Psoriasis is a chronic inflammatory skin disease in which epidermal proliferation is closely associated with excessive microvascular expansion within the papillary dermis. Angiopoietins have recently been identified as the major ligands of the endothelial-specific receptor Tie2. Angiopoietin 1 induces Tie2 signaling as a receptor activator and maintains blood vessel formation, whereas angiopoietin 2 destabilizes vessels by blocking Tie2 signaling as an antagonist of angiopoietin 1 and acts with vascular endothelial growth factor to initiate angiogenesis. In this study we examined the potential role of angiopoietins and the Tie2 receptor in vascular changes of psoriasis. Angiopoietin 1, angiopoietin 2, and Tie2 were up-regulated in involved psoriasis skin compared to uninvolved psoriasis skin, healthy skin, and chronic spongiotic dermatitis skin. Angiopoietin 1 was expressed by stromal cells in the highly vascularized papillary dermis of involved psoriasis skin. Angiopoietin 2 was expressed by endothelial cells in the vicinity of the proliferating epidermis that abundantly expressed vascular endothelial growth factor. Vascular endothelial growth factor and basic fibroblast growth factor, which were overexpressed in involved psoriasis skin, enhanced angiopoietin 2 and Tie2 expression in dermal microvascular endothelial cell cultures. Thus, our findings suggest that upregulation of angiopoietin 1, angiopoietin 2, and Tie2 is closely associated with the development of microvascular proliferation in psoriasis, and that the angiopoietin–Tie2 system may act coordinately with vascular endothelial growth factor and basic fibroblast growth factor to promote neovascularization in psoriasis. Moreover, successful antipsoriatic treatment was accompanied by noticeable reduction of angiopoietin 2 expression, suggesting that alteration of angiopoietin 2 expression may be particularly important in controlling vascular proliferation in the treatment of psoriasis. Key words: angiogenesis/basic fibroblast growth factor/vascular endothelial growth factor. J Invest Dermatol 116:713–720, 2001

Psoriasis is a common, chronic skin disease characterized by recurrent erythematous skin plaques that exhibit epidermal hyperplasia, inflammatory cell accumulation, and abnormalities of the papillary dermal vasculature (Weinstein et al., 1985; Mordovtsev and Albanova, 1989; Baker and Fry, 1992). Microvessels in the papillary dermis of psoriatic plaques are tortuous and dilated (Ragaz and Ackerman, 1979), show increased endothelial surface areas (Macdonald et al., 1989), and exhibit endothelial cell proliferation (Braverman and Sibley, 1982; Morganroth et al., 1991). In vivo angiogenesis assays have demonstrated the ability of the lesional epidermis to induce neovascularization (Malhotra et al., 1989). Furthermore, overexpression of strong angiogenic inducers, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), has also been demonstrated in psoriasis (Yaguchi et al., 1993; Detmar et al., 1994).

Recently, angiopoietin 1 (Ang1) and angiopoietin 2 (Ang2) were identified as ligands of the Tie2 (Tek) receptor, a novel endothelium-specific receptor tyrosine kinase (Sato et al., 1995; Davis et al., 1996; Suri et al., 1996; Maisonnier et al., 1997). Ang1 induces Tie2 autophosphorylation as a receptor activator, whereas Ang2 blocks Tie2 signaling as a naturally occurring antagonist of Ang1.

Ang1–Tie2 signaling is essential for correct vascular formation. Mouse embryos lacking either Ang1 or Tie2 show the absence of capillary sprouts and abnormal vascular networks, leading to embryo lethality (Dumont et al., 1994; Sato et al., 1995; Suri et al., 1996). Ultrastructural analyses in Ang1 knockout mice demonstrated the loss of adhesion between endothelial cells and associated pericytes (Suri et al., 1996). This suggests that Ang1–Tie2 signaling helps maintain and stabilize newly formed vessels by regulating the interaction between endothelial cells and surrounding support cells, such as smooth muscle cells and pericytes (Suri et al., 1996). Furthermore, Ang1 may act directly on endothelial cells as an effective apoptosis survival factor (Kwak et al., 1999). In addition, Ang1 has a crucial function in vascular expansion as Ang1 overexpression in mouse skin results in increased vascularity (Suri et al., 1998). In vitro experiments demonstrated that Ang1 induced only weak proliferation in endothelial cells, but strongly supported sprouting (Davis et al., 1996; Koblizech et al., 1998) and chemotactic responses (Witzenbichler et al., 1998).
In contrast, Ang2 promotes vessel destabilization by blocking the Ang1–Tie2 receptor interaction. Transgenic overexpression of Ang2 leads to a lethal phenotype reminiscent of embryos lacking either Ang1 or Tie2, with severe disruption of vascular development (Maisonpierre et al., 1997). During embryogenesis, Ang2 has been demonstrated primarily at sites of vascular remodeling. Ang2 is expressed together with VEGF at the invading front of vessel sprouting, or in the absence of VEGF at sites of vessel regression (Maisonpierre et al., 1997). These expression patterns suggest that vessel destabilization by Ang2 in the absence of VEGF leads to frank vascular regression, whereas such destabilization in the presence of angiogenic inducers such as VEGF strongly facilitates angiogenic responses. Recently, it has been shown that the sequential and cooperative expression of Ang2 and VEGF is essential in tumor angiogenesis (Stratmann et al., 1998; Holash et al., 1999). Thus, these findings indicate that Ang2 is a crucial factor in the initiation of angiogenesis and that Ang2 and VEGF have distinct yet complementary roles in vascular development.

To elucidate the potential role of the angiopoietin/Tie2 system in vascular changes of psoriasis, we examined the expression and localization of Ang1, Ang2, and Tie2 in psoriasis skin and studied the cytokine-mediated regulation of these molecules in cell cultures.

MATERIALS AND METHODS

Skin specimens Six millimeter punch biopsies from clinically uninvolved and involved skin of 14 different patients with untreated, chronic plaque-type psoriasis were obtained according to a protocol approved by the Institute Review Board of Mount Sinai School of Medicine. Patients ranged in age from 36 to 54 y. Skin biopsies were divided in two and used for histopathologic examination and reverse transcriptase polymerase chain reaction (RT-PCR) analysis. Expression during antipsoriatic therapy was analyzed for seven patients, of whom five received psoralen plus ultraviolet A light (PUVA) treatment and two received topical tazarotene (a retinoid acid receptor β/γ selective retinoid) treatment as described previously (Hecker et al., 1999).

Normal adult skins obtained from reconstructive surgery were used as healthy controls. In addition, punch biopsies from four untreated patients with chronic spongiotic dermatitis were included in this study.

Cell cultures Human microvascular endothelial cells of dermal origin (HMVEC) were isolated from clotted normal adult skins obtained from reconstructive surgery. Cells were grown in endothelial cell basal medium (EBM, Clonetics) containing human epidermal growth factor (10 ng per ml), hydrocortisone (1 μg per ml), 5% fetal bovine serum (FBS), and bovine brain extract (12 μg per ml). HMVECs were used between passages 4 and 6. Human dermal fibroblasts were derived by explant outgrowth from normal adult skins as described previously (Kuroda et al., 1999) and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 2 mM glutamine and 10% FBS. Fibroblasts at four to seven passages were used for experiments. Human recombinant VEGF were purchased from R&D systems (Minneapolis, MN). Human recombinant bFGF, platelet-derived growth factor (PDGF), and transforming growth factor β1 (TGF-β1) were from Roche Molecular Biochemicals (Indianapolis, IN).

RNA preparation and cDNA synthesis Total RNA from skin specimens and cell cultures was isolated using the RNeasy kit (Quiagen, Chatsworth, CA). After desoxynucleosine “I” treatment, Life Technologies, Grand Island, NY), total RNA was reverse transcribed into cDNA for PCR amplification using oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase (Life Technologies).

Quantitative RT-PCR Competitive RT-PCR was performed by mixing a constant amount of the target cDNA with varying copy numbers of competitive DNA fragments. Target cDNA was amplified with known amounts of competitor in 30 μl reaction mixtures containing 1 × PCR buffer, 200 nM dNTP, 1.5 mM MgCl2, 500 nM primers, and 1.25 U Platinum Taq DNA polymerase (Life Technologies). Cycling parameters were as follows: denaturation, 94°C for 30 s; annealing, 60°C (for VEGF and bFGF) or 65°C (for all other molecules) for 1 min; extension, 72°C for 1 min, with 30–40 cycles. Competitive DNA fragments were designed to contain the same primer binding sequences as the target cDNA for each primer set and were constructed using the competitive DNA construction kit (Takara Biomedicals, Kusatsu, Japan) according to the manufacturer’s instructions.

As the ratios of target to competitor PCR product amplifications were proven constant from 20 to 40 cycles in preliminary experiments, target cDNAs and competitive fragments were confirmed to be amplified with equal efficacy. The following primers were used for DNA amplification: Ang1, 5′-GGGGGAGGTTGGACTGTAAT-3′ and 5′-TTGGCACATACA-3′; Ang2, 5′-GGGAGGGTCTCTGAGCTCATGTA-3′ (362 bp PCR product); Ang2, 5′-GGATCCTGGGAGAGGAAC-3′ and 5′-CTCTGACCCAGCTATCGTA-3′ (535 bp); Tie2, 5′-ATCTCATTTGCAAGAATGCTGTCGCC-3′ and 5′-TGTGAAGGCTCTCAGGCTCAGATTG-3′ (512 bp); VEGF, 5′-TCCAGGAGTACCTCTGAGAC-3′ and 5′-TCCACATTGATGCTCAGTG-3′ (203 bp); bFGF, 5′-CCAGCTGACTGCCAA-3′ and 5′-TCTTCTGGCCATGGAATTG-3′ (270 bp); platelet-endothelial cell adhesion molecule 1 (PECAM), 5′-CTGAGGTTGAAGGTGATGAG-3′ and 5′-AGATTATTGCTTCTGGGACAC-3′ (296 bp); β-actin, 5′-GTATGGTGGCATGTAG-3′ and 5′-TATGTCACCGCAGTTC-3′ (510 bp). All primer sets were tested to ensure that genomic DNA was not amplified under the conditions used. PCR products were electrophoresed in 3% agarose gel, visualized by ethidium bromide staining, and photographed with Polaroid 665 film. Band intensities were then quantified by densitometry (NIH Image 1.55 Program, National Institutes of Health, Division of Computer Research and Technology, Bethesda, MD). To determine the number of competitor template molecules equal to the starting number of the target DNA molecules, the ratios of the band intensities of target and respective serially diluted competitor PCR products were plotted against the starting number of competitor molecules. The copy numbers of target cDNA were then calculated from the graph. The results are presented as the number of transcripts per μg total RNA and are normalized with respective β-actin amplifications.

Immunohistochemical staining Seven micron frozen sections were fixed in 4% paraformaldehyde for 10 min and incubated with primary antibody overnight at 4°C. The following antibodies were used for detection: anti-Ang1 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Ang2 (Santa Cruz Biotechnology), anti-Tie2 (Santa Cruz Biotechnology), anti-PECAM (Chemicon, Temecula, CA), anti-type IV collagen (d1 chain (a generous gift from Dr. Y. Sado, Division of Immunology, Shigeri Medical Research Institute), anti-α-smooth muscle actin (αSMA) (Sigma, Saint Louis, MO), and anti-VEGF (Santa Cruz Biotechnology). Biotinylated secondary antibodies and streptavidin-peroxidase or -alkaline phosphatase conjugates (Vector Laboratories, Burlingame, CA) were used to detect primary antibodies. Reactions were developed using diaminobenzidine (DAB) or Fast Red (Zymed Laboratories, South San Francisco, CA) as chromogenic substrates, and some sections were counterstained with hematoxylin. Controls included omission of the primary and/or secondary antibodies and preincubation with Ang1, Ang2, and Tie2 antibodies with respective immunizing peptides (Santa Cruz Biotechnology).

In situ hybridization For the preparation of Ang1 and Ang2 RNA probes, T7 RNA polymerase promoters were incorporated into the antisense and sense primers. RT-PCR was performed using one primer with T7 RNA polymerase promoter and the unmodified primer of the pair (Birk and Grimm, 1994). Labeled probes were synthesized by in vitro transcription with T7 polymerase in the presence of digoxigenin labeled dUTP (Roche) using the PCR products as a template. Paraformaldehyde-fixed, paraffin-embedded specimens were used for in situ hybridization. Sample pretreatment and hybridization was performed as described previously (Kuroda et al., 1998) with some modifications. Briefly, the deparaffined sections were incubated with proteinase K, rinsed in 4% paraformaldehyde, incubated with glycine, and dipped in 0.2 N HCl. After prehybridization, sections were hybridized with antisense or sense RNA probes in a solution containing 50% formamide, 10% dextran sulfate, 1 × Denhardt’s solution, 200 μg per ml yeast tRNA, 600 mM NaCl, 1 mM ethylenediamine tetracetic acid, 0.25% sodium dodecyl sulfate (SDS), and 10 mM Tris-HCl (pH 7.6), and incubated for 18 h at 30°C. Sections were washed in 2 × sodium citrate/chloride buffer (SSC), treated with RNase A, and washed with 0.2 × SSC at 50°C. Immunologic detection was performed with antidigoxigenin antibody conjugated with alkaline phosphatase (Roche). 5-Bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) was used as chromogenic substrate.

Northern blot analysis An aliquot of total RNA (5 μg per lane) was size fractionated by 1% agarose gel electrophoresis and transferred to a nylon filter. Blots were prehybridized, hybridized, autoradiographed, and stripped for rehybridization as previously described (Kuroda and Shinkai, 1997). Ang2 and Tie2 human cDNA probes used for hybridization were prepared by RT-PCR as described above using total RNA isolated from...
human placenta (Clontech Laboratory, Palo Alto, CA) and were labeled radioactive by nick translation kit with $\alpha$-32P-dCTP (Amersham Pharmacia Biotech, Piscataway, NJ). As a control of constitutive expression, a 0.8 kb human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used. The intensities of specific mRNAs were quantified with densitometry.

**Immunoblotting analysis**

Protein extracts from cultured cells were prepared using lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1% Nonidet P-40). Equal amounts of the protein extracts were separated on SDS-polyacrylamide gels under reducing conditions. For cell culture media, proteins were precipitated by the addition of trichloroacetic acid, and samples corresponding to the same cell numbers were electrophoresed. Proteins were transferred to polyvinylidene fluoride membranes, detected by incubation with anti-Ang2 or Tie2 polyclonal antibodies (Santa Cruz Biotechnology), and visualized by the enhanced chemiluminescence detection system (Amersham Pharmacia). Relative protein levels were quantified by densitometry.

**Statistics**

The Mann–Whitney U test was used to compare gene expression in skin samples from psoriasis patients and healthy controls. Cytokine regulation in cell cultures was analyzed using the Student’s t test. Changes in gene expression before and during therapy were analyzed by the Wilcoxon single-rank test. p-values of less than 0.05 were considered as significant. All values were represented as mean ± SD.
mRNA levels of Ang1, Ang2, and Tie2 in involved psoriasis skin were higher than those observed in dermatitis.

We also examined PECAM mRNA expression as a marker of endothelial cell proliferation in psoriasis. As expected, PECAM transcripts were significantly increased in involved skin (625 ± 484) compared to uninvolved skin (157 ± 76, p < 0.001) and healthy skin (71 ± 38, p < 0.01). As endothelial cells can express most angiogenic regulators, we compensated Ang1 and Ang2 mRNA levels with PECAM mRNA levels (i.e., as a value per 100 PECAM transcripts) to exclude discrepancies in vascular density. These analyses confirmed that copy numbers of Ang1 transcripts were significantly higher in involved psoriasis skin (157 ± 122) than in uninvolved (46 ± 39, p < 0.001) and healthy (36 ± 30, p < 0.01) skin. Likewise, copy numbers of Ang2 transcripts were also higher in involved psoriasis skin (34 ± 28) than in uninvolved (4.4 ± 3.9, p < 0.001) and healthy (3.6 ± 3.1, p < 0.01) skin. Thus, increases in Ang1 and Ang2 expression in psoriasis were the result of specific upregulation rather than the consequence of increased vascular density.

To investigate the recently proposed hypothesis that angiopoietins can produce complementary effects on angiogenesis in the presence of other angiogenic molecules (Maisonpierre et al, 1997; Gale and Yancopoulos, 1999), we examined VEGF and bFGF expression in psoriasis. VEGF and bFGF mRNAs were present at significantly higher levels in involved psoriasis skin compared to uninvolved and healthy skin (Fig 4). Increased VEGF mRNA levels were particularly noticeable, with copy numbers of 12,581 ± 880 in involved psoriasis skin compared to 88 ± 45 in uninvolved skin and 85 ± 35 in healthy skin. This suggests that angiopoietins together with VEGF, bFGF, or both, are coordinately expressed in psoriasis.

Localization of Ang1, Ang2, and Tie2 expression in psoriatic skin.

To further evaluate the pathologic implications of increased Ang1, Ang2, and Tie2 expression in psoriasis, we examined the spatial distribution of these molecules in involved and uninvolved specimens from psoriasis patients and in healthy skin specimens using immunohistochemistry and in situ hybridization. Baseline membrane (type IV collagen) and PECAM staining clearly showed vascular proliferation in the papillary dermis of involved psoriasis skin compared to uninvolved and normal healthy skin (Fig 5A–D). Ang1 staining was undetectable in healthy and uninvolved psoriasis skin specimens but clearly detected in the papillary dermis of involved skin (Fig 5E–H). In involved psoriasis skin, Ang1 signals were found in stromal cells, such as mononuclear inflammatory cells, dermal dendritic cells, or fibroblasts. Consistent with protein expression results, Ang1 mRNA signals were also detected in the papillary dermis of involved psoriasis skin (Fig 5I–L). Neither Ang2 protein nor mRNA were observed in healthy and uninvolved psoriasis skin specimens but were clearly expressed in the papillary dermis of involved skin (Fig 5M–T). Ang2 signals were observed in endothelial cells. Tie2 was expressed by proliferating endothelial cells in the papillary dermis as well as by endothelial cells in the deep dermis in involved psoriasis skin (Fig 6A–C). Healthy and uninvolved psoriasis skin vessels also showed Tie2 staining. Preabsorption of primary antibodies with their respective immunizing peptides resulted in no specific staining patterns (data not shown).

As angiopoietins are believed to play a key role in the recruitment of pericytes, we examined the expression of αSMA to detect possible alterations of pericytes in psoriasis. Proliferating vessels in the papillary dermis of involved skin showed αSMA staining as well as larger vessels in the deep dermis in the same specimens (Fig 6D–G). Increased numbers of αSMA-positive vessels were found in the papillary dermis of involved skin compared to uninvolved and healthy skin, but no differences in staining intensities of each vessel were observed between skin samples. These findings suggest that pericytes are properly recruited to proliferating vessels in psoriasis.
Figure 5. Distribution of Ang1 and Ang2 expression in psoriasis. Type IV collagen immunostaining in involved psoriasis skin (A) and uninvolved skin (B) and PECAM immunostaining in involved psoriasis skin (C) and healthy skin (D) (DAB/hematoxylin). Involved psoriasis skin shows vascular proliferation in the papillary dermis. Immunohistochemistry for Ang1 in involved skin (E, F), uninvolved skin (G), and healthy skin (H) (Fast Red/hematoxylin). In situ hybridization for Ang1 antisense probe in involved skin (I, J) and uninvolved skin (K) and for Ang1 sense probe in involved skin (L) (BCIP/NBT; no counterstain). Ang1 protein and mRNA signals are observed on cells in the papillary dermis of involved skin but not in uninvolved skin or healthy skin. Immunohistochemistry for Ang2 in involved skin (M, N), uninvolved skin (O), and healthy skin (P) (Fast Red/hematoxylin). In situ hybridization for Ang2 antisense probe in involved skin (Q, R) and uninvolved skin (S) and for Ang2 sense probe in involved skin (T) (BCIP/NBT; no counterstain). Ang2 protein and mRNA signals are observed on endothelial cells (arrows) in the papillary dermis of involved skin but not in uninvolved skin or healthy skin. Sense probes for in situ hybridization show no specific staining patterns (L, T).

Figure 6. Immunohistochemistry of Tie2, αSMA, and VEGF. Tie2 staining in the papillary dermis (A) and the deep dermis (B) of involved psoriasis skin and healthy skin (C) (Fast Red/hematoxylin). Proliferating vessels in the papillary dermis of involved skin show positive staining for Tie2 as well as vessels in the deep dermis or in healthy skin. αSMA staining in the papillary dermis (D, E) and the deep dermis (F) of involved psoriasis skin and healthy skin (G) (DAB/hematoxylin). Vessels in the papillary dermis of involved skin show positive staining for αSMA as well as vessels in the deep dermis or in healthy skin. Arrowhead (F) shows an eccrine duct. VEGF staining in involved psoriasis skin (H), uninvolved skin (I), and healthy skin (J) (Fast Red; no counterstain). VEGF staining was observed in the proliferating epidermis of involved skin and in some mononuclear inflammatory cells infiltrating the upper dermis. VEGF signals were not detectable or only faintly detectable in uninvolved skin and healthy skin.
VEGF staining was abundantly observed in keratinocytes in proliferating epidermis in involved skin, but signals were not detectable or only faintly detectable in healthy skin or uninvolved skin samples (Fig 6H–J). This suggests that angiopoietins are expressed in the presence of high levels of VEGF.

Cytokine regulation of Ang1, Ang2, and Tie2 To identify molecules that may regulate Ang1 and Ang2 expression, we investigated the effects of various cytokines in HMVEC and dermal fibroblast cultures by quantitative RT–PCR. The cytokines were chosen according to their ability to affect cell growth and differentiation of endothelial cells or fibroblasts (Herrmann and Bohme, 1993; Bouck et al., 1996). As shown in Fig 7, VEGF and bFGF noticeably elevated Ang2 mRNA levels in HMVEC whereas PDGF, bFGF, and TGF-β showed no or minor inhibitory effects on Ang1 expression in fibroblast cultures. To more closely determine the effects of VEGF and bFGF on Ang2 expression, we performed northern blot and immunoblot analyses on HMVEC cultures (Fig 8). VEGF and bFGF enhanced Ang2 mRNA levels up to 6.2 ± 1.2-fold (n = 4, p < 0.01) and 8.1 ± 1.1-fold (n = 4, p < 0.01), respectively. Both cytokines used in combination showed an 11.4 ± 2.8-fold increase (n = 4, p < 0.01). At the protein level, VEGF upregulated Ang2 expression by 4.2 ± 0.7-fold (n = 4, p < 0.05), bFGF by 4.6 ± 1.0-fold (n = 4, p < 0.05), and VGEF and bFGF by 7.2 ± 1.8-fold (n = 4, p < 0.05).

To evaluate the cytokine regulation of the Tie2 receptor, we examined the effects of VEGF and bFGF in HMVEC by northern blot and immunoblot analyses (Fig 9). VEGF and bFGF increased Tie2 mRNA levels up to 3.0 ± 0.5-fold (n = 4, p < 0.01) and 3.2 ± 0.7-fold (n = 4, p < 0.01), respectively. Tie2 protein expression was also increased 2.8 ± 0.4-fold by VEGF (n = 4, p < 0.05) and 3.0 ± 0.8-fold by bFGF (n = 4, p < 0.05).

Ang1, Ang2, and Tie2 expression in psoriatic skin during antipsoriatic treatment To determine whether an improvement in skin lesions was correlated with changes in angiopoietins and Tie2 expression, we examined mRNA levels of these molecules in serial biopsies from psoriatic patients who had undergone successful antipsoriatic therapy. Skin biopsies were taken from skin lesions of five psoriatic patients treated with PUVA and two patients treated with topical tazarotene. All patients showed marked clinical improvement during antipsoriatic therapy, reaching minimal residual activity after 4–8 wk of treatment. After 8 wk of therapy, Ang1, Ang2, Tie2, and PECAM mRNA levels in involved skin were significantly reduced (Fig 10). The reduction in Ang2 was particularly noticeable. Ang2 mRNA levels in involved skin were reduced to levels observed in uninvolved skin after 8 wk of therapy, whereas Ang1, Tie2, and PECAM expression levels in clinically improved psoriasi lesions were still higher compared to uninvolved skin. Moreover, in three patients tested, marked reduction of Ang2 in involved skin was already evident at week 4 of therapy.
DISCUSSION

In this study, we have shown that the expression levels of Ang1, Ang2, and its specific receptor, Tie2, were elevated in involved psoriasis skin compared to uninvolved skin, healthy skin, and chronic dermatitis skin. Angiopoietins and Tie2 receptor are thought to play a key role in angiogenesis during embryogenesis. This system has also been shown to participate in the development of pathologic neovascularization, including tumor angiogenesis, ocular vascular disease, and wound healing (Wong et al., 1997; Peters et al., 1998; Stratmann et al., 1998; Otani et al., 1999; Zaggag et al., 1999). Therefore, our results suggest that upregulation of angiopoietins and Tie2 is closely associated with vascular expansion in psoriasis.

Our histopathologic studies revealed the different localization of Ang1 and Ang2 expression in psoriasis. Ang1 was expressed by stromal cells in the highly vascularized papillary dermis of involved skin. Ang2 was also present in the papillary dermis of involved psoriasis skin, but the signals were limited to endothelial cells. The distinct but overlapping expression pattern suggests that the elevated expression of the two molecules may have different implications for the development of vascular expansion in psoriasis. Ang2 is thought to be a crucial initiator for angiogenesis in which VEGF is involved. In the rat glioma model, Ang2 was highly induced in co-opted host vessels around tumor cells immediately before robust angiogenesis (Holash et al., 1999). These findings indicate that upregulation of Ang2 in endothelial cells could be an initial sign of vascular expansion in psoriasis, as well as of tumor angiogenesis. Moreover, a recent study revealed that Ang2 expression was downregulated by Ang2 itself in bovine microvascular endothelial cell cultures (Mandriota and Pepper, 1998). This suggests that, shortly after overproduction, Ang2 expression in psoriasis lesions may be normalized via a negative feedback mechanism. Thus, it is possible that, at the onset of neovascularization in a psoriatic lesion, interaction between Ang1 and the Tie2 receptor is blocked by Ang2 upregulation, resulting in endothelial cell destabilization and the initiation of vascular proliferation in the presence of high VEGF levels. Subsequently, local diminution of Ang2 with high expression of Ang1 would reestablish Tie2 signaling and maintain the newly formed proliferating vessels. As Ang1 signaling is believed to participate in pericyte–endothelial cell interactions, the upregulation of Ang1 may contribute to the recruitment of pericytes to proliferating vessels in psoriasis. Consistent with this hypothesis, our immunohistochemistry results for αSMA showed that pericytes were not evenly distributed in proliferating vessels of involved psoriasis skin. In addition, Ang1 may also promote vascular expansion in the presence of high VEGF levels in psoriasis, as studies have reported that Ang1 was able to synergize with VEGF to induce neovascularization (Asahara et al., 1998) and sprout formation (Kohlizek et al., 1998) and augment antipapoptotic effects (Kwak et al., 1999; Papapetropoulos et al., 1999). As shown in Fig 11, alternate binding of Ang1 and Ang2 to the Tie2 receptor may occur incessantly, maintaining vascular expansion in an active psoriatic lesion.

Cytokine studies clearly demonstrated that both VEGF and bFGF upregulated Ang2 expression in HMVEC cultures, which is in general agreement with a previous report (Mandriota and Pepper, 1998). In addition, our study showed that these two growth factors synergistically enhanced the expression of Ang2. Because VEGF and bFGF are present at high levels in involved psoriasis skin, these growth factors may be involved in the induction of Ang2 expression.

Tie2 was expressed by endothelial cells in the highly vascularized papillary dermis as well as by endothelial cells in other areas including the deep dermis. Because Tie2 receptor signaling is assumed to have a role in the stabilization of newly formed vessels, Tie2 receptor upregulation may be crucial for vascular expansion in psoriasis and would reflect the increase in new vessels. Our study also showed that VEGF and bFGF enhanced the expression of the Tie2 receptor on endothelial cells, suggesting that VEGF and bFGF are involved in the maintenance of proliferating vessels through the upregulation of Tie2 receptor signals.

This study showed an association between improvement in skin lesions as a result of antipsoriatic therapy and decreased levels of angiopoietins and Tie2 in skin lesions. In particular, successful antipsoriatic treatment dramatically reduced Ang2 expression in involved skin to levels of uninvolved skin, and this reduction...
Figure 11. Altered expression of Ang1 and Ang2 in the development and resolution of microvascular proliferation in psoriasis. (A) Endothelial cells in uninvolved psoriasis skin. Proper balance between Ang1 and Ang2 expression controls the integrity of blood vessels through the Tie2 receptor in uninvolved skin, as well as in healthy skin. (B) Ang2 expression in endothelial cells at the onset of neovascularization in a psoriatic lesion. Local overproduction of Ang2 in endothelial cells by VEGF and/or bFGF blocks the Ang1 signal, resulting in endothelial cell destabilization and initiation of vascular proliferation in the presence of other angiogenic inducers such as VEGF and/or bFGF. (C) Papillary dermis of an active psoriatic lesion. Ang2 overexpression may be downregulated via a negative feedback mechanism. Ang1 expressed by stromal cells may then reestablish Tie2 signaling and maintain the newly formed proliferative vessels. Moreover, in some endothelial cell Ang2 may again increase and bind to Tie2 receptor by overcoming high Ang1 levels, which promotes the recommencement of neovascularization. Alternate binding of Ang1 and Ang2 to Tie2 may maintain vascular expansion in the psoriatic lesion. (D) The papillary dermis of a resolving psoriatic lesion. Successful antipsoriatic treatment downregulates Ang2 expression and may stop the formation of new vessels, thereby normalizing vessels in a psoriatic lesion.

appears to occur early during therapy. These observations suggest that alteration of Ang2 expression levels may be particularly important in controlling vascular proliferation in psoriasis. PUVA and retinoid treatments have been shown to affect psoriatic keratinocytes or activated T cells (Esgleyes-Ribot et al., 1994; Vallat et al., 1994). Our studies suggest that suppression of Ang2 expression in endothelial cells of the papillary dermis is another possible molecular basis for these antipsoriatic therapies.

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