

Rhinophyma - unusual expression of simple-type keratins and S100A in sebocytes and abundance of VIP receptor-positive dermal cells

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Summary. Rhinophyma represents a severe variant of rosacea, a common mid-facial erythematous dermatosis. Increased blood flow and pooling in skin are thought to be involved in its pathogenesis. Since neuropeptides and their receptors are responsible for local blood flow regulation, immunolocalization for the vasoactive intestinal peptide (VIP)-receptor(R) was performed in slice biopsies taken from five patients with glandular rhinophyma. Additional immunostainings included intermediate filaments (keratin, vimentin) and neuroglandular antigen (NGA). In contrast to controls, rhinophyma disclosed not only a more dense distribution of VIP-R positive cells within the endothelium but immunoreactive perivascular large cells. The immature sebocytes stained positive with monoclonal antibody Cam5.2 against glandular antigens and polyclonal anti-S100A. Elastotic connective tissue in the dermis showed a strong immunoreactivity for vimentin and NGA. From these results we suggest that, (a) ligands of the VIP-R may contribute to vascular and dermal alterations in rosacea and (b) immature sebocytes show an unusual antigen expression of S100A and glandular keratin.

Key words: Rosacea, Rhinophyma, Neuropeptides, Vasoactive intestinal peptide-receptor, Sebocytes, Glandular keratin

Introduction

Rosacea is a centrofacial disease with characteristic flushing and blushing. Although not uncommon among adults and in patients with acquired immunodeficiency of all ages, virtually nothing is known about pathogenesis. The major symptom is mid-facial erythema, which can be accomplished by papules, pustules, cysts and sometimes granulomas and may affect the eyes (Marks, 1989; Plewig and Kligman, 1993; Wilkin,

1994).

Flushing, swelling and relapsing, rosacea can be provoked by various factors including sunlight in some patients and topical or systemic drugs in others. Demodicosis has been supposed to play a role in facial and extrafacial rosacea (Rufli and Bücher, 1984) but the mite itself is no *conditio sine qua non* for rosacea (Sibenge and Gawkrödger, 1992). Studying the flush reaction in patients with erythematous rosacea, Wilkin (1981) found that oral uptake of coffee induces a heat-exchange mechanism in the carotids, which signals the hypothalamus to cause vasodilatation. Patients with the carcinoid syndrome may also develop cutaneous signs of rosacea (Findley and Simson, 1977).

Sibenge and Gawkrödger (1992) investigated the facial blood flow by laser-Doppler flowmetry in 25 patients with rosacea and found a mean percent flux value of 18.52 units, which is more than 4 times higher than in control subjects (mean 4.13 units). This argues for a dilatation of the papillary dermal vasculature. Motly et al. (1989) investigated teleangiectasia by light and electron microscopy and found a prominent dilatation of small dermal blood vessels and lymphatics. The vessel walls were thickened and showed an increased endothelial cell labelling index. The dermal and perivascular connective tissue appeared to show fragmentation. The authors conclude that teleangiectasias in rosacea are due to a dermal abnormality and causes vascular pooling.

Since the local blood flow is regulated by neuropeptides like substance P (SP) released from cutaneous nerve endings (Hägermark et al., 1978) it has been suggested that they may be involved in rosacea pathogenesis. Indeed, SP levels in peripheral blood are raised in at least some patients with rosacea (Powell et al., 1989). Others have found an increased cutaneous innervation by SP-immunoreactive nerve fibres (Kürkcüoğlu and Alaybeyi, 1991). It was demonstrated, that SP stimulates fibroblast growth in vitro (Nilsson et al., 1985) and can be involved in inflammation by induction of histamine release from cutaneous mast cells (Hägermark et al., 1978; Hartschuh et al., 1983).

Vasoactive intestinal peptide (VIP) is another interesting neuropeptide, involved in local blood flow regulation and inflammation. VIP has been shown to stimulate the growth of keratinocytes and other epithelial cells in vitro (Haegerstrand et al., 1989; Wollina et al., 1992a,b). Since VIP effects are mediated by receptor binding, we investigated the distribution of VIP-receptor (-R)-positive cells in a severe rosacea subtype, rhinophyma, and compared our results to other immunohistochemical findings in this disease.

Materials and methods

Patients

Five male patients with glandular type rhinophyma have been included (age 58-76 years, mean 70.5 years). All of them underwent surgical therapy by shaving with a shaw scalpel and local analgesia. We applied a hydrocolloid dressing on the wound area and observed a rapid re-epithelialization during the next two weeks.

The immunohistochemical results were compared with a control group of patients (n=7), who were surgically treated for melanocytic nevi on their nose.

Tissue processing

Slide biopsies were frozen immediately in liquid nitrogen and stored until used. Unfixed frozen sections cut at 5 µm were immunostained.

Antibodies

The monoclonal antibody 109.10 (Immunotech, Marseille, France) was employed for immunolocalization of VIP-R protein (working dilution 1:50). To further characterize the tissue sections, polyclonal rabbit broadspectrum antikeratin (Dakopatts; working dilution 1:100), monoclonal mouse IgG2a antibody Cam 5.2 against glandular cytokeratins #8, 18, 19 (Becton-Dickinson; 1:2), monoclonal mouse IgG1 antibody Vim 9(1) against vimentin (Monosan, 1:10), polyclonal rabbit antiserum against S100A (Dakopatts, ready for use), and monoclonal mouse IgG2a antibody against neuroglandular antigen LS59 (generously provided by Dr. Jerry, Calgary, Canada; 1:100) have been additionally used.

Staining method

Immunoperoxidase technique with amino ethyl carbazole was used according to our standard protocol (Wollina et al., 1992c, 1995).

Results

All rhinophyma patients disclosed comparable immunohistochemical findings. Antibody 109.10 gave a considerable high percentage of vascular and peri-

vascular labelled cells in rhinophymas vs. controls. Higher numbers of immunoreactive cells were observed around sebaceous glands, in the vascular sheet of the hair bulbs and in the papillary body. The epithelial tissue of the glands, hair follicles and epidermis was completely negative. The staining pattern was homogeneous cytoplasmic. Two cell types could be differentiated, a larger one mostly perivascular, and a smaller one mostly endothelial. The distribution of 109.10-positive endothelial cells was mostly scattered, partly clustered (Fig. 1).

Though the general distribution of 109.10-positive endothelial cells was not different from controls, clustering was found only in rhinophyma which was accompanied by a higher density of labelled cells and a significant percentage of perivascular larger cells.

Keratin and vimentin expression in the epidermis and the hair follicles was comparable in both groups of patients. Basal epidermal Cam 5.2-positive cells were observed, which are thought to represent Merkel cells. In sebaceous glands, the undifferentiated basal cells gave positive reactions with monoclonal Cam 5.2 against glandular keratins and with polyclonal S100A (Fig. 2). Both Vim9(1) and LS59 disclosed an almost identical distribution of dermal staining, which showed the formation of a more dense connective tissue in rhinophyma. Especially around vessels and sebaceous glands, the staining intensity was remarkably high, consistent with the histopathological feature of elastosis (Fig. 3). Vim9(1) additionally labelled dendritic suprabasal epidermal cells, i.e. Langerhans cells, without obvious differences between both groups of patients.

Discussion

Recurrent flushing is the earliest component of rosacea to be apparent. So the first definitive stage of the disease is vascular. The erythema represents the increased number of erythrocytes in a mildly inflamed dermal vasculature. There is an increased frequency of flushing in rosacea and a correlation of the severity of ocular rosacea and flushing (Wilkin, 1994). Patients with flushing disorders like carcinoid syndrome and mastocytosis can develop rosacea (Findley and Simson, 1977; Wilkin, 1994).

Peptidergic nerve endings of skin are involved in the regulation of local blood flow (Bloom and Polack, 1983). In rosacea, the local blood flow is increased as well as the number of peptidergic nerve endings (Kürkcüoglu and Alaybeyi, 1991; Sibenge and Gawkrödger, 1992).

VIP is a major neuropeptide of cutaneous nerve endings, often colocalized with SP, which has also been located in Merkel cells and dermal mast cells (Hartschuh et al., 1983; Björklund et al., 1986). Both peptides, VIP and SP, have growth factor-like activities for human skin cells (Nilsson et al., 1985; Hagerstrand et al., 1989; Wollina et al., 1992a,b). Both have been considered in rosacea pathogenesis (Powell et al., 1989; Kürkcüoglu

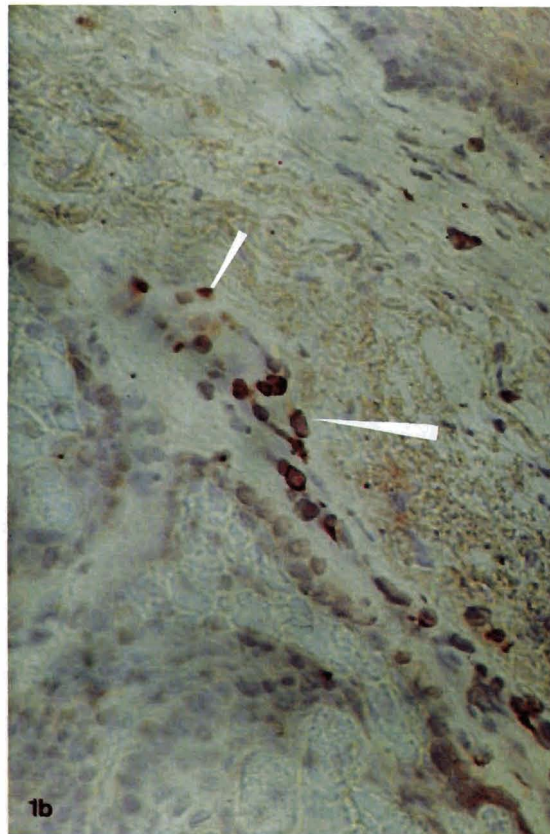
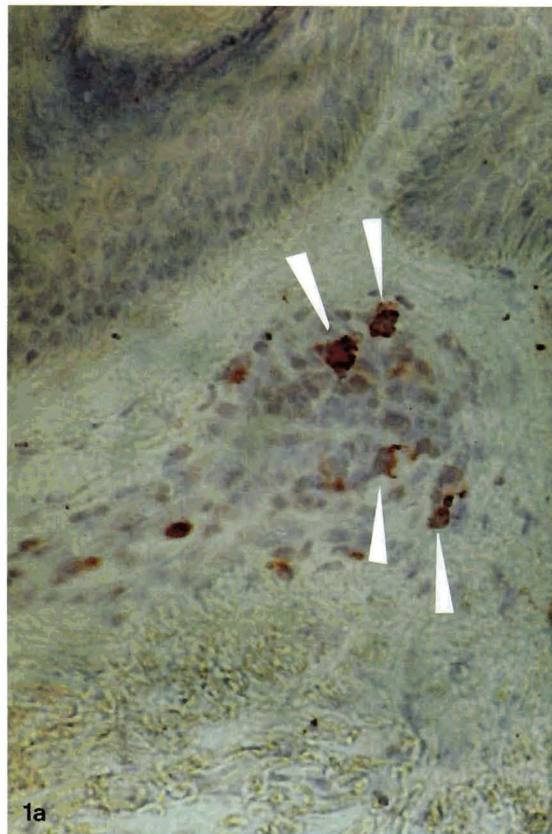


Fig. 1. Rhinophyma. Immunostaining with monoclonal antibody 109.10 against the VIP-receptor: **(a)** positively-stained cells in the papillary body (arrows) and **(b)** periadnexal within the endothelium and perivascular. Larger (long arrow) and smaller cells (short arrow) can be differentiated. x 640

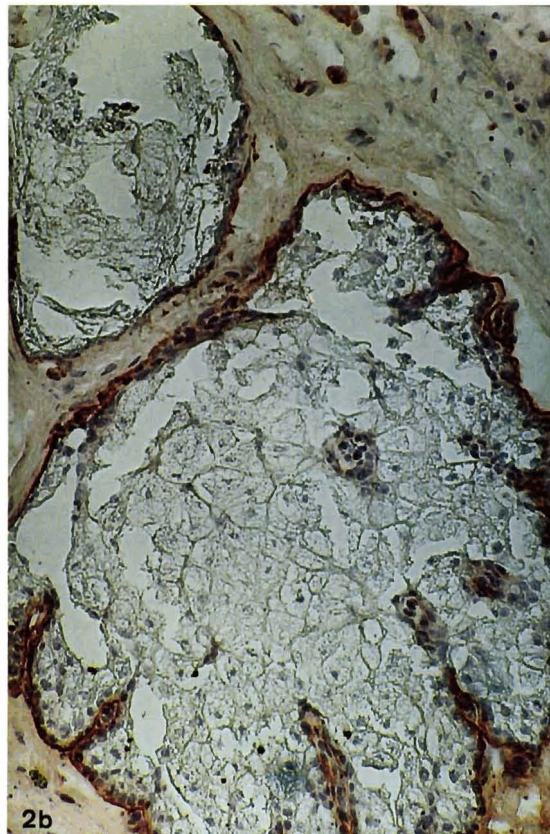
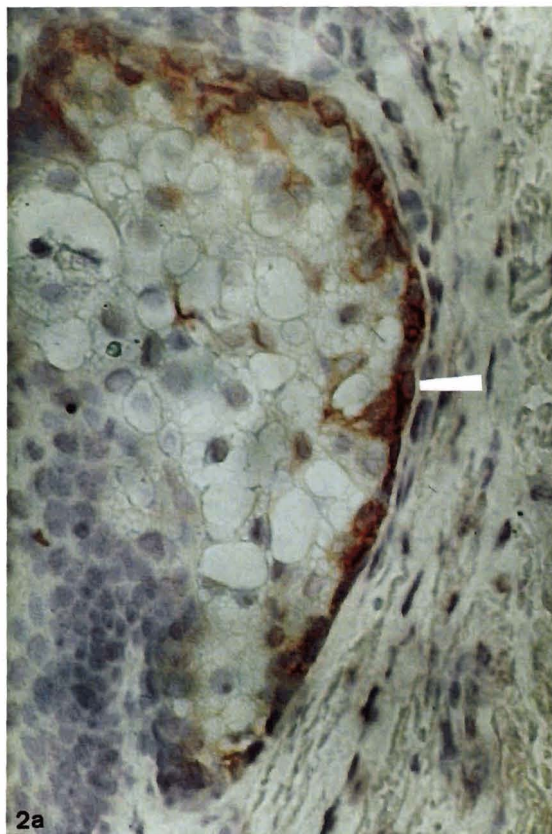


Fig. 2. Rhinophyma. Immunostaining of sebaceous glands: **(a)** Cam 5.2 (arrow) and **(b)** anti-S100A label immature sebocytes. x 640

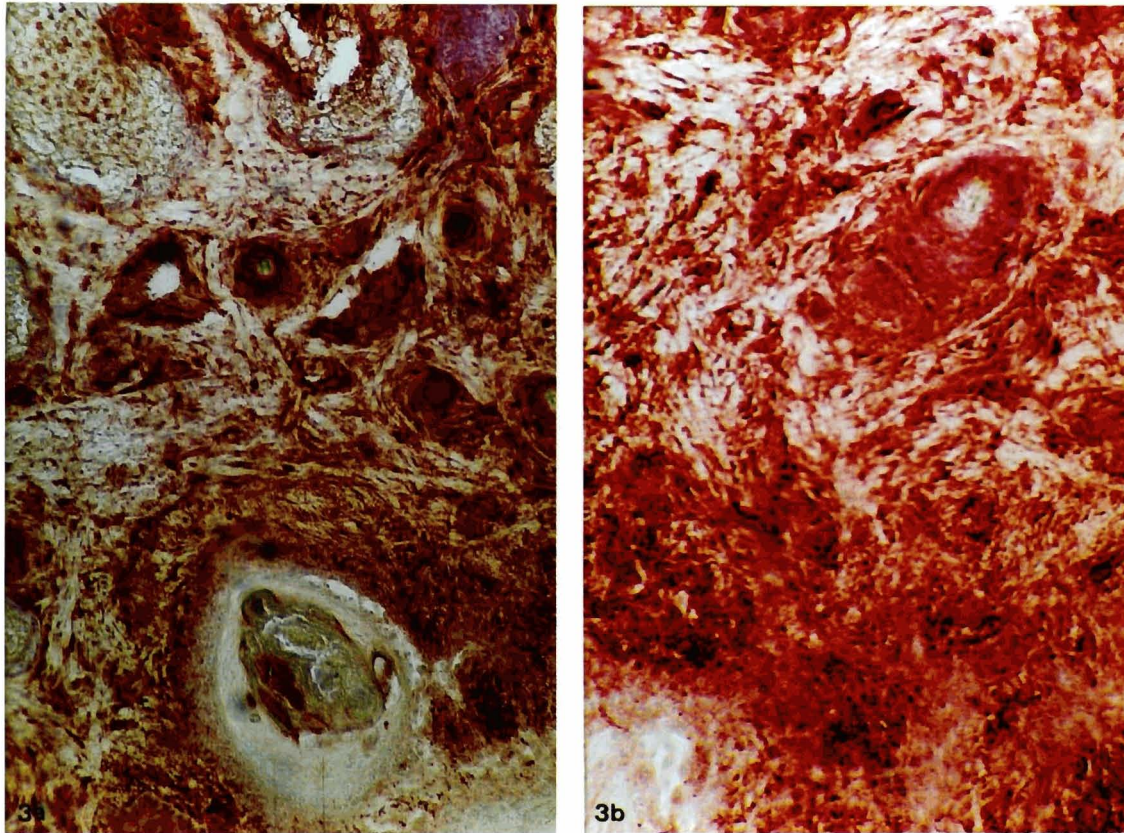


Fig. 3. Rhinophyma. Immunostaining of dermal connective tissue: (a) Vim9(1) and (b) LS59 show an almost identical distribution. x 200

and Alaybeyi, 1991; Grosshans, 1993).

Autoradiographic SP-R localization in human skin has been published recently by Pincelli et al. (1993). They found weak signals in the epidermis and higher densities in the papillary body and around adnexal structures. In the present paper, the VIP-R has been immunolocalized with a monoclonal antibody 109.10 (Pichon et al., 1983). The antibody labels in normal skin the eccrine duct epithelium and scattered endothelial cells (Herbst, 1991; Wollina, 1991). VIP immunoreactivity has been demonstrated in nerve fibres of the dermal vasculature (Hartschuh et al., 1983; Björklund et al., 1986). VIP belongs to a superfamily of peptides including secretin, glucagon, glucagon-like peptides, etc. showing sequence homologies. These peptides may interact with the VIP-R (Robberecht et al., 1990; Couvineau et al., 1994). Thus, the demonstration of VIP-receptor protein does not necessarily implicate that VIP is the only ligand in rhinophyma tissue. On the other hand, overexpression of VIP-R may be the consequence of deficiency of ligands. In contrast to control subjects, rhinophyma patients disclosed a high number of VIP-R positive cells clustering in dermal vessels. Additionally, especially larger immunoreactive cells have been localized in the perivascular tissue.

The rhinophyma skin disclosed some additional peculiar features: the dermal tissue showed an elastotic connective tissue, positively stained with monoclonal antibodies against vimentin and neuroglandular antigen,

particularly dense around dermal vessels and adnexes. The immature basal cells of sebaceous glands stained positive for S100A and Cam 5.2. Kurokawa et al. (1989) investigating normal, seborrheic and acne skin, reported that sebaceous gland epithelium is completely negative for cytokeratins 18 and 19. This, Cam 5.2 staining seems to be a distinct feature in rhinophyma.

The higher number of VIP-receptor bearing dermal cells may be involved in abnormal vascular regulation and edema formation in rosacea/rhinophyma (Bloom and Polak, 1983; Grosshans, 1993). The data obtained by immunostainings provide further arguments for a possible involvement of neuropeptide-mediated regulation of the typical altered vascular responsibility in rosacea.

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