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Aberrant Production of Interleukin-8 and Thrombospondin-1 by Psoriatic Keratinocytes Mediates Angiogenesis

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Psoriasis is a common inherited skin disease that is characterized by hyperproliferation of epidermal keratinocytes and excessive dermal angiogenesis. A growing body of evidence supports a key pathogenetic role for activated keratinocytes in the angiogenic response that accompanies psoriasis. We investigated the role of psoriatic epidermis in the aberrant expression of angiogenesis by examining the ability of pure populations of multipassaged keratinocytes obtained from the skin of normal individuals and psoriatic patients to induce angiogenesis* in vivo *in the rat corneal bioassay and endothelial cell chemotaxis* in vitro. *Media conditioned by keratinocytes from psoriatic patients, including both symptomless skin and psoriatic plaques, induced vigorous angiogenic responses in over 90% of corneas tested and potently stimulated directional migration of capillary endothelial cells* in vitro. *In contrast, conditioned medium from normal keratinocyte cultures was weakly positive in less than 10% of corneas assayed and failed to stimulate endothelial cell chemotaxis. Furthermore, keratinocytes from psoriatic skin exhibited a 10- to 20-fold increase in interleukin-8 production and a sevenfold reduction in thrombospondin-1 production. The angiogenic activity present in keratinocyte-conditioned media from psoriatic patients was suppressed by adding either highly purified thrombospondin-1 (125 ng) or following the addition of either normal keratinocyte-conditioned media or neutralizing interleukin-8 antibody. We conclude that psoriatic keratinocytes are phenotypically different from normal keratinocytes with respect to their angiogenic capacity and that this aberrant phenotype is attributable to a defect in the overproduction of interleukin-8 and a deficiency in the production of the angiogenesis inhibitor thrombospondin-1. (Am J Pathol 1994, 144:820–828)

Psoriasis is a common skin disease, linked to both genetic and environmental triggering factors.[1] It is characterized pathologically by excessive growth of epidermal keratinocytes (Krs), inflammation, and dermal neovascularization, which apparently results from complex and reciprocal molecular cross-talk between activated epidermal and dermal cells.[2] Twenty years ago, Folkman[3] suggested that hyperkinetic psoriatic Krs produced an angiogenic factor mediating the neovascularization of the upper dermis.[4] Using fresh human psoriatic lesional tissue (no animal model for psoriasis exists), which was separated into epidermal and dermal components, the angiogenic activity associated with the lesion was found by two different groups to reside in the epidermal compartment.[5,6] However, as pointed out in an accompanying editorial,[7] this experimental approach could not conclusively demonstrate whether the source of the angiogenic activity were the Krs, which are known to produce a variety of proangiogenic cytokines such as basic fibroblast growth factor, interleukin-1 (IL-1),

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transforming growth factor-α, or was derived from the recruited intraepidermal leukocytes producing tumor necrosis factor-α.

To preclude this problem of different potential cell sources and to further dissect the cytokine network in psoriasis,[2] we have taken advantage of our ability to cultivate serially pure populations of keratinocytes from psoriatic skin in a serum-free medium to investigate the molecular basis responsible for angiogenesis in psoriasis.[8] We were particularly interested in the psoriatic Krs essentially for two reasons: first, abnormalities that develop in psoriatic Krs *in vivo* precede the development of dermal neovascularization; second, we and others have reported previously that psoriatic Krs overproduced IL-8 *in vivo*, a recently described proangiogenic cytokine. In addition, Krs are an abundant source of thrombospondin-1 (TSP-1), an extracellular matrix molecule that has been shown to be an important negative regulator of angiogenesis.[9–15] We hypothesize that a defect in the overproduction of angiogenic mediators and/or a deficiency in anti-angiogenic factors such as TSP-1 may be responsible for the aberrant expression of angiogenesis in psoriasis. In this report, we test this hypothesis by assaying for angiogenic activity present in the conditioned media (CM) of cultured psoriatic Krs and correlating this activity to levels of IL-8 and TSP-1.

Materials and Methods

Cell Culture and Preparation of CM

Multipassaged human Krs were obtained from 6-mm punch biopsies of human skin after obtaining informed consent and approval of the University of Michigan Human Subjects Committee. Biopsies were obtained from the buttocks of normal healthy adult individuals without any skin disease and on no medication or from the buttocks or lower back of psoriatic patients with active untreated disease. Samples included normal skin (NN), clinically symptomless or lesion-free skin (PN) at least 10 cm away from a lesion; and biopsies of the active advancing margin of expanding psoriatic plaques (PP). Psoriatic patients included male and female adults between the ages of 24 and 49 years old. The epidermis was immediately removed from the dermis by using 0.3% trypsin and 0.1% ethylenediaminetetraacetic acid, and a single-cell suspension of epidermal cells was produced by vigorous pipetting in a low-calcium (0.15 mmol/L) serum-free Kr basal medium (KBM, Clonetics Corp., San Diego, CA). Krs were seeded onto plastic petri dishes (Corning Inc., Corning, NY) and grown in the basal medium supplemented with epidermal growth factor (10 ng/ml), insulin (5 mg/ml), and 0.4% (V/V) bovine pituitary extract (designated KGM), as previously described.[8] When Krs reached confluency, they were removed from the petri dish using 0.03% trypsin, 0.01% ethylenediaminetetraacetic acid and serially passaged multiple times. Passage 1 cells represent the initial splitting of Krs obtained from the original epidermal sheet. Krs were maintained at 37 C in a humidified incubator containing 5% CO_2 and used between passage numbers 2 and 4. After multiple passages, confluent Krs grown in 60-mm petri dishes using KGM were washed four times with 4 ml of KBM over a 6-hour period. Next either fresh KBM or KGM was added (4 ml/dish) and the resultant 2-hour or 48-hour CM was aspirated from the dish, centrifuged, and concentrated 20-fold by centrifugation using Centricon-10 filters (Amicon, Inc., Beverly, MA). CM was stored at −80 C for up to 1 week.

Fibronectin (FN), TSP-1, and IL-8 Enzyme-Linked Immunosorbent Assays

The 2-hour cell-free CM was analyzed directly for production of three secreted proteins: FN, TSP-1, and IL-8. FN (GIBCO, Grand Island, NY), TSP-1, purified from human platelets as previously described,[15] and IL-8 (human recombinant, R & D Systems, Minneapolis, MN) were used to establish standard curves in a sensitive enzyme-linked immunosorbent assay procedure previously described by us.[13,14] Briefly, CM were diluted in 0.05 mol/L carbonate buffer, pH 9.6; 50 μl of the diluted fluids were added to each well of a 96-well microtiter plate and allowed to incubate for 3 hours at 37 C. Purified FN, TSP-1, or IL-8 diluted in the same buffer were as positive controls and culture media alone were used as negative controls. The wells were washed three times with phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 (PBS-T). Antibodies to FN, TSP-1, or IL-8 were diluted in PBS-T, added to the wells (50 μl/well), and incubated for 30 minutes at 37 C. The wells were again washed in PBS-T, and 50 μl of the appropriately-diluted alkaline phosphatase-conjugated second antibody was added to each well. After 30 minutes of incubation with the second antibody at 37 C, the wells were again washed three times with PBS-T. Finally, 100 μl of a 1 mg/ml solution of *p*-nitrophenyl phosphate in 0.05 mol/L carbonate buffer, pH 9.6, was added to each well and the absorbance (OD 405) of the wells was measured at various time intervals with a Titertek Multiskan instrument (Flow Laboratories,

McClean, VA). For add-back experiments, highly purified TSP-1 was used in the indicated amounts.

IL-8 Neutralization

Neutralization of IL-8 was accomplished by incubating 1 ml of concentrated CM (5 μg of protein) with goat anti-human IL-8 neutralizing antibody (R & D Systems) diluted 1:500 in PBS for 2 hours at 37 C on a rocking platform. This dilution of antibody was found to suppress endothelial cell chemotaxis induced by 50 ng pure human recombinant IL-8 (R & D Systems) by approximately 70% Samples were then passed through a protein A column according to the manufacturers directions (Pierce, Rockford, IL) to remove antibody bound IL-8, and media was reconcentrated then assayed in rat corneas or in the endothelial cell chemotaxis assay.

Corneal Neovascularization Bioassay

Angiogenic activity present in control (KBM and KGM media) and in PP, PN, and NN CM, was examined after 48 hours in the avascular cornea of F344 female rats eyes (Harlan Laboratories, Madison, WI), as previously described.[16] Briefly, each sample of CM (5 μg of total protein) was combined with an equal volume of sterile Hydron casting solution (Interferon Sciences, New Brunswick, NJ), and 5 μl aliquots were pipetted onto the surface of 1-mm diameter Teflon rods (Dupont Corporation, Wilmington, DE) glued to the surface of a glass petri dish. The resulting pellets were air-dried in a laminar hood for 1 hour and refrigerated overnight. Just before the pellets were implanted, they were rehydrated with a drop of lactated ringers solution then placed in a surgically created intracorneal pocket approximately 1.5 mm from the limbus. Corneas were observed for a period of 7 days. Animals were then perfused with a colloid carbon solution, eyes were enucleated, and fixed in 10% neutral buffered formalin overnight. The following day, corneas were excised, flattened, and photographed. A positive neovascularization response was recorded only if sustained directional ingrowth of capillary sprouts and hairpin loops toward the implant was observed. Negative responses were recorded when either no growth was observed or when only an occasional sprout or hairpin loop showing no evidence of sustained growth was detected. Representative corneas were examined histologically, and except for occasional neutrophils found in the limbus of both control (angiogenic negative) and test (angiogenic positive) corneas, nonspecific inflammation was not a contributing factor in any of the corneal responses (data not shown).

Endothelial Cell Chemotaxis

Endothelial cell chemotaxis was performed in a 48-well, blind well chemotaxis chambers (Nucleopore Corp., John Cabin, MD) as previously described.[17,18] Nucleopore chemotaxis membranes (5-μ pore size) were prepared by soaking them sequentially in 3% acetic acid overnight and for 2 hours in 0.1 mg/ml gelatin. Membranes were rinsed in sterile water, dried under sterile air, and stored at room temperature for up to 1 month. Bovine adrenal gland capillary endothelial cells, maintained in gelatin-coated flasks in Dulbecco's modified Eagles medium (Gibco) with 10% fetal bovine serum were used as target cells. Twenty-four hours before use bovine adrenal gland capillary endothelial cells were serum starved by growing cells in Dulbecco's modified Eagle's medium with 0.1% bovine serum albumin (Gibco). Twenty-five μl of cells, at a concentration of 1×10^6/ml in Dulbecco's modified Eagle's medium with 0.1% bovine serum albumin, were dispensed into each of the bottom wells. A chemotaxis membrane was positioned atop the bottom wells, chambers were sealed, inverted, and incubated for 2 hours to allow cells to adhere to the membrane. Chambers were then reinverted, 50 μl test media (48-hour CM) was dispensed into the top wells and reincubated for an additional 2 hours. Membranes were fixed and stained with Diff-Quick staining kit (Baxter Healthcare Corp., McGaw Park, IL) to enumerate membrane bound cells and cells that had migrated through the membrane to the opposite surface were counted. Four replicates, 10 fields per replicate, were tested for each sample, and experiments were repeated at least twice. Results were expressed as the total number of endothelial cells that migrated across the filter in 10 high-powered (400×) fields. Differences between controls and the various test groups were analyzed using the Student's *t*-test.

Results

Production of TSP-1 and IL-8 by Cultures of Normal and Psoriatic Keratinocytes

As per our previous experience with a different set of NN, PN, and PP Krs, no differences in the rate of growth of Krs obtained from different patients was observed using KGM as the growth medium.[8] However,

Table 1. *Production of FN, TSP-1, and IL-8 by Krs from NN, PP, and PN Skin*

Source of CM	ng/ml/10^6 cells		
	FN	TSP-1	IL-8
NN 1†	ND	778	ND
NN 2	720	556	ND
NN 3	819	523	ND
NN 4	ND	ND	0.95
NN 5	ND	ND	0.97
NN 6	ND	ND	1.32
PN 2	784	173	ND
PP 1	ND	266	ND
PP 2	ND	225	ND
PP 3	880	165	ND
PP 4	846	116	ND
PP 5	ND	ND	29.9
PP 6	ND	ND	20.3
PP 7	ND	ND	35.6
PP 8	ND	ND	22.9
PP 9	ND	ND	11.9

* Values were obtained by enzyme-linked immunosorbent assay for 2 hour-CM (SEM < 15% for all indicated values). ND = not determined.

† Number refers to patient number.

when the CM was analyzed for secreted levels of extracellular matrix molecules, differences were observed between NN and PP Krs with respect to TSP-1 levels, but not FN levels (Table 1). For the FN levels, both NN and PP Krs produced approximately equivalent amounts ranging from 720 to 880 ng/ml/10^6 cells. However, whereas NN Krs produced between 523 to 778 ng/ml/10^6 cells of TSP-1, the PP Krs (including four different PP cultures and one PN culture) underproduced TSP-1 (ranging from 116 to 266 ng/ml/10^6 cells). These low levels of TSP-1 production by psoriatic cells are also approximately 50% lower than our two previously published results using a different series of NN Krs in which we observed levels of TSP-1 in the range of 450 to 500 ng/ml/10^6/cells.[11,12] In addition, several PP and PN cultures were found to contain elevated levels of IL-8 (12 to 36 ng/ml/10^6 cells) when compared to NN cultures (1 to 2 ng/ml/10^6 cells) (Table 1). Because TSP-1 has been shown to suppress angiogenesis and IL-8 is a known mediator of angiogenesis,[17–20] we wanted to determine if this reduction in the level of TSP-1 and/or overproduction of IL-8 was responsible for the enhanced angiogenic activity in the CM of PP and PN cells. This was done by directly assaying their CM for angiogenic activity using the rat corneal bioassay and for the ability of CM to stimulate endothelial cell chemotaxis *in vitro*.

Angiogenic and Endothelial Cell Chemotactic Activity of CM from Cultured NN and Psoriatic Krs

Because KGM contains growth supplements that are capable of inducing angiogenesis, we assayed both KGM and KBM for angiogenic activity in rat corneas. Table 2 shows that KGM itself elicited only an occasional and inconsistent angiogenic response in three of 19 tested corneas. KBM was completely devoid of any angiogenic potential in three separate experiments using a total of 17 corneas. In a similar fashion, CM obtained from NN Krs maintained in either KGM or KBM produced only a sporadic angiogenic response. For NN cells in KBM, none of 12 tested corneas was positive, and using KGM only two of 11 corneas were considered positive. However, when either PN or PP Kr CM was examined using cells maintained either in KGM or KBM, consistent angiogenic responses were observed (Table 2). Figure 1 (lower panel) reveals a representative negative response using NN Kr CM, whereas the middle panel demonstrates a positive response induced after 7 days by PN Kr CM, and the top panel shows a vigorous neovascular response using PP Kr CM. It should be noted that even though a few tested corneas were scored as positive using CM from NN Krs grown in KGM, the extent of neovascularization induced by PN and PP Krs was considerably more striking.

We then tested aliquots of these same media for their ability to induce chemotaxis of capillary endothelial cells. CM from PP and PN Krs potently stimulated endothelial cell migration, whereas media from NN Krs, as well as cell-free control CM (KGM and KBM) exhibited a markedly reduced ability to stimulate endothelial cell chemotaxis (Figure 2).

Suppression of Psoriatic Kr-Induced Angiogenesis in Vivo *and Endothelial Cell Chemotaxis* in Vitro *by TSP-1, NN Kr CM, or IL-8 Neutralizing Antibody*

Because we observed elevated production of IL-8 and a deficiency in the levels of TSP-1 by PP Krs and because both of these mediators have been previously shown to participate in the regulation of angiogenesis,[17–20] we next explored whether the angiogenic activity produced by PP Krs could be reversed by addition of either highly purified TSP-1, CM from NN Krs, or following incubation of CM with IL-8 neutralizing antibody. Previously, it was determined that 125 ng of TSP-1 was sufficient to completely inhibit corneal neovascularization induced by basic fibroblast growth factor.[17] When 125 ng of TSP-1 added to PP Kr CM (using either PN or PP Krs maintained in either KBM or KGM), all but one of the 12 positive angiogenic responses was negated (Table 3). Using PP Kr CM, which was highly angiogenic, a dose response curve was observed in which adding

Table 2. *Angiogenic Responses Induced by Krs from NN, PP, and PN Skin*

Conditioned media (CM)	Proportion of positive responses (%)
Control media	
Basal media (KBM)*	0/17 (100)
Growth media (KGM)*	3/19 (16)
NN Krs	
KBM	0/12 (0)
KGM	2/11 (18)
PN Krs	
KBM	13/18 (72)
PP Krs	
KBM	26/29 (86)
KGM	16/17 (94)

* Results are from two separate experiments.
Keratinocytes were derived from individual patients and 48-hour CM was generated by cells grown in either basal (KBM) or growth (KGM) media.

back less than 125 ng of TSP-1 resulted in either partial (for 50 and 10 ng) or no inhibition (for 1 ng) of angiogenesis. Similarly CM for NN Krs known to contain TSP-1 was equally capable of blocking PP angiogenic activity. When either polyclonal or monoclonal antibodies to TSP-1 were mixed with NN Kr CM (data not shown), these media were no longer able to block the angiogenic activity in PN or PP Kr CM nor was any angiogenic activity detected in NN CM. This suggests that TSP-1 is a major Kr-derived inhibitor of angiogenesis. The potential role of IL-8 as a positive regulator of angiogenesis in psoriasis was revealed when CM from PN or PP Krs was incubated with neutralizing antibody to IL-8 then implanted in corneas. Table 4 shows that in three separate preparations of CM from PN or PP Krs, neutralizing antibody to IL-8 was able to substantially reduce the angiogenic activity present in these media. Inhibitory activity was also observed *in vitro* (Figures 2 to 4) where TSP-1 at a concentration of 125 ng/ml, NN CM, or IL-8 neutralizing antibody were able to block effectively endothelial cell chemotactic activity present in PN or PP Kr CM.

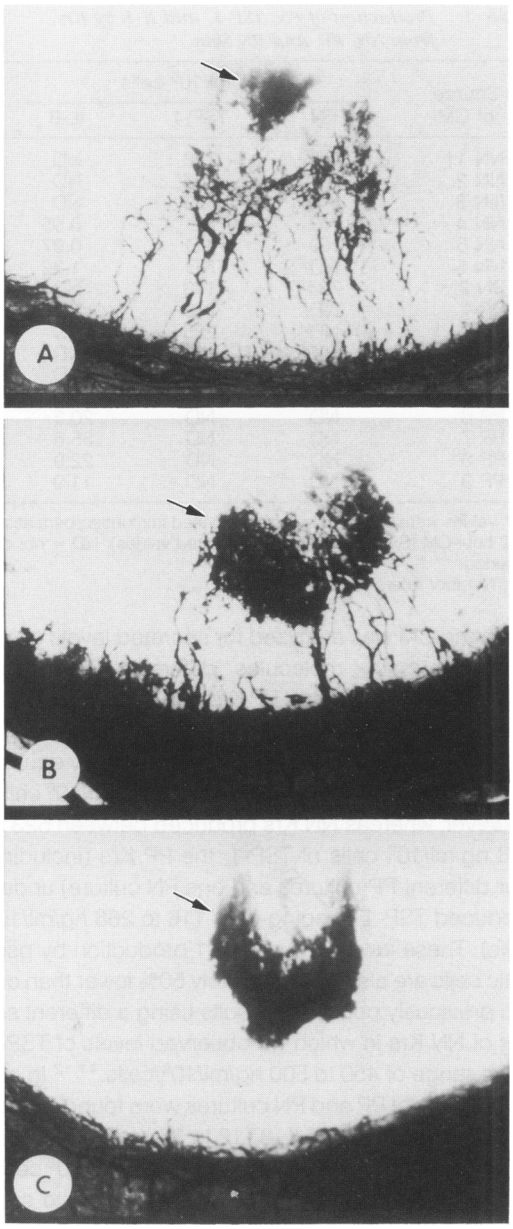

Figure 1. *Colloidal carbon perfused rat corneas demonstrating positive or negative neovascular responses following implantation of Hydron pellets containing CM from PP* (**A**), *PN* (**B**), *or NN Krs* (**C**). *Note the brushlike ingrowth of capillary sprouts induced by PP Krs in* **A**, *and a positive although less vigorous response induced by CM from PN Krs in* **B**. *A negative response to NN Kr CM is shown in* **C**. *Note the virtual absence of new capillaries in the cornea. The blackened areas (arrows) are shadows cast by the Hydron implant. Magnification, 30×.*

Discussion

The formation of new capillary blood vessels is a distinctive feature of a number of important physiological and pathological processes.[22,23] Normally, angiogenesis is encountered infrequently and is tightly controlled in both time and space. Angiogenesis that is encountered in physiological settings such as in chronic inflammation, wound healing, ovulation, and embryogenesis, is rapidly induced and down-regulated in a timely and coordinate fashion. The consequences of inappropriate angiogenesis leading to excessive or prolonged capillary growth is readily apparent when one looks at the increasing number of disease processes that are now classified as angiogenesis dependent.[24] In addition to solid tumors, chronic inflammatory diseases such as rheumatoid

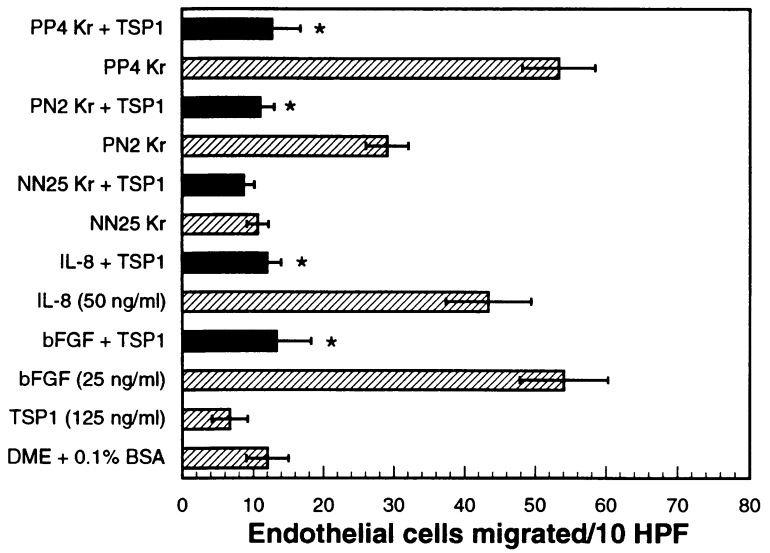

Figure 2. *TSP-1-mediated inhibition of endothelial cell migration in response to CM from PP and PN Krs (hatched bars). TSP-1 is also effective in blocking endothelial migration induced by IL-8 and basic fibroblast growth factor. Mean ± SD of three independent experiments,* *P ≤ .001 *by Students* t*-test.*

arthritis and psoriasis are two examples where excessive angiogenesis has been shown to contribute significantly to the pathogenesis of these disorders.[3–7,25,26]

In the present investigation, we examined the mechanisms underlying the unregulated angiogenesis that accompanies the chronic inflammatory skin disorder psoriasis. Our results demonstrate that PP Krs play a central role in the aberrant expression of angiogenesis that manifests in this disorder and that these cells seem to have a combined defect in both the overproduction of the proangiogenic cytokine IL-8 and the underproduction of the recently described angiogenesis inhibitor TSP-1.[17,18,27] It is well-established that inflammatory cells, in particular macrophages, play a central role in the induction of angiogenic responses in chronic inflammatory diseases, such as rheumatoid arthritis[20,26] and that macrophages are a rich source of both IL-8 and TSP-1.[20,28] Although we are unable to exclude the participation of these as well as other dermal-derived inflammatory cell population in psoriatic angiogenesis, they do not seem to be a major contributor in this clinical setting inasmuch as previous studies by Malhotra et al[6] have shown that fragments of psoriatic dermis implanted into rabbit corneas failed to stimulate neovascularization. Also based on an earlier study, the majority of the IL-8 detected at the protein level by immunostaining, and the messenger RNA level by Northern blot hybridization was localized in the epidermal Krs of psoriatic plaque.[11] Previous studies by us and others have described differences between normal and PP Krs with respect to their growth response[8] and immunomodulatory capacity.[21] We can now include among these aberrant phenotypes a defect in the production of both pro- and anti-angiogenic cytokines that governs the orderly development of new capillary blood vessels.

CM from NN Krs grown in either basal (KBM) or growth factor-supplemented (KGM) media consistently failed to induce an angiogenic response in the

Table 3. *Inhibition of PP and PN Kr-Mediated Angiogenesis by TSP-1 and CM from NN Krs*

CM ± TSP-1 or NN Kr CM	Proportion of positive responses (%)
	−TSP-1 +125 ng TSP-1
PN 1	3/3 (100) 0/3 (0)
PP 1	2/2 (100) 0/2 (0)
PP 3	3/3 (100) 1/7 (14)
	TSP-1:250–1 ng
PP 4 + 250 ng TSP-1	0/5 (0)
PP 4 + 125 ng TSP-1	0/3 (0)
PP 4 + 50 ng TSP-1	1/4 (25)
PP 4 + 10 ng TSP-1	2/4 (50)
PP 4 + 1 ng TSP-1	3/3 (100)
	−NN Kr CM +NN Kr CM
PP 1	2/2 (100) 0/4 (0)
PP 2	2/2 (100) 0/5 (0)
PP 3	3/3 (100) 1/4 (25)
PP 4	2/2 (100) 0/4 (0)

Table 4. *Inhibition of PP and PN Krs Induced Angiogenesis by IL-8 Neutralizing Antibody*

Conditioned media (CM)±IL-8 neutralizing antibody (Ab)	Proportion of positive responses (%)
PN 1	2/3 (67)
PN 1 + IL-8 Ab	1/3 (33)
PP 1	2/2 (100)
PP 1 + IL-8 Ab	1/3 (33)
PP 3	4/4 (100)
PP 3 + IL-8 Ab	2/5 (40)

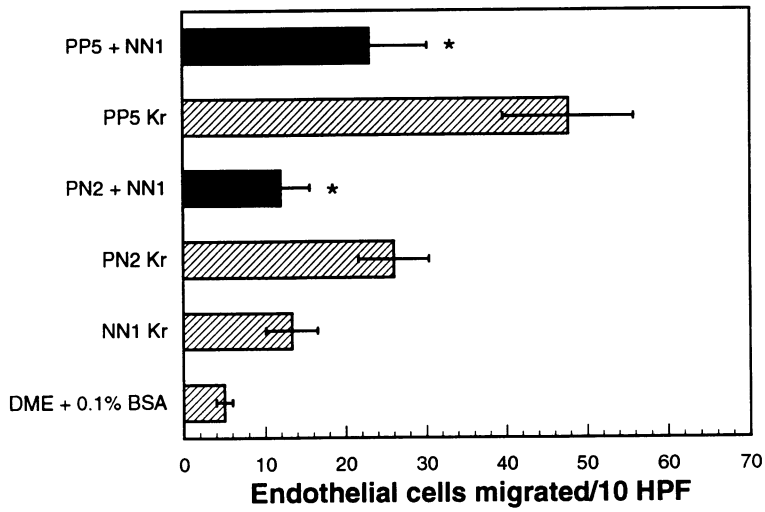

Figure 3. *Inhibition of PP and PN (hatched bars) Kr-induced endothelial cell migration by NN Kr CM (solid bars). Mean ± SD of three independent experiments, *P ≤ .05 by Students t-test.*

rat corneal bioassay. In contrast, CM from PP or PN Krs was potently angiogenic in this model system. Although PN Krs failed to display many of the phenotypic properties of established psoriatic cells, their CM nevertheless induced angiogenic responses as potent as established PP Krs. In this regard, the angiogenic activity displayed by PN Krs parallels the behavior of preneoplastic cells where we and others have shown that expression of the angiogenic phenotype frequently precedes the development of other traits that contribute to the neoplastic phenotype.[29–31]

Of particular interest were the results that showed a marked reduction in the level of TSP-1 and overproduction of IL-8 in diseased Krs. Pure TSP-1, CM from NN Krs that contain TSP-1, and neutralizing antibody to IL-8 were all able to block or reduce the angiogenic activity present in PP Kr CM. In view of the recently described role for these two mediators in the control of angiogenesis, we then asked whether PP Krs in addition to overproducing IL-8, might also exhibit a deficiency in the production of TSP-1.[13,14] When the levels of two matrix glycoproteins, FN and TSP-1, in the CM of NN and PP Kr CM were examined, it was found that whereas levels of FN in both types of cells were indistinguishable, TSP-1 levels were markedly reduced in PP Krs. This suggested that a deficiency in TSP-1 production in combination with the overexpression of the angiogenic mediator IL-8 contributes to the aberrant neovascularization that accompanies this disorder.

The properties of TSP-1, a matrix glycoprotein, have been well described. TSP-1 is a 450-kd trimeric, multifunctional molecule of mosaic composition that is present in great abundance in the platelet α granules and is secreted by a wide variety of epithelial and mesenchymal cells.[32–35] TSP-1 was first identified as an inhibitor of angiogenesis when an anti-angiogenic

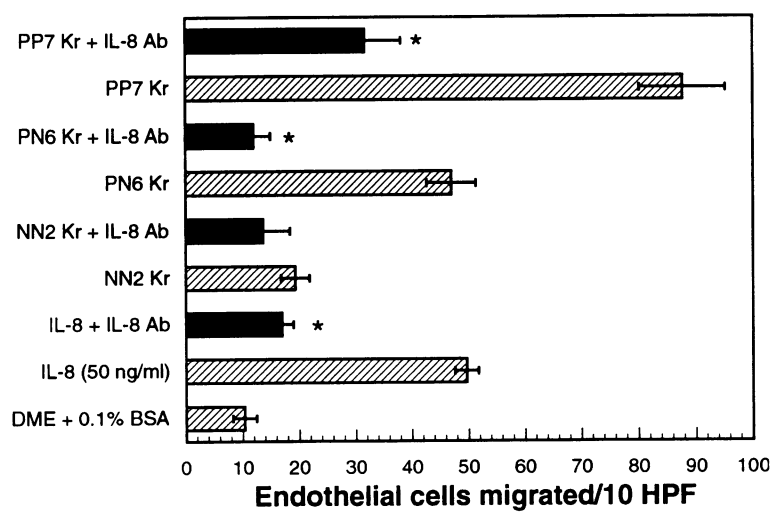

Figure 4. *IL-8 antibody (solid bars) blocks endothelial migration stimulating activity of PP and PN CM (hatched bars). One μg of CM or 50 ng/ml of human recombinant IL-8 (positive control) was mixed with a monoclonal antibody to IL-8 at a 1:500 dilution and incubated at 37 C for 2 hours. Samples were then passed through a protein A column as described in Materials and Methods, and media was assayed for chemotactic activity. Mean ± SD of three independent experiments, *P ≤ .001 by Students t-test.*

hamster protein, whose secretion was controlled by a tumor suppressor gene, was found to have an amino acid sequence similar to human platelet TSP-1.[17] A role for TSP-1 in the inhibition of angiogenesis is supported by several observations. It is present adjacent to mature quiescent vessels and is absent from actively growing sprouts both *in vivo* and *in vitro*.[27,36] Hemangiomas that consist of rapidly proliferating endothelial cells fail to make detectable TSP-1.[37] Antibodies to TSP-1 added to endothelial cell cultures decrease the formation of endothelial sprouts *in vitro*,[27] and endothelial cells in which TSP-1 production has been blocked following the introduction of anti-sense TSP-1 exhibit an accelerated rate of growth, enhanced chemotactic activity, and an increase in the number of capillary-like cords (DiPietro et al, submitted for publication). We have also observed a correlation between the level of TSP-1 production by monocytes during wound repair and the timely down-regulation of wound neovascularization (LA DiPietro and PJ Polverini, unpublished observations). More recently, we have reported that the angiogenic inhibitory activity of TSP-1 is restricted to two domains of the central stock region of the molecule: the procollagen homology region and the properdin-like repeats. The mechanism(s) underlying anti-angiogenic effect of TSP-1 is not well understood. It has been proposed that the anti-angiogenic domains of TSP-1 may bind to receptors on endothelial cells and interfere with positive signals generated by angiogenic factors.[38]

In comparison, less is known about the mechanism underlying the angiogenic activity of IL-8. Originally identified as a potent chemoattractant for neutrophils and lymphocytes,[39–41] it is now known to have a wide spectrum of functions in the inflammatory response. More recently it has been shown to be a mediator of neovascularization in the rabbit cornea and is present in abundance in rheumatoid synovial fluids and in the CM of rheumatoid synovial tissue macrophages.[19,20]

The pathogenesis of psoriasis is multifaceted and includes among others the feature of inappropriate angiogenesis. It is now well-established that angiogenesis is a tightly regulated process that is under complex positive and negative control. It is also apparent that a feature common to most chronic inflammatory diseases such as psoriasis is uncontrolled angiogenesis. Although the complement of positive and negative regulators of angiogenesis may vary among different physiological and pathological settings, the recognition of this dual mechanism of control is necessary if we are to gain a more thorough understanding of this complex process and its significance in disease. The recent elucidation of the cooperative interaction among positive and negative regulatory molecules during normal physiological angiogenesis, and the apparent disruption of this program in disorders such as rheumatoid arthritis and psoriasis, suggest that future studies of pathological angiogenesis must focus on the interaction of both positive and negative regulators of this process.

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