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Immunology

Mononuclear phagocytes: A major population of effector cells responsible for rejection of allografted tumor cells in mice

(peritoneal exudate cells/adherent phagocytes/nonself-specific cytotoxicity)

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ABSTRACT **To understand the *in situ* mechanism of immunological response of recipient animals to allografted tumor cells, the types of cells that infiltrated into the rejection site were examined. When Meth A cells (H-2^d) were given i.p. to an allogeneic [C57BL/6 (H-2^b)] strain of mouse, the tumor cells ceased to grow on the 6th day, accompanied by an i.p. infiltration of leukocytes. The tumor cells were totally eliminated from the peritoneal cavity around the 12th day. The highest cytotoxic activity against Meth A cells was obtained with the peritoneal exudate cells harvested on day 8. On this day, the exudate cells consisted of three populations when examined by flow cytometry, and each was isolated by sorting. Each of them appeared to be homogeneous, and they were morphologically identified as lymphocytes; granulocytes; and medium-sized, mononuclear, less-granular cells. The cytotoxic activity was confined exclusively to the last population. The effector cells (H-2^b) were cytotoxic against not only Meth A cells (H-2^d) but also concanavalin A-stimulated allogeneic spleen cells [C3H/He (H-2^k), CBA/N (H-2^k), A/J (H-2^a), BALB/c (H-2^d), and DBA/2 (H-2^d) strains of mouse]. The effector cells were totally inert against concanavalin A-activated syngeneic spleen cells [C57BL/6 (H-2^b) and C57BL/10 (H-2^b) strains of mouse]. The effector cells were phenotypically (Thy-1.2^- $CD3^-$ Lyt-1^- Lyt-2^- L3T4^- immunoglobulin$^-$ asialo GM1^-), morphologically, and functionally distinct from cytotoxic T cells, natural killer cells, and lymphokine-activated killer cells but were adherent mononuclear phagocytes.**

The idea that lymphocytes are essential for allograft rejection is supported by a considerable body of evidence (1–3). Recently, there has been significant debate concerning the roles played by the phenotypically distinct Lyt-1^+ and Lyt-2^+ T-lymphocyte subpopulations in initiating and effecting graft rejection in a major histocompatibility complex restricted (4–8) or unrestricted (9–12) manner. The major histocompatibility complex-unrestricted cytotoxic activities *in vitro* are also mediated by natural killer cells, lymphokine-activated killer cells, and macrophages (13, 14). Very little information, however, is available on the *in situ* effector cells mediating the destruction of the graft.

In 1988, after an i.p. transplantation of Meth A cells, we succeeded in isolating cellular infiltrates among whole peritoneal cells (leukocytes and Meth A cells) by differential centrifugation (15). The present report describes the isolation and characterization of effector cells in the peritoneal exudate cells (PEC) responsible for rejection of i.p. allografted tumor cells. The cytotoxicity was found to be unique; the effector (H-2^b) cells obtained from Meth A (H-2^d) cell-sensitized mice were cytotoxic against various (H-2^a, H-2^d, and H-2^k) allogeneic Con A blasts but not against syngeneic (H-2^b) Con A blasts. Flow cytometry, complement-dependent lysis with specific antibodies, and morphological or functional examination showed that the isolated effector cells were distinct from cytotoxic T cells, natural killer cells, and lymphokine-activated killer cells but were adherent phagocytes. Comparison of the effector cells with previously reported macrophages is also discussed.

MATERIALS AND METHODS

Chemicals. $Na_2{}^{51}CrO_4$ (10.5 GBq/mg) was purchased from New England Nuclear. RPMI 1640 medium and Eagle's minimum essential medium were obtained from Nissui Seiyaku (Tokyo). Fetal calf serum (FCS) was purchased from HyClone and was used after heat inactivation. Penicillin, streptomycin, and L-glutamine were obtained from GIBCO. Trypan blue and Turk solution were purchased from Wako Pure Chemical (Osaka). Con A was obtained from Sigma. Polystyrene particles, 2.020 ± 0.0135 μm in diameter, were purchased from Dow. All other chemicals were of reagent grade.

Antibodies and Complement. Monoclonal antibody to mouse CD3 (145-2C11), originally obtained from J. A. Bluestone (Ben May Institute, Department of Pathology, University of Chicago), was kindly donated by Y. Asano (Department of Immunology, Faculty of Medicine, University of Tokyo). The antibody was used as a conjugate to fluorescein isothiocyanate (FITC), prepared by the method of Sasaki *et al.* (16) with some modifications. Monoclonal antibodies to Thy-1.2 antigen, Lyt-1 antigen (FITC-labeled), and Lyt-2 antigen (FITC-labeled) as well as FITC-labeled goat anti-mouse immunoglobulin antiserum were purchased from Becton Dickinson. FITC-labeled goat anti-mouse μ-chain $F(ab')_2$ fragment was obtained from Cappel Laboratories. Monoclonal antibody to L3T4 antigen was obtained from Cedarlane Laboratories (Hornby, ON, Canada). Rabbit anti-asialo GM1 antiserum was purchased from Seikagaku Kogyo (Tokyo). Monoclonal antibody to mouse macrophage (M1/70.15) (FITC-labeled) was obtained from Caltag Laboratories (South San Francisco, CA).

Animals. Male, specific pathogen-free C57BL/6, C57BL/10, CBA/N, C3H/He, A/J, DBA/2, and BALB/c mice (7 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). Mice were raised under specific pathogen-free conditions in an air-conditioned room at 25 ± 2°C controlled to 50% humidity at the Osaka Bioscience Institute.

Tumor Cells. Meth A cells were kindly provided by S. Muramatsu (Department of Zoology, Faculty of Science, Kyoto University). Meth A cells were maintained by i.p. passage of 8×10^6–1×10^7 cells into a syngeneic strain (BALB/c) of mouse.

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Abbreviations: PEC, peritoneal exudate cells; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter.

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Preparation of Spleen and Thymus Cells. Mice were sacrificed by cervical dislocation, spleens and thymuses were removed aseptically, and single-cell suspensions were prepared as described (15).

Enrichment of Peritoneal Leukocytes. At various time intervals, two mice were sacrificed in each experiment after an i.p. transplantation of Meth A cells (3×10^6 cells per mouse). The leukocyte-rich fraction among whole peritoneal cells was purified as described (15). The recovery of leukocytes was >90%, and the purity {[(whole peritoneal cells − tumor cells)/(whole peritoneal cells)] × 100} was >95%.

Cell Sorting. With the gate set in the forward scattering–side scattering mode, the cells were sorted by using a fluorescence-activated cell sorter (FACS) (FACStar; Becton Dickinson). The yield of cells varied between 65% and 85%.

FACS Analysis. A mixture of PEC was obtained from C57BL/6 mice treated with Meth A cells, and control spleen cells were obtained from untreated animals. The cells (5×10^5) suspended in 25 μl of cold phosphate-buffered saline (PBS) containing 2% FCS and 0.1% sodium azide were stained with labeled antibodies and analyzed by flow cytometry with a FACS. Dead cells and erythrocytes were gated out on the basis of light scattering as the data were collected.

Target Cells for Cytotoxic Assay. Meth A cells maintained by serial passage in the ascites form in BALB/c mice were cultured for 48–72 hr in RPMI 1640 with 10% FCS. ^{51}Cr-labeled Meth A cells were prepared by the method of Ascher *et al.* (17). In addition to Meth A cells, ^{51}Cr-labeled Con A blasts were also used as target cells. Con A blasts were prepared as reported by Ascher *et al.* (17).

Cytotoxicity Assay. The cytotoxic activity against ^{51}Cr-labeled target cells was determined by the method of Ascher *et al.* (17). The spontaneous release of ^{51}Cr from incubation was <10% in 6 hr and <25% in 18 hr. Percent specific lysis was calculated by the following formula: percent specific lysis = $[(cpm_{exp} - cpm_{spon})/(cpm_{max} - cpm_{spon})] \times 100$ (where exp = experimental and spon = spontaneous). Maximal release (cpm_{max}) was determined from supernatants of cells subjected to detergent lysis with 0.5% Triton X-100.

Cytotoxicity with Antibody Plus Complement. To determine the surface antigens on the infiltrating leukocytes, the cells were treated with various antibodies in the presence of nontoxic rabbit complement in a two-step procedure (18).

Analysis by Phase-Contrast Microscopy. Cells for light microscopic examination were prepared by using a cytocentrifuge (Cytospin 2; Shandon Southern Products, Cheshire, U.K.). Cells ($2–5 \times 10^4$) suspended in 150 μl of PBS plus 5% FCS were deposited on a slide by centrifugation at 400 rpm for 5 min. The cells were dried on the slide, fixed in a solution of acetic acid/ethanol, 5:95 (vol/vol), and analyzed with a phase-contrast microscope.

Test for Endocytosis. An *in vitro* test for endocytic activity was performed on adherent cell populations. Immunoglobulin-coated polystyrene particles, 2.020 ± 0.0135 μm in diameter (at a final concentration of 0.1% solids), were administered for 1.5 hr at 37°C after which the cells were washed, fixed, and then examined by phase-contrast or Nomarski reflection microscopy.

Myeloperoxidase Staining. Cells ($2–5 \times 10^4$) suspended in 150 μl of PBS plus 5% FCS were deposited on a slide by using a cytocentrifuge and stained by the method reported by Kaplow (19).

Cell Number and Viability. Cell number was determined with a hemocytometer using Turk solution. In the case of adherent cells on coverslips, the cell number was estimated by the addition of nuclear counting solution (20). The viability of the cells was determined by the trypan blue exclusion method.

RESULTS

Cytotoxicity of Infiltrating Leukocytes Against Meth A Cells. When Meth A cells (3×10^6 cells per mouse) were introduced i.p. into an allogeneic (C57BL/6) strain of mouse, the growth of Meth A cells commenced within 3 days after transplantation as in syngeneic animals, but the tumor cells ceased to grow on the 6th day (Fig. 1). Rapid elimination of the tumor cells from the peritoneal cavity began around the 8th day, and on the 14th day none were found. The leukocytes infiltrating into the peritoneal cavity showed increased cytotoxicity against Meth A cells with time after transplantation, reaching a peak (≈39%) on day 8 (18-hr incubation for the assay). Even in a 6-hr assay (*Inset*), the cytotoxicity was 20.5% ± 8.0% (mean ± SD, $n = 4$). Thereafter, the cytotoxic activity decreased, and almost no cytotoxicity remained against Meth A cells on day 14.

Morphology of Cells Infiltrating into the Peritoneal Cavity. On examination by flow cytometry, the isolated PEC consisted mainly of two population types (a and b) on day 0 (Fig. 2*A*), three types (a, c, and d) on days 3–11 (Fig. 2*B*), and three types (a, c, and e) on days 13 and 14 (Fig. 2*C*). On day 18 or later (Fig. 2*D*), the population types of PEC were essentially the same as those of untreated animals. The three population types (a, c, and d) of PEC on day 8, when the highest cytotoxic activity was observed (Fig. 1), can be morphologically distinguished by phase-contrast microscopy of acetic acid/ethanol-fixed cell preparations (Fig. 3). The FACS-purified cells in these fractions appeared to be homogeneous. The cells in population a were found to be lymphocytes: lymphocytes generally assume circular shapes with smooth cell surfaces and possess a high nuclear-to-cytoplasmic ratio as shown in Fig. 3*A*. The cells in population c were granulocytes: in mice mature granulocytes frequently have a doughnut-shaped nucleus (Fig. 3*B*). The cells in population d were round, mononuclear, less-granular, and medium-sized (almost the same size as granulocytes) (Fig. 3*C*). The nucleus was large and kidney- or horseshoe-shaped. Therefore, the cells in population d were neither typically lymphocytes nor typically macrophages in appearance, but the type of cells could not be readily identified. The cells in two other populations, population b on day 0 and population e on day 14, were morphologically identified as macrophages and large granular cells, respectively (Fig. 3 *D* and *E*). On morphological or flow cytometric examination, the characteristics of

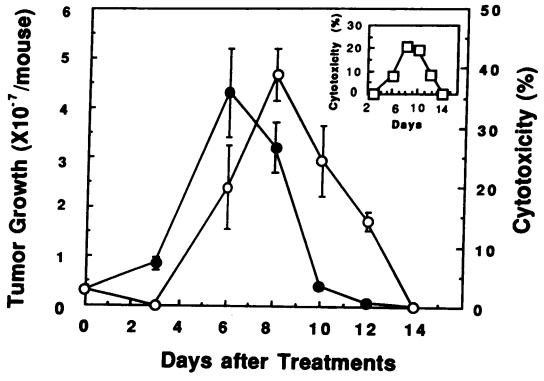

FIG. 1. Growth of Meth A cells and cytotoxicity of PEC against Meth A cells. Meth A cells (3×10^6 cells per mouse) were given i.p. to C57BL/6 mice. At appropriate time intervals, peritoneal cells were recovered, and the numbers of tumor cells (●) were determined. The leukocyte-rich fraction was obtained, and the cytotoxic activity against ^{51}Cr-labeled Meth A cells with an effector-to-target ratio of 50 was determined after a 6-hr (*Inset*) or 18-hr (○) incubation. The cytotoxic activity was augmented as the effector-to-target ratio was increased (data not shown for the ratios of 5, 10, and 20). Each value represents the mean ± SD of four experiments.

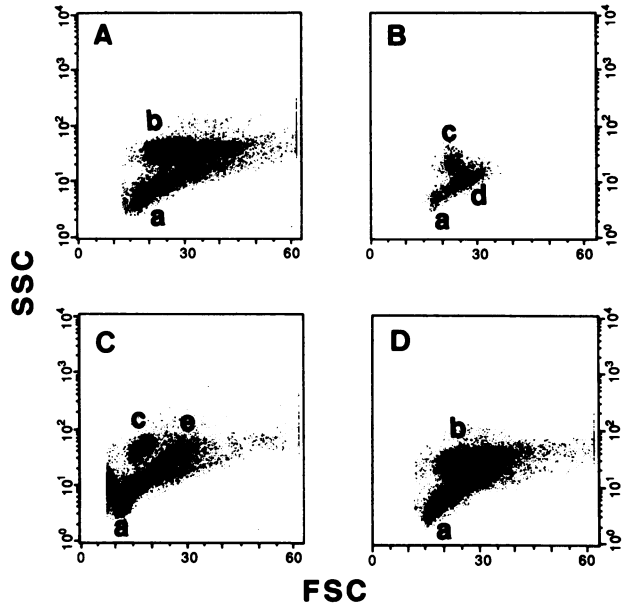

FIG. 2. Flow cytometric characteristics of cells infiltrating into the peritoneal cavity. Meth A cells (3×10^6 cells per mouse) were given i.p. to C57BL/6 mice. At various time intervals, all PEC were analyzed by flow cytometry [10,000 cells per sample except for *B* (2000 cells per sample)] in which forward scattering is plotted linearly against the logarithm of side scattering. (*A*) Day 0. (*B*) Day 6. (*C*) Day 14. (*D*) Day 18. The population types (a–e) are indicated.

cells in population b on day 18 were essentially identical with those of cells in population b on day 0, whereas typical macrophages were rarely (≤2% of all PEC) seen in the PEC harvested on days 3–11.

Cytotoxicity by Specific Population Type of PEC. To establish which population type(s) of PEC is cytotoxic against Meth A cells, the cytotoxic activities of three purified population types (a, c, and d) were determined on day 8, when the highest cytotoxic activity was observed with all PEC (Fig. 1). As shown in Table 1, the cytotoxic activity was exclusively localized in population d, whereas there was almost no activity in the two other population types (a and c). The cytotoxic activity of the isolated population d was enhanced 2- to 3-fold compared with that of all PEC. This is quite reasonable because the cells in population d (≈50% of the cell number) in all PEC were purified ≈2-fold to apparent homogeneity (≈100% cell number) by sorting. Furthermore, the cytotoxic activity of all PEC was essentially reconstituted by the mixture of three populations with their percent cell numbers (data not shown). These results indicate that the cytotoxic activity of all PEC against Meth A cells is attributable exclusively to that of population d and that the two other population types (a and c) have little, if any, activity.

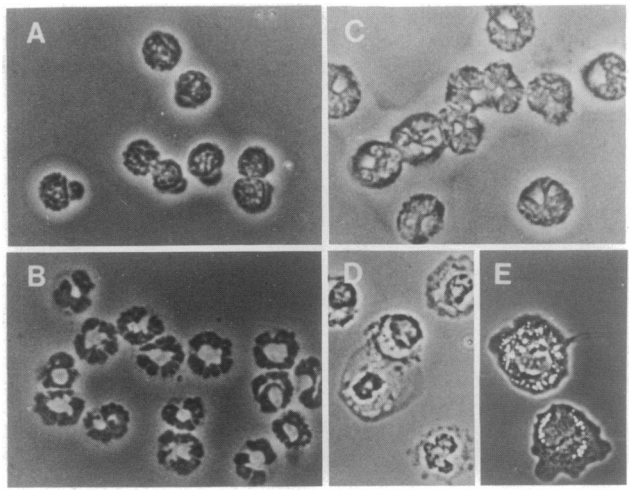

FIG. 3. Morphological characteristics of infiltrating leukocytes. On day 8 after i.p. transplantation of Meth A cells, PEC (leukocyte rich) were separated, and the cells making up the three population types (a, c, and d) were isolated by sorting. Population types b and e were isolated by sorting from peritoneal cells obtained on days 0 and 14, respectively. Following fixation, the cells were analyzed with a phase-contrast microscope. (*A*) Cells in population a in Fig. 2. (*B*) Cells in population c. (*C*) Cells in population d. (*D*) Cells in population b. (*E*) Cells in population e. (×640.)

Cytotoxic Repertoire of Effector Cells. To understand the cytotoxic repertoire of the effector cells, Con A-induced blasts from various strains of mouse were used as target cells. As shown in Fig. 4, the effector cells (H-2^b) isolated from Meth A (H-2^d) cell-sensitized mice were cytotoxic not only against Meth A (H-2^d) cells but also against Con A-induced BALB/c (H-2^d) or DBA/2 (H-2^d) blasts with essentially the same activity, suggesting that the effector cells may not recognize tumor antigens specific for Meth A cells. The effector cells also showed cytotoxic activity against Con A-stimulated CBA/N (H-2^k), C3H/He (H-2^k), or A/J (H-2^a) blasts. However, no killing was seen against Con A-activated blasts from syngeneic animals [C57BL/6 (H-2^b) or C57BL/10 (H-2^b)]. These results suggest that the cells (H-2^b) in population d isolated from Meth A (H-2^d) cell-sensitized mice were cytotoxic against allogeneic, but not against syngeneic, cells.

Phenotypic Analyses of Effector Cells. To ascertain the identity of the effector cells, the surface antigens were phenotypically analyzed. When the cells in population d were incubated with FITC-labeled anti-CD3 (a well-defined T-cell antigen) antibody, the cells virtually (94.5 ± 4.9%, $n = 4$) lacked CD3 (Fig. 5*A*), whereas ≈30% of the spleen cells from untreated C57BL/6 mice were clearly stained (Fig. 5*B*). The cytotoxic activity (62.3 ± 4.4%, $n = 3$) in the FACS-purified $CD3^-$ population was essentially the same as that (65.4 ± 7.2%, $n = 3$) of the untreated effector cells, suggesting that the vast majority (≥94%) of cytotoxicity was present in the $CD3^-$ effector cells.

Flow cytometric staining for Lyt-1 or Lyt-2 antigen or immunoglobulin on the cells in population d was clearly and consistently negative (data not shown). In addition, anti-Thy-1.2, anti-asialo GM1, or anti-L3T4 antibody almost completely lysed thymus lymphocytes in the presence of complement (data not shown), but the majority (≥83%) of effector cells were viable under the same conditions (Fig. 6*A*).

To exclude the possibility that a small fraction of cells in population d, which was specifically lysed by the treatment with complement and antibody, retained a high cytotoxicity, the cytotoxic activity of effector cells was determined after the incubation of the effector cells with antibody and com-

Table 1. Cytotoxic activities of all PEC and of three population types of cells against Meth A cells

Effector cells*	% cell number†	% cytotoxicity‡
All PEC	100	29.7 ± 2.1
Population a	26.5 ± 1.6	9.3 ± 2.5
Population c	23.2 ± 2.0	9.8 ± 1.0
Population d	50.3 ± 3.4	72.1 ± 7.6

*All PEC were recovered on day 8 after Meth A transplantation. Three population types (a, c, and d) of PEC were isolated by sorting.
†With the gate set in the forward scattering–side scattering mode, the data were collected, and the percent cell number of each population in all PEC was calculated with a Hewlett–Packard 9153B computer. Each value represents the mean ± SE of six experiments.
‡Percent cytotoxicity of all PEC or the purified population was determined with an effector-to-target ratio of 50. Each value represents the mean ± SE of six experiments.

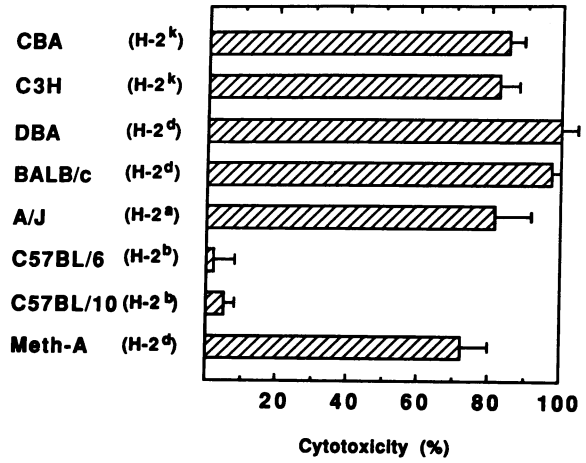

FIG. 4. Cytotoxic repertoire of effector cells. Cells in population d on day 8 after Meth A transplantation were isolated by sorting, and the sorted cells were used as effector cells. ^{51}Cr-labeled Con A blasts or Meth A cells were used as target cells in a ^{51}Cr-release cytotoxic assay with an effector-to-target ratio of 50 after an 18-hr incubation. Each value represents the mean ± SE of six experiments.

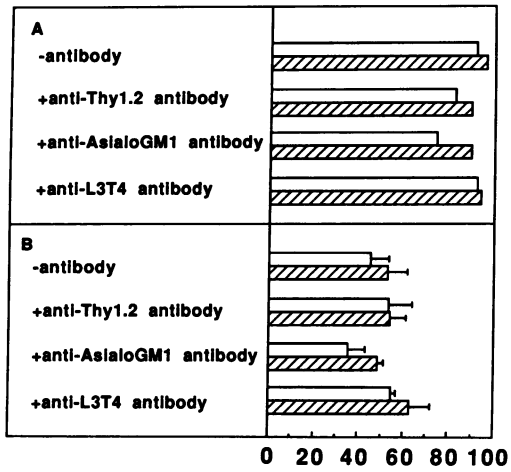

FIG. 6. Complement-dependent lysis with specific antibodies. (*A*) Percent viability. Cells in population d on day 8 after Meth A transplantation were isolated by sorting. The cells were incubated with various antibodies in the presence (□) or absence (▨) of nontoxic rabbit complement. The viability of cells was judged by trypan blue exclusion. Each value represents the mean of duplicate experiments. (*B*) Percent cytotoxicity. After the incubation of cells in population d with various antibodies in the presence (□) or absence (▨) of complement (*A*), the cytotoxic activities against ^{51}Cr-labeled Meth A cells were determined with an effector-to-target ratio of 50 after an 18-hr incubation. Each value represents the mean ± SD of three experiments.

plement. As shown in Fig. 6*B*, 75–99% of the cytotoxic activity of effector cells, which had been treated with antibody alone, remained after the treatment.

These results indicate that the FACS-purified effector cells are Thy-1.2$^-$ CD3$^-$ Lyt-1$^-$ Lyt-2$^-$ L3T4$^-$ immunoglobulin$^-$ asialo GM1$^-$.

Effector Cells Are Adherent Phagocytes. Although the cells in population d were not typically macrophages in appearance, the nucleus was large and kidney- or horseshoe-shaped. Therefore, the possibility still remains that the effector cell is of monocyte/macrophage lineage. When the effector cells were cultured on a coverslip, the vast majority of cells were found to be adherent (namely, 82.8%, 77.9%, and 72.5% of the cells initially plated were left on the coverslips after 2 hr, 14 hr, and 2 days of incubation, respectively). Under the same conditions, however, essentially no lymphocytes or granulocytes were left on the coverslips. The cytotoxicity of the adherent effector cells was 78.9% ± 5.0% (n = 8), which was comparable to or somewhat higher than that (74.0% ± 5.5%, n = 8) of control cells, which were kept on ice for 2 hr. Furthermore, <40% of freshly isolated effector cells were dimly fluorescent with FITC-labeled anti-mouse Mac-1 antibody, whereas after a 1-day culture on a coverslip, >95% of effector cells were brightly fluorescent with the antibody. Under the conditions, however, flow cytometric staining for Thy-1.2, Lyt-2, or immunoglobulin on the effector cells was clearly negative (data not shown).

To determine if the effector cell is a phagocyte, the monolayers on the coverslips were cultured with immunoglobulin-coated polystyrene particles. As shown in Fig. 7, it is evident that almost all adherent cells phagocytosed large numbers of particles. Fig. 7 also shows that the effector cells are adherent and have large pseudopods. Moreover, the effector cells exhibited very weak peroxidase activity as compared to granulocytes, whereas resident macrophages were almost unstained (data not shown).

These results taken together demonstrate that the effector cells responsible for rejection of allografted Meth A cells are adherent mononuclear phagocytes.

DISCUSSION

A variety of cell types have been shown to infiltrate allografts undergoing rejection, including granulocytes, monocytes, and lymphocytes (21–24). The distribution of cell types varies according to the system studied and according to the time after transplantation. The precise role of each cell type in graft rejection has not been defined, although in the past considerable emphasis has been placed on direct T-cell-mediated cytotoxicity. In this study, we have isolated effector cells responsible for rejection of allografted tumor cells from the peritoneal cavity (Fig. 3*C* and Table 1). Morphologically, the effector cells were apparently homogeneous (Fig. 3*C*) and were medium-sized, mononuclear, less-granular cells (Figs. 2 and 3). Phenotypically, the effector cells were Thy-1.2$^-$ CD3$^-$ Lyt-1$^-$ Lyt-2$^-$ L3T4$^-$ immunoglobulin$^-$ asialo GM1$^-$ (Figs. 5 and 6). Functionally, the cytotoxicity was unique in that the effector cells (H-2^b) were

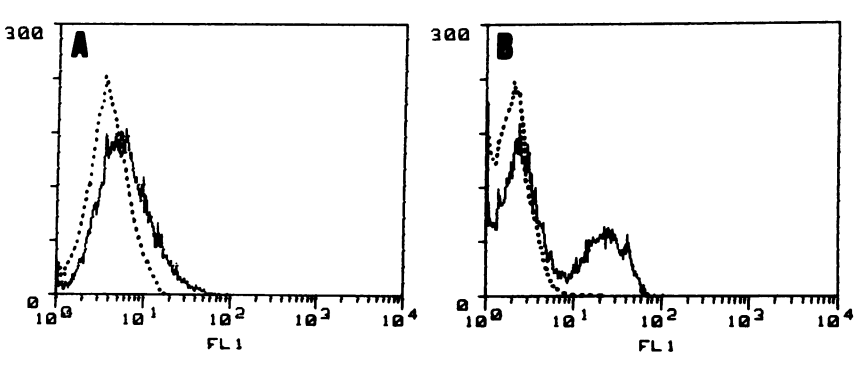

FIG. 5. Phenotypic analyses of effector cells. Cells in population d on day 8 after Meth A transplantation were isolated by sorting. Spleen cells were prepared from untreated C57BL/6 mice and used as a control. Cytofluorographic analyses were carried out using a FACS in the presence and absence of FITC-labeled anti-CD3 antibody. Vertical and horizontal axes represent the cell number and relative logarithmic fluorescence intensity, respectively. (*A*) Effector cells in the presence (—) and absence (····) of antibody. (*B*) Spleen cells in the presence (—) and absence (····) of antibody.

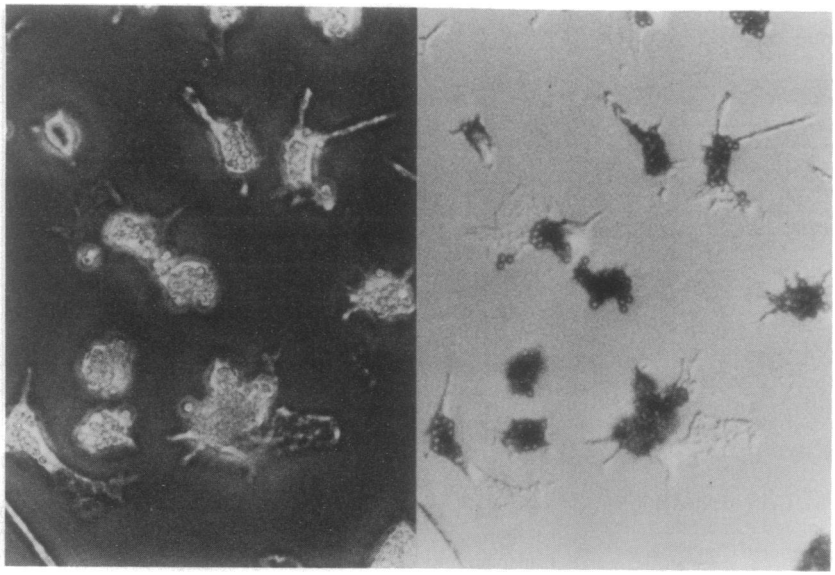

FIG. 7. Effector cells are adherent phagocytes. Cells in population d on day 8 after Meth A transplantation were isolated by sorting. The sorted cells were applied on a coverslip (≈10^5 cells per coverslip), and the endocytic activity was examined by phase-contrast (*Left*) and Nomarski reflection (*Right*) microscopy. Two pictures were taken in the same field. (×360.)

cytotoxic against nonself (Fig. 4). Finally, we clearly demonstrated that the effector cells were adherent mononuclear phagocytes (Fig. 7).

An interesting feature of the cellular infiltrate after tumor transplantation was the high proportion of cell population d, which reached a peak of ≈50% on day 8 after tumor transplantation (Table 1). Since the majority of cells in population d were adherent and phagocytic (Fig. 7), these results suggest that the effector cells may be the previously reported activated macrophages. However, the following argues against this conclusion. (*i*) In a 6-hr assay, the percent cytotoxicity of all PEC on day 8 was 20.5% ± 8.0% (mean ± SD, $n = 4$) (Fig. 2), suggesting ≈40% cytotoxicity in population d, as described in Table 1. Even in the case of macrophages activated by interferon γ and lipopolysaccharide, however, the incubation with target cells for 48 hr or more is necessary to achieve such a level of cytotoxicity (25, 26). (*ii*) Preliminary experiments in our laboratory have demonstrated that, in an 18-hr assay, the cytotoxic activities of two other kinds of purified peritoneal macrophages (interferon γ- and lipopolysaccharide-activated thioglycolate-induced macrophages and bacillus Calmette–Guérin-stimulated macrophages) against ^{51}Cr-labeled Meth A cells were ≈11% and ≈25%, respectively. The cytotoxic activities of these activated macrophages, however, were specific for tumor cells so far tested but totally inert (≤2%) against both syngeneic and allogeneic Con A-activated blasts. Furthermore, the cytotoxic activity of peripheral mononuclear leukocytes or monocytes against ^{51}Cr-labeled Meth A cells was <10%.

Hancock *et al.* (27) and von Willebrand and Häyry (28) have found that the infiltrating cells isolated from rejecting human grafts were 45–52% mononuclear phagocytes, 24–32% lymphocytes, ≈20% polymorphs, and several percent plasma cells. Reitamo *et al.* (29) and Hancock *et al.* (27) have also reported that macrophages formed the largest component of infiltrating cells in acute rejection. The mononuclear phagocytes (or macrophages) that have been described by others as a major population of cells infiltrating into human grafts may be essentially the same kind of cells as the effector cells described here.

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