The Mouse Vitronectin Receptor Is a T Cell Activation Antigen

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Summary

In this report, we demonstrate that the T cell activation antigen, recognized by monoclonal antibody H9.2B8, is the murine homologue of the vitronectin receptor (VNR) and, thereby, we provide initial evidence that VNR is expressed on lymphoid cells. VNR is expressed on a variety of T cell lines, tumors, and Con A–activated splenocytes, but not resting T cells, and is capable of binding to the extracellular matrix proteins fibronectin, fibrinogen, and vitronectin, via the tripeptide sequence RGD. There was no evidence of novel β chains pairing with the VNR α chain, as has been demonstrated in some human cells. In view of recent studies demonstrating that this molecule functions as an accessory molecule in T cell activation, the VNR may play an important role in mouse T cell functions.

Integrins comprise a complex family of cell adhesion molecules involved in cell-cell and cell–extracellular matrix protein (ECMP)[1] interactions (1). At least three subfamilies have been defined on the basis of the association of any of a number of α chains with a common β chain (2). We have previously characterized two mAbs (H9.2B8 and 8.18E12) that identified a novel integrin expressed on murine γ/δ and α/β T cell lines and hybridomas as well as on T cells activated for prolonged periods of time (7–21 d) by mitogens or alloantigens. This integrin mediated adhesion to fibronectin, vitronectin, and fibrinogen, and the two mAbs in combination, as well as the tetrapeptide RGDS alone, could inhibit the binding of cell lines to these ECMP (3). In the present report, we make use of a number of antisera to the vitronectin receptor (VNR) and demonstrate that this novel integrin is the murine homologue of the human VNR, thereby demonstrating for the first time that the VNR is present on mouse T lymphocytes and that the expression of this β3 integrin can be upregulated by activation.

Materials and Methods

Cell Lines. Dendritic epidermal T cell (DETC) line T195 and its corresponding hybridoma (T195/BW) derived from fusion to BW5147 have been previously described (4–6).

Antibodies. mAb H9.2B8 is a hamster mAb obtained after immunizations with mouse DETC lines (3). Rabbit anti-human VNR serum was purchased from Telios Pharmaceuticals, Inc. (La Jolla, CA). Rabbit antiserum to human GpIIIa (anti-β3 chain) was a gift from Dr. R. McKever (Oklahoma University, Norman, OK) and has previously been shown to react with mouse β3 integrin chains (7). Clone M18/2.a.8, secreting rat IgG reactive with mouse β2 integrin chains (8), was purchased from the American Type Culture Collection (Rockville, MD). Rat mAb to mouse LFA-1 (from clone M17/5.2) was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). mAb H1.2F3, which is reactive with a T cell activation antigen, has been described previously (9).

Antipeptide Sera. Rabbit anti–peptide sera against the COOH-terminal sequences of human β1 (anti-β1) (NPKYEGK) (10) and VNR α chain (anti-VNRα) (CEQLQPHENGEGNSET) (11) were prepared as previously described (12).

Con A Stimulation of Splenocytes. Con A–activated spleen cells from 8-wk-old female C3H/HeN mice were prepared as previously described (3).

Radioiodination and Immunoprecipitation Analysis. Cells were surface labeled with ^{125}I using the previously described lactoperoxidase method (13). Immunoprecipitations and immunodepletion experiments were performed as previously described (3). Precipitates were analyzed on SDS-PAGE (7% acrylamide) slab gels in the presence of 5% 2-ME.

Two-dimensional Electrophoresis. Nonequilibrium pH gradient electrophoresis (NEPHGE) was performed according to O'Farrell et al. (14) under nonreducing conditions using pH 4–6.5 ampholines (Pharmacia Fine Chemicals, Piscataway, NJ) in the first dimension and SDS-PAGE (7% acrylamide) in the second dimension.

Ligand Binding. Rat fibronectin (Calbiochem-Behring Corp., La Jolla, CA), rat fibrinogen (Sigma Chemical Co., St. Louis, MO), human vitronectin (Calbiochem-Behring Corp.), and rat collagen I (Sigma Chemical Co.) were coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals). Peptide GRGDSPC (Telios Pharmaceuticals, Inc.) was coupled to thiopropyl-activated Sepharose

[1] *Abbreviations used in this paper:* DETC, dendritic epidermal T cells; ECMP, extracellular matrix proteins; NRS, normal rabbit serum; VNR, vitronectin receptor.

4B (Pharmacia Fine Chemicals). Magnetic laminin agarose was a gift from Advanced Magnetics (Cambridge, MA). Precipitation experiments were performed by incubating 250 μl packed beads with 100 μl cell lysate for 60 min room temperature. The beads were washed in 0.5% NP-40 buffer (3) and integrins eluted with RGDS peptide (2 mg/ml) in 0.5% NP-40 buffer for 60 min. Immunoprecipitations using anti-VNRα sera were performed with the eluate as previously described (3).

Adhesion Assay. 96-well plates (Costar, Cambridge, MA) were coated with vitronectin (2.0 μg/ml) by incubating wells overnight at 37°C and then washing three times with binding buffer (PBS supplemented with 2.5 mg/ml BSA [Sigma Chemical Co.]). Remaining sites were blocked by adding 200 μl binding buffer and incubating for 2 h at 37°C.

Resting T cells and Con A–activated lymphoblasts (10^7 cells/ml) were labeled with 300 μCi ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 h at 37°C. The cells were then washed with HBSS and resuspended in 10 ml RPMI supplemented with 10% FCS for 30 min. After two washes, the cells were resuspended (5×10^5/ml) in binding buffer. The cell suspension (100 μl) was added to plates precoated with human vitronectin (Telios Pharmaceuticals, Inc.). Adhesion was evaluated in the presence of RGDS or RGES (Peninsula Laboratories, Inc., Belmont, CA) or mAbs by adding these agents directly to the wells (100 μl). After a 2-h incubation at 37°C, the wells were washed three times with binding buffer. The level of adhesion was subsequently evaluated by the addition of 200 μl of 0.5% Triton X-100 and counting the lysate in a gamma counter. The percent cell adhesion is expressed as a percentage of the total counts per minute added to the well. Background binding represents the percent adhesion to plates coated with albumin alone.

Results and Discussion

mAb H9.2B8 Recognizes Mouse VNR. We have previously shown with DETC cells that mAbs H9.2B8 and 8.18E12 recognize the α chain of an integrin-like heterodimer of 95 kD (β chain) and 140 kD (α chain) under nonreducing conditions, and that, upon reduction, the α chain is cleaved into 116- and 24-kD components (3). To analyze the relationship of this heterodimer to other integrins, we performed a series of sequential immunoprecipitations. Cell lysates (T195 or T195/BW) were precleared of material reactive with antisera to β3 or VNRα chains or, as a negative control, normal rabbit serum (NRS). These precleared lysates were then immunoprecipitated with various integrin-reactive antisera followed by analysis on SDS-PAGE under reducing conditions (Fig. 1). After preclearing material reactive with NRS, anti-β1 serum precipitated a heterodimer with an α chain of 140 kD (intense band) and a β chain of 135 kD (faint band) (Fig. 1, lane *2*). Antibody to integrin β2 chains precipitated heterodimers of 98 kD (β chain) and 180 kD (α chain) (Fig. 1, lane *3*) that were identical in size to the chains precipitated by a mAb reactive with the α chain of LFA-1 (Fig. 1, lane *4*). Three antisera to the VNR were used. Anti-β3 and anti-VNRα chain sera, as well as antiserum to purified human VNR, all precipitated a heterodimer with a β chain of 98 kD and an α chain of 116 kD (Fig. 1, lanes *5*, *6*, and *8*). The 24-kD component of the α chain can be visualized on 12% acrylamide gels (not shown). The molecular sizes of the component VNR chains were similar to those of the integrin recognized by mAb H9.2B8 (Fig. 1, lane *7*).

To determine if mAb H9.2B8 recognizes VNR, T195/BW cell lysates were cleared of material reactive with antisera to β3 chains or VNRα chains by repeated immunoprecipitations. Subsequent precipitations revealed that both anti-β3 and anti-VNRα sera cleared the integrin recognized by mAb H9.2B8 (Fig. 1, *B* and *C*), thus proving that mAb H9.2B8 recognizes the VNR. Other immunodepletion experiments demonstrated that mAb H9.2B8 precleared the material recognized by anti-VNR sera (data not shown). The process of

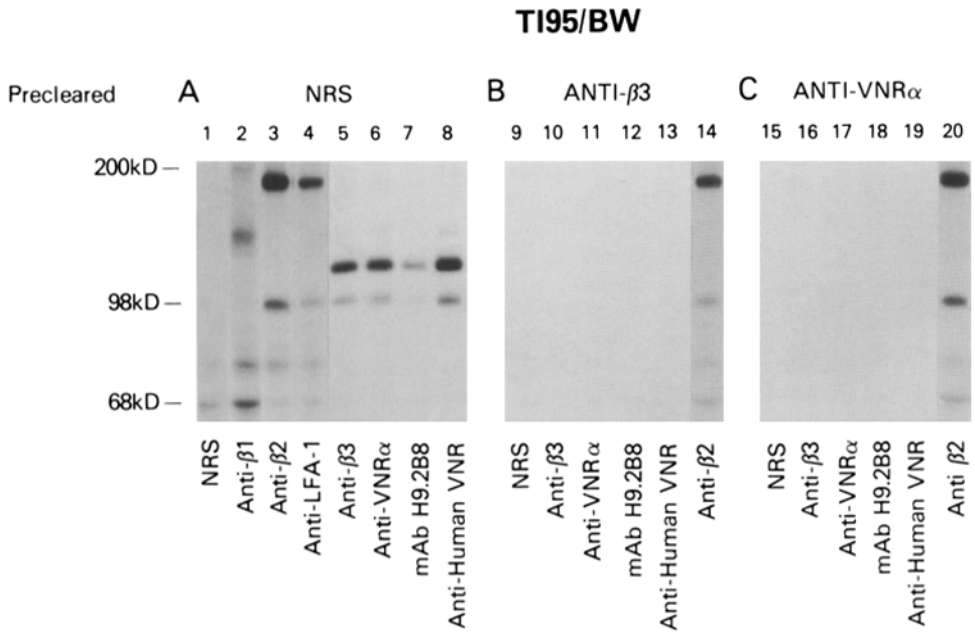

Figure 1. mAb H9.2B8 recognizes the vitronectin receptor. Lysates of ^{125}I cell surface–labeled T195/BW were precleared by serial immunoprecipitations with antiserum to the β3 chain or the VNRα chain, or as a negative control, NRS. Precleared cell lysates were immunoprecipitated with NRS, anti-β1 chain peptide serum, mAb M18/2.a.8 to the β2 chain, mAb M17/5.2 to LFA-1 α chain, anti-β3 chain serum, anti-VNRα serum, anti–human VNR serum, and mAb H9.2B8. Precipitates were analyzed by SDS-PAGE with 7% acrylamide under reducing conditions. The position of prestained molecular mass markers is indicated on the left. The bands below the 98-kD molecular mass marker in lanes *2*, *3*, *4*, *14*, and *20* are background bands as evidenced by their presence in lane *1*. Identical results were obtained with the parent T195 DETC cell line.

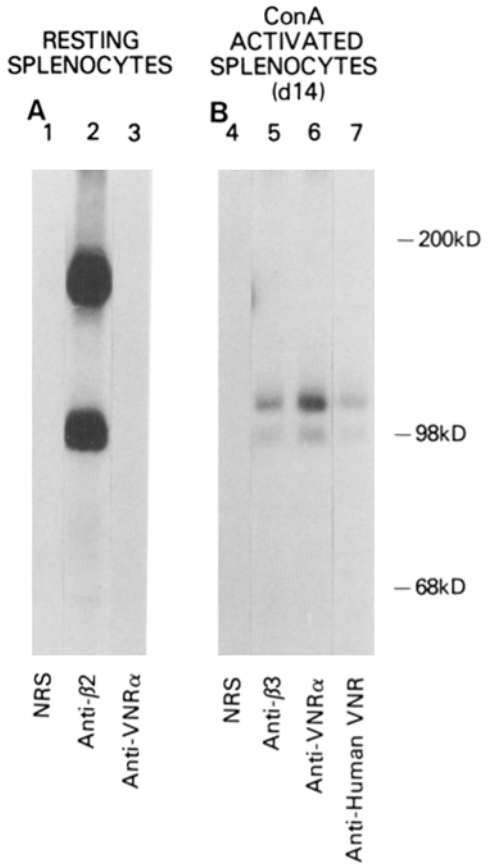

Figure 2. VNR is expressed by activated T cells. Spleen cells from C3H/HeN mice were grown in the presence of Con A (3 μg/ml) for 4 d and IL-2 (20 U/ml) for a further 10 d. These, and fresh spleen cells as nonactivated controls, were surface labeled with ^{125}I and cell lysates immunoprecipitated with NRS, mAb M18/2.a.8 to β2 chain, anti-β3 chain serum, anti-VNRα serum, and anti-human VNR serum. Immunoprecipitates were analyzed by SDS-PAGE with 7% acrylamide under reducing conditions. The position of prestained molecular mass markers is indicated on the right. The cells that proliferated in response to Con A were found by FACS analysis to be >95% Thy-1$^+$.

immunodepletion did not interfere with subsequent immunoprecipitations, as β1 integrins (not shown) and LFA-1 (Fig. 1, lanes *14* and *20*) could still be precipitated from precleared lysates.

Con A–activated Splenocytes Express VNR. We have previously shown by FACS® analysis that the α chain recognized by mAb H9.2B8 (here shown to be VNRα) is present on a population of activated splenocytes (3). In view of the fact that VNR has not previously been reported to be expressed by mouse lymphoid cells, we examined the structure of the receptor expressed by Con A–activated T cells. As shown in Fig. 2, no material could be immunoprecipitated from lysates of resting lymphocytes with the anti-VNRα peptide serum, while the typical LFA-1 heterodimer was seen with the anti-β2 precipitate (*A*). In contrast, when similar studies were performed on lysates of T cells after 14 d of activation with Con A, all three anti-VNR sera immunoprecipitated a 116- and 98-kD dimer (Fig. 2 *B*). To determine if the VNR expressed by the activated splenocytes was functional, quantitative cell attachment studies were performed. About 30% of splenic T cells activated in vitro with Con A and IL-2 adhered to wells coated with vitronectin, and this binding could be completely inhibited by mAb H9.2B8 and by the RGDS peptide, but not by the RGES peptide or the control mAb H1.2F3 (Table I). No binding of resting T cells to vitronectin-coated plates could be detected.

Previous studies with human VNR have shown the VNR α chain may associate with β chains other than β3, namely β1 (15, 16), βx (now named β5) (17), βs (18), and β3b (19). Some of these novel β chains are not easily distinguished by their mobilities in one-dimensional SDS-PAGE, but can be clearly distinguished from β3 chains by pI (17, 19). To examine whether some of the murine lymphoid VNR α chains might be associated with chains other than β3, we compared immunoprecipitations from T195/BW and Con A–activated splenocytes by NEPHGE analysis (Fig. 3). It can be seen that both T195/BW and Con A–activated splenocytes apparently express only a single heterodimer when examined after precipitation with anti-β3 or anti-VNRα sera, indicating that the VNR expressed by Con A–activated splenocytes is composed solely of the VNRα/β3 heterodimer.

VNR Expression by Activated T Cells Bind Multiple ECMP. Integrins have been shown to bind various ECMP (20), and the key amino acid sequence in many of these binding specificities has been shown to be the RGD motif (1). To determine the specificity of murine VNR, lysates from cells were reacted with ECMP- or RGD-containing peptide coupled to

Table 1. *The Binding of Activated Murine T Cells to Vitronectin Is Inhibited by RGDS and mAb H9.2B8*

		Inhibitor (μg/ml)							
		RGDS		RGES		H9.2B8		H1.2F3	
	Media	100	10	100	10	50	5	50	5
Day 10 lymphoblasts	32.1	4.5	7.4	29.0	26.8	7.6	7.3	23.5	23.5
Uncultured	7.9	4.7	5.9	5.5	4.8	5.5	4.5	5.9	4.7

The results are expressed as the percent cell adhesion. The background binding for the lymphoblasts was 7.3% and for the uncultured cells was 3.8%.

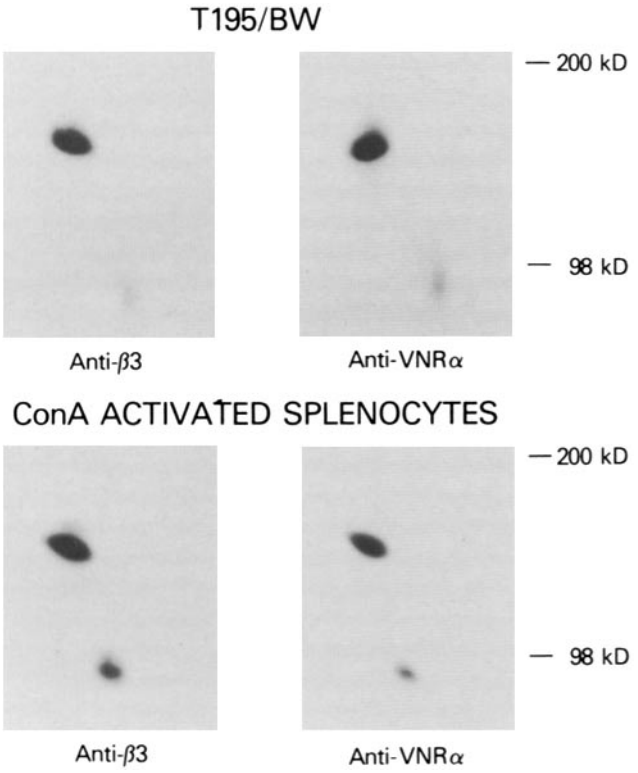

Figure 3. NEPHGE analysis of VNR. T195/BW and Con A–activated splenocytes were surface labeled with ^{125}I and cell lysates immunoprecipitated with anti-β3 serum or anti-VNRα serum. Immunoprecipitates were first separated by NEPHGE using pH 4–6.5 ampholines in the first dimension, then SDS-PAGE with 7% acrylamide under nonreducing conditions in the second dimension. The position of prestained molecular mass markers is indicated on the right.

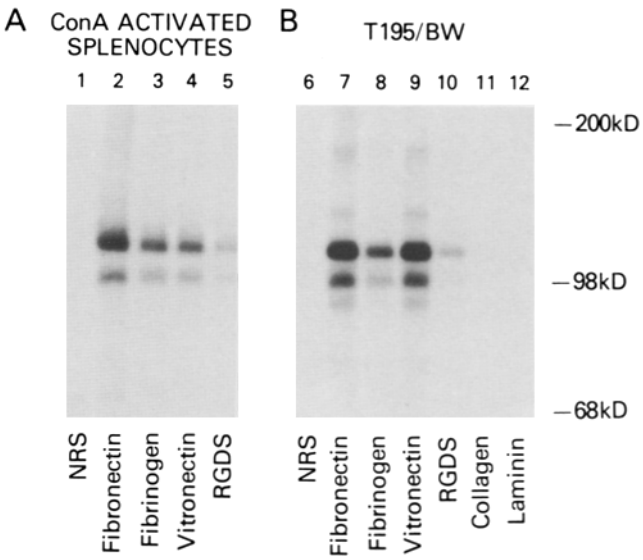

Figure 4. Binding of VNR to extracellular matrix proteins. T195/BW and Con A–activated splenocytes were cell surface labeled with ^{125}I and cell lysates precipitated with fibrinogen, fibronectin, vitronectin, laminin, or collagen bound to Sepharose 4B. Integrins were eluted with 2 mg/ml RGDS peptide and immunoprecipitated with NRS or anti-VNRα serum. Precipitates were analyzed at 7% SDS-PAGE under reducing conditions. The faint bands of 90, 130, and 170 kD were only seen in precipitates of material eluted from extracellular matrix protein-coupled beads. Their identities are currently under investigation. The position of prestained molecular mass markers is indicated on the right.

Sepharose beads, eluted with RGDS peptide, and immunoprecipitated with anti-VNRα sera. The results of such analyses shown in Fig. 4 indicate that VNR from Con A–stimulated splenocytes bound to RGD, fibronectin, fibrinogen, and vitronectin. Binding to all ligands was blocked by the presence of RGDS peptide (data not shown). A similar binding pattern was seen with VNR expressed by T195/BW (Fig. 4).

Our previous studies (3), together with the results of this report of the mouse VNR, demonstrate that it is similar to the human VNR (21). Both integrins comprise an α chain, which is cleaved upon reduction, and a β chain, which has an apparently larger molecular mass upon reduction than when nonreduced, indicative of intrachain disulfide bonds. The mouse VNR differs from the human VNR in that it is capable of binding to fibronectin in addition to fibrinogen and vitronectin. Binding to all three ECMP is mediated by the VNRα/β3 heterodimer, and we have not been able to demonstrate any other chains associated with VNRα or β3 that may contribute to this unique binding specificity. In the case of human cells, adherence to fibronectin is associated with VNRα paired with the β1 chain (15, 16) and the βx chain (17, 22); however, the VNRα/β1 heterodimer does not mediate adherence to vitronectin, and the VNRα/βx heterodimer does not bind fibrinogen. One possible explanation for the difference is that the studies in man have characterized the VNR on nonlymphoid cells, whereas our studies in the mouse have characterized a VNR found on lymphoid cells.

Previous studies have shown that activation is required for integrin expression and/or function on T lymphocytes. Integrins that are expressed constitutively are only able to bind their ligand after T cell activation (20, 23–25), whereas integrins not normally expressed by resting T cells, such as VLA-1 and VLA-2, are induced after T cell activation. In this study, we have shown that only activated murine T cells express the VNR. These results differ from those of Klingemann and Dedhar (26), who demonstrated VNR expression on resting human T cells. Although a species difference in the regulation of expression of the VNR on lymphoid cells may exist, these latter studies were performed with polyclonal antisera to the human VNR and have not, as yet, been confirmed with mAbs.

The function of VNR on activated T lymphocytes is still unclear; however, it seems likely that VNR functions as a costimulatory molecule (27), as has been recently described for several other integrins (LFA-1, VLA-4, -5, -6) (23–25).

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