RPMI or AIM V media in 24-well cluster plates overnight. Supernatants were harvested and frozen at −20°C before assay for lymphokines. Wells of 24-well cluster plates were incubated overnight at 37°C with 1.0 ml of ECM protein or mAb in PBS. Wells were subsequently washed three times with PBS before use.

Lymphokine Assays. IL-2 was assayed on CTLL cells responsive to both IL-2 and IL-4 as previously described (29). IL-4 was specifically assayed using the CT.4S cell that is responsive to IL-4, but not IL-2 (29). Granulocyte/macrophage CSF (GM-CSF) was assayed using DA-1 cells. Supernatants were titrated by doubling dilutions in 96-well microtiter plates before adding the indicator cells (5,000 cells/well, total volume of 200 μl). After 48 h, 1.0 μCi of [^{3}H]TdR was added and plates were harvested 5 h later. Units were defined as the reciprocal of the dilution of supernatant (×100) required to induce a half-maximal proliferative response of the indicator cell line.

Production of TCR Loss Mutants of T195/BW. TCR loss mutants of T195/BW were prepared by exposing cells to ethyl methane sulphonate (Sigma Chemical Co.), 200 μg/ml for 24 h at a cell concentration of 2.5×10^5/ml. Mutagenized cells were grown in CM-RPMI for 7 d before selection using the F10/56 antibody (1/100 diluted ascites) in the presence of rabbit complement (1/10 dilution; Cedarlane Laboratories, Hornby, Ontario). Negative selection with F10/56 and complement was repeated on three successive days to yield a preparation that was 20–30% positive by staining with F10/56. These cells were then cloned by limiting dilution and the clones were subsequently screened by staining with F10/56-FITC and analysis using a FACSCAN (Becton Dickinson & Co., Mountain View, CA).

Purification of mAbs. The anti-clonotypic mAb F10/56 was generated by immunization of a hamster with T195/BW cells. Further characterization of this mAb will be presented elsewhere (Roberts, K., and E. M. Shevach, manuscript in preparation). The mAbs H1.2F3 (18), 2C11 (31) and H9.2 (20) were purified from hybridoma supernatants on protein A–Sepharose affinity columns (Pharmacia Fine Chemicals, Piscataway, NJ). M1/9.3.4.HL.2 (M1/9; 32) and 8.18 (20) were purified on a mouse anti-rat κ (MAR 18.5) affinity column. The hamster mAb F10/56 was purified on a mouse anti-rat κ (RG7/7, cross-reactive with hamster; 33) affinity column. Antibody was subsequently eluted from the columns at pH 4.3 and dialyzed against PBS. Contaminant fibronectin was removed by passage of affinity pure antibody preparations over heparin agarose columns (Sigma Chemical Co.) before use in functional experiments.

Results

Inhibition of the Constitutive Production of Lymphokines by mAbs Against the TCR. In an attempt to evaluate the activation requirements and growth properties of γ/δ T cells, a panel of murine DETC lines as well as T cell hybridomas derived from those lines were tested for their ability to produce a variety of lymphokines. Surprisingly, 3 of 12 lines/hybridomas tested produced lymphokines when the cells were incubated in the absence of exogenous growth factors or stimulatory ligands. When tested on factor-dependent cell lines in the presence of specific antilymphokine mAbs, two of the cell lines (Y93A and Y245) produced GM-CSF, but not IL-2 or IL-4, while one of the cell lines (T195) produced only IL-4 (data not shown). Interestingly, all three of the T cell lines/hybridomas that were capable of spontaneous cytokine production have closely related TCRs insofar as they are known to express both Vγ1.1 Cγ4 and Vδ6 chains (25, 27, 28). T195 and T195/BW (a hybridoma made by fusion of this line to BW5147) only express the Cγ4, Vδ6 receptor, while Y93A and Y245 also have functional mRNA for Cγ1 and a second TCR composed of Cγ1, Vδ6 chains can be immunoprecipitated from surface-labeled cells (28). The nine other DETC lines and hybridomas that did not produce lymphokines in the absence of stimulation all express the Vγ3Cγ1 and Vδ1 chains (27).

Since these data suggested a correlation between the expression of the Cγ4, Vδ6 TCR and the phenotype of constitutive lymphokine production, we sought further evidence

Table 1. *Inhibition of Constitutive Lymphokine Production by Soluble Antibodies to the TCR*

Exp.	DETC line	Medium	Anti-CD3 (sol.)	Anti-CD3 (imm.)	Anti-CD69 (sol.)	Anti-Clono (T195) F10/56 (sol.)
1	Y93A	35,160	18,021	43,384	ND	ND
	Y245	71.494	34,721	75,287	ND	ND
2	T195/BW	22,751	1,798	25,559	ND	ND
3	T195/BW	40,016	753	ND	51,090	13,149
	T195	69,696	8,164	ND	77,287	9,915

Supernatants from DETC cultures were assayed for lymphokine. Results are expressed as cpm and represent [^{3}H]TdR incorporation of indicator cells using 6.25% DETC supernatant. DA-1 cells were used in Exp. 1 and CTLL used in Exps. 2 and 3. In Exps. 1 and 2, purified mAbs were either added to the culture medium or immobilized on plates (used at 20 μg/ml in both cases). In Exp. 3, antibodies were added to the culture media (5.0 μg/ml).

that the TCR was involved. Inhibition of GM-CSF production by both Y93A and Y245 cell lines was observed when these lines were cultured in the presence of soluble, but not immobilized, anti-CD3 (Table 1, Exp. 1). Similarly, IL-4 production by the T195 cell line as well as by the T195/BW hybridoma was markedly inhibited when the cells were cultured in the presence of soluble anti-CD3 mAb, but not when the cells were exposed to the same mAb immobilized on plastic (Table 1, Exp. 2). It would therefore seem unlikely that engagement of the TCR per se inhibits the production of lymphokine by these cells. Moreover, a mAb (F10/56) reactive with clonotypic determinants of the T195 TCR-γ/δ also inhibited IL-4 production when added in soluble form (Table 1, Exp. 3). These results confirm and extend to nontransformed cell lines the observations of O'Brien et al. (15) who demonstrated an association between the expression of Cγ4 and Vδ6 in a large panel of T cell hybridomas derived from newborn thymus and spontaneous IL-2 production and a similar inhibition by soluble anti-CD3. Although stimulation via the TCR may lead to growth arrest of T cell hybridomas (34), the proliferation of T195/BW was identical in the presence or absence of anti-CD3. In subsequent experiments, T195/BW was used exclusively because of its ease of growth, its susceptibility to inhibition by anti-CD3, and the availability of the anti-clonotypic mAb F10/56.

Activation Properties of T195/BW Cultured in Serum Free Medium. The ability of anti-CD3 and the anti-clonotypic mAb to inhibit IL-4 production by T195/BW when used in soluble, but not plate-bound, form raised the possibility that the anti-CD3/TCR mAbs inhibited T cell activation by blocking the recognition of antigen present in the culture medium or present on the T cell hybridoma itself. T195/BW was therefore adapted to "serum-free" culture conditions (AIM V medium) to assess the possible involvement of an antigen present in FCS. Cells grown in AIM V proliferated as well as those grown in RPMI containing 10% FCS or AIM V supplemented with FCS (data not shown). However, no IL-4 was detected in the supernatants of T195/BW grown in AIM V, while supplementation of the AIM V with FCS resulted in IL-4 production (Table 2). In contrast to the lack of effect of immobilized anti-CD3 on T195/BW cells grown in serum, the hybridoma cells grown in AIM V without FCS could be induced to produce levels of IL-4 with either immobilized anti-CD3 or the clonotypic mAb, F10/56, comparable to those observed by serum stimulation (Table 2). Immobilized mAbs specific for the VNR (H9.2) and the murine CD45 homologue (M1.9), antigens expressed by the hybridoma, failed to induce lymphokine production.

The Serum Components Responsible for IL-4 Production are ECM Proteins. One of the most striking differences between T195/BW cells grown in serum and T195/BW grown in AIM V was that the former grew in a strongly adherent monolayer, while the latter grew in suspension culture. Furthermore, addition of serum to the AIM V media induced rapid adhesion of the T195/BW cells to plastic and subse-

Table 2. *IL-4 Production by T195/BW After Stimulation by Immobilized Antibodies*

Culture conditions	IL-4 production
	U/ml
AIM V	0
Anti-CLA (M1.9)	0
Anti-VNR (H9.2)	0
Anti-VNR (8.18)	0
Anti-CD3	425
Anti-clonotype(F10/56)	222
FCS	250
PMA + Ionomycin	1,597

T195/BW cells were cultured in AIM V on plates coated with antibodies (20 μg/ml) or in the presence of FCS (10%) or PMA (10 ng/ml.) and ionomycin (400 ng/ml.). Supernatants were harvested after 18 h and assayed on CT.4S for IL-4 content.

quent spreading. It has been previously demonstrated (20) that this hybridoma adheres to fibronectin, fibrinogen, and vitronectin, all of which are present in serum. Moreover, precoating plates with serum was just as effective at inducing IL-4 production as including it in the culture media (Table 3). Collectively, these observations raised the possibility that cell adherance was related to lymphokine production in FCS containing media. To address this issue, T195/BW cells, which had been grown in AIM V for 3 wk, were cultured in AIM V on plates coated with various ECM proteins. IL-4 production was observed (Table 3) when the hybridoma was incubated overnight on plates coated with human fibronectin, mouse fibronectin, or human vitronectin (and fibrinogen, data not shown), but not with rat collagen or BSA. Thus, the specificity of the induction of IL-4 production by the ECM proteins is identical to the previously described (20) specificity of the adhesion of T195/BW to ECM proteins mediated by the integrin identified by mAb H9.2.

It has previously been demonstrated that the amino acid tripeptide, RGD, is one of the cell attachment sites on the fibronectin, fibrinogen, and vitronectin molecules (35, 36). To further evaluate whether ECM proteins containing RGD were the only components in serum that mediated the activation of T195/BW, we examined the effect of addition of the synthetic peptide RGDS on the constitutive production of IL-4 by this cell when it is maintained in serum containing

Table 3. *Induction of IL-4 Secretion by T195/BW by Serum and ECM Proteins*

	FCS concentration		
	10.0	1.0	0.1
		%	
Soluble	95*	1,515	1,887
Plate bound	250	1,250	1,316
	ECM protein concentration		
	10.0	1.0	0.1
		mg/ml	
Fibronectin (mouse)	426	735	0
Fibronectin (human)	556	1,220	0
Vitronectin (human)	384	870	0
Collagen type IV (rat)	<1	0	0
Albumin (bovine)	0	0	0

T195/BW cell in AIM V were incubated (18 h) on plates precoated with different concentrations of ECM proteins. Supernatants were subsequently assayed for IL-4 as in the legend to Table 2. T195/BW hybridoma cells incubated in AIM V alone made 0 U/ml IL-4.
* Results are expressed as units per milliliter of IL-4.

Table 4. *Peptide Inhibition of IL-4 Production by T195/BW*

Exp.	Stimulus	Peptide		Peptide Concentration 0.625	2.5	10.0	40.0
					μg/ml		
1	FCS	Medium	299				
		RGES		–	294	286	286
		RGDS		–	250	310	<1
2	Fibronectin	Medium	327				
		RGES		328	317	317	305
		RGDS		161	<1	<1	0
	FCS	Medium	190				
		RGES		167	152	189	189
		RGDS		122	43	0	0
	Anti-CD3	Medium	304				
		RGES		345	323	323	294
		RGDS		303	345	351	328

T195/BW cells grown in RPMI containing 10% FCS were washed and then recultured in RPMI containing 10% FCS (Exp. 1). In Exp. 2, T195/BW cells grown in AIM V were cultured in AIM V on fibronectin (1.0 μg/ml) coated plates, in AIM V supplemented with 5% FCS, or in AIM V on plates coated with anti-CD3 (2C11, 20 μg/ml). peptides were added to the culture medium at the concentrations indicated. After 18 h of culture, supernatants were assayed for IL-4 content as in the legend to Table 2.
* Results are expressed as units per milliliter of IL-4.

medium. The addition of RGDS, but not the control peptide RGES, inhibited by >95% the production of IL-4 by T195/BW in serum containing medium (Table 4, Exp. 1). Thus, RGDS-containing proteins are responsible for almost all of the effects of serum on the activation of T195/BW. Very similar results were obtained when the T195/BW cells that had been adapted to AIM V were stimulated with plate-bound fibronectin, or serum, but not immobilized anti-CD3 (Table 4, Exp. 2). Since activation by immobilized anti-CD3 was not affected by the RGDS peptide, these results demonstrate that the inhibition of the response to serum or ECM proteins by RGDS is specific, and further demonstrate that RGDS induced inhibition of IL-4 production is not secondary to the delivery of a negative signal.

The RGDS Sequence Is Sufficient to Stimulate the T195/BW Hybrid. The minimum portion of the fibronectin molecule required to stimulate T195/BW was investigated using proteolytic fragments of human fibronectin (Table 5, Exp. 1). Costar wells were coated with each of the fibronectin fragments and then blocked with BSA. Any fragment containing the cell attachment domain was sufficient to trigger IL-4 production and these fragments were as efficient as the whole fibronectin molecule. Fragments containing the gelatin binding site or the heparin binding site failed to stimulate; a very low level of stimulation was observed using the 29-kD NH_2-terminal fragment.

The stimulatory sequence in fibronectin was further defined by using a commercial peptide of 2,000 daltons molecular weight (Pep Tite 2000) that contains the RGDS cell attachment sequence and a highly charged sequence of amino acids that facilitate its binding to plastic. Plates coated with Pep-Tite 2000 (1.0 μg/ml) were as effective as plates coated with the intact fibronectin molecule (1.0 μg/ml) in the induction of IL-4 production by T195/BW (Table 5, Exp. 2). This result is most consistent with the view that sequences outside the cell attachment site on ECM proteins are not required to trigger T195/BW. Furthermore, it is also very unlikely that the TCR plays any direct role in the recognition of the ECM proteins as both the integrin and the TCR would then be required to simultaneously bind to the RGDS sequence.

The VNR Identified by mAb H9.2 Mediates the Stimulation of T195/BW by ECM Proteins. One likely candidate for the integrin responsible for the activation of T195/BW cells was the heterodimer recognized by mAbs H9.2 and 8.18, which we have previously shown to be expressed on many of the γ/δ DETC cell lines as well as on T195/BW hybridoma cells (20). T195/BW cells maintained in serum free medium were stimulated with immobilized murine fibronectin or by supplementing the medium with FCS (5% final). IL-4 production induced by either fibronectin or serum was completely inhibited by mAb H9.2 (Table 6), while mAb 8.18 which recognizes a distinct site on the 120-kD fragment of the α chain of the integrin heterodimer produced much less inhibition. This result differs from the effects of these mAbs on cell adhesion where the simultaneous addition of both H9.2 and 8.18 was required to block adhesion (20). The addition of anti-CD3 also resulted in complete inhibition of IL-4 production, while mAbs H1.2F3 (anti-CD69) and M1.9 (anti-CD45, data not shown), which are directed to other cell surface antigens expressed on the hybridoma, failed to inhibit. IL-4 production could also not be inhibited by anti–MHC class I, anti–MHC class II, or anti–LFA-1 mAbs (data not shown).

Table 5. *Induction of IL-4 Production by T195/BW After Stimulation with Proteolytic Fragments of Fibronectin Containing the Cell Attachment Site or PepTite 2000*

Exp.	Culture conditions	IL-4 production
		U/ml
1	Whole fibronectin	8,333
	NH_2 terminus fragment	67
	Gelatin binding fragment	2
	Heparin binding fragment	5
	Cell attachment site fragment	26,315
	PMA + Ionomycin	5,000
	FCS	714
	Media alone	4
2	Fibronectin (1.0 μg/ml)	1,818
	Fibronectin (10.0 μg/ml)	1,099
	PepTite 2000 (1.0 μg/ml)	1,380
	PepTite 2000 (10.0 μg/ml)	925
	PMA + Ionomycin	2,439
	FCS	513
	Media alone	0

In Exp. 1, T195/BW cells grown in AIM V media were cultured in AIM V on plates precoated with 1.0 μg/ml of whole or proteolytic fragments of human fibronectin. One aliquot of cells was stimulated with PMA/Ionomycin and a second aliquot was cultured in 5% FCS. In Exp. 2, cells were stimulated with either murine fibronectin or PepTite 2000. IL-4 production was assayed in both experiments as in Table 2.

Table 6. *The Fibronectin and Serum Induction of IL-4 Production by T195/BW is Inhibited by Antibodies to the VNR*

Culture Conditions	Fibronectin	FCS
Media alone	588*	800
Anti-CD69 (H1-2F3)	800	1,600
Anti-CD3 (2C11)	0	0
Anti-VNR (H9.2)	0	0
Anti-VNR (8.18)	200	400

T195/BW cells adapted to grow in AIM V were stimulated in AIM V on plates coated with murine fibronectin (1.0 μg/ml) or in the presence of FCS (5.0%). Soluble mAbs (5 μg/ml) were added to the culture media and culture supernatants harvested following an 18-h incubation. IL-4 content of the supernatants was assayed as in the legend to Table 2.
* Units per milliliter of IL-4.

Co-Expression of the TCR Is Required for Integrin-mediated T Cell Activation. Although these studies strongly implicate the integrin defined by mAb H9.2 as the signal-transducing molecule involved in the induction of IL-4 production by ECM proteins, they fail to explain why this response is so readily inhibited by anti-CD3 or the anti-clonotypic mAb. To more accurately define the role of the TCR in this process of activation, TCR loss mutants were generated from the T195/BW hybrid by chemical mutagenesis followed by negative selection with the anti-clonotypic mAb F10/56 and complement. Five cloned lines were selected on the basis of their lack of reactivity with the anti-clonotypic mAb by FACS analysis and all expressed levels of the integrin comparable to the parental cell line (data not shown). The clones appeared to retain the phenotypic characteristics of the parental line as they continued to grow adherent and to spread on plastic. Northern blot analysis revealed that all five mutant lines had lost expression of the TCR γ chain (data not shown). None of the mutants produced IL-4 constitutively (Table 7) or after stimulation with immobilized F10/56, but all were capable of IL-4 production after stimulation with the combination of PMA and ionomycin. These results demonstrate an absolute requirement for coexpression of the TCR for activation of T195/BW by ECM proteins.

Discussion

The studies described in the present report are an extension of our previous reports on the characteristics of γ/δ T cell lines derived from murine skin (25–27) and have specifically addressed the mechanisms responsible for the production of cytokines by several DETC lines in the absence of exogenous stimulation. 3 of 12 DETC lines generated from C3H mouse skin constitutively produced lymphokine. On closer investigation, two of the lines (Y93A and Y245) were shown to produce GM-CSF and one (T195) IL-4. After adaptation of the T195/BW hybridoma to serum-free culture conditions, cessation of the "spontaneous" production of IL-4 was observed and IL-4 production could then be induced by the addition of RGD-containing ECM proteins to the culture. The response to the ECM proteins could be completely inhibited by a mAb to the murine VNR. However, the induction of IL-4 production could also be inhibited by anti-CD3 and by an anti-clonotypic mAb to the TCR-γ/δ of T195. As TCR-γ/δ loss mutants of T195/BW also failed to respond to ECM proteins, these data demonstrate that engagement of the VNR by its ligand is necessary, but not sufficient, for the induction of IL-4 production and that the TCR-γ/δ also plays a role in the response to ECM proteins.

Table 7. *Production of IL-4 by Parental and TCR Loss Mutants of T195/BW*

	TCR loss mutant					
Culture conditions	5.13	1.1	5.5	5.4	3C	Parental
FCS	0.1*	0.0	0.0	0.0	0.0	1,333
Anti-clonotype (F10/56)	0.0	0.0	0.0	0.0	0.0	435
PMA + Ionomycin	7,143	1,852	571	1,176	833	200

Five mutants failing to express the TCR epitope recognized by F10/56 were prepared as described. The TCR loss mutants and parental line were incubated overnight in RPMI containing 10% FCS, plates coated with F10/56 (20 μg/ml), or PMA/Ionomycin for 18 h. Culture supernatants were then analyzed for IL-4 content as described in Table 2.

* Units per milliliter of IL-4.

One possible explanation for our findings was that the TCR-γ/δ expressed by T195 recognized a non-RGD site on the ECM proteins as its ligand, and that engagement of this site by the TCR together with engagement of the RGD site by the VNR were both required for the induction of IL-4 production. The active components in serum were found to be the ECM proteins, vitronectin, fibronectin, and fibrinogen (data not shown). Since the RGDS peptide was efficient at inhibiting lymphokine production by the hybridoma grown in the presence of serum, it would appear that proteins containing the RGDS sequence are the only components of serum that were responsible for triggering T195/BW. It is rather unlikely that the ECM proteins were serving as antigen for the TCR-γ/δ since fibronectin and vitronectin share minimal sequence homologies other than RGDS. Moreover, fibronectin only stimulated when firmly immobilized on plastic and no evidence could be obtained that soluble fibronectin could be processed and presented by the hybridoma cells (data not shown). The ability of the synthetic 20 amino acid RGDS-containing peptide, PepTite 2000, to efficiently activate IL-4 production is consistent with the view that RGDS is both necessary and sufficient for both adhesion and cell activation. Although it is formally possible that the VNR and the TCR could both recognize the RGDS sequence, we regard this possibility as unlikely.

The ability of mAb H9.2 to completely inhibit the production of IL-4 by T195/BW strongly suggests that the integrin identified by this mAb is involved in the process of T cell activation. We believe that this integrin is the VNR because it is reactive with an antiserum to the human VNR and is not reactive with antisera specific for the human integrin $\beta1$ or $\beta2$ COOH-terminal amino acid sequences; furthermore, in detergent extracts of T195/BW, all of the molecules reactive with mAb H9.2 are also reactive with an antiserum to the human $\beta3$ COOH-terminal sequence (24a).

Adhesion of the cells to the culture dish was insufficient to induce T cell activation as adhesion mediated by an immobilized mAb to the common leukocyte antigen (CD45) failed to induce IL-4 production. Thus, it is likely that the interaction and engagement of the VNR with immobilized, but not soluble, ECM proteins, lead to signal transduction mediated by the integrin. Integrins have been implicated as playing a role in adhesion, motility, hemostasis, development, induction of differentiation, and cytoskeletal reorganization (21, 37). It is likely that integrins function as signal transducers because they interact with molecules of both the extracellular matrix and with the cytoskeleton and bind the

cytoskeletal protein talin (38). Although the biochemical basis for this interaction has not yet been determined, the failure of mutant integrin $\beta 1$ chains that lack a cytoplasmic domain to localize efficiently in focal contacts even though the mutant integrins are exported to the cell surface and bind fibronectin (39) strongly implicates the cytoplasmic portion of the β chain in this process.

Although some evidence exists for the role of integrins in the enhancement of phagocytosis of opsonized particles by monocytes (40), the induction of the respiratory burst in human neutrophils (41), and the regulation of stromelysin gene expression in fibroblasts (42), the ability of the integrins to function as signal transducers in the process of T cell activation and lymphokine production has not been exhaustively analyzed. Wacholtz et al. (43) have demonstrated that co-stimulation of resting $CD4^+$ T cells with immobilized anti-CD3 can be enhanced by co-stimulation with an anti-LFA-1 mAb and that crosslinking of LFA-1 and CD3 simultaneously produced a prolongation in the increase in $[Ca^{2+}]_i$ and enhancement of both IL-2 production and proliferation. Similarly, Matsuyama et al. (44) have shown that fibronectin synergizes with suboptimal concentrations of anti-CD3 to induce a proliferative response of resting $CD4^+$ T cells and that the stimulatory effects of fibronectin could be inhibited by RGDS and a mAb to VLA-5. In contrast to our findings, fragments of fibronectin containing the cell adhesion site alone were not as effective as the intact molecule in inducing T cell activation.

Although the ability of ECM proteins to activate T195/BW was mediated by the VNR, expression and engagement of the VNR were not sufficient to induce IL-4 production. The inhibitory effects of soluble anti-CD3 and the anti-clonotypic mAb to the T195 TCR raised the possibility that the TCR/CD3 complex also played an active role in the regulation of cytokine production by the DETC lines. Furthermore, the coexpression of the TCR on the surface of the T195/BW cells was required for activation as mutants that lacked the Cγ4, Vδ6 TCR failed to respond to stimulation by serum, ECM proteins, or PepTite 2000.

There are a number of possible explanations for the requirement of TCR expression for integrin-mediated activation. First, it is unlikely that the VNR and the TCR complex are physically associated since the VNR does not comodulate with the TCR (data not shown). A second possibility is that activation via the VNR is analogous to activation via other T cell surface antigens that mediate so-called alternative pathways of activation. Thus, mAbs to Thy-1 (45, 46), Ly-6 (47), and CD2 (48, 49) are all capable of stimulating lymphokine production by murine or human T cells and their abilities to induce lymphokine production are absolutely dependent on coexpression of the TCR, but engagement of the TCR by its ligand is not required. However, it is important to note that the VNR identified by mAb H9.2 is expressed by many other T cell clones (both γ/δ and α/β), none of which produce lymphokines constitutively (20; and Roberts, K., and E. M. Shevach, Manuscript in preparation).

An alternative model that is most compatible with our data is that not only is coexpression of the VNR and TCR required for the induction of IL-4 production, but that the TCR must also be engaged by its ligand, most likely a cell surface antigen expressed by the hybridoma itself. A specific correlation between the spontaneous production of IL-2 and expression of a TCR-γ/δ using Cγ4 and frequently Vδ6 has been described (15, 50) in a large panel of T cell hybrids derived from newborn thymus. In the present report, the three cell lines that produced lymphokines "constitutively" in culture also expressed a TCR utilizing the Cγ4 and Vδ6 gene products. Taken together, these observations strongly favor the view that the combination of Cγ4 with a restricted number of δ chains (particularly Vδ6) results in expression of a TCR that is likely to recognize an autoantigen. However, the failure of the cell to be triggered in serum-free medium even under high density culture conditions suggests that the TCR expressed by T195/BW is of low affinity and requires the VNR to function as an "accessory molecule" to produce a high affinity interaction in a manner similar to that proposed for CD4/CD8 and MHC class II/class I, CD2 and LFA-3, as well as LFA-1 and ICAM-1 (51). However, in the case of T195/BW the ligand for the VNR accessory molecule need not be present on the APC.

It is very difficult to address the question of the expression of the VNR on Cγ4, Vδ6 expressing T cells in vivo. No mAbs are yet available that would facilitate the isolation of this T cell subset and we were unable to identify the VNR on unfractionated thymocytes, lymph node cells, spleen cells (20), DETC in situ, and on populations of adult thymocytes enriched for "double-negative" T cells that contained a high percentage of γ/δ cells. On the other hand, since the VNR is inducible on many α/β T cells by chronic activation in vitro, the manner in which its expression is regulated in vivo may be complex and it is difficult to exclude the possibility that a very small number of activated cells express this molecule both in the thymus and in peripheral lymphoid tissues. In addition, ECM patients are actively synthesized by inflammatory cells and T cells expressing the VLA-1 and VLA-2 antigens can be found in sites of inflammation (52); it is therefore possible that the VNR and other members of the integrin family may function as accessory molecules by recognizing ECM proteins on the surface of APCs and assist the TCR in the recognition of processed antigens on the surface of the same cell. Further studies of the mechanisms by which the VNR is induced and the possible role of cytokines in the induction process should shed light on the role that it plays in the function of both α/β and γ/δ T cells.

Lastly, the nature of the autoantigen recognized by T cells that express the Cγ4Vδ6 TCR has not been defined. O'Brien et al. (15) demonstrated that many of the hybridomas generated from newborn thymus that spontaneously produced IL-2 were also reactive with PPD from *Mycobacterium tuberculosis* and with a member of the heat shock family of proteins. Although these observations were interpreted as indicating that the physiologic ligand of the TCR-γ/δ is likely to be an autologous heat shock protein, probably a member of the GroEL family (17), it is also possible that certain subpopulations of

γ/δ T cells might recognize bacterial antigens as their physiologic ligand and use the VNR or another member of the integrin family as accessory molecules. Their reactivity with autologous heat shock proteins would then be considered to be a crossreaction. It is of particular interest that a number of bacteria have been shown to interact with ECM proteins. Both fibronectin and fibrinogen bind to *Staphylococcus aureus* (53–55) as well as Calmette-Guerin bacillus (BCG) (56) and vitronectin binds to streptococci (57). It has also recently been shown that the integrin $\beta 1$ chains are receptors for invasin, a protein involved in the penetration of bacteria into nonphagocytic cells (58). The combined use of certain γ/δ receptors together with the integrins may prove to be an important mechanism for the generation of host resistance to bacterial antigens. The VNR may play a particularly important role in this process as it is a promiscuous integrin that is capable of interacting with bacteria coated with one of several ECM patients or even bacterial antigens that contain an RGD sequence.

We thank Drs. W. E. Paul and D. Margulies for careful review of the manuscript and Dr. J. Czop for her generous gift of the fibronectin fragments. We thank Ms. S. Starnes for preparation of the manuscript.

Address correspondence to Dr. Ethan M. Shevach, Cellular Immunology Section, NIAID, National Institutes of Health, Bldg. 10, Room 11N315, Bethesda, MD 20892.

Received for publication 21 August 1990 and in revised form 26 September 1990.

References

1. Raulet, D.H. 1989. The structure, function, and molecular genetics of the γ/δ T cell receptor. *Annu. Rev. Immunol.* 7:175.
2. Bluestone, J.A., R.Q. Cron, B.A. Cotterman, B.A. Houlden, and L.A. Matis. 1988. Structure and specificity of TCR $\gamma\delta$ receptors on major histocompatibility complex antigen specific $CD3^+$, $CD4^-$, $CD8^-$ T lymphocytes. *J. Exp. Med.* 168:1899.
3. Rivas, A., J. Koide, M.L. Cleary, and E.G. Engleman. 1989. Evidence for involvement of the γ/δ T cell antigen receptor in cytotoxicity mediated by human alloantigen-specific T cell clones. *J. Immunol.* 142:1840.
4. Matis, L.A., A.M. Fry, R.Q. Cron, M.M. Cotterman, R.F. Dick, and J.A. Bluestone. 1989. Structure and specificity of a class II MHC alloreactive $\gamma\delta$ T cell receptor heterodimer. *Science (Wash. DC).* 245:746.
5. Matis, L.A., R. Cron, and J.A. Bluestone. 1987. Major histocompatibility complex–linked specificity of $\gamma\delta$ receptor-bearing T lymphocytes. *Nature (Lond.).* 330:262.
6. Bonneville, M., K. Ito, E.G. Krecko, S. Itohara, D. Kappes, I. Ishida, O. Kanagawa, C.A. Janeway, D.B. Murphy, and S. Tonegawa. 1989. Recognition of a self major histocompatability complex TL region product by γ/δ T-cell receptors. *Proc. Natl. Acad. Sci. USA.* 86:5928.
7. Porcelli, S., M.B. Brenner, J.L. Greenstein, S.P. Balk, C. Terhorst, and P. Bleicher. 1989. Recognition of cluster of differentiation 1 antigens by human $CD4^-$ $CD8^-$ cytolytic T lymphocytes. *Nature (Lond.).* 341:447.
8. Kozbor, D., G. Trinchieri, D.S. Monos, M. Isobe, G. Russo, J.A. Haney, C. Zmijewski, and C.M. Croce. 1989. Human TCR-$\gamma^+\delta^+$ T lymphocytes recognize tetanus toxoid in an MHC-restricted fashion. *J. Exp. Med.* 169:1847.
9. Vidovic, D., M. Roglic, K. McKune, S. Guerder, C. MacKay, and Z. Dembic. 1989. Qa-1 restricted recognition of foreign antigen by a γ/δ T-cell hybridoma. *Nature (Lond.).* 340:646.
10. Modlin, R.L., C. Pirmez, F.M. Hoffman, V. Torigan, K. Uyemura, T.H. Rea, B.R. Bloom, and M.B. Brenner. 1989. Lymphocytes bearing antigen-specific $\gamma\delta$ T cell receptors accumulate in human infectious disease lesions. *Nature (Lond.).* 339:544.
11. Janis, E.M., S.H.E. Kaufmann, R.H. Schwartz, and D.M. Pardoll. 1989. Activation of $\gamma\delta$ T cells in the primary immune response to Mycobacterium tuberculosis. *Science (Wash. DC).* 244:713.
12. Augustin, A., R.T. Kubo, and G.-K. Sim. 1989. Resident pulmonary lymphocytes expressing the γ/δ T-cell receptor. *Nature (Lond.).* 340:239.
13. Haregewoin, A., G. Soman, R.C. Hom, and R.W. Finberg. 1989. Human γ/δ T cells respond to mycobacterial heat-shock protein. *Nature (Lond.).* 340:309.
14. Holoshitz, J., F. Koning, J.E. Coligan, J. De Bruyn, and S. Strober. 1989. Isolation of $CD4^-CD8^-$ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. *Nature (Lond.).* 339:226.
15. O'Brien, R.L., M.P. Happ, A. Dallas, E. Palmer, R. Kubo, and W.K. Born. 1989. Stimulation of a major subset of lymphocytes expressing T cell receptor $\gamma\delta$ by an antigen derived from Mycobacterium tuberculosis. *Cell.* 57:667.
16. Rajasekar, R., G.-K. Sim, and A. Augustin. 1990. Self heat shock and $\gamma\delta$ T-cell reactivity. *Proc. Natl. Acad. Sci. USA.* 87:1767.
17. Born, W., M.P. Happ, A. Dallas, C. Reardon, R. Kubo, T. Shinnick, P. Brennan, and R. O'Brien. 1990. Recognition of heat shock proteins and $\gamma\delta$ cell function. *Immunol. Today.* 11:40.
18. Yokoyama, W.M., F. Koning, P.J. Kehn, G.M.B. Pereira, G. Stingl, J.A. Coligan, and E.M. Shevach. 1988. Characterization of a cell surface-expressed disulfide-linked dimer involved in murine T cell activation. *J. Immunol.* 141:369.
19. David, V., H. Bachelez, G. Leca, L. Degos, S. Boumsell, and A. Besussan. 1990. Identification of a novel 110-kilodalton structure expressed on a subset of T cell receptor-$\gamma\delta$-bearing cloned lymphocytes. *J. Immunol.* 144:1.
20. Maxfield, S.R., K. Moulder, F. Koning, A. Elbe, G. Stingl, J.E. Coligan, E.M. Shevach, and W.M. Yokoyama. 1989. Murine T cells express a cell surface receptor for multiple extracel-

lular matrix proteins: identification and characterization with monoclonal antibodies. *J. Exp. Med.* 169:2173.
21. Hynes, R.O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48:549.
22. Freed, E., J. Gailit, P. van der Greer, E. Ruoslahti, and T. Hunter. 1989. A novel integrin β subunit is associated with the vitronectin receptor α subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:2955.
23. Cheresh, D.A., J.W. Smith, H.M. Cooper, and V. Quaranta. 1989. A novel vitronectin receptor integrin ($\alpha_v\beta x$) is responsible for distinct adhesive properties of carcinoma cells. *Cell.* 57:59.
24. van Kuppevelt, T.H.M., L.R. Languino, J.O. Gailit, A. Suzuki, and E. Ruoslahti. 1989. An alternative cytoplasmic domain of the integrin $\beta 3$ subunit. *Proc. Natl. Acad. Sci. USA.* 86:5415.
24a. Moulder, K., K. Roberts, E.M. Shevach, and J.E. Coligan. 1991. The mouse vitronectin receptor is a T cell activation antigen. *J. Exp. Med.* In press.
25. Stingl, G., K.C. Gunter, E. Tschachler, H. Yamada, R.I. Lechler, W.M. Yokoyama, G. Steiner, R.N. Germain, and E.M. Shevach. 1987. Thy-1$^+$ dendritic epidermal cells belong to the T-cell lineage. *Proc. Natl. Acad. Sci. USA.* 84:2430.
26. Yokoyama, W.M., F. Koning, G. Stingl, J.A. Bluestone, J.E. Coligan, and E.M. Shevach. 1987. Production of a T cell hybridoma that expresses the T cell receptor γ/δ heterodimer. *J. Exp. Med.* 165:1725.
27. McConnell, T.J., W.M. Yokoyama, G.E. Kikuchi, G.P. Einhorn, G. Stingl, E.M. Shevach, and J.E. Coligan. 1989. δ-chains of dendritic epidermal T cell receptors are diverse but pair with γ-chains in a restricted manner. *J. Immunol.* 142:2924.
28. Koning, F., W.M. Yokoyama, W.L. Maloy, G. Stingl, T.J. McConnell, D.I. Cohen, E.M. Shevach, and J.E. Coligan. 1988. Expression of Cγ4 T cell receptors and lack of isotype exclusion by dendritic epidermal T cell lines. *J. Immunol.* 141:2057.
29. Hu-Li, J., J. Ohara, C. Watson, W. Tsang, and W.E. Paul. 1989. Derivation of a T cell line that is highly responsive to IL-4 and IL-2 (CT.4R) and of an IL-2 hyporesponsive mutant of that line (CT.4S). *J. Immunol.* 142:800.
30. Czop, J.K., J.L. Kadish, D.M. Zepf, and K.F. Austen. 1985. Identification with monoclonal antibodies of different regions of human plasma fibronectin including that which interacts with human monocyte fibronectin receptors. *Immunology.* 54:407.
31. Leo, O., M. Foo, D.H. Sachs, L.E. Samelson, and J.A. Bluestone. 1987. Identification of a monoclonal antibody specific for murine T3. *Proc. Natl. Acad. Sci. USA.* 84:4586.
32. Springer, T., G. Galfre, D.S. Secher, and C. Milstein. 1978. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. *Eur. J. Immunol.* 8:539.
33. Springer, T., A. Bhattacharya, J.T. Cardoza, and F. Sanchez-Madrid. 1982. Monoclonal antibodies specific for rat IgG1, IgG2a, and IgG2b subclasses, and kappa chain monotypic and allotypic determinants: reagents for use with rat monoclonal antibodies. *Hybridoma.* 1:257.
34. Sussman, J.J., T. Saito, E.M. Shevach, R.N. Germain, and J.D. Ashwell. 1988. Thy-1 and Ly-6-mediated lymphokine production and growth inhibition of a T cell hybridoma require coexpression of the T cell antigen receptor complex. *J. Immunol.* 140:2520.
35. Pierschbacher, M.D., and E. Ruoslahti. 1984a. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature (Lond.).* 309:30.
36. Pierschbacher, M.D., and E. Ruoslathi. 1984b. Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. *Proc. Natl. Acad. Sci. USA.* 81:5985.
37. Hemler, M.E., C. Huang, and L. Schwarz. 1987. The VLA protein family characterization of five distinct cell surface heterodimers each with a common 130,000 M_r, subunit. *J. Biol. Chem.* 262:3300.
38. Horwitz, A., K. Duggan, C. Buck, M.C. Beckerle, and K. Burridge. 1986. Interaction of plasma membrane fibronectin with talin—a transmembrane linkage. *Nature (Lond.).* 320:531.
39. Solowska, J., J.-L. Guan, E.E. Mercantonio, J.E. Trevithick, C.A. Buck, and R.O. Hynes. 1989. Expression of normal and mutant avian integrin subunits in rodent cells. *J. Cell. Biol.* 109:853.
40. Pommier, C.G., S. Inada, L.F. Fries, T. Takahashi, M.M. Frank, and E.E. Brown. 1983. Plasma fibronectin enhances phagocytosis of opsonized particles by human peripheral blood monocytes. *J. Exp. Med.* 157:1844.
41. Nathan, C., S. Srimal, C. Farber, E. Sanchez, L. Kabbash, A. Asch, J. Gailit, and S.D. Wright. 1989. Cytokine-induced respiratory burst of human neutrophils: Dependence on extracellular matrix proteins and CD11/CD18 antigens. *J. Cell. Biol.* 109:1341.
42. Werb, Z., P.M. Tremble, O. Behrendtsen, E. Crowley, and C.H. Damsky. 1989. Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J. Cell Biol.* 109:877.
43. Wacholtz, M.C., S.S. Patel, and P.E. Lipsky. 1989. Leucocyte function-associated antigen 1 is an activation molecule for human T cells. *J. Exp. Med.* 170:431.
44. Matsuyama, T., A. Yamada, J. Kay, K.M. Yamada, S.K. Akiyama, S.F. Schlossman, and C. Morimoto. 1989. Activation of CD4 cells by fibronectin and anti-CD-3 antibody; a synergistic effect mediated by the VLA-5 fibronectin complex. *J. Exp. Med.* 170:1133.
45. Gunter, K.C., T.R. Malek, and E.M. Shevach. 1984 T cell-activating properties of an anti-Thy-1 monoclonal antibody. *J. Exp. Med.* 159:716.
46. Gunter, K.C., R.N. Germain, R.A. Kroczek, T. Saito, W.M. Yokoyama, C. Chan, A. Weiss, and E.M. Shevach. 1987. Thy-1-mediated T-cell activation requires co-expression of CD3/Ti complex. *Nature (Lond.).* 326:505.
47. Malek, T.R., G. Ortega, C. Chan, R.A Kroczek, and E.M. Shevach. 1986. Role of Ly-6 in lymphocyte activation. II. Induction of T cell activation by monoclonal anti-Ly-6 antibodies. *J. Exp. Med.* 164:709.
48. Alcover, A., C. Alberini, O. Acuti, L.K. Clayton, C. Transy, C. Spagnoli, P. Moingeon, P. Lopez, and E.L. Reinherz. 1988. Interdependence of CD3-Ti and CD2 activation pathways in human T lymphocytes. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1973.
49. Breitmeyer, J.B., J.F. Daley, H.B. Levine, and S.F Schlossman. 1987. The T11 (CD2) molecule is functionally linked to the T3/Ti T cell receptor in the majority of T cells. *J. Immunol.* 139:2899.
50. Happ, M.P., R.T. Kubo, E. Palmer, W.K. Born, and R.L. O'Brien. 1989. Limited receptor repertoire in a mycobacteria-reactive subset of $\gamma\delta$ T lymphocytes. *Nature (Lond.).* 342:696.
51. Shevach, E.M. 1989. Accessory molecules. *In* Fundamental Immunology. Second Edition. W.E. Paul, editor. Raven Press Ltd. New York. 413–441.
52. Hemler, M.E., D. Glass, J.S. Coblyn, and J.G. Jacobson. 1986. Very late activation antigens on rheumatoid synovial fluid T

lymphocytes. Association with stages of T cell activation. *J. Clin. Invest.* 78:696.
53. Kuusela, P. 1978. Fibronectin binds to *Staphylococcus aureus*. *Nature (Lond.).* 276:718.
54. Kuusela, P., T. Bartio, M. Buento, and E.B. Myhre. 1985. Attachment of staphylococci and streptococci on fibronectin, fibronectin fragments and fibrinogen bound to a solid phase. *Infect. Immunol.* 50:77.
55. Mosher, D.F., and R.A. Proctor. 1980. Binding and factor $XIII_a$-mediated cross-linking of a 27-kilodalton fragment of fibronectin to staphylococcus aureus. *Science (Wash. DC).* 209: 927.
56. Kavoussi, L.R., E.J. Brown, J.K. Richey, and T.L. Ratliff. 1990. Fibronectin-mediated Calmette-Guerin Bacillus attachment to murine bladder mucosa: Requirement for the expression of an antitumor response. *J. Clin. Invest.* 85:62.
57. Valentin-Weigand, P., J. Grulich-Henn, G.S. Chhatwal, G. Muller-Berghaus, H. Blobel, and K.T. Preissner. 1988. Mediation of adherence of streptococci to human endothelial cells by complement S protein (Vitronectin). *Infect. Immunol.* 56: 2851.
58. Isberg, R.R., and J.M. Leong. 1990. Multiple $\beta 1$ chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. *Cell.* 60:861.