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Immunohistochemical Localization of α_2-Macroglobulin Receptors in Human Skin

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The localization of receptors for the proteinase inhibitor α_2-macroglobulin was studied in human skin by immunohistochemistry using a monoclonal mouse antibody. No epidermal staining was identified. α_2-Macroglobulin receptors were identified on dermal fibroblasts and dermal dendritic cells. Endothelial cells did not stain with the antibody, but positive staining cells were concentrated around dermal vessels. The myoepithelial layer of eccrine glands exhibited receptors; however, there was no staining of the eccrine epithelial layer. The distribution of α_2-macroglobulin receptors correlates with the reported distribution of α_2-macroglobulin: both are present in the dermal connective tissue and absent in epithelium and endothelium. The distribution of α_2-macroglobulin and its receptor in the dermis is consistent with a possible role in regulation of dermal proteolytic activity. ***Key words: Dermis; Collagenase.***

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α_2-Macroglobulin is a proteinase inhibitor named for its electrophoretic mobility and large size. It is capable of inhibiting endoproteinases of all classes (serine, cystine, aspartic, and metalloproteinases), whether of endogenous or exogenous origin (1). α_2-Macroglobulin is an excellent inhibitor of mammalian collagenase and accounts for 95% of the collagenase inhibitory activity of human plasma (2). Inhibition of proteinases by α_2-macroglobulin requires cleavage of the α_2-macroglobulin "bait region"; in this reaction, α_2-macroglobulin is a 150-fold better substrate for collagenase than is type I collagen (3). In studies of the inhibition of collagenase performed in the presence of both α_2-macroglobulin and tissue inhibitor of metalloproteinase (TIMP), a specific inhibitor of collagenase, collagenase is bound to α_2-macroglobulin preferentially (4).

α_2-Macroglobulin is present in many connective tissues (5). Studies examining skin consistently demonstrate α_2-macroglobulin in the dermis (6, 7). One study reported immunolocalization of α_2-macroglobulin in epidermis (6). Production of α_2-macroglobulin by cultured skin fibroblasts has also been demonstrated (8). In considering the regulation of dermal collagenase, it was suggested that α_2-macroglobulin is present in skin only during inflammatory events (9); however, there currently is no justification for this conclusion.

The binding of proteinases by α_2-macroglobulin is an irreversible process. In the circulation, α_2-macroglobulin that has reacted with proteinase is cleared by hepatocytes through a receptor-mediated pathway (10). The receptor recognition site on α_2-macroglobulin is expressed only after reaction with proteinase (11); it is not expressed on the native inhibitor. Cultured fibroblasts, in contrast to many cultured epithelial and endothelial cells, have been shown to express α_2-macroglobulin receptors (12). α_2-Macroglobulin receptors in skin could function to remove α_2-macroglobulin-proteinase complexes which form in skin as a result of inhibition of either collagenase, other endogenous proteinases released in inflammatory conditions, or exogenous proteinases for which specific endogenous inhibitors do not exist. This study explores the distribution of α_2-macroglobulin receptors in normal human skin.

MATERIALS AND METHODS

Human skin was obtained with Institutional Review Board approval from excess tissue of surgical pathology specimens. Frozen sections and formalin-fixed paraffin-embedded material were used and showed similar findings. A mouse monoclonal anti-α_2-macroglobulin-receptor antibody was generously supplied by Dr Dudley Strickland (American Red Cross Blood Services Laboratory, Rockville, MD). Production and characterization of this antibody has been described previously (13). Rabbit polyclonal antibody to S-100 protein was obtained from Biogenex (San Ramon, CA).

Paraffin-embedded sections required treatment with trypsin (0.2%, 45°C, 10 min) prior to incubation with the anti-α_2-macroglobulin-receptor antibody. Sections were incubated with the antibody in dilutions of 1:50 to 1:400 at 4°C overnight. Optimal staining with minimal background was obtained with a 1:200 dilution. Bound antibody was detected with an anti-mouse IgG Vectastain ABC kit (Vector Laboratories, Burlingame, CA) using the protocol supplied by the manufacturer and aminoethylcarbazole as substrate for the immunoperoxidase reaction. Similar findings were obtained in frozen and formalin-fixed, paraffin-embedded material. Anti-S-100 antibody was used in a 1:80 dilution and was detected with an anti-rabbit IgG Vectastain ABC-AP kit. Photomicrographs were taken using a Nikon Microphot-FX equipped with GIF filter and Kodak Technical Pan film.

RESULTS

α_2-Macroglobulin receptor immunoreactivity was not observed in epidermis (Fig. 1A), consistent with an absence of α_2-macroglobulin receptors on keratinocytes, melanocytes and Langerhans' cells. The presence of melanocytes and Langerhans' cells in the tissue was demonstrable with S-100 staining. Dendritic cells in the papillary dermis and fibroblasts in the reticular dermis were strongly immunoreactive (Fig. 1A,B). In control experiments in which the primary anti-α_2-macroglobulin-receptor antibody was not added there was no immunoreactivity.

Vascular endothelium did not stain, but immunoreactive cells were present around dermal vessels (Fig. 1C). Adnexal

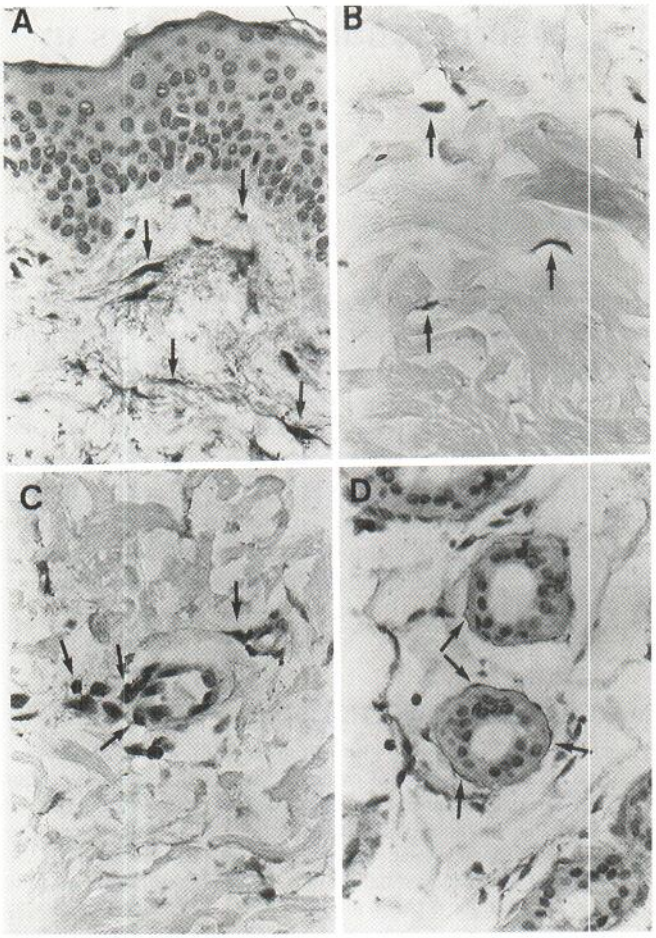

Fig. 1. Immunohistochemical staining of trypsinized, formalin-fixed, paraffin-embedded human skin with a mouse monoclonal anti-α_2-macroglobulin-receptor antibody (antibody dilution 1:200, high magnification). (A) The epidermis exhibits no immunoreactive material. In the papillary dermis there are deeply stained dendritic cells. (B) In the reticular dermis, the antibody stains fusiform fibroblasts. (C) The endothelium of dermal vessels does not stain for α_2-macroglobulin receptors. There are immunoreactive perivascular cells. (D) The myofibroblast layer of the eccrine glands is reactive with the antibody.

epithelium did not stain; there was staining of the myofibroblast layers of eccrine glands (Fig. 1D). Piloerector muscle did not exhibit immunoreactivity.

DISCUSSION

The distribution of α_2-macroglobulin receptors in skin is consistent with previous data showing binding and uptake of labelled α_2-macroglobulin by cultured skin fibroblasts but not by endothelial or epithelial cells. This distribution also correlates well with those studies demonstrating α_2-macroglobulin in normal dermis but not in epidermis. The presence of both α_2-macroglobulin and its receptor in skin suggests that this proteinase inhibitor may have a role in regulation and clearance of proteinases in dermal connective tissue.

Other functional roles for the α_2-macroglobulin/α_2-macroglobulin-receptor system may be suggested. α_2-Macroglobulin binds many cytokines. Transforming growth factor-β (TGF-β) binds specifically to α_2-macroglobulin-proteinase complexes and not to native α_2-macroglobulin (14). The receptor-mediated clearance of the TGF-β/α_2-macroglobulin-proteinase complex is hypothesized to regulate the levels of this cytokine (14). The α_2-macroglobulin receptor is homologous to the low density lipoprotein receptor and binds apoprotein E-enriched lipoproteins *in vitro* (13, 15). This receptor, therefore, may have a role in lipoprotein homeostasis and could be involved in lipid uptake in cutaneous processes. The binding of α_2-macroglobulin to its receptor results in regulation of macrophage effector mechanisms, including superoxide generation and proteinase production (16); similar effects could occur with dermal dendritic cells and/or fibroblasts. α_2-Macroglobulin may have a role in control of fibroblast proliferation, as *in vitro* synergy with platelet-derived growth factor has been noted (17).

The presence of α_2-macroglobulin receptors on both dermal dendritic cells and fibroblasts but not keratinocytes or melanocytes suggests its use as a marker for "fibrohistiocytic" spindle cell proliferations of skin. Further studies will examine this issue. α_2-Macroglobulin receptors are a marker for cells of monocyte/macrophage lineage (18). In this respect, it is interesting that staining of epidermal Langerhans' cells was not observed. The absence of α_2-macroglobulin receptors in areas lacking α_2-macroglobulin suggests a connection in their expression. This, too, may be a focus for future study.

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