American Journal of Pathology, Vol. 142, No. 3, March 1993

Macrophages and Fibroblasts Express Embryonic Fibronectins During Cutaneous Wound Healing

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Fibronectins (FN) comprise a family of adhesive glycoproteins that are prominent components of wound healing. These proteins arise by alternative splicing of a single gene transcript at three sites, termed EIIIA, EIIIB, and V. Extravasated plasma FN, which lacks the EIIIA and EIIIB domains, along with fibrin, comprise the "provisional" matrix that forms within minutes of tissue injury. By 2 days after cutaneous excisional wounding in rats, total FN messenger RNA (mRNA) expression is increased locally and dramatically within the surrounding dermis, in the subjacent muscle (panniculus carnosus) and, notably, at the wound margins. Moreover, in contrast to normal skin, 2-day wounds express EIIIA- and EIIIB-containing "embryonic" FN mRNAs. To identify the cells responsible for synthesizing the various FN isoforms, we performed in situ hybridization with probes for the various FN mRNAs. Collagen and lysozyme probes were employed to distinguish fibroblasts from macrophages. At early intervals (2 days) after wounding, macrophages were the principal cells that expressed FN mRNA. Moreover, many of these cells expressed embryonic FN mRNAs. At 7 to 10 days, when the wound defect was maturing, fibroblasts were the major cells synthesizing these embryonic FNs. It is widely accepted that wound macrophages phagocytose debris and provide degradative enzymes and cytokines essential for early stages of tissue repair. Our findings suggest an additional function for wound macrophages—synthesis of embryonic FNs providing an extracellular matrix that facilitates wound repair, perhaps by promoting cell migration. (Am J Pathol 1993, 142:793–801)

Wound healing is comprised of a succession of overlapping stages that include acute and chronic inflammation, cell migration, matrix deposition, and tissue remodeling.[1] In each of these stages, cells function in patterns that resemble those of normal embryonic development.[2] Thus, cell-to-cell interactions, transmission of diffusible signals, and complex interactions among epithelial cells, mesenchymal cells, and the extracellular matrix are all features common to both embryogenesis and adult tissue repair. Fibronectin (FN) is a prominent matrix component that promotes cell migration in development and has important functions in several stages of wound healing, e.g., platelet aggregation, epidermal cell migration and differentiation, collagen matrix assembly, and wound contraction.[3–8]

The FNs comprise a family of dimeric glycoproteins found both in blood plasma and in the extracellular matrices of many tissues.[8–12] Molecular cloning has demonstrated that FN exists in multiple forms that arise from a single messenger RNA (mRNA) transcript that can be alternatively spliced in three regions: EIIIA (or ED-A), EIIIB (ED-B), and V (or IIICS). Whereas the EIIIA and EIIIB domains are either included or excluded, processing of the V region is more complex.[13] Plasma FN (pFN) lacks the EIIIA and EIIIB domains, but the cellular FNs, synthesized by cultured cells, are a mixture of forms with and without these regions. The mechanisms responsible for regulating FN splicing *in vivo* and the functions of the EIIIA and EIIIB domains are unknown. The V region contains at least one cell-binding site, suggesting that splicing within this domain may alter cell-FN attachment.[14] EIIIA- and EIIIB-containing FN mRNAs predominate in the early embryo, whereas, in the adult, most normal tissue FNs lack these domains.[15–19] Recently, we reported that normal

Supported by grants from the National Institutes of Health (CA28471 to H. F. Dvorak and GM36812 to L. Van De Water) and the American Cancer Society (JFRA 198 to L. Van De Water).

Accepted for publication September 4, 1992.

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adult rat skin expressed FN mRNAs that lacked the EIIIA and EIIIB domains.[20] However, embryonic FN mRNAs containing the EIIIA and EIIIB domains were expressed during cutaneous wound healing in a temporally and spatially defined pattern.[2,20] Unfortunately, the suboptimal morphology afforded by *in situ* hybridization in paraffin sections did not allow us to distinguish macrophages from fibroblasts and, therefore, to identify the specific cell types responsible for synthesizing FNs at successive stages of wound healing.

We have now carried these studies a step further by employing probes for lysozyme and type I collagen mRNAs that allowed reliable distinction of macrophages from fibroblasts. We report that macrophages are the first cells in healing wounds to express increased amounts of FN mRNA locally and do so as early as 2 days after injury; some of these macrophage FN mRNAs contain the EIIIA and EIIIB domains. However, at a late (7-day) interval after wounding, granulation tissue fibroblasts assumed the role of expressing FNs in both adult and embryonic forms.

Materials and Methods

Probes

Probes for rat FN employed in either *in situ* hybridization and RNAse protection assays (EIIIA and EIIIB) have been described.[20] The templates used to generate probes for the rat EIIIA and EIIIB domains of FN mRNA were the kind gift of R.O. Hynes.[21] A complementary DNA probe (438 bp) representing murine lysozyme mRNA[22,23] was prepared by polymerase chain reaction using sense (CTCAAGCTTGTCTATGAACGTTGTGAG) and anti-sense (GGCGAATTCCCGAATGAGCTGCAGTAG) primers, together with a λgt11 mouse macrophage library (Clontech, Palo Alto, CA) and a Thermal Cycler (Perkin-Elmer/Cetus) programmed (30 cycles) at 95 C for 1 minute, 55 C for 1 minute 72 C for 2 minutes. The resulting product was subcloned into pGEM-3 (Promega Biotec, Madison, WI) using established procedures.[24] A *Pst*1/*Eco*RI fragment (600 bp) of type I collagen was excised from pα1R2[25] (a kind gift of B. Kream and D. Rowe), gel purified, and subcloned into PGEM-3.

Preparation of Tissue and Cells for in Situ *Hybridization*

Partial thickness punch biopsy wounds (4 mm diameter) were placed on the flanks of adult female (150 to 200 g) CD rats (Charles River Laboratories, Inc., Wilmington, MA) anesthetized with 25 mg/kg ketamine HCl and 5 mg/kg xylazine. At various intervals after wounding, rats were sacrificed and tissues harvested, fixed, embedded, and prepared for in situ hybridization as described previously.[20]

Peritoneal exudate cells were elicited by intraperitoneal injection of 5 ml (CD rats) or 1 ml (female Balb/c mice) 3% thioglycollate. Four days later, peritoneal cells were harvested by lavage with Hanks' balanced salt solution (rats, 60 ml; mice, 10 ml), washed once, and attached to poly-L-lysine-coated slides by centrifugation (700 rpm, 4 minutes) of cell suspensions (1×10^5 cells/slide) in a Shandon Cytocentrifuge. For some experiments, rat embryo fibroblasts (Rat-1, ATCC) were cultivated in Lab-Tek chambers (1 to 2×10^5 cells cm^{-2}) in Dulbecco's minimal essential medium with penicillin and streptomycin, supplemented with fetal calf serum (5% v/v). For *in situ* hybridization, slides were fixed in ice-cold 5% glacial acetic acid, 4% formaldehyde, and 85% ethanol for 10 minutes and stored in ethanol (70%) before processing. All procedures utilizing animals had the approval of the Beth Israel Hospital IACUC.

In Situ *Hybridization*

Single-stranded RNA probes, with either sense or anti-sense orientation, were synthesized to a specific activity of $\sim 10^8$ cpm/μg with [^{35}S]-UTP and purified on polyacrylamide gels.[20] The DNA inserts were 270, 213, 209, 250, 438, and 600 nucleotides for FN-C, EIIIA, EIIIB, V95, lysozyme, and α2(I) collagen, respectively, and were used without any reduction in length.

In situ hybridization was carried out as described previously.[20] Preliminary experiments established the specificity of the probes for lysozyme and type I collagen. The anti-sense probe for mouse lysozyme reacted with mouse (Figure 1A) and rat (not shown) peritoneal macrophages but not with lymphocytes, neutrophils, or mast cells. Virtually all cells with the nuclear morphology of macrophages were identified as positive with the lysozyme probe; in contrast, no peritoneal cells were positive with "sense" probes (Figure 1B). To confirm the specificity of the lysozyme probe, mouse peritoneal macrophage or rat fibroblast RNA was fractionated by electrophoresis on agarose gels, transferred to nitrocellulose, and hybridized with a [^{32}P]-labeled lysozyme probe. Hybridization was observed to an appropriately sized, 1.6 kilobase mRNA, present in macrophages but not in fibroblasts (results not shown).[23] When *in situ* hybridization was carried out for type I collagen, no labeling was observed in peritoneal exudate cells

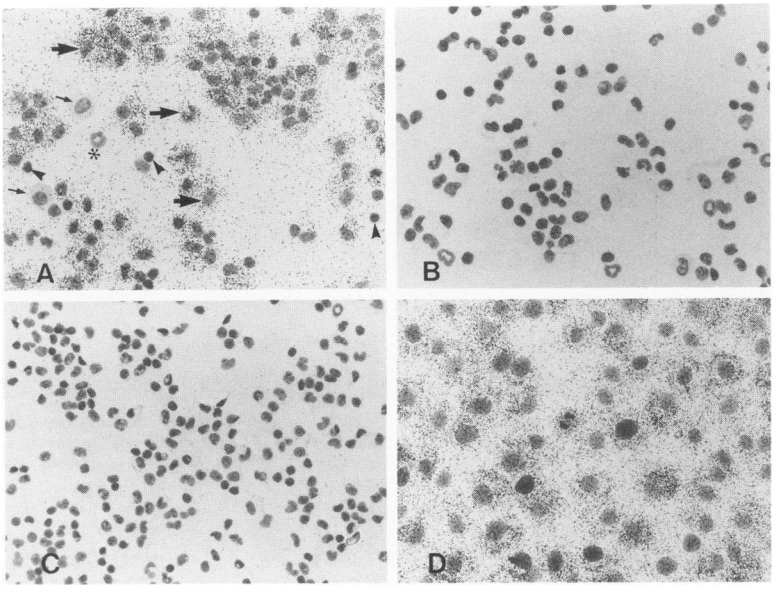

Figure 1. *In situ hybridization demonstration of specificity of lysozyme and collagen probes in mouse peritoneal exudate cells* (**A** *to* **C**) *or in cultured rat fibroblasts* (**D**). *Cytospin preparations of freshly isolated, thioglycollate-elicited peritoneal cells* (**A** *to* **C**) *were hybridized with the lysozyme* (**A**) *or α2(I) collagen* (**C**) *anti-sense or lysozyme sense* (**B**) *probes. Macrophages (large arrows) labeled with the lysozyme anti-sense probe* (**A**), *whereas neutrophils (asterisk), mast cells (small arrows), and lymphocytes (arrowheads) did not. Peritoneal cells also did not label with the lysozyme sense probe* (**B**) *or with the probe to collagen* (**C**). *The collagen probe reacted strongly with cultured rat embryo fibroblasts* (**D**). *Hematoxylin stain, brightfield* (**A**, **B**, **D**, ×*300*; **C**, ×*240*).

(Figure 1C). However, the same probe reacted with virtually all cultured rat embryo fibroblasts (Figure 1D), and significant levels of collagen mRNA were found in fibroblasts of uninjured dermis, indicating that our collagen probe labeled fibroblasts in tissue sections as well as in culture. These cells did not react with a collagen probe transcribed in the sense orientation (not shown). Sections were viewed on a Zeiss Axiophot microscope. Black and white photographs were taken with transmitted light and color photographs were with incident light from a 200 watt mercury lamp using a polarizer filter set (Zeiss 487960, Carl Zeiss Co, Oberkochen, West Germany) in the reflector slider.

Preparation of Cellular RNAs from Tissues and Cells in Culture

Freshly prepared rat liver, Rat-1 cells (ATCC, Rockville, MD) cultivated in FCS_5 at a density of 1 to 2 × 10^5 cells/cm^2 or rat or mouse peritoneal cells were immediately extracted in guanidinium isothiocyanate and centrifuged through CsCl.[26]

RNAse Protection Assays

These were carried out as described.[27] In brief, uniformly labeled RNA probes were prepared by *in vitro* transcription from all templates using T7 polymerase with [^{32}P]-UTP (800 Ci nmol/L^{-1}) included in the reaction mixture. Transcripts were purified on denaturing polyacrylamide gels, and a molar excess of probe was added to cellular RNA and to carrier yeast transfer RNA. Mixtures were heated and hybridization carried out overnight at 37 C. Unhybridized probes were removed by digestion with a mixture of RNAses A and T1 for 30 minutes at 37 C. The samples were then extracted with phenol/chloroform and analyzed on a denaturing 6% polyacrylamide gel.

Results

Macrophages Are Responsible for the Majority of FN mRNA Synthesized in Early Phases of Wound Healing

We previously reported that the expression of total FN messenger RNA increases progressively in the first week after wounding[20] and that embryonic forms of FN messenger RNAs appear in some of the granulation tissue cells. Our present investigation sought to identify the cell type(s) responsible for FN synthesis at both early and late intervals after wounding. Closely adjacent sections of 2-day rat wounds were hybridized with anti-sense [^{35}S]-labeled RNA probes for lysozyme, type I collagen, and FN-C mRNAs (Figure 2). FN mRNA expression was concentrated primarily in cells (arrows) at the lateral wound margins (Figure 2A) or superficial wound bed. These FN-producing cells were positive for lysozyme, a macrophage-specific marker (Figure 2B) but were negative for collagen mRNA expression (Figure 2C). Individual macrophages in the superficial wound bed varied in the amounts of FN mRNA they expressed; approximately half were heavily labeled, whereas others were lightly labeled or unlabeled (Figure 3A). Macrophages in the deeper wound bed expressed FN in a similar pattern (Figure 3B). Neutrophils (arrowheads), readily identified by nuclear morphology, were consistently negative for FN mRNA.

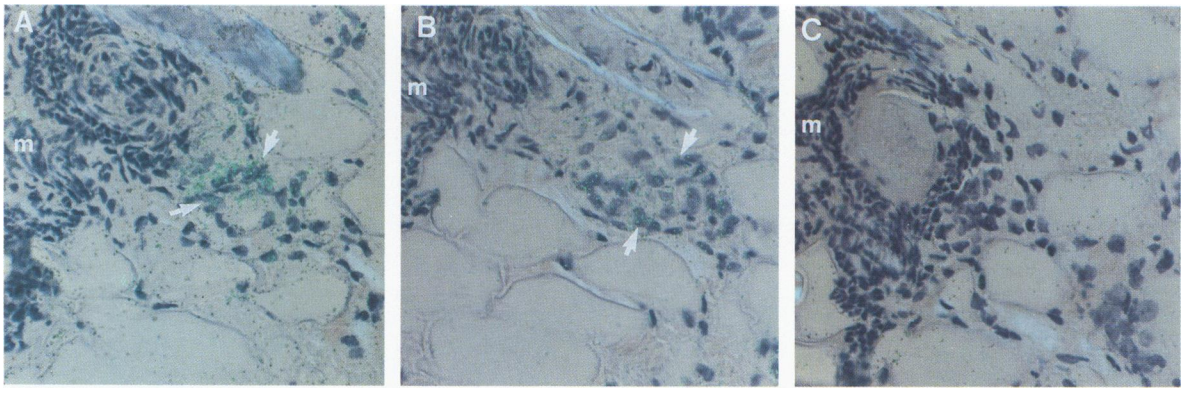

Figure 2. *Macrophage FN mRNA expression in 2-day wounds. Wound sections were hybridized with FN* (A), *lysozyme* (B), *or α2(I) collagen* (C) *anti-sense probes. Cells that labeled positively (arrows) with FN and lysozyme were found in nests at the margin of the wound; these cells did not label with a probe to α2(I) collagen. Transillumination of hematoxylin-stained paraffin sections (×720). Silver grains stain green.*

Macrophages Express Differentially Spliced Forms of FN mRNA at Early Stages of Wound Healing

To determine which forms of FN were synthesized by wound macrophages, adjacent sections of 2-day wounds were hybridized with probes reactive with total FN mRNA (FN-C) and with mRNAs containing the EIIIA and EIIIB domains. Included among the prominent clusters of FN-C mRNA–positive cells in the superficial wound bed (Figure 4A) were cells that expressed FN mRNAs containing the EIIIA (Figure 4C) and EIIIB (Figure 4B) domains. Macrophages in subjacent muscle were also labeled with FN-C probes (Figure 4D), but only occasional cells labeled with FN-A (Figure 4F) and EIIIB labeling was equivocal (Figure 4E).

Fibroblasts Produce Differentially Spliced FN mRNAs at Later Stages of Wound Healing

At later stages of healing (7 to 10 days), the wound defect was filled with maturing granulation tissue containing numerous fibroblasts as judged both by morphological criteria in 1 μm Epon sections (Figure 5F) and by hybridization with a type I collagen probe. There was no significant labeling with a lysozyme probe (Figure 5D). Both EIIIA- (Figure 5B) and EIIIB- (Figure 5C) as well as FN-C- (Figure 5A) labeled cells were prominent and co-localized over cells identical in appearance to those that labeled with the type I collagen probe (Figure 5E). Thus, embryonic FN mRNAs continued to be produced but now predominantly by fibroblasts.

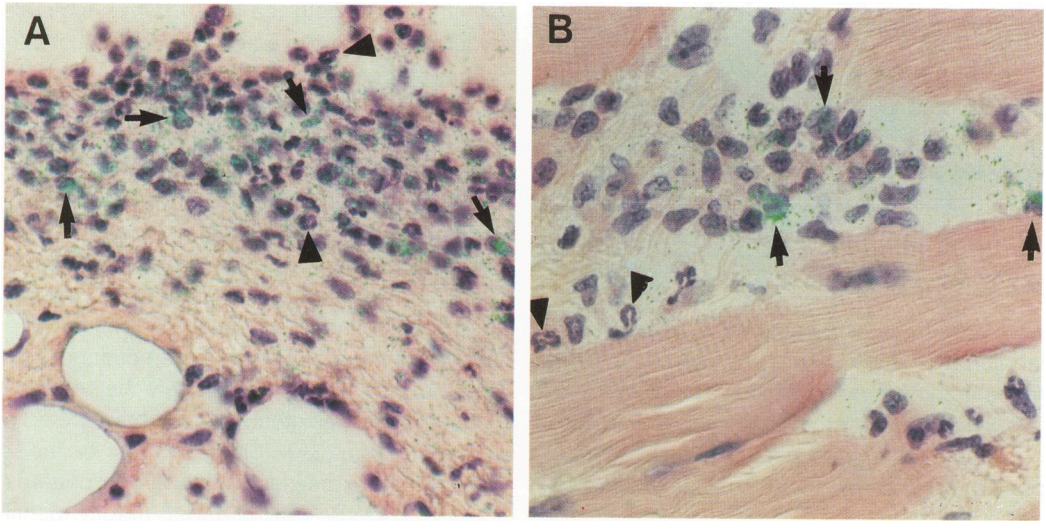

Figure 3. *FN mRNA expression in the superficial* (A) *and deep* (B) *beds of 2-day wounds using a probe (FN-C) that reacted with all forms of FN mRNA. Macrophages (arrows) expressed FN mRNA superficially* (A) *and within the subjacent muscle* (B). *Neutrophils (arrowheads) did not label. Silver grains stain green. Transillumination of hematoxylin- and eosin-stained paraffin sections (×480).*

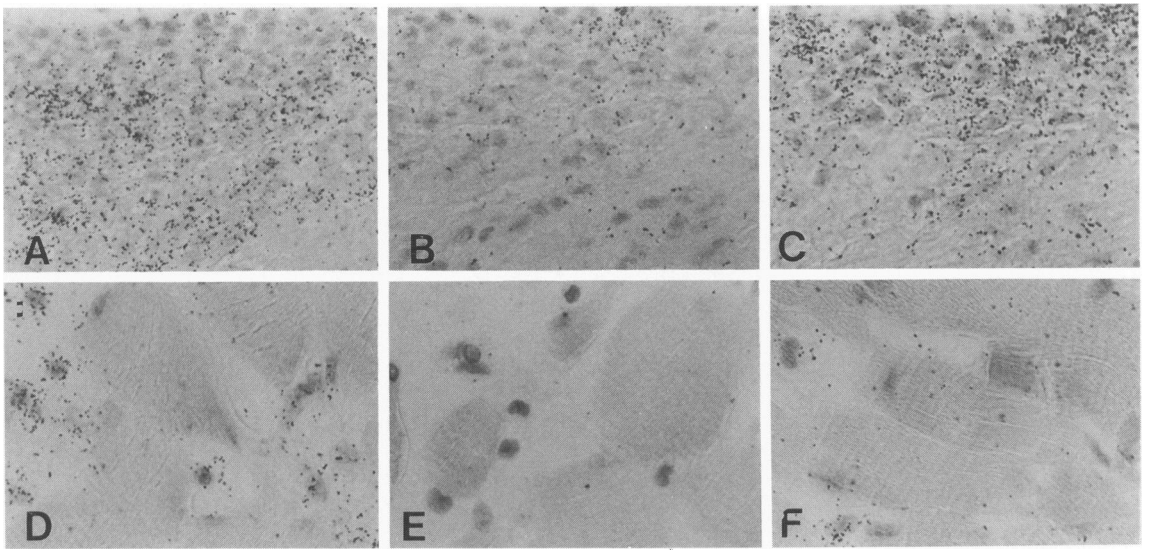

Figure 4. *Expression of different alternatively spliced forms of FN by macrophages in the superficial* (A *to* C) *and deep* (D *to* F) *beds of 2-day wounds. Sections were hybridized with probes that recognize all forms (FN-C) of FN mRNA* (A, D), *EIIIB-containing FN mRNA* (B, E), *or EIIIA-containing FN mRNA* (C, F). *All probes were of similar size and were labeled to the same approximate specific activity and sections and were exposed to autoradiographic emulsion for identical intervals. Labeling (silver grains are black dots) is evident in the superficial wound bed* (A, C) *with all three probes (FN-C, EIIIA, EIIIB). In subjacent muscle* (D *to* F), *only occasional EIIIA-positive cells were found and EIIIB labeling was equivocal. Hematoxylin- and eosin-stained paraffin sections, brightfield (×640).*

Isolated Peritoneal Macrophages and Cultured Fibroblasts Express Alternatively Spliced Forms of FN

Our *in situ* hybridization results indicated that 2-day wound macrophages expressed embryonic FNs. To obtain additional support for this finding, we performed nuclease protection assays on thioglycollate-elicited peritoneal cells isolated from rats. Macrophages produced a mixture of FN mRNAs, some of which contained whereas others lacked the EIIIA domain (Figure 6A, lane 3); however, rat peritoneal cells did not express significant amounts of FN mRNA that contained the EIIIB domain (Figure 6B, lane 3). Identical results were obtained with resident peritoneal macrophages isolated from animals not previously injected with thioglycollate. Established lines of rat embryo fibroblasts produced FN in its several variant forms (Figure 6, A, lane 4, and B, lane 2).

In situ hybridization gave identical results. As noted earlier, fibroblasts identifiable with a type I collagen probe (see Figure 1), were not found in freshly isolated peritoneal exudates. Peritoneal macrophages (Figure 7A, arrows) expressed FN mRNA whereas lymphocytes, granulocytes, and mast cells did not. We observed considerable heterogeneity in the proportion and overall levels of FN mRNA expression; ~50% of the macrophages observed labeled positively, but the number of grains per cell varied widely from <10 to >50. Peritoneal macrophages also expressed EIIIA-containing FN mRNA, again with considerable heterogeneity in terms of grain number per cell. However, in no instance was EIIIB mRNA expressed at detectable levels (Figure 7B).

Discussion

Only low levels of FN mRNA are expressed in normal adult rat skin and these lack detectable EIIIA and EIIIB domains. In contrast, healing skin wounds are characterized by greatly increased local expression of cellular FNs, at least some of which include the EIIIA and EIIIB domains.[20] In the present investigation, we identified the specific cells that synthesized FN messenger RNAs at early and late intervals after wounding, using specific probes to lysozyme and type I collagen to identify macrophages[23] and fibroblasts, respectively (Figure 2). At an early interval (2 days) after wounding, lysozyme-expressing macrophages were the principal cells expressing both FN-C and embryonic forms of FN mRNA (Figure 4). It is probable that wound macrophages also synthesized and secreted FN protein because FN mRNA and protein expression have been closely correlated in isolated macrophages.[28] Although earlier *in vitro* work had demonstrated that macrophages are capable of synthesizing FN (and other matrix components such as thrombospondin and osteopontin),[29–34] the present study is the first to demonstrate such synthesis in an *in vivo* setting. As wound healing progressed and granulation tissue replaced the provisional fibrin wound matrix (days 7 to 10), fibroblasts

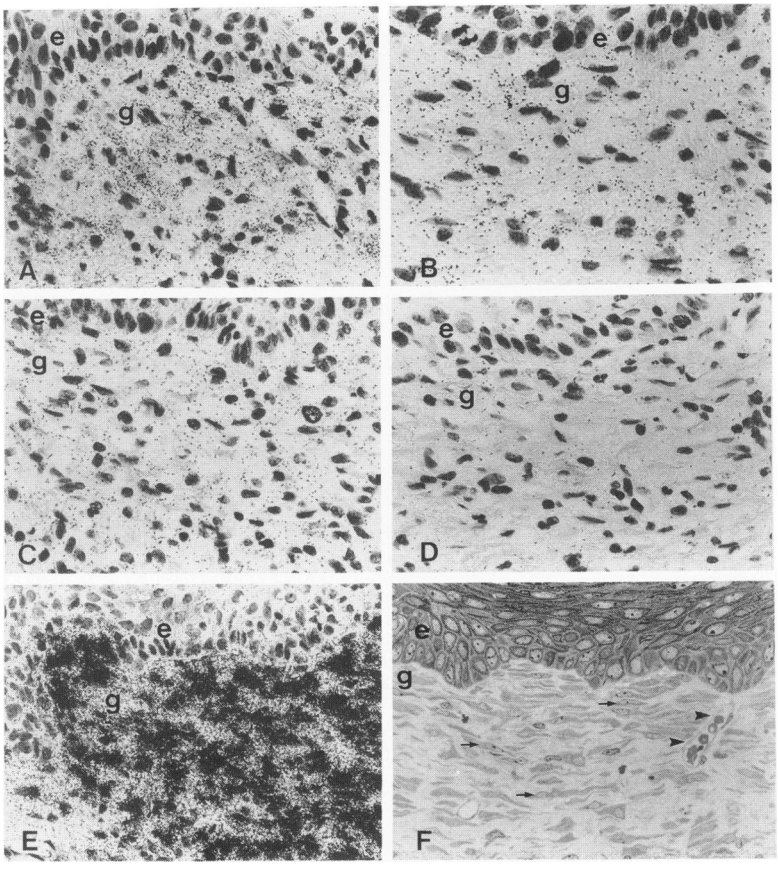

Figure 5. *Histology* **(F)** *and FN, collagen, and lysozyme mRNA expression* **(A** *to* **E)** *in 7-day wounds. FN mRNAs containing the FN-C region* **(A)**, *the EIIIA* **(B)**, *and EIIIB* **(C)** *domains were expressed by cells in granulation tissue (g) but not in the epidermis (e). Few of these cells expressed lysozyme mRNA* **(D)**, *whereas most expressed* $\alpha 2(I)$ *collagen mRNA* **(E)**. *F: Histology of 7-day wound. Note restored epidermal covering, elongate fibroblasts (arrows) oriented parallel to the surface, and newly-formed blood vessels (arrowhead) characteristic of maturing granulation tissue* **(A** *to* **E)**. *Hematoxylin- and eosin-stained paraffin sections, coated with emulsion so that silver grains appear as black dots in brightfield:* **(F)**, *Giemsa-stained 1 μm Epon section* **(A, C, D,** $\times 360$; **B,** $\times 470$; **E, F,** $\times 300$).

became the major source of cellular FN mRNA synthesis (Figure 5); like macrophages, fibroblasts expressed FNs that included the EIIIA and EIIIB domains. Thus, in healing skin wounds, both macrophages and fibroblasts synthesized increased amounts of total and embryonic FN mRNAs but at distinctly different times. Other cells participating in wound healing (neutrophils, lymphocytes, mast cells, and keratinocytes) did not synthesize detectable FN mRNA of any type (Figures 1, 3, and 5 and ref. 20).

Whereas wound macrophages expressed FN mRNAs that contained both the EIIIA and EIIIB domains, peritoneal exudate macrophages expressed FN mRNAs containing EIIIA but not EIIIB (Figures 6, 7). To account for this result it seems fair to conclude that expression of the EIIIA and EIIIB domains is controlled independently and that different sets of regulatory signals present in wounds and in the peritoneal cavity account for the different types of FN expressed. Circulating blood monocytes, the precursors of tissue macrophages, do not express detectable levels of FN mRNA or protein.[28,35] Therefore, because wound macrophages express FN as early as 1 to 2 days after wounding, monocyte differentiation into FN-expressing macrophages must be rapid and likely results from signals encountered during or shortly after diapedesis. Signaling could reflect macrophage contact with endothelial cell surface proteins, with matrix molecules and/or with soluble growth factors. Growth factors such as TGF-β alter the FN splicing patterns of cultured fibroblasts and airway epithelial cells[36,37] and are present in wounds as products of both activated platelets and macrophages.[38,39] It is known that TGF-β stimulates cultured fibroblasts to synthesize increased amounts of both FN and collagen[40–42]; however, the signals that regulate macrophage expression and alternative splicing of FN mRNAs have not yet been determined. On the other hand, recent evidence indicates that simple culture of bone marrow monocyte precursors leads to phenotypically distinct macrophage subsets that elaborate distinct cytokines.[43,44] Thus, the pattern of FN splicing we have observed in macrophages at an early stage of wound healing could result from the selective attraction from the circulation of monocyte subsets that have been preprogrammed in the bone marrow to express one or another FN mRNA. Available data are insufficient to decide between these alternatives, i.e., whether wound macrophages are derived from monocytes preprogrammed to synthesize only certain discrete FN isoforms or whether the monocytes attracted to wounds

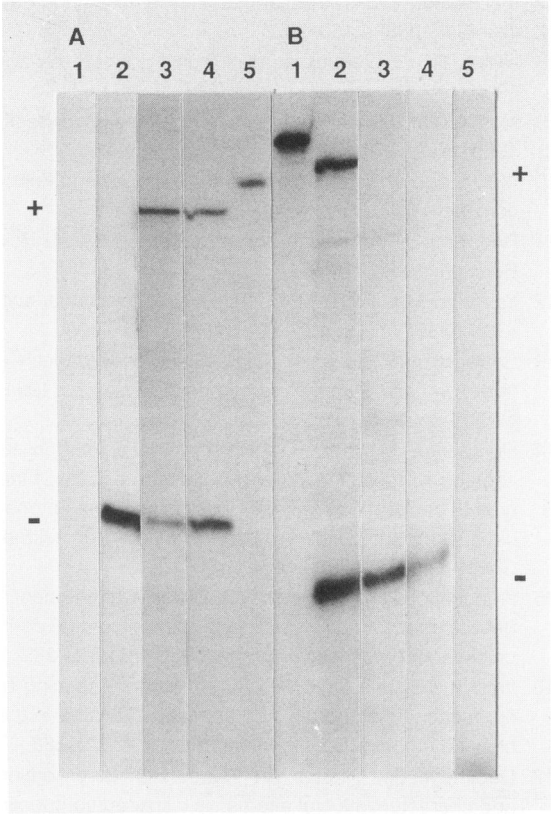

Figure 6. *Alternative splicing of FN by rat peritoneal macrophages and fibroblasts demonstrated by RNAse protection assays. Assays were carried out with separate probes for EIIIA* (A) *and EIIIB* (B). *Macrophages* (A, *lane 3*) *expressed FN mRNA species that both included (+) and excluded (−) the EIIIA domain; however, FN mRNA species excluding (−) the EIIIB domain* B, lane 3) *were predominant. For comparison, rat fibroblasts* (A, lane 4, *and* B, lane 2) *express FN mRNAs that both include and exclude the EIIIA and EIIIB domains whereas rat liver* (A*2*, B*4*) *mRNA exclude both EIIIA and EIIIB domains. Yeast RNA* (A, lane 1, *and* B, lane 5), *which contains no FN mRNA, served as a negative control. Probes* (A, lane 5, *and* B, lane 1) *were applied to gels without RNAse treatment and contained short plasmid sequences that caused them to migrate slightly above the included (+) bands. Identical results were obtained with resident or elicited peritoneal macrophages.*

have the potential to synthesize multiple FN isoforms as determined by local signals.

The function(s) of individual FNs in wound healing are poorly understood. The abundance of FN present and the appearance of new, embryonic forms of the protein together suggest that these functions are biologically significant. One likely possibility is that FN has a structural function. Thus, it is known that pFN is covalently crosslinked into fibrin (by transglutamination with clotting factor XIIIA[45] and in this manner becomes an integral component of the early or provisional extracellular matrix. At later stages of wound healing, as the provisional matrix is replaced by granulation tissue, FN fibrils are thought to link interstitial collagens to the surfaces of fibroblasts.[46] *In vitro* studies have shown that both plasma and cellular FNs become incorporated into the extracellular matrices around cultured fibroblasts.[7,47,48] Recently it has been observed that cellular FNs are more efficiently incorporated into pericellular matrices than pFN, whereas cellular FNs are less efficiently incorporated into fibrin gels than pFN.[48,49] These data would suggest that cellular FNs, including those containing the EIIIB and EIIIA domains, contribute to the establishment of pericellular matrices, perhaps facilitating the generation and/or remodeling of mature matrix as part of granulation tissue formation.

In embryonic development, the prominent expression of EIIIA/EIIIB-containing FNs is associated with cell migration,[2,15,16,20] and it would not be surprising if these FNs had a similar role in wound healing where cell migration is also a prominent feature; e.g., the sequential extravasation of neutrophils and monocytes into the fibrin-rich provisional wound matrix, the migration of fibroblasts and endothelial

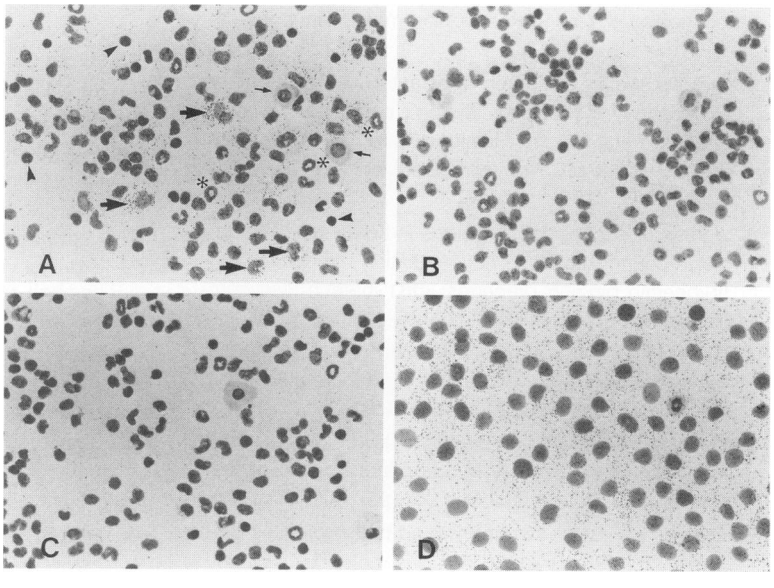

Figure 7. *Expression of FN mRNA by cytospun rat peritoneal exudate cells* (A *to* C) *and by cultured, adherent rat fibroblasts* (D). *Freshly isolated thioglycollate-elicited peritoneal exudate cells were hybridized with probes complementary to FN-C* (A), *to EIIIB-containing FN mRNA* (B), *or with the sense FN-C probe* (C)*). Fibroblasts were hybridized with FN-C* (D). *Macrophages* (A, *large arrows*) *and rat fibroblasts* (D) *expressed FN mRNA, whereas neutrophils* (A, *asterisks*), *lymphocytes* (A, *black arrowhead*), *and mast cells* (A, *small arrow*) *did not. None of the peritoneal cells tested expressed the EIIIB domain* (B) *or hybridized to a FN-C sense probe* (C) *(×300).*

cells from the neighboring connective tissue, and the lateral and upward migration of keratinocytes from neighboring epidermis and from skin appendages to cover the wound defect.[1] Whether newly synthesized cellular FNs facilitate cell migration awaits experimental testing. If they do, it will be of interest to determine whether these FNs affect all migrating cells or only the two found to make FNs in healing wounds, i.e., macrophages and fibroblasts. Arguing in favor of the possibility that FNs affect the behavior of non-FN-producing cells is the finding that migrating proliferating keratinocytes express fibronectin receptors[50] whereas differentiating keratinocytes do not.[3] The possible role of FN in endothelial cell migration in granulation tissue is more complicated because FN has been reported to promote[51] or arrest[52] endothelial cell migration in different models. More recent studies examining FN splicing *in situ* have shown that cellular FNs are expressed locally in regions of endothelial cell migration in balloon-injured arteries (Dubin et al, submitted).

In summary, the extracellular matrix is thought to provide an appropriate context for cell inflammatory sites.[53] By elaborating growth factors, cytokines, and, as here shown, cellular FNs, macrophages aid in the recruitment of fibroblasts and new blood vessels that transform the provisional fibrin-pFN matrix into the collagen-cellular FN matrix of granulation tissue. Alternative splicing of FN by both macrophages and fibroblasts may provide an important control point in wound healing and one that may be susceptible to therapeutic modulation.

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