Epiligrin, A Component of Epithelial Basement Membranes, Is An Adhesive Ligand for $\alpha3\beta1$ Positive T Lymphocytes

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Abstract. The cutaneous T cell lymphomas (CTCL), typified by mycosis fungoides, and several chronic T cell mediated dermatoses are characterized by the migration of T lymphocytes into the epidermis (epidermotropism). Alternatively, other types of cutaneous inflammation (malignant cutaneous B cell lymphoma, CBCL, or lymphocytoma cutis, non-malignant T or B cell type) do not show evidence of epidermotropism. This suggests that certain T lymphocyte subpopulations are able to interact with and penetrate the epidermal basement membrane. We show here that T lymphocytes derived from patients with CTCL (HUT 78 or HUT 102 cells), adhere to the detergent-insoluble extracellular matrix prepared from cultured basal keratinocytes (HFK ECM). HUT cell adhesion to HFK ECM was inhibitable with monoclonal antibodies (mAbs) directed to the $\alpha3$ (P1B5) or $\beta1$ (P4C10) integrin receptors, and could be up-regulated by an activating anti-$\beta1$ mAb (P4G11). An inhibitory mAb, P3H9-2, raised against keratinocytes identified epiligrin as the ligand for $\alpha3\beta1$ positive T cells in HFK ECM. Interestingly, two lymphocyte populations could be clearly distinguished relative to expression of $\alpha3\beta1$ by flow cytometry analysis. Lymphokine activated killer cells, alloreactive cytotoxic T cells and T cells derived from patients with CTCL expressed high levels of $\alpha3\beta1$ ($\alpha3\beta1^{high}$). Non-adherent peripheral blood mononuclear cells, acute T or B lymphocytic leukemias, or non-cutaneous T or B lymphocyte cell lines expressed low levels of $\alpha3\beta1$ ($\alpha3\beta1^{low}$). Resting PBL or $\alpha3\beta1^{low}$ T or B cell lines did not adhere to HFK ECM or purified epiligrin. However, adhesion to epiligrin could be up-regulated by mAbs which activate the $\beta1$ subunit indicating that $\alpha3\beta1$ activity is a function of expression and affinity. In skin derived from patients with graft-vs.-host (GVH) disease, experimentally induced delayed hypersensitivity reactions, and CTCL, the infiltrating T cells could be stained with mAbs to $\alpha3$ or $\beta1$ and were localized in close proximity to the epiligrin-containing basement membrane. Infiltrating lymphocytes in malignant cutaneous B disease (CBCL) did not express $\alpha3\beta1$ by immunohistochemical techniques and did not associate with the epidermal basement membrane. The present findings clearly define a function for $\alpha3\beta1$ in T cells and strongly suggest that $\alpha3\beta1$ interaction with epiligrin may be involved in the pathogenesis of cutaneous inflammation.

LYMPHOCYTE-mediated cutaneous dermatoses can be broadly categorized (Kurtin et al., 1986; Knobler and Edelson, 1986) into those which involve the superficial dermis and epidermis (T cell type) or those that involve the deep or reticular dermis (B cell type). The so-called T cell pattern is histologically characterized by a lichenoid infiltrate whereby the T cells are concentrated in a bandlike pattern in the papillary dermis and epidermis often obscuring the basement membrane zone (Knobler and Edelson, 1986; Kurtin et al., 1986; Murphy and Mihm, 1986). Lichen planus, graft vs.-host disease and the group of cutaneous T cell lymphomas (CTCL)[1] typified by mycosis fungoides are examples of cutaneous T cell disorders which have a classic T cell pattern of infiltration. In addition, in CTCL significant epidermotropism (migration into the epidermis) occurs and Pautrier microabscesses containing T cells are often observed in the epidermis (Knobler and Edelson, 1986). Migration of T cells into the epidermis is also characteristic of other dematoses such as contact dermatitis. In contrast, cutaneous B cell lymphomas (CBCL) and the group of non-malignant disorders referred to as pseudolymphoma or lymphocytoma cutis are not characterized by the migration of cells into the epidermis (Kurtin et al., 1986). Rather, these disorders show

1. *Abbreviations used in this paper*: AAT, adenine/aminopterin/thymidine; ALL, acute lymphocytic leukemias; BMZ, basement membrane zone; CBCL, cutaneous B cell lymphoma; CTCL, cutaneous T cell lymphoma; CTL, cytotoxic T lymphocyte; ECM, extracellular matrix; EPI, epidermis; HFF, human foreskin fibroblast; HFK, human foreskin keratinocyte; ICAM, intercellular adhesion molecule; KGM, keratinocyte growth medium; LECCAM, lectin-like cell adhesion molecule; MF, mycosis fungoides; PBL, peripheral blood lymphocyte; VCAM, vascular cell adhesion molecule.

perivascular and interstitial cellular aggregates within the reticular dermis often with relative sparing of the papillary dermis or the subepidermal collagenous (grenz) zone (B cell type). The mechanisms operating to determine the specificity of T or B cell localization in the skin are presently unknown.

Integrins are a family of heterodimers that are involved in a variety of cell–cell and cell–matrix interactions (Hynes, 1992). In hematopoietic cells the $\beta 1$ (VLAs) and $\beta 2$ (LFA, Mac-1, p150/95) integrins are widely expressed and mediate the adhesion of such cells to extracellular matrix proteins (ECM) (collagens, fibronectins, laminins) and the vascular cell adhesion molecules (ICAMs 1-3, VCAM) (reviewed by Hemler, 1990; Osborn, 1991; Lasky, 1992; Hynes, 1992). In the skin, integrins, and their ligands/co-receptors have been implicated in keratinocyte desmosome and hemidesmosome function (Carter et al., 1990*a*, *b*; Larjava et al., 1990; Symington et al., 1993), vascular emigration of neutrophils ($\beta 2$/ICAM) and T lymphocytes ($\alpha 4\beta 1$/VCAM) (Wysocki and Issekutz, 1991; reviewed by Lasky, 1992), and the $\beta 2$/ICAM pathway has been implicated in the retention of T lymphocytes in inflammatory epidermis (reviewed by Walsh et al., 1990; Barker, 1991; Barker and Nickoloff, 1992). However, the migration of T cells into the epidermis necessarily requires such cells to penetrate the epidermal basement membrane. ICAM or VCAM interactions cannot account for this since these integrin co-receptors are not known to be constituents of the epidermal basement membrane. Recently, the $\alpha 3\beta 1$ integrin has been reported to be the receptor used by keratinocytes to adhere to epiligrin, a newly described component of epithelial basement membranes (Carter et al., 1991). However, the mechanisms T cells used to adhere to and subsequently penetrate the epidermal basement membrane are completely unknown.

The epidermal basement membrane is synthesized by basal keratinocytes and many of its components can be detected in the detergent insoluble matrix deposited by such cells in culture. Therefore, in the present paper, we examined the ability of various T (or B) cell populations to adhere to the ECM synthesized by cultured human foreskin keratinocytes (HFK). The results of these studies show clearly that cultured cells from patients with CTCL exhibited a clear preference for matrix derived from keratinocytes. Inhibition studies with mAbs directed to integrin receptors and keratinocyte matrix molecules revealed that CTCL adhesion to HFK ECM was a function of $\alpha 3\beta 1$ interaction with epiligrin. Immunoperoxidase staining analysis of cutaneous T cell lymphoma and other inflammatory dermatoses revealed that the infiltrating T cells expressed $\alpha 3\beta 1$ and could be detected in the basement membrane zone in close association with epiligrin. In contrast, infiltrating cells in CBCL did not stain with mAbs to $\alpha 3$ and did not associate with the basement membrane or epidermis. The present findings clearly define a function for $\alpha 3\beta 1$ in T cells and suggest a mechanism for T lymphocyte migration into the epidermis that may involve $\alpha 3\beta 1$ interaction with adhesion molecules such as epiligrin in the epidermal basement membrane.

Methods and Materials

Materials

Protein-A and -G agarose, PMSF, N-ethylmaleimide (NEM) and BSA were from Sigma Immunochemicals (St. Louis, MO). Peroxidase- and fluorescein-conjugated (goat) anti-mouse IgG (H and L chains) or peroxidase- and rhodamine-conjugated (goat) anti-rabbit IgG and IgM (H and L) were obtained from Vector Laboratories (Vecta-Stain Elite Kit; Burlingame, CA) and Tago, Inc. (Burlingame, CA). [^{51}Cr]sodium chromate was from New England Nuclear (Boston, MA) and [^{35}S]methionine and cysteine were from Amersham Corp. (Arlington Heights, IL). Phycoerythrin-conjugated avidin was from Cal-Tag (San Francisco, CA). N-hydroxysuccinimide-biotin was from Pierce (Rockford, IL).

Cells and Cell Culture

Normal newborn HFKs were prepared as described (Carter et al., 1991) and were maintained in serum-free keratinocyte growth medium (KGM; Clonetics, San Diego, CA). KGM contained insulin, EGF, hydrocortisone, and bovine pituitary extract. Primary cultures of human foreskin fibroblasts (HFFs) were prepared by collagenase digestion of neonatal foreskins as described (Carter et al., 1991). Plastic non-adherent peripheral blood lymphocytes were obtained from normal donors and were prepared as described (Wayner et al., 1988, 1989). Acute lymphocytic leukemias (ALL), T or B, were obtained from the peripheral blood of individuals undergoing treatment at the Fred Hutchinson Cancer Center (Seattle, WA) and were the generous gift of Dr. Bob Andrews (Seattle, WA). The ALL were frozen (FBS 90% and DMSO 10%) and stored in liquid nitrogen before use.

The monoclonal C1C4 HLA B7-specific cytotoxic T lymphocyte (CTL) cell line has been described (Wayner et al., 1988, 1987). C1C4 cells were maintained in 10 U/ml recombinant IL-2 (Dr. D. Urdal, Immunex, Seattle, WA) and were routinely used 7-10 d after antigen stimulation with an HLA B7-positive EBV-transformed B cell line (ST-1). Lymphokine-activated killer cells or LAK cells were generated by incubating freshly derived PBMC in RPMI-1640 supplemented with 10% FBS and 500 U/ml IL-2 for 7-10 d (Wayner et al., 1988). Molt 4 (human non-cutaneous CD4+ T cell leukemia), HUT 78 (human CD4+ T cell lymphoma isolated from a patient with Sezary's syndrome), HUT 102 (human cutaneous CD4+ T cell lymphoma isolated from a patient with mycosis fungoides), WI-38 (diploid lung fibroblasts), Ramos (human Burkitt lymphoma), U937 (human monocytic leukemia), HT1080 (human fibrosarcoma), PC3 (pancreatic carcinoma), and A431 (epidermoid carcinoma) cells were obtained from the ATCC. The human KCA (B lymphoblastoid) cell line was a generous gift from Dr. Eugene Butcher (Stanford University, Stanford, CA), the K562 (erythroleukemia) cell line was a generous gift from Dr. Yoshi Takada (Scripp's Clinic, La Jolla, CA), the UCLA P3 (carcinoma) cell line was a generous gift from Dr. David Cheresh (Scripp's Clinic), and the A375 (melanoma, low metastatic variant) and H2981 (lung carcinoma) cells were a generous gift from Dr. Diane Horn (Oncogen, Bristol-Myers Squibb, Seattle, WA). The Jurkat cell line (human non-cutaneous CD4+ T cell leukemia) was as described (Wayner et al., 1988, 1989). Unless stated otherwise, all cell lines were maintained in RPMI-1640 supplemented with 10% FBS (Hyclone).

Preparation of ECM Adhesive Ligands

Pepsinized human laminin from placenta was a generous gift from Dr. Helena Hessle (Telios Corp., La Jolla, CA). Human plasma fibronectin and collagens types I and III were prepared as described (Wayner and Carter, 1987; Wayner et al., 1988). Epiligrin was immunoaffinity purified from HFK culture supernatant as described (Carter et al., 1991).

Preparation of ECM

ECM from human foreskin keratinocytes or carcinoma cell lines was prepared by growing cells for 3-4 d in KGM (HFK) or RPMI 10% FBS (carcinomas) on 48-well tissue culture plates (Costar Corp., Cambridge, MA) as described (Carter et al., 1991). The cell layer was removed by sequential extraction with 1% vol/vol Triton X-100 detergent in PBS, 2 M urea in 1 M NaCl, and 8 M urea. All extraction buffers contained 1 mM PMSF and 2 mM NEM as protease inhibitors. The ECM was further digested with 10 μg/ml DNAase for 30 min in 1% heat-denatured BSA before use in cell adhesion assays. ECM derived from WI-38 fibroblasts was prepared exactly as described (Wayner et al., 1988).

mAbs

mAbs to Basement Membrane Components. Monoclonal anti-laminin was a generous gift from Dr. Eva Engvall (La Jolla Cancer, La Jolla, CA), monoclonal anti-entactin (A9) was a generous gift from Dr. Alfred Fish (Department of Pediatric Nephrology, University of Minnesota, Min-

neapolis, MN) and monoclonal anti-type IV collagen was obtained from Dako Corp. (Carpinteria, CA). mAbs to epiligrin (P1E1, P3H9-2, P3E4) and fibronectin (P1H11, P3D4) were produced by the methods of Oi and Herzenberg (1980) and Taggart and Samloff (1983) as described (Wayner and Carter, 1987; Wayner et al., 1988, 1989; Garcia-Pardo et al., 1992) using cultured keratinocytes or purified plasma fibronectin as immunogens. Spleen cells from immune RBF/Dn mice were fused with NS-1/FOX-NY myeloma cells. Viable heterokaryons which secreted antibodies specific for epiligrin or fibronectin were selected in RPMI 1640 medium supplemented with adenine/aminopterin/thymidine (AAT). mAb P1E1 (anti-epiligrin) has been described (Carter et al., 1991) and was used to immuno-affinity purify epiligrin from HFK-conditioned cultured medium.

mAbs to Integrin Receptors. mAbs to the integrin receptors $\beta1$ (P4C10), $\alpha2\beta1$ (P1H5), $\alpha3\beta1$ (P1B5, P1F2), $\alpha4\beta1$ (P4C2, P4G9), $\alpha5\beta1$ (P1D6), and $av\beta5$ have been described (Wayner and Carter, 1987; Wayner et al., 1988, 1989, 1991; Carter et al., 1990*a*). mAb P4H9 is an anti-$\beta2$ (CD18) mAb that was raised against normal PBL. In immune precipitation experiments it preclears a prototype anti-$\beta2$ reagent, 60.3 (Beatty et al., 1983). P4H9 completely inhibits several $\beta2$-dependent functions: CTL- and NK-mediated cytotoxicity, MLR reactivity and homotypic B cell aggregation. The activating anti-$\beta1$ mAb P4G11 was raised against U937 cells and was selected for its ability to up-regulate hematopoietic cell adhesion to surfaces coated with plasma fibronectin. Monoclonal anti-$av\beta3$ was a generous gift from Dr. David Cheresh (Scripps Clinic) and anti-$\alpha1$ (TS2/7) was a generous gift from Dr. Martin Hemler (Dana Farber).

mAbs were purified from culture supernatant or ascites fluid (athymic mice) on a protein G sepharose column (Sigma Immunochemicals). FITC-conjugated T cell (CD3, CD4, CD7, CD8, CD45RO) and B cell (CD19) specific antibody reagents were purchased from Dako Corp. The anti-CD45RO reagent, UCHL-1, has been used to define a subpopulation of "memory" T cells (Akbar et al., 1988; Merkenschlager et al., 1988).

Adhesion Assays

The cell substrate adhesion assay using ECM (HFK or WI-38) or purified ECM ligands and ^{51}Cr-labeled cells was exactly as previously described (Wayner and Carter, 1987; Wayner et al., 1989; Carter et al., 1991). Non-adherent cells were removed by washing with PBS supplemented with 1 mM $CaCl_2$ and the adherent cells were dissolved in SDS/NaOH and quantitated in a gamma counter.

Modulation of cell-substrate adhesion with mAbs. The effects of modulating antibodies to integrin receptors on T cell adhesion were determined as previously described (Wayner et al., 1989; Wayner and Kovach, 1992). Cells were labeled with $Na_2{}^{51}CrO_4$ (50 μCi/ml for 2–4 h) and allowed to adhere to protein- or matrix-coated surfaces for 30–60 min in the presence (or absence) of mAbs known to perturb integrin activity.

Flow Cytometry Analysis

Expression of $\alpha3$ and $\beta1$ by cultured hematopoietic cells in suspension was evaluated by indirect immunofluorescence staining and flow cytometry. The data were analyzed using CONSORT 30 software on a FACScan (Becton Dickinson, Mountain View, CA). Positive fluorescence was determined on a four decade log scale and fluorescence intensity (log FI) was expressed as the mean channel number of 5,000 cells. In tabulated data, the background fluorescence was subtracted. Analysis of $\alpha3$ expression on freshly derived peripheral blood lymphocyte subpopulations or frozen acute lymphocytic leukemias (T or B) was accomplished by dual-label immunofluorescence staining and flow cytometry. For dual-label studies peripheral blood T lymphocytes, "memory" T lymphocytes or B lymphocytes were identified with FITC-conjugated mAbs to CD3, CD45RO or CD19 (Dako Corp., respectively). $\alpha3$ was detected with biotinylated P1F2 and phycoerythrin-conjugated avidin (Cal-Tag Labs, San Francisco, CA).

Biopsy Specimens and Immunohistochemical Staining

Biopsy specimens (4-mm punch) from patients with established contact hypersensitivity reactions (n = 5) (Waldorf et al., 1991), epidermotropic cutaneous T cell lymphoma (patch and plague-stage MF; n=10), chronic cutaneous GVH disease (n=2), and cutaneous B cell lymphoma (n=5) were snap frozen in liquid nitrogen or first embedded in OCT, and then snap frozen in isopentane. Sections were prepared with a cryostat at 5- or 6-μm intervals. Normal scalp skin was a generous gift from Dr. Maria Hordinsky (Department of Dermatology, University of Minnesota). The GVH tissues were derived from patients with acute disease and were the generous gifts of Dr. George Sale (Fred Hutchinson Cancer Center). The infiltrating mononuclear cells were determined to be "activated or memory" T lymphocytes by standard immunohistochemical techniques ($CD3^+$, $CD4^+$, $CD8^+$, $CD45RO^+$, $CD45RA^-$). In other studies, serial sections were stained by immunohistochemistry with the following mAbs: Leu 1 (CD5), Leu 2 (CD8), Leu 3 (CD4), Leu 4 (CD3), Leu 5 (CD2), Leu 6 (CD1), Leu 12 (CD19), and anti-κ and anti-λ immunoglobulin light chains (Becton Dickinson). All cases were also stained for $\alpha2$ (P1H6), $\alpha3$ (P1F2), and $\beta1$ (P4C10). Selected cases (at least two from each category) were also stained for $\alpha4$ (P4G9) and $\alpha5$ (P1D6). Isotype-matched irrelevant mAbs served as negative controls. Dual-label immunofluorescence staining of snap frozen tissue was performed with: (*a*) UCHL-1 anti-CD45RO or anti-CD3 detected with rhodamine conjugated goat anti-mouse; and (*b*) biotinylated P1E1 anti-epiligrin detected with FITC-conjugated avidin. In three cases of MF and two cases of contact dermatitis, conventional transmission EM was also performed to assess spatial relationships between infiltrating lymphocytes and the basement membrane zone.

Immune Precipitation, Sequential Immune Precipitation, and SDS-PAGE

Viable cells were surface labeled with ^{125}I or NHS-biotin or metabolically labeled with [^{35}S]methionine and cystine (Wayner and Carter, 1987; Wayner et al., 1991) followed by extraction with 1% Triton X-100 detergent in PBS supplemented with 1 mM $CaCl_2$. 1 mM PMSF and 1 mM NEM were used as protease inhibitors. Immune precipitations, sequential immune precipitations, and SDS-PAGE were carried out exactly as previously described (Wayner and Carter, 1987; Wayner et al., 1989). The identity of the proteins immune precipitated from detergent extracts of cells by the putative upregulating anti-$\beta1$ mAb, P4G11, was determined by sequential immune precipitation or pre-clearing analysis with a known anti-$\beta1$ mAb, P4C10 (Carter et al., 1990*a*,*b*).

Results

Adhesion of T Cells to HFK Matrix

The epidermal basement membrane is thought to be synthesized by basal keratinocytes and many of its components can be detected in the detergent insoluble ECM deposited by such cells in culture. Therefore, we examined the ability of the ECM deposited by cultured human foreskin keratinocytes (HFK) to support the adhesion of various T and B cell populations.

T cells differed dramatically in their ability to adhere to HFK ECM (Table I). Of the tumor cell lines we examined, only T lymphocytes established from patients with CTCL (HUT 102 or HUT 78) could adhere to HFK matrix (Table I). T cells established from patients with non-cutaneous leukemia (Molt 4) or lymphoma (Jurkat) did not attach to HFK matrix. With regard to non-malignant T cell populations, unactivated, plastic non-adherent peripheral blood mononuclear cells (85% T cells) did not adhere to HFK ECM while cultured alloantigen specific cytotoxic T cells (CTL) and IL-2-activated LAK cells exhibited significant binding. As we have previously reported, HT1080 cells adhere to HFK matrix and are included as a positive control. Interestingly, none of the B cell lines we examined adhered to HFK matrix. All of the T and B cell populations attached to matrix derived from WI-38 fibroblasts (not shown) demonstrating that the failure of non-cutaneous T cells and B cells to bind HFK ECM was not due to an inability to bind secreted matrix. WI-38 matrix, as we have previously reported, contains multiple adhesive ligands including fibronectin and collagen (Wayner et al., 1988).

Lymphocyte Adhesion to HFK ECM Is Correlated with Expression of $\alpha3\beta1$

As we have already reported, keratinocytes and other non-

Table I. Adhesion of Lymphoid Cells to HFK ECM and Expression of the α3β1 Integrin Receptor

T cells	ADHESION (HFK ECM)*	α3 (MFI)‡	β1 (MFI)
HUT 78 (Sezary's)	89	48	61
HUT 102 (Mycosis fungoides)	76	79	156
CTL (cytotoxic T lymphocyte)	42	32	141
LAK (IL-2 activated killer cell)	22	46	189
HSB-2 (non-cutaneous leukemia)	12	13(Sh)§	118
Jurkat (non-cutaneous lymphoma)	2	10(Sh)	174
Molt 4 (non-cutaneous leukemia)	4	9(Sh)	118
ALL-T (3)	ND	–	98
B cells			
ST-1 (EBV transformed cell line)	0	8(Sh)	28
Ramos (Burkitt's lymphoma)	2	–	76
KCA (B lymphoblastoid)	8	11(Sh)	51
ALL-B (3)	ND	–	46
Other			
PBMC (T and B)	0	6.0(Sh)	66‖
K562 (erythroleukemia)	0	–	19
HT1080 (fibrosarcoma)	108	446	584
PC3 (carcinoma)	92	184	278
A375 (melanoma)	86	23	62

* Adhesion expressed as percent of Con A (5 μg/ml) control.
‡ MFI, mean fluorescence intensity calculated as mean channel number minus the mean channel number for the negative non-immune IgG control.
§ Sh indicates a shift in the positive direction of the entire population and a (–) indicates no detectable fluorescence.
‖ Two subpopulations ($\beta1^{high}$ and $\beta1^{low}$). The value shown in the table is the MFI calculated for the whole population.

Adhesion of T or B cell populations to ECM derived from HFK: correlation with α3 expression. For matrix production, the HFK were grown on 48-well tissue culture plates. HFK matrix and the adhesion assay were exactly as previously described (Carter et al., 1991). Surface expression of α3 was detected by flow cytometric analysis of cells stained with an α3-specific mAb (P1B5) exactly as described (Wayner et al., 1988; Wayner et al., 1989). Histograms were generated on a four log scale, analyzed with Consort 30 software and are expressed as MFI. Fc receptors were blocked with purified goat IgG (50 μg/ml). Staining for flow cytometry analysis was carried out at 4°C in the presence of 0.02% sodium azide and 1% normal goat serum.

epithelial cell populations use the integrin α3β1 to bind to their secreted matrix (Carter et al., 1991). α3β1 expression on lymphocytes, however, is highly restricted (Wayner et al., 1988; Hemler, 1990). Therefore, we performed the following experiments to see if T cell adhesion to HFK ECM was correlated with expression of α3β1.

The cells in Table I could be clearly divided into two populations relative to α3β1 expression ($\alpha3^{high}$ and $\alpha3^{low}$). Only those cells able to adhere to HFK matrix expressed high levels of α3β1: CTCL (HUT 78 or HUT 102), LAK, and CTL were $\alpha3\beta1^{high}$ while non-cutaneous T cells (Molt 4 and Jurkat), freshly derived PBMC, and all of the B cell populations we examined were $\alpha3\beta1^{low}$. The cultured T or B cell lines could not be so easily distinguished based on surface expression of other integrins such as α4β1 or α5β1 (not shown). All plastic non-adherent PBMC were weakly positive for α3 (Table I) and in some individuals, there appeared to be a subpopulation of cells which stained more brightly with mAbs to α3. Interestingly, in such individuals, this sub-population also stained with UCHL-1 (anti-CD45RO) identifying it as a "memory" T cell subset (Akbar et al., 1988). The $\alpha3\beta1^{high}$ phenotype was also correlated with the expression of the CD45RO epitope in cultured T cell populations (HUT 78, CTL, LAK, but not Jurkat or Molt 4). As we and others have reported, HT1080 fibrosarcoma cells, A375 melanoma, and several carcinoma cell lines (A431 and PC3 cells) also expressed high levels of α3β1. Immunoprecipitation analysis with anti-α3 revealed that in CTL and HUT 78 cells, the integrin α3 chain was found in the form of a heterodimer containing a β1-like subunit (Fig. 1). The presence of β1 was confirmed by preclearing T cell detergent extracts with an anti-β1 mAb (P4C10) before immune precipitating with anti-α3 (P1B5). Under these conditions no α3 could be precipitated (not shown). Jurkat cells, which are $\alpha3\beta1^{low}$ by flow cytometry, and HT1080 cells, which as we have already reported, are $\alpha3\beta1^{high}$ (Wayner and Carter, 1987) are included as controls. The conjugates of α1, α2, α4 (p80/70, cleaved form), α5, and α6 complexed with β1 could also be immune precipitated from CTL, and α1, α4 (predominantly

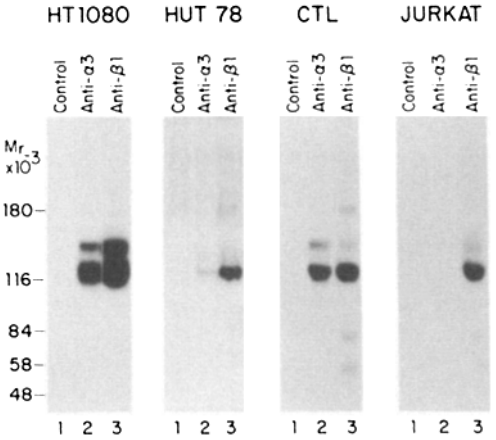

Figure 1. Immunoprecipitation of α3β1 from CTL, HT1080, HUT 78, or Jurkat cells. Surface labeled cells were extracted with 1% Triton X-100 in PBS, pH 7.4, containing 1 mM $CaCl_2$ and protease inhibitors as previously described (Wayner and Carter, 1987; Wayner et al., 1989). Precipitation of integrins from detergent extracts of cells with mAbs bound to protein A-sepharose was carried out exactly as previously described (Wayner and Carter, 1987) and subjected to autoradiography. The antibodies used were: *Control*, SP2 myeloma culture supernatant (lane *1*); *anti-α3*, P1B5 (lane 2); *anti-β1*, P4C10 (lane *3*).

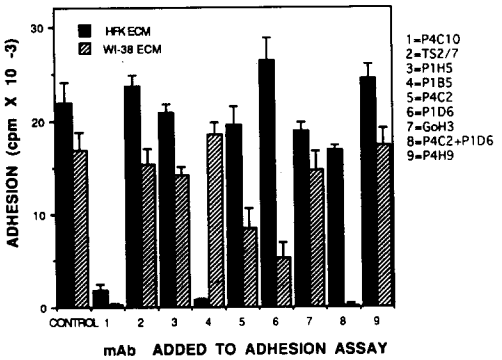

Figure 2. Hut 78 cell adhesion to ECM derived from HFK or WI-38 fibroblasts: effect of anti-integrin mAbs. Cell adhesion to matrix in the presence of anti-integrin mAbs was exactly as described for Table I. mAbs used were: *P4C10*, $\beta 1$ (inhibitory), *TS2/7*, $\alpha 1$ (non-functional), *P1H5*, $\alpha 2$ (inhibitory), *P1B5*, $\alpha 3$ (inhibitory); *P4C2*, $\alpha 4$ (inhibitory), *P1D6*, $\alpha 5$ (inhibitory); *GoH3*, $\alpha 6$ (inhibitory); *P4H9*, $\beta 2$ (inhibitory).

p150, uncleaved form) and $\alpha 5$ complexed with $\beta 1$ could be precipitated from HUT 78 cells (not shown).

T Cells Use $\alpha 3\beta 1$ to Bind HFK and Carcinoma Cell Matrix

To directly test the possibility that T cells use $\alpha 3\beta 1$ as the receptor for HFK matrix, we examined the attachment of HUT 78 cells to HFK ECM in the presence of mAbs to inhibitory epitopes on various integrin receptors (Fig. 2). The data summarized in Fig. 2 show that HUT 78 cell adhesion to HFK matrix (*solid bars*) can be completely abrogated by antibodies to $\alpha 3$ (P1B5) or $\beta 1$ (P4C10). Inhibitory antibodies to $\alpha 2$ (P1H5), $\alpha 4$ (P4C2), $\alpha 5$ (P1D6), $\alpha 6$ (GoH3), $\alpha v/\beta 3$ (LM609), $\alpha v/\beta 5$ (P3G2), or $\beta 2$ (P4H9) either alone or in combination (for $\alpha 4$ and $\alpha 5$) had no effect on HUT 78 cell adhesion to HFK matrix clearly indicating that the T lymphocyte receptor for HFK matrix is the functional conjugate of $\alpha 3$ with $\beta 1$. HUT 78 cells could also adhere to matrix derived from WI-38 fibroblasts (Fig. 2, *striped bars*). However, this interaction was mediated primarily by fibronectin as mAbs to $\beta 1$ (P4C10) or the lymphocyte fibronectin receptors, $\alpha 4\beta 1$ or $\alpha 5\beta 1$ (P4C2 and P1D6, respectively) when used in combination completely inhibited this process. Interestingly, HUT 78 (Fig. 3) or HUT 102 (not shown) cells adhered to the ECM deposited by several malignant epithelial cell lines (Fig. 3, A431, HT29, UCLA P3). Adhesion of HUT cells to the ECM deposited by carcinoma cells could also be specifically inhibited by mAbs to $\alpha 3$ (Fig. 3, P1B5) or $\beta 1$ (not shown). These findings strongly suggest that the ECM derived from keratinocytes and epithelial tumor cell lines is unique and capable of supporting the adhesion of $\alpha 3\beta 1$-positive T lymphocytes.

T Cell Adhesion to HFK ECM via $\alpha 3\beta 1$ Can Be Inhibited by Anti-Epiligrin mAbs

Since epiligrin is the component in HFK matrix responsible for $\alpha 3\beta 1$-dependent keratinocyte adhesion (Carter et al., 1991) it was of interest to determine whether HUT 78 cells could attach to surfaces coated with purified epiligrin (Fig. 4 *A*). As expected, these cells adhered to epiligrin and mAbs to $\alpha 3$ or $\beta 1$ (Fig. 4 *B*) completely inhibited this. mAbs to other integrin receptors ($\alpha 2$, P1H5; $\alpha 4$, P4C2; $\alpha 5$, P1D6;

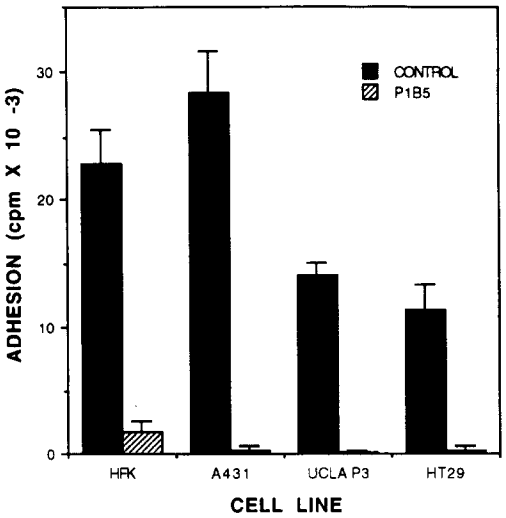

Figure 3. Adhesion of HUT 78 cells to ECM derived from HFK or epithelial cell lines. All epithelial cells were grown to confluency in 48-well plates. They were extracted as previously described and blocked with PBS supplemented with HBSA (Wayner et al., 1989). ^{51}Cr-labeled HUT 78 cells were allowed to adhere to ECM-coated plates in the presence of purified mAb directed to $\alpha 3$ (*P1B5*, 10 μg/ml) for 30 min. Bound HUT 78 cells were quantitated as described in the Materials and Methods and the legend to Table I.

$\alpha 6$, GoH3; $\beta 2$, P4H9; $\alpha v\beta 3$, LM609; $\alpha v\beta 5$, P3G2) had no effect. $\alpha 3\beta 1^{low}$ lymphocyte populations (such as Jurkat or ST-1 cells) did not adhere to surfaces coated with purified epiligrin (not shown) although such cells could adhere to surfaces coated with fibronectin (not shown).

These data (Fig. 4 *A*) strongly suggest that T cell adhesion to HFK ECM is a function of $\alpha 3\beta 1$ interaction with epiligrin. However, in addition to epiligrin, HFK ECM contains adhesion molecules such as fibronectin and laminin (Fig. 5 *A*, P1H11 and 4C7) that have also been reported to be ligands for $\alpha 3\beta 1$ (Gehlsen et al., 1990; Elices et al., 1990). Therefore, to determine the factor responsible for HUT 78 cell adhesion in HFK ECM we developed mAbs to HFK cells and screened them for: (*a*) their specific reactivity with HFK ECM; and (*b*) their specific ability to inhibit HUT 78 cell adhesion to secreted matrix. Of these, P3H9-2 and P3E4 reacted strongly with HFK ECM (Fig. 5 *A*) but not with WI-38 ECM, purified human fibronectin, human placental laminin or collagen types I or III (not shown). P3H9-2 completely inhibited HUT 78 cell adhesion to intact HFK ECM (Fig. 5 *B*) or purified epiligrin (not shown) but had no effect on adhesion of these cells to WI-38 ECM or purified fibronectin (not shown). P3H9-2 also inhibited HUT 78 cell adhesion to ECM prepared from A431 cells (not shown) strongly suggesting that T cell adhesion to carcinoma ECM is also epiligrin dependent. Immune precipitation analysis with [^{35}S]methionine-labeled HFK-conditioned culture medium revealed that P3H9-2 immune precipitated an epiligrin complex identical to that precipitated by our previously described anti-epiligrin, mAb P1E1 (Fig. 6). In sequential immune precipitation experiments P3H9-2 precleared the epiligrin complex recognized by P1E1 from HFK conditioned culture medium (not shown). In tissue, P3H9-2 and P1E1 had a similar, if not identical, staining pattern (Fig. 7, *A* and *C*). Interestingly, we observed extensive epithelial and endothelial basement membrane staining

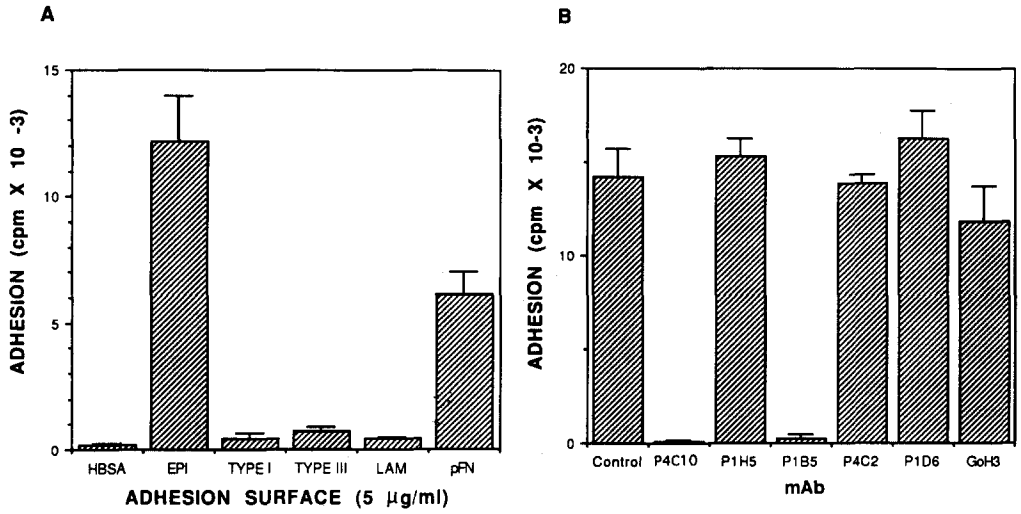

Figure 4. Adhesion of HUT 78 cells to purified epiligrin and role of $\alpha3\beta1$. (*A*) Adhesion of HUT 78 cells to purified epiligrin or other ECM adhesive ligands. *HBSA*, heat-denatured BSA (Wayner et al., 1989); *EPI*, P1E1 affinity-purified epiligrin; *type I*, human pepsinized type I collagen from placenta; *type III*, human pepsinized type III collagen from placenta; *laminin*, human-pepsinized laminin from placenta; *pFN*, human plasma fibronectin. All adhesive ligands were used at 5 μg/ml coating concentration. (*B*) Inhibition of HUT 78 cell adhesion to P1E1 affinity-purified epiligrin (Carter et al., 1991) with anti-integrin mAbs. Adhesion to purified epiligrin (5 μg/ml coating concentration) was carried out in the presence of the indicated mAbs (ascites at 1/100).

with P3H9 in normal skin (Fig. 7, *A* and *C*, scalp) and tonsil (not shown).

T Cell Adhesion to HFK ECM and Epiligrin Can Be Up-regulated by mAbs to β1

Several T lymphocyte β1 integrins ($\alpha4\beta1$, $\alpha5\beta1$, $\alpha6\beta1$) have been shown to require activation for ligand binding to occur (reviewed by Shimizu and Shaw, 1991). Activation can be achieved with T cell receptor cross-linking, phorbol esters, and mAbs to the integrin β1 subunit. However, it is not yet known whether $\alpha3\beta1$ is similar and can also be activated for ligand binding. Therefore, we examined the effects of several activating mAbs to β1 (P4G11) for their ability to up-regulate adhesion of HUT 78 ($\alpha3\beta1^{high}$) or Jurkat ($\alpha3\beta1^{low}$) cells to epiligrin coated surfaces. Activating mAbs (data shown for P4G11) were selected for their ability to up-regulate adhesion of hematopoietic cells to fibronectin-coated surfaces. In sequential immunoprecipitation experiments they preclear the β1 complex from detergent extracts prepared with surface labeled HUT 78 cells (Fig. 8).

Although resting HUT 78 cells express basal epiligrin adhesion, such adhesion could be clearly up-regulated by activation with P4G11 (Fig. 9). Pretreatment with an inhibitory anti-β1 (P4C10), however, had the opposite effect. Interestingly, adhesion of P4G11-pretreated HUT 78 cells to epiligrin could be inhibited by mAbs to α3 (P1B5, not shown) showing clearly that up-regulated binding was mediated by the functional complex of $\alpha3\beta1$. Interestingly, adhesion of Jurkat cells to purified epiligrin could be slightly up-regulated by P4G11 consistent with the low levels of $\alpha3\beta1$ expressed by these cells (Table I). These findings (Fig. 9)

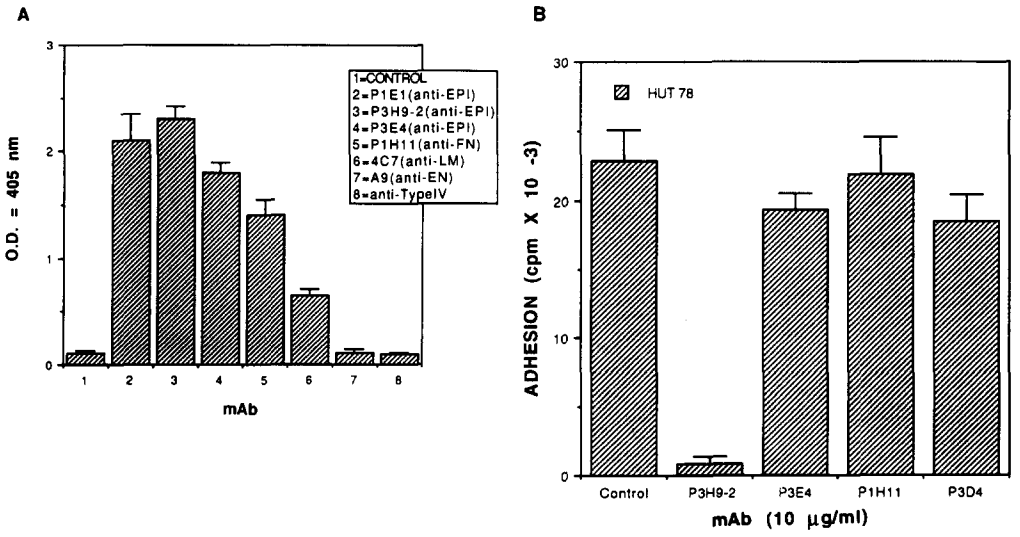

Figure 5. Characterization of inhibitory anti-epiligrin mAb, P3H9-2. (*A*) Composition of HFK matrix by ELISA and reactivity with mAbs P3H9-2 and mAbs to epiligrin (P1E1), fibronectin (P1H11), laminin (4C7), entactin (A9), and type IV collagen (Dako). HFKs were grown to confluency in 96-well plates and extracted as described (Carter et al., 1991). The matrix was stained with the indicated mouse mAbs and detected with HRP-conjugated rabbit anti-mouse secondary antibody (1/1,000) and ABTS substrate (Kirkgaard and Perry). (*B*) Inhibition of HUT 78 cell adhesion to HFK matrix with mAb P3H9-2. All mAbs were purified from culture supernatant and were used at 10 μg/ml (*control*, 10 μg/ml purified mouse IgG). HFK matrix was prepared as described in the Methods and Materials. P3H9-2 reacts with P1E1 purified epiligrin by ELISA. P1H11 and P3D4 are mAbs directed to the central cell binding domain and the carboxy-terminal cell binding domain of fibronectin, respectively. P3D4 inhibits lymphocyte adhesion to fibronectin (Garcia-Pardo et al., 1992).

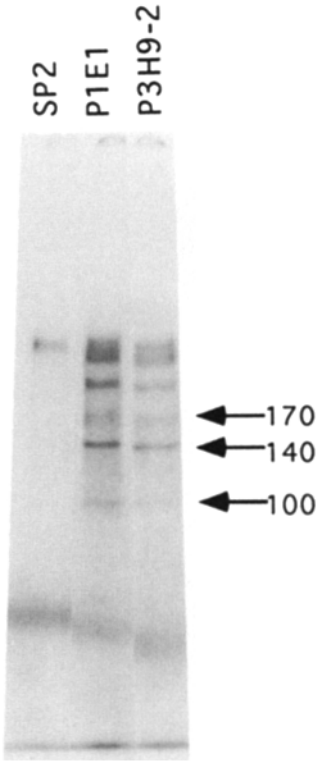

Figure 6. Immune precipitation of P1E1- or P3H9-2-reactive material from the conditioned culture medium of [^{35}S]methionine and cysteine-labeled HFKs. Immune precipitations were analyzed on a 7% SDS-polyacrylamide gel under reducing conditions. *SP2*, Myeloma culture supernatant. The uppermost bands not indicated by molecular weight represent the laminin-like complex that co-precipitates with epiligrin (Carter et al., 1991). Three bands composing the epiligrin complex are indicated (M_r = *170*, *140*, or *100*).

show clearly that adhesion of T cells to epiligrin requires the participation of an active $\alpha3\beta1$ complex and that the failure of $\alpha3\beta1^{low}$ cell populations to bind HFK ECM is a function of both $\alpha3\beta1$ surface expression and activation.

Co-localization of T Cells with the Epiligrin-containing Basement Membrane

Together, the above data suggest that $\alpha3\beta1$ interaction with epiligrin may be involved in T cell adhesion to basement membranes in situ. Therefore, we examined biopsy specimens from several inflammatory or malignant skin conditions for the presence and localization of $\alpha3\beta1$-positive T cells. Graft-vs.-host disease is often associated with T cell infiltration of the basal epidermis. In several cases of cutaneous GVH we examined there was a clear tendency for CD3+ T cells to be localized in the basement membrane zone (Fig. 10, *C* and *E*) in close association with the epiligrin containing basement membrane (Fig. 10, *B* and *D*). Dual label studies with mAbs directed to well-characterized T cell antigens (CD3, CD8, CD45RO) revealed that the infiltrating cells in close association with epiligrin (Fig. 11 *B*) were activated or "memory" cytotoxic T cells (Fig. 11 *A*, *A* and *B*, same field). Due to the intense reactivity of basal keratinocytes with mAbs to $\alpha3$ and the close association of T cells with the basal layer (Fig. 11, *A* and *B*) it was difficult to detect T cell specific $\alpha3$ staining in these specimens even with dual label techniques.

Immunohistochemical Comparison of T or B Lymphocyte Infiltrates in Skin

We have shown that $\alpha3\beta1^{high}$ T cells bind epiligrin in HFK matrix, but that non-cutaneous T cells ($\alpha3\beta1^{low}$) and B cells ($\alpha3\beta1^{low}$) do not bind epiligrin with high affinity even when the $\beta1$ complex is activated with mAb P4G11. To better define

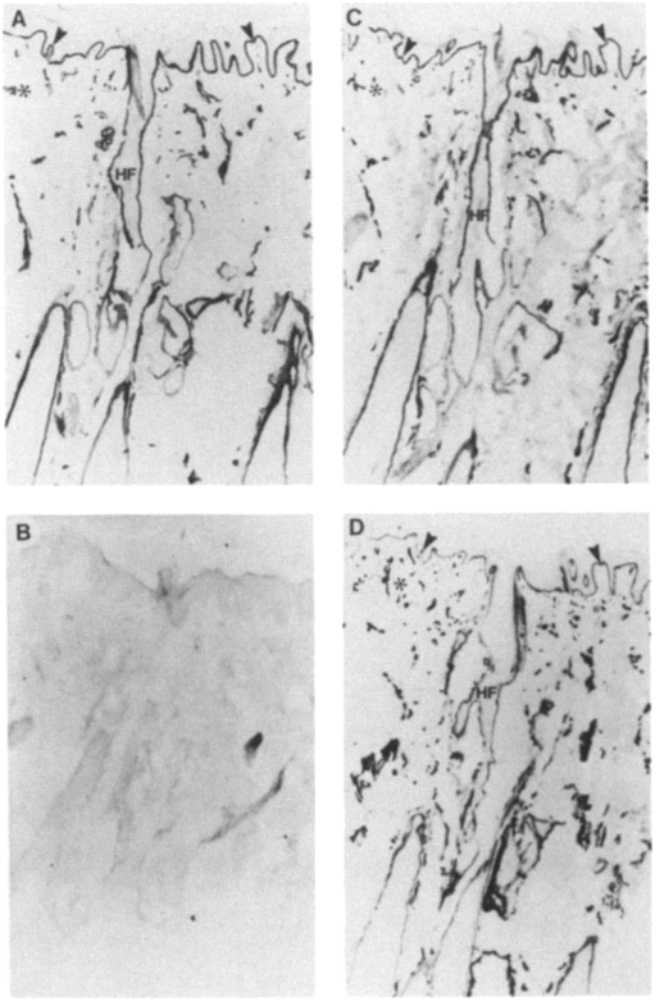

Figure 7. Comparison of immunoperoxidase staining patterns generated in normal scalp skin with the mAbs P3H9-2, P1E1, or anti-laminin. (*A*) P1E1 reactivity in normal scalp. (*B*) Negative control stained with normal mouse IgG. (*C*) P3H9-2 reactivity in same tissue. (*D*) Laminin detected with 4C7 anti-laminin (Telios). *, Indicates region rich in endothelium, *HF*, hair follicle; *arrowheads*, epidermal basement membrane.

these apparent associations relative to disease pathogenesis and localization of T or B cells within the skin, we examined the correlative immunohistochemistry in adjacent tissue sections of benign T cell infiltrates (contact dermatitis) or malignant T cell infiltrates (CTCL) and compared them to malignant B cell (CBCL) infiltrates (Fig. 12). High-resolution light microscopy (plastic embedded, 1-μm sections; Fig. 12, *A* and *B*) revealed frequent localization of T lymphocytes in the basal layer directly above the epidermal basement membrane in contact dermatitis (Fig. 12 *A*, *arrows*). In Fig. 12 *B*, the infiltrating cells, in direct contact with the basement membrane, are stained with a T cell specific marker, Leu 1. Transmission EM (Fig. 12 *C*) confirmed the close association of Leu 1 positive lymphocyte plasma membranes (asterisks) with the basement membrane where epiligrin is found. Similar features were observed in CTCL biopsies (Fig. 12 *D*) and contrasted sharply with B cell infiltrates that did not infiltrate the epidermis or superficial dermal layers (Fig. 12 *E*). In Fig. 12 *D*, the mycosis fungoides (MF) T cells can be seen in association with the BMZ and throughout the

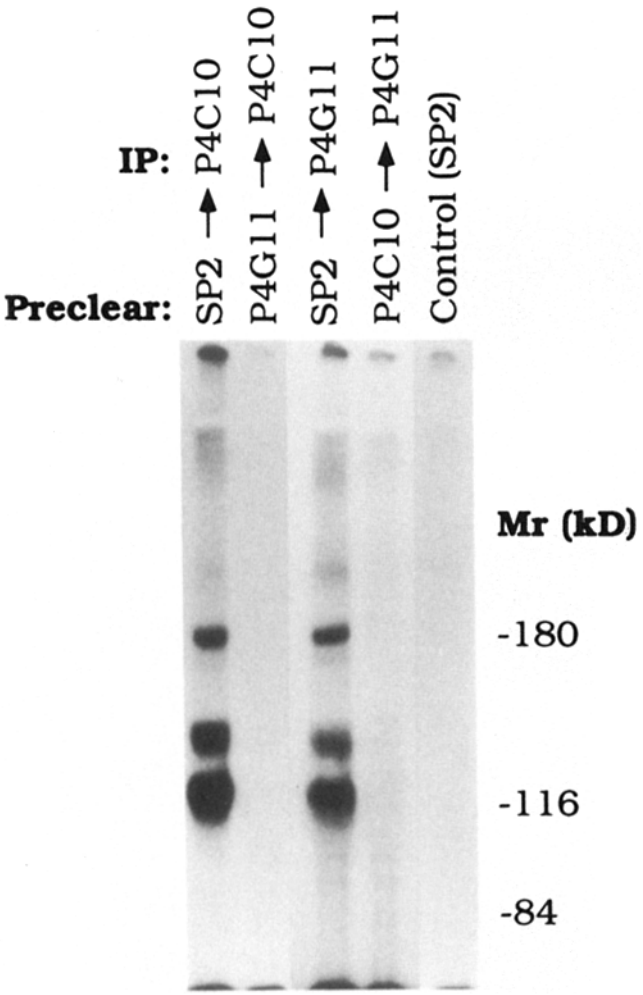

Figure 8. Identification of activating mAb P4G11 as anti-$\beta 1$ by sequential immune precipitation analysis with known anti-$\beta 1$ P4C10. HUT 78 cells were surface labeled and extracted with 1% Triton X-100 in PBS as previously described in the legend for Fig. 1. Sequential preclearing was performed with the indicated mAbs. Four rounds of preclearing (*Preclear*) were carried out for each mAb or control supernatant (*SP2*). After preclearing, the lysates were immune precipitated (*IP*) with the indicated mAbs. P4C10 is a previously characterized inhibitory anti-$\beta 1$ (Carter et al., 1990*a*,*b*).

epidermis (EPI), whereas in Figure 12 *E*, the BMZ and EP are clearly free of B cell infiltrates. In MF, the intraepidermal infiltrating cells were identified by immunoreactivity with anti-Leu 1 (Fig. 12 *F*). Expression of $\alpha 3$ on such cells was difficult to differentiate from the intense basal cell reactivity with PIF2 (anti-$\alpha 3$) in all conditions examined. However, $\beta 1$ expression by these cells was more intense and in some specimens, T cells in Pautrier microabscesses could be clearly distinguished by reactivity with P4C10 (Fig. 12 *G*). This technical problem was not encountered, however, with dermal T cells identified by anti-Leu 1 in MF (Fig. 12 *H*) which in serial sections were shown to demonstrate convincing $\alpha 3$ reactivity (Fig. 12 *I*). The staining observed in Fig. 12 *I* is the result of intense reactivity of dermal T cells with PIF2 as the resident dermis (except for the endothelium lining the blood vessels) is essentially negative for $\alpha 3$ reactivity (see Fig. 12 *K*). In contrast, dermal B cell infiltrates that expressed B cell antigens and monotypic immunoglobulin light

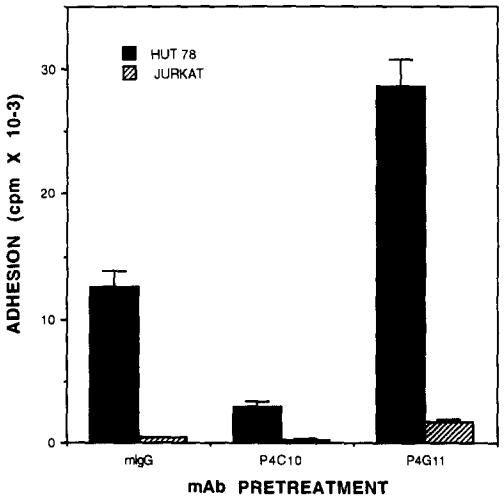

Figure 9. Modulation of HUT 78 cell adhesion to epiligrin by mAb P4G11. HUT 78 cells ($\alpha 3\beta 1^{high}$) or Jurkat cells ($\alpha 3\beta 1^{low}$) were preincubated with P4G11 (20 μg/ml), P4C10 (20 μg/ml), or control IgG (20 μg/ml non-immune mouse IgG) at 5×10^5 cells per ml for 30 min at 37°C. At the end of this time they were washed and allowed to adhere to epiligrin coated surfaces for 30 min at 37°C.

chains (Fig. 12 *J*) failed to express $\alpha 3$ when adjacent serial sections were stained under identical conditions (Panel K). Compare the intense T cell $\alpha 3$-immunoreactivity in Fig. 12 *I* with the complete lack of dermal B cell $\alpha 3$-immunoreactivity in Fig. 12 *K*.

Discussion

We have recently described a novel cell adhesion molecule (epiligrin) synthesized by basal keratinocytes (Carter et al., 1991). In tissue, epiligrin is localized to the lamina lucida of the basement membrane. In culture, epiligrin is a primary adhesive ligand synthesized by rapidly growing basal keratinocytes and is deposited into the detergent-insoluble ECM. The receptor for epiligrin used by keratinocytes and other non-epithelial cell populations is the $\alpha 3\beta 1$ integrin (Carter et al., 1991). The results of the present studies show unequivocally that T lymphocytes also use $\alpha 3\beta 1$ to adhere to epiligrin in the ECM deposited by normal dermal keratinocytes or carcinoma cells.

Although it has been known for some time that $\alpha 3\beta 1$ is an activation-dependent T cell receptor, its function in T cells has until now been largely unknown. It is interesting that although $\alpha 3\beta 1$ has been reported to be a laminin, collagen, and a fibronectin receptor in non-lymphoid cell populations (Wayner and Carter, 1987; Gehlsen et al., 1989; Dang et al., 1990; Elices et al., 1991) HUT 78 cells and CTL appeared to use $\alpha 3\beta 1$ exclusively to adhere to HFK matrix or epiligrin. In this regard, the Dang et al. (1990) study, in particular, deserves comment since these workers reported that mAb J143 (anti-$\alpha 3$) inhibited interstitial collagen-induced CD4+ T cell activation. Our studies contrast with those reported in this previous paper. Although HUT 78 cells expressed abundant cell surface $\alpha 3$ complexed with $\beta 1$, they did not adhere to collagen types I or III. Furthermore, the results of inhibition studies with PIB5 (anti-$\alpha 3$) and PIH5 (anti-$\alpha 2$) which are well-characterized inhibitory reagents (Wayner and Carter, 1987; Wayner et al., 1988; Takada et

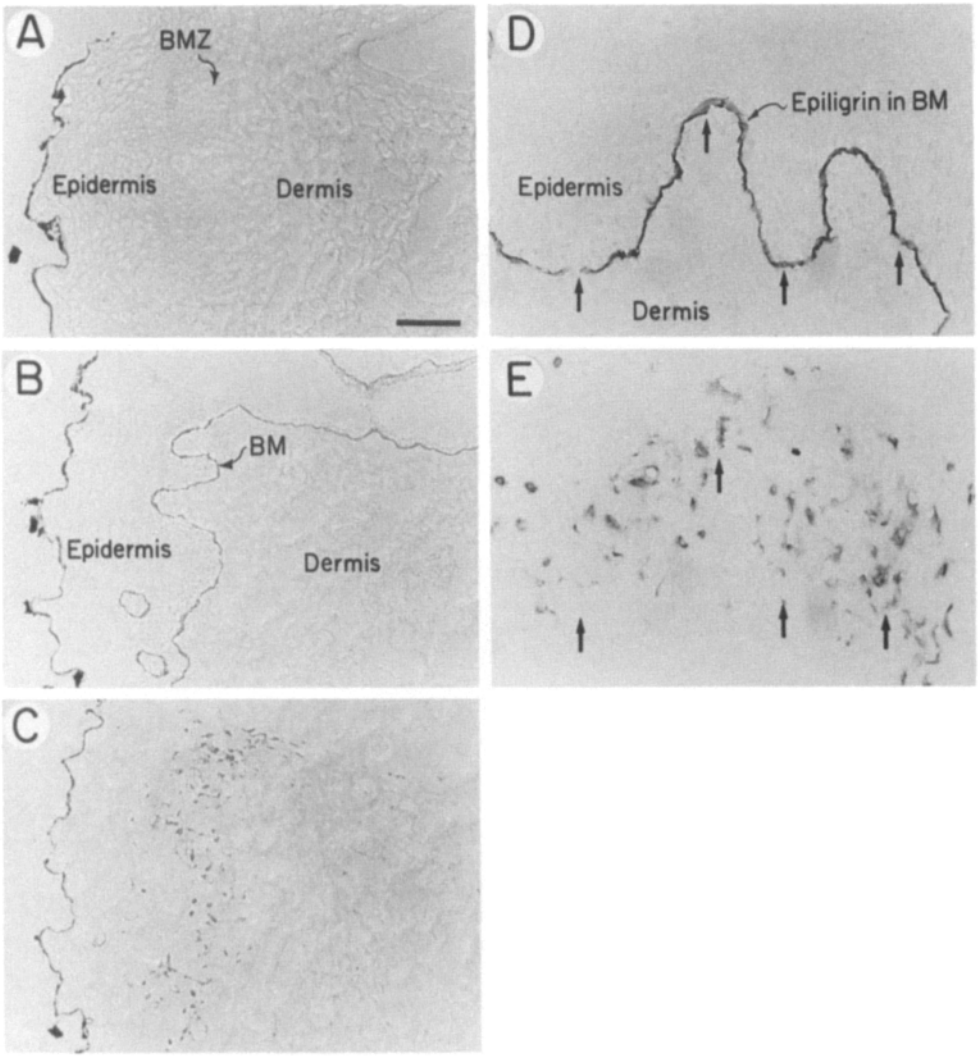

Figure 10. Immune peroxidase localization of epiligrin or CD3+ T lymphocytes in cryostat sections of skin derived from a patient with cutaneous graft-vs.-host (GVH) disease. (*A*, *B*, and *C* are serial sections. The four large arrows in *D* and *E* (400×) are included to indicate reference points for comparison. (*A*) Phase contrast micrograph of GVH skin stained with non-immune mouse IgG (2 μg/ml). The background staining with mouse IgG is negligible. *BM*, Epidermal basement membrane; *BMZ*, basement membrane zone. (*B*) Immune peroxidase staining of epiligrin with mAb P1E1 (Carter et al., 1991). Epiligrin staining is coincident with the dermal–epidermal boundary or basement membrane (*BM*, *arrow*). (*C*) Immunolocalization of CD3+ T lymphocytes in GVH skin. T lymphocytes are concentrated in the vicinity of the basement membrane in close proximity to the epiligrin. *D* and *E* are higher power views of *B* and *C* (400×). Bar (*A–C*), 100 μm.

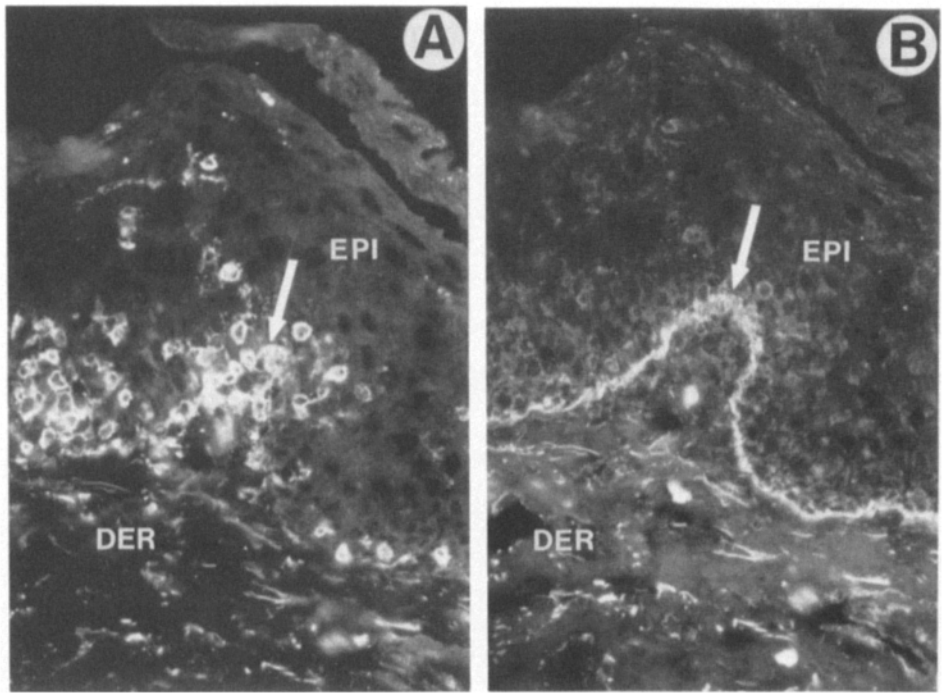

Figure 11. Co-localization of CD45RO+ T cells and epiligrin in human GVH skin. (*A*) Immunofluorescence staining with UCHL1 anti-CD45RO+. mAb UCHL1 was detected with rhodamine conjugated goat anti-mouse. (*B*) Immunofluorescence localization of epiligrin. Biotinylated P1E1 (anti-epiligrin) was detected with FITC anti-avidin. The tissue is the same as that shown in Fig. 10. The BMZ is clearly infiltrated with large numbers of CD45RO+ T cells (*CD3+*, Fig. 10). In Fig. 11 *A*, the T cells can be seen in intimate contact with the epiligrin containing basement membrane (*A* and *B* are the same field, arrow marks identical spot in each photograph for comparison).

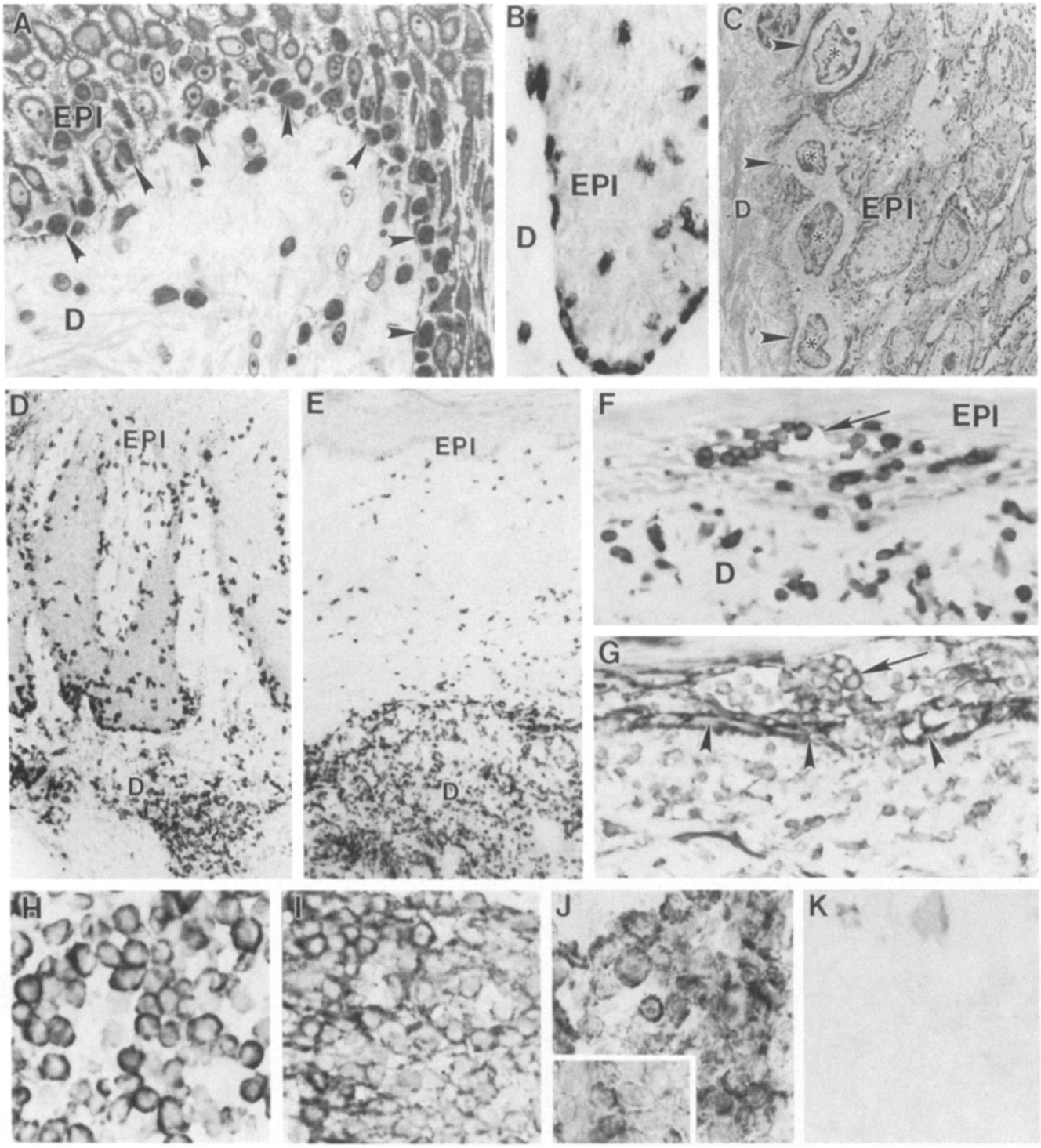

Figure 12. Immunohistochemical localization of T and B lymphocytes in various cutaneous malignant and inflammatory disorders. (*A–C*) Localization of T cells within lower epidermis (*EPI*, epidermis; *D*, dermins) in allergic contact dermatitis. (*A*) One micron-thick, plastic-embedded section, *arrowheads*, representative lymphocytes above basement membrane (400×); (*B*) immunoperoxidase stain, Leu 1 (pan T cell antigen) (400×); (*C*) transmission electron micrograph, (*) lymphocytes; (*arrowheads*) points of close approximation between lymphocyte plasma membranes and basement membrane; (*EPI*) epidermis; (*D*) dermis (×5000). (*D–K*) Comparison of infiltrate patterns and antigenic phenotypes between mycosis fungoides (*MF*) and cutaneous B cell lymphoma (*CBCL*). (*D*) MF; (*E*) CBCL; note prominent migration of T cells (Leu 1+) into hyperplastic epidermis (*EPI*) in *D*, and sparing of epidermis (*EPI*) and superficial dermis (*D*, dermis) by B cells (Leu 12+) in *E* (*D*, Leu 1, ×100; *E*, Leu 12, ×100). (*F*) Clusters of Leu 1-positive T cells (*arrow*) within epidermis (*EPI*) in MF; (*G*) section adjacent to F stained for $\beta 1$ with apparent staining of T cell cluster (*arrow*) and adjacent basal keratinocytes (*arrowheads*) (*F* and *G*, ×400). (*H*) Cluster of Leu 1-positive dermal T cells; (*I*) section adjacent to H showing $\alpha 3$ expression by the T cells. (*J*) Cluster of Leu 12-positive dermal B cells inset showing monotypic expression of surface κ-type immunoglobulin light chains; (*K*) section adjacent to *J* showing relative absence of $\alpha 3$ immunoreactivity on dermal B lymphocytes (*H–K*, ×800).

al., 1988; Kunicki et al., 1988; Languino et al., 1989; Carter et al., 1991) show that in T cells which express both $\alpha2\beta1$ and $\alpha3\beta1$ collagen adhesion is mediated by $\alpha2\beta1$ (Goldman et al., 1992; Wayner, E. A., manuscript in preparation). The results presented here also argue against a role for $\alpha3\beta1$ as a receptor for fibronectin or laminin. HUT 78 cells which express $\alpha3\beta1$ do not adhere to mouse laminin or human laminin derived from placenta even after activation of the $\beta1$ complex. HUT 78 cells did adhere to fibronectin-coated surfaces. However, as we and others have reported (Garcia-Pardo and Ferreira, 1990; Wayner and Kovach, 1992) adhesion of HUT 78 cells to fibronectin involved the cooperative interaction of $\alpha5\beta1$ and $\alpha4\beta1$. mAbs to $\alpha3$ (PlB5) had no effect on T (or B) cell adhesion to fibronectin even if the $\beta1$ complex was in an active conformation.

The adhesive ligand for T cells in HFK ECM was identified as epiligrin. This conclusion was based on the results of several experiments carried out with a monoclonal antibody selected for its ability to inhibit T cell adhesion to HFK matrix. (*a*) P3H9-2 reacted only with HFK or carcinoma cell matrix and PlE1 purified epiligrin; (*b*) P3H9-2 specifically inhibited HUT 78 cell adhesion to HFK ECM or purified epiligrin; (*c*) P3H9-2 and PlE1 immune precipitated identical complexes from HFK-conditioned media; and (*d*) the pattern of P3H9-2 immuno-reactivity in tissue was similar to that observed for PlE1 and distinct from that observed for laminin, type IV collagen, or fibronectin. Recently, $\alpha3\beta1$ has been reported to be a receptor for entactin (Dedhar et al., 1992), an adhesive component of the lamina densa (Chung and Durkin, 1990). However, the matrix we prepared from HFK and carcinoma cells, that could support the efficient adhesion of $\alpha3\beta1^{high}$ T cells, did not contain entactin and P3H9-2 did not react with purified entactin. Furthermore, HUT 78 cells ($\alpha3\beta1^{high}$) do not adhere to surfaces coated with purified recombinant entactin (Yi, X. Y., and E. A. Wayner, manuscript in preparation). Together, the present findings show clearly that in T cells the primary function of $\alpha3\beta1$ is as a receptor for epiligrin.

Two T lymphocyte populations could be distinguished based on surface $\alpha3\beta1$ expression. Non-cutaneous T cell lines (Molt 4 or Jurkat), resting peripheral blood mononuclear cells, and all of the cultured B cell populations we examined were $\alpha3\beta1^{low}$ while antigen or IL-2 activated (CTL, LAK), or cutaneous T cells (HUT 78, HUT 102) were $\alpha3\beta1^{high}$. All of the $\alpha3\beta1^{high}$ T cells adhered to epiligrin. Furthermore, adhesion of HUT 78 cells to epiligrin could be upregulated with activating mAbs directed to $\beta1$. This suggests that regulation of $\alpha3\beta1$ function in T cells is similar to other $\beta1$ integrins. It was interesting that most of the B cell populations we examined expressed little $\alpha3\beta1$ nor did they adhere to HFK matrix suggesting that unactivated B cells may not be able to interact with epiligrin in epidermal basement membranes. This could explain the failure of such cells to associate with the basement membrane in situ. Failure to interact with the basement membrane in the absence of the appropriate activating signals could lead to the "trapping" and localization of $\alpha4\beta1+$, $\alpha5\beta1+$, $\alpha3\beta1^{low}$ B cells within the fibronectin-rich reticular dermis. Indeed, the in situ immunohistochemistry in this study consistently demonstrated a correlation between $\alpha3\beta1$ expression (T cells in reactive infiltrates and MF) and a tendency for intraepidermal migration. Malignant B cells that were $\alpha3\beta1^{low}$ in situ did not show epidermal or superficial dermal infiltration. Since epiligrin is confined to the lamina lucida of the basement membrane (Carter et al., 1991), these findings indicate that keratinocytes synthesize additional adhesion molecules, such as ICAM-1 (reviewed by Walsh et al., 1990; Barker et al., 1991; Barker and Nickoloff, 1992), that might promote the epidermotropism characteristic of cutaneous T cell disease.

Interestingly, in the present experiments mAb P3H9-2 to epiligrin-stained endothelial as well as epithelial basement membranes. We have now observed epiligrin immunoreactivity in the endothelium of several tissues including skin, lymph node, tonsil, thymus, and lung. These data suggest that $\alpha3\beta1$ positive T cells may interact with epiligrin in subendothelial basement membranes as well as the epidermal basement membrane. Furthermore, since we have shown that endothelial cells express $\alpha3\beta1$ (Languino et al., 1990), these findings suggest that epiligrin may also be involved in endothelial cell–basement membrane interactions. In several experiments using mAb PlE1 we did not detect epiligrin in endothelial basement membranes (Fig. 10 *B*). This may be due to differences in antibody access to the antigen. However, data to be presented elsewhere indicates that the epiligrin forms expressed in epidermal and endothelial basement membranes are not identical.

$\alpha3\beta1$ interaction with epiligrin has been proposed to be a key mechanism used by keratinocytes to adhere to the basement membrane (Carter et al., 1991). The results of several recent studies support this concept. Epiligrin is similar if not identical to another recently described epithelial basement membrane protein, kalanin (Rousselle et al., 1991; Marinkovich et al., 1992). mAbs to kalanin induce skin fragments to de-epithelialize in culture. Furthermore, epiligrin has been shown to be a target in acquired autoimmune and inherited blistering skin diseases and is identical to the BM600 antigen which is absent from the skin of patients with lethal junctional epidermolysis bullosa (Domloge-Hultsch et al., 1992). Together, these findings suggest an important role for $\alpha3\beta1$ and epiligrin in determining the integrity of the skin. Our present findings further suggest a primary role for $\alpha3\beta1$ and epiligrin in determining T lymphocyte activities in the basement membrane zone. In addition to epidermal migration, vacuolar alteration, satellite cell necrosis of basal keratinocytes, and blistering are key features of T lymphocyte–mediated inflammation. We propose that the interaction of activated or memory T cells with epiligrin via $\alpha3\beta1$ contributes to their adhesion to the basement membrane in certain pathological cutaneous T cell disorders, such as GVH, chronic eczematous dermatitis, and mycosis fungoides (CTCL).

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