Specificity of Macrophage Receptors (35208)

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Several cell types participate in the immune response. The role of macrophages is controversial and they have been described both as active antibody producers (1) and as passive scavengers (2). It is clear, however, that macrophages are important in the primary processing of antigens (3–7), but the antigen recognition mechanism involved has not been explained. Rowley (8) maintained that coating of any foreign particle with globulin is a prerequisite for phagocytosis. Thus it is feasible to anticipate that macrophages may have receptor sites specific for gamma globulin. Indeed, many specific receptor sites have been postulated and a few have been described (9–13). In this work the specificity of gamma globulin receptors on mouse peritoneal macrophages was examined.

Material and Methods. Peritoneal macrophages. Mouse peritoneal macrophage suspensions were prepared by injecting 4.0 ml of medium 199 containing heparin (10 μ/ml) into the peritoneal cavity of normal inbred mice (Albany strain). After 10–15 min the animals were sacrificed, the abdomen massaged gently, the peritoneal cavity opened, and the fluid collected. Cells from 3–4 mice were pooled for the daily work.

Microchambers. The microchambers (12 $\times$ 5 mm) were formed by a glass ring affixed to clean microscopic slides.

Rosette formation. 0.2 ml of the macrophage suspension was placed in the microchambers and incubated at 37° for 20 min to allow for adherence of macrophages to the glass. After incubation, the supernatant fluid was aspirated and replaced with the different proteins examined. Medium 199 was added to positive and negative controls. Slides were reincubated for 20 min and the supernatant fluid was removed and replaced with a 2% suspension of sheep erythrocytes (SRBC), sensitized with rabbit anti-SRBC IgG. Washed nonsensitized SRBC were used in the negative controls. After additional incubation, the slides were washed free of nonadherent erythrocytes with cold Hanks' balanced salt solution, dried, stained with Wright's stain, and mounted.

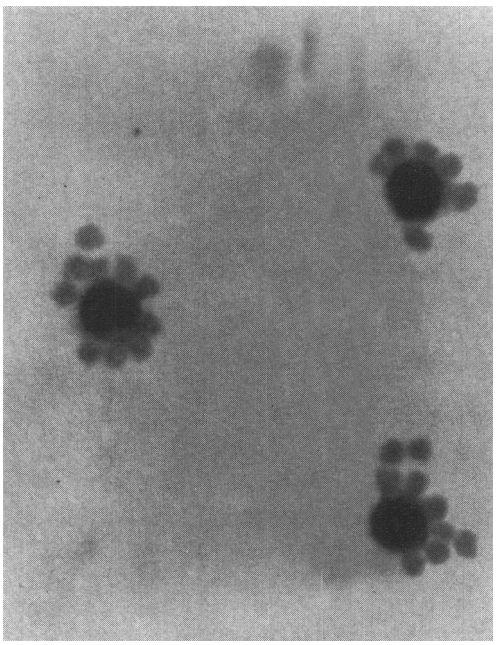

FIG. 1. Sensitized sheep red blood cells forming a rosette around mouse peritoneal mononuclear cells. Smear stained with Wright stain.

Results. 200–300 consecutive mononuclear cells were counted and those with three or more adherent SRBC [rosette (Fig. 1)] were scored. Triplicate slides were examined for each determination.

In the absence of homologous or heterologous protein, *i.e.*, positive controls, 70–80%

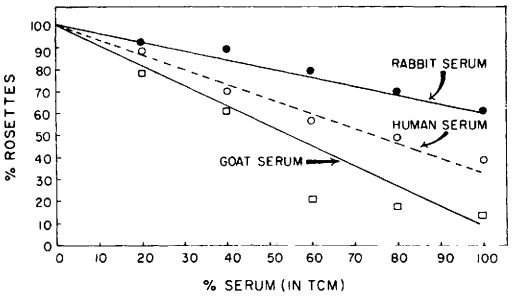

FIG. 2. The inhibitory effect of increasing concentration of heterologous sera on rosette formation: (●) rabbit serum; (○) human serum; (□) goat serum.

of the mononuclear cells had three or more adherent red blood cells. The addition of rabbit serum or IgG inhibited rosette formation. The inhibition was proportional to the concentration of the protein in the microchamber. Goat and human sera blocked rosette formation and were more effective inhibitors than rabbit serum (Fig. 2). Mouse serum, on the other hand, did not have any inhibitory effect.

Electrophoresis of the different sera revealed that the blocking effect was a function of the gamma globulin concentration (Fig. 3).

Normal mouse and rabbit sera of similar globulin concentrations were adjusted to 25 mg of protein/ml and mixed in varying proportions. In spite of the constant protein concentration in each microchamber, pure rabbit serum inhibited rosette formation, pure mouse serum enhanced it, and mixed sera

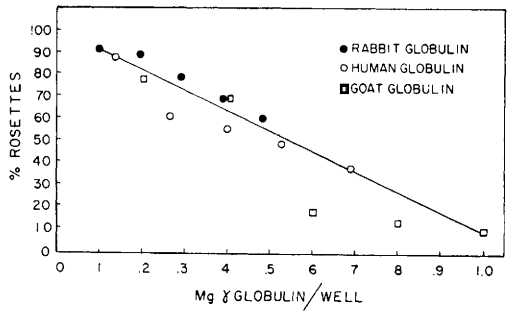

FIG. 3. The inhibitory effect of different concentration of heterologous sera on rosette formation: (●) rabbit globulin; (○) human globulin; (□) goat globulin.

affected the rosette formation according to the relative concentration of each (Fig. 4).

Sera were fractionated on Sephadex G-200 and DEAE-cellulose columns to obtain IgG and IgM fractions. The purity of the different fractions was ascertained by immunoelectrophoresis. IgG was found to possess an inhibitory effect similar to that of unfractionated serum while IgM had no effect. Rabbit, goat, and human IgG showed the same blocking efficiency as their respective sera, but when the percentage of rosette formation was plotted against the actual milligram concentration of different IgG per chamber, no significant difference was observed between the three heterologous globulins (Fig. 3). F(c) fragment (14), but not F(ab), of rabbit IgG blocked rosette formation in direct correlation to the concentration of the piece in the well (9, 15).

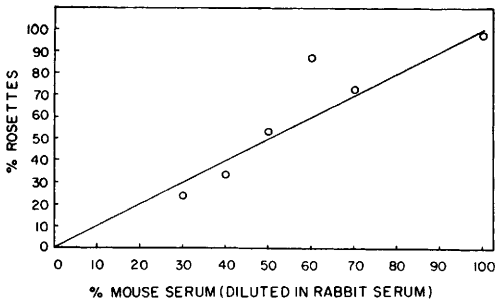

FIG. 4. The effect of increased concentration of mouse serum on rosette formation. (Total protein concentration is constant 25 mg/ml).

Discussion. Peritoneal macrophages from normal inbred mice possess, on their surface, receptor sites specific for heterologous IgG. These receptors differentiate between self and foreign IgG, but not between IgG from heterologous species. Human and goat IgG block the attachment of SRBC sensitized with rabbit IgG as efficiently as rabbit IgG. Mouse IgG, on the other hand, does not have any blocking effect and seems to have an adjuvant effect on rosette formation. The recognition of the IgG is specific and cannot be blocked by other proteins such as albumins, IgM, or alpha-2 macroglobulin.

This specificity is self-oriented; it can differentiate between heterologous and homologous IgG, which are similar in molecular size

and configuration, although it cannot differentiate heterologous IgG.

The recognition of the globulin is apparently mediated through F(c) fragment and so concurs with previous observations reported in relation to cytophilic antibodies. These IgG receptors, present in normal nonimmunized animals can recognize a group rather than just a single foreign molecule. Possibly these receptors facilitate recognition of foreign molecules for either phagocytosis or antibody production.

Summary. Mouse peritoneal macrophages possess receptors specific for IgG. These receptors differentiate between self and foreign IgG but not between IgG from different heterologous species.

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