Two Antigenically Different Types of Macrophages (35862)

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Phagocytic cells in mammals are usually considered in two different groups, namely fixed or tissue, and free or circulating, macrophages (1–3). Structural (4), metabolic (5), and functional (6) differences between these two cell types have been documented. In addition, there is evidence that circulating monocytes act as precursors of free (7) and also of some forms of fixed (8, 9), macrophages. Using an antibody prepared against rat peritoneal macrophages and immunofluorescent techniques we have studied the distribution of the antigen(s) in several cell types of the rat, as well as in other related species. The results of this work suggest that free macrophages are antigenically different from fixed macrophages, and also that the antigen(s) lack species specificity.

Methods. Antiserum to rat peritoneal macrophages was obtained by the immunization of several rabbits during 5 weeks with two weekly subcutaneous injections of 8×10^6 cells each, obtained from the peritoneal cavity of rats stimulated 4 days previously with 5 ml of 10% proteose peptone in sterile saline. The proportion of macrophages in several preparations varied between 80 and 90%. In some rabbits, the rat peritoneal cells were injected emulsified in an equal volume (0.25 ml) of complete Freund's adjuvant. After completion of the 5 weeks the rabbits were left undisturbed for 1 month, then an intraperitoneal booster of the same number of rat peritoneal cells was given and 1 week later they were bled. The antisera obtained were tested in immunoelectrophoresis against complete rat serum; only some of the rabbits that received Freund's adjuvant showed faint precipitate lines against some components. Also, rat red cell agglutination was positive with undiluted antiserum but disappeared entirely after 1:8 dilution. Nevertheless, all rabbit antisera were absorbed with complete rat serum and rat red blood cells before testing on other cells. Antisera were heated to 56° for 20 min; and IgG was obtained by precipitation with 15% saturation of Na_2SO_4 and DEAE column chromatography.

The presence of antimacrophage antibody was established by immunohistochemical studies: smears of rat peritoneal macrophages were stained by both direct and indirect techniques (10), using for the latter a fluorescein-conjugated globulin prepared in our laboratory from goat antirabbit IgG antiserum. Appropriate dilutions (1:10) eliminated unspecific staining by normal rabbit IgG. Direct staining of smears of rat peritoneal macrophages and other cells with goat antirabbit IgG antiserum was also negative.

The distribution of the antigen(s) in other cells from various tissues of the rat, as well as in several organs of mice, guinea pigs, and rabbits, was examined in smears and/or touch preparations. Frozen sections were attempted early in the study but were abandoned because of the high degree of unspecific fluorescence. The various cell types present in the slides were identified and differential cell counts were made of paired preparations stained with Wright's mixture. Other than peritoneal macrophages, phagocytic cells were identified as alveolar macrophages in lung preparations, as Kupffer cells in the liver, and as reticulum cells in spleen, lymph nodes, and thymus. It was assumed that although an unspecified number of the phagocytic cells present on the slides were not of tissue but of hematogenous origin, it would be very unlikely that they could represent more than a small fraction of the total number of cells. We believe that a reasonable estimate of the number of hematogenous phagocytic cells in a given smear and or/

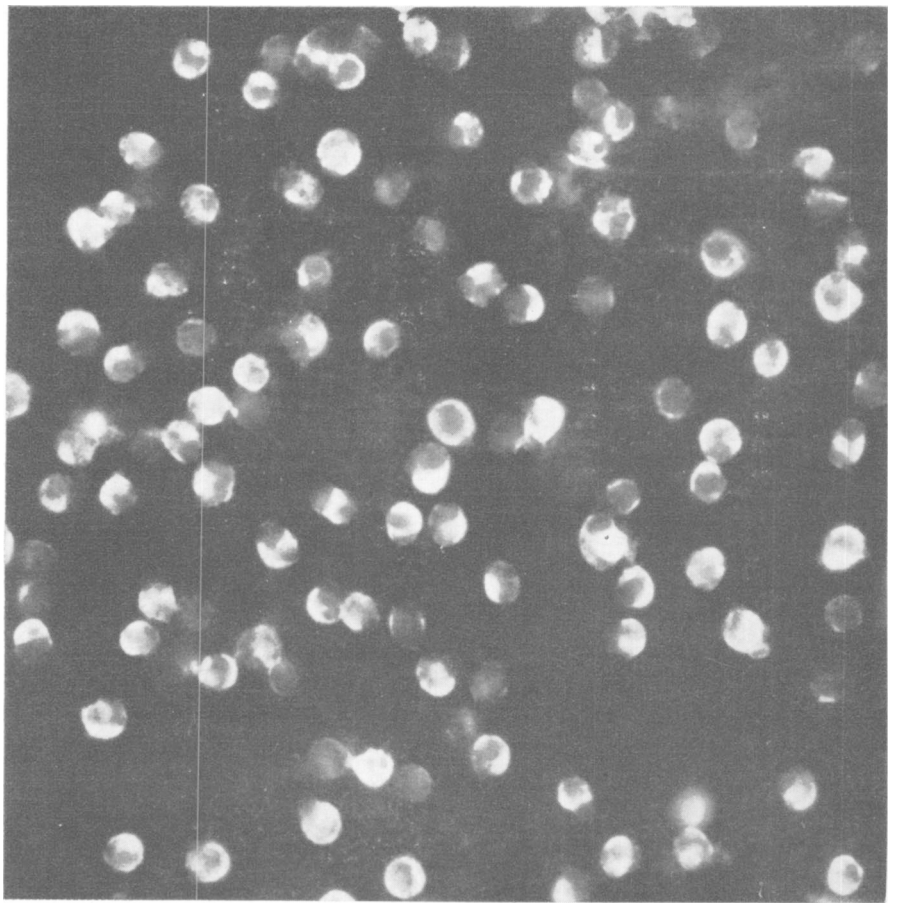

FIG. 1. Rat peritoneal macrophages showing positive membrane fluorescent staining. Smears of unstimulated peritoneal cells were air dried; washed with buffered saline (pH 7.3); covered with a solution of either antimacrophage or control IgG with 0.1 mg of protein/ml for 10 min at room temperature; washed thoroughly with the same buffered saline; and covered with a solution of fluorescein-conjugated globulin prepared from goat antirabbit IgG antiserum, also with 0.1 mg of protein/ml. After 10 min the slides were washed with buffered saline and covered with glycerin for microscopic examination under fluorescent light.

touch preparation of a solid organ such as liver or lymph node may be obtained from the degree of contamination by other blood cells, namely polymorphonuclear leukocytes and erythrocytes. Such contamination can be greatly reduced by drying the cut surface of the organ with filter paper before making the smear or touch preparation.

Results and Discussion. The antibody was localized in unstimulated rat peritoneal macrophages as homogeneous cytoplasmic or peripheral rings of yellow green fluorescence (Fig. 1). The results were similar when smears of rat peritoneal macrophages were prepared 4 days after stimulation of the peritoneal cavity with either proteose peptone or carrageenan (0.5% in saline). Lymphocytes, mast cells, and some polymorphonuclear leukocytes present in the smears were consistently negative. The only difference detected between the two staining methods was the brighter fluorescence obtained with the indirect technique. For this reason, most of the observations were made with this method.

The presence or absence of the antigen(s) in macrophages from various tissues in rat, guinea pig, and mouse was then surveyed by indirect immunofluorescence staining, and in

TABLE I. Distribution of Rat Peritoneal Macrophage Antigen(s) in Other Macrophages in the Rat and Other Species.[a]

Source of macrophages	Rat	Mouse	Guinea pig	Rabbit
Peritoneum	+	+	+	+
Bone marrow	+	+	+	+
Blood smear (monocytes)	+	+	+	+
Postpartum uterus	+	NT[b]	NT	NT
Granuloma	+	NT	+	NT
Lung	—	—	—	—
Liver	—	—	—	—
Spleen	—	—	—	—
Lymph node	—	—	—	—
Thymus	—	—	—	—

[a] Smears and/or touch preparations of the various organs listed from the four animal species were treated as described in Fig. 1. The few positive cells present in slides marked as negative were considered monocytes derived from the circulation. Positive slides revealed more than 90% of macrophagic cells with fluorescent staining.

[b] NT, not tested.

rabbits by direct fluorescence staining of smears and/or touch preparations of the organs listed in Table I. Again the positive fluorescence staining was limited to cells identified as free macrophages, whereas other cell types such as lymphocytes or polymorphonuclear leukocytes remained unstained. Of special interest was the finding that most of the cells identified as fixed macrophages in touch preparations of lung, liver, spleen, lymph nodes, and thymus failed to stain with the antimacrophage antibody. The few positive phagocytic cells in these preparations were considered as hematogenous in origin, since their number was usually correlated with the degree of contamination by other blood cells.

The results clearly indicate that there are two antigenically different types of macrophages, that the antigen(s) is present in free, and absent in fixed, macrophages, and that each of these two groups resembles more the interspecies homologous elements than the macrophages of the opposite group in the same species. Of course, there may be more than two antigenic types, specially among fixed macrophages, which we have characterized only by the absense of rat peritoneal macrophage antigen(s), and this possibility is now being explored.

The specificity of all antimacrophage antisera tested by us and included in this study has been restricted mostly to macrophages; some degree of red blood cell agglutination, also reported by others (11, 12), can be easily removed by absorption. No reactivity has been shown against lymphocytes, polymorphonuclear leukocytes, or other cell types present in smears or touch preparations of various organs. This finding is in agreement with several other authors (12–15), who have used antimacrophage antisera generated against highly purified macrophagic preparations. Nevertheless, some workers have reported different degrees of cross reactivity of antimacrophage antisera with other cells such as lymphocytes or even polymorphonuclear leukocytes (16, 17). We have made similar observations with some antisera obtained from rabbits immunized for prolonged periods and with rat peritoneal macrophages contaminated with more than 10 to 20% lymphocytes and/or polymorphonuclear leukocytes. Nevertheless, when purer cell preparations are used and antisera are obtained early in the immunization period (5 weeks), the antimacrophage activity is highly specific.

Conversely, it is well known that many antilymphocyte antisera show various effects on macrophages (18–20). This is not surprising, since the cell suspensions used to stimulate antilymphocyte antibody production are seldom purified and often contain significant numbers of macrophages. Another possible explanation for the different spectrum of specificity between some antimacrophage and most antilymphocyte antisera might be the absence (or relatively low concentration) of lymphocytic antigens in macrophages; whereas the lymphocyte would carry antigens common to both itself and the macrophage.

The differences found in the distribution of rat peritoneal macrophage antigen(s) in the various types of phagocytic cells in the rat could indicate modulations of differentiation in the same cell line; on the other hand, these differences may represent a clue for the important problem of the single versus mul-

tiple origin of the various forms of macrophages present in a single organism. The finding that antigenic identity is preserved in the same cell types in different animal species suggests that the molecule(s) bearing the antigenic determinant(s) may play a role in some widely distributed function of free macrophages in rodents.

Summary. Using stimulated rat peritoneal macrophages, a specific antimacrophage antibody has been prepared in rabbits. Immunofluorescent studies reveal that the antigen(s) is present in free, and absent in fixed, phagocytic cells in the rat. The antigen(s) has also been identified in free macrophages of guinea pigs, mice, and rabbits. Fixed macrophages in the same animal species are consistently negative. The results indicate that free macrophages are antigenically different from fixed phagocytic cells, and that the antigen(s) lacks species specificity.

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