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Immunohistochemical Studies from Vitiligo
-Comparison between active and inactive lesions-

Sung Ku Ahn, Eung Ho Choi, Seung Hun Lee[1], Jin Hyung Won, Seung Kyung Hann[1] and Yoon-Kee Park[1]

Vitiligo is an acquired, progressive depigmenting disorder of unknown etiology. In this study, to clarify pathogenesis of vitiligo, the marginal skin of actively spreading and stable vitiligo was examined using ICAM-1, HLA-DR, CD4 and CD8 monoclonal antibodies. In immunohistochemical study, ICAM-1 was expressed in four of five epidermis in active lesions, but not in stable lesion. Dermal ICAM-1 was also expressed in all active and stable lesions. HLA-DR was also expressed in all active epidermis in active lesions, but two of five epidermis in stable lesion. Dermal HLA-DR was also expressed in all active and stable lesion. CD4 lymphocytes were expressed more strongly in active lesion, but CD8 lymphocytes were not different in both lesions. There was no significant difference of degree of positivity with CD4 and CD8 in normal control specimens. In conclusion, we think that ICAM-1 and HLA-DR expression, cytokines released from keratinocytes, melanocytes or lymphocytes and infiltration of activated T-lymphocytes play an important role in disease activity.

Key Words: Vitiligo, ICAM-1, HLA-DR, CD4, CD8

Vitiligo is an acquired, progressive depigmenting disorder of unknown etiology. To explain the etiology and pathogenesis, various theories have been put forward, but these theories can not explain all the variants in vitiligo. Among the many hypothesis on vitiligo, the immunologic and keratinocytes involvement (Breathnach *et al.* 1966; Bhawan and Bhutani, 1983; Moellmann *et al.* 1982) have been the important main theories. Hann *et al.* (1992) performed light and electron microscopic studies on the vitiliginous and adjacent, normal appearing skin from 97 patients with actively spreading vitiligo and 19 patients with stable vitiligo. In addition to degenerative changes in melanocytes, epidermal degenerative changes, epidermal and dermal infiltration of lymphocytes and melanophages in the upper dermis were also seen in the normal appearing skin adjacent to vitiliginous skin. These epidermal and dermal changes were more prominent in the skin of actively spreading vitiligo than stable vitiligo.

The importance of adhesion molecules and cytokines produced by immune cells in normal as well as pathologic conditions have been well recognized. The ICAM-1 plays a critical role in lymphocyte interaction with a variety of cell types and in cell migration. Additionally, cytokines such as interferon gamma are involved ICAM-1/LFA-1 interaction and in the induction of HLA-DR by keratinocytes (Albadri *et al.* 1993; Gilhar *et al.* 1993).

The aim of the present study is to clarify the immunohistochemical difference between active and stable vitiligo using several mono-

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Department of Dermatology, Yonsei University Wonju College of Medicine and Department of Dermatology, Yonsei University College of Medicine[1]
Address reprint reguest to Dr.S K Ahn, Department of Dermatology, Yonsei University Wonju College of Medicine, Wonju, 220-701, Korea

clonal antibodies including ICAM-1, HLA-DR, CD4 and CD8.

MATERIALS AND METHODS

Skin biopsies from vitiliginous and adjacent normal appearing skin were obtained from five patients with actively spreading vitiligo and five patients with stable vitiligo. We also obtained biopsy specimens from two normal controls.

The activity of vitiligo was determined by a questionare (Do you think that vitiliginous patches are spreading and newly formed now?) on the history of the disease taken during each patient's first visit to our dermatologic clinic. We did not include anyone who had treatment history within one year prior to visiting our clinic in this study. There was no clinical evidence of inflammation. The biopsies were perfomed under local anesthesia and taken as boat shape incision by scapel.

The skin biopsies were divided into two halves. One half was fixed in 10% neutral buffered formalin, and paraffin blocks were made. The sections were stained with hematoxylin-eosin and Fontana-Masson. The other half was immediately frozen with liquid nitrogen and stored at −70℃ until further use. Cryostat sections, 5㎛ thick, were air dried for 30 minutes, fixed in aceton at 4℃ for 10 minutes. A avidin-biotin peroxidase technique (Vectastain ABC Kits; Vector Laboratories, Inc., Burlingame, CA.) with monoclonal antibodies was used with 3-amino-9-ethylcarbazole as the chromogen and counterstained with 1% hematoxylin.

Table 1. Comparison between active and stable vitilligo

Case	ICAM-1	HLA-DR	CD4	CD8
Active vitiligo				
Case 1 epidermis	+	+		
dermis	+	++	+	+
Case 2 epidermis	+	++		
dermis	+	++	+	+
Case 3 epidermis	−	+		
dermis	+	++	+	−
Case 4 epidermis	+	+		
dermis	+	+	+	++
Case 5 epidermis	+	+		
dermis	+	+	+	+
Stable vitiligo				
Case 1 epidermis	−	++		
dermis	+	++	−	+
Case 2 epidermis	−	++		
dermis	+	++	−	+
Case 3 epidermis	−	−		
dermis	+	+	−	+
Case 4 epidermis	−	−		
dermis	+	+	−	+
Case 5 epidermis	−	−		
dermis	+	++	+	+

−: negative staining
0: 1~2% of cell staining
±: 2~5% of cell staining
+: 6~25% of cell staining
++: 26~50% of cell staining
+++: 51~75% of vell staining
++++: 76~100% of cell staining

The slides were assessed under an Olympus microscope by two observers simultaneously. Positive cells were evaluated insame high power fields (×200) on 10 sections for each antibody. Only cells displaying membrane or nuclear stainings were counted.

The extent of staining was graded by the relative percentage of the epidermal cells, or endothelial cells in the dermis stained on a scale from − to 4+ : − ; negative staining, 0; 1~2% of cell staining, ± ; 2~5% of cell staining,+ ; 6~25% of cell staining, ++ ; 26~50% of cell staining, +++ ; 51~75% of cell staining, ++++ ; 76~100% of cell staining

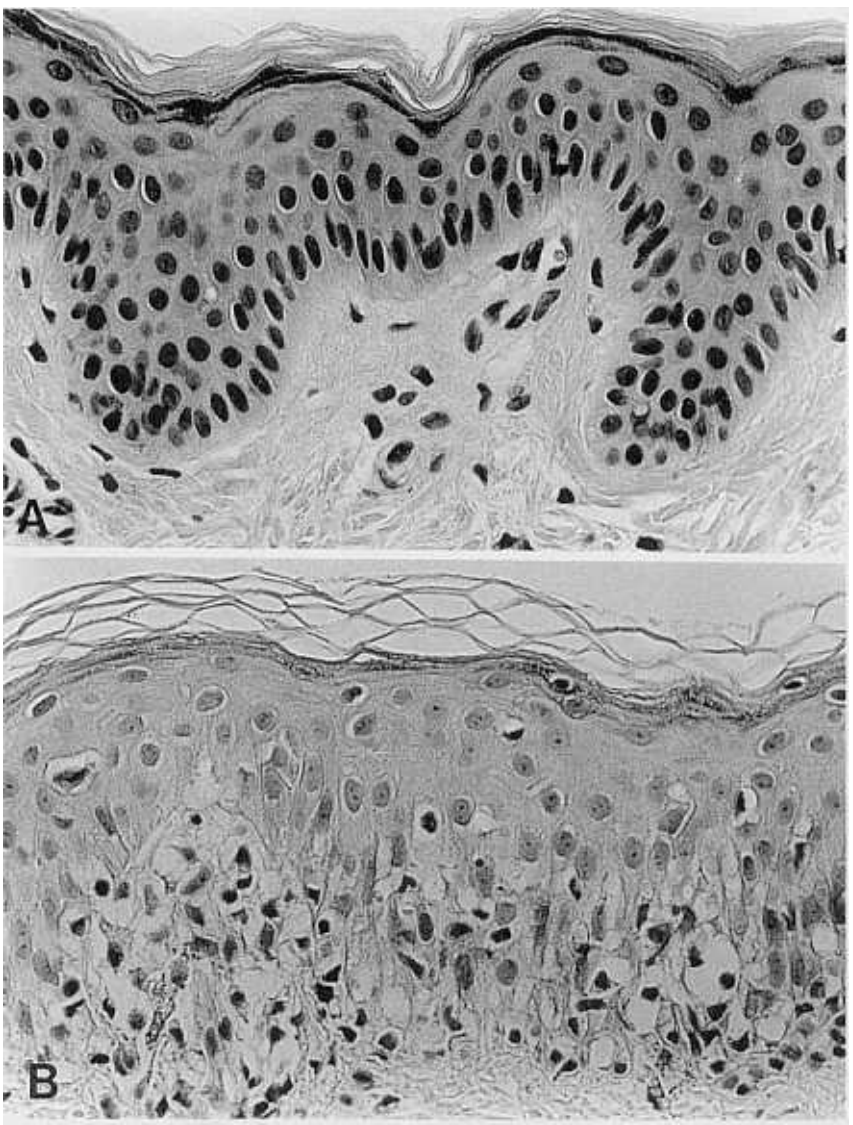

Fig. 1. *Epidermal changes were unremarkable in stable vitiligo (A; H & E stain, ×400). In active vitiligo, moderate lymphocyte infiltration of epidermis, vacuolar degeneration of basal cells, lymphocyte infiltration around vasculatres and melanophage deposition in upper dermis were observed (B; H & E stain, ×400).*

RESULTS

In light microscopic study of the normal appearing skin adjacent to vitiliginous areas, mild to moderate focal lymphocyte infiltration of epidermis and focal vacuolar degeneration of basal cells with infiltrating lymphocytes were more prominent in actively spreading vitiligo than stable vitiligo. Some spongiosis including both vacuolar degeneration of basal cells and lymphocyte infiltration and dyskeratosis were only seen in actively spreading vitiligo, but not in stable vitiligo. The microvesicles, severe form of spongiosis were not seen in this study. The focal lymphocyte infiltration around vasculatures and melanophage deposition in upper dermis were more prominent in actively spreading vitiligo than stable vitiligo. The vitiliginous skin in actively spreading and stable vitiligo did not show remarkable changes except loss of pigment and melanocytes (Fig. 1). In immunohistochemical study, ICAM-1 was expressed in four of five epidermis, mainly basal cells, in active lesions, but not in stable lesions. All active and stable lesions expressed ICAM-1 in dermis, mainly perivascular areas of upper dermis (Fig. 2). HLA-DR was diffusely expressed in entire layers of epidermis in all active lesions, but two of five epidermis, mainly localized in basal cell layers, in stable lesions. All active and stable lesions expressed HLA-DR more widely than ICAM-1 mainly in upper dermal perivascular areas (Fig. 3). CD4 lymphocytes were expressed in all active lesions, but one of five specimens in inactive lesions. CD8 lymphocytes were expressed in all

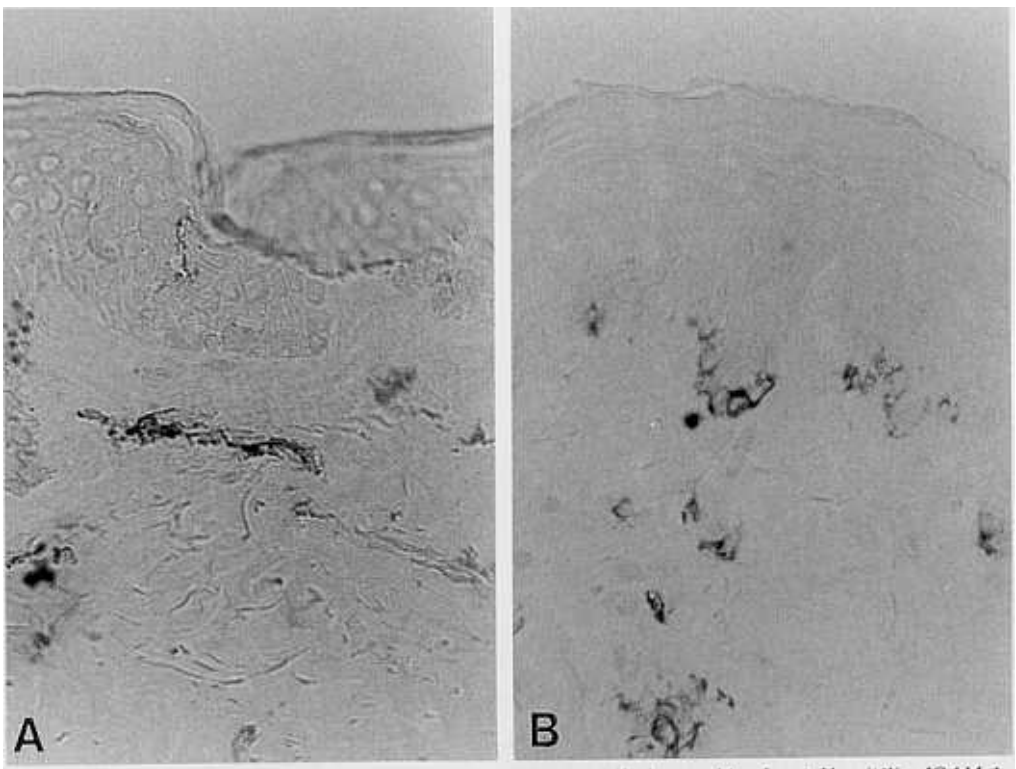

Fig. 2. Immunohistochemical staining for ICAM-1 expression on vitiliginous skin. In stable vitiligo, ICAM-1 expression is absent from epidermal cells (A, ×400). In active vitiligo, there is prominent ICAM-1 expression by epidermal cells (B, ×400).

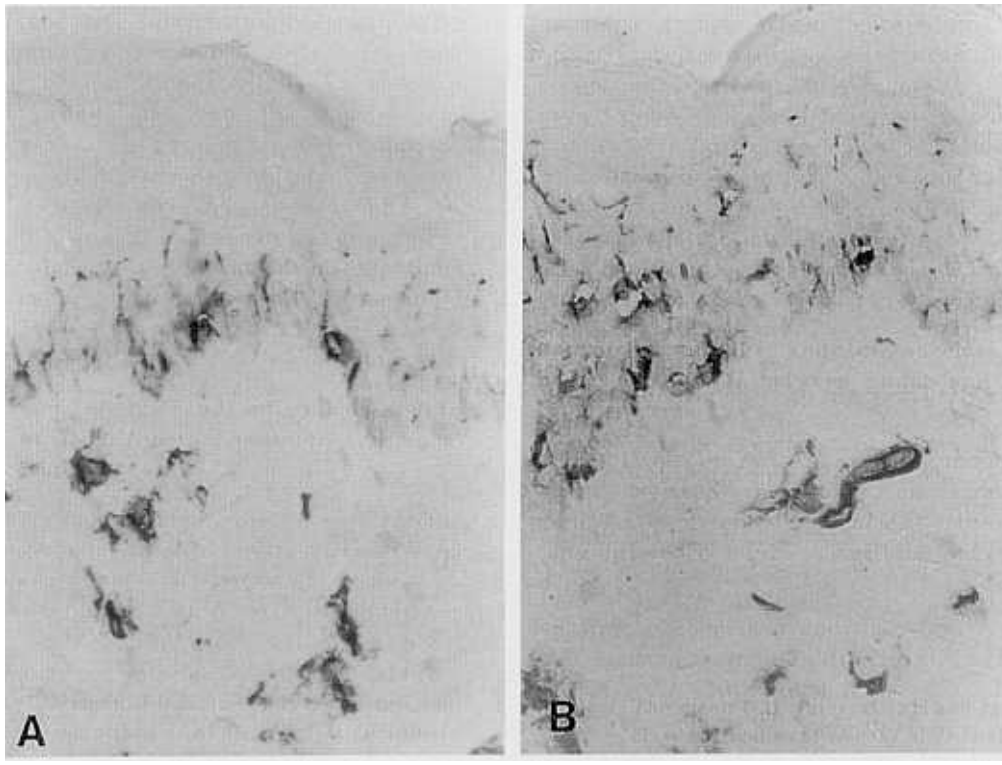

Fig. 3. *Immunohistochemical staining of HLA-DR expression on vitiliginous skin. In stable vitiligo, HLA-DR was expressed in lower epidermis (A, ×400). In active vitiligo, HLA-DR was expressed by entire epidermis (B, × 400).*

active and inactive lesions except only one active lesion.

In normal control specimens, there was no significant difference of degree of positivity with CD4 and CD8. HLA-DR and ICAM-1 were expressed more weakly or negatively in infiltrated cells and blood vessels of upper dermis and epidermal cells.

DISCUSSION

On review of vitiligo literatures, we could not find comparative immunohistochemical studies between marginal skins of active and stable vitiligo. We chose marginal skin in both active and stable vitiligo and found more epidermal changes including lymphocytic infiltration, vacuolar degeneration of basal cells, spongiosis and dyskeratosis and dermal changes including lymphocytic infiltration and melanophage deposition in active lesions than stable ones as our previous study revealed (Hann *et al.* 1992). In our previous light and electron microscopic study (Hann *et al.* 1992), we observed that the epidermal lymphocyte infiltration and the melanocyte degeneration in marginal normal appearing skin of active vitiligo were related with disease activity, and suggested that the disease activity was resulted from the cellular immunity. Many inflammatory dermatoses such as psoriasis, atopic and contact dermatitis, and lichen planus show similar histopathologic findings. In these inflammatory dermatoses, infiltrating activated T lymphocytes into epidermis were predominently memory T lymphocytes (CD4+/CD45RA-) (Barker and Macdonald, 1992) and

ICAM-1 was expressed on epidermal keratinocytes (Singer *et al.* 1989). In our study, we could find increased epidermal expression of ICAM-1, HLA-DR and CD4 lymphocytes in active vitiligo as other inflammatory dermatoses.

A recent report (Albadri *et al.* 1993) presented abnormal expression of MHC class II molecules by perilesional melanocytes in thirteen of 21 patients with vitiligo and six-fold increase in number expressing the ICAM-1. In their study, MHC class II expression by melanocytes was not observed in unaffected skin of vitiligo patients, normal skin and psoriatic lesion, but ICAM-1 expression was observed in all lesions though the positive rate was different. No correlation was found between the number of melanocytes expressing MHC class II or ICAM-1 and the number of T lymphocytes at the margins of vitiligo lesions. They suggested that MHC class II was absent and ICAM-1 only slightly upregulated in the melanocytes in psoriasis, suggesting that interferon gamma (INF-γ), which induces the expression of these molecules in cultured melanocytes, may not have the same effect when secreted in vivo by activated T cells infiltrating the skin. Gilhar *et al.* (1993) injected recombinant INF-γ intradermally in the vitiliginous and normal pigmented skin of each vitiligo patients. HLA-DR and ICAM-1 expression by the epidermal cells (mainly keratinocytes) were observed in all sites injected with INF-γ. In our opinion, the expression of ICAM-1 and HLA-DR on melanocytes significantly occur in vitiligo, but not in other inflammatory dermatoses. Therefore the epidermal infiltrating cells damage melanocytes and contribute to the depigmentation. The anatomical relationship between keratinocytes and melanocyte, termed the epidermal melanin unit (Quevedo *et al.* 1987), suggests that the changes of keratinocytes may affect melanocytes and pigmentation. The keratinocytes have been reported to synthesize and secrete cytokines such as interleukin (IL-1, IL-6, IL-8, tumor necrosis factor (TNF)-α, and transforming growth factor-α, and β (Luger, 1989). The immune cytokines, IL-1α, IL-6 and TNF-α inhibit the proliferation and the melanogenesis of normal human melanocytes (Swope *et al.* 1991). The melanocytes may participate in inflammatory processes by the production of chemotactic factors such as IL-8 and monocyte chemotactic and activating factor (MCAF) and respond synergistically to TNF-α and IFN-γ to produce IL-8 and probably MCAF (Zachariae *et al.* 1991). IL-8 was known to have T-lymphocyte chemotactic activity (Larsen *et al.* 1989). Also the melanocytes increase their expression of ICAM-1 upon stimulation with IL-1α, TNF-α, and IFN-γ (Yohn *et al.* 1990)

In summary, keratinocytes showing infiltration of lymphocytes, spongiosis, dyskeratosis and vacuolar degeneration as well as melanocyte may contribute to the depigmentation of vitiligo. What is the initiation factor of melanocyte destruction ? We do not know the exact etiologic factor in vitiligo but we think that ICAM-1 and HLA-DR expression, cytokines released from kerainocytes, melanocytes or lymphocytes and activated T-lymphocyte play an important role in disease activity.

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