Mediation of Systemic Vascular Hyperpermeability in Severe Psoriasis by Circulating Vascular Endothelial Growth Factor

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Background: Severe forms of psoriasis can be complicated by systemic microvascular hyperpermeability. Vascular endothelial growth factor (VEGF) possesses potent vascular permeability activity. We suggest that VEGF enters the systemic circulation and acts on microvessels to mediate hyperpermeability.

Objectives: To quantify renal microvascular permeability and circulating VEGF concentration in severe psoriasis, and to investigate the relationship between plasma VEGF concentration and skin and joint involvement.

Design: Inception cohort studies of patients with generalized pustular psoriasis and plaque psoriasis.


Patients: Twenty-two patients (15 men and 7 women) with moderate and severe psoriasis were recruited (age range, 29-77 years; mean age, 47 years); 5 had generalized pustular psoriasis, 2 had erythrodermic psoriasis, and 15 had moderate-severe plaque psoriasis. An age- and sex-matched control group of 17 individuals (10 men and 7 women) was recruited (age range, 29-69 years; mean age, 42 years).

Results: There was pathological proteinuria in patients with relapsing generalized pustular psoriasis, (4-fold increase in urinary protein excretion rate in relapse compared with remission). In patients with moderate and severe psoriasis, mean plasma VEGF concentration during relapse was approximately 2.5 times greater than during remission (mean VEGFremission=103 pg/mL; mean VEGFrelapse=257 pg/mL; P<.01). There was a correlation between extent of skin involvement and plasma VEGF level (mean VEGFsevere psoriasis=365 pg/mL; mean VEGFmoderate psoriasis=149 pg/mL; P=.03). There was a correlation between presence of psoriatic arthritis and plasma VEGF level (mean relapse VEGFmonarthritis=277 pg/mL; mean relapse VEGFnonarthritis=103.5 pg/mL; P=.03).

Conclusions: Generalized pustular psoriasis is accompanied by pathological proteinuria and elevated plasma VEGF levels. Plasma VEGF concentration is significantly elevated in patients with extensive skin and joint involvement and may act on renal microvasculature to induce hyperpermeability.

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PATIENTS, MATERIALS, AND METHODS

PATIENTS

Patients participated in this study after regional and hospital ethics committee approval had been obtained. Of the 22 patients (15 men and 7 women; age range, 29-77 years; mean age, 47 years) with active psoriasis in this study, 5 had GPP, 2 had erythrodermic psoriasis, and 15 had moderate-severe plaque psoriasis (Table 1). Psoriasis Area and Severity Index (PASI) scoring was used to assess disease activity in patients with plaque psoriasis and in those with GPP in remission. For purposes of comparison, severe psoriasis was defined as GPP or plaque disease with a PASI score greater than 30, whereas moderate psoriasis was defined as plaque disease with a PASI score less than 30. Ten of 22 patients had active psoriatic arthritis at the time of relapse of their skin disease. Patients were classified as having active arthritis if they had morning stiffness for more than 45 minutes, 5 swollen joints, and 5 tender joints. Venous blood samples were taken from all 22 patients for VEGF analysis during relapse and remission. Blood samples were taken from premenopausal women at times outside menstruation. Urine specimens were taken from patients with GPP for protein analysis during relapse and remission. Relapse was defined as a flare of disease activity characterized by a PASI score increase greater than 70%, and remission was defined as a nadir of disease activity after treatment characterized by a PASI score decrease greater than 70%.

A control group of 17 individuals (10 men and 7 women; age range, 29-69 years; mean age, 42 years) was recruited. Venous blood samples were obtained from each control for VEGF analysis. Again, in premenopausal women, blood samples were obtained at times outside menstruation. A separate group of 8 patients (5 men and 3 women; age range, 24-69 years; mean age, 42 years) with active psoriatic arthritis was enrolled, psoriatic arthritis being defined according to the criteria of Moll and Wright. All patients had monoarthritis or oligoarthritis with involvement of at least 1 knee joint. Synovial fluid samples for VEGF analysis were obtained from knee joints displaying clinical signs of active synovitis (Table 2).

For RNA experiments, two 6-mm punch biopsy samples were taken from active plaques in 4 patients. Treatment was limited to emollients alone in the 2 weeks preceding biopsy. Normal skin tissue for RNA extraction was obtained from operative mammoplasty procedures (n = 3).

URINARY PROTEIN EXCRETION

Urinary protein excretion rates (UPERs) in 5 patients with GPP were quantified in relapse and remission. Timed 24-hour samples were collected, and protein was assayed using a dye-binding colorimetric method (Biotrol Urine Proteins; Diagnostics Merck-Biotrol, Nogent-sur-Marne, France). The assay uses a molybdate–pyrogallol red complex that reacts with protein in acidic solution to form a blue-purple complex that absorbs at 600 nm. The color intensity measured at 600 nm is directly proportional to the protein concentration in the sample.

VEGF ENZYME-LINKED IMMUNOSORBENT ASSAY

Venous blood samples were immediately anticoagulated with sodium heparin, 10 U/mL, in sterile, endotoxin-free tubes and centrifuged at 400g for 10 minutes, supernatant removed, and stored at −70°C until required. Synovial fluid from knee effusions was drained using a sterile technique, and samples were separated, as indicated for venous blood samples, into a cellular and supernatant fraction. The 100-µL samples of plasma and synovial fluid were immunosayed in duplicate for human VEGF using a commercially available quantitative enzyme-linked immunosorbent assay kit that measures VEGF165 (Quantikine; R & D Systems, Oxford, England). Although all 4 VEGF species have biological activity, VEGF165 is soluble compared with VEGF121 and VEGF189, which remain cell associated and, therefore, of relevance in this study. The Quantikine kit uses a quantitative sandwich enzyme immunoassay method and has a minimum level of detection of 9 pg/mL.

RNA PREPARATION AND RIBOPROBE CONSTRUCTION

Psoriatic and normal skin specimens were homogenized using a manual microhomogenizer. The RNA was prepared using a method adapted from Chomczynski and Sacchi. A VEGF riboprobe was designed to protect the full length of the smallest isoform (VEGF121, yielding a 471-base band, with a lower band of 427 bases representing the remaining isoforms). This 520-base probe was generated by linearizing the full-length complementary DNA for VEGF121 (including 26 bp of 3′ untranslated sequence) cloned into pBluescript SK with EcoRV and transcribed with T7 RNA polymerase.

RIBONUCLEASE PROTECTION ANALYSIS

A minimum of 100,000 cpm of each antisense riboprobe was hybridized overnight at 55°C to each sample with transfer RNA as a negative control. The RNase digestion of the unhybridized RNA fragments was achieved by adding RNase digestion buffer containing RNAs A and T1 to each sample. RNases were inactivated with 12.5 µL of a mixture containing 16% sodium dodecylsulphate solution with proteinase K, 4 µg/µL. After phenol extraction and ethanol precipitation, the samples were resuspended and loaded onto 3% polyacrylamide/urea sequencing gels followed by autoradiography. In each hybridization, an antisense transcript corresponding to human DNA topoisomerase transcibed from a construct was included as an internal control. Positive control messenger RNA from a breast carcinoma was loaded onto each gel. The resulting bands were quantitated densitometrically using a standard Gel Plotting macro and a software program (NIH Image 1.61; National Institutes of Health, Bethesda, Md). Vascular endothelial growth factor signals were normalized to the internal control (DNA topoisomerase).

vascular hyperpermeability in situations characterized by widespread capillary leak, such as the ovarian hyperstimulation syndrome. In patients with extensive, active psoriasis, systemic disturbance is not uncommon; fever, fluid imbalance, and thermoregulatory dysfunction are recognized complications. In chronic plaque psoriasis, micro-
albuminuria indicates subclinical renal microvascular hyperpermeability,\(^18\) whereas further studies\(^19\) have demonstrated that the extent of albuminuria reflects the degree of psoriatic skin involvement. Microalbuminuria in psoriasis may result from the activity of a circulating permeability factor produced by lesional tissue. Following our reported\(^20\) observation of elevated plasma VEGF in erythrodermic psoriasis, we hypothesize that in severe psoriasis, VEGF, elaborated by lesional psoriatic tissue, enters the systemic circulation and acts in an endocrine fashion on renal microvasculature to induce clinically significant hyperpermeability.

There are several reports\(^21,22\) of severe pulmonary edema occurring in patients with generalized pustular psoriasis (GPP), the edema accumulating as a direct consequence of increased pulmonary microvascular permeability. Hypoalbuminemia is a common complication of GPP, and this association may again reflect microvascular hyperpermeability with protein loss into the gastrointestinal or renal tracts. In the present study, renal microvascular permeability and circulating VEGF have been quantified in patients with GPP during relapse and remission. In a larger group of patients with moderate and severe psoriasis, correlation has been sought between the extent of skin involvement and plasma VEGF concentration and between the presence of psoriatic arthritis and plasma VEGF concentration. Lesional skin and joint fluid has been assayed to identify the source of circulating VEGF in psoriasis.

**RESULTS**

**URINARY PROTEIN EXCRETION RATES**

All 5 patients with GPP demonstrated pathological UPERs during relapse (range, 0.15-1.59 g/24 h; mean, 0.55 g/24 h; reference value, <0.15 g/24 h). During remission, 4 of 5 UPER values returned to within reference values (range, 0.09-0.20 g/24 h; mean, 0.14 g/24 h; reference value, <0.15 g/24 h) (**Figure 1**). There was a mean 4-fold increase in UPER in relapse compared with remission.

**PLASMA VEGF ANALYSIS**

The 5 patients with GPP demonstrated mean plasma VEGF levels 2.6-fold greater in relapse compared with remission (**Figure 2**). In the larger group of 22 patients (5 with GPP, 2 with erythrodermic psoriasis, and 15 with moderate-severe plaque psoriasis), mean plasma VEGF concentration was approximately 2.5 times greater than VEGF\(_{\text{remission}}\) (mean±SEM VEGF\(_{\text{relapse}}\) = 257±49 pg/mL and VEGF\(_{\text{remission}}\) = 103±6.7 pg/mL; \(P<.01\), 2-sample t test) (**Figure 3**). Plasma VEGF concentration in an age- and sex-matched control group (n = 17) was significantly lower than VEGF\(_{\text{relapse}}\) and VEGF\(_{\text{remission}}\) (mean±SEM

### Table 1. Plasma VEGF Concentrations and PASI Scores in 22 Patients With Psoriasis During Relapse and Remission

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Psoriasis Type</th>
<th>Arthritis</th>
<th>PASI Score</th>
<th>VEGF, pg/mL</th>
<th>PASI Score</th>
<th>VEGF, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 GPP</td>
<td>–</td>
<td>.</td>
<td>1003.5</td>
<td>14.4</td>
<td>545.3</td>
<td></td>
</tr>
<tr>
<td>2 GPP</td>
<td>+</td>
<td>.</td>
<td>472.7</td>
<td>10.0</td>
<td>132.2</td>
<td></td>
</tr>
<tr>
<td>3 GPP</td>
<td>–</td>
<td>.</td>
<td>525.5</td>
<td>11.2</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>4 GPP</td>
<td>+</td>
<td>.</td>
<td>391.9</td>
<td>3.3</td>
<td>174.5</td>
<td></td>
</tr>
<tr>
<td>5 GPP</td>
<td>+</td>
<td>.</td>
<td>194</td>
<td>8.2</td>
<td>98.5</td>
<td></td>
</tr>
<tr>
<td>6 EdP</td>
<td>–</td>
<td>.</td>
<td>42.0</td>
<td>121.1</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>7 EdP</td>
<td>+</td>
<td>.</td>
<td>31.8</td>
<td>96.8</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>8 PIP</td>
<td>+</td>
<td>.</td>
<td>42.2</td>
<td>47.0</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>9 PIP</td>
<td>+</td>
<td>.</td>
<td>42.0</td>
<td>215</td>
<td>4.3</td>
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</tr>
<tr>
<td>10 PIP</td>
<td>+</td>
<td>.</td>
<td>35.3</td>
<td>308.7</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>11 PIP</td>
<td>–</td>
<td>.</td>
<td>33.0</td>
<td>211.4</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>12 PIP</td>
<td>–</td>
<td>.</td>
<td>28.7</td>
<td>45</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>13 PIP</td>
<td>+</td>
<td>.</td>
<td>27.8</td>
<td>536</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>14 PIP</td>
<td>+</td>
<td>.</td>
<td>27.0</td>
<td>235.1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>15 PIP</td>
<td>–</td>
<td>.</td>
<td>25.1</td>
<td>92.6</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>16 PIP</td>
<td>–</td>
<td>.</td>
<td>20.4</td>
<td>86.1</td>
<td>8.3</td>
<td></td>
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<tr>
<td>17 PIP</td>
<td>+</td>
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<td>18.9</td>
<td>159.6</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>18 PIP</td>
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<td>.</td>
<td>17.8</td>
<td>67.6</td>
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<td></td>
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<tr>
<td>19 PIP</td>
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<td>.</td>
<td>17.6</td>
<td>206.8</td>
<td>1.9</td>
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<tr>
<td>20 PIP</td>
<td>–</td>
<td>.</td>
<td>16.7</td>
<td>73.1</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>21 PIP</td>
<td>–</td>
<td>.</td>
<td>14.0</td>
<td>53.2</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>22 PIP</td>
<td>–</td>
<td>.</td>
<td>12.6</td>
<td>78.1</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

*VEGF indicates vascular endothelial growth factor; PASI, Psoriasis Area and Severity Index; GPP, generalized pustular psoriasis; –, no arthritis; ellipses, not applicable; +, active arthritis present; EdP, erythrodermic psoriasis; and PIP, plaque psoriasis.

### Table 2. Clinical Details and Synovial VEGF Concentrations in 8 Patients With Psoriatic Arthritis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Arthritis</th>
<th>Synovial VEGF, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Oligoarthritis</td>
<td></td>
<td>2528.6</td>
</tr>
<tr>
<td>2 Oligoarthritis</td>
<td></td>
<td>2411.1</td>
</tr>
<tr>
<td>3 Monoarthritis</td>
<td></td>
<td>3284.5</td>
</tr>
<tr>
<td>4 Oligoarthritis</td>
<td></td>
<td>463.8</td>
</tr>
<tr>
<td>5 Oligoarthritis</td>
<td></td>
<td>1258.6</td>
</tr>
<tr>
<td>6 Oligoarthritis</td>
<td></td>
<td>1225.0</td>
</tr>
<tr>
<td>7 Oligoarthritis</td>
<td></td>
<td>2614.4</td>
</tr>
<tr>
<td>8 Oligoarthritis</td>
<td></td>
<td>1990.2</td>
</tr>
</tbody>
</table>

*VEGF indicates vascular endothelial growth factor.
VEGFremission=103±6.7 pg/mL, VEGFcontrol=24.7±6.7 pg/mL; P < .001, 2-sample t test) (Figure 3).

Comparison of plasma VEGF levels in patients with severe psoriasis (GPP+PASI score >30) (n=11) vs those with moderate psoriasis (PASI score <30) (n=11) demonstrated significantly higher VEGF levels in the severe group (mean±SEM VEGFsevere =365±78 pg/mL, VEGFmoderate =149±43 pg/mL; P = .03, 2-sample t test) (Figure 4).

A relationship was demonstrated between circulating VEGF levels and the presence of active psoriatic arthritis (mean±SEM relapse VEGFarthritis =277±53 pg/mL [n=10], mean relapse VEGFnonarthritis =103.5±19.0 pg/mL [n=12]; P = .03, 2-sample t test) (Figure 5).

PSORIATIC SYNOVIAL FLUID VEGF ANALYSIS

In another group of 8 patients with active psoriatic arthritis, synovial fluid VEGF was assayed using enzyme-linked immunosorbent assay (Table 2). The VEGF enzyme-linked immunosorbent assay has a minimum level of detection for VEGF165 of 9 pg/mL. High synovial VEGF concentrations (mean±SEM, 1972±221.1 pg/mL) were identified in each case.

RIBONUCLEASE PROTECTION ANALYSIS

Ribonuclease protection assays for VEGF are shown in Figure 6. Short (48-hour) exposure demonstrated a strong signal for VEGF in all 4 psoriasis samples. In normal skin (n=3) at the same exposure, VEGF signals are of low intensity. To obtain values of fold-change in messenger RNA levels, messenger RNA abundance was quantitated from autoradiographic data by scanning laser densitometry. Signals from the VEGF messenger RNA were normalized to those of the internal topoisomerase control. Quantification by this method showed an approximate 4-fold increase in VEGF signal in lesional vs normal skin.

Our findings demonstrate that GPP is accompanied by pathological proteinuria and markedly elevated concentrations of plasma VEGF. During remission, urinary protein excretion normalizes and circulating VEGF levels return to control values. In a larger group of patients (including those with GPP, erythrodermic psoriasis, and plaque psoriasis), plasma VEGF concentration is consistently higher in relapse than in remission and is significantly elevated in patients with extensive skin involvement and active joint disease. We suggest that VEGF, synthesized in psoriatic skin and synovium, enters the systemic circulation and may act on renal microvasculature to induce hyperpermeability with consequent proteinuria.

Renal microvascular hyperpermeability permits the escape of larger protein molecules and those of smaller mo-
molecular weight (eg, albumin), which pass into the glomerular filtrate and are clinically measurable as proteinuria. Microalbuminuria, defined as mildly elevated levels of proteinuria (30-200 mg/L), has been reported in patients with mild-moderate psoriasis by Cecchi et al.19 The mean UPER in their cohort of patients with a PASI score greater than 11 was 28.8 mg/24 h, whereas the mean relapse UPER in our GPP group was 560 mg/24 h, which reduced to 140 mg/24 h during remission (reference value, <150 mg/24 h). The results of Cecchi and colleagues and our own data suggest the presence of renal microvascular hyperpermeability in psoriasis that increases with intensity of skin disease but reverses with successful treatment.

Within the papillary dermis of lesional, psoriatic skin, the superficial microvasculature is characterized by an angiogenic and hyperpermeable phenotype, features that contribute to the development and persistence of skin lesions in psoriasis. Microvascular hyperpermeability at any site can be mediated by several biologically active substances, including VEGF, which has permeability activity 40000 times greater than histamine on a molar basis.29 Detmar et al11 initially identified VEGF overproduction in psoriatic epidermis, and they subsequently demonstrated the central role of keratinocyte-derived VEGF in changes to underlying superficial dermal microvasculature. In addition to acting locally to induce angiogenesis and microvascular hyperpermeability, circulating VEGF has been implicated in systemic capillary permeability associated with conditions such as ovarian hyperstimulation syndrome and tumor ascites.17,31 Other studies have demonstrated up-regulation of VEGF in pathological conditions characterized by proteinuria and increased renal microvascular permeability.32

Bhushan et al11 reported an association among VEGF concentration in lesional skin, extent of psoriatic skin involvement (PASI), and VEGF concentration in peripheral blood. In our experiments, the finding that patients with severe psoriasis (GPP+PASI score >30) had significantly higher levels of plasma VEGF compared with patients with moderate psoriasis (PASI score <30) again suggests that circulating levels of VEGF reflect the extent of psoriatic skin involvement. Further evaluation revealed that patients with active psoriatic arthritis had significantly higher levels of circulating VEGF than those without arthropathy, whereas separate experiments demonstrated high concentrations of VEGF in the articular fluid of involved psoriatic joints. High synovial VEGF concentrations in psoriatic synovial fluid were initially reported by Fearn et al,31 and our results are consistent with their data. These findings indicate that an articular source may contribute, along with the cutaneous source, to circulating VEGF concentration in patients with active psoriasis.

We hypothesize that there may be a causal relationship between renal microvascular hyperpermeability in patients with severe psoriasis and high circulating VEGF levels. Reports of pulmonary edema in GPP secondary to the capillary leak syndrome suggest the involvement of pulmonary microvascular hyperpermeability, which may be mediated by a circulating vasoactive cytokine, such as VEGF.18,17 Although the renal and pulmonary vasculature can respond...
to circulating permeability signals in severe psoriasis, other microvascular beds seem to be resistant to systemic hyperpermeability factors. Organ-dependent variations in response to VEGF may be explained by a lack of accessibility of bioactive VEGF in certain sites or because of qualitative or quantitative differences in VEGF receptors.

Plasma VEGF analysis in patients with severe psoriasis may be a useful predictor of clinical outcome and affect management. In addition, VEGF and VEGF-mediated pathways may represent potential targets in the development of future therapeutic strategies in psoriasis.

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