MACROPHAGE–FIBROBLAST INTERACTION AND ITS POSSIBLE ROLE IN THE REGULATION OF COLLAGEN METABOLISM DURING WOUND HEALING

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The role of macrophages in the resorption of the denatured collagen of wound detritus was established by transmission and scanning electron microscopy of healing skin wounds. In the period preceding active collagen synthesis and fibrillogenesis a phenomenon of close cellular contact between macrophages and fibroblasts of the granulation tissue was found. It is postulated that the role of macrophage–fibroblast interaction consists of the transmission of specific information with the aid of processed collagen breakdown products on the basis of feedback between collagen catabolism and biosynthesis. This feedback may be part of the homeostatic mechanism regulating the development of connective tissue.

KEY WORDS: wound healing; fibroblast; macrophage; cellular contact.

The principal connective-tissue cell, responsible for both the biosynthesis and the catabolism of collagen, is the fibroblast [2, 8]. The molecular and ultrastructural aspects of collagen metabolism have been adequately studied. The mechanisms controlling its metabolism are less clear, especially in developing connective tissue during wound healing, inflammation, sclerosis, etc. At certain stages of these processes the predominant cell type is the macrophage, the functions of which include cleansing of the zone of injury. However, the macrophage also undoubtedly has a role in the subsequent transformation when the predominant process is proliferation of fibroblasts and the formation of collagen fibers. Reducing the number of macrophages in the wound considerably by the action of antimacrophagal serum caused a sharp delay in healing [6].

The object of this investigation was to study macrophage–fibroblast interaction and its role in the autoregulatory mechanisms of connective tissue development.

EXPERIMENTAL METHOD

Full-thickness wounds (4 cm^2) were inflicted on the back of August rats. The wounds in 20 animals healed under a scab, but a Teflon ring, covered with a cellophane film to prevent scab formation, was sutured into the wound of the other 20 rats. The animals were killed between 1 and 30 days later; the granulation tissue was fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, and embedded in Durcupan. Semithin sections were stained with toluidine blue; ultrathin sections, stained with uranyl acetate and lead oxide, were examined in the IEM-100B microscope. Samples of tissue for scanning electron microscopy were fixed in glutaraldehyde, dehydrated in acetone, and fractured in liquid nitrogen. The cleavage surface, and also dewaxed and frozen sections, were sprayed with carbon and silver and examined in the ISM-50A microscope with a magnification of between 100 and 30,000×.

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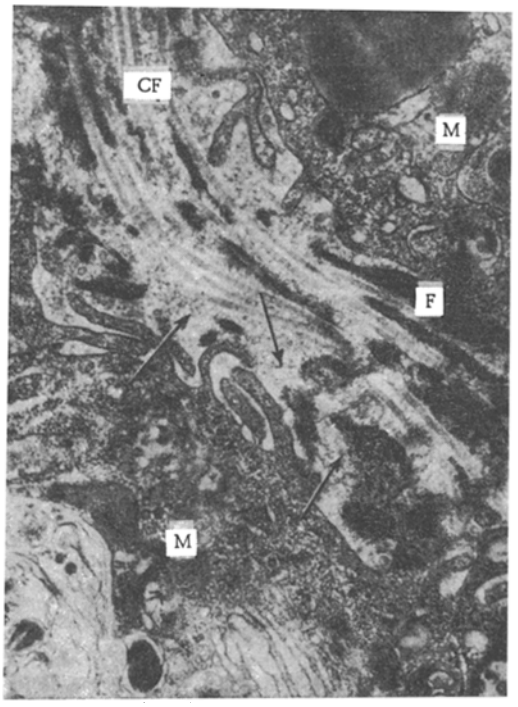

Fig. 1

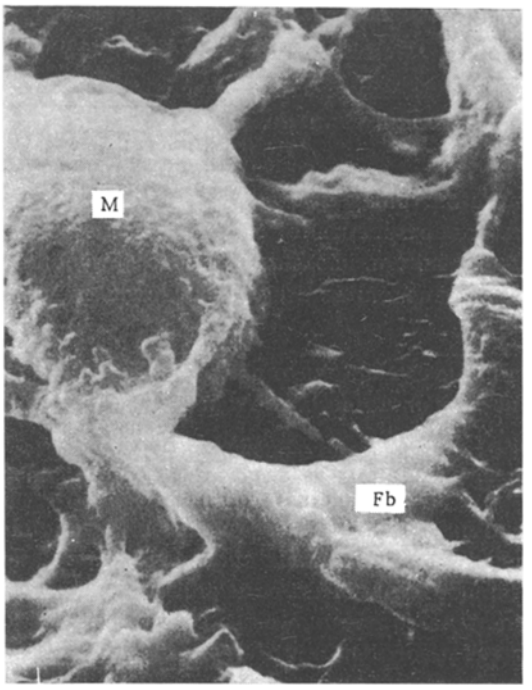

Fig. 2

Fig. 1. Macrophages (M), absorbing detritus consisting of fibrin (F) and collagen fibrils (CF), which have lost their characteristic structure in some places (arrows) (32,000×).

Fig. 2. Scanning electron micrograph of macrophage (M) and fibroblast (FB) in contact with each other (18,000×).

EXPERIMENTAL RESULTS

During the first few hours neutrophils and monocytes from the blood migrated into the wound. Macrophages differentiate from the monocytes, and on the second to third day after wounding they became the predominant cell form. The ultrastructure of the actively functioning macrophages differed considerably from that of the less differentiated forms. The nucleus was condensed, lost its nucleoli, became bean-shaped or lobular, and was often situated eccentrically. The lamellar complex and endoplasmic reticulum were partly reduced. Cytoplasm of the macrophages became denser and contained numerous phagocytic vacuoles, primary and secondary lysosomes, and residual bodies. Multiple projections formed on the cell surface (Figs. 1 and 2).

The function of the macrophages during this period is cleansing the wound from disintegrating cells (including neutrophils) and fibrin. At the edges and in the floor of the wound the tissue detritus consisted chiefly of collagen fibers, which are also absorbed by macrophages (Fig. 1). The collagen fibrils in the detritus retained their characteristic striation for a long time, but they gradually lost it and underwent granular degeneration and lysis. This denatured collagen was phagocytosed by macrophages. This confirms the writers' previous observations showing that macrophages, unlike fibroblasts, participate in the catabolism of collagen only when it has lost its fibrillary structure [2].

Fibroblasts appeared in the wound by the end of the first day, but appreciable numbers of them were not observed until the fourth to fifth day with the ingrowth of granulation tissue capillaries. At that time definite equilibrium existed between the number of macrophages and fibroblasts. Some of the fibroblasts had a pale, round nucleus with a large nucleolus, numerous free ribosomes and polysomes in their cytoplasm, and a moderately developed granular endoplasmic reticulum with narrow tubules. Other fibroblasts had ultrastructural signs of active collagen secretion: a well-developed lamellar complex with saccular dilatations and an abundant endoplasmic reticulum, the cisterns of which often occupied practically all of the cytoplasm. Under the scanning electron microscope the bodies of the fibroblasts were fusiform, cylindrical, or slightly flattened. Collagen fibrils formed around the cells. No significant difference in the formation of granulation tissue depending on the experimental conditions was observed.

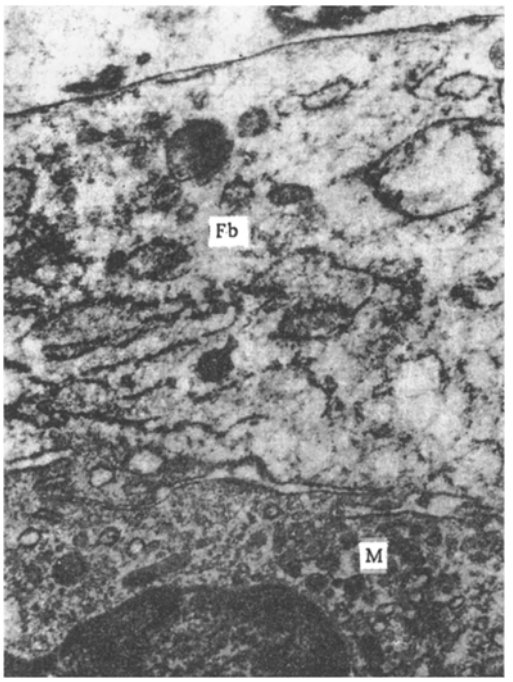

Fig. 3. Close contact between cell bodies of macrophage (M) and fibroblast (FB) (28,000×).

From the writers' point of view the phenomenon of direct contact between fibroblasts and macrophages revealed in these experiments is particularly important; these contacts could be seen on both light and scanning electron microscopy (Fig. 2), but they were particularly demonstrative on transmission electron microscopy (Fig. 3). The macrophage made close contact with the cell body of the fibroblasts by its projecting processes or the two cells were in close contact and in some parts their outer membranes were not clearly distinguishable. Mature actively phagocytosing macrophages and also fibroblasts usually with no marked features of increased collagen synthesis took part in these contacts. This phenomenon appeared at the beginning of the fibroblastic phase (fourth to fifth day) in the region where cells were loosely arranged and was absent in the zones of compact cells, evidence against its artifactual nature. No such contacts were observed under these conditions between the other cells of the granulation tissue, other than the well-known fusion of macrophages during the formation of a giant multinuclear cell. On the subsequent days (7th, 10th, 15th, and so on), despite the closer arrangement of the cells, no intercellular contacts were formed, the number of macrophages decreased, and fibroblasts became the predominant cell form.

When the functional purpose of this phenomenon is examined, the transmission of information from macrophage to fibroblast, like the transmission of immune information during macrophage–lymphocyte interaction, cannot be ruled out. The relative rarity of the phenomenon is perhaps evidence of the short duration of these contacts. According to data in the literature, macrophages evidently produce a certain factor (possibly of lysosomal origin) which stimulates proliferation of fibroblasts [6, 7]. Leibovich and Ross [6] likewise do not rule out inactivation of inhibitors of fibroblast proliferation by macrophages. Experiments have shown that a fibrogenetic factor, stimulating collagen production in a culture of fibroblasts, is produced in macrophages phagocytosing SiO_2 [4].

Ever since the work of Carrel and Ebeling [3], various hypothetical substances formed in the wound during cell disintegration and promoting its healing have been postulated (wound hormones, trephones, desmones, cytopoietins), but so far they have not been identified. Tissue breakdown products perhaps induce reparative processes through the intermediary of macrophages phagocytosing these products.

On the basis of their previous observations the writers postulated that besides nonspecific fibrogenetic factors, a more specific mechanism of feedback between collagen breakdown and synthesis may act in wounds, inflammation, and fibrosis [1] to regulate the metabolism of collagen during the formation of new connective tissue. The first stage of this feedback, according to this hypothesis, is resorption of disintegrating collagen fibrils of the wound detritus by macrophages, the processing of the molecules in the lysosomes of the macro-

phage, and the supplying of breakdown products of a certain size (peptides) to the fibroblasts, where they stimulate collagen biosynthesis.

The fact that cell contacts are found only at a certain stage of connective tissue development (before intensive synthesis and fibrillogenesis of the collagen) and that the fibroblasts participating in them are already relatively well differentiated, and not their possible precursors (mononuclear cells or pericytes), is evidence that macrophage–fibroblast interaction is aimed chiefly not at stimulating proliferation of the cells themselves, but at stimulating collagen formation in them. These hypotheses are also confirmed by experimental and clinical observations of the more rapid growth of connective tissue and of wound healing after implantation of collagen materials or injection of collagen solutions into the wounds [1, 5, 9]. Injection of fibrin into the wound in these experiments did not stimulate healing, thus indicating that this phenomenon is relatively specific.

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