Gottron’s papules exhibit dermal accumulation of CD44 variant 7 (CD44v7) and its binding partner osteopontin: a unique molecular signature

Jessica S. Kim, MDa,b,c, Muhammad M. Bashir, PhDb,c, and Victoria P. Werth, MDb,c

a New York University School of Medicine, New York, NY
b Philadelphia V.A. Medical Center, Philadelphia, PA
c University of Pennsylvania, Philadelphia, PA

Abstract

The accumulated mucin in non-Gottron’s dermatomyositis (DM) lesions is primarily chondroitin-4-sulfate (C4S), which is immunomodulatory in vitro. Gottron’s papules are a particularly resistant manifestation of DM that often persist after other lesions have resolved with therapy. We examined non-Gottron’s DM lesions and Gottron’s papule skin biopsies for C4S, CD44v7, a CS-binding isoform causally implicated in autoimmunity, and osteopontin, a CD44v7 ligand implicated in chronic inflammation. Gottron’s papule dermis contained more C4S and CD44v7 than non-Gottron’s lesions. Normal skin showed less CD44v7 over joints relative to Gottron’s lesions. All DM dermis had increased osteopontin compared to healthy skin. Mechanically stretching cultured fibroblasts for six hours induced CD44v7 mRNA and protein, while IFN-γ treatment induced OPN mRNA and protein. Osteopontin alone did not induce CD44v7, but stretching dermal fibroblasts in the presence of osteopontin increased THP-1 monocyte binding, which is blunted by anti-CD44v7 blocking antibody. C4S, CD44v7, and osteopontin are three molecules uniquely present in Gottron’s papules that contribute to inflammation individually and in association with one another. We propose that stretch-induced CD44v7 over joints, in concert with dysregulated osteopontin levels in the skin of DM patients, increases local inflammatory cell recruitment and contributes to the pathogenesis and resistance of Gottron’s papules.

Introduction

Dermatomyositis (DM) is an autoimmune disorder with characteristic skin findings including Gottron’s papules over the extensor surfaces of large and small joints, most frequently of the hands (Callen, 2010). The pathogenesis of these skin findings remains unexplained.

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

Corresponding Author: Victoria P. Werth, M.D. Department of Dermatology, University of Pennsylvania, 2 Maloney Building, 36th and Spruce Sts, Philadelphia, Pennsylvania 19104 Telephone number: 215-823-4208, FAX number: 215-349-8339 werth@mail.med.upenn.edu.

Conflict of Interest No conflict of interest to report.
Histology of DM skin lesions includes upper dermal deposition of mucin that is strongest where it co-localizes with the mononuclear infiltrate (Janis and Winkelmann, 1968). Recently, our group molecularly characterized the accumulated mucin in non-Gottron’s DM lesions as primarily chondroitin-4-sulfate (C4S) (Kim and Werth, 2011). Published reports indicate that C4S has immunomodulatory effects in vitro, which may contribute to inflammation via monocyte activation or counteract it by blocking the effects of TNFα and IL-1β (Fioravanti and Collodel, 2006; Rachmilewitz and Tykocinski, 1998; Xu et al., 2008).

In the current study, we focused on Gottron’s papules, a particularly resistant manifestation of DM that often persists after other lesions have resolved with therapy. Two reports to date describe histologically similar findings in Gottron’s papules as in other DM lesions (Hanno and Callen, 1985; Mendese and Mahalingam, 2007), but these similarities cannot explain the divergent clinical courses. Because C4S has not previously been reported in Gottron’s papules, we now hypothesized that this molecule and its associated binding partners might differ between Gottron’s papules versus photodistributed non-Gottron’s DM skin lesions.

**Results**

**Gottron’s papules contain increased content of chondroitin-4-sulfate and CD44v7 compared to other active DM lesions or location-matched healthy controls**

Immunohistochemical analysis of Gottron’s biopsies of DM patients demonstrated a similar distribution of papillary dermal C4S as in all other DM skin lesions, but with a striking density exceeding that of samples from other locations. Compared to healthy control biopsies (Figure 1a), samples taken from non-Gottron’s DM lesions (Figure 1b) exhibited a mean 2.9-fold increased density of C4S. Compared to healthy control IP biopsies (Figure 1c), samples from Gottron’s (Figure 1d) exhibited a 4.6-fold increased density of C4S (both p<0.001). Gottron’s had a mean 1.6-fold C4S density compared to biopsies from other non-Gottron’s DM lesions (p<0.01). C4S was not present in the epidermis of any samples.

We next investigated the Gottron’s biopsies for dermal expression of proteins that interact with CS. Many proteins covalently or non-covalently attach to CS, but of these proteins, only the variant 7 (v7) isoform of CD44 has also been causally implicated in autoimmunity (Farkas et al., 2005; Hoffmann et al., 2007; Marhaba et al., 2003; Wittig et al., 2000; Wittig et al., 2002). The v7 domain, as well as one region within the ‘standard’ CD44 (CD44s) that lacks any of the variant domains, act as covalent attachment sites for CS, while CD44v6-7 has also been shown to non-covalently bind CS (Keller et al., 2007; Sleeman et al., 1997). Thus, we examined our samples for expression of CD44s and variant isoforms CD44v3, v6 and v7 (Kim and Werth, 2011). Consistent with previous reports, we observed strong epidermal expression of CD44s, CD44v3, and CD44v6 in healthy control skin, particularly within the basal layer and stratum spinosum, but scant dermal matrix expression beyond staining of infiltrating leukocytes (Leigh et al., 1996; Seiter et al., 1998; Seiter et al., 1996). Non-Gottron’s and Gottron’s DM lesions exhibited qualitatively stronger CD44s and CD44v3 staining within vessels compared to healthy controls; however dermal staining patterns of CD44s, CD44v3, and CD44v6 in healthy controls, non-Gottron’s and Gottron’s did not show significant differences (data not shown). We found no detectable CD44v6 in
any dermal sample, despite abundant pericellular keratinocyte staining in the epidermis in the same slides, which served as an internal positive control.

In contrast, Gottron’s biopsies demonstrated a mean 4.5-fold denser accumulation of CD44v7 within the papillary dermal matrix compared to healthy control non-IP biopsies (p<0.001), and a mean 2.5-fold CD44v7 density compared to the dermis of non-Gottron’s lesions (p<0.01) (Figure 2). Non-Gottron’s lesions did not differ from controls and this differential expression was specific to CD44v7.

To study the effect of location versus disease in our patient samples, we acquired biopsies from the skin overlying the fourth proximal IP (PIP) joint of five healthy volunteers with no history of autoimmune disease. Immunohistochemistry of healthy IP skin was compared with Gottron’s samples to reveal a 4.5-fold greater density of C4S (p<0.001) and a 2.8-fold greater density of CD44v7 (p<0.01) in the IP dermis of patients with disease. For within-person comparisons, we also obtained a second biopsy from either the shoulder or the arm of two healthy volunteers. C4S density was not significantly different in the skin overlying the IP joint compared to non-IP skin of either volunteer. CD44v7 density in IP skin, however, demonstrated a modest but significant 1.6-fold (t-test p=0.0149) and 1.4-fold (t-test p=0.0307) increase compared to that in non-IP skin in the two volunteers, respectively.

**DM dermis contains increased osteopontin protein**

Osteopontin is a known binding ligand for CD44v7, and this specific interaction has been implicated in chronic inflammation (Denhardt et al., 2001). Osteopontin is present in the spinous and granular layers of normal epidermis, and around sweat glands, hair follicles, and sebaceous glands of the dermis (Chang et al., 2008). Our examination of healthy control skin revealed similar patterns (Figure 3a). However, compared to location-matched healthy controls, biopsies taken from non-Gottron’s (Figure 3b) and Gottron’s (Figure 3c) exhibited increased accumulation of osteopontin diffusely distributed throughout the dermis. Quantitative analysis of dermal osteopontin revealed a mean 3.3-fold greater density in non-Gottron’s samples compared to healthy control samples (p<0.01) and a 2.5-fold osteopontin density in Gottron’s compared to healthy controls (p<0.05) (Figure 3d). Osteopontin in Gottron’s was not found to be significantly different from that in non-Gottron’s DM lesional skin, nor did osteopontin in healthy control IP skin differ from healthy control non-IP skin.

**Mechanical stretching of dermal fibroblasts in vitro induces CD44v7 mRNA expression**

Because mechanical stretching has been reported to induce expression of CD44v7 and v8 in porcine ocular trabecular meshwork cells (Keller et al., 2007), we hypothesized that the increased CD44v7 seen in the IP skin of DM patients and healthy volunteers is induced by chronic stretching of the skin over joints. Thus, we stretched primary dermal fibroblasts on cell culture membrane inserts and then determined CD44v7 mRNA expression by quantitative real-time PCR (RT-PCR). We found that mechanical stretching of dermal fibroblasts induced a significant 1.6-fold increase in CD44v7 mRNA expression at six hours (p<0.01), which returned to a baseline expression by twelve hours (Figure 4a). CD44v7 protein in the supernatant of stretched fibroblasts at 6 hours increased 3.3 ± 1.3 times relative to control fibroblasts (p<0.05) (Figure 4b,c). Osteopontin alone did not induce
CD44v7 mRNA (data not shown). These results were reproducible in three repeat experiments. In contrast, mechanical stretching did not have a significant effect on fibroblastic osteopontin mRNA expression.

**Interferon-γ induces osteopontin mRNA expression in cultured dermal fibroblasts**

Interferon-γ has been reported to stimulate osteopontin expression in monocytes (Li et al., 2003), but this induction has not been studied in dermal fibroblasts. Because interferon-γ is present in the skin of DM patients (Caproni et al., 2005; Wenzel et al., 2005) and mutations in the interferon-γ pathway have been associated with DM (Chinoy et al., 2007), we hypothesized a role for interferon-γ in the dysregulation of osteopontin expression in the skin. Treatment of human primary dermal fibroblasts with interferon-γ (10ng/mL) induced a 4.4-fold mean OPN mRNA expression compared to untreated control cells in vitro (p<0.001) (Figure 5a). Interferon-γ (10ng/mL) induced an 8.1-fold increase in OPN protein in fibroblast supernatant (p<0.001) (Figure 5b). Osteopontin mRNA and protein in the fibroblast supernatant were not induced by the pro-inflammatory cytokines IL-1α or interferon-α, indicating specificity (Figure 5a, 5b). Neither interferon-γ nor osteopontin treatment had a significant effect on fibroblast CD44v7 mRNA expression (data not shown).

**Mechanical stretching of dermal fibroblasts treated with osteopontin increases THP-1 monocyte adhesion**

Based on these findings, we hypothesized that the increased CD44v7 in association with its ligand osteopontin could play a crucial role in the localized inflammation of Gottron’s papules. To study the functional effect of increased CD44v7 ligation with osteopontin on immune cell recruitment, we incubated dermal fibroblast monolayers overnight, without or with stretching, without or with recombinant human osteopontin (rOPN), followed by rinsing. Fluorescently labeled THP-1 monocytes were then co-cultured with the prepared fibroblast monolayers, followed by rinsing to remove non-adherent monocytes, and the adherent monocytes were visualized with an inverted fluorescent microscope. We found that overnight incubation of fibroblast monolayers in medium supplemented with rOPN had no effect on THP-1 adhesion in the absence of stretch (Figure 6). Stretching the fibroblasts in the absence of rOPN supplementation resulted in an increase in the number of adherent THP-1 cells 1.8 times unstretched, no-rOPN controls (Figure 6, p<0.01). Stretching fibroblasts overnight in medium supplemented with rOPN resulted in an increase in THP-1 adhesion 2.2 times that of stretched fibroblasts without rOPN (p<0.001) and 2.3 times that of unstretched, no-OPN controls (p<0.001).

To demonstrate the specific effect of fibroblast CD44v7 on THP-1 adhesion, we incubated prepared fibroblast monolayers with anti-CD44v7 blocking antibody or isotype control IgGκ for one hour immediately before THP-1 co-culture. Stretched, rOPN-treated monolayers blocked with anti-CD44v7 antibody demonstrated a 41% reduction in THP-1 adhesion compared to identically prepared fibroblast monolayers incubated with control isotype IgGκ (Figure 6, p<0.001).
Discussion

In the current study, we found a unique molecular signature of Gottron’s papules that consists of a dense dermal deposition of C4S, abundant amounts of a C4S-binding molecule, CD44v7, that has been causally linked to autoimmunity, and co-localized deposits of osteopontin, a cytokine-like factor that cooperates with CD44v7 to recruit inflammatory cells. In contrast, non-Gottron’s DM lesions had far less C4S and exhibited levels of CD44v7 that were indistinguishable from the low amounts in skin from healthy controls. Osteopontin was present in non-Gottron’s DM lesions, but the ability of this molecule to recruit immune cells remains unproven in the near-absence of CD44v7, its major binding partner. The combination of mechanical stretch to induce CD44v7 over the extensor surfaces of joints with interferon-γ, a factor that induces osteopontin expression and occurs more generally in the skin of DM (Caproni et al., 2005; Wenzel et al., 2005), may explain the co-localization of these molecules in Gottron’s papules.

The CD44v7 chondroitin-sulfate proteoglycan is of particular interest in autoimmunity as a demonstrated mediator of cell homing, adhesion, and survival. The soluble pattern of CD44v7 seen in our samples is likely the result of CD44 shedding and sequestration through its association with the cell-associated matrix; shed CD44 also competes with cell surface CD44 for ligand binding, modulation or disruption of CD44-dependent cell-cell and cell-matrix adhesion (Cichy and Pure, 2003). In a mouse model of experimental colitis, anti-CD44v7 antibody treatment blocks T cell extravasation and recruitment to the intestinal mucosa thus curing colitis (Farkas et al., 2005; Wittig et al., 2002). Blockade of CD44v7 also induces apoptosis of mononuclear cells that had been extracted from the inflamed mucosa of patients with Crohn’s disease (Wittig et al., 2002).

The role of osteopontin in autoimmunity is an area of active investigation. Osteopontin gene polymorphisms associate with autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis, and multiple sclerosis, and plasma osteopontin concentration is correlated with SLE susceptibility, serum cytokine profiles, and disease activity (Forton et al., 2002; Han et al., 2008; Kariuki et al., 2009; Wong et al., 2005). A polymorphism for osteopontin in a cohort of newly diagnosed juvenile DM patients was associated with increased serum interferon-α level (Niewold et al., 2010).

Demonstrated immunologic functioning of osteopontin includes polyclonal B cell and macrophage activation in autoimmune murine models and activation-induced T-cell death (Chiocchetti et al., 2004; Lampe et al., 1991; Singh et al., 1990; Weber and Cantor, 2001). In the skin, osteopontin secreted by activated T cells is thought to enhance Th1-mediated immunity through interaction with immune cells: osteopontin enhances production of interferon-α in plasmacytoid dendritic cells (PDCs), has chemotactic properties for macrophages, DCs and T cells, and osteopontin encountered by DCs in the dermal matrix activates and polarizes Langerhans cells and myeloid type DCs toward a Th1 phenotype, directing them to draining lymph nodes (Giachelli et al., 1998; Kawamura et al., 2005; O’Regan et al., 2000; Renkl et al., 2005; Weiss et al., 2001). The specific, dose and integrin-dependent binding of osteopontin to CD44 is implicated with roles in adhesion, migration, modulation of cytokine secretion, anti-apoptotic signaling and tumor invasion.
(Anborgh et al., 2010). For example, osteopontin induces CD44-dependent chemotaxis and down-modulates anti-inflammatory IL-10 secretion through interactions with CD44, and is hypothesized as an activator of the CD44 variant survival signal in lymphocytes by the extracellular matrix of the gut mucosa (Ashkar et al., 2000; Denhardt et al., 2001; Katagiri et al., 1999; Weber et al., 1996)

Intriguingly, CD44v7, a principal CD44 isoform sufficient for osteopontin binding (Katagiri et al., 1999), is the isoform present in our Gottron’s papules. We have previously shown CD44v7 expression in DLE skin (Kim and Werth, 2011). However, osteopontin is not found in abundance within DLE lesions, and accordingly the osteopontin/CD44v7 complex is not seen.

C4S and CD44 are known to contribute to cellular adhesion (Charbonneau et al., 2007; Murai et al., 2004). Our THP-1 cell adhesion assay mimics the co-expression of CD44v7 and osteopontin in active areas of active inflammation in Gottron’s papules of dermatomyositis, and its results demonstrate the adhesive interactions regulated by stretch-induced CD44v7 on dermal fibroblasts complexed with osteopontin. Stretching of dermal fibroblasts may induce upregulation of a variety of molecules, but the inhibitory effect of anti-CD44v7 monoclonal antibody implicates a functional role specific to this molecule in adhesion.

Thus, the present study identifies C4S, CD44v7, and osteopontin as three molecules that contribute to inflammation individually and in association with one another and are uniquely present together in Gottron’s papules. We propose that stretch-induced expression of CD44v7 overlying joints, in concert with dysregulated levels of osteopontin generally in the skin of DM patients, increases local recruitment of leukocytes and contributes to the pathogenesis and resistance of Gottron’s papules. A greater understanding of these molecules and the consequence of their accumulation in DM skin lesions may ultimately provide novel therapeutic targets.

### Materials and Methods

#### Skin specimen collection

Lesional skin biopsies from patients with dermatomyositis (DM, n=10) were obtained in the autoimmune skin disease clinic at Penn. Five biopsies were taken from Gottron’s papules over the PIP or MCP joints and five unmatched biopsies were taken from active lesions on the arm, shoulder, or chest. Healthy control skin was provided by volunteers (n=10) without a history of autoimmune skin disease; five biopsies were from skin overlying the PIP of the third digit, and five biopsies were from sun-protected arm. Two volunteers provided both a PIP and arm or shoulder sample. All biopsies were obtained with written, informed patient consent and adherence to the Helsinki Guidelines according to our approved protocol under the UPenn Institutional Review Board. Biopsies were fixed in 10% neutral buffered formalin overnight, embedded in paraffin, and cut into 4-μm sections.
Immunostaining

Immunostaining for chondroitin-4-sulfate was performed on human skin as described previously (Chang et al., 2011a) by predigestion with chondroitinase AC II (Sigma, St. Louis MO), followed by incubation with an anti-deltaDi-4S monoclonal antibody that recognizes a terminal glucuronic acid residue adjacent to N-acetylgalactosamine-4-sulphate at the non-reducing end of chondroitin sulfate chains in proteoglycans produced after chondroitinase digestion of chondroitin sulphate chains (clone 2-B-6, Seikagaku Biobusiness Corporation, Japan). We also performed immunostaining using monoclonal antibodies against a fusion protein of glutathione S-transferase with individual variant domains (v3, v6, and v7) of human CD44 (AbD Serotec, Raleigh NC). Staining for CD44 v6 required heat-induced antigen retrieval, whereby slides were incubated for ten minutes at 70°C in Target Retrieval Solution (Dako, Carpinteria CA). Osteopontin was detected using a rabbit polyclonal antibody recognizing full-length as well as thrombin and MMP-cleaved osteopontin (AbCam, Cambridge MA). Sections were then incubated with biotinylated secondary antibody and streptavidin-horseradish peroxidase (Dako, Carpinteria CA), followed by Vector NovaRED substrate (Vector, Burlingame CA). Negative controls included mouse IgG κ isotype antibody (Sigma, St. Louis MO) and PBS in place of chondroitinase AC II enzyme digestion.

Systematic Quantification of Immunostaining

All high-power fields in three sections of each 3-5mm skin biopsy were examined at 10X magnification. Among all fields examined, one high-powered field appearing most representative of all sections was selected from each biopsy to measure matrix intensity, and this field was photographed at 10X magnification. An intensity channel was applied to photograph files using commercial software to isolate staining intensity (Image-Pro Plus v5.0). One-hundred 5 m circular manual tags were selected by systematic uniform random sampling of the area of interest (e.g. within the upper dermis for CD44v7) (Mayhew 2008). The mean intensity of staining within the total area selected by the manual tags is reported as the representative staining intensity value for each specimen.

Cell culture, mechanical stretch and cytokine treatment

Normal adult, human fibroblasts were obtained from the American Type Culture Collection (Rockville MD). Mechanical stretching experiments were performed by seeding primary human dermal fibroblasts onto membrane inserts (6-well format, 23.1-mm diameter, 3.0-μm pore size, BD Falcon #353091, BD Biosciences, Franklin Lakes, NJ) and culturing the cells to confluence (Keller et al., 2007). Next, a smooth 5-mm glass bead was placed in the dish beneath the center of the insert membrane, and constant weight was applied to the lid of the insert to force the edge of the insert down onto the bottom of the dish. This produced distortion of the membrane, increasing its surface area and thereby mechanically stretching the cells and their extracellular matrix. Stretching was continued for 6 or 12 hours, and then each stretched membrane insert was cut out, vortexed in RNAeasy lysate buffer, and RNA purified, followed by quantitative PCR (q-PCR).

For cytokine stimulation, fibroblasts were plated in 100-mm diameter dishes and grown to confluence. Fibroblasts were treated for six hours with 10ng interferon-γ/mL (R&D
Systems, Minneapolis, MN), 100U interferon-α/2 mL (PBL Interferon Source, Piscataway NJ), or 20ng recombinant human interleukin (IL)-1α/mL, before lysis with RNAeasy mini kit lysate buffer and processing RNA (Qiagen, Valencia CA). Experiments were performed in triplicate and repeated three times with reproducible results.

Quantitative Real-Time RT-PCR

Quantification of specific mRNAs was performed as previously described (Chang et al., 2011a). Total RNA was reverse transcribed using the SuperScript® First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad CA) with random hexamer primers. Real-time PCR was performed using Taqman® Gene Expression Assays for human osteopontin (OPN) (Taqman Assay ID Hs00960942_m1) and PPIA (cyclophilin A), the housekeeping control (Taqman Assay ID Hs99999904_m1) (Applied Biosystems, Foster City CA). Custom-made sequences for detection of human CD44 exon 11 transcripts, which encode exclusively the v7 motif, were 5′-tgc aag gaa gga caa cac caa-3′ (sense primer) and 5′-ggg tgt gag att ggg ttg aag aaa t-3′ (antisense primer). All reactions were performed in triplicate. The Ct method was employed to analyze the differential gene expression in cytokine-treated fibroblast cultures compared to controls, as described previously (Chang et al., 2011b).

Western blot

Serum-free supernatant from control and stretched fibroblasts were collected after 6 hours. The supernatants were centrifuged at 10,000g for 1 minute. Equal amounts of protein (10 μg) were separated on a 4-12% bis-Tris gel (Invitrogen), transferred to a membrane (Millipore MA, USA), and stained with 1:2000 dilution of mouse anti-human CD44v7 (AbD Serotec UK). For loading control, the washed membrane was stained with 0.1% Coomassie R-350 (GE Healthcare). Western blot results were recorded by X-ray films (Kodak Scientific Imaging USA) and quantified by ImageJ (Collins, 2007).

Osteopontin protein determination by ELISA

Conditioned media from interferon-γ, IL-1α, or interferon-α-treated cultured fibroblasts were collected and concentrated two-fold. The protein was quantitated by CB-Protein-Assay-Kit (G-Biosciences, St Louis, MO) and volume was adjusted to 100 ul, so that equal protein was used for each sample. Osteopontin protein was quantitated by commercial ELISA (R&D Systems Inc).

Adhesion Assay

Primary dermal fibroblasts were cultured to confluence on 23.1-mm polyethylene terephthalate membrane inserts and then stretched, or not, with 5-mm glass beads. Here, stretching lasted overnight and was combined with treatment with 0 or 5.0μg of recombinant OPN/ml. Unbound OPN was rinsed with warmed PBS prior to the assay of monocyte adherence. Fibroblasts were maintained in serum-free, phenol red-free DMEM for the remainder of the assay. For inhibition studies, 25 g of mouse anti-human CD44 variant 7 (AbD Serotec, Raleigh NC) per mL or mouse anti-human IgGk isotype antibody control (Sigma, St. Louis MO) per mL was added to stretched monolayers after rinsing of unbound OPN for 1 hour, then washed. Living THP-1 monocytes labeled with 5-
chloromethylfluorescein diacetate (CellTracker™ Green CMFDA, Invitrogen, Eugene, OR) were added \(5 \times 10^5\) /insert, followed by a 30-min incubation at 37°C. Unbound monocytes were then removed by four rinses, and the number of monocytes firmly bound to the fibroblast monolayers was counted under fluorescent microscopy in 6 random high-power fields near the centers of the stretched membranes, where the stretching was the greatest. Experiments were repeated four times with reproducible results.

**Statistical analysis**

Comparison of several groups simultaneously was performed by initially using analysis of variance (ANOVA; GraphPad Prism v5.01). When ANOVA indicated differences amongst the groups, pairwise comparisons between experimental groups were performed using the Student-Newman-Keuls q statistic and are indicated in Figures 1-6. Unless otherwise indicated, summary statistics are reported as mean ± SEM.

**Acknowledgments**

We thank Dr. Ming-Lin Liu for expert technical assistance. We also thank Dr. Kevin Jon Williams for guidance with final manuscript refining and editing. A preliminary report of this work was presented at the Society for Investigative Dermatology (May 2010, Atlanta, GA) (Kim and Werth, 2010).

This material is based upon work supported by the Lupus Foundation of America and in part by a Merit Review Grant from the Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development) and by the National Institutes of Health (NIH K24-AR 02207) to VPW.

**Abbreviations used**

- DM: dermatomyositis
- C4S: chondroitin-4-sulfate
- CD44v7: CD44 variant isoform 7
- CD44s: CD44 standard
- IP: interphalangeal
- rOPN: recombinant human osteopontin

**References**

Anborgh PH, Mutrie JC, Tuck AB, Chambers AF. Role of the metastasis-promoting protein osteopontin in the tumour microenvironment. J Cell Mol Med. 2010; 14:2037–44. [PubMed: 20597997]

Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, et al. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. Science. 2000; 287:860–4. [PubMed: 10657301]

Callen JP. Cutaneous manifestations of dermatomyositis and their management. Curr Rheumatol Rep. 2010; 12:192–7. [PubMed: 20425525]

Caproni M, Torchia D, Cardinale C, Volpi W, Del Bianco E, D’Agata A, et al. Infiltrating cells, related cytokines and chemokine receptors in lesional skin of patients with dermatomyositis.[see comment]. BrJDermatol. 2005; 151:784.
Chang LM, Maheshwari P, Werth S, Schaffer L, Head SR, Kovarik C, et al. Duplicate of 6981. Identification and molecular analysis of glycosaminoglycans in cutaneous lupus erythematosus and dermatomyositis. J Histochem Cytochem. 2011a; 59:336–45. [PubMed: 21378287]

Chang LM, Maheshwari P, Werth S, Schaffer L, Head SR, Kovarik C, et al. Identification and molecular analysis of glycosaminoglycans in cutaneous lupus erythematosus and dermatomyositis. J Histochem Cytochem. 2011b; 59:336–45.

Chang PL, Harkins L, Hsieh YH, Hicks P, Sappayatosok K, Yodsanga S, et al. Osteopontin expression in normal skin and non-melanoma skin tumors. J Histochem Cytochem. 2008; 56:57–66. [PubMed: 17938278]

Charbonneau C, Gautrot JE, Hebert MJ, Zhu XX, Lerouge S. Chondroitin-4-sulfate: a bioactive macromolecule to foster vascular healing around stent-grafts after endovascular aneurysm repair. Macromol Biosci. 2007; 7:746–52. [PubMed: 17457946]

Chinoy H, Salway F, John S, Fertig N, Tait BD, Oddis CV, et al. Interferon-gamma and interleukin-4 gene polymorphisms in Caucasian idiopathic inflammatory myopathy patients in UK. Ann Rheum Dis. 2007; 66:970–3. [PubMed: 17405833]

Chiocchetti A, Indelicato M, Bensi T, Mesturini R, Giordano M, Sametti S, et al. High levels of osteopontin associated with polymorphisms in its gene are a risk factor for development of autoimmunity/lymphoproliferation. Blood. 2004; 103:1376–82. [PubMed: 14592838]

Cichy J, Pure E. The liberation of CD44. J Cell Biol. 2003; 161:839–43. [PubMed: 12796473]

Collins TJ. ImageJ for microscopy. Biotechniques. 2007; 43:25–30. [PubMed: 17936939]

Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest. 2001; 107:1055–61. [PubMed: 11342566]

Farkas S, Hornung M, Sattler C, Anthuber M, Gunthert U, Herfarth H, et al. Short-term treatment with anti-CD44v7 antibody, but not CD44v4, restores the gut mucosa in established chronic dextran sulphate sodium (DSS)-induced colitis in mice. Clin Exp Immunol. 2005; 142:260–7. [PubMed: 16232212]

Fioravanti A, Collodel G. In Vitro Effects of Chondroitin Sulfate. Adv Pharmacol. 2006; 53:449.

Forton AC, Petri MA, Goldman D, Sullivan KE. An osteopontin (SPP1) polymorphism is associated with systemic lupus erythematosus. Hum Mutat. 2002; 19:459. [PubMed: 11933203]

Giachelli CM, Lombardi D, Johnson RJ, Murry CE, Almeida M. Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli in vivo. Am J Pathol. 1998; 152:353–8. [PubMed: 9466560]

Han S, Guthridge JM, Harley IT, Sestak AL, Kim-Howard X, Kaufman KM, et al. Osteopontin and systemic lupus erythematosus association: a probable gene-gender interaction. PLoS One. 2008; 3:e0001757. [PubMed: 18335026]

Hanno R, Callen JP. Histopathology of Gottron’s papules. J Cutan Pathol. 1985; 12:389–94. [PubMed: 4056162]

Hoffmann U, Heilmann K, Hayford C, Stalmach A, Wahnschaffe U, Zeitz M, et al. CD44v7 ligation downregulates the inflammatory immune response in Crohn’s disease patients by apoptosis induction in mononuclear cells from the lamina propria. Cell Death Differ. 2007; 14:1542–51. [PubMed: 17479111]

Janis JF, Winkelmann RK. Histopathology of the skin in dermatomyositis. A histopathologic study of 55 cases. Arch Dermatol. 1968; 97:640–50. [PubMed: 4172448]

Karikari SN, Moore JG, Kirou KA, Crow MK, Utset TO, Niewold TB. Age- and gender-specific modulation of serum osteopontin and interferon-alpha by osteopontin genotype in systemic lupus erythematosus. Genes Immun. 2009; 10:487–94. [PubMed: 19339987]

Katagiri YU, Sleeman J, Fuji H, Herrlich P, Hotta H, Tanaka K, et al. CD44 variants but not CD44s cooperate with beta1-containingintegrins to permit cells to bind to osteopontin independently of arginine-glycine-aspartic acid, thereby stimulating cell motility and chemotaxis. Cancer Res. 1999; 59:219–26. [PubMed: 9892210]

Kawamura K, Iyonaga K, Ichiyasu H, Nagano J, Suga M, Sasaki Y. Differentiation, maturation, and survival of dendritic cells by osteopontin regulation. Clin Diagn Lab Immunol. 2005; 12:206–12. [PubMed: 15643009]
Keller KE, Kelley MJ, Acott TS. Extracellular matrix gene alternative splicing by trabecular meshwork cells in response to mechanical stretching. Invest Ophthalmol Vis Sci. 2007; 48:1164–72. [PubMed: 17325160]

Kim J, Werth VP. Gottron’s papules exhibit accumulation of CD44 variant 7 (CD44v7) and its binding partner osteopontin: a unique molecular signature. J Invest Dermatol (abstr). 2010; 130(Suppl 1):S36.

Kim JS, Werth VP. Identification and regulation of specific chondroitin sulfate species in discoid lupus erythematosus and dermatomyositis. J Histochem Cytochem. 2011; 59:789–90. [PubMed: 21804080]

Lampe MA, Patarca R, Iregui MV, Cantor H. Polyclonal B cell activation by the Eta-1 cytokine and the development of systemic autoimmune disease. J Immunol. 1991; 147:2902–6. [PubMed: 1918998]

Leigh CJ, Palechek PL, Knutson JR, McCarthy JB, Cohen MB, Argenyi ZB. CD44 expression in benign and malignant nevomelanocytic lesions. Hum Pathol. 1996; 27:1288–94. [PubMed: 8958300]

Li X, O’Regan AW, Berman JS. IFN-gamma induction of osteopontin expression in human monocytoïd cells. J Interferon Cytokine Res. 2003; 23:259–65. [PubMed: 12804068]

Marhaba R, Bourrouba M, Zoller M. CD44v7 interferes with activation-induced cell death by up-regulation of anti-apoptotic gene expression. J Leukoc Biol. 2003; 74:135–48. [PubMed: 12832452]

Mendese G, Mahalingam M. Histopathology of Gottron’s papules—utility in diagnosing dermatomyositis. J Cutan Pathol. 2007; 34:793–6. [PubMed: 17880586]

Murai T, Sougawa N, Kawashima H, Yamaguchi K, Miyasaka M. CD44-chondroitin sulfate interactions mediate leukocyte rolling under physiological flow conditions. Immunol Lett. 2004; 93:163–70. [PubMed: 15158613]

Niewold TB, Kariuki SN, Morgan GA, Shrestha S, Pachman LM. Gene-gene-sex interaction in cytokine gene polymorphisms revealed by serum interferon alpha phenotype in juvenile dermatomyositis. J Pediatr. 2010; 157:653–7. [PubMed: 20605164]

O’Regan AW, Hayden JM, Berman JS. Osteopontin augments CD3-mediated interferon-gamma and CD40 ligand expression by T cells, which results in IL-12 production from peripheral blood mononuclear cells. J Leukoc Biol. 2000; 68:495–502. [PubMed: 11037970]

Rachmilewitz J, Tykociński ML. Differential effects of chondroitin sulfates A and B on monocyte and B-cell activation: evidence for B-cell activation via a CD44-dependent pathway. Blood. 1998; 92:223–9. [PubMed: 9639520]

Renkl AC, Wussler J, Ahrens T, Thoma K, Kon S, Uede T, et al. Osteopontin functionally activates dendritic cells and induces their differentiation toward a Th1-polarizing phenotype. Blood. 2005; 106:946–55. [PubMed: 15855273]

Seiter S, Schadendorf D, Tilgen W, Zoller M. CD44 variant isoform expression in a variety of skin-associated autoimmune diseases. Clin Immunol Immunopathol. 1998; 89:79–93. [PubMed: 9756727]

Seiter S, Tilgen W, Herrmann K, Schadendorf D, Patzelt E, Moller P, et al. Expression of CD44 splice variants in human skin and epidermal tumours. Virchows Arch. 1996; 428:141–9. [PubMed: 8688968]

Singh RP, Patarca R, Schwartz J, Singh P, Cantor H. Definition of a specific interaction between the early T lymphocyte activation 1 (Eta-1) protein and murine macrophages in vitro and its effect upon macrophages in vivo. J Exp Med. 1990; 171:1931–42. [PubMed: 2351930]

Sleeman JP, Kondo K, Moll J, Ponta H, Herrlich P. Variant exons v6 and v7 together expand the repertoire of glycosaminoglycans bound by CD44. J Biol Chem. 1997; 272:31837–44. [PubMed: 9395530]

Weber GF, Ashkar S, Glimcher MJ, Cantor H. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). Science. 1996; 271:509–12. [PubMed: 8560266]

Weber GF, Cantor H. Differential roles of osteopontin/Eta-1 in early and late lpr disease. Clin Exp Immunol. 2001; 126:578–83. [PubMed: 11737079]
Weiss JM, Renkl AC, Maier CS, Kimmig M, Liaw L, Ahrens T, et al. Osteopontin is involved in the initiation of cutaneous contact hypersensitivity by inducing Langerhans and dendritic cell migration to lymph nodes. J Exp Med. 2001; 194:1219–29. [PubMed: 11696588]

Wenzel J, Scheler M, Bieber T, Tuting T. Evidence for a role of type I interferons in the pathogenesis of dermatomyositis. Br J Dermatol. 2005; 153:462–3. author reply 3-4. [PubMed: 16086781]

Wittig BM, Johansson B, Zoller M, Schwarzler C, Gunthert U. Abrogation of experimental colitis correlates with increased apoptosis in mice deficient for CD44 variant exon 7 (CD44v7). J Exp Med. 2000; 191:2053–64. [PubMed: 10859330]

Wittig BM, Stallmach A, Zeitz M, Gunthert U. Functional involvement of CD44 variant 7 in gut immune response. Pathobiology. 2002; 70:184–9. [PubMed: 12571424]

Wong CK, Lit LC, Tam LS, Li EK, Lam CW. Elevation of plasma osteopontin concentration is correlated with disease activity in patients with systemic lupus erythematosus. Rheumatology (Oxford). 2005; 44:602–6. [PubMed: 15705633]

Xu CX, Jin H, Chung YS, Shin JY, Woo MA, Lee KH, et al. Chondroitin sulfate extracted from the Styela clava tunic suppresses TNF-alpha-induced expression of inflammatory factors, VCAM-1 and iNOS by blocking Akt/NF-KB signal in JB6 cells. Cancer Letters. 2008; 1:93. [PubMed: 18295395]
Figure 1. Gottron’s papules exhibit unusually dense deposits of chondroitin-4-sulfate in the papillary dermis

Biopsies are stained with an antibody against chondroitin 4-sulfate (C4S). a. Healthy control skin (HC, non-IP); b. DM lesions (DM, non-GP); c. the PIP of healthy volunteers (HC, IP); d. Gottron’s papules (GP). Representative photomicrographs are shown, original magnification x5, bar = 100 m; e. Systematic quantification of C4S staining intensity within the papillary dermal matrix. Every data point is displayed; wide and short horizontal bars represent mean and SEM, respectively. P<0.0001 by ANOVA; (***, P<0.001; **, P<0.01; n.s., not significant).
Figure 2. Gottron’s papules, but not non-Gottron’s DM lesions, exhibit abundant accumulation of CD44v7 in the papillary dermis
Sequential sections taken from the same biopsies shown in Figure 1 are stained with an antibody specific for CD44v7. a. Healthy control skin (HC, non-IP); b. Non-Gottron’s DM lesions (DM, non-GP); c. The PIP of healthy volunteers (HC, IP); d. Gottron’s papules (GP). Representative photomicrographs are shown, original magnification x5, bar = 100 m; e. Systematic quantification of CD44v7 staining intensity within the papillary dermal matrix. Every data point is displayed; wide and short horizontal bars represent mean and SEM, respectively. P=0.0003 by ANOVA; (***, P<0.001; **, P<0.01; n.s., not significant).
Figure 3. Both Gottron’s papules and non-Gottron’s DM lesions exhibit abundant accumulation of osteopontin, a CD44v7 binding partner, in the papillary dermis
Sequential sections taken from the same biopsies shown in Figures 1 and 2 are stained with an antibody specific for osteopontin. a. Healthy control skin (HC, non-IP); b. Non-Gottron’s DM lesions (DM, non-GP); c. The PIP of healthy volunteers (HC, IP); d. Gottron’s papules (GP). Representative photomicrographs are shown, original magnification x5, bar = 100 m; e. Systematic quantification of osteopontin staining intensity within the papillary dermal matrix. Every data point is displayed; wide and short horizontal bars represent mean and SEM, respectively. P=0.0036 by ANOVA; (**, P<0.01; *, P<0.05; n.s., not significant).
Figure 4. Mechanical stretching induces CD44v7 in cultured human dermal fibroblasts
Confluent cultured human dermal fibroblasts grown on flexible membranes were subjected to tonic mechanical stretching. All cells were plated simultaneously and stretched for the final 6 or 12 h of incubation, after which RNA was harvested simultaneously. a. CD44v7 mRNA levels were assayed by qRT-PCR, normalized to PPIA mRNA levels, and then expressed relative to unstretched controls. Every data point from a representative experiment is displayed; wide and short horizontal bars represent mean and SEM, respectively. P=0.0017 by ANOVA; (**, P<0.01; n.s., not significant); b. CD44v7 protein in the supernatant of stretched fibroblasts at 6 hours were assayed by western blot and expressed relative to unstretched controls; c. Mean CD44v7 protein detected in one representative experiment; horizontal bar represents SEM. P=0.0321 by ANOVA (*, p<0.05).
Figure 5. Interferon-γ, but not interferon-α or interleukin-1α, induces osteopontin in cultured dermal fibroblasts

Confluent cultured human dermal fibroblasts were incubated for 6h in control medium or in medium supplemented with IFNγ (10ng/mL), IFNα (100U/ml), or IL-1α (20ng/ml), after which RNA or conditioned media for ELISA were harvested; data from representative experiments are displayed. a. Osteopontin (OPN) mRNA levels were assayed by qRT-PCR, normalized to PPIA mRNA levels, and expressed relative to control; points denote mean OPN/PPIA mRNA for each well (sampled in triplicate), wide and short horizontal bars represent mean and SEM of triplicate conditions, respectively. P<0.0001 by ANOVA (***, P<0.001; n.s., not significant); b. Osteopontin protein in control and conditioned media was quantitated by ELISA; mean osteopontin protein detected (ng/ml) is indicated, horizontal bar represents SEM of triplicate conditions. P=0.0003 by ANOVA; (***, P<0.001; n.s., not significant).
Figure 6. Mechanical stretching of fibroblasts, particularly in combination with osteopontin treatment, augments the adhesion of THP-1 monocytes, in a process dependent on CD44v7

Confluent monolayers of cultured human dermal fibroblasts grown on flexible membranes were incubated overnight without or with osteopontin (OPN), without or with stretch, as indicated. Unbound OPN was rinsed away, and then adherence of fluorescently labeled THP-1 monocytes onto these monolayers was assessed. Wells were incubated for one hour following treatment with anti-CD44v7 blocking antibody or isotype control Ig, and then washed just before addition of the labeled monocytes. Representative fluorescent micrographs of adherent monocytes (green dots) are shown. a. Control fibroblasts; b. Mechanically stretched fibroblasts; c. Mechanically stretched fibroblasts pretreated with recombinant OPN; d. Counts of adherent, fluorescent monocytes per high-power field (HPF; means±SEM, n=6), P<0.0001 by ANOVA; (***, P<0.001; **, P<0.01; *, P<0.05; n.s., not significant).