Promotion of wound repair in old mice by local injection of macrophages

DAVID DANON*, MARY ANN KOWATCH†, AND GEORGE S. ROTH†

*Weizmann Institute of Science, Rehovot, Israel; †Molecular Physiology and Genetics Section, Gerontology Research Center, National Institute on Aging, Francis Scott Key Medical Center, Baltimore, MD 21224

Communicated by Christian B. Anfinsen, December 9, 1988

ABSTRACT Application of peritoneal macrophages to experimentally induced cutaneous wounds of old mice accelerates healing to levels almost comparable to those of untreated young animals. Slightly greater acceleration is observed when macrophages are obtained from young as opposed to old donors. These findings are consistent with a defect in macrophage function as a cause of impaired wound healing in senescence and suggest a possible therapeutic strategy.

Macrophages play an important role in wound repair (1) and produce substances that stimulate the synthesis of collagen by fibroblasts (2–4). Furthermore, macrophages produce a substance that causes proliferation of fibroblasts in vitro (5). Activated macrophages have been reported to induce vascular proliferation (6). Although attempts were made to characterize the substances produced by macrophages that enhance wound repair, a simpler way to accelerate the healing process was devised. It consisted of mobilizing the macrophages to the wound area and activating them. The effects of topical application of a variety of substances on the repair process of skin wounds in mice were examined. It was found that promotion of wound repair could be achieved in mice by application of glucan (7). The promotion of wound healing by glucan was evaluated later on single animals, by treating an experimental wound on one rear leg (the wound on the other leg serving as a control) in mice, rats, and guinea pigs. Glucan was found to be effective by accelerating the mobilization of macrophages to the wound (7, 8). All these experiments were carried out on young animals or on cells in culture. It is generally accepted that cutaneous wounds heal slower in the elderly as compared with the young. This has been confirmed in clinical studies (9–11) and in experimental investigations on mice and rats (12–16, †, §). Wound healing rate has even been used as a biological marker of age in mice.† Previous results suggested that altered estrogen or androgen levels or altered sensitivity to these steroids could account, at least partially, for the slower wound repair in old rats (16). The macrophage has been reported to be an estrogen-responsive cell type.§ Moreover, we demonstrated recently that the application of anti-macrophage serum to the experimental wounds in young mice resulted in slower wound healing, similar to that of old mice, during the period of greatest activity—i.e., the first 4–5 days after wounding (17). In the present study we investigated the possibility of accelerating wound healing in old mice by injecting peritoneal macrophages directly into the wound area.

MATERIALS AND METHODS

Wounding and Measurement Procedure. C57BL/6J male mice were obtained and maintained as described (19). Animals were anesthetized by methoxyflurane inhalation and clipped and shaved on the back. Four cutaneous wounds were made on each animal by lifting the shaved skin perpendicularly to the length of the mouse and punching twice (once anteriorly on the right side and once posteriorly on the left side) with a leather punch (circumference, 4.7 mm), resulting in two wounds on each side. Wounds were photographed immediately and at subsequent times from a fixed distance with the wound surface perpendicular to the axis of the lens. After development of the film, wound images were projected onto paper at a fixed distance, traced with a pencil, and cut out with sharp scissors. The relative sizes of the wounds were assessed by weighing the cut-out paper tracings. Healing was assessed from the mean decrease in wound area for the four wounds on each mouse.

Preparation and Application of Peritoneal Macrophages. Mice were anesthetized and 0.5 × 1.0-cm oval incisions were made abdominally. A 20-gauge needle was inserted 3–5 mm through the abdominal muscle and 2.5 ml of sterile phosphate-buffered saline (PBS; 0.15 M NaCl/0.1 M phosphate, pH 7.4) was injected into the abdominal cavity. After 2–3 min, a small curved forceps was used to open the incision without bleeding, and a 5-ml syringe with a 20-gauge needle, on which a 3-cm length of polyethylene tubing was mounted, was inserted and used to withdraw the fluid exuding from the abdominal cavity. Between 2 and 2.5 ml of the injected PBS was recovered, containing about 10⁶ cells with very few erythrocytes. After the fluid was centrifuged at 500 × g for 4 min, the supernatant was discarded and the sediment was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.
resuspended in Dulbecco's modified Eagle's medium (DMEM) containing penicillin, streptomycin, and 5% fetal bovine serum. The cells were incubated in 5-cm plastic Petri dishes and incubated for 2 hr at 37°C with 5% CO₂. After 2 hr the supernatant was removed, and the adherent cells were washed with DMEM, removed with a "policeman," and collected by adding 2 ml of PBS. Aggregates of cells were dispersed by passage through a 3-cm, 22-gauge needle, centrifuged as before, and resuspended in PBS to 2–3 × 10⁶ cells per ml. Differential counts indicated that cell preparations consisted of at least 80% monocytes and did not differ between age groups.

The two wounds on the right side of each recipient mouse were injected subcutaneously with 0.2 ml of macrophage suspension each. The two wounds on the left side were injected similarly with PBS. Sterile, water-soluble bacteriostatic gel (Surgilub, Forger, Melville, NY) was applied to the open wounds to minimize drying of the solutions. Wound healing was then assessed on days 0, 1, 3, and 5.

**RESULTS**

**Effect of Age on Wound Healing.** Fig. 1 shows that healing of cutaneous wounds induced by the present procedure is significantly slower ($P < 0.0001$ by analysis of variance) in old mice than in young mice. Times required for 50% closure were approximately 3 and 6 days for the young and old groups, respectively.

**Effect of Peritoneal Macrophage on Wound Healing.** Our previous studies, using a slightly different wounding procedure, had yielded similar results and suggested that a defect in macrophage function might account for age differences during the first few days of healing (17). Therefore, we next examined the effect of direct macrophage administration in the wound area. Fig. 2 illustrates the results of experiments in which macrophages obtained from young mice were transferred to either young or old recipients, or macrophages from old mice were transferred to old recipients. Macrophages were applied to wounds on the right side of the back only. Each mouse served as its own control, with PBS applied to wounds on the left side of the back. In all cases examined, right-side wounds healed significantly faster than left-side wounds ($P < 0.005$ by repeated measures analysis of variance). Fifty-percent healing times were reduced to approximately 3 days in old animals and 2 days in young. Macrophages obtained from young donors appeared to be somewhat more effective than those from old donors in

![Graph](image1)

**Fig. 2.** Application of peritoneal macrophages accelerates wound healing in mice. Wounds were induced as for Fig. 1. Just prior to the experiment, peritoneal macrophages were prepared and applied to right-side wounds. Left-side wounds were treated similarly with PBS. Wound healing was assessed on days 0, 1, 3, and 5. (Top) Young (5- to 8-month) macrophages applied to young mice. (Middle) Young macrophages applied to old (24- to 27-month) mice. (Bottom) Old macrophages applied to old mice. □, PBS-treated; ○, macrophage-treated.

![Graph](image2)

**Fig. 3.** Numbers of mice with wounds healing faster on the macrophage-treated right side (R) or saline-treated left side (L) or healing equally well (E). The animals used in Fig. 2 were inspected to determine the side on which the wounds healed faster. Values represent the numbers of mice with smaller wounds on the designated side on days 1, 3, and 5.
accelerating healing in old recipients (Fig. 3). When the numbers of mice healing faster on the macrophage- or PBS-treated sides were compared as a function of time, those old mice receiving young macrophages consistently showed a higher percentage of animals with faster healing on the right side (75–77%) than those old animals receiving old macrophages (50–60%). As in Fig. 2, it can be seen that all macrophage-treated groups healed faster than PBS-treated groups.

Fig. 4 shows an old mouse on days 0 and 5 after treatment with young macrophages. The contrast between the size of the left (PBS-treated) and right (macrophage-treated) wounds after 5 days is quite striking, with markedly faster healing on the right side.

**DISCUSSION**

Our previous studies showed (i) that the rate of cutaneous wound healing is reduced in aged mice and (ii) that antimacrophage serum administered to young mice slows healing times to those of old counterparts (17). Thus, it appeared possible that an age-related decline in some macrophage-dependent function(s) might be at least partially responsible for impaired wound healing during aging.

The results presented here demonstrate that direct macrophage treatment of cutaneous wounds can significantly accelerate wound healing in mice. In addition, such treatment is capable of reducing the time for 50% wound closure in old mice to approximately 3 days, a value similar to that for untreated young mice. Macrophages obtained from young donors appear to be slightly more effective than those from old donors for acceleration of healing. Nevertheless, the substantial acceleration afforded even by old macrophages suggests that such manipulation may provide a useful therapeutic strategy for stimulation of impaired wound healing in the elderly.

The precise age-associated impairment in macrophage function remains to be determined. Preliminary findings suggested that slower healing rates in aged mice were not due to reduced presence of macrophages in the wound area (17), although the rate of arrival of macrophages to the wound area was not evaluated. On the other hand, the acceleration of wound healing by macrophages from old mice injected into the wounds of old mice may suggest that macrophage homing capacity may be reduced in advanced age. Thus, some aspect of macrophage function, in addition to migratory capacity, would seem to be affected with age. It is now important, therefore, to examine macrophages from aged animals directly, especially with respect to their capacity for regulation by humoral factors and ability to produce trophic agents necessary for the healing process. This may be particularly important in light of the tendency (Fig. 3) for PBS-treated wounds to begin to approach healing rates of macrophage-treated wounds on day 5, possibly due to diffusion of such factors.

We thank Dr. Donald K. Ingram for statistical consultation and Mrs. Rita Wolferman for typing the manuscript.