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Cytokine Effects and Role of Adhesive Proteins and Fc Receptors in Human Macrophage-Mediated Antibody Dependent Cellular Cytotoxicity

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Abstract Mononuclear phagocytes participate in host immunological defense against tumors. We have investigated the role of selected recombinant cytokines on human macrophage-mediated tumor cytotoxicity in vitro utilizing a human colon cancer cell line target, SW1116, and murine monoclonal antibody 17-1A. Blood monocytes were kept in continuous culture to allow differentiation into macrophages. Maximum antibody dependent cellular cytotoxicity (ADCC) as measured in a ^{3}H-thymidine release assay occurred after culturing the monocytes for 5–7 days. Human recombinant macrophage colony stimulating factor (CSF) (1,000 U/ml) did not increase ADCC above control levels whereas recombinant human granulocyte-macrophage colony stimulating factor, interleukin 4, and interleukin 3 were all capable of increasing ADCC. Antibodies to the CD11/CD18 integrin receptors did not significantly inhibit ADCC. When the ADCC incubation occurred in the presence of antibodies to the human Fc receptors, ADCC was inhibited significantly only by anti-FcRIII (3G8). A role for tumor necrosis factor alpha or other soluble mediators of cytotoxicity was not demonstrable in this system. These studies suggest avenues for manipulation and augmentation of macrophage-mediated antitumor ADCC.

Key words: ADCC, cytokine, macrophages, tumors, human colon cancer

Macrophages are important effector cells in host defense against metastatic tumors [1,2]. How activated macrophages recognize and kill diverse tumor target cell types is uncertain. Some studies have suggested that direct macrophage-target cell contact involving lysosomal enzyme transfer results in tumor cell destruction [3], but other studies have implicated macrophage release of secretory products which mediate target cell lysis [4].

In addition to their role in nonspecific cytotoxicity, macrophages can also participate in antibody-dependent cellular cytotoxicity (ADCC). Murine monoclonal antibodies of the IgG2a and IgG3 isotypes can effectively mediate cell killing and have found clinical use in tumor immunotherapy [5]. Such antibodies have made possible in vitro studies of macrophage tumor cell killing through Fc receptor activation and studies of the variables important for effective cytotoxic interactions between tumor cells, macrophages, and monoclonal antibodies. Conditions necessary for monocyte/macrophage activity in ADCC have not been fully defined.

In order to better understand human macrophage-mediated tumor cytotoxicity, we have utilized an in vitro cytotoxicity assay in which a human colon cancer cell line, SW1116, is lysed by cultured macrophages reacting with a murine IgG2a monoclonal antibody, 17-1A (Centocor, Inc., Malvern, PA) [6]. In this system, we have examined the influence of hematopoietic cytokines known to enhance monocyte precursor maturation and/or macrophage effector functions. These include macrophage-colony stimulating factor (M-CSF), granulocyte-mac-

Abbreviations used: ADCC, antibody dependent cellular cytotoxicity; CSF, colony stimulating factor; M-CSF, macrophage CSF; GM-CSF, granulocyte-macrophage CSF; IL, interleukin; TNF, tumor necrosis factor; LPS, lipopolysaccharide, FcRs, Fc receptors; E:T ratio, effector:target ratio; Moab, monoclonal antibody; PBS, phosphate buffered saline; FBS, fetal bovine serum; BSA, bovine serum albumin; HABS, pooled human AB serum; rHu, recombinant human, SD, standard deviation, SEM, standard error of the mean.

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rophage-colony stimulating factor (GM-CSF), andinterleukins (IL) 1, 3, 4, and 6 [7]. In addition, we have examined the role of the leukocyte adhesion molecules of the CD11/CD18 family [8] in this macrophage-mediated ADCC system. Previously, a role for the LFA-1 (CD11a) antigen in macrophage capture of tumor cells has been found [9]. The contribution of individual human Fc receptors (FcRs) to ADCC in this system has also been examined.

METHODS

Preparation of Monocytes/Macrophages

Mononuclear cells were separated from heparinized buffy coat preparations from normal American Red Cross donors by density gradient centrifugation on Isopaque-Ficoll (Pharmacia, Piscataway, NJ). After washing, mononuclear cells were layered onto 75 cm^2 tissue culture flasks (Corning) for 1–2 hours to allow adherence of monocytes. The flasks were then washed 5–8 times until removal of visible nonadherent cells. The adherent cells were cultured in RPMI (Gibco, Grand Island, NY) medium with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) with or without lymphokines until they were harvested for cytotoxicity assays. At the time of harvest, greater than 90% of cells were macrophages based on Wright's [10] or nonspecific esterase staining [11]. These cells were LeuM3 (CD14) and LeuM5 (CD11c) positive and approximately 7–10% stained with antibody to FcRIII indicating monocyte-macrophage maturation and differentiation [12].

Target Cells

The SW1116 cell line, derived from a human colon carcinoma, was obtained from the ATCC (Rockville, MD). It is an adherent line and was grown in RPMI with 10% FBS and passaged weekly after trypsinization. This line was not contaminated with *Mycoplasma pneumoniae* as assessed with Gen-Probe Rapid Detection System (San Diego, CA). Cell viabilities of both effector and target cells were determined with Trypan Blue (Sigma, St. Louis, MO) before cytotoxicity assay.

Cytokines and Other Chemicals

Recombinant human (rHu) M-CSF was provided by Cetus (Emeryville, CA) at 6.5×10^5 U/ml and was purchased from Cellular Products (Buffalo, NY) at 10^5 U/ml. Both preparations contained less than .1 ng/ml endotoxin. rHu GM-CSF, rHuIL-1 alpha, rHuIL-3, rHuIL-4, and rHuIL-6 were purchased from Genzyme (Boston, MA). All Genzyme preparations contained $\leq$ .03 ng/ml endotoxin. In some experiments, GM-CSF (9.3×10^6 U/mg) generously provided by Steve Clark, Genetics Institute (Cambridge, MA) was utilized. In some experiments with IL-6, a preparation supplied at 2 mg/ml by Larry Souza, Amgen (Thousand Oaks, CA) was used. *Salmonella typhimurium* Lipopolysaccharide (LPS) and fraction V bovine serum albumin (BSA) were purchased from Sigma Chemical Co., St. Louis, MO. Fibronectin from human plasma and laminin (Collaborative Research, Bedford, MA) were used to coat tissue culture flasks at 4–5 $\mu g/cm^2$.

Antibodies

17-1A, a murine monoclonal antibody (moab) specific for an antigen expressed on human gastrointestinal tumor cells [6], was a gift of Centocor, Inc. (Malvern, PA). It was supplied at 9.3 mg/ml and was diluted in endotoxin-free phosphate-buffered saline (PBS) with 15% BSA. It was utilized in these experiments at a final concentration of 5 $\mu g/ml$, a concentration which was saturating in cytotoxicity titration experiments. Monoclonal antibodies to the three human Fc receptors were obtained from R. John Looney (University of Rochester, Rochester, NY); 3G8 (IgG1), which recognizes FcRIII was used as a supernatant. Moab 32 (IgG1), which recognizes FcRI was used at a 1:5 dilution of hybridoma supernatant. Purified IV3 (IgG2b), which recognizes FcRII was used at 10–100 $\mu g/ml$; 10F12 (anti-CD18, against the integrin β_2 chain), L1 (anti-LFA-1 alpha, CD11a), 44a (anti-Mo1, CD11b), and L-29 (anti-p150,95, CD11c) were obtained from M. Amin Arnaout, Massachusetts General Hospital, Boston, MA. All were used at saturating dilutions of ascites preparations. 84H10, anti-CD54 (ICAM-1) was purchased from AMAC (Westbrook, ME) and was used at 2 $\mu g/ml$. Appropriate isotype specific control antibodies were purchased from Coulter (Hialeah, FL) and used at saturating dilutions. Polyclonal rabbit anti-human TNF-alpha for neutralization was purchased from Genzyme and diluted with PBS/0.1% BSA prior to use. A neutralizing moab to TNF-alpha of IgG1 subtype was kindly provided by Genentech (San Francisco, CA [13]). Its neutralization titer was $> 5 \times 10^5$ neutralizing units/ml. Polyclonal rabbit antisera to human actin was obtained from

Dr. Charles H. Packman, University of Rochester, Rochester, NY. Neutralizing monoclonal antibodies to GM-CSF and IL-3 and a neutralizing polyclonal antibody to IL-4 were purchased from Genzyme.

Cytotoxicity Assay

Assays were performed in 96-well round bottom tissue culture plates. SW1116 cells were labeled with 1 μCi/ml ^{3}H-TdR (Amersham Arlington Heights, IL) for 18–24 h, and 10^4 washed trypsinized cells were placed in each well in 100 μl of medium. For cells to be assessed in an ADCC assay, 17-1A was added to the SW1116 cells prior to plating.

Macrophages were harvested with a cell scraper, washed, and added at appropriate cell numbers in 100 μl final volume to yield the desired effector:target ratios (E:T). The final volume of supernatant in each well was 200 μl. The plates were then incubated for the planned time at 37°C/5% CO_2 after which 100 μl of cell-free supernatant was removed, added to liquid scintillation fluid, and counted. Spontaneous release was determined by sampling supernatant from wells containing SW1116 target cells ± antibody without macrophage effectors. The spontaneous release was always <10% of the maximum release. Maximum release was determined by adding 10% SDS to control aliquots of SW1116 cells. Percentage cytotoxicity was determined from the following standard equation:

$$\text{Percent cytotoxicity} = \frac{\text{sample cpm} - \text{spontaneous cpm}}{\text{maximum released cpm} - \text{spontaneous cpm}} \times 100.$$

Each condition was assayed in at least triplicate fashion. Standard deviations were <20% of mean values. In assay conditions assessing the effect of added antibody, macrophage effector cells were treated with the antibody in question at 0°C for 30–40 min prior to addition to 96-well plates.

Immunofluorescence and Flow Cytometry

Cells were stained with saturating dilutions of primary antibodies at 0°C for 30 min, washed ×3 in PBS/1% BSA/.1% azide, and then stained 30 min at 0°C with 2° antibody, FITC-conjugated goat anti-mouse IgG, Tago (Burlingame, CA). They were then washed again ×3, resuspended in PBS, and fixed with 1% paraformaldehyde. Cells were analyzed in routine fashion on an EPICS V flow cytometer (Coulter) using log green fluorescence and 90° vs. forward angle light scatter.

RESULTS

Monocyte-Macrophage Differentiation

Blood monocytes cultured in fetal bovine serum alone underwent morphologic changes characteristic of in vitro differentiation to macrophages with increase in cell size and increase in cell vacuolization. Such morphologic changes were not augmented in the presence of M-CSF, IL-3, or GM-CSF but were enhanced with human serum.

Time Course for Development of Monocyte Cytotoxicity

Maximum antitumor cytotoxicity was not seen until after 5–7 days of monocyte culture. Figure 1 shows a typical time course of development of capability for ADCC of macrophages cultured in RPMI with 10% FBS. Macrophages were harvested on the indicated day after initial culture and were co-incubated with SW1116 targets and Moab 171A for 18–20 h. In experiments where monocytes were cultured up to 14 days prior to harvest for use in cytotoxicity assays, levels of antibody dependent tumor cell killing comparable to those achieved by day 7 macrophages were seen (40–55%).

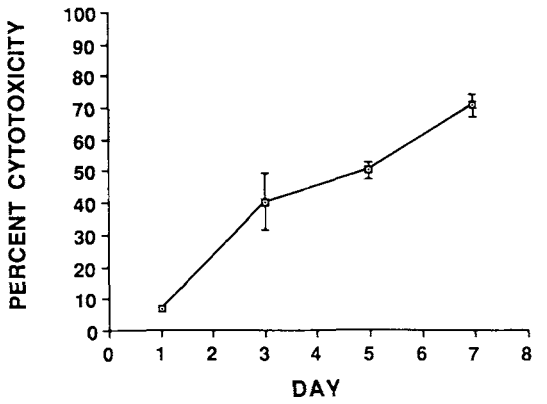

Fig. 1. Shown is a representative evolution of cytotoxicity as a function of time of culture of effector monocyte/macrophages in days. Percent cytotoxicity was determined as described in the Methods section. Each point represents the mean ± SD percent cytotoxicity of ≥3 replicate samples at an E:T ratio of 50:1. Cells were harvested on the indicated day and coincubated with target cells for 20 h.

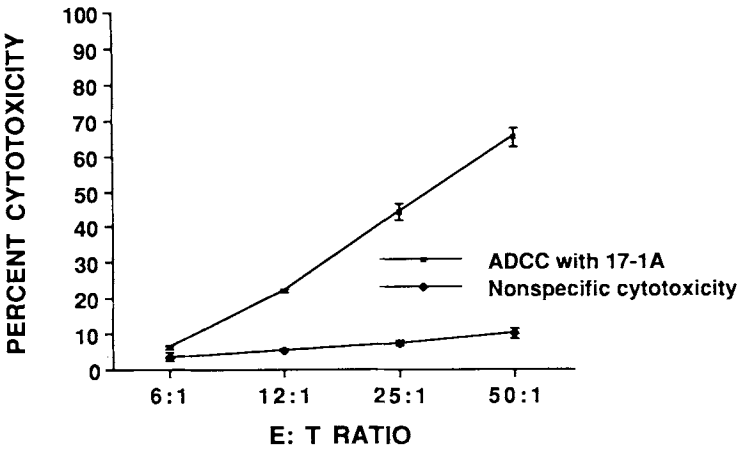

Fig. 2. Shown is the influence of E:T ratio on both ADCC and nonspecific cytotoxicity in a representative experiment. Monocytes were used as effectors after 7 days of culture in a 20 h coincubation assay. Data are reported as mean ± SD percent cytotoxicities.

Effector:Target Ratios

ADCC increased at E:T ratios of 6:1 to 50:1 with a plateau effect noted in some donors between 30:1 and 50:1. The amount of target cell killing achieved varied considerably between donors (range 9% to 66%, n = 11, E:T ratio 25:1). Antibody-independent killing was substantially less than ADCC (7.4% ± 1.7% nonspecific cytotoxicity vs. 43% ± 6% specific cytotoxicity, n = 11, E:T ratio 25:1). Figure 2 shows a representative E:T dose response with blood monocytes cultured 7 days and then used as effector cells.

Effect of Coincubation Times on ADCC Development

To assess whether the cytotoxicity generated in this system followed the relatively slow development of macrophage-mediated cytotoxicity or the rapid development of maximum ADCC seen with natural killer cells [14], the effect of cytotoxicity after various durations of effector and target cell coincubation times was measured. At an E:T ratio of 50:1, specific cytotoxicity increased in a nearly linear fashion between 6 and 24 h as shown in Figure 3. Cell mediated specific cytotoxicity also increased from 24 to 48 h but little increment in ADCC was noted thereafter.

Hematopoietic Cytokine Effects on Ability of Macrophages to Mediate ADCC

Hematopoietic cytokines which influence monocyte precursor maturation or end-stage macrophage effector function were studied for possible effects on the ability of macrophages to mediate cytotoxicity in this system. Cytokines in question were added to monocyte cultures at their inception and were washed at the time of cell harvest for cytotoxicity assays. Figure 4 shows the effects of cytokines tested on ADCC. Macrophage effectors were cultured for 5–7 days prior to use in cytotoxicity assays and were used at E:T ratios of 50:1 in 18 h incubations. M-CSF, 1,000 U/ml; IL-1 alpha, 5 U/ml; and IL-6, 100 U/ml did not alter the degree of ADCC seen. IL-1 alpha was inactive from 1 U/ml to 20 U/ml, and IL-6 was inactive in concentrations up to 300 U/ml (data not shown). GM-CSF, 50 U/ml, IL-3, 50 U/ml, and IL-4, 100 U/ml were capable of significantly increasing ADCC ($P < .05$).

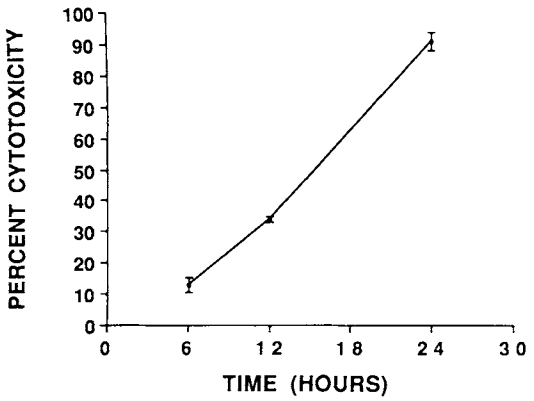

Fig. 3. Shown is the effect of coincubation time of macrophages with effector cells on ADCC development. At an E:T ratio of 50:1, cytotoxicity increased in a nearly linear pattern between 6 and 24 h. Spontaneous release was determined separately at each time point. Data are shown as mean ± SD percent cytotoxicity.

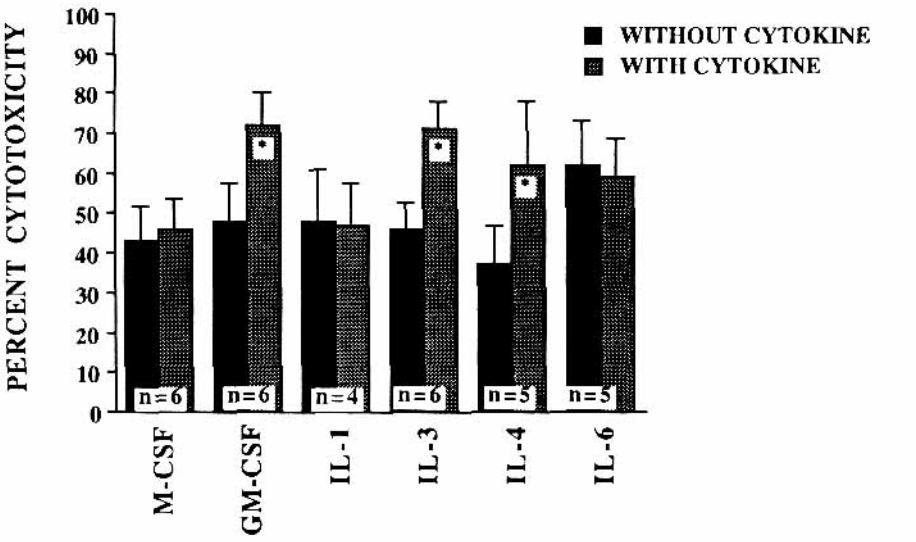

Fig. 4. Data represent mean ± SEM percent cytotoxicities from experiments in which macrophages cultured for 7–8 days with or without cytokine were used as effectors at an E:T ratio of 50:1 in 18 h incubations. Cytokine concentrations used were M-CSF, 1,000 U/ml; GM-CSF, 50 U/ml; IL-1 alpha, 5 U/ml; IL-3, 50 U/ml; IL-4, 100 U/ml; and IL-6, 100 U/ml. For experiments with GM-CSF, IL-3, and IL-4, the increase in ADCC observed was significant at the $P < .05$ level by a paired t test (*).

M-CSF did not increase ADCC at any E:T ratio tested and was inactive at doses from 10 U/ml to 1,000 U/ml (Fig. 5a). In contrast, GM-CSF increased cytotoxicity significantly at E:T ratios of 25:1 and 50:1, n = 8 (Fig. 5b) and was active in a dose-response manner at concentrations of 10–50 U/ml (Fig. 6).

Neutralizing antibodies to GM-CSF, IL-3, and IL-4, when added at the inception of monocyte culture along with the appropriate cytokine abrogated the increase in ADCC observed with each of these cytokines (Table I). GM-CSF, 50 U/ml, IL-3, 100 U/ml, and IL-4, 100 U/ml, did not change percent specific cytotoxicity with 17-1A when added only at the time of the cytotoxicity assay. LPS at .01 ng/ml to 100 ng/ml did not increase cytotoxicity when added at the beginning of monocyte cultures.

Role of LFA-1 Antigens in Macrophage-Mediated ADCC

To determine whether or not the adhesive proteins of the CD11/CD18 integrin family were important in macrophage target cell interactions, cytotoxicity was studied in the presence of antibodies to these receptors. As shown in Table II, functional blocking antibodies to CD18 (common β chain) and to each of the three CD11 alpha chain antigens did not significantly inhibit macrophage-mediated ADCC against the SW1116 cell line. For these assays, monocytes were treated with concentrations of the antibody up to ten-fold higher than that required to saturate binding for 30 min at 4°C prior to cell addition to the ADCC assay. Whether antibody was washed or not washed prior to addition of target cells did not affect results. ICAM-1 (CD54), a ligand for the LFA-1 (CD11a) antigen [15], was present on the SW1116 cell line as assessed by flow cytometry (data not shown).

Macrophages cultured in the presence of GM-CSF for 5 days demonstrated increased CD11b/CD18 as compared with control cells [16] but antibody to CD11b did not prevent the increased cytotoxicity noted with GM-CSF.

Role of Other Matrix Components in ADCC

Culturing macrophages over flask surfaces coated with 4 $\mu g/cm^2$ fibronectin or 4 $\mu g/cm^2$ laminin did not significantly influence the degree of cytotoxicity observed against the SW1116 cell line: control plastic flasks; 34 ± 13%, fibronectin-coated flasks; 29 ± 9.4%, and laminin-coated flasks; and 38 ± 5.8% (Mean ± SEM percent cytotoxicity, n = 5).

Effect of Human Serum and FcR Antibodies on Macrophage-Mediated ADCC

Table III indicates that treatment of 6-day macrophage effector cells with the 3G8 antibody significantly depressed ADCC, indicating a role for the FcRIII in this effector cell-antibody coated

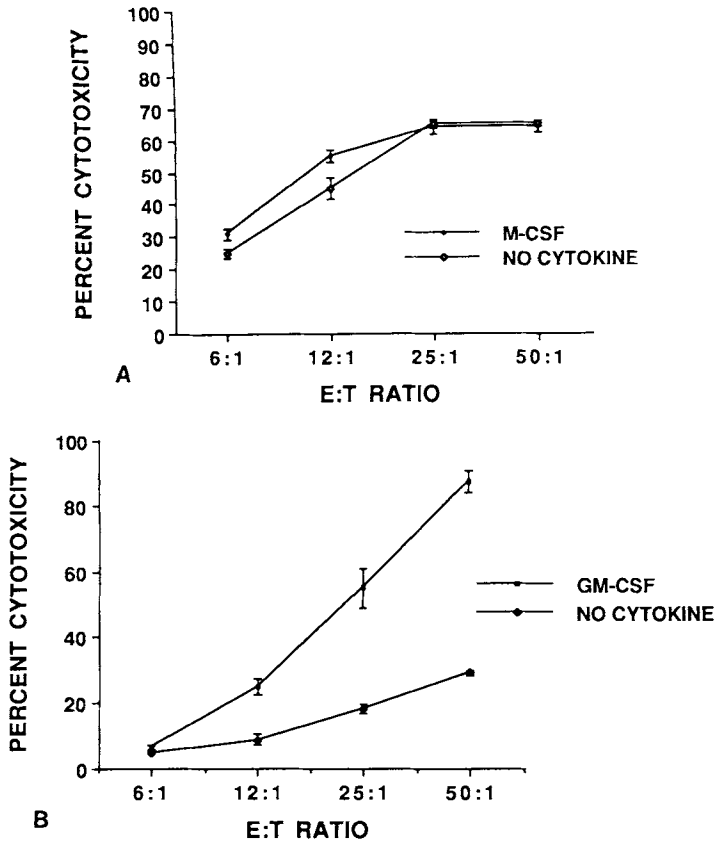

Fig. 5. A and B. Shown are the effects of 1,000 U/ml M-CSF (**A**) and 50 U/ml GM-CSF (**B**) at various E:T ratios in individual experiments. Data represent mean ± SD for triplicate samples.

target cell interaction. Graded dilutions of 3G8 hybridoma supernatant to 1:100 resulted in measurable inhibition of ADCC in a dose-response fashion. Since blocking of this FcR did not decrease levels of cytotoxicity to the range of nonspecific cytotoxicity, this suggested that other FcRs may be operative. Also, human serum, which contains monomeric IgG, when present during the effector/target cell coincubation decreased ADCC to a significant degree (Table IV). When concentrations of human serum, which were only partially inhibitory to specific cytotoxicity, were added to samples containing saturating concentrations of 3G8, greater inhibition beyond that seen with Moab 3G8 alone was seen (45% ± 11% greater inhibition, n = 4, at a serum concentration of 1%). This finding thereby suggested participation of other Fc receptors capable of binding serum IgG in this ADCC system.

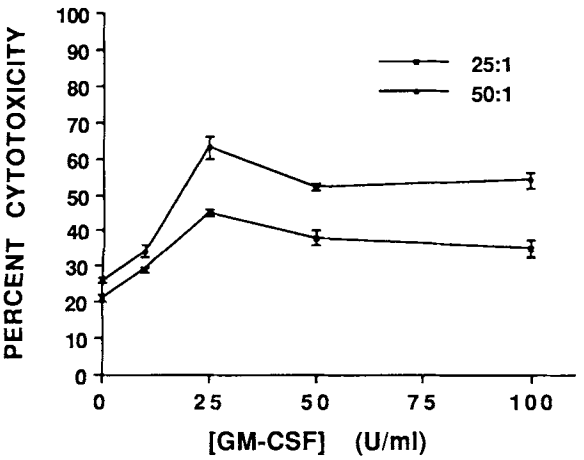

Fig. 6. Shown is the effect of GM-CSF concentration on ADCC (mean percent cytotoxicity ± SD) at E:T ratios of 50:1 and 25:1. Macrophages were cultured for 7 days prior to use in cytotoxicity assays.

Role of Soluble Mediators

Conditioned medium from macrophages cultured for 6 days in FBS did not demonstrate any cytotoxicity against the SW1116 target cell line. Figures 7 and 8 indicate that neutralizing poly-

TABLE I. Antibody Neutralization of Cytokine Effects on Cytotoxicity*

Cytokine	Macrophage alone	Macrophage + cytokine	Macrophage + cytokine + antibody
50 U/ml GM-CSF	13 ± 7	18 ± 13	10 ± 5
100 U/ml IL-3	39 ± 14	47 ± 16	30 ± 12
100 U/ml IL-4	66 ± 6	86 ± 7	77 ± 10

*Shown is the mean ± SEM percent cytotoxicity in three experiments for each indicated cytokine in control cultures, cultures with the indicated concentration of cytokine, and cultures with cytokine plus amounts of antibody known to neutralize the added cytokine in progenitor colony assays. Antibody alone did not decrease cytotoxicity significantly below control levels. Anti-GM-CSF was used at 1 μg/ml, anti-IL-3 at 2.5 μg/ml, and anti-IL-4 (BL-4P) at 1 μg/ml.

clonal and monoclonal antibodies to TNF alpha did not inhibit ADCC mediated by human macrophages against the SW1116 cell line.

DISCUSSION

Cells of the monocyte-macrophage system are capable of killing many tumor cell types in vitro. In the system we evaluated, generation of effective cytotoxicity against the human colon cancer cell line, SW1116 in the presence of the murine IgG2a monoclonal antibody, 17-1A, by human blood monocytes required culture duration sufficient for monocyte to macrophage differentiation. Development of such cytotoxicity also required coincubation of effector and target cells for approximately 24 h, in keeping with the slow development of macrophage-induced cytotoxicity noted by others [14]. The E:T ratios most effective are consistent with other studies [17,18].

Anti-tumor ADCC in the system described here could be increased by certain hematopoietic cytokines. Whereas the total amount of target cell lysis obtained varied amongst donors, these cytokine effects were consistent in all donors tested. M-CSF, a growth and differentiation factor for bone marrow progenitor cells of monocyte lineage which also promotes survival and functional activities of mature macrophages, was ineffective in augmenting ADCC in this system over a wide dose range. Recombinant human M-CSF has been found to have divergent effects in various cytotoxicity systems. Nakoinz [19] found that human M-CSF alone had little effect but could enhance ADCC to murine tumor targets when used as a costimulant with interferons alpha, beta, gamma, and IL-2. Mufson et al. [20] found that M-CSF-

TABLE II. Role of CD11/CD18 Antigens in Macrophage-Mediated ADCC*

	Percent cytotoxicity (mean ± SEM)		
	Test mab	Control ab	n
Anti-CD18	48 ± 3.5	53 ± 3.4	5
Anti-CD11a	44 ± 13	44 ± 9.2	3
Anti-CD11b	46 ± 6.4	53 ± 4.2	7
Anti-CD11c	40 ± 8.6	53 ± 9.9	6

*To assess whether blocking antibodies to the functional sites of CD11/CD18 antigens would affect the degree of macrophage-mediated ADCC against the SW1116 cell line, monocytes treated with these antibodies for 30 min at 4°C were compared to control macrophages. Shown is the mean ± SEM percent cytotoxicity in n experiments. The decreased cytotoxicity observed with anti-CD11c was not significant by the paired t test. Each antibody demonstrated saturable binding at a concentration of 1:500 or 1:1,000. In these blocking experiments, antibodies were used at a 1:100 concentration.

Table III. Effect of Antibodies to Fc Receptors on Macrophage Mediated ADCC†

	Percent cytotoxicity (Mean ± SEM)		
	Test mab	Control	n
mab 32 (Anti-FcRI)	67 ± 17	68 ± 19	4
mab IV.3 (Anti-FcRII)	59 ± 11	56 ± 10	7
mab 3G8 (Anti-FcRIII)	39 ± 10*	61 ± 13	7*

†Macrophage effector cells cultured for 7 days were pretreated with excess concentrations of hybridoma supernatants of the FcR antibodies. Shown is the effect on cytotoxicity against the SW1116 cell line at an E:T of 25:1 in n experiments.
*$P < .001$ by the paired t test.

TABLE IV. Effect of Pooled Human AB Serum on ADCC*

Serum	Percent cytotoxicity (mean ± SEM)
10% FCS	53.9 ± 11.7
10% HABS	9.5 ± 4.4

*Cytotoxicity of ^{3}HTdR labeled SW1116 cells by human monocytes (E/T 25:1) was evaluated in medium containing 10% fetal calf serum (FCS) or 1% pooled human AB serum (HABS.) Results represent mean ± SEM of percent cytotoxicity from four experiments with each condition performed in at least triplicate fashion.

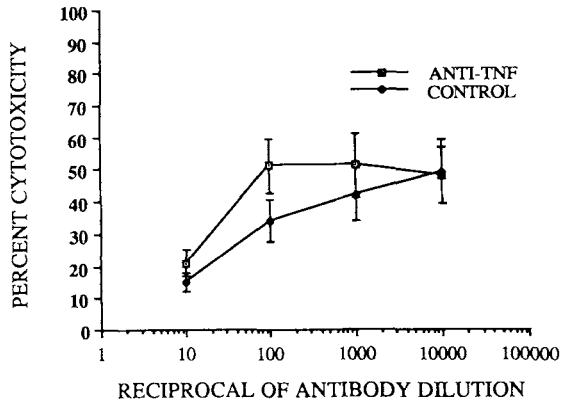

Fig. 7. Shown are the means ± SEMs of cytotoxicity of monocytes/macrophages cultured for 7 days at an E:T ratio of 50:1 at various concentrations of a polyclonal neutralizing rabbit antibody to human TNF-alpha or the same concentration of irrelevant rabbit polysera.

augmented ADCC by human blood monocytes against a human colon cancer cell line. Utilizing the murine WEHI-3 cell line as targets, Sampson-Johannes and Carlino [21] found that M-CSF induced enhanced cytotoxicity by monocytes in 40% of donors but reproducibly enhanced it when 2° activators such as LPS or gamma interferon were present. Suzu et al. [17] found that nonspecific cytotoxicity toward leukemic cell lines was enhanced by 50–100 ng/ml M-CSF.

Cheung and Munn [22] found that antitumor cytotoxicity mediated by human blood monocytes was enhanced by M-CSF. Target cells were found to be phagocytosed with their radioisotope markers intact by cultured monocytes. Whether such a phagocytic mechanism may be operative in the system reported here is not known. Others have also suggested that macrophages exposed to M-CSF may inhibit tumor proliferation (cytostasis) but not cause lysis (cytotoxicity) of tumor cells. Only lysis would be detected in chromium or thymidine release studies [23].

IL-1 and IL-6 did not affect macrophage ability to mediate ADCC in this system, whereas GM-CSF, IL-3, and IL-4 exposure resulted in significantly increased cytotoxicity. That IL-1 and IL-6, which act on early hematopoietic progenitors, would not affect an end-stage cell function such as ADCC is not unexpected. Similarly, some studies of tumor-mediated cytotoxicity have found enhancing roles for GM-CSF, IL-3, and IL-4 [24–26], while others failed to demonstrate a role for either IL-3 or GM-CSF [18,19]. The positive enhancing effect of cytokines in the system described herein was dose responsive, and effects were seen particularly at E:T ratios $>25:1$.

Hibbs has suggested that direct macrophage-target cell contact involving lysosomal enzyme transfer results in tumor cell destruction [3]. Johnson and others [27] have shown that physical blockade of effectors from target cells can abrogate cytolysis. Still other studies have suggested that macrophage release of secretory products may mediate target cell lysis [4,28], suggesting that cell-cell contact may not be essential. In the macrophage-mediated ADCC system described here, cytotoxicity occurred indepen-

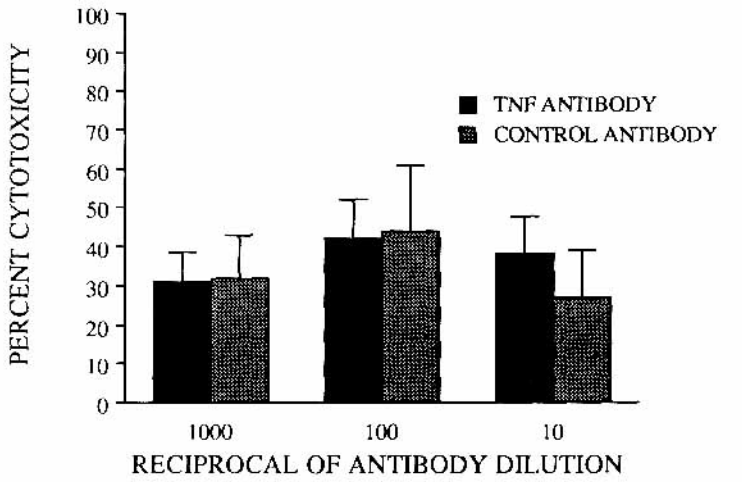

Fig. 8. Shown is the mean ± SEM percent cytotoxicity of monocytes/macrophages cultured for 7 days against SW116 at an E:T ratio of 50:1 in the presence of a blocking monoclonal antibody to TNF-alpha or in the presence of an irrelevant isotype specific control antibody.

dently of β_2 integrin family function as indicated by negative antibody blocking studies. This is in keeping with the work of Strassman et al. [9], which showed that antibody to LFA-1 decreased binding (contact/capture) to tumor targets without affecting extent of cytotoxicity. Also, since CD11b declines on cells cultured in vitro [29], it is unlikely to play a role in cytotoxicity which is most effective after 5–7 days of culture. It is thus also unlikely that cytokines such as GM-CSF and IL-4, which have been found to upregulate the CD11a/CD11b antigen on monocyte/macrophages [16,30], enhance cytotoxicity by increasing target cell capture through this mechanism.

These data do not rule out the possibility that other matrix binding proteins may be important in macrophage-target cell interactions. Monocytes cultured on collagen have been found to be less cytotoxic than those cultured on glass, for example [31], and a recent report has suggested a role for thrombospondin in monocyte killing of human squamous epithelial cells [32]. In the preliminary studies described here, no role for laminin or fibronectin substrates was observed in influencing the degree of monocyte-macrophage cytotoxicity.

Finally, neutralizing polyclonal and monoclonal antibodies to TNF (alpha) failed to block macrophage ADCC in this system. The inability of TNF antibodies to block cytotoxicity does not completely rule out a possible function for this cytokine in effecting ADCC since membrane-bound TNF [33] could be involved, and this TNF could be in a form not accessible to anti-TNF antibody [28].

In this ADCC system mediated by macrophages and an IgG2a murine monoclonal antibody, antibodies to FcRIII inhibited ADCC, thus indicating a role for this receptor. The development of effective ADCC at 5–7 days corresponds with the late development of this antigen on cultured monocytes as reported by Fleit et al. [34]. Since ADCC was not completely blocked by 3G8, other FcR(s) presumably participate. This is possibly FcRI given the fact that Moab32 does not block monomeric IgG binding [35] and given the observation that human serum was effective in blocking ADCC, indicating competition of human IgG1 and IgG3 with the murine IgG2a for this receptor [36]. Erbe et al. [37] have recently reported that various cytokines affect ADCC by myeloid cells in an Fc receptor-specific manner. Whether cytokines might enhance ADCC through increased expression of FcRs or by a change in their affinity for the murine monoclonal antibody is at present undetermined.

In vitro studies of antibody-mediated monocyte-macrophage cytotoxicity against tumor cells such as the SW1116 colon cancer cell line described here may have significance for in vivo use of these monoclonal antibodies as used to treat human malignancies. Other investigators have reported that cytokines such as GM-CSF administered in vivo to cancer patients can enhance monocyte cytotoxicity as measured in in vitro systems [38]. The studies presented here lend support to augmenting macrophage ADCC through effector cell activation with cytokines selected for their effectiveness in in vitro systems and for manipulating macrophage-mediated ADCC through modulation of Fc receptor number or function.

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