Resident's Page

In vivo antinuclear antibodies of the skin

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INTRODUCTION

Direct immunofluorescence (DIF) testing occupies an important place in the diagnosis and evaluation of many diseases. It is most commonly employed on skin biopsies to diagnose the autoimmune bullous disorders of the skin as well as systemic connective tissue diseases (SCTD), especially lupus erythematosus and vasculitis, including leukocytoclastic vasculitis and Henoch-Schonlein purpura. By DIF, presence of immune complexes in the skin biopsy at various locations, e.g., at the dermoepidermal junction (DEJ), upper dermal blood vessels, cytoid bodies, and intraepidermal intercellular spaces, etc., helps us to arrive at a definite diagnosis. "Lupus band test" (LBT) is most common pattern observed on DIF examination of skin biopsies of patients suffering from SCTDs. Broadly, it is the deposition of immunoglobulins (Igs) at the DEJ in lesional and nonlesional skin with IgM being the most frequent deposit. In addition, DIF microscopy of the skin has also disclosed antibodies bound to epidermal cell nuclei in several connective tissue disorders also known as *in vivo* ANA (antinuclear antibody) phenomenon or epidermal nuclear staining (ENS) which presents as keratinocyte nuclear fluorescence [Figure 1]. In 88% of the cases, connective tissue disorders could be predicted by the presence of antibodies against the epidermal cell nuclei of the skin.[1] Circulating ANAs are commonly found in patients with SCTDs.

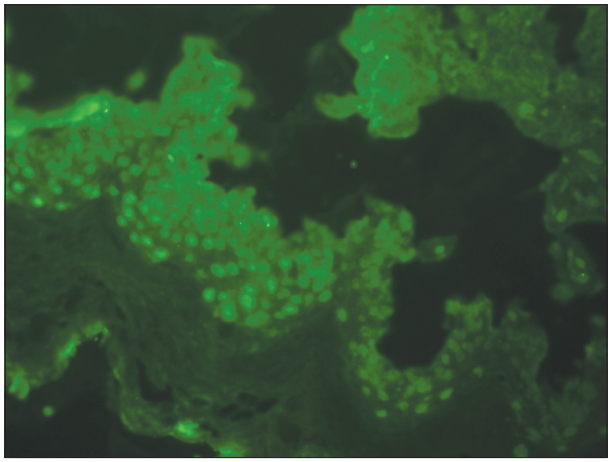

Figure 1: Direct immunofluorescence photomicrograph of skin biopsy showing IgG reactive 2+ diffuse nuclear staining in epidermal cells (ANA *in vivo*) (×400)

IMMUNOFLUORESCENCE CHARACTERISTICS

ANA *in vivo* has been observed in both lesional skin and normal skin. In addition to skin, *in vivo* ANAs can also be seen in diseased kidney, oral mucosa, and lung tissues in SCTDs.[2] IgG class of antibody is the most common type of Ig found in ANA *in vivo;* however, less commonly, IgM and IgA can also be found.[3] Four different patterns of ENS (viz. speckled, homogenous, nucleolar, and rim) have been reported in the literature, with speckled pattern being the commonest type.[4] The pattern of *in vivo* ANA can provide some diagnostic information. The homogeneous *in vivo* ANA pattern, though seldom found, occurs exclusively in systemic lupus erythematosus (SLE). The nucleolar pattern is very specific for scleroderma. Except for homogeneous pattern, *in vivo* ANAs do not discriminate better between the various SCTDs than do serum antibodies.

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CLINICAL CORRELATION

The frequency with which *in vivo* ANA in skin occurs in various SCTDs varies between 2.6 and 17.8% in different studies.[5,6] It occurs in 19% of cases with SLE, in 32% of mixed connective tissue disease, in 22% of scleroderma, in 20% of cutaneous vasculitis, in 18% of polymyositis, in 33% of Sjogren's syndrome, but is absent in cases with rheumatoid arthritis.[7] The diagnostic value of *in vivo* ANA in differentiating between the various connective tissue disorders is low with the exception of SLE as mentioned above.[1] In SLE patients with *in vivo* ANA, the incidence of nephropathy is significantly lower ($P<0.01$), regardless of LBT positivity.

SEROLOGICAL CORRELATION

Serologically, 98% of patients showing ENS have circulating ANAs by indirect immunofluorescent testing. The patterns of ENS in the skin biopsy specimens correlate with that of serum ANA in the majority of cases.[3,8] The speckled pattern of ENS is found to be most often associated with serum antibodies to either nuclear ribonucleoprotein (RNP) or Smith (Sm) antigen.[1,9] These two antigens are constituents of extractable nuclear antigen (ENA). ENA is a saline-soluble (extractable) nuclear antigen with several distinct antigenic sites. One is nuclear RNP that is RNase sensitive and the other is resistant to RNase and is identical to Sm antigen.

It has also been noted that some patients with serum antibodies to ENA did not display *in vivo* ANA on skin biopsies and vice versa.[4] Some authors have also demonstrated *in vivo* nuclear staining to be present in one tissue but absent in other tissues of same patients even when biopsies were performed at the same time and processed in the same way.[2] Moreover, no difference was detected between diseased and normal skin for the occurrence of *in vivo* ANA and also no association has been observed between this phenomenon with immune deposits at DEJ or in subepidermal vessels.[3]

PATHOGENESIS OF ANTINUCLEAR ANTIBODY *IN VIVO*

The exact pathogenesis of ANA *in vivo* remains obscure, but the explanation that it is a simple artifact seems to be quite untenable. Tuffanelli in 1975 proved that the phenomenon is not an artifact, as shown by its repeated observations at various time points in the same patient.[10] Gilliam (1975) and Iwatzuki *et al*. (1982) maintained that it is an *in vitro* phenomenon occurring only in relation to high titers of anti-RNP antibody in the blood and attributed ENS to tissue contamination occurring during excision of the skin specimen.[11,12] Izuno hypothesized that certain permeability-enhancing co-factors, in addition to consistently high titers of RNP antibodies, may be necessary to permit penetration of anti-RNP antibody into the nuclei of living epidermal cells.[13] However, it was later shown that ANA *in vivo* occurs with Igs other than IgG and not only with low titers of circulating ANA, but even in their absence.[14,15] The *in vivo* speckled nuclear staining for Igs within keratinocytes of lesional and nonlesional skin is correlated to antibodies to nuclear RNP and Ro. Relocation of nuclear and cytoplasmic Ro antigens to the cell surface has been implicated as a key event in permission of binding of autoantibodies. Ultraviolet light exposure, viral infection, and estrogen treatment of cultured keratinocytes have been shown to displace Ro antigen. The selective association between *in vivo* ANA in the skin and non-histone nucleoprotein antibodies in blood suggests it to be a true *in vivo* phenomenon.

CONCLUSION

The presence of *in vivo* ANA in clinically healthy skin is a phenomenon with a high predictive value for SCTDs.[1] However, compared with circulating ANAs, its diagnostic value in discriminating between the various SCTDs is very low. Furthermore, deposition of IgG in epidermal cell nuclei in speckled pattern appears to correlate with high-titres of serum antibody to ENA and is an immunopathologic marker for a subset of SCTDs. These findings re-emphasize the importance of cutaneous immunopathology in the diagnosis and management of patients with SCTDs.

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