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S100A8 and S100A9 are messengers in the crosstalk between epidermis and dermis modulating a psoriatic milieu in human skin

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ABSTRACT

S100A8 and S100A9 are members of the S100A8 protein family that exist as homodimers and heterodimers in neutrophils, monocytes, and macrophages. Recent studies have shown the pivotal roles of S100A8 and S100A9 in the propagation of inflammation and keratinocyte proliferation in psoriasis. We found significant up-regulation of S100A8 and S100A9 secretion from keratinocytes in psoriatic lesions. To mimic the *in vivo* secretory conditions of S100A8 and S100A9 from psoriatic epidermal keratinocytes, we used the culture medium (CM) of S100A8 and S100A8/A9 adenovirus-transduced keratinocytes to investigate the functions of S100A8 and S100A9. We detected increased levels of various pro-inflammatory cytokines in the CM, including IL-8 and TNF-α, which are involved in aggravating psoriatic skin lesions, and IL-6 and members of the CXCL family of pro-angiogenic cytokines. The CM increased immune cell migration and increased angiogenesis in human umbilical vein endothelial cells. In conclusion, we found that the upregulated production of S100A8 and S100A9 by psoriatic epidermal keratinocytes activated adjacent keratinocytes to produce several cytokines. Moreover, S100A8 and S100A9 themselves function as pro-angiogenic and chemotactic factors, generating a psoriatic milieu in skin.

1. Introduction

Psoriasis is a multisystemic disease orchestrated by complex interactions between keratinocytes and immune cells [1]; however, its exact pathogenesis has not been elucidated. Recent findings have brought back the classical view of the disease; i.e., that keratinocytes are primarily responsible for the onset of disease. Zenz et al. [2] reported that transgenic mice, from which the AP-1 family members JunB and c-Jun had been constitutively deleted from basal keratinocytes, showed psoriasis-like skin inflammation and arthritis. Prior to disease onset, S100A8 and S100A9 were strongly induced in the keratinocytes. S100A8 and S100A9 are members of the S100 protein family and are considered to be characteristic of several inflammatory conditions, including inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, inflammatory dermatosis, and psoriasis [3]. S100A8 and S100A9 are expressed in the human epidermis and in cultured keratinocytes, and their distinct biological functions in psoriasis are now being investigated. Benoit et al. [4] demonstrated the upregulated secretion of S100A8 and S100A9 in psoriatic skin, and suggested an extracellular role for S100A8 and S100A9 in psoriasis, rather than intracellular calcium-dependent signaling. Nukui et al. [5] showed the secretion of S100A8 and S100A9 from cultured human keratinocytes, and recombinant S100A8/A9 induced the up-regulation of cytokines in psoriatic skin. Here, we present further data on the involvement of S100A8 and S100A9 in the pathogenesis of psoriasis, including the direct chemotactic effect of S100A8, S100A9, and the S100A8/A9 complex on various immune cells involved in the pathogenesis of psoriasis and induction of pro-inflammatory cytokine production by keratinocytes. Moreover, S100A8, S100A9, and S100A8/A9 induced pro-angiogenic mediator production by keratinocytes and directly mediated angiogenesis, which may not only be a co-factor, but also an inducer of psoriasis development.

2. Materials and methods

2.1. Patients

Biopsies were taken from the psoriatic plaque lesions and normal opposite sites of four psoriasis patients, together with normal skins of unaffected individuals as normal controls. Skin samples were obtained in accordance with the ethical committee approval process of Chungnam National University.

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2.2. RT-PCR

Total RNAs were prepared using an Easy blue RNA isolation kit (Intron, Daejeon, Korea) according to the manufacturer's protocol. Two μg of total RNAs were reverse transcribed with M-MLV reverse transcriptase (Promega, Madison, WI). Aliquots of the RT mixture were subjected to PCR with primers: S100A8, forward 5′- GCTGTCTTTCAGAAGACCTGG and reverse 5′- CTCTGGGCCCAGTA-ACTCAG; S100A9, forward 5′- CAGCTGAGCTTCGAGGAGTT and reverse 5′- CCACAGCCAAGACAGTTTGA.

2.3. Recombinant protein

Recombinant S100A8 and S100A9 proteins were expressed in BL21 (DE3) pLys competent cells (Promega). After IPTG induction, the bacteria were centrifuged and the pellet was resuspended in binding buffer (0.5 mM NaCl, 20 mM Tris–HCl, pH 7.9, 5 mM imidazole) and lysed by sonication for 10 min. Recombinant His-Tag S100A8 and S100A9 proteins were purified using nickel column. Bound S100A8 and S100A9 were freed using elution buffer (1 M imidazole, 20 mM Tris–HCl, pH 7.9, 0.5 M NaCl). Eluted solution was dialysed to remove imidazole using 20 mM Tris–HCl, pH 7.9 and 150 mM NaCl, and concentrated using Centricon Plus-70 (Millipore, Billerica, MA).

2.4. Recombinant adenovirus

The replication-incompetent adenoviruses were created using Virapower adenovirus expression system (Invitrogen), as previously reported [6]. Briefly, site-specific recombination between entry vectors and adenoviral destination vector pAd/PL-DEST was achieved by LR clonase (Invitrogen). The resulting adenoviral expression vectors were transfected into 293A cells using Lipofectamine 2000 (Invitrogen). Cells were grown until 80% Cytopathic Effect (CPE) was seen.

2.5. Immunoprecipitation and Western Blot

To harvest cell lysates for immunoblotting, cells were lysed in RIPA buffer (150 mM NaCl, 50 mM Tris, pH 7.4, 1 mM EDTA, and 1% NP-40). For co-immunoprecipitation, lysates were incubated with 2 μg anti-HA antibody (Santa Cruz Biologicals, Santa Cruz, CA) for overnight. Immunoprecipitates were then isolated on protein A agarose beads (ELPIS biotech, Daejeon, Korea). The following primary antibodies were used for Western Blot: GFP (Santa Cruz, sc-8334, rabbit polyclonal), HA (Santa Cruz, sc-7392, mouse monoclonal), S100A8 (Santa Cruz, sc-8112, goat polyclonal), S100A9 (Santa Cruz, sc-8114, goat polyclonal), and actin (Sigma, A5060, rabbit polyclonal).

2.6. ELISA

The CMs were obtained after adenoviral transduction. Levels of TNF-α and IL-8 were quantified using ELISA kits (Biosource International, Camarillo, CA), according to the manufacturer's recommended protocols.

2.7. Migration assay

The directional migration was measured in a modified Boyden's chamber (Costar, Cambridge, MA). Briefly, polycarbonate filters with 5 μm pores were used. Raw 264.7 cells, Jurkat cell or THP1 were seeded on the upper wells, and the lower wells contained CMs. After incubation for 24 h for Raw 264.7 cells, the migrated cells on the lower surface of the filter were fixed and stained with crystal violet. For Jurkat and THP1, incubation was shortened for 6 h and the migrated cells in the lower chambers were counted using Flow cytometry (FACS canto II ; Becton Dickinson, San Diego, CA) and Flow Jo (Version 4.5, Treestar, Ashland, OR).

2.8. Cytokine arrays

CMs after keratinocytes transduced with S100A8 and S100A9 expressing adenoviruses were used for the cytokine arrays. The cytokine arrays were performed by RayBiotech according to their protocols for the RayBio human angiogenesis antibody array 1 (Cat# AAH-ANG-1, RayBiotech, Norcross, GA) and RayBio human cytokine antibody array 3 (Cat# AAH-CYT-3, RayBiotech, Norcross, GA).

2.9. HUVEC cell proliferation and viability assay

HUVECs were cultured in gelatin coated 6-well plates in growth media. After 24 h in culture, the culture media was replaced with CMs from keratinocytes with GFP, S100A8, S100A9, S100A8 plus S100A9 adenovirus infected, and VEGF (20 ng/mL) and incubated for 48 h. Cell proliferation was quantified by direct cell counting. Cell Viability was assayed as previously described [7].

2.10. Endothelial cell migration and capillary-like tube formation assay

The chemotactic motility of HUVECs and tube formation were assayed as described previously [8]. CMs and VEGF (20 ng/mL) was placed in the lower wells. HUVECs were loaded into each of the upper wells and the chamber was incubated at 37 °C for 4 h. Cells were fixed and stained with hematoxylin and eosin. Chemotaxis was quantified by counting the number of cells in each well from six random fields using 100 × magnification on a microscope. For the tube formation, 250 μL of growth factor-reduced Matrigel (10 mg protein/mL) was pipetted into a 16 mm tissue culture well and polymerized for 30 min at 37 °C. HUVECs were plated onto the Matrigel layer at a density of 1.5×10^5 cells/well and CMs and VEGF (20 ng/mL) was added. After 24 h, the cultures were photographed (40 × magnification). The area covered by the tube network was measured using an optical imaging technique in which pictures of the tubes were scanned in Adobe Photoshop and quantified with the Image J program (NIH, Bethesda, US).

2.11. Statistical analysis

Statistical analysis was performed with the SPSS Version 12.0 (SPSS Inc., Chicago, IL, US) for Windows. The *t*- test and one-way ANOVA tests were used for comparison between groups, with values of $p < 0.05$ being regarded as significant. Data are presented as means ± SD.

3. Results

3.1. Expression of S100A8 and S100A9 in psoriatic epidermis

Immunohistochemical staining clearly showed dramatic induction of S100A8 and S100A9 in active, involved psoriatic regions and gradually decreasing expression as the epidermis began to express a normal histologic appearance. A normal uninvolved skin region expressed a negligible quantity of S100A8 and S100A9 (Fig. 1a). To confirm the association of S100A8 and S100A9 expression with psoriatic lesions, we obtained skin tissues from four psoriasis patients and compared the mRNA levels by RT-PCR. In all cases, S100A8 and S100A9 were markedly up-regulated in psoriatic plaque lesions (I, involved region) as compared with non-lesion skin

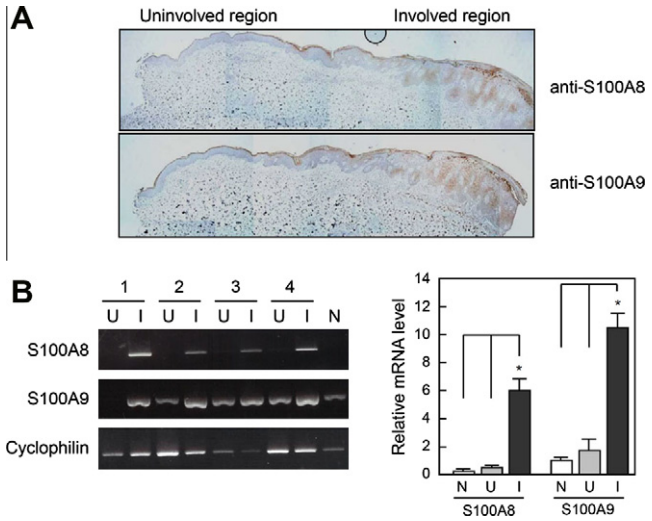

Fig. 1. S100A8 and S100A9 are upregulated in the epidermis of psoriatic skin. (A) Immuno histochemical staining of uninvolved skin from a psoriasis patient. (B) Skin samples were obtained from four psoriasis patients and the mRNA levels of S100A8 and S100A9 determined by RT-PCR. Right panel: relative mRNA levels, which were calibrated to an internal control (cyclophilin). Four skin samples obtained from volunteers were used as normal controls. N, normal skin; U, uninvolved region of a psoriatic patient; I, involved region of a psoriatic patient. The statistical significance was determined using Student's *t*-test ($^{*}p < 0.05$).

(U, uninvolved region) from both patients and normal controls (N) (Fig. 1b).

3.2. Homodimer and heterodimer formation by S100A8 and S100A9

To confirm the formation of dimeric complexes between S100A8 and S100A9 *in vivo*, we carried out immunoprecipitation after transient transfection in 293 cells. S100A8 and S100A9 were co-immunoprecipitated, confirming their protein–protein interaction *in vivo* (Fig. 2a). To further verify dimer formation, we produced His-tagged recombinant proteins in *E. coli*. Recombinant S100A8 and S100A9 were mixed together and analyzed by SDS–PAGE under reducing and non-reducing conditions. Under reducing conditions, S100A8 and S100A9 migrated in accordance with their expected molecular masses (14 and 18 kDa). However, under non-reducing conditions, all expected forms, including the S100A8 and S100A9 monomers, S100A8 and S100A9 homodimers, and S100A8/A9 heterodimer, were detected (Fig. 2b). These results suggest that S100A8 and S100A9 interact non-covalently, and that they function coordinately and/or independently as homodimers or heterodimers, depending on the microenvironment.

3.3. Secretion of S100A8 and S100A9 from adenovirus-overexpressed cultured human keratinocytes

To investigate the functional role of S100A8 and S100A9 in keratinocytes, we created recombinant adenoviruses expressing GFP-fused S100A8 and DsRed-fused S100A9. Adenovirus GFP-S100A8- and DsRed-S100A9- transduced keratinocytes expressed the exogenously introduced gene, as determined by Western Blot analysis (Fig. 2c). Previous reports have indicated that S100A8 and S100A9 are secreted from keratinocytes and that extracellular S100A8 and S100A9 have multifunctional roles in various cellular processes [9]. We speculated that the increased S100A8 and S100A9 in epidermal keratinocytes from psoriatic skin lesions are secreted into the extracellular space of the epidermis and into the dermis; here, they may function as deterioration factors inducing a psoriatic milieu. We assessed whether overexpressed S100A8 and S100A9 were secreted from keratinocytes. After adenoviral transduction, samples of the culture medium (CM) were used for Western Blotting. As expected, S100A8 and S100A9 were detected in the medium, confirming that S100A8 and S100A9 were secreted from the keratinocytes (Fig. 2d).

3.4. Effect of S100A8 and S100A9 on cytokine production in keratinocytes and exogenous S100A8 and S100A9 on immune cells

In psoriasis, keratinocytes are also the source of pro-inflammatory cytokines and pro-angiogenic mediators [10]. The CM was sampled after the transduction of GFP-S100A8 and DsRed-S100A9 to detect its autocrine stimulatory effect to induce pro-inflammatory cytokines. Cytokine expression (e.g., growth-related genes [GROs], GRO-α, IL-6, IL-8, and TNF-α) was detected in CM samples using a human cytokine and angiogenesis antibody array (Fig. 3a). We then determined the TNF-α and IL-8 levels in the CM by ELISA. The levels of secreted TNF-α and IL-8 were significantly increased by the overexpression of S100A8 and S100A9 (Fig. 3b).

Because keratinocyte-infiltrated immune cell interactions are essential for promoting psoriasis, we examined the chemotactic effect of S100A8 and S100A9 on macrophages, lymphocytes, and monocytes. It is accepted that psoriasis is a T-cell-dependent disease, and recent studies have emphasized the importance of macrophages in its pathogenesis [11,12]. Human keratinocytes were transduced with GFP-S100A8 and DsRed-S100A9 overnight and the media replenished, followed by further incubation for 24 h. The spent CM was placed in the lower wells of Boyden's chamber to mimic the presence of S100A8 and S100A9 in the extracellular space. Jurkat or THP1 (human acute monocytic leukemia cell line) cells were added to the upper chamber and allowed to migrate through the membrane. The CM of S100A8-, S100A9-, or S100A8/A9- transduced keratinocytes dramatically increased the migration of Jurkat and THP1 cells. But, there was no synergistic effect of S100A8/A9- co-transduction on Jurkat and THP1 cell migration (Fig. 3c and d). These results suggest that the increased infiltration of monocytes, macrophages, and lymphocytes in psoriatic lesions may be partly due to the secretion of S100A8 and S100A9 from psoriatic keratinocytes.

3.5. S100 proteins increase the proliferation, migration, capillary-like tube formation, and survival of human endothelial cells

Vessel formation proceeds in early psoriasis before the skin lesion appears; these enhanced vessels provide routes for inflammatory cells to move from the blood into the lesion. Thus, angiogenesis plays an important role in psoriasis [13,14]. To determine whether S100 proteins derived from keratinocytes are associated with enhanced angiogenesis, their capacity as endothelial activators was assessed using *in vitro* angiogenesis models. As shown in Fig. 4, a significant increase in proliferation, migration, and capillary-like tube formation was observed in primary cultured human umbilical vein endothelial cells (HUVECs) incubated with CM from S100A8-, S100A9-, and S100A8/A9- transduced keratinocytes. The effects were comparable with those of the optimal concentration of VEGF, the most powerful pro-angiogenic factor. In terms of capillary-like tube formation, 48 h of incubation with CM from S100A8- or S100A8/A9- transduced keratinocytes maintained extensive tube networks, and generated more cell connections than did VEGF treatment. In addition, the CM protected the endothelial cells from serum deprivation-induced apoptosis. These results suggest that S100 proteins derived from keratinocytes have potent angiogenic activity leading to the formation of blood vessels in psoriatic epidermis.

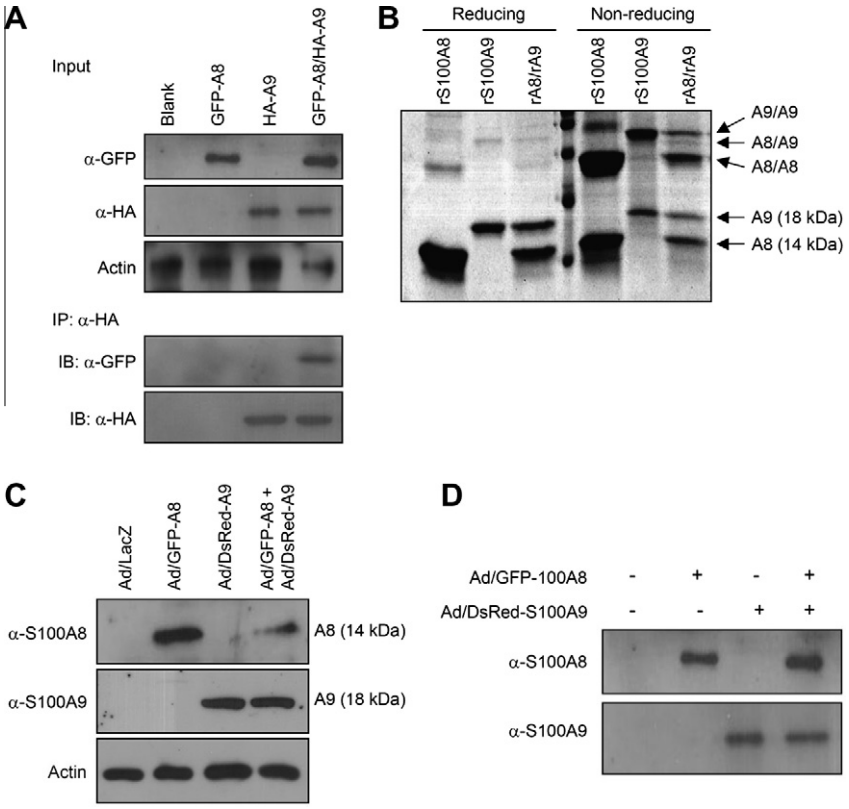

Fig. 2. Secretion of S100A8 and S100A9 from adenovirus-overexpressing cultured human keratinocytes. (a) HEK 293 cells were transfected with blank vector or an expression vector for GFP-tagged S100A8 (GFP-A8) and HA-tagged S100A9 (HA-A9). Cell extracts were incubated with anti-HA antibodies then precipitated with protein A agarose beads. Samples were resolved by SDS–PAGE, transferred to nitrocellulose membranes and incubated with anti-GFP or -HA antibodies. In GFP-A8 and HA-A9 double transfectants, the co-immunoprecipitated band is shown. (b) Homodimer and heterodimer formation between S100A8 and S100A9 proteins. Recombinant proteins were mixed, resolved by SDS–PAGE, and visualized by silver staining. Under non-reducing conditions, the reducing agent mercaptoethanol was omitted from the sample buffer. (c) Keratinocytes were transduced with 10 MOIs of adenovirus expressing GFP-tagged S100A8 and DsRed-tagged S100A9; ectopically expressed proteins were then evaluated by Western Blotting. (d) Secretion of S100A8 and S100A9 from keratinocytes. Keratinocytes were transduced with 10 MOIs of recombinant adenovirus overnight. After washing, the medium was replenished and the cells again incubated. At the indicated time points, the culture medium was collected and fractionated by SDS–PAGE. Secreted S100A8 and S100A9 were then detected by Western Blotting. rS100A8, recombinant S100A8 protein; rS100A9, recombinant S100A9 protein; A8/A8, S100A8 homodimer; A9/A9, S100A9 homodimer; A8/A9, heterodimer of S100A8 and S100A9.

4. Discussion

Many researchers have attempted to identify the genes responsible for psoriasis, which is an epidermal disorder related to abnormal keratinocyte differentiation and proliferation. Of these, S100 proteins are closely related with abnormal keratinocyte differentiation located within the epidermal differentiation complex on human chromosome 1q21 [15].

Among the many S100 proteins that are up-regulated in epidermal diseases, S100A8 and S100A9 are increased under inflammatory conditions and stimulate the growth of keratinocytes. Nukui et al. [8] suggested that excessive keratinocyte growth is induced by a positive feedback loop involving S100A8/A9 in keratinocytes, and they suggested the role of S100A8/A9 as inducers of cytokine production in psoriatic epidermis. The significance of S100A8 and S100A9 in psoriasis pathogenesis was suggested by their elevated levels in the serum of patients with severe skin lesions [4]. However, the role of S100A8 and S100A9 in the pathogenesis of psoriasis remains unclear. The initial steps in the pathogenesis of psoriasis remain a mystery, but current models suggest that interactions between keratinocytes and immune cells are responsible for disease onset. Moreover, angiogenesis in the superficial dermal microvasculature is important in psoriasis development [16].

We hypothesized that crosstalk among immune cells, epidermal keratinocytes, and dermal vessels are responsible for generating a psoriatic milieu in skin. Increased secretion of S100A8 and/or S100A9 in psoriatic keratinocytes may facilitate crosstalk among immune cells, epidermal keratinocytes, and dermal vessels.

To determine the functions of S100A8 and S100A9, we overexpressed S100A8 and/or S100A9 by recombinant adenovirus transduction into keratinocytes to evaluate the chemoattractant and angiogenic capacity of secreted S100A8 and/or S100A9. As expected, S100A8 and S100A9 were detected in the CM of keratinocytes transduced with adenovirus expressing S100A8 and/or S100A9 (Fig. 2). We used the CM of S100A8- and/or S100A9-expressing adenovirus-transduced keratinocytes because we hypothesized that this would most closely mimic psoriatic epidermis since S100A8 and S100A9 undergo post-translational, oxidative modifications [17]. Recently the receptor for advanced glycation and end products (RAGE) was identified as ligand of S100A8 and S100A9 [18]. We hypothesized the epidermal keratinocytes in psoriasis might be responding in an autocrine manner to their own secreted S100A8 and/or S100A9 and the adjacent keratinocytes or inflammatory cells in a paracrine manner via RAGE pathway and activated keratinocytes produce other molecules, including cytokines resulting in aggravation of skin lesions and play a central pathogenetic role in psoriasis.

As expected, the CM of S100A8- and/or S100A9- expressing adenovirus-transduced keratinocytes contained various cytokines that could aggravate or induce psoriatic skin lesions and angiogenesis. Human cytokine and angiogenesis antibody arrays elucidated the novel role of S100A8 and/or S100A9 in psoriasis pathogenesis

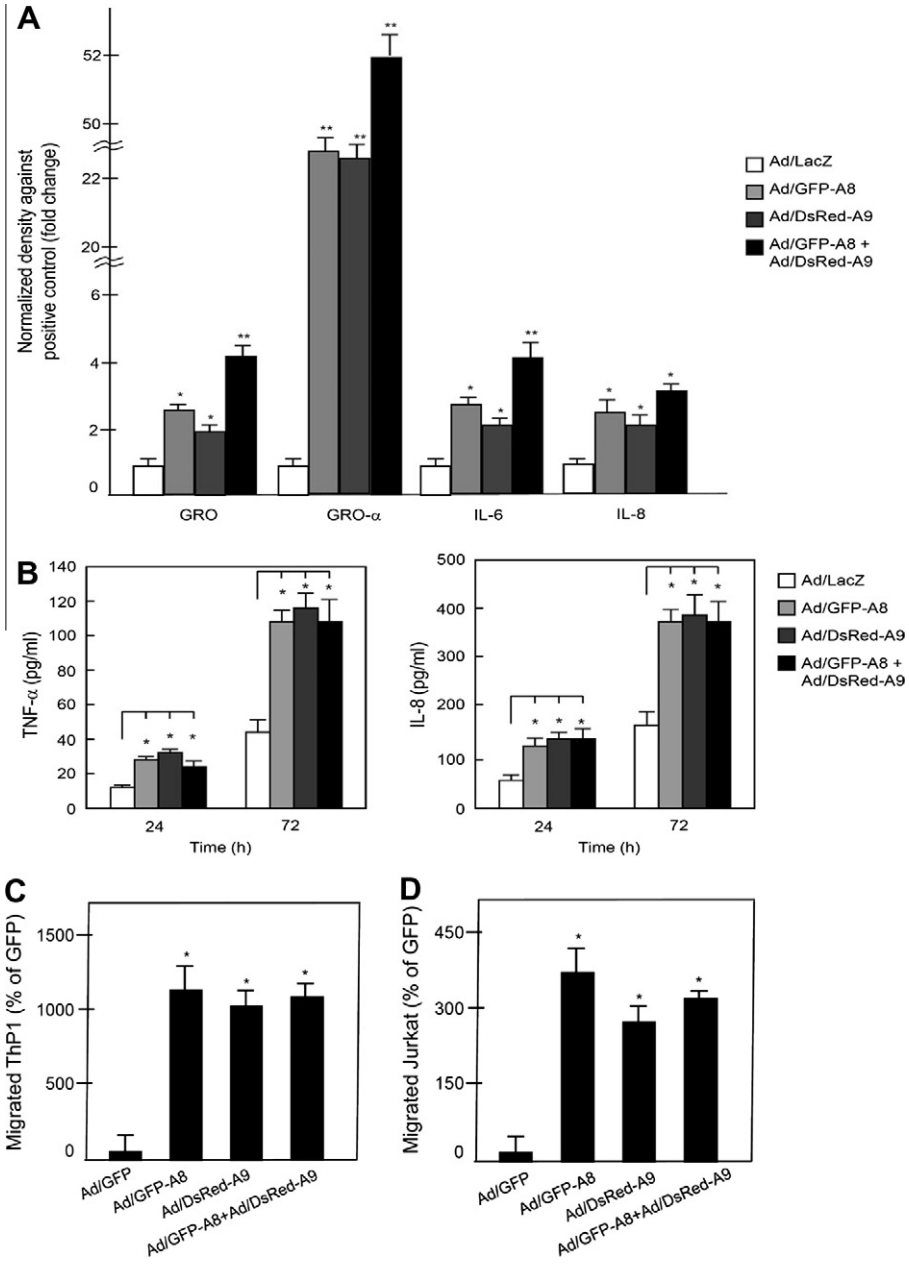

Fig. 3. Effect of S100A8 and S100A9 on cytokine production in keratinocytes and of exogenous S100A8 and S100A9 on immune cells. (A) Keratinocytes were transduced with S100A8- and S100A9- expressing adenovirus at the indicated MOIs overnight. After washing, the medium was replenished and the cells again incubated for 48 h. The culture medium was collected after 48 h and the cytokine levels determined using human cytokine and angiogenesis antibody arrays. (B) After adenoviral transduction, the culture medium was collected at the indicated time points and secreted cytokine levels determined by ELISA. (C) Culture media of S100A8- and/or S100A9- expressing adenovirus-transduced keratinocytes were placed in the lower wells of a Boyden's chamber, and RAW264.7 macrophages were seeded on the upper wells. After 24 h of incubation, the migrated cells on the lower surface of the filter were fixed and stained. For quantification, the migrated cells were counted at × 100 magnification. Four independent fields were counted in each experiment. (D) Culture media of S100A8- and/or S100A9- expressing adenovirus-transduced keratinocytes were placed in the lower wells of a Boyden's chamber, and THP1 or (E) Jurkat cells were seeded in the upper wells. After 6 h of incubation, the migrated live cells in the lower chambers were counted by FACS analysis. The migration assay was repeated at least three times. The values represent the means ± SEM of three independent determinations ($^{*}p < 0.05$, $^{**}p < 0.01$ versus Ad/GFP).

through the induction of pro-angiogenic and proinflammatory cytokines by keratinocytes, including IL-6, IL-8, GROs, and GRO-α [19]. This in turn explains the induction by S100A8 and/or S100A9 of an inflammatory and angiogenic milieu in psoriatic skin lesions. Of the pro-angiogenic cytokines, TNF, IL-8, and IL-17 are expressed during psoriasis, and increased CXCL1 is responsible for the direct recruitment of neutrophils to psoriatic lesions [20,21]. With regard to immune cells, S100A8 and S100A9 are constitutively expressed in neutrophils, monocytes, and macrophages; in terms of function, they act as chemoattractants and help retain immune cells at inflammatory sites [22]. The S100A8 and S100A9 exist in homo- or heterodimers, but their functional difference has not been fully elucidated. The culture media of S100A8- and/or S100A9- expressing adenovirus-transduced keratinocytes were strong chemoattractants for THP1 and Jurkat cells but the S100A8/A9 failed to show the synergistic effect *in vitro*. These results suggest S100A8 and/or A9 dimers, coordinately and/or independently, are responsible for attracting immune cells to the dermis in psoriatic skin *in vivo*.

Secreted S100A8 and/or S100A9 may migrate to the papillary dermis and are responsible for inducing capillary proliferation shown in psoriasis. We investigated the effect of the CM of

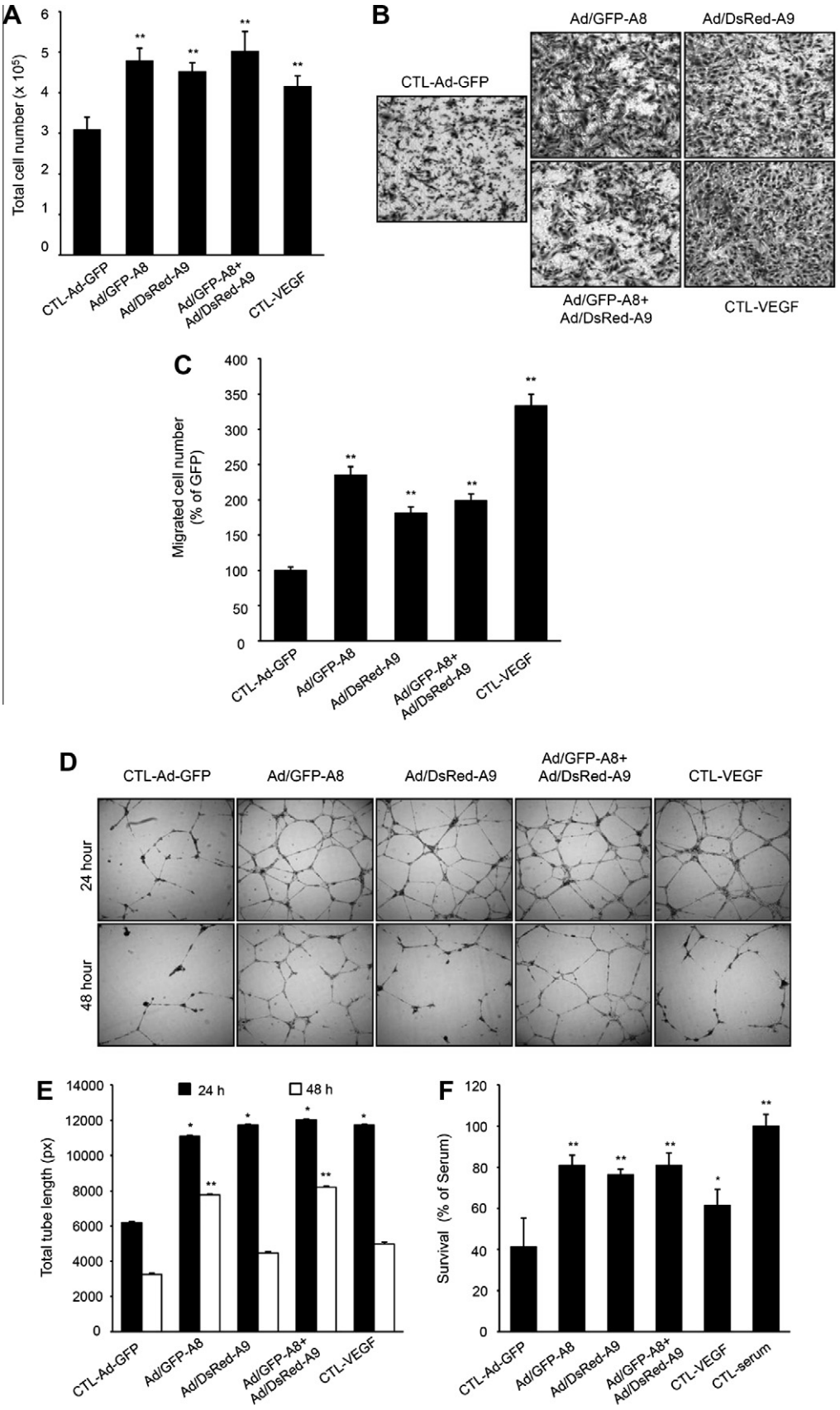

Fig. 4. S100 proteins increase the proliferation, migration, capillary-like tube formation, and survival of human endothelial cells. Proliferation (a), migration (b, c), and capillary-like tube formation (d, e) assays were performed using culture media from keratinocyte cells expressing GFP, S100A8, S100A9, S100A8 + S100A9 adenovirus-infected, and VEGF (20 ng/ml) as described in the materials-methods. (f) HUVECs were plated onto six-well plates in 1 ml of M199 containing 20% FBS. The next day, the cells were switched to serum-free M199 with or without 5% FBS, CMs, and VEGF (20 ng/ml). After 36 h, cell viability was assessed by trypan blue exclusion. Two independent experiments were performed, each using triplicate samples. The data are presented as the mean ± SD. $^{*}p < 0.01$, $^{**}p < 0.001$ versus GFP.

S100A8- and/or S100A9- expressing adenovirus-transduced keratinocytes on endothelial cells. Of the members of the S100 protein family, only S100A4 is yet known to stimulate angiogenesis. A capillary tube formation assay showed good tubular network formation by HUVECs treated with CM from S100A8- and/or S100A9-expressing adenovirus-transduced keratinocytes after 24 h. After 48 h, prolonged capillary tubule formation was observed in HUVECs treated with CM from S100A8 and S100A8/A9 transduced keratinocytes, suggesting that S100A8 has greater angiogenic capacity than S100A9. The angiogenesis antibody arrays showed increases in IL-6, IL-8, GROs, and GRO-α in the CM of S100A8 and/or S100A9 adenovirus-transduced keratinocytes, and no increases in the levels of major pro-angiogenic cytokines, including VEGF, thrombopoietin, or angiogenin. Based on these data, we assumed that S100A8 and/or S100A9 over-expressed in psoriatic epidermis may induce angiogenesis in psoriatic dermal papilla.

In summary, our results suggest that S100A8 and/or S100A9 function to generate a psoriatic milieu in human skin. i.e., 1) as secretory proteins from psoriatic epidermal keratinocytes, S100A8 and/or S100A9 may signal via positive feedback to adjacent keratinocytes to produce pro-inflammatory and pro-angiogenic cytokines, thus exacerbating psoriatic skin lesions; 2) secreted S100A8 and/or S100A9 attract immune cells, thus facilitating the complex interaction with keratinocytes; and 3) S100A8 and/or S100A9 promote endothelial cell proliferation, survival, and angiogenesis both directly and by inducing keratinocytes to produce pro-angiogenic cytokines.

This study provides new insight into the key role of S100A8 and/or S100A9 in the crosstalk between immune cells, keratinocytes, and angiogenesis in psoriatic skin, and suggests that inhibiting S100A8 and/or S100A9 may be a novel therapy targeting various molecular events involved in the initiation of psoriasis.

Acknowledgment

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