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Appearance of tenascin in healing skin of the mouse: possible involvement in seaming of wounded tissues

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ABSTRACT Distribution of the extracellular matrix glycoprotein tenascin during wound healing in mouse skin was studied immunohistochemically. Within 24 hours after wounding, and preceding the formation of granulation tissue, tenascin appeared in the basement membranes beneath epidermis and hair follicles adjacent to the wound edges and in the wounded edges of cutaneous muscle layer. Granulation tissue began to form in the wound space at about 1-2 days and was immediately covered by epidermis. Tenascin first appeared in the periphery of the granulation tissue beneath healing epidermis and around the wounded edges of cutaneous muscle layer. Then the tenascin-positive area extended into the inner region of granulation tissue. At about 5-7 days, all of the granulation tissue was intensely stained with anti-tenascin serum. Tenascin immunoreactivity decreased as granulation tissue was replaced with reconstructed dermal tissue at 7-14 days. In most cases, tenascin staining persisted longest in the dermis beneath the healing epidermis and at the juncture of healing edges of cutaneous muscle layer. It disappeared at about 10-14 days after wounding. These findings suggest that tenascin may play an important role in the seaming of wounded tissues.

KEY WORDS: *tenascin, wound healing, granulation tissue, adhesion, skin*

Introduction

The extracellular matrix glycoprotein tenascin was originally found at the junction between muscle fibers and tendon (Chiquet and Fambrough, 1984a, b; Chiquet-Ehrismann *et al.*, 1986). It is identical to glioma mesenchymal extracellular matrix protein (GMEM) (Bourdon *et al.*, 1983, 1985), hexabrachion (Erickson and Inglesias, 1984), cytotactin (Grumet *et al.*, 1985) and J1 (Kruse *et al.*, 1985). Thereafter, temporally and spatially restricted distribution of this molecule has been reported in various embryonic tissues (Faissner *et al.*, 1988; Ekblom and Aufderheide, 1989), including the mesenchyme of developing palate where bilateral outgrowths of maxillary process fuse medially, resulting in continuous secondary palate (Ferguson, 1988); mesenchyme at the site of vaginal separation from urogenital sinus (Takeda *et al.*, 1988); and mesenchyme at the site of urethral separation from ventral surface epithelium of developing penis (Murakami *et al.*, 1990). In these cases, seaming and reconstruction of mesenchyme seem to be essential for the completion of morphogenesis. The wound healing of skin is naturally thought to involve seaming and reconstruction of mesenchyme. These findings prompted us to examine the distribution of tenascin in healing wounds of skin.

During the course of this study, Mackie *et al.* (1988) reported the appearance of tenascin in healing skin wounds of rats. In addition to confirming some of their results, we obtained some further observations which shed new light on the role of tenascin in wound healing.

Results

Razor wounds were made on the skin of the mouse back and later examined by anti-tenascin immunofluorescence as described in the Matherials and Methods section. Within 24 hours, the wound space was filled with loose mesenchymal tissue containing fibroblasts, macrophages, leucocytes and debris of fibrin clots. Wounded edges of epidermis began to migrate on the mesenchymal tissue (Fig. 1a). At the same time, tenascin staining appeared in the basement membranes beneath epidermis and hair follicles adjacent to the edges of the wound and in the wounded edges of cutaneous muscle layer (Fig. 1b). The wound

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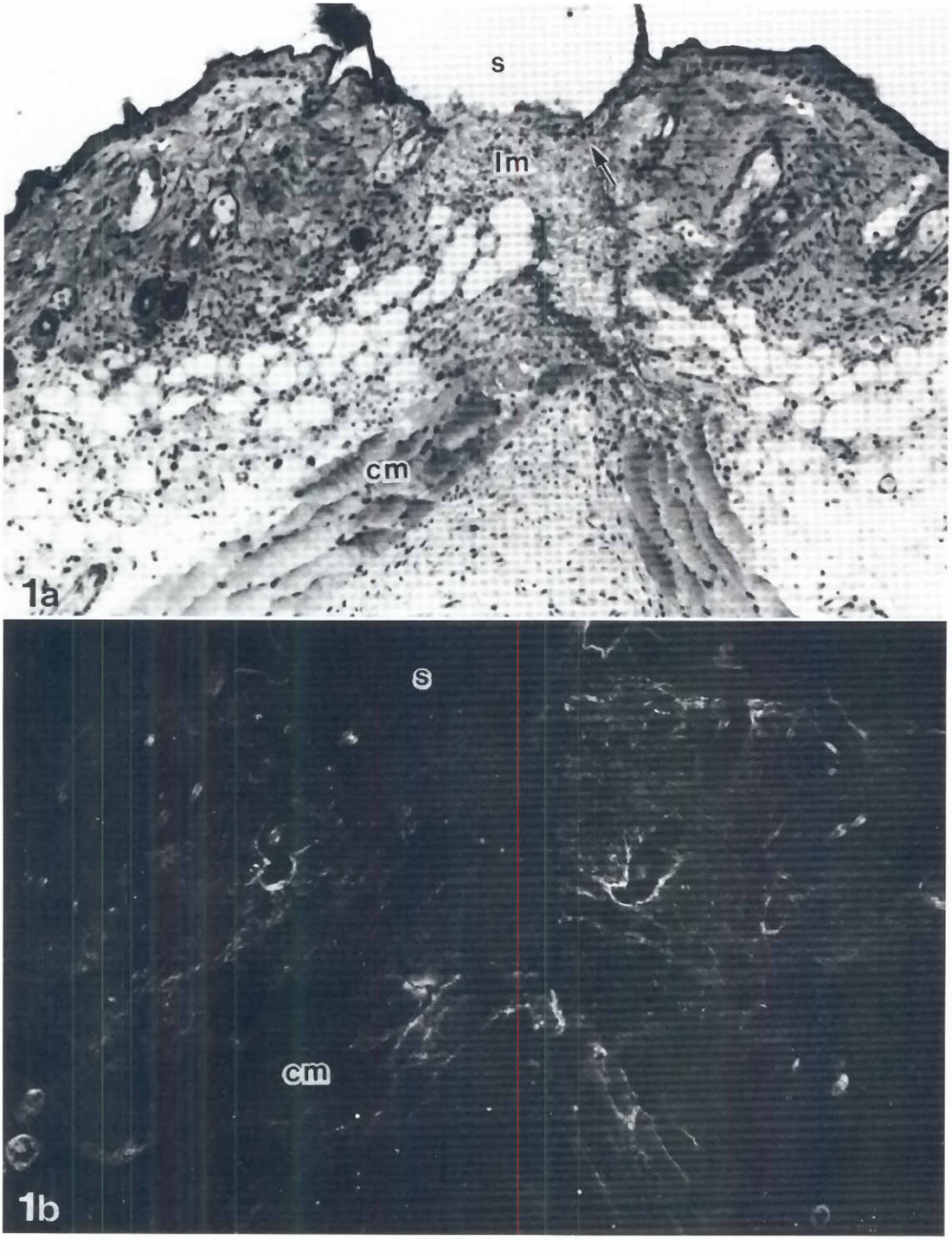

Fig. 1. Cross section of a wound at 24 hours after wounding. (a) *Epidermis has began to migrate (arrow) on the loose mesenchyme (lm) filled in the wound space. cm, cutaneous muscle; s, scab (mostly peeled off during histological procedure). x140.* **(b)** *Anti-tenascin immunostaining of a section of the same wound. Tenascin has appeared in the basement membranes of epidermis and hair follicles on the wound edges, and in the wounded edges of cutaneous muscle (cm). s, scab. x140.*

surface was completely covered with healing epidermis within 2 days. Shortly after the beginning of epidermal migration (1-2 days), granulation tissue consisted of myofibroblasts and blood vessels had begun to form in the wound space (Fig. 2a). Tenascin immunoreactivity appeared first in the periphery of the granulation tissue beneath healing epidermis, around the wounded edges of cutaneous muscle layer, and in some cases, in the periphery of granulation tissue contacting intact dermis. Thereafter, the tenascin-positive area gradually extended into the inner region of granulation tissue (Fig. 2b). The epidermis which covered the granulation tissue became hypertrophic (Fig. 2). At about 5-7 days, the wound space was filled with mature granulation tissue (Fig. 3a) which was intensely

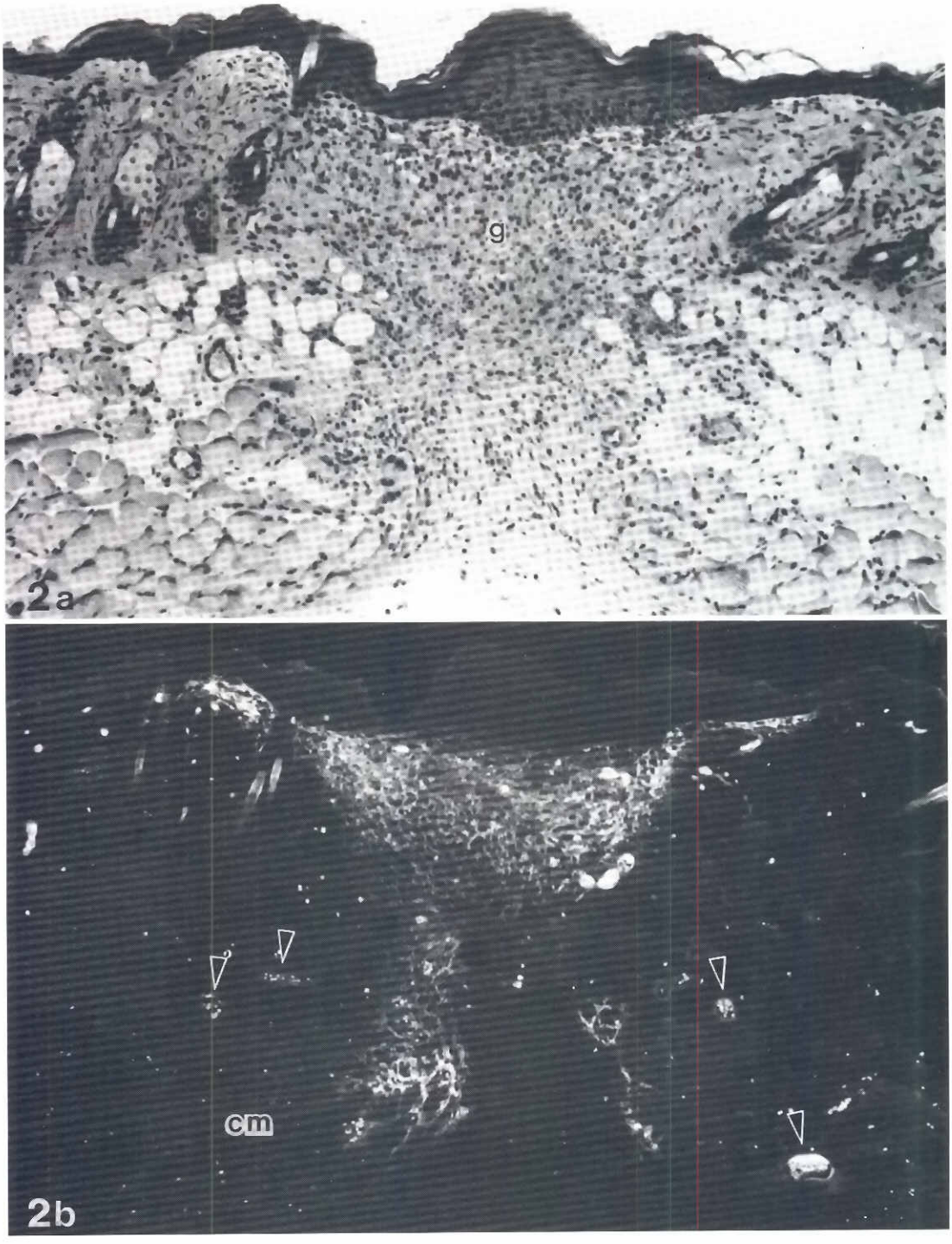

Fig. 2. Cross section of a wound at 4 days after wounding. (a) *Granulation tissue (g) developed in the wound space. x140.* **(b)** *Anti-tenascin immunostaining of a section of the same wound. Regions of granulation tissue beneath healing epidermis and around the wounded edges of cutaneous muscle (cm) were stained. Fluorescence of erythrocytes (arrowheads) is autofluorescence. x140.*

stainable with anti-tenascin serum (Fig. 3b). At about 7-10 days, the granulation tissue began to be replaced with reconstructed dermal tissue, and the wounded edges of cutaneous muscle layer began to adhere to each other (Fig. 4a, arrow). The tenascin-positive area decreased along with the decreasing granulation tissue. In most cases, tenascin staining persisted longest in the dermis beneath healing epidermis and at the junction between the edges of healing cutaneous muscle layer (Fig. 4b). Some cells whose cytoplasm was stained intensely and granularly with anti-tenascin serum were often observed around the wounds during the healing processes, especially at about 4-7 days after wounding (Fig. 4b, arrow and inset). These cells have not yet been identified. Approximately 10-14 days after

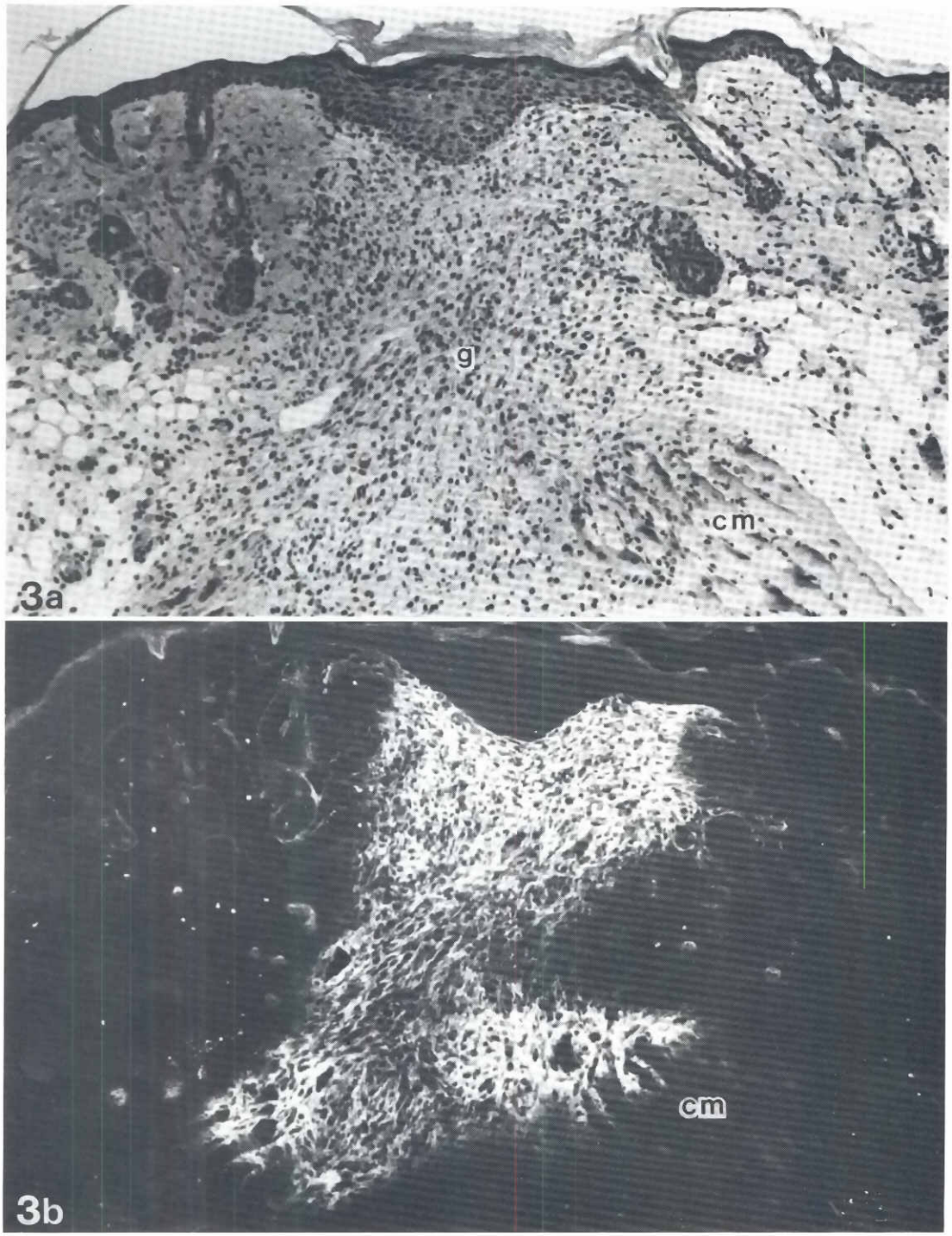

Fig. 3. Cross section of a wound at 5 days after wounding. (a) *Wound space was filled with mature granulation tissue (g). cm, cutaneous muscle. x140.* **(b)** *Anti-tenascin immunostaining of a section of the same wound. Whole granulation tissue was intensely stained. cm, cutaneous muscle. x140.*

wounding, wound healing was complete in most wounds. That is, the granulation tissue had been completely replaced with reconstructed dermal tissue, and hairs began to develop on epidermis. Tenascin was scarcely present in the completely healed skin. The time length required for these processes depended on the severity of the wound.

Discussion

In the present study, tenascin appeared transiently in healing skin wounds: in the wounded edges of epidermis, hair follicles, and cutaneous muscle layer, and in the granulation tissue. Tenascin is a highly adhesive molecule

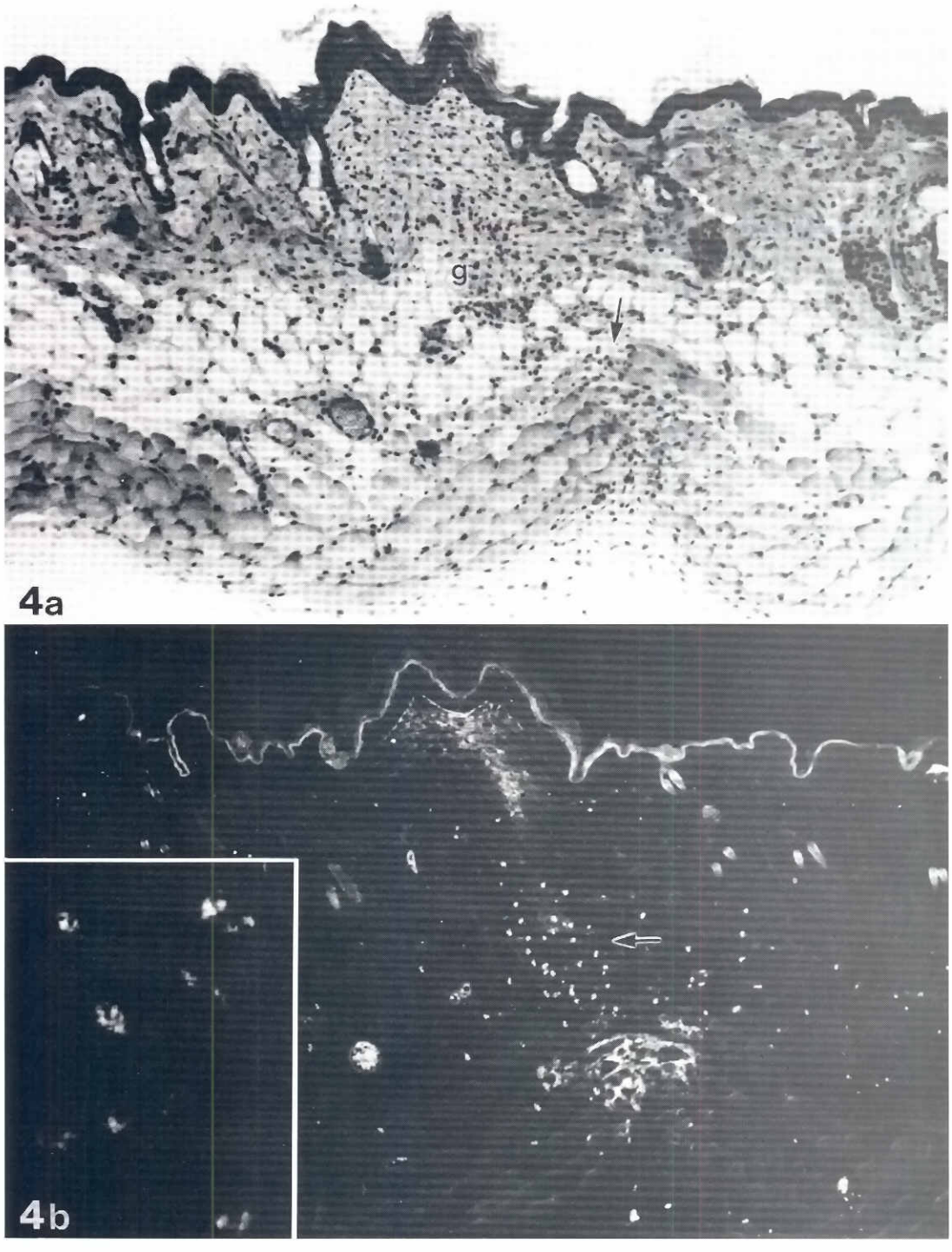

Fig. 4. Cross section of a wound at 7 days after wounding, in a later stage of healing process. (a) *Granulation tissue (g) has decreased. Wounded edges of cutaneous muscle have adhered to each other (arrow). x140.* **(b)** *Anti-tenascin immunostaining of a section of the same wound. Granulation tissue remaining beneath the healed epidermis and the adhesion site of wounded edges of cutaneous muscle are still positive. Some unidentified cells around the wound were intensely positive for tenascin (arrow and inset). x140. (inset, x850).*

which can bind to cell surface and other extracellular matrix components including fibronectin and proteoglycan (Chiquet and Fambrough, 1984b; Hoffman *et al.*, 1988). The adhesive nature of this molecule and its distribution pattern in healing wounds strongly suggest that tenascin may work as a kind of bond to promote seaming of wounded tissues. In our previous study (Murakami *et al.*, 1990), we demonstrated a close spatial relationship between the distribution of tenascin and actin filaments in non-muscular mesenchyme of developing urethra and proposed that tenascin may work as an adhesion molecule and/or mechanical link in contractile mesenchyme. Involvement of tenascin in cytoskeletal organization has been suggested by its inhibitory effects on integrin-mediated cell attachment (Chiquet-Ehrismann *et al.*, 1988) and by the inhibitory effects of RGD peptides on tenascin-mediated cell attach-

ment (Bourdon and Ruoslahti, 1989). Inasmuch as the granulation tissue of healing wounds also contains myofibroblasts (i.e. contractile fibroblasts rich in actin filaments) which are thought to cause wound contraction (Majno *et al.*, 1971; Gabbiani *et al.*, 1972), it is proposed that tenascin and actin are functionally related in wound healing.

During the course of the present study, the appearance of tenascin in healing skin wounds of rat and the possible importance of this molecule in wound healing were reported by Mackie *et al.* (1988). The spatial and temporal distribution of tenascin presented here is generally the same as theirs, though the distribution of tenascin around wounded muscle layer was not described in their paper. These investigators suggested that the healing epidermis may induce tenascin production on dermal tissue. Tenascin did indeed appear first at the dermal-epidermal junctions and extended later into inner granulation tissue. However, we found that the wounded edges of cutaneous muscle layer also produced tenascin prior to the development of granulation tissue between epidermis and muscle layer. This finding suggests that the production of tenascin in wounded edges of cutaneous muscle layer is not induced by healing epidermis, but is an intrinsic response of this tissue to wounding.

Fibronectin also has been known to appear during wound healing (Kurkinen *et al.*, 1980; Grinnel *et al.*, 1981). The distribution pattern of fibronectin is quite different from that of tenascin. During wound healing, fibronectin appears in fibrin clots and the loose mesenchymal tissue fills in the wound space and persists in the granulation tissue throughout the healing process. Moreover, even the intact dermal tissue is always stained with anti-fibronectin serum. It is possible that fibronectin plays a role in the formation of granulation tissue, whereas tenascin may be necessary for the function of mature granulation tissue, wound contraction and dermal reconstruction.

Materials and Methods

Female C57BL/6 strain mice were anesthetized with ether, and their backs shaved with a razor blade. Transverse razor cuts about 5 mm in length were made down to the level of cutaneous muscle layer on the skin of the back. Most of the mice were given 2-4 wounds of different stages, and 1-4 mice were examined at each stage. The mice were anesthetized with ether and killed by cervical dislocation at 1, 2, 3, 4, 5, 7, 10, or 14 days after wounding, and the skins were excised, fixed with 95 % ethanol, embedded in paraffin and sectioned. Deparaffinized sections were stained by immunofluorescence, as described previously (Murakami *et al.*, 1990) using rabbit anti-tenascin serum prepared to chick tenascin, a gift of Dr. Chiquet-Ehrismann (Friedrich-Miescher-Institute, Basel). Some sections were treated with 0.5% testicular hyaluronidase (Type I-S, Sigma) in 0.2 M acetate buffer, pH 4.8, for 1 hour at room temperature, which increased immunoreactivity of the sections. Some sections were stained with haematoxylin and eosin.

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