Chemokinetic Response of Activated Macrophages to Soluble Products of Neoplastic Cells

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ABSTRACT

The rate of lateral movement of normal (unelicited) and pyran-activated murine (C57BL/6) peritoneal macrophages was used as a parameter of the macrophage response to exposure to the soluble by-products of neoplastic cells. Macrophages suspended in nutrient medium (5 x 10⁶ cells/ml) were injected into Sykes-Moore chambers, and their movement was recorded by time lapse cinematography for 37 hr. After 14 hr of filming, the nutrient medium was removed and replaced with fresh nutrient medium or cell-free conditioned media from syngeneic Lewis lung carcinoma or 2182 fibrosarcoma cells or syngeneic mouse embryo fibroblasts. Activated macrophages in nutrient medium moved about 2.0 times faster than did normal macrophages. When the medium was replaced with cell-free medium from the nonneoplastic mouse embryo fibroblasts, the rate of movement did not change. In contrast, cell-free conditioned media from the neoplastic cell lines caused a 2- to 3-fold increase in the rate of lateral movement of pyran-activated macrophages. The conditioned media did not induce any rate change when added to normal macrophages. Thus, activated macrophages responded cytrotropically to soluble by-products of the neoplastic cells tested.

INTRODUCTION

It is well documented in animal model systems that the migration and phagocytic functions of Mφ are inhibited in vivo by the presence of a syngeneic tumor (18, 19). Accordingly, the in vitro chemotactic responsiveness of Mφ is also depressed by serum-borne factors from tumor-bearing animals and man (13, 14). Seemingly, neoplasms thus abrogate the immunosurveillance role of the reticuloendothelial system in early detection and destruction of neoplastically transformed cells. Indeed, this idea is substantiated by the fact that monocyte function is significantly restored by the surgical removal of a neoplasm (3). However, immunoadjuvant-mediated tumor cell killing is frequently associated with the influx of Mφ into a tumor site. Mφ migration and cytotoxicity can be modulated in favor of the tumor-bearing host by activation with adjuvants such as Mycobacterium bovis, Corynebacterium parvum and pyran copolymer (4, 6, 20). Recent in vitro studies with the use of time lapse cinematography have shown that during coincubation with neoplastic cells the rate of random migration of Mφ that were activated by BCG or pyran is much greater (3 to 5 times) than that of normal Mφ (16, 25).

Normal Mφ obtained from mice by peritoneal lavage migrated similarly whether tumor cells were present or absent (25). However, when activated Mφ were coincubated with carcinoma cells, their movement varied as a function of whether they were in physical contact with a target tumor cell. When not in contact with a tumor cell, their rate of movement tripled. This suggested a cytropic response to a soluble product of the neoplastic cells. The experiments reported in this paper were designed to test this hypothesis. Activated and normal Mφ were exposed to cell-free media from cultures of neoplastic or nonneoplastic cells. The rate of lateral movement of those Mφ was then determined by time lapse cinematography.

MATERIALS AND METHODS

Target Cell Populations. The LL and 2182 cell lines are syngeneic in C57BL/6 mice and were used in this study as the tumor target cell populations. These cell populations were maintained locally as continuous cultures and were chosen as representing distinct classes of tumors, a carcinoma and a fibrosarcoma. Syngeneic secondary MEF’s were used as the normal target cell population. Previous studies have demonstrated that activated Mφ are cytoidal to LL and 2182 cells but not to MEF’s (11). The MEF’s were derived from dissociated 16-day C57BL/6 mouse embryos and were stored in 10% dimethyl sulfoxide in nutrient medium at −90°.

Effector Cells. Mφ were activated by i.p. injection of 25 mg of pyran copolymer, XA124-177 (Hercules Chemical Corp., Wilmington, Del.). On Day 7 peritoneal exudate cells were removed by lavage with Hanks’ balanced salt solution. Peritoneal exudate cells of several pyran-treated or normal (control) mice were pooled, centrifuged, and resuspended in EMEM in sterile Petri dishes (11). Mφ were purified by 2 hr of adherence followed by removal of the nonadherent cells by washing with 3 changes of EMEM. The adherent Mφ were then removed with a rubber policeman. Viability was determined by trypan blue exclusion (5), and the cells were resuspended in nutrient medium at 5 x 10⁶ cells/ml and were injected into Sykes-Moore chambers.

Media. The nutrient medium used for maintaining the
various target cell populations or derived from the target cells as conditioned medium for the experiments as well as for maintaining the effector cells was EMEM. This medium contained Earle's balanced salt solution, 2× amino acids, vitamins, 100 units penicillin per ml, 100 μg streptomycin per ml, and 20% heat-inactivated fetal bovine serum.

The various conditioned media were prepared by discarding the nutrient medium from established cultures of each of the target cells, rinsing the cells 3 times, and adding fresh EMEM. After 24 to 48 hr, the conditioned media were decanted, centrifuged to remove cells and cell debris, and used to replace the medium on 14-hr cultures of activated and normal MΦ.

**Time Lapse Cinematography.** A Wild M40 inverted phase-contrast microscope enclosed in a custom incubator was used for cinematography. During filming, the specimens were maintained at 37° in a moist atmosphere of 5% CO₂ in air. A Bellex Pollard 16-mm camera was regulated with a Sage Series 500 cinematographic apparatus (Sage Instruments, White Plains, N. Y.). Exposures were made at 0.4 sec/exposure and 1.0 exposure/min with Kodak Plus-X reversal 16-mm film. Cells were filmed with a ×10 phase-contrast objective lens and a ×5 projection lens. All experiments were repeated and filmed in duplicate. The rate of lateral movement of MΦ was measured by mapping the position of individual cells at 10-min intervals (as shown in Chart 1) and measuring the distance traveled with a Numonics Model 250 planimeter (Numonics Corp., Lansdale, Pa.). For graphic illustration of these data, the rates of movement of 25 to 40 cells during 1-hr periods were statistically evaluated. Student's t test was used to assess the significance of differences. Significant differences were considered to be p < 0.05.

**Protocol.** Normal as well as activated peritoneal MΦ that were suspended in nutrient medium were injected into Sykes-Moore chambers and time lapse cinematographic recording was begun. After 14 hr the nutrient medium was removed from these cultures and replaced with cell-free conditioned media from the target cell populations. Cinematography was continued for an additional 23 hr, and the rate of movement of the MΦ under the varying media conditions was then determined from maps that were made from the time lapse film.

**RESULTS**

The rate of lateral movement of peritoneal MΦ varied depending on whether they were activated and on the source of cell-free supernatants. This is illustrated qualitatively in Chart 1. The chart is composed of maps of the

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**Chart 1.** Maps illustrating MΦ movement during the tenth hr after changing their media. a, normal MΦ, fresh EMEM; b, normal, LL-conditioned medium; c, normal, 2182-conditioned medium; d, activated MΦ, EMEM; e, activated, LL-conditioned medium; f, activated, 2182-conditioned medium.
movement of several Mφ from various experimental groups during the tenth hr after changing their nutrient medium to 1 of the conditioned media. The migration of normal Mφ was similar whether they were placed in nutrient medium or in tumor-conditioned media (Chart 1, a, b, c). However, in all situations the movement of normal Mφ was clearly less than that of the pyran-activated Mφ (Chart 1, d, e, and f). Under these conditions the movement of the activated Mφ was less in control than in tumor-conditioned media. The rate of movement of the Mφ shown in Chart 1 is illustrated quantitatively in Chart 2. Activated Mφ in control nutrient medium moved at a rate approximately twice that of normal Mφ (0.46 versus 0.22 μm/min; p < 0.001). This difference was even greater when the cells were in tumor-conditioned media (0.96 versus 0.20 μm/min, p < 0.001, with the supernatant of LL; 0.71 versus 0.27 μm/min, p < 0.001, with 2182 supernatant). In contrast to the increased rate of movement of activated Mφ in either of the tumor supernatants, no significant change (p > 0.100) occurred in the rate of movement of normal Mφ whether nutrient medium or tumor-conditioned media were placed on them. This is not to say, however, that the normal Mφ were unresponsive to the tumor-conditioned media. Both normal and activated Mφ tended to spread and develop ruffled borders in the presence of the tumor-conditioned media but not in the presence of nutrient medium or MEF-conditioned medium. The extent of ruffling of the borders of activated Mφ was in general greater than that of normal Mφ. Many of the activated cells developed ruffled borders within 15 min after the addition of a tumor supernatant, whereas this process took as long as 2 hr for normal Mφ. These actively motile borders usually formed the leading edge in the direction of movement.

The rate of movement of Mφ over the total time period and under the various experimental conditions is illustrated in Charts 3 to 5. The rate of migration of the activated Mφ in nutrient medium was initially about twice that of normal Mφ. This difference gradually decreased; the rate of movement of the activated cells progressively declined with time, whereas that of normal Mφ remained constant (Chart 3). The change to fresh control nutrient medium at 14 hr seemed to have a slight stimulating effect on the activated Mφ but not on the normal ones. Conditioned medium taken from cultures of syngeneic MEF’s, a nonneoplastic cell population, had no significant effect on the rate of migration of the activated Mφ (Chart 3). However, conditioned supernatants from the neoplastic cell lines induced an immediate and precipitous increase in the rate of movement of the activated but not the normal Mφ (Charts 4 and 5). During the first 14 hr, the activated Mφ illustrated in

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**Chart 2.** Rate of movement ± S.E. of normal (●) and activated (□) Mφ illustrated in Chart 1. The media had been changed to fresh EMEM (A), LL-conditioned medium (B), and 2182-conditioned medium (C).

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**Chart 3.** MEF-conditioned media and nutrient media controls. Rate of movement of activated and normal Mφ. Arrow at 14 hr, the removal of nutrient medium and the addition of fresh nutrient medium to normal Mφ (●) and to activated Mφ (□) or the addition of MEF-conditioned medium to activated Mφ (△). The results represent the mean ± S.E. Micra, linear distance of 10^-4 m.

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**Chart 4.** LL-conditioned media. The arrow at 14 hr, the removal of nutrient medium and the addition of supernatant from cultures of LL cells to activated Mφ (●) and to normal Mφ (○) or the addition of fresh nutrient medium to activated Mφ (□) and to normal Mφ (●). Mean rates of movement ± S.E. Micra, linear distance of 10^-4 m.

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**Chart 5.** 2182-conditioned media. Arrow, the removal of nutrient medium and the addition of supernatant from cultures of 2182 cells to activated Mφ (●) and to normal Mφ (○) or the addition of fresh nutrient medium to activated Mφ (□) and normal Mφ (●). Mean rates of movement ± S.E. Micra, linear distance of 10^-4 m.
with pyran copolymer, which are known to be cytotoxic for tumor cells (11), respond chemokinetically to cell-free supernatants of cultured neoplastic cells. This response is selective for the neoplastic cells, but it is not tumor antigen specific. Conditioned media from 2 dissimilar tumor cell lines each elicited an increase in the rate of movement of activated Mφ, while conditioned medium from nonneoplastic cells had no effect. Preliminary studies (21) suggest that, as others (15) have demonstrated with BCG-activated Mφ, pyran-activated Mφ also respond chemotactically to tumor cell-conditioned media.

Mφ "activation" has come to refer to a modulated state wherein the functional capacities of Mφ have been stimulated to include nonspecific cytotoxicity that is selective for neoplastically transformed cells (9, 11). In animal systems a wide variety of i.p. administered inflammatory agents will elicit Mφ that are metabolically stimulated. However, only certain of these compounds (such as endotoxin, BCG, C. parvum, or pyran copolymer) will elicit cells that are also nonspecifically cytotoxic for tumor cells (1, 11, 16, 23). Karnovsky et al. (12) have distinguished between normal, elicited, and activated Mφ. While metabolic distinctions between the latter 2 populations are not clear-cut, a specific membrane antigen was recently demonstrated on pyran- and C. parvum-activated mouse Mφ that also express tumor cytotoxicity (10). The activated Mφ has been shown by many investigators to be a principal effector cell in certain forms of active tumor immunotherapy, particularly when granulomatous agents such as BCG and C. parvum are used (7, 21–23). If activated Mφ respond chemokinetically to soluble tumor-derived substances in vivo, as we have demonstrated in vitro, then one could reason that these cells would have an increased incidence of migration out of the vascular system to an appropriate stimulus. The studies of Ando et al. (2) with BCG-treated Mφ suggest that this is the case. Chemotactic sensitivity to soluble tumor products would tend to cause newly emigrated activated Mφ to remain at the tumor site. In vitro correlates wherein Mφ were added to established cultures of carcinoma cells suggest that this may be the case (25). Activated Mφ moved slowly and interacted vigorously at the plasmalemmal interface when they were in physical contact with carcinoma cells. Remarkably, their rate of lateral movement tripled when they lacked contact with a tumor cell, suggesting a cytotoxic response to the presence of tumor cells.

In the present study and in previous studies, we have shown that normal (unelicited) Mφ were unresponsive to conditioned media or to the presence of carcinoma cells (25). While we observed that the rate of lateral movement of normal Mφ did not change, Normann and Sorkin (17) have demonstrated that the chemotactic responsiveness of normal mouse Mφ was depressed by tumor-conditioned media. Similarly, recent studies have shown the presence of circulating factors in the tumor-bearing host that can depress a variety of Mφ functions, such as phagocytosis (8, 18, 19) and migration to inflammatory sites (8, 29). Additionally, peripheral blood monocytes from cancer patients frequently have depressed chemotactic responsiveness (8, 28). Thus, one might postulate suppression of Mφ function as a mechanism for newly transformed neoplastic cells to escape immunosurveillance destruction by host cells (20, 28). However, the alteration of Mφ function through nonspecific activation appears to change the response of these cells to tumor-derived substances, providing increased host resistance to neoplasia. Animal models with granuloma-producing activating compounds introduced to attract activated Mφ to the tumor site (i.e., intrallesional BCG) have proven successful in providing nonspecific resistance to tumor growth and metastasis (7, 24, 30). Otu et al. (19) have recently shown that Mφ functions are depressed in C57BL/6 mice after LL cells were inoculated s.c. However, we have previously shown with the same animal model system that pyran-activated Mφ effectively suppress tumor growth and metastasis (27). Moreover, our studies suggest that these activated Mφ respond both chemokinetically and chemotactically to the LL cells and that the activated Mφ are cytotoxic to these target cells (11, 21, 25, 26). The generalized nature of this response is indicated in recent reports that demonstrate that activated Mφ respond cytotropically to soluble molecules derived from a variety of different tumors (15, 26). Thus, during the process of activation Mφ susceptibility to tumor-derived effector molecules appears to be modulated from suppression to attraction.

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REFERENCES

6. Hama, M. G., Jr., Zbar, B., and Rapp, H. J. Histopathology of Tumor Regression after Intrallesional Injection of Mycobacterium Bovis. I. Tu-
MΦ Chemokinesis

References:

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