RETICULUM CELL SARCOMA: AN EFFECTOR CELL IN ANTIBODY-DEPENDENT CELL-MEDIATED IMMUNITY

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A transplantable, murine reticulum cell sarcoma is described which exhibits the cytologic, adherence, and phagocytic properties of macrophages. It forms specific rosettes with erythrocytes in the presence of the corresponding anti-serum. The ascites cells mediate antibody-dependent cellular immunity as assayed by release of radioactivity from $^{51}$Cr-labeled erythrocytes. The contribution of contaminating host cells in the cytotoxic reaction was ruled out by growing the tumor in F1 mice and removing the host cells by anti-H2 serum and complement. The tumor cells have receptors for IgG2a and IgG2b immunoglobulins. The availability of a pure population of effector cells in the immune system allows study of the biochemical processes pursuant to lysis of foreign cells.

Cell types other than lymphocytes are involved in a number of reactions in the immune system. These range from participation in the induction of humoral and cell-mediated immune responses to effector functions of phagocytosis and lysis. Immunogens are rapidly collected at the dendritic processes of reticular cells in the follicles of lymphoid organs (1). Antigen bound in this way abuts on most of the follicular lymphocytes and is postulated to increase the efficiency of induction of B cells (2) to antibody production (2). In addition certain antigens which have been ingested by macrophages show increased immunogenicity (3). The adherent population which includes macrophages is required in vitro for the induction of humoral immunity (4) and T cell cytotoxicity (5). Such adherent cells are reported to possess receptors for T cell monomeric IgM which allow them to “present” antigen (6) or inductive signals (7) to precursors of antibody-forming cells.

Macrophages and other phagocytic cells (8) also play a key role in effector mechanisms of the immune system. Cells in the adherent population mediate cytotoxicity dependent on humoral antibody (9, 10). The effector cells showing activity comparable to adult spleen cells are already present in fetal liver and newborn spleen organs well before immune function appears (9, 11). Macrophages are also reported to exert cytostatic and cytotoxic effects on syngeneic and allogeneic tumor cells in the presence of specific T lymphocyte factors (12, 13). Another type of cell which mediates antibody-dependent cytotoxicity has been described as non-phagocytic (11, 14) or nonadherent (15), with some characteristics of monocytes (16) and of lymphocytes (11). This killing mechanism is effective against microorganisms (17), xenogeneic cells (9–11, 14), and allogeneic (13, 18) and syngeneic tumor cells (19).

In this paper we characterize a reticulum cell sarcoma with the morphologic, adherent, and phagocytic properties of macrophages. This ascites tumor line bears receptors for IgG2a and IgG2b immunoglobulins and mediates antibody-dependent cytolysis. In view of the heterogeneity which exists in lymphoid and peritoneal cell populations, the availability of such tumors which can be used to replace a normal cell type will be invaluable in analyzing immune processes.

METHODS AND MATERIALS

Mouse tumor lines. J774 tumor arose in a female BALB/c/NH mouse in 1968 during a plasmacytoma induction program (20). The initial neoplasm was ascitic, with solid abdominal tumors also found. The tumor was passaged subcutaneously in which case it metastasized to the liver, or as ascites. Experiments with J774A ascites were performed with cells from passages 7–24, showing greater than 90% viability by trypan blue exclusion.

Staining and microscopy. Direct smears of ascitic fluid were fixed in methanol and stained with Giemsa. Tissues removed at necropsy were fixed in neutral buffered 10% formalin, imbedded in paraffin, sectioned and stained with hematoxylin and eosin (courtesy of Monique Lacorbiere). For electron microscopy, cells were fixed in osmium tetroxide, embedded in Vestopol-W, sectioned and stained with uranyl acetate and lead citrate (courtesy of Marlene Bajak).

Antisera and antigens. Four-month-old BALB/c females were immunized with 0.2 ml of a 1% suspension of either sheep (SRBC) or burro (BRBC) erythrocytes (Colorado Serum Co., Denver, Colo.) incorporated into complete Freund’s adjuvant. Immunizations were repeated after 4 weeks in incomplete Freund’s adjuvant. After an additional 4 weeks a single i.v. injection of 0.2 ml of 1% RBC in saline was given. Sera were prepared from mice 10 days after the last injection. Hemagglutination titers of BALB anti-SRBC and BALB anti-BRBC were 4000 to 8000 against the homologous antigen, and less than 4 against the heterologous antigen. BALB/c anti-H2b sera were prepared by 8 weekly i.p. injections of 107 live EL4 ascites cells, a C57BL leukemia (21). The cytotoxic titer of this antiserum to EL4 cells with rabbit complement was 1:1000.

Erythrocyte antigen-binding assay. Ascites cells were harvested into cold Eagle’s medium without serum, containing 5 I.U. sodium heparin per milliliter. Cells were washed by low speed centrifugation three times with cold Eagle’s medium to remove red cells and debris. Next 1 x 10⁸ viable cells were plated in 2-ml tissue culture dishes (Falcon 3001) in Eagle’s medium minus serum. Cells were allowed to incubate overnight in order to adhere to the culture dishes. Adherent cells...
were washed twice with phosphate-buffered saline (PBS). One milliliter of immune or normal sera, diluted in PBS, was added to each dish followed by the addition of 1.0 ml of a 0.25% RBC suspension. The dishes were then incubated for 20 min in a humidified CO₂ incubator at 37°C. After incubation the dishes were washed four times with 2 ml volumes of PBS. Finally 1 ml H₂O was added to lyse the remaining adherent erythrocytes, and the supernatant was removed after agitation. Supernatants were either diluted in H₂O or read directly at 415 nm in a Zeiss spectrophotometer to quantitate erythrocyte binding to J774.

**Antibody directed cytotoxicity mediated by cells.** The chromium release assay was used as described for the assay of cellular immunity in vitro (9). 10⁵ ⁵¹Cr-labeled RBC were used as targets. Cells tested for killer function in the presence of antisera were 10⁵ spleen cells from 2-month-old female BALB/c mice, or 2 × 10⁶ cells from J774A ascites, MOPC 70A ascites (a BALB/c myeloma secreting IgG1) or P388 ascites (a DBA/2 leukemia). Cells were cultured at 37°C in a total volume of 1 ml of Dulbecco modified Eagle’s medium plus 5% fetal calf serum in Falcon 3001 tissue culture dishes with rocking. Specific antisemur or control serum was added in a 10-μl volume. After 6 hr, the contents of dishes were suspended by scraping, centrifuged, and the percentage of radioactive chromium released to the supernatant was determined. Specific chromium release was calculated by correcting for background release in the absence of antisemur or test effector cells (9). Assays were performed in duplicate and standard errors were <5% of the means.

**Removal of host cells from J774A ascites preparations.** J774A was grown as ascites in (C57BL/6 × BALB/c)F1 mice. We suspended 4 × 10⁷ cells in siliconized tubes in balanced salt solution containing absorbed rabbit complement (1:10 final concentration) and anti-H₂ serum (1:100), and incubated them for 45 min at 37°C. Less than 10% of the cells were killed by this treatment, mostly the small host cells present in the preparation. Control incubations used complement and normal BALB/c serum at 1:100. Fl spleen cells were similarly treated; incubation with anti-H₂ serum caused over 80% lysis of nucleated cells, whereas incubation with normal serum and complement killed less than 20% of cells. After incubation cells were washed twice with PBS and tested for effector cell cytotoxicity.

**Aggregated myeloma proteins.** The following purified proteins were used: IgM, MOPC104E; IgG1, MOPC21; IgG2a, ADJPC5; IgG2b, GPC5; IgG3, J606; and IgA, S107. Samples were aggregated with bis-diazoized benzidine (23), dialyzed, and centrifuged 1 min at 10,000 × G. The supernatants containing moderately aggregated protein which showed absorbance of 0.1 to 0.5 at 400 nm for a protein concentration of 1 mgN/ml were used (see Table V in Reference 22). Samples were iodinated by the chloramine-T method (23) with °I to a specific activity of 10⁴ to 10⁵ cpm/μg.

**RESULTS**

**Characterization of tumors.** At autopsy, mice carrying J774A tumors were found to have metastases of cells to liver, lymph nodes, uterus, ovaries, and lungs. If injected subcutaneously, tumors did not develop at the injection site, but rather metastasized to the liver. Neoplastic cells were not found in the peripheral blood.

Microscopic examination showed metastasis to all abdominal organs and lungs (Figs. 1, and 2a). Silver impregnation of tissue sections revealed abundant reticulin fibers within tumor foci (Fig. 2b). The ascites cells are about 30 μm in diameter, adhere firmly to plastic dishes, and contain ample lightly-staining cytoplasm with numerous vacuoles (Fig. 3). Most of the cells develop long processes after 24-hr incubation. The cells rapidly take up neutral red stain and carboxyl iron and form rosettes with glutaraldehyde-fixed RBC, as do macrophages (24). By gross and microscopic morphology, J744A is identical to the type A reticulum cell sarcoma previously described by Dunn (25), and belongs to the general category of histiocytic lymphomas.

**Binding of antigen-antibody complexes by J774A.** Retention of erythrocytes by J774A cells in the presence of anti-erythrocyte serum is shown in Figures 4 and 5. At the higher antigen-erythrocyte ratios, each tumor cell had bound at least 10 erythrocytes, as seen in the microscope after extensive washing. Fibroblasts failed to bind erythrocytes under the same conditions. If the adherent J774A cells were washed after incubation with immune sera, no antigen was seen to be bound after a second incubation with antigen alone (Fig. 5a). Retention of erythrocytes was specific in that incubation of J774A cells with BALB/c anti-burro erythrocytes failed to induce binding of sheep erythrocytes (Fig. 5b), and vice versa (not shown).

**Antibody-directed cell lysis mediated by J774A ascites cells.** Since J774A cells bind antigens in the presence of the appropriate antisemur, we tested for killing of the target cells by the chromium release assay. Cells harvested from J774A ascites fluid were highly efficient in the lysis of °I-labeled erythrocytes, releasing 50 to 77% of the radioactive chromium by 6 hr of incubation together with either rabbit or mouse anti-SRBC (Table I). The magnitude of lysis by 2 × 10⁷ J774A cells was similar to that observed with 10⁸ normal spleen cells. In control cultures incubated with normal rabbit or mouse serum (Table I), or with reticulum ascites cells replaced by myeloma or lymphoma ascites (Table II), only negligible release of radioactivity over background values was observed. Occasionally the J774A cells showed a slight lysis of erythrocytes in the absence of antiserum, usually less than 10% of the optimal lysis in the presence of specific antiserum. As with killing by normal spleen cells, the reticulum cell sarcoma was effective at very low concentrations of antiserum, up to 10⁻⁴ dilution. The cytotoxicity does not appear to require complement since the same lysis of SRBC occurs in medium with or without fetal calf serum, as shown for spleen effector cells (9).

The J774A ascites preparations include various numbers (1 to 10%) of small, nucleated cells, presumably normal peritoneal cells. The antibody-dependent cytotoxicity demonstrated here appears to be mediated by the large tumor cells rather than contaminating normal peritoneal cells since killing did not correlate with the degree of contamination, and ascites forms of other tumors were not effective (Table II). Preliminary experiments with normal peritoneal cells and peritoneal exudates indicate that the number of host cells would have to approximate the numbers of tumor cells in ascites preparations to give the cytotoxicity seen here.

In order to prove more directly that the J774A cells were the effector cells, the BALB/c tumor was passaged in (C57BL/6 × BALB/c)F1 mice and the host F1 cells in the ascites population killed by anti-H₂ serum plus complement. This treatment abolished the antibody-dependent cytotoxicity of normal F1 spleen cells, while leaving intact the function of ascites tumor cells to lyse SRBC in the presence of anti-SRBC (Table III). The ascites fluid contained mostly H₂⁺ tumor cells resistant to
anti-H2b killing, whereas there was a massive lysis of the F1 spleen cells which might block the function of surviving F1 effector cells. Therefore a mixing experiment was performed, which showed that surviving J774 effector cells could still function after incubation in the presence of antibody lysis of spleen cells (Table III).

**Specificity of immunoglobulin receptor on J774A cells.** Myeloma proteins with known antigen-binding activity were tested for their ability to mediate rosetting with erythrocytes coupled with the corresponding antigen. The systems tested were IgM: MOPC104E with dextran; IgA: J558 and dextran; IgG3: J606 and levan (26). Despite hemagglutination titers of 1000 to 8000 against the homologous antigen, no rosette formation occurred (data not shown). Myeloma proteins were then tested for inhibition of the binding of antibody-coated erythrocytes to J774A. Amounts of IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA myeloma proteins up to 1 mg/ml did not diminish adherence of erythrocytes when preincubated 10 to 30 min with tumor cells before addition of RBC.

Since myeloma proteins did not prevent antigen-antibody complex binding by competition, a positive experiment was performed to determine the specificity of the immunoglobulin receptor on J774 cells. Myeloma proteins and normal immunoglobulins of the proper class frequently gain biologic activity nonspecifically by aggregation, comparable to the corresponding antigen-antibody complex. Thus when aggregated, IgG induces increased capillary permeability and consequent skin reaction and fixes complement; IgM fixes complement only; IgE gives the skin reaction only; and IgA and IgD are inactive (27). Since antibody directing binding and lysis of foreign targets has a much higher affinity for the effector cell when complexed with antigen (Fig. 5a; References 9, 10), aggregated myeloma proteins were used to mimic this situation. Consequently we tested 125I-labeled, aggregated myeloma proteins

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*Figure 1.* Metastatic lesion of the reticulum cell sarcoma in the lung of a BALB/c mouse. Tumor cells are quite compact, having deeply basophilic nuclei. Note cellular pleomorphism within an isolated nodule (H & E, × 320).
Figure 2. A, section of intra-abdominal tumor which was adherent to omentum, peritoneum and bowel (H & E, × 160); B, same area as 2A (Gomori’s silver stain, × 160).

Table I

Antibody-dependent lysis of SRBC by reticulum cell sarcoma

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Dilution</th>
<th>% Specific Lysis</th>
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<td></td>
<td></td>
<td>Spleen cells</td>
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<tr>
<td>NRS</td>
<td>10^{-4}</td>
<td>1</td>
</tr>
<tr>
<td>RaSRBC</td>
<td>10^{-4}</td>
<td>56</td>
</tr>
<tr>
<td>NMS</td>
<td>10^{-3}</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NMS</td>
<td>10^{-4}</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MaSRBC</td>
<td>10^{-2}</td>
<td>82</td>
</tr>
<tr>
<td>MaSRBC</td>
<td>10^{-4}</td>
<td>79</td>
</tr>
<tr>
<td>MaSRBC</td>
<td>10^{-4}</td>
<td>32</td>
</tr>
<tr>
<td>MaSRBC</td>
<td>10^{-8}</td>
<td>7</td>
</tr>
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</table>

Each incubation contained 10^8 51Cr-labeled SRBC and either 10^7 nucleated spleen cells from 2-month-old normal unimmunized female BALB/c mice or 2 x 10^8 J774A cells, as described in Materials and Methods. Rabbit anti-SRBC (RaSRBC, Pentex) or normal rabbit serum (NRS) was added (10 μl of 1:100) to a final dilution of 1:10000 (10^{-4}), as indicated. To other incubations, 10 μl of dilutions of normal BALB/c mouse serum (NMS) or BALB/c anti-SRBC (MaSRBC) were added to obtain the final dilutions indicated. Radioactivity released to the supernatant was determined after 6 hr. Results are corrected for background release of 51Cr with spleen cells (11%) or J774A (12%) in the absence of rabbit or mouse serum.

Discussion

The reticulum cell sarcoma line described here has a number of properties characteristic of macrophages. The J774A cells are readily adherent, phagocytic, and able to bind specifically antibody-coated erythrocytes. The sarcoma cells function very well as effector cells in antibody-dependent cytotoxicity. J774A cells are as active as 5 times as many spleen cells in lysing erythrocytes in the presence of the appropriate antiserum (Tables I, II). Since the J774A cells are not adapted to growth in culture, all experiments were performed with ascites preparations. The small numbers of host cells in the ascites fluid could not account for the killing reaction, since when the tumor concentration spleen cells bound only small amounts of IgG2a and IgG2b. A θ+ leukemia, EL4, showed no significant binding of immunoglobulin.

Table II

J774A as the effector cell in ascites preparations

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Dilution</th>
<th>Cytotoxicity of Effector Cells</th>
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<tr>
<td></td>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td>MaSRBC</td>
<td>10^{-4}</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NMS</td>
<td>10^{-4}</td>
<td>64</td>
</tr>
</tbody>
</table>

* Lysis of 51Cr-labeled SRBC was determined as in Table I, using 10^7 BALB/c spleen cells or 2 x 10^8 J774A, P888, or MOPC70A ascites cells. Results were corrected for background release of 51Cr from SRBC (10%) in the presence of spleen cells but no antiserum.
was grown in F₁ hosts, treatment with complement and antiserum to F₁ cells blocked all cytotoxicity by host spleen cells but left the ascites tumor activity intact (Table III).

In considering the signaling of an effector cell by antibody-antigen complexes, the interaction of free immunoglobulin with cell Fc receptors must be considered. If the binding constant for free immunoglobulin (or a cytophilic subclass) approximates its serum concentration, there may be competition for binding of antibody-antigen complexes. In the case of the basophil or mast cell which mediates anaphylactoid reactions via histamine release, the affinity of the cell for free immunoglobulin of the IgE class is high. However, its dissociation constant (~10⁻¹⁰ M) is equal to or higher than the serum level of IgE which is ~10⁻⁹ M (28). Consequently, these cell receptors are saturated with IgE only in unusual cases (28, 29), and normally IgE cannot compete effectively with an antigen-antibody complex.

In the present case of phagocytosis or cell-mediated lysis, the affinity of monomeric (non-aggregated) immunoglobulin for the adherent cell is low. The normal level of the IgG classes which arm these cells is of the order of 10⁻⁸ M (approximately 1 mg/ml). The dissociation constant is not measurable and might be ≥10⁻⁹ M, so that these Fc receptor sites are also not normally saturated. In both the macrophage and mast cell, the effector function is triggered only if the cell-bound antibody is aggregated on the surface, normally by interaction with a multideterminant antigen. For complexes of several antibodies bound to a multivalent antigen, it is likely that the complex has a much higher binding constant than free antibody.

In part these considerations explain the difficulty in establishing the class of antibody directing non-T cell cellular immunity. The use of competition experiments with myeloma proteins to define the class of antibody normally mediating this type of cellular immunity can be misleading. First the concentration of the competing monomeric (non-aggregated) immunoglobulin must be at least 10-fold above the binding constant (~10⁻⁴ M) to see an inhibiting effect. Second, nonspecific interactions between the effector cell and the antigen-antibody complex, e.g., charge, could stabilize the interaction irreversibly once the complex is fixed to the cell via the antibody Fc.
Figure 4. Binding of SRBC to J774A cells in the presence of anti-SRBC. An erythrocyte has apparently been phagocytosed, although phagocytic vacuoles were not observed (electron micrograph, × 7500).

Figure 5. a, Binding of BRBC to the reticulum cell sarcoma in the presence of antiserum. J774A was incubated with anti-BRBC 20 min at 37°C before addition of antigen (O), as described in Methods and Materials. In another series of dishes, after incubation with anti-BRBC, the adherent J774A cells were washed three times with saline prior to the addition of BRBC (●). Absorbed erythrocytes, as measured by hemoglobin absorption at 415 nm, is plotted against dilutions of anti-BRBC serum. Each point is the average of triplicate incubations with the range indicated by bars. Absorbance at 415 nm with antiserum replaced by PBS = 0.04. b, Specificity of antigen binding.

In spite of these difficulties, mouse IgG2b appears to be most active in blocking the killing by nonadherent effector cells (16). We have shown that the adherent J774A effector cells have receptors for both IgG2 classes (Fig. 3). The participation of IgG2a and IgG2b antibody in cytotoxicity is curious. Both classes fix complement (31). Although external complement components are not required in the reaction (9, 32), it is possible that the effector cell itself synthesizes one or more complement components which are secreted and activated locally to effect cytotoxicity. Synthesis of complement components by macrophages has been reported (33). J774A cells do not bind mouse IgG3, and apparently macrophages do not have receptors for this class (34). Thus although IgG3 immunoglobulin is transmitted to the fetus more efficiently than other IgG


**TABLE III**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytotoxicity of Effector Cells*</th>
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<tbody>
<tr>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td>NMS + C</td>
<td>45 (&lt;1)</td>
</tr>
<tr>
<td>Anti-H2b + C</td>
<td>&lt;1 (&lt;1)</td>
</tr>
</tbody>
</table>

* (C57BL/6 × BALB/c)F1 spleen cells, J774A ascites cells grown in F1 mice, or a mixture of these two populations were incubated with complement and either normal mouse serum (NMS + C) or anti-H2b serum (anti-H2b + C) to kill F1 cells, as described in Materials and Methods. 10^8 control or treated spleen cells or 2 × 10^4 control or treated J774, based on cell numbers before serum plus complement treatment, were tested for cytotoxicity with 10^{-4} dilution of BALB/c complement and either normal mouse serum (NMS + C) or anti-H2b serum (anti-H2b + C) to kill F1 cells, as described in Materials and Methods. 10^8 control or treated spleen cells or 2 × 10^4 control or treated J774, based on cell numbers before serum plus complement treatment, were tested for cytotoxicity with 10^{-4} dilution of BALB/c complement and either normal mouse serum (NMS + C) or anti-H2b serum (anti-H2b + C) to kill F1 cells, as described in Materials and Methods.

**Figure 6.** J774A ascites, BALB/c spleen or EL4 ascites cells were washed four times in balanced salt solution (BSS). 10^5 cells (open bars) or 2 × 10^6 cells (hatched bars) were incubated with 1 μg aggregated, ^125I-labeled myeloma proteins (described in Methods and Materials) in BSS in siliconized tubes. After 30 min incubation on ice, the cells were washed four times, transferring to new tubes after the second wash. Radioactivity remaining with the cells was determined and is shown as the percentage of initial protein of IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA classes.

classes (34), it is not responsible for the antibody-dependent cellular immunity which we have shown to be derived from the maternal circulation (9).

There are contradictions in the literature which need explanation. For example, mouse macrophages have receptors demonstrating predominately IgG2a specificity, however, some but not all IgG1 myeloma proteins block macrophage rosette formation as efficiently as IgG2 molecules (35). Either there are two subclasses of IgG1 or some of the IgG1 preparations were denatured. All IgG immunoglobulin classes both bind to mast cells and block passive cutaneous anaphylaxis. Yet only IgG1 and IgE molecules will mediate this reaction (36).

Since J774A cells resemble macrophages, we considered the possibility that these cells would exhibit the function of adherent cells in the induction of immune responses in vitro. When tested at 10^2 to 10^3 J774A cells/1 ml cultures containing 10^7 spleen cells depleted of adherent cells (24), there was no restoration of the induction of antibody formation (unpublished results of P. R., J. Watson, and E. Trenkner). In fact, when 10^3 to 10^4 J774A cells were added to normal spleen cultures, the normal induction of antibody-forming cells was prevented. The tumor cells do not grow in culture. However, they may exert an inhibitory effect on cultures which could override the manifestation of an adherent cell cooperating function. Activated macrophages (37-40) and leukocytes (41) are generally toxic to many other cell types. On the other hand, we have presented evidence elsewhere (24) that pure populations of macrophage-like cells derived from bone marrow cultures in the presence of colony-stimulating factor also do not replace adherent cells in immune responses in vitro. A number of adherent or phagocytic cell types in lymphoid organs have been distinguished (42, 43). A virus-transformed macrophage cell line has been described which restores the immune response of nonadherent spleen cells (44). It is therefore likely that there are several classes of macrophages or adherent cells with different functions.

**Acknowledgments.** We thank Dr. R. Hyman for advice and encouragement, Drs. J. Watson and J. Andersson for helpful discussion, Drs. M. Weigert, R. Riblet, and B. Slade for purified myeloma proteins, and Ms. I. Nakoinz for expert assistance.

**Note added in proof:** Walker and Demus have recently described a virally transformed macrophage cell line which exhibits extensive phagocytosis and moderate lysis of chicken erythrocytes in the presence of specific antisera (J. Immunol., this issue, p. 765). We have now adapted J774A ascites to growth in culture. The cell line shows mainly phagocytosis, in contrast to the original ascites line or ascites-passed culture line in which lysis predominates.

**REFERENCES**