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Letter to the Editor

Autoreactivity to sweat and sebaceous glands and skin homing T cells in lupus profundus

Lupus erythematosus profundus or lupus erythematosus panniculitis (LEP) is a clinical variant of lupus erythematosus, which involves the deep dermis and subcutaneous fat tissue. Here we illustrate some previously undescribed findings in LEP by direct immunofluorescence (DIF) and by immunohistochemistry (IHC). These include autoreactivity to the sweat and sebaceous glands that were in close proximity to the inflamed panniculus, and a strong expression to ezrin, radixin and moesin; (ERM) with colocalization of CD44, CD3, CD4, CD8, CD45 and HLA DP,DR,DQ. Based on our findings, ERM seem to contribute to the polarization of the extracellular matrix to allow T lymphocyte homecoming locomotion within the skin, including sweat and sebaceous glands and panniculus in LEP.

Report

A 48-year-old African American female presented with a three-month history of multiple, asymptomatic, erythematous plaques and nodules on the leg. She denied arthralgia, arthropathy, myalgia, fatigue, fever, Raynaud’s phenomenon, gastrointestinal symptoms, and lesions occurring on the head, trunk, and upper extremities. We found no associated systemic symptoms. At the physical exam we detected multiple, erythematous nodules and indurated plaques on the lower right thigh. Lesions were skin-colored, plaques. Laboratory data shows and erythrocyte sedimentation rate of 3 mm/h. A complete blood count with differential was normal. Antinuclear antibody titer was normal. Antibodies to double-stranded DNA, SS-A, and SS-B were negative. Anti-streptococcal antibody titers were negative. Levels of antinuclear antibodies and its secondary we used Alexa Fluor® 647-conjugated anti-mouse (both from Invitrogen, USA).

DIF

In brief, 4 μm thick skin cryosections were partially fixed on 3% paraformaldehyde, and incubated with the secondary antibodies all FITC-conjugated. The following antibodies were raised on rabbit directed to a) anti-human IgG (γ chain), b) anti-human IgA (α chains), c) anti-human IgM (μ chain), d) anti-human fibrinogen, and e) anti-human albumin (all at either 1:20 to 1:40 dilutions), all from Dako, Carpenteria, California, USA. In addition to anti-ezrin antibodies and its secondary we used Alexa Fluor® 647-conjugated anti-mouse.

IHC

To study the possible correlation of the [ezrin, radixin and moesin; (ERM)] and the homecoming immune cells to the skin, we performed IHC by using a dual endogenous peroxidase blockage, according to the Dako (Denmark) insert, with the addition of Envision dual link. Furthermore, we applied 3, 3 diaminobenzidine and counterstained with hematoxylin. The samples were run in a Dako Autostainer Universal Staining System. We tested for goat anti-human CD44, CD3, CD4, CD8, CD45, HLA DP,DR,DQ and anti-human IgM. Our tests showed positive staining by both DIF and IHC of the sweat as well as the sebaceous by various antibodies. In addition we detected overexpression of ezrin with colocalization of antibodies directed to CD44, CD3, CD4, CD8, CD45 and autoreactivity with IgM. See Figures 1 and 2. Following the diagnosis of LEP, the patient was treated with plaquenil, 200 mg/day and the response to this therapy was characterized by slow regression of the inflammatory lesions.

Discussion

Only few cases in the world have described autoreactivity to sweat and sebaceous glands in lupus panniculitis as shown in our case. We tested for ERM and CD44 based on the fact that these molecules have been shown to promote T cell activation [1]. CD44 is a binding partner for the membrane–cytoskeleton cross-linker protein ezrin. The transmembrane receptor CD44 conveys important signals from the extracellular microenvironment to the cytoplasm, a phenomenon known as "outside–in" signaling [1–3]. As described for other authors, we demonstrated that infiltrating T lymphocytes seem to be directed by a signal from the CD44, a receptor for extracellular matrix...
proteins and glycosaminoglycans [1–3]. These findings suggest that migratory polarity of some immune cells including some T cell subpopulation may depend on the recruitment of ERM proteins by the intracellular domain of CD44 and other cells including CD3, CD4, CD8, CD45 and HLA DP,DR, DQ. Other studies have shown other immunologic roles of ERM based on the fact that CD44–ezrin–actin is an important modulator of Fas-mediated apoptosis signal pathway [4,5].

LEP is characterized by a predominately lobular, B-cell lymphocytic and plasmacytoid infiltration in the subcutaneous adipose tissue, with progressive fibrosis and septal
scarring. LEP is an unusual presentation of lupus erythematosus, characterized by deep subcutaneous nodules, most commonly localized to the upper limbs and face [6,7]. The clinical and histologic diagnoses of LEP may be difficult in cases in which involvement of subcutaneous fat tissue is the only manifestation of the disease. LEP has also been reported to associate with discoid lupus erythematosus (DLE) [7]. In our case, in addition to positive classic lupus band test, we detected autoreactivity to sweat and sebaceous glands by DIF as well as a strong autoreactivity directed to ERM, both at the inflamed glands as well as the epidermis above the panniculitis and under the panniculitis.

In a related case of lupus erythematosus treated with cyclophosphamide, other authors have reported that DIF showed not only positive lupus band test but also a autoreactivity two eccrine sweat glands also occurred as described in our case [8]. Other authors have described a novel autoantibody reactive with carbonic anhydrase, which is present in sera from patients with systemic lupus erythematosus (SLE) [9]. This autoantibody is directed against sweat glands, as well as kidney [9]. In a further large study, the skin biopsies from 62 lupus erythematosus patients were examined by DIF to determine the presence of IgG, IgM, IgA and C3 among different structures of the skin [10,12]. In addition to positive lupus band test, autoreactivity was observed to some collagen fibers, dermal capillaries, the basement membrane zone of selected hair follicles, sweat glands and arrector pili muscles [5].

As related in our case, other authors reported of a 53-year-old woman suffering with SLE with vasculitis and overexpression of ezrin in her renal biopsy, and the presence of a significant number of T cells in the renal interstitial [11]. The T cells in the kidney were found to express CD44 and phosphorylated ezrin thus accounting for their renal homing [10]. In addition, the authors reported that T lymphocytes in SLE display increased levels of CD44; ERM-phosphorylation; actin polymerization, and chemotactic migration, when compared with T cells in patients with rheumatoid arthritis and normal individuals. The significance of our findings in DIF and IHC in regards of the presence of ERM in proximity with the affected lesions and colocalization with CD3, CD4, and CD8, CD45, CD44 and HLA DP,DR,DQ antibodies in addition to autoreactivity detected when using IgM against sweat and sebaceous glands maybe indicative that CD44–ERM CD4, CD8, CD45 HLA DP,DR,DQ signaling pathway may be involved in the homing of T cells to the skin in LEP.

Conflicts of interest
None.

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References


Figure 1  (a) In this Figure, are the clinical, H & E, IHC and DIF findings of LEP, with specific attention to the overexpression of [ezrin, radixin and moesin; (ERM)] on epidermis and in the sweat glands in proximity to the LEP phenomenon. (a) An infiltrate consisting mostly of lymphocytes and histiocytes around eccrine sweat and sebaceous glands (yellow arrows) (100×). (b) By DIF, we observed positive immunostaining of the eccrine sweat glands, with IgG-FITC-conjugated antibodies (green) (red arrows) (400×). In c, d, g, h, the nuclei were counterstained using Dapi (blue staining) (white arrows). (c) The DIF also showed a positive immunostaining with the eccrine sweat glands when FITC-conjugated anti-human fibrinogen antibodies were used (green staining) (red arrows) (400×). (d) In addition, positive staining was observed around the inflamed eccrine sweat glands when an anti-ezrin monoclonal antibody was used at a 1:30 dilution (red staining) (dark yellow arrows). The red arrow shows positive stain of the sweat glands when using the antiserum anti-human fibrinogen FITC conjugated. (e) IHC shows a positive stain of CD8 cells around the sweat gland. (f) A DIF revealed the positive lupus band test for IgA, IgG, IgM, and C3, in which all FITC-conjugated antibodies showed a linear distribution at the dermal/epidermal junction (red arrow). In addition, some deposits by the same antibodies were seen between epidermal cells (pink arrow). (f) Another DIF using FITC-conjugated anti-human IgA antibodies revealed deposits of IgA at the dermal/epidermal junction in a linear pattern (red arrow). In addition, some deposits of clustered IgA were seen between several epidermal cells (pink arrow) (400×). (g) By DIF, a strong granular circular staining (circular around the epidermal cells) was seen when FITC-conjugated anti-human-albumin antibodies were used (yellow arrows). (h) A strongly positive ezrin was seen at all levels of the epidermis, as well as in some foci within the superficial dermis (red) (yellow arrows) (400×). (i) H & E staining demonstrates a predominately lobular panniculitis with infiltration of lymphocytes and histiocytes in the deep dermis (yellow arrow). In addition, there is a lobular panniculitis with a dense infiltrate of lymphocytes, plasma cells, and macrophages. Focal hyalinization of the adipocytes is present. (j and k) Positive CD45 and CD8 cells around the panniculitis. (l) A clinical picture of the panniculitis.
Figure 2 IHC of the skin. The red arrows show positivity as following: (a) Positive IgM around the sweat glands. (b) Positive CD44 around the sweat glands (colocalizing with the ezrin overexpression), as well as in some superficial vessels. (c) CD45 positive cells were seen around the superficial vessels. (e) H & E lymphohistocytic infiltrated around the sweat glands (blue arrow) and under them (white arrow). (f) Positive IHC staining of the same lymphohistocytic inflammatory using anti-human IgM antibody. (g and h) CD8 and CD4, respectively positive in the deep panniculus surrounding the sweat glands and their ductus. (i) HLA DP,DR,DQ, positive in the lymphohistocytic infiltrated above the panniculitis. (j) Positive staining around the sweat (white arrow) and the sebaceous glands (red arrows) with IgM by IHC. (k and l) IgM positive around the pilosebaceous complex and on the panniculitis respectively.


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