American Journal of Pathology, Vol. 147, No. 6, December 1995
Copyright © American Society for Investigative Pathology

Altered Vascular Endothelium Integrin Expression in Psoriasis

Daniel Creamer,* Michael Allen,* Ana Sousa,† Robin Poston,† and Jonathan Barker

From St. John's Institute of Dermatology, UMDS, St. Thomas' Hospital Campus, and Department of Experimental Pathology,† UMDS, Guy's Hospital Campus, London, United Kingdom*

Considerable evidence indicates that microvascular changes observed in psoriasis are a result of vascular proliferation. A critical step in the sequence of events leading to neovascularization involves interactions between endothelial cells and extracellular matrix proteins mediated in part by the integrin family of adhesion molecules. A number of endothelial integrins have been shown to participate in neovascularization, including members of the $\beta1$, $\beta3$, and $\beta4$ subfamilies. To investigate the role of these integrins in psoriasis, specimens of lesional and nonlesional skin were taken from 10 patients with active, untreated plaque disease. Vascular endothelium was labeled with monoclonal antibodies specific for $\alpha2$, $\alpha5$, $\alpha6$, $\beta1$, $\alpha v\beta3$, and $\beta4$ integrins. The use of image analysis permitted quantification of immunoperoxidase staining and comparison of endothelial labeling in lesional and nonlesional skin. There was a significant increase in endothelial staining of $\alpha v\beta3$ integrin in lesional compared with nonlesional skin, both in superficial and deep vasculature. In contrast, there was a significant decrease in endothelial $\beta4$ staining in lesional compared with nonlesional superficial dermal vessels. $\alpha2$, $\alpha5$, $\alpha6$, and $\beta1$ staining showed no significant difference between the two groups. These results demonstrate an important role of $\alpha v\beta3$ and $\beta4$ integrins in the microvascular changes of psoriatic lesions. (Am J Pathol 1995, 147:1661–1667)

Psoriasis is a common, chronic skin disorder characterized by hyperproliferation of the epidermis, inflammatory cell accumulation, and increased tortuosity and dilatation of dermal papillary blood vessels.[1] The morphological changes observed in psoriatic vessels result in an expanded microvasculature within lesional skin.[2] Evidence indicates that this expansion results from vascular proliferation mediated by angiogenic factors.[3]

Under normal, physiological circumstances, angiogenesis is limited to embryonic development, the endometrial cycle, and wound healing.[4] However, vascular proliferation is an integral part of the pathophysiology of a number of inflammatory diseases, such as rheumatoid arthritis, as well as being important for the growth and metastatic capacity of tumors.[4] Studies of angiogenesis have shown that the process occurs via a series of sequential steps. Initial proteolytic enzyme dissolution of the endothelial basement membrane is followed by cell migration and division to form sprouts that penetrate the perivascular connective tissue. As sprouts elongate, canalization develops proximally, and the resulting hollow vascular tubes anastomose to form capillary loops.[5] The angiogenic pathway involves a variety of interactions between endothelial cells and extracellular matrix proteins,[6] modulated by a number of mitogenic factors.[7] Integrins, a family of cell surface molecules, play a key role in angiogenesis through mediating adhesion to neighboring cells and extracellular matrix and initiating intracellular signaling.[8] Structurally, integrins are composed of α- and β-glycoprotein chains forming heterodimers that are expressed on the cell surface and linked to the cytoskeleton.[9] These receptors bind to extracellular matrix molecules through their external domain and may directly attach to cytoskeletal elements through their cytoplasmic domain. The ligand specificity and signaling ability are determined by varying combinations of α- and β-integrin subunits.[9] There are 14

Supported by a research fellowship from the Special Trustees of St. Thomas' Hospital (DC).

Accepted for publication August 8, 1995.

Address reprint requests to Dr. J. Barker, St. John's Institute of Dermatology, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK.

known α-subunits and 8 known β-subunits, and of these, members of the β1, β3, and β4 subfamilies have been shown to be expressed on endothelial cells and to be important in angiogenesis.[10-13] In particular, studies of human granulation tissue have demonstrated an increased expression of $\alpha v\beta 3$ integrin on proliferating vascular endothelium.[13] Furthermore, *in vivo* studies have demonstrated inhibition of angiogenesis by blocking this integrin.[13]

To determine the role of integrins in the vascular changes of psoriasis we have compared the expression of α2, α5, α6, β1, β4, and $\alpha v\beta 3$ integrins on endothelium in lesional and nonlesional psoriatic skin with a standard immunohistochemical technique. Quantification of immunoperoxidase staining was achieved using hue-saturation-intensity (HSI) color image analysis.

Materials and Methods

Patients

Ten patients with plaque psoriasis (seven males and three females) between 31 and 77 years of age (mean, 56 years) participated in this study after ethical approval had been obtained. None of the patients were receiving systemic therapy and topical treatment was limited to emollients alone for 2 weeks preceding biopsy. Paired specimens were obtained with 4-mm-punch biopsies from active plaque edges and uninvolved skin. Nonlesional skin was biopsied at least 4 cm from the edge of the nearest plaque. Specimens were embedded in OCT compound, snap frozen in liquid nitrogen, and stored until required.

Immunohistochemistry

Five-micron cryostat sections of tissue were mounted on 3-aminopropyltriethoxysilane-coated glass slides, air dried, and fixed with acetone. Nonspecific antibody binding was blocked by pretreatment of sections with 20% normal swine serum. Sections were then labeled with a standard peroxidase anti-peroxidase technique.[14]

Primary murine monoclonal antibodies (MAbs) were used against α2 integrin (clone HAS6, kind gift of Dr. F. Watt, Keratinocyte Laboratory, Imperial Cancer Research Foundation, Lincoln's Inn Fields, London; used at a concentration of 10 μg/ml),[15] α5 and α6 integrins (clones SAM 1 and GoH3, respectively, both obtained from Immunotech, Marseilles, France; used at a concentration of 2 μg/ml), β1 integrin (clone PL 18.5, obtained from Takara Biomedicals, Ltd.; used at a concentration of 1 μg/ml), β4 integrin (clone 3E1, obtained from Life Technologies, Ltd.; used at a dilution of 1:10,000), and $\alpha v\beta 3$ integrin (clone 23C6, kind gift of Dr. M. Horton, Haemopoiesis Research Group, Imperial Cancer Research Foundation, St. Bartholomew's Hospital, London; used at a concentration of 1.3 μg/ml).[16] MAb LM 609, which also recognizes $\alpha v\beta 3$ integrin[13] was the generous gift of Dr. D. A. Cheresh (Scripps Institute, La Jolla, CA) and was used at a concentration of 5 μg/ml. Isotype controls of irrelevant specificity were included in each assay.

Double immunofluorescence labeling was applied to serial sections prepared as above. Sections were incubated with two antibodies, one specific for von Willebrand factor (rabbit polyclonal, obtained from Dako, Carpinteria, CA) and the other specific for one of β1, β4, or $\alpha v\beta 3$ integrins (see above). This step was followed by sequential incubations with secondary antibodies; trimethylrhodamine isothiocyanate (TRITC)-conjugated rabbit anti-mouse (Dako) and fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit (Jackson Immunoresearch Laboratories, West Grove, PA).

Image Analysis

The HSI method of color image analysis was performed with a JVC 3 chip color TV camera linked to a personal computer containing color image processing boards (Data Translation, Marlborough, MA) with Colour Freelance software (Foster-Findlay Associates, Newcastle on Tyne, UK).[17]

HSI color image analysis is largely independent of light intensity and allows reliable and reproducible detection of a particular color, in this case the brown immunoperoxidase reaction product. The method relies on a thresholding technique, detecting peroxidase staining at a given point (pixel) in an all-or-none manner. Biological variation in concentration of labeled molecule means that, in areas of elevated concentration, a greater proportion of the pixels exceed the detection thresholds and are registered as positive. In calibration experiments with media containing a uniform concentration of antigen, a sigmoid relation was observed between the percentage of the area stained and the concentration of antigen.[18]

Quantification of vascular staining entailed initial manual outlining of the vessel under analysis with a mouse facility. Detection of total vascular tissue within the outlined zone (excluding vessel lumen) and calculation of its area in pixels was performed automatically. The system then detected those pixels in the vessel wall that were within the appropriate

range of HSI values for the reaction product. For each vessel studied, the number of positive pixels were divided by the total number of pixels within that vessel. This value, expressed as the percentage of endothelial staining, gave quantitative expression to the concentration of labeled molecule and thus to observed staining intensity.

Coded slides were analyzed with the single operator blind to the diagnosis. In each section, 10 vessels in the superficial dermis and 4 in the mid/deep dermis were randomly selected. The percentage of endothelial staining was calculated for each vessel and a mean value obtained for each subject. The mean percentage of staining for β1, β4, and αvβ3 integrins was calculated from the summation of mean values from all 10 subjects.

Statistics

Data were expressed as mean ± SEM. Statistical analysis of the data was performed by the Wilcoxon signed-rank test. Differences were taken as significant when $P < 0.05$.

Results

αvβ3 Integrin

Anti-αvβ3 MAbs (23C6 and LM 609) labeled endothelium widely in all sections studied (Figure 1). Relative uniformity in the intensity of staining was seen within different zones of the vascular plexus. Smooth muscle of the pilosebaceous unit also stained with 23C6 and LM609. Application of image analysis to sections labeled with MAb 23C6 demonstrated a 3-fold increase in the mean percentage of endothelial staining of 42.35 ± 4.28 in lesional superficial vessels, compared with 15.44 ± 2.10 in nonlesional superficial vessels ($P < 0.01$, Figure 2). There was a less significant increase in the percentage of endothelial staining of deep vessels of 38.91 ± 4.97 in lesional skin, compared with 23.11 ± 10.91 in nonlesional skin ($P < 0.05$).

For all integrins studied there was no quantitative difference in endothelial staining between deep and superficial vessels within lesional or nonlesional groups (results not shown).

β4 Integrin

Anti-β4 MAb (3E1) labeled the basement membrane zone of all structures including epidermis and endothelium (Figure 1). As previously reported, β4 labeled basal and suprabasal keratinocytes in lesional and nonlesional skin.[19] There was a significant decrease in the mean percentage of endothelial β4 staining of superficial vessels in lesional skin, 44.22 ± 2.51, compared with nonlesional skin, 59.61 ± 3.82 ($P < 0.01$; Figure 2). Image analysis did not demonstrate a significant difference in deep vessel β4 staining between lesional and nonlesional specimens.

α2, α5, α6, and β1 Integrins

The integrin subunits α2, α5, and α6 commonly associate with β1 to form the complexes α2β1, α5β1, and α6β1. Immunohistochemical staining with MAbs specific for α2 (HAS6), α5 (SAM1), α6 (GoH3) and β1 (PL18.5) all labeled vascular endothelium without detectable difference in intensity of staining between the two groups. In view of this, image analysis to quantify staining intensity was performed on those sections labelled with β1 alone (fig 1). The mean percentage endothelial staining in nonlesional superficial vessels was 61.77 ± 6.03 SEM, compared with 49.15 ± 5.14 SEM in lesional superficial vessels (fig 2). This difference was not statistically significant. Similarly, there was no significant difference in deep vessel β1 staining between the two groups (results not shown). Pilosebaceous smooth muscle and basal keratinocytes were also labelled with PL18.5. Basal keratinocyte staining with anti-β1 integrin was noticably less intense in lesional skin compared with nonlesional skin, as previously described.[19]

Immunofluorescence Double Labeling

Immunofluorescence double labeling with polyclonal antibody specific for von Willebrand factor confirmed vascular endothelial labeling by anti-integrin antibodies (Figure 3).

Discussion

Our data comparing the expression of endothelial integrins on dermal microvasculature in psoriasis demonstrate a significant increase in αvβ3 expression and a significant decrease in β4 expression in lesional *versus* nonlesional skin. There was no significant change in α2, α5, α6, or β1 integrin expression between these two groups.

Considerable evidence indicates that microvascular changes observed in psoriasis are a result of angiogenesis. A number of known angiogenic factors have been shown to be overexpressed in le-

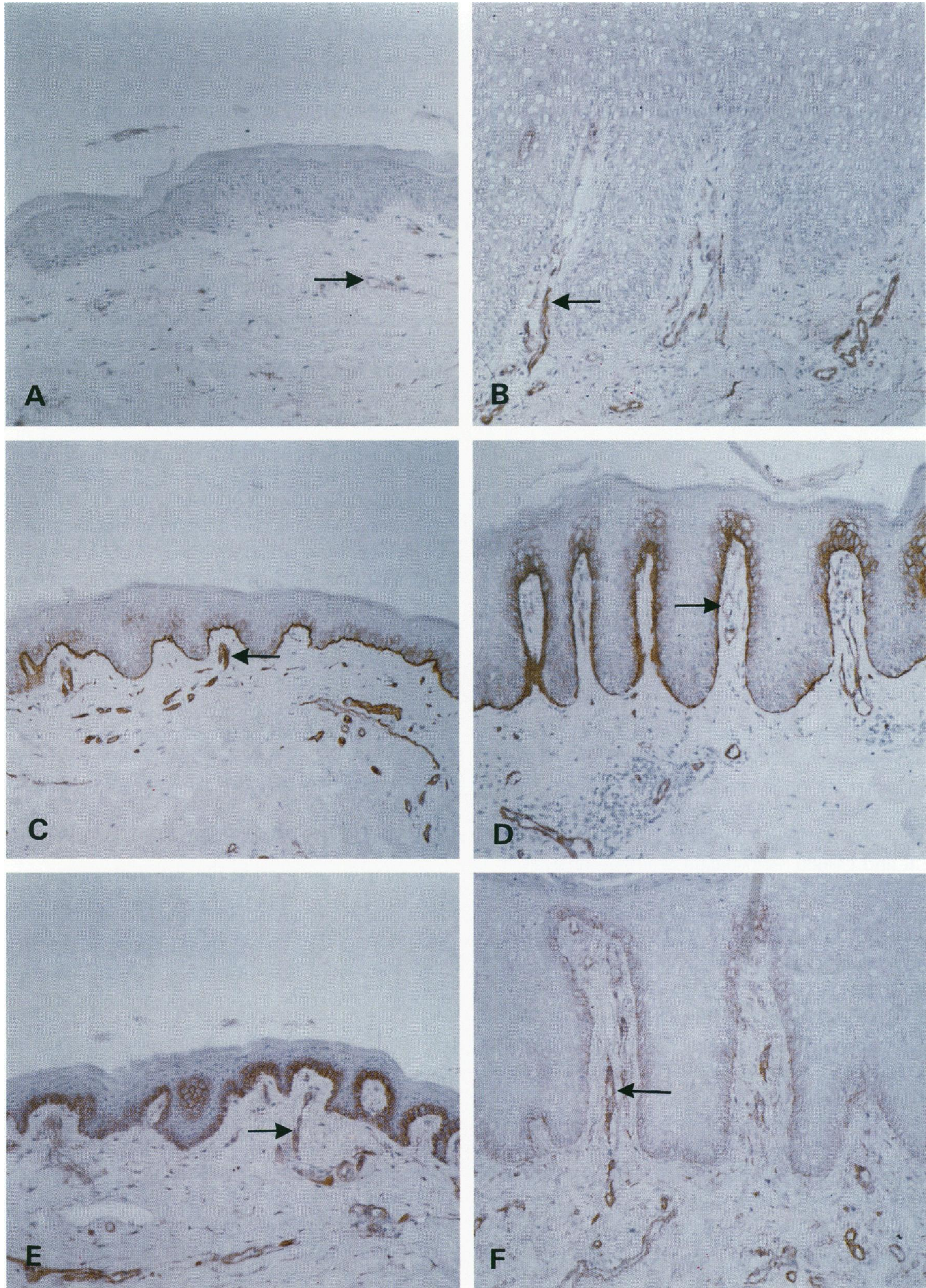

Figure 1. *Expression of αvβ3, β4, and β1 integrins on superficial cutaneous microvasculature* (arrows) *of lesional and nonlesional skin in psoriasis. Immunohistochemical staining of paired frozen sections with monoclonal antibody (MAb) 23C6, specific for αvβ3 integrin, demonstrates an increased endothelial expression in lesional* (B) *compared with nonlesional* (A) *skin. MAb 3E1, specific for β4 integrin, demonstrates a decreased endothelial expression in lesional* (D) *compared with nonlesional* (C) *skin. MAb PL18.5, specific for β1 integrin, demonstrates similar expression in nonlesional* (E) *and lesional* (F) *skin. Magnification, ×28.*

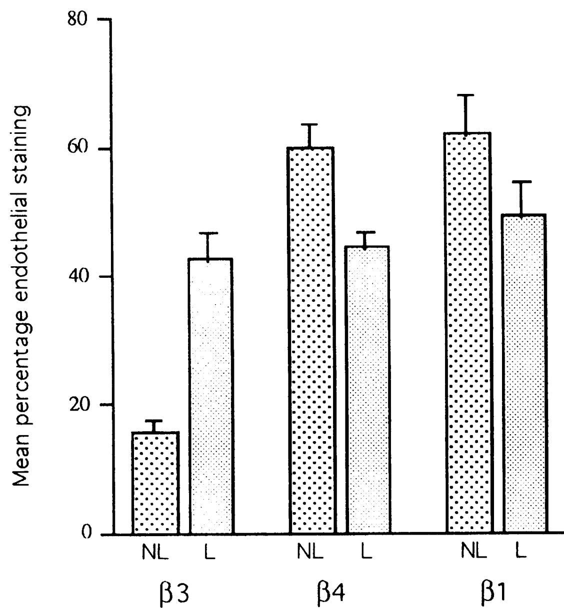

Figure 2. *Percentage of endothelial staining of superficial dermal vessels in nonlesional (NL) and lesional (L) psoriatic skin labeled with MAbs specific for αvβ3, β4, and β1 integrins. The mean percentage values (plus SEM) of vascular staining quantified by HSI color image analysis from paired biopsies in 10 patients with psoriasis are shown.*

sional epidermis in psoriasis including transforming growth factor-α,[20] interleukin-8,[21,22] vascular endothelial growth factor,[23] and tumor necrosis factor-α.[24] In addition, psoriatic keratinocytes in culture produce increased amounts of interleukin-8 and decreased expression of the angioinhibitory factor thrombospondin.[22] Although these mitogenic factors possess activity on a wide range of cell types, the above studies strongly support the hypothesis that vascular proliferation in psoriasis is driven by keratinocyte-derived soluble mediators.

The cell-cell and cell-matrix interactions that accompany angiogenesis are mediated in part by surface-bound integrins.[8] Consequently, phenotypic changes in angiogenic vasculature will be reflected in endothelial integrin expression. *In vivo* and *in vitro* studies have demonstrated the importance of a number of endothelial cell-bound integrins in angiogenesis including α2, α6, αv, β1, β3, and β4.[10–13]

An *in vitro* study of angiogenesis has shown that cultured endothelial cells can be completely inhibited from tube formation by MAb to β1 integrin.[10] However, immunohistochemical studies showed no increased expression of β1 integrin on proliferating vessels of human granulation tissue or on basic fibroblast growth factor-induced neovasculature in the chick chorioallantoic membrane assay of angiogenesis.[13] These latter findings correlate with our data, which show no significant change in β1 expression or in the expression of α2, α5, and α6 integrins on psoriatic vessels.

β4 integrin associates with α6 to form a complex that recognizes laminin and is a major component of hemidesmosomes, structures involved in the attachment of epithelial cells to basement membranes. The role of β4 integrin in endothelial cell/basement membrane interactions is unclear, although a similar anchoring role is postulated.[25] Confocal microscopic examination of angiogenic sprouts in human neonatal foreskin endothelium has shown an increased expression of α6 and β4 integrins.[12] These observations are surprising because endothelial cells leave the laminin-rich milieu of the basement membrane during the migratory phase of angiogenesis. In addition, *in vitro* studies have demonstrated that dermal microvascular cells do not synthesize significant amounts of A-chain laminin, the ligand for α6β4.[12] Cultured human dermal microvascular endothelial cells demonstrate decreased expression of β4 integrin after stimulation with basic fibroblast growth factor, a potent angiogenic factor.[26] Our observations also demonstrate a significant decrease in endothelial β4 expression in involved psoriatic microvasculature. During angiogenesis, endothelial cells must dissociate from the laminin-rich basement membrane, which may be facilitated, in part, by down-regulation of β4 integrin expression.

The β3 subfamily of integrins consists of the β3 chain associated with αIIb or αv chains. On nucleated cells, β3 is associated with a 150-kd chain, αv, found to be identical to a protein previously described as a constituent of the vitronectin receptor.[27] As well as vitronectin, αvβ3 integrin is capable of binding to a number of ligands including fibrinogen, thrombin, von Willebrand factor, and thrombospondin. Immunohistochemistry studies of wound healing have demonstrated an increased expression of αvβ3 integrin on proliferating vessels.[13] On the chick chorioallantoic membrane, angiogenesis is also accompanied by an increased expression of αvβ3 integrin. Blockade of αvβ3 integrin with MAb in this assay leads to an inhibition of angiogenesis.[13] Basic fibroblast growth factor increases the expression of αvβ3 on cultured human dermal microvascular endothelial cells with concomitant morphological changes and increased cellular binding to vitronectin-coated surfaces.[28,29] Our studies of integrin expression in psoriasis have shown a significant increase in endothelial αvβ3 integrin expression in lesional compared with nonlesional dermal microvasculature. This provides additional evidence to support the involvement of angiogenesis in psoriasis and indicates that endothelial αvβ3 integrin may

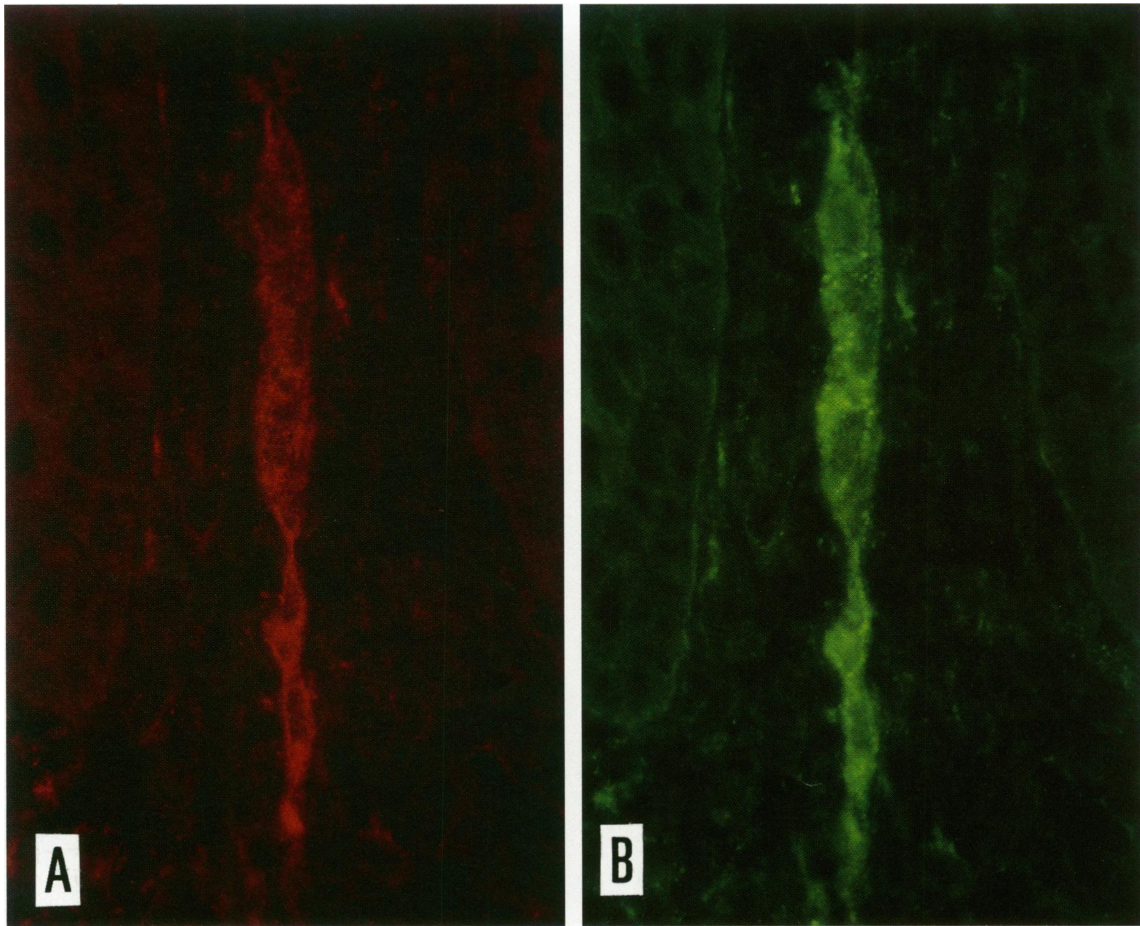

Figure 3. *Expression of αvβ3 integrin complex on microvascular endothelium in psoriasis. Double immunofluorescence of lesional psoriatic skin demonstrates localization of αvβ3 integrin* (**A**; *using MAb LM 609 labeled with TRITC*) *with vascular endothelium identified with von Willebrand factor* (**B**; *FITC labeled*). *Magnification, ×560.*

have an important role in the pathophysiology of this disease.

The importance of investigating vascular proliferation in psoriasis not only furthers our understanding of pathogenetic mechanisms but also assists the development of new therapeutic strategies. There is evidence to suggest that current treatments including methotrexate, cyclosporin A, photochemotherapy, and Goeckerman's therapy act, in part, via normalization of psoriatic microvasculature.[30,31] Similarly, the therapeutic effect of razoxane in psoriasis is thought to be attributable to its action on abnormal vessels.[32] Further elucidation of the angiogenic process, such as the role played by proteolytic enzymes,[33] may reveal possible targets for pharmacological intervention. Angiogenic inhibitors, such as suramin analogues and the fumagillin derivative AGM-1470, are currently being evaluated for development as anti-cancer drugs.[34] A parallel approach to develop angiosuppressive agents in psoriasis may yield more effective therapies for this disease.

References

1. Barker JNWN: Pathophysiology of psoriasis. Lancet 1991, 338:227–230
2. Barton SP, Abdullah MS, Marks R: Quantification of microvascular changes in the skin in patients with psoriasis. Br J Dermatol 1992, 126:569–574
3. Wolf JE: Angiogenesis in normal and psoriatic skin. Lab Invest 1989, 62:139–142
4. Folkman J, Shing Y: Angiogenesis. J Biol Chem 1992, 267:10931–10934
5. Folkman J, Haudenschild C: Angiogenesis in vitro. Nature 1980, 288:551–556
6. Ingber DE: Extracellular matrix as a solid-state regulator in angiogenesis: identification of new targets for anti-cancer therapy. Semin Cancer Biol 1992, 3:57–63
7. Folkman J, Klagsbrun M: Angiogenic factors. Science 1987, 235:442–447
8. Luscinskas FW, Lawler J: Integrins as dynamic regulators of vascular function. FASEB J 1994, 8:929–938
9. Hynes RO: Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992, 69:11–25

10. Bauer J, Margolis M, Schreiner C, Edgell C-J, Azizkhan J, Lazarowski E, Juliano RL: In vitro model of angiogenesis using a human endothelium-derived permanent cell line: contributions of induced gene expression, G-proteins, and integrins. J Cell Physiol 1992, 153: 437–439
11. Gamble JR, Matthias LJ, Meyer G, Kaur P, Russ G, Faull R, Berndt MC, Vadas MA: Regulation of in vitro capillary tube formation by anti-integrin antibodies. J Cell Biol 1993, 121:931–943
12. Enenstein J, Kramer RH: Confocal microscopic analysis of integrin expression on the microvasculature and its sprouts in the neonatal foreskin. J Invest Dermatol 1994, 103:381–386
13. Brooks PC, Clark RAF, Cheresh DA: Requirement of vascular integrin $\alpha v\beta 3$ for angiogenesis. Science 1994, 264:569–571
14. Allen MH, Markey AC, MacDonald DM: The development of a reproducible immunocytochemical technique for co-localizing cutaneous antigens. Am J Dermatopathol 1991, 13:221–227
15. Tenchini ML, Adams JA, Gilberty C, Steel J, Hudson DL, Malcovati M, Watt FM: Evidence against a major role for integrins in calcium-dependent intercellular adhesion of epidermal keratinocytes. Cell Adhes Commun 1993, 1:55–66
16. Horton MA, Lewis D, McNulty K, Pringle JAS, Chambers TJ: Monoclonal antibodies to osteoclastomas (giant cell bone tumours): definition of osteoclast-specific cellular antigens. Cancer Res 1985, 45:5663–5669
17. Poston RN, Gall NP: Hue-saturation-intensity colour image analysis for the quantitation of immunoperoxidase staining. Transactions of the Royal Microscopical Society, vol 1: Micro 90. Edited by HY Elder. New York, Adam Hilger, 1990, pp 525–528
18. Sousa AR, Lane SJ, Atkinson BA, Poston RN, Lee TH: The effects of prednisolone on the cutaneous tuberculin response in patients with corticosteroid-resistant bronchial asthma. J Allergy Clin Immunol 1995 (in press)
19. Pellegrini G, De Luca M, Orecchia G, Balzac F, Cremona O, Savoia P, Cancedda R, Marchisio PC: Expression, topography, and function of integrin receptors are severely altered in keratinocytes from involved and uninvolved psoriatic skin. J Clin Invest 1992, 89:1783–1795
20. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey RJ, Ellingsworth L, Derynck R, Voorhees JJ: Overexpression of transforming growth factor alpha in psoriatic epidermis. Science 1989, 243:811–814
21. Shroder JM, Christophers E: Identification of C5a and an anionic neutrophil-activating peptide (ANAP) in psoriatic scale. J Invest Dermatol 1986, 87:53–58
22. Nickoloff BJ, Mitra RS, Varani J, Dixit VM, Polverini PJ: Aberrant production of interleukin-8 and thrombospondin-1 by psoriatic keratinocytes mediates angiogenesis. Am J Pathol 1994, 144:820–828
23. Detmar M, Brown LF, Claffey KP, Yeo K-T, Kocher O, Jackman RW, Berse B, Dvorak HF: Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. J Exp Med 1994, 180:1141–1146
24. Ettehadi P, Greaves MW, Wallach D, Aderka D, Camp RDR: Elevated tumour necrosis factor-alpha (TNF-α) biological activity in psoriatic skin lesions. Clin Exp Immunol 1994, 96:146–151
25. Kennel SJ, Godfrey V, Ch'ang LY, Lankford TK, Foote LJ, Makkinje A: The beta 4 subunit of the integrin family is displayed on a restricted subset of endothelium in mice. J Cell Sci 1992, 101:145–150
26. Sepp NT, Cornelius LA, Romani N, Li L-J, Wright Caughman S, Lawley TJ, Swerlick RA: Polarised expression and basic fibroblast growth factor-induced down-regulation of the $\alpha 6\beta 4$ integrin complex on human microvascular endothelial cells. J Invest Dermatol 1995, 104:266–270
27. Albelda SM: Endothelial and epithelial adhesion molecules. Am J Respir Cell Mol Biol 1991, 4:195–203
28. Swerlick RA, Brown EJ, Xu Y, Lee KH, Manos S, Lawley TJ: Expression and modulation of the vitronectin receptor on human dermal microvascular endothelial cells. J Invest Dermatol 1992, 99:715–722
29. Sepp NT, Li L-J, Lee KH, Brown EJ, Caughman SW, Lawley TJ, Swerlick RA: Basic fibroblast growth factor increases expression of the $\alpha v\beta 3$ integrin complex on human microvascular endothelial cells. J Invest Dermatol 1994, 103:295–299
30. Braverman IM, Sibley BA: Role of the microcirculation in the treatment and pathogenesis of psoriasis. J Invest Dermatol 1982, 78:12–17
31. Norrby K: Cyclosporine is angiostatic. Experientia 1992, 48:1135–1138
32. Atherton DJ, Wells RS, Laurent MR, Williams YF: Razoxane (ICRF 159) in the treatment of psoriasis. Br J Dermatol 1980, 102:307–317
33. Hanemaaijer R, Koolwijk P, le Clercq L, de Vree WJ, van Hinsbergh VW: Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells: effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. Biochem J 1993, 296:803–809
34. Auerbach W, Auerbach R: Angiogenesis inhibition: a review. Pharm Ther 1994, 63:265–311