Case Report

Vimentin compartmentalization in discoid lupus

[1]Ana Maria Abreu-Velez, M.D. Ph.D, [2]Graham Smith, Jr., M.D., [1]Michael S. Howard, M.D.

[1]Georgia Dermatopathology Associates, Atlanta, Georgia, USA.
[2]Diagnostic and Medical Clinic/Dermatology, Mobile, Alabama, USA.

Abstract

Context: Discoid lupus erythematosus (DLE) is a chronic skin condition, often presenting inflammatory, scarring lesions predominating on sun exposed areas of the face and scalp. **Case Report:** A 46-year-old black female was evaluated for possible DLE. Biopsies for hematoxylin and eosin (H&E) and immunohistochemistry (IHC) examination, as well as for direct immunofluorescence (DIF) analysis were performed. The H&E staining demonstrated mild epidermal atrophy with focal follicular plugging. A mild interface infiltrate of lymphocytes and histiocytes and a superficial and deep, perivascular and periadnexal dermal infiltrate of lymphocytes, histiocytes and plasma cells was observed The DIF revealed strong deposits of immunoglobulins IgG, IgM, fibrinogen and Complement/C3, present in a granular pattern at the basement membrane junction (BMZ) of the skin as well as in the BMZ of the sebaceous glands. In addition, deposits of IgA surrounding the superficial dermal blood vessels were appreciated. The IHC displayed compartmentalization of vimentin around the BMZ of both the superrficial skin and sebaceous gland BMZs, as well as similar patterns of deposits of the same immunoglobulins, complement, and fibrinogen as visualized by DIF. **Conclusions**: Minimal attention has been given to the process of compartmentalization of the dermis in inflammatory skin conditions, including DLE. However, it seems that in addition to the classical immunoglobulin and complement "lupus band" deposits at the BMZ, an additional, orchestrated immunologic reorganization of the dermis surrounding the inflammatory process is also present. Such an immunologic reorganization of the dermis could play a significant role in the pathophysiology of this disorder.

Keywords: Vimentin, discoid lupus erythematosus, autoantibodies.

Correspondence to: Ana Maria Abreu-Velez, M.D., Ph.D., Georgia Dermatopathology Associates, 1534 North Decatur Rd. NE; Suite 206, Atlanta, GA 30307-1000, USA, Tel.: (404) 3710027, Fax: (404) 3711900**,** Email: abreuvelez@yahoo.com.

Introduction

Lupus erythematosus is an autoimmune disease. The cause of lupus is unknown, and the disease may present in multiple clinical forms. These clinical forms include 1) systemic lupus erythematosus (SLE) may affect any body organ, and 2) discoid lupus erythematosus (DLE), which is often clinically less severe, and classically affects only the skin [1, 2]. About 5–10% of patients with DLE will progress to systemic lupus. Other forms of lupus may also occur [1, 2].

DLE is a chronic skin condition of inflammatory plaques with scarring, favoring the face, ears, and scalp and at times on other body areas [1, 2]. These lesions develop as a red, inflamed patch with a scaling and crusty appearance. The patch center may appear lighter than normal skin, with the patch rim areas darker than normal skin. When lesions occur in areas such as the beard or scalp, permanent hair loss and scarring may occur [1, 2]. Sometimes, the trunk as well as the extremities is more extensively involved. The skin lesions may vary in appearance; a red bump or patch may appear first, and be painless or only slightly itchy [1, 2]. The area may be scaly, or even wart-like. With time, the center of the lesion may become white and scarred. A small percentage of patients with DLE may develop disease sequelae within internal organs [1, 2]. Children, as well as people with multiple skin lesions are at increased risk of these internal disease

developments. If a physician wishes to evaluate a patient for lupus, a skin biopsy should be done to confirm the diagnosis. If the skin biopsy shows DLE, then further blood testing may be indicated.

The exact cause of DLE is unknown; however, the disorder is believed to be autoimmune in nature, with the patient's immune system pathologically assaulting normal skin areas [1, 2]. Further, DLE often runs in families, and females with DLE outnumber males 3 to 1. In some patients with DLE, sunlight and cigarette smoking may incite development of lesions. Cortisone ointment applied to the skin in the involved areas will often improve the lesions, and slow their progression. Cortisone injections into the lesions may also be utilized to treat DLE, and typically are more effective than cortisone ointment [1, 2]. Alternatively, calcineurin inhibitors, pimecrolimus cream or tacrolimus ointment may be used. Imiquimod has also been reported to be helpful in a few patients. Plaquenil (hydroxychloroquine) will also often improve the condition [1, 2].

Patients on plaquenil need eye exams once a year to prevent damage to the retina of the eye, as well as periodic blood work. Drugs closely related to plaquenil (such as chloroquine and quinacrine hydrochloride) may be more effective in addressing the clinical lesions, but also display more side effects. Other drugs, such as isotretinoin and acitretin, were also be used [1, 2]. Patients whose condition is sensitive to sunlight need to wear ultraviolet A and/or B (UVA/UVB) blocking sunscreens daily, as well as a hat or other covering while outdoors [1, 2]. Follow-up with the doctor is important every six months to a year to prevent disease spread and to minimize scarring.

Case Report

A 46-year-old black female presented with clinical lesions consistent with DLE. She denied systemic symptoms of arthralgia, arthropathy, myalgia, fatigue, Raynaud's phenomenon and gastrointestinal complaints. Lesions were present on the head, trunk, and upper extremities, and presented as reddish-purple, atrophic plaques. Laboratory data showed a normal complete blood count (CBC) with differential analysis, and a normal erythrocyte sedimentation rate. Antiphospholipid antibody testing was negative; serum electrolytes, blood urea nitrogen, creatinine, and liver function tests, as well as urinalysis and chest radiographs were within normal limits. The antinuclear antibody (ANA) titer was normal. Specific ANA screening yielded negative results for anti-Smith, anti-double stranded DNA (dsDNA), and anti-histone antibodies. Tests for anti ribonuclease-sensitive antigen (RNAse), extractable nuclear antigen (ENA), small nuclear antigen) (sn), ribonucleoproteins (RNP), and U1, and U2 complexes were negative, as was testing for anti-SS-A (anti-Ro) and anti-SS-B (anti-La). Levels of Complement C3 and C4 were normal. Biopsies of the lesions were performed, with subsequent Hematoxylin and Eosin (H&E) staining, multicolor direct immunofluorescence (DIF) and immunohistochemistry

(IHC) studies.

H & E: Routine H&E staining was performed, and examination of the tissue sections revealed mild epidermal atrophy with focal follicular plugging. A mild interface infiltrate of lymphocytes and histiocytes was noted. Within the dermis, a superficial and deep, perivascular and periadnexal infiltrate of lymphocytes, histiocytes, and plasma cells was observed. Neutrophils and eosinophils were rare. Increased dermal mucin was not found; disorganization of the dermis was observed on H & E (Figs. 1 and 2).
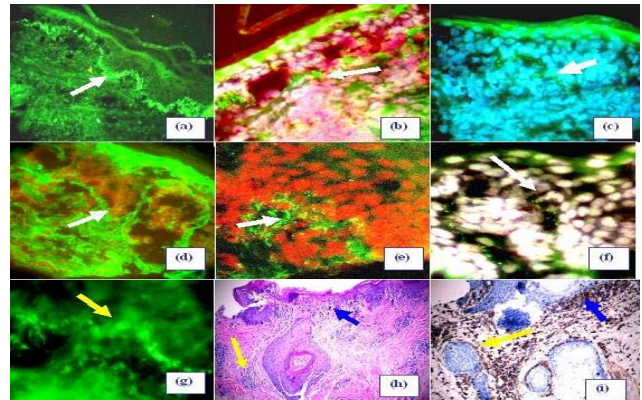
Fig. 1 (a) through (f). DIF. All of these sections were partially fixed using 3.5% paraformaldehyde to improve the visibility of any lipidic antigens. (a), Reactivity to the BMZ of the skin is displayed utilizing FITC conjugated anti-human IgM (green BMZ staining, white arrow), without counterstaining. In (b), we performed identical primary staining, and counterstained the nuclei with TO-PRO®-3/DNA (pinkish) for 30 minutes. In (c), we performed identical primary staining, and counterstained the nuclei with DAPI (blue). In (d), we performed identical primary staining, and counterstained with TO-PRO®-3/DNA five minutes (pink/orange). In (e), we performed identical primary staining, and counterstained with TO-PRO®-3/DNA for 20 minutes (pink/red). Finally, in (f), we performed identical primary staining, and counterstained the nuclei with Hoechst H1399 (white). In (g), staining was performed as in (a), but without prefixation with paraformaldehyde; please note that the lupus BMZ band is less well defined (yellow arrow). In (h), H & E staining demonstrates mild epidermal atrophy with focal follicular plugging. A mild interface infiltrate of lymphocytes and histiocytes is noted. Within the dermis, a superficial and deep, perivascular and periadnexal infiltrate of lymphocytes, histiocytes, and plasma cells is observed. Histologic alteration of the base membrane zone of the skin is noted (blue arrow), as well as a mild perifollicular lymphocytic infiltrate (yellow arrow) (200X). In (i), from the same H & E block, an IHC stain was performed using anti-human vimentin antibody, confirming the alterations in the BMZ (blue arrow) and mild aggregation of vimentin around the sebaceous glands and hair follicles (yellow arrow).
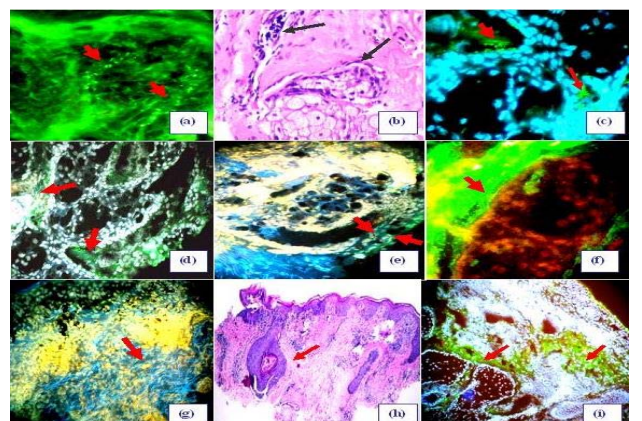
Fig 2 (**a**). **DIF** shows punctuate deposits of FITC conjugated anti-human fibrinogen deposited at the BMZ of the skin, which could be confused with background immunofluorescence (red arrows). In (**b**), H & E staining displays peri-sebaceous gland inflammation, predominately by lymphohistiocytic cells (black arrows) (200X). In (**c**) through (**g**) and (**i**), prefixation of the sections for DIF was performed utilizing paraformaldehyde In (**c**), the nuclei were stained with DAPI (blue), and the resultant color contrast aids in the visualization of green immunostaining around the sebaceous glands (green staining) (red arrows). In (**d**), we counterstained the nuclei with Hoechst H1399 (white), and again, excellent contrast was obtained between pathologic autoreactivity and background staining around the sebaceous glands (red arrows). In (**e**), Positive staining is noted around a sebaceous gland BMZ using FITC conjugated anti human IgM antibody (yellowish-white staining) (red arrows). In (**f**), we counterstained the nuclei of the sebaceous glands with TO-PRO®-3/DNA (brown staining) and the deposits of the FITC conjugated anti-fibrinogen are thus highlighted outside the sebaceous glands (strong green staining) (red arrow). In (**g**), we performed DIF and counterstained the nuclei with Hoechst 33342 (yellow staining). The color contrast helped to identify dermal disorganization in the superficial as well as the reticular dermis. In this case, we utilized anti-human vimentin conjugated with Pacific blue (blue staining) (red arrow). (**h**) H & E staining shows lymphohistiocytic infiltration around the sebaceous glands (red arrows). Finally, in (**i**), we observe green autoreactivity around the sebaceous glands when utilizing FITC conjugated anti-human IgM (red arrows).

DIF: Skin cryosections were prepared, and incubated with multiple fluorescein isothiocyanate (FITC)-conjugated secondary antibodies. The secondary antibodies were of rabbit origin and included: 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 used at 1:20 to 1:60 dilutions and obtained from Dako (Carpinteria, California, USA). We also utilized secondary antibodies of goat origin, including: a) anti-human IgE antiserum (Vector Laboratories, Bridgeport, New Jersey, USA) and b) anti-human C1q (Southern Biotech, Birmingham, Alabama, USA). Finally, monoclonal anti-human IgG4 FITC conjugated antibody was used to test for possible intercellular staining between keratinocytes (Sigma-Aldrich Saint Louis, Missouri, USA). The slides were then counterstained with 4',6-diamidino-2-phenylindole (Dapi) (Pierce, Rockford, Illinois, USA) or TO-PRO®-3/DNA combined with (ANA Hoesch H1399. We also counterstained with Hoechst 33342 (Invitrogen Corporation, Carlsbad, California USA). Slides were then washed, coverslipped, and dried overnight at 4°C. In Figures 1 and 2, we demonstrate some of the most prominent H&E and DIF patterns observed, as well as the improvement of the quality and power of DIF staining by the use of simultaneous multicolor fluorescence and counterstaining of cell nuclei. The DIF results were classified as follows: (0, negative; +, weak positive; ++ and +++, positive; and ++++, maximum positivity). We found 1) deposits of IgG, in a granular pattern at the basement membrane zone (BMZ) of the dermal/epidermal junction, as well as at the BMZ of the sebaceous glands (+++); 2) deposits of IgA surrounding the superficial dermal blood vessels (++); 3) deposits of IgM in a granular pattern at the BMZ of the skin (+++); 4) deposits of complement C3 in a granular pattern at the BMZ of the skin and of the sebaceous glands (+++) and 5) deposits of fibrinogen, in a granular pattern at the BMZ of the skin (++).

IHC: To study correlation with our vimentin DIF data, we performed IHC utilizing a Dako dual endogenous peroxidase blockage system, with the addition of an Envision dual link. We utilized Dako technical instructions for staining. We applied 3, 3 diaminobenzidine as an IHC chromogen, and counterstained with hematoxylin. Fig. 3 highlights our results, including the compartmentalization of vimentin around several skin appendices where the DIF autoreactivty was detected. The degree of compartmentalization of vimentin by IHC parallels the deposition of immunoglobulins, complement and other immunosurfactants (such as fibrinogen) by DIF.
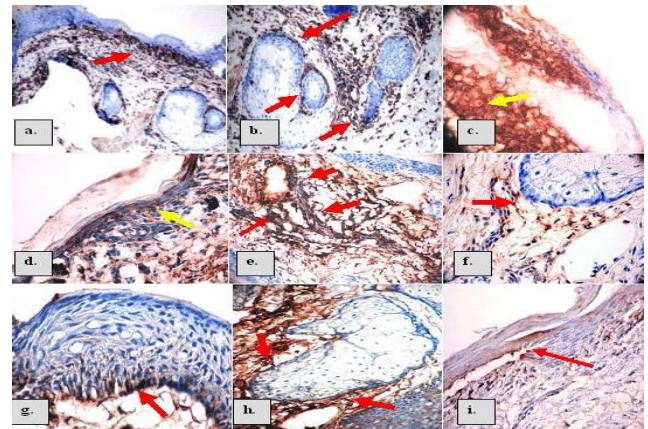
Fig. 3 IHC findings. a, Shows compartmentalization of vimentin at the BMZ of the skin (red arrow), and in **b** around the BMZ of the sebaceous glands at higher magnification (red arrows). **c**. Strong positive staining of the keratinocytes around the hair follicle of a pilosebaceous unit with albumin (yellow arrow). **d**. Positive deposits of fibrinogen at the altered BMZ (yellow arrow). **e**. Deposition of fibrinogen around a sebaceous gland, with concurrent damage to the sebaceous gland (red arrows). **f.** Positive staining of vessels around a sebaceous gland utilizing IgM (red arrow). **g**. Positive staining at the skin BMZ with Complement/C3 (red arrow) and, in **h**, against the BMZ of a sebaceous gland (red arrows). **i**. Positive deposits of IgM are noted along the altered BMZ of the skin (red arrow).

Discussion

The classic hallmark in the diagnosis of DLE is the presence of a positive lupus band test (LBT) [3-5]. This test utilizes immunofluorescence to determine the presence and extent of immunoglobulin and complement deposits at the dermal-epidermal junction of skin specimens from patients with DLE or systemic lupus erythematosus. The lupus band test is performed with direct immunofluorescence (DIF) staining; in a positive test, both IgG and complement deposition are found at the dermal-epidermal junction [3-5]. The LBT test can be helpful in distinguishing SLE from DLE, in that the lupus band test will be positive in both clinically involved and uninvolved skin in SLE, whereas in DLE only the involved skin will test positive [3-5]. A lupus band test that reveals granular deposition of immunoglobulins and complement at the base memebrane zone of the dermal/epidermal junction is characteristic, but not pathognomonic for lupus erythematosus. When strict, linear deposition criteria for a positive lupus band test are used, a positive result is highly specific for the diagnosis of lupus erythematosus. Notably, a positive LBT is

infrequently found in clinically normal skin of patients with other connective tissue disorders [3-5]. Cutaneous lupus may be difficult to separate from other morphologically similar clinical disorders. Thus, the lupus band test is often useful in narrowing the differential diagnosis because other disorders have either a different, specific pattern of immunoglobulin deposition, or no known immunoglobulin deposition [3-5]. Although in DLE much diagnostic attention has been directed at the lupus band test, we recognized when performing our case workup that the extracellular matrix surrounding the main inflammatory process at the base membrane zone of the skin and the sebaceous glands demonstrated a strong accumulation and reorganization of vimentin. In relation to this finding, we suggest that inflammatory cells are likely required to move into the inflamed area utilizing the structural plasticity of the dermal extracellular matrix. We found minimal scientific literature focus on the "reorganization and compartmentalization" of the extracellular matrix in the presence of inflammatory and/or autoimmune processes, and minimal correlation of this process with the fact that dermal mesenchymal cells express vimentin as an intermediate filament [6-8]. Interestingly, vimentin is an antigen targeted in selected rheumatological autoimmune diseases [6-8]; further, it has been shown that IgG binding to cytoskeletal intermediate filaments activates the complement cascade [7].

We observed a spatial arrangement of vimentin. Intermediate filaments are not polar, and there is only a very small pool of non-filamentous proteins interfacing with filamentous vimentin formation. It appears that intermediate filaments are formed by two mechanisms: 1) cotranslation around the nucleus, and 2) rapid formation from a small pool of monomers. The precise function(s) of intermediate filaments remains one of the unsolved enigmas in cell biology. However, a number of suggestions have been offered, including 1) mechanical integrators of space, 2) cell regulation, and 3) modulators of nuclear function.   In addition, some studies have demonstrated of the cytoskeleton in inflammation, including during oxidative stress [8, 9]. During activation of neutrophils at an inflammatory site, and/or following hypoxic reperfusion events, reactive oxygen species are generated; superoxide (O2) is generated from NADH oxidase and can dismutate to form hydrogen-peroxide (H_2O_2). Hydrogen peroxide may then react with superoxide when catalytic iron (or another oxidation/reduction active metal) is present to produce the highly reactive hydroxyl radical by means of the Fenton reaction. Receptor-cytoskeleton interactions and membrane traffic may regulate chemoattractant-induced superoxide production in human granulocytes [8, 9]

Autoantibodies to cytoskeletal proteins occur in rheumatoid arthritis and in other connective tissue disorders, such as Sjogren's syndrome and SLE [6, 10]. Antibodies to intermediate filaments are widespread in autoimmune disorders, with antibodies to vimentin occurring in up to 80% of patients with rheumatoid arthritis and a vimentin cross-reactive epitope of type 12

streptococcal M protein documented in patients with rheumatic fever and cardiac autoimmunity [6, 10]. A subset of antibodies from patients with SLE reacts with both DNA and vimentin [10]; these antibodies are specific for the IgM class [11].

Any use of vimentin autoantibodies for diagnostic purposes may be hampered by a lack of specificity, as they occur in 12% of apparently normal subjects. The naturally occurring vimentin antibodies are of the IgG and IgM classes [3, 4]. The biological relevance of these antibodies remains obscure, as does the mechanism by which they arise. Indeed, whether they are protective or damaging is unknown. These antibodies may prevent the overactivation of complement by aiding opsonisation and removal of selected intermediate filaments. Conversely, the antibodies may direct the immune system to such filaments. In acute proliferative glomerulonephritis, which may occur after streptococcal infection, cross reactive antibodies are present that react with a different epitope on type 1M and type 12M streptococcal proteins, as well as with vimentin.

Vimentin may play a role in cell/complement activation; specifically, vimentin may act to bring inflammatory cells into proximity with the lupus band, where the primary tissue damage occurs in DLE. Some experiments have shown that when tissue sections of fixed cells are incubated with normal serum, specific binding of serum complement components (Clq, C3, and C4) to the intermediate filaments occurs. The binding is rapid for Clq, and causes complement activation through the classical pathway. Accumulation of C3 occurs; the C3 binding may be through the alternative pathway, and seems to be independent of antibodies. The activation of complement causes degradation of the intermediate filaments, which are highly sensitive to proteolysis [12, 13] Intermediate filaments are, therefore, endogenous activators of complement. It has been shown that monocytes can bind to cells which have been previously incubated with serum and that such monocyte binding is closely associated with intermediate filament structures [12, 13]. It has been also shown by Western blotting that vimentin filaments (but not monomeric vimentin) possess high affinity saturable binding sites for IgG. The binding occurs through the Fc portion of the IgG molecule, and most likely in the Cy2 domain [13]. In endothelial cells, IgG aggregates have been shown to be closely associated with intermediate filaments *in vivo*. Furthermore, intermediate filaments activate complement by formation of complexes with IgG and Clq [13]. Therefore, monocyte binding may help in the *in vivo* removal of exposed intermediate filaments or non-viable, damaged cells by opsonisation. A prerequisite for this mechanism is damage to the plasma membrane; thus, viable cells would be unaffected, producing a self regulating system [13]. When tissue damage occurs, monocyte recruitment is needed, and might occur as a result of the release of chemotactic factors generated during complement activation. In summary, the presence of large quantities of vimentin intermediate filaments may be important in DLE as a recruiter of monocytes, which could in turn play multiple pathophysiologic roles.

By utilizing several techniques, we were able to correlate the presence of vimentin in close proximity to areas where autoreactivities were observed in a patient with a DLE. DLE is a chronic autoimmune skin disease that usually affects the sun-exposed skin, but eyelid involvement around the meibomian glands (modified sebaceous glands in the eyelids) sometimes occurs [14, 15]. Lipid antigens (and/or antigens closely associated with the BMZ of the sebaceous glands, including lupus anticoagulants) on the phospholipid membrane surface are associated with the pathophysiology of this disorder. Since lipid antigens are better identified by prefixation of the samples in paraformaldehyde, ethanol or methanol, we suggest prefixation of biopsy samples for several minutes in patients possibly affected by DLE, since these antigens could then be unmasked for analysis [14, 15].

In a recent study of systemic lupus erythematous utilizing a multiplex approach to disease autoantigen definition, five distinct clusters of IgG autoreactivity were noted in the sera of one of the patients. The main autoantigens included IgG clusters directed to 1) DNA/chromatin/glomeruli and 2) laminin/myosin/matrigel/vimentin/heparan sulphate [12]. We have described for the first time vimentin autoreactivity and compartmentalization at and near the base membrane zones of the skin and the sebaceous glands in DLE, colocalizing with other antibodies including IgG, IgM, fibrinogen, albumin, and C3.

Conclusion

The lupus band test (LBT) is a direct immunofluorescent technique for demonstrating a band of localized immunoglobulins at the dermal/epidermal junction in the skin of patients with lupus erythematosus (LE). Although these immunoglobulins and complement are present at the dermal/epidermal junction in systemic lupus and in discoid lupus, we encourage diagnostic laboratories performing DIF to routinely test for the presence of vimentin within the dermis of these patients. The compartmentalization may also be displayed utilizing periodic acid-Schiff (PAS) staining. Thus, our findings may provide valuable assistance in establishing the diagnosis of DLE in selected patients.

References

1. Tuffanelli DL. Discoid lupus erythematosus. Clin Rheum Dis. 1982; 8:327-341.
2. Husz S, Szabó E, Berkò G, Hunyadi J, Dobozy A, Simon N. Immunological studies in patients with discoid lupus erythematosus. Diagn Histopathol. 1981; 4:149-155.
3. Monroe, EW. Lupus Band Test. Arch Dermatol.1977; 113:830-834.
4. Burnham TK, Neblett TR, Fine G. Immunoglobulins in the skin of patients with lupus erythematosus. Acta Derm Venereol. 1966; 46:356.
5. Burnham TK, Neblett TR, Fine G. The application of the fluorescent antibody technique to the investigation of lupus erythematosus and various dermatoses. J Invest Dermatol. 1963; 41:451-456.
6. Rogers KR, Morris C J, Blake DR. Autoantibodies to vimentin reacts with a 38 kD central domain region. Br J Rheumatol 1990; 29: 19.
7. Hansson GK, Langerstedt E, Bengtsson A, Heideman M. IgG binding to cytoskeletal intermediate filaments activates the complement cascade. Exp Cell Res 1987; 170: 338-350.
8. Jesaitis AJ, Tolley J0, Allen RA. Receptor-cytoskeleton interactions and membrane traffic may regulate chemoattractant- induced superoxide production in human granulocytes. Cell Biol 1986; 261:13662-13669.
9. Hansson GK, Starkebaum GA, Benditt EP, Schwartz SM. Fc-mediated binding of IgG to vimentin type intermediate filaments in vascular endothelial cells. Proc Natl Acad Sci USA.1984; 81: 3103-3107.
10. Sanchez A, Osorio C, Alvaro-Gracia JM, Padilla R, Avila J. A subset of antibodies from the sera of patients with systemic lupus erythematosus reacts with vimentin and DNA. Rheumatol; 1990; 17:205-209.
11. Kraus W, Sevder JM, Beachev EH. Vimentin cross-reactive epitope of type 12 streptococcal M protein. Infect Immun 1989; 57:2457-2461.
12. Li QZ, Xie C, Wu T, et al. Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays. Clin Invest. 2005; 115:3428-3439.
13. Linder E, Chang C, Edgington T S. Complement-mediated binding of monocytes to intermediate filaments *in vitro*. Am J Pathol 1983;112:267-277.
14. Mseddi M, Marrekchi S, Meziou TJ, et al Discoid lupus erythematosus with eyelid involvement. A series of nine patients. J Fr Ophtalmol. 2007;30:247-249.
15. Acharya N, Pineda R 2nd, Uy HS, Foster CS. Discoid lupus erythematosus masquerading as chronic blepharoconjunctivitis. Ophthalmology, 2005; 112:19-23.