Lymphocyte Adhesion to Psoriatic Dermal Endothelium: Mechanism and Modulation

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Psoriasis is characterized by the hyperproliferation of keratinocytes in the epidermis and the accumulation of activated CD4+ T lymphocytes in the upper dermis. We have recently tested the hypothesis that the abnormal endothelial proliferation in the dermal papillae of psoriatic lesions may be mechanistically linked to the expression of endothelial ligands capable of promoting lymphocytes binding and extravasation. The results indicated that specialized endothelial cells lining the post-capillary venules of psoriatic lesions are capable of promoting the selective adherence of human CD4+ T cells and its memory subset. In contrast, B cells, CD8+ T cells, and CD45RA+ T cells are deficient in their capacities to bind. The adhesion process is energy and calcium dependent and involves tissue-specific lymphocyte receptors, with LFA-1 molecules playing an accessory role. We concluded that transformation of the dermal endothelium into a lymphocyte-receptive phenotype by defined growth factors or cytokines may represent a positive feedback mechanism promoting lymphocyte migration into the diseased sites. *J Invest Dermatol 95:29S–31S, 1990*

The migration of lymphocytes into various lymphoid organs and chronic inflammatory lesions of the body is a carefully regulated process. Lymphocytes possess adhesion molecules that mediate their homing patterns through peripheral lymph nodes and gut-associated lymphoid tissues. The adhesion molecules, functionally defined as homing receptors, are expressed on the surface of recirculating lymphocytes and display high affinity for organ-specific ligands that are expressed on the lumenal surface of high endothelial cells lining the post-capillary venules (HEV) of the lymphoid tissues (reviewed in [1]).

Lymphocyte migration into chronic inflammatory dermatoses is also non-random. Histologic examination of the skin of patients with atopic dermatitis, lichen planus, and psoriasis has revealed dense dermal lymphocytic infiltrates within the lesions. Interestingly, these infiltrates are composed predominantly of CD4+ T lymphocytes, and many of these cells express HLA-DR antigen [2]. Presumably, these activated lymphocytes represent a subset of recirculating lymphocytes that may be skin-seeking, although little is known of the mechanisms regulating lymphocyte entry into the lesions.

In order for lymphocytes to gain access to the dermis and epidermis, they must first enter the dermal environment from the blood vasculature. At the start of the migratory process, lymphocytes pass into the tissue by adhering to the endothelium of dermal post-capillary venules, migrating through the blood vessel wall and subsequently distributing along chemotactic gradients to the characteristic microenvironments within the epidermis. It is likely that the key cellular event that determines whether a circulating lymphocyte migrates into the skin is the adhesive interaction between lymphocyte and the endothelial cell (EC) of the dermal vessel wall.

We have recently studied the mechanism promoting lymphocyte trafficking into psoriatic lesions. Psoriasis is characterized by excessive epidermal proliferation and dense dermal infiltrates that are composed largely of T cells and macrophages. Dilatation of capillaries and activation of endothelium in the dermal papillae have been reported to be an early manifestation of an erupting psoriatic lesion and are associated with epidermal influx of lymphocytes and Langerhans cells. Interestingly, a significant increase in the blood flow at the active edge of a psoriatic plaque has been reported recently, and this change often precedes the characteristic increase in T-cell infiltrates into the skin [3]. In addition, high endothelial venules, which are adapted to support lymphocyte migration into lymphoid parenchyma, are also observed in psoriatic skin. These structures may represent sites for recruitment of lymphocytes into the dermis [4]. We therefore tested the hypothesis that the endothelial proliferation in psoriasis may be mechanistically linked to the expression of surface ligands capable of mediating lymphocyte adhesion.

In our study, the migration of lymphocytes from the blood into the dermis was investigated using an in vitro lymphocyte-frozen psoriatic-skin-section adherence assay. Human peripheral blood mononuclear cells isolated from normal volunteers or psoriatic patients adhered specifically to the papillary dermis when overlaid onto frozen sections of psoriatic plaques [5]. However, we found that the capacity of circulating peripheral blood lymphocytes to adhere to the psoriatic endothelium was not identical for all lymphocyte subpopulations. Although both human T and B lymphocytes were capable of adhering to the papillary dermis, they exhibit significantly different cell dose-response relationships, suggesting that these populations differ significantly in their psoriatic skin binding potentials. Optimal binding of T cells occurred at 2×10^6 cells overlaid.

Because CD4+ T cells predominate in the chronic psoriatic le-

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Abbreviations:
EC: endothelial cells
GM-CSF: granulocyte-macrophage colony stimulating factor
HEV: high endothelial venules
LFA-1: lymphocyte function-associated antigen 1
LN: lymph node
PP: Peyer's patch
TGF-β: transforming growth factor-beta

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sion, the adhesive properties of these cells were compared with the $CD8^+$ subset. We isolated the T-cell subset by fluorescence-activated cell sorting with the OKT4 and OKT8 antibodies and examined the capacity of each population to bind. The results demonstrated that binding of $CD4^+$ T cells was approximately 4 times that of the $CD8^+$ T cells and twice that of unfractionated T cells when the cells were each overlaid at a concentration of 2×10^6 cells. Similar results were obtained when $CD4^+$ and $CD8^+$ T cells were isolated by negative selection of T cells stained with the reciprocal antibody in order to avoid non-specific interference of lymphocyte binding by antibody molecules bound to the cells.

In the next series of experiments, T lymphocytes were further subdivided into the functionally distinct suppressor-inducer subset ($CD45R^+$) and the helper-inducer subset ($CDw29^+$), and the relative capacity of each to bind to psoriatic tissue sections was tested. For this purpose, T cells isolated from buffy coats obtained from randomly selected normal donors were labeled with the monoclonal antibodies 4B4 or 2H4, followed by FITC-conjugated goat anti-mouse Ig staining and sorting into $CDw29^+$ and $CD45R^+$ T cells, respectively. When overlaid onto psoriatic tissue sections, $CDw29^+$ T cells adhered significantly better than $CD45R^+$ T cells. In four different experiments, the adhesion of $CDw29^+$ T cells was approximately 4 times that of $CD45R^+$ T cells [16].

There is considerable evidence in humans that the $CDw29^+$ T-cell subset provides help for B cells in pokeweed mitogen-driven Ig productions (reviewed in [7]). These lymphocytes also proliferated vigorously when restimulated with an antigen to which the donor was primed in vitro. Recent observations have suggested that the $CDw29^+$ and $CD45R^+$ T cells do not represent a distinct lineage of lymphocytes but rather represent $CD4^+$ T cells at different maturational stages. Regardless, these cells differed functionally in their activation requirements and lymphokine secretion patterns, which might have distinct effects on lymphocyte migration, possibly through actions on the adhesiveness of endothelial cells and chemotaxis of mononuclear cells. For example, the $CDw29^+$ subsets of peripheral T cells have been shown to secrete interferon gamma and interleukin-3, which may contribute to the pathogenesis of disease by activating Langerhans cells and keratinocytes and enhancing leukocyte traffic into the psoriatic epidermis.

Our observation that $CDw29^+$ T cells exhibit a greater affinity than $CD45R^+$ T cells to psoriatic plaques may be a general feature of chronic inflammatory infiltrates. Recently, an increase of $CDw29^+$ T cells has been reported in a variety of pathologic conditions, such as in thyroid tissues in Graves diseases, synovium in rheumatoid arthritis, and the dermis in atopic dermatitis [8]. These observations raise the possibility that $CDw29^+$ T cells also adhere selectively to the vascular endothelium lining the blood vessel in these tissues. The implication is that changes in the local vasculature to promote lymphocyte adhesion may not be tissue specific, but rather are a common feature in sites of chronic lymphocytic infiltration.

The biochemical and metabolic requirements for human T lymphocyte adhesion to psoriatic endothelial cells were studied with enzymes that modify lymphocyte surface structures and pharmacologic agents that alter metabolic processes. The results of these experiments indicate that the binding process requires energy, is calcium but not magnesium dependent, involves microfilament but not microtubule, and is sensitive to brief treatment with trypsin and/or glycosidase. These requirements are similar to those for lymphocyte adhesion to high endothelial venules of lymphoid tissues. However, pretreatment of lymphocytes with monoclonal antibodies against lymphocyte homing receptors for lymph node and Peyer's patch high endothelium had no effect on lymphocyte binding to psoriatic endothelium, suggesting that the adherence may be mediated by a tissue-specific receptor-ligand interaction.

Leukocyte adhesion to vascular endothelium is mediated in part by the integrin family of adhesive receptors. Additional experiments have also shown that the lymphocyte function-associated antigen-1 (LFA-1) molecules may function as an accessory adhesion mechanism responsible for lymphocyte binding to psoriatic endothelium. Pretreatment of lymphocytes with saturating amounts of 60.3 MoAb (specific for the β-heterodimer of LFA-1 molecules) or MHM-24 MoAb (specific for the α-heterodimer) resulted in partial inhibition ($\sim 40\%$) of binding [16].

The results of these experiments provided strong support for the concept that specialized endothelia lining dermal vessels in involved skin are capable of supporting lymphocyte binding in a fashion similar to high endothelium in lymphoid tissues, and may also represent a means whereby selective lymphocyte subsets can be recruited from the bloodstream into chronic inflammatory loci in pathologic conditions. The receptor revealed in these studies permits $CD4^+$ T cells and the $CDw29^+$ subset to bind to endothelial cells by an LFA-1-independent process. However, because psoriatic skin is frequently inflamed and often infected, an LFA-1-dependent mechanism may be central in promoting other leukocyte migration into the skin and may be responsible for an acute inflammatory response.

The studies described thus far have concentrated on lymphocyte adhesion to psoriatic plaques and show continued clinical progression. Although the exact mechanism(s) remains to be elucidated, ultraviolet radiation can exert significant effects on the immune system; one of these is to alter the distribution of subpopulations of circulating lymphocytes. The data we have obtained thus far has provided evidence that human lymphocytes from patients undergoing UV light therapy (8-methoxy psoralen and UVA or tar and UVB) failed to adhere to the psoriatic endothelium. Moreover, psoriatic lesions from PUVA- or UVB-treated patients also failed to support binding of normal lymphocytes [9]. In addition, recent studies have also shown that lymphocytes did not adhere to clinically and histologically resolved lesions treated with corticosteroids, indicating that the adhesion process can be modulated in vivo by treatment modalities.

Interestingly, recent data have also suggested that transforming growth factor-β (TGF-β) might have an important role in decreasing lymphocyte adhesion to psoriatic endothelium. TGF-β is known to affect immune function in vitro through several mechanisms. TGF-β inhibits IL-1 and IL-2 dependent T-cell proliferation, and it exhibits lymphokine-activated killer cells and cytotoxic T-cell generation. TGF-β also exhibits opposing effect on macrophages. It is reported as a chemo-attractant for monocytes and has been shown to stimulate IL-1 mRNA expression and growth factor secretion by these cells. On the other hand, TGF-β de-activates macrophages and inhibits macrophage precursor cell proliferation. In our earlier studies, exposure of circulating lymphocytes with picomolar TGF-β in the picomolar range for 8 to 24 h inhibited the binding of these cells to lymph node and Peyer's patch high endothelium in vitro. Subsequently, we found that preincubation of human T cells with TGF-β also blocked lymphocyte binding to the psoriatic endothelium in the frozen tissue section assay. The effect was dose dependent and was effective at a concentration of 0.1 to 0.5 ng/ml per 5×10^6 cells and exposure of 6–18 h. The way TGF-β produces these inhibitory effects is not known, but a modulation of the receptors for the dermal endothelial ligands may be involved.

The data we have obtained thus far provided evidence that the endothelial component of psoriatic skin, especially in the dermal papillae, is capable of controlling the magnitude and specificity of lymphocyte migration into this tissue. Because the receptor/ligand interaction could be demonstrated in vitro only with the involved lesion, and not with normal skin, one reasonable hypothesis would be that the endothelium found in involved dermis of psoriatic patients could be upregulated to express the ligands for lymphocyte adhesion by selective cytokines. The cytokines responsible for induction of specialized endothelium may be secreted directly by activated T lymphocytes or indirectly by macrophages and keratinocytes.

This concept is supported by the demonstration that several agents, including interferon-gamma (IFN-γ), interleukin-1, tumor necrosis factor (TNF), and bacterial polysaccharide, stimulate umbilical vein endothelial cell adhesiveness for lymphocytes in vitro. In addition, recent experiments indicated that TNF-α, IFN-γ, and granulocyte-macrophage colony stimulating factor (GM-CSF) can

increase adhesiveness of cultured LN and Peyer's patch HEV cells. For lymphocytes, the increased adhesiveness of HEV cells can be blocked by pretreatment of lymphocytes with antibodies against the homing receptors, suggesting that the homing receptor/endothelial ligand interaction functioned in this system. In addition, although large vessel endothelial cells have also been shown to become more adhesive for neutrophils and monocytes in response to cytokine stimulation, the HEV cells isolated from LN and PP, when stimulated with TNF-α or IFN-γ, retained the capacity to adhere lymphocytes selectively [10]. This observation suggests heterogeneity in the adhesion molecules that are induced between the different endothelial cell types. The understanding gained from these two approaches supports the idea that cytokines released in the psoriatic lesions may lead to the development of specialized endothelium that is physiologically and functionally suited to mediate the extravasation of $CD4^+$ T cells and the $CDw29^+$ subset. Research in this area is now focused on the identification of the putative skin-specific receptor/ligands and the regulatory effects of cytokines on the lymphocyte recruitment process.

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