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Differential Effect of Cytokine Treatment on Fcα Receptor I- and Fcγ Receptor I-Mediated Tumor Cytotoxicity by Monocyte-Derived Macrophages

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Macrophages represent an important effector cell for Ab-mediated tumor therapy. Previous studies have documented that cytokines can influence Fc receptor (FcR) expression and function. In this study we examined the tumoricidal activities of the type I receptors for IgG (FcγRI) and the IgA FcR (FcαRI) on monocyte-derived macrophages (MDM) cultured in the presence of IFN-γ, M-CSF, or GM-CSF. Bispecific Abs were used to target a Her2/neu breast carcinoma cell line, SKBR-3, to FcαRI or FcγRI on MDM. Although FcαRI and FcγRI share a common signaling pathway contingent on association with the γ-chain (FcγRII subunit), a marked difference in their efficiency in mediating tumoricidal functions was seen in response to specific cytokines. M-CSF- and GM-CSF-treated MDM mediated efficient phagocytosis of SKBR-3 cells by FcαRI and FcγRI; however, IFN-γ-treated MDM phagocytosed tumor cells only with the FcγRII-direct bispecific Abs. Similarly, IFN-γ-cultured MDM lysed tumor cells more efficiently via FcγRI then by FcαRI as measured in Ab-dependent cellular cytotoxicity assays. Conversely, GM-CSF-treated MDM mediated more efficient lysis of tumor cells via FcαRI than FcγRI, while M-CSF-cultured MDM were relatively less efficient in mediating Ab-dependent cellular cytotoxicity through either receptor. With the exception of IFN-γ-mediated enhancement of FcγRII expression and FcγRI γ-chain complexes, the regulation of FcγRII- or FcαRI-mediated activity occurred without significant change in either receptor expression or total complexes with γ subunit. These data suggest that the efficiency of Ab-mediated tumor therapy, which depends on FcR effector cell functions, may be modified by the use of specific cytokines. The Journal of Immunology, 2000, 164: 5746–5752.

Macrophages are a critical part of the immune system, participating in both natural and specific immunity. Macrophages can function as effector cells to eliminate pathogens and as accessory cells that recruit and activate other immune cells. Furthermore, macrophages have been implicated to be critical in mediating Ab-dependent tumor regression in some animal models (1, 2). Many of these effector and accessory functions are mediated through Fc receptors (FcR) (2) that bind Ag-complexed Igs. Human macrophages constitutively express the Fc receptor for IgA (FcαRI), and the high and low affinity Fc receptors specific for IgG (FcγRI, FcγRIIA, and FcγRIIIA). These FcR mediate effector functions that are well documented, including Ab-dependent cellular cytotoxicity (ADCC) and phagocytosis (3–7).

In contrast, polymorphonuclear cells (PMN), an other myeloid effector cell with phagocytic and cytolytic capacity, constitutively express FcRI, but not FcγRI. However, FcγRI expression can be induced in vitro with IFN-γ treatment or in vivo with either G-CSF or IFN-γ (8–10). The relative importance of these two FcR types in promoting cytotoxic effector functions of various myeloid populations (monocytes, macrophages, and PMN) has not been clearly delineated.

FcγRI (CD64) binds monomeric human IgG1 and IgG3 with high affinity (3, 4), and FcγRIIIA (CD16) has intermediate affinity for monomeric IgG. FcγRIIa (CD32) efficiently binds to IgG immune complexes and IgG-opsonized particles, but not to monomeric IgG (3). On macrophages, a single class of IgA Fc receptor, FcαRI (CD89), has been characterized and binds both Ag-complexed and monomeric IgA1 and IgA2 (5, 11). This suggests that in vivo FcαRI may be saturated with monomeric IgA in the same manner as FcγRI and FcγRIIIA are significantly occupied with IgG.

mAb have been developed that bind to FcγRI (mAb 32.2 and 22) and FcαRI (mAb A77) at sites distinct from their ligand binding domains (11–13). ADCC mediated by bispecific Abs (BsAb) prepared using these anti-FcR Abs is not blocked by human IgG or IgA (6, 7, 14–16). In addition to triggering effector functions under physiologically relevant conditions, BsAb made from these mAbs bind exclusively to the targeted FcR and provide a suitable method to study the capacity of individual FcR in a variety of functional assays. Both FcγRI and FcαRI have been shown to be functionally associated with the γ subunit, which mediates signaling events following receptor clustering (17, 18). Although these receptors share a common signaling component (the γ subunit), their expression on the surface of effector cells is differentially regulated. FcαRI expression on monocytes can be enhanced by TNF-α (19, 20), IL-1β, GM-CSF, and bacterial LPS (20), whereas TGF-β1 has been shown to decrease FcαRI expression (21). Monocyte FcγRI expression is up-regulated with IFN-γ and IL-10, and can be down-regulated with IL-4 (8, 9, 22, 23).
Previous studies have demonstrated that treatment of monococyte-derived macrophages (MDM) with M-CSF or IFN-γ differentially regulates FcγR-mediated phagocytosis and lysis of tumor cells (22, 24, 25). These data showed that M-CSF-cultured MDM were proficient in mediating phagocytosis of tumor cells via FcγRII and FcγRIII, whereas cells incubated with IFN-γ were ineffective at mediating phagocytosis of tumor cells via these two low affinity IgG receptors. The reverse has been demonstrated with regard to ADCC. MDM propagated in IFN-γ appear to be more efficient at mediating FcγRII- and FcγRIII-dependent tumor cell lysis than untreated or M-CSF-cultured MDM (22, 24). However, the FcγRI-mediated effector functions of MDM (ADCC and phagocytosis) can be enhanced with IFN-γ (15, 26).

The cytotoxic capacity of the macrophage FcγRI has not been fully explored. Recently, we and Valeriou et al. reported that FcγRII on monocytes, PMN, and macrophages is a potent trigger molecule for ADCC and phagocytosis of tumor cells (6, 7). Because of the role of IgA and FcγRII in mucosal immunity, the potential for this receptor to mediate anti-tumor activity through systemic treatment has been largely overlooked. In this study we report for the first time the ability of MDM to mediate ADCC via the FcγRII. In addition, we have investigated the impact of several immune-modulating cytokines on the ability of MDM to kill tumor cells by phagocytosis or ADCC through FcγRI and FcγRII.

Materials and Methods

Abs and cell lines

FcγRI-specific mAb A77, anti-HER2/neu mAb 520C9, anti-FcγRII (IV.3), anti-CD33 (251), anti-CD14 (AML-2-23), and anti-FcγRII (H22, 32.2) mAbs were purified from culture supernatants by protein A chromatography. The HER2/neu-specific mAb 251.3 was provided by Dr. Paul Guyre, Dartmouth Medical School (Hanover, NH). The SKBR-3 cell line that overexpresses HER2/neu protooncogene was obtained from American Type Culture Collection (Manassas, VA) and cultured in RPMI supplemented with 10% FBS.

Bispecific Abs

The preparation of the BsAb A77 × 520C9 and H22 × 520C9 has previously been described in detail (7, 15). All the BsAb preparations were tested for endotoxin contamination by the chromogenic Limulus amebocyte lysate assay (BioWhittaker, Walkersville, MD) and were found to be free of endotoxin contamination (assay sensitivity limit, 0.1 EU/ml).

Phagocytosis assay

BsAb-mediated phagocytosis of SKBR-3 cells by (MDM) was examined by a modification of the method described by Munn and Cheung (27). Briefly, monocytes, purified from normal adult source leukopaks (Advanced Biotechnologies, Columbia, MD), were differentiated in 24-well plates in macrophage serum-free medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS and either M-CSF (2 ng/ml; R&D Systems, Minneapolis, MN), GM-CSF (10 ng/ml; R&D Systems), or IFN-γ (1000 U/ml; Genzyme, Cambridge, MA) for 5–10 days. The cultures were washed and centrifuged at 15,000 g for 30 min at 4°C, and the supernatants were collected and frozen at −80°C. The lysates were centrifuged at 12,000 g at 4°C for 1 h and the supernatants were adjusted to 1.0 mg/ml protein, then frozen at −80°C.

Confocal imaging

After fixation and flow cytometric analysis of the phagocytosis samples, dual-positive cells were sorted with a FACStarPlus™ flow cytometer (Becton Dickinson, Mountain View, CA) and examined with a Bio-Rad MRC1024 laser scanning confocal microscope (Hercules, CA). Cells were scanned for fluorescence using the 488-nm line from a 15-nW Kr/Ar laser and two photodetectors (522/32-nm dichroic for FITC fluorescence and 585-nm longpass for PKH-26 fluorescence). A ×63 Plan-Apo 1.4 NA objective (Carl Zeiss, Thornwood, NY) was used in conjunction with an iris setting of 2.5, which allowed for detection of optical sections of the fluorescence image that were ~1.5 μm thick. A minimum of 100 cells/sample was examined for quantitative evaluation of phagocytosis.

Tumor cell survival assay

To determine the tumor cell survival following phagocytosis, samples were obtained from the trypsin-harvested cells and plated in 96-well tissue culture plates. Live tumor cells were allowed to adhere overnight in RPMI containing 10% FBS. The medium was removed from each well, and the cells were washed with 0.25% glutaraldehyde. Plates were blocked with 5% BSA solution, then reacted with mAb 251.03, which binds HER2/neu at a different site than 520C9 mAb. The 251.03 mAb was detected with goat anti-mu IgG Fc-specific alkaline phosphatase probe (The Jackson Laboratory, Bar Harbor, ME). The plates were developed with p-nitrophenyl phosphate and read at a wavelength of 405–650 nm. The relative number of SKBR-3 cells was calculated from the formula: % cell survival = ([sample OD − MDM only OD]/SKBR-3 only OD − MDM only OD) × 100%. Samples were analyzed in duplicate.

Ab-dependent cellular cytotoxicity

SKBR-3 cells were cultured as described above and used as targets for lysis by MDM. Targets were labeled with 100 μCi of 51Cr for 1–2 h before combining with effectors cells and BsAb in a U-bottom microtiter plate. After incubation for 16–18 h at 37°C supernatants were collected and analyzed for radioactivity. Cytotoxicity was calculated by the formula: % lysis = (experimental cpm − target leak cpm)/detergent lysis cpm − target leak cpm) × 100%. Specific lysis = % lysis with BsAb − % lysis without BsAb. Assays were performed in triplicate.

FcγR expression

Monocytes were differentiated in 24-well or 150-cm² plates in the presence or the absence of IFN-γ, M-CSF, or GM-CSF as described for the phagocytosis assays. The cells were harvested with trypsin and incubated at 4°C for 60 min with 10 μg/ml of IV.3, 32.2, A77, or 251 mAbs to stain for CD32, CD64, CD89, and CD33, respectively. After incubation with anti-murine IgG-FITC probe, cells were washed and fixed in 1% paraformaldehyde, and their fluorescence was analyzed by FACSscan.

γ Subunit association with FcγRI and FcγRII

Monocytes from three donors were prepared and differentiated into macrophages exactly as described for the phagocytosis experiments, except that they were cultured in 175-cm² tissue culture flasks. Cells were harvested by scraping using cold 0.02% EDTA solution, then were washed with cold PBS. A portion of these cells was analyzed by flow cytometry. Macrophages were adjusted to 10³/ml of lysis buffer (1% digitonin, 20 mM triethanolamine, 150 mM NaCl, 0.12% Triton X-100, 2 mM PMSF, and 0.5 mM trypsin-inhibitory unit/ml aprotonin) and incubated for 1 h on ice. The lysates were centrifuged at 15,000 × g for 30 min at 4°C, and the supernatants were adjusted to 1.0 mg/ml protein, then frozen at −80°C. The lysates were added directly to microtiter wells coated with A77 F(ab)², M22 F(ab)², or 520C9 F(ab)² and incubated overnight at 4°C. After washing, the wells were incubated for 2 h at ambient temperature with a 1/2,000 dilution of γ subunit-specific rabbit serum (donated by Dr. Don Durden, Indiana University School of Medicine, Indianapolis, IN). The assay was developed with an alkaline phosphatase-conjugated goat anti-rabbit IgG probe with p-nitrophenyl phosphate and read at a wavelength of 405–650 nm.

Statistics

Statistical analysis was performed using Student’s t test and calculated by SIGMAPLOT software (Jandel, San Francisco, CA). p < 0.05 was considered significant. All experiments were performed a minimum of three times.

Results

The role of tumor cell phagocytosis by macrophages in vivo remains speculative, yet in vitro studies clearly demonstrate therapeutic potential for this form of anti-tumor activity (24–28). The
Development of stable dyes and new methods has been essential to study this phenomenon in vitro. We have adapted a flow cytometry method, initially described by Munn and Cheung (27), to study tumor cell phagocytosis by MDM FcαRI and FcγRI. This two-color method employs a red lypophilic dye to stain SKBR-3 carcinoma cells, and FITC-conjugated mAbs to label MDM. Phagocytosis can be evaluated by quantitative analysis of the number of single-colored cells (red or green) and the number of dual-colored cells, which represent tumor cells engulfed by MDM. Initial experiments using confocal microscopy were performed to confirm that dual-positive cells represented phagocytosis of target cells and not merely binding of target with effector cells. The BsAb A77×520C9 and H22×520C9, which specifically targeted the HER2/neu-expressing SKBR-3 cells to FcαRI and FcγRI, respectively, were used to mediate phagocytosis. These BsAb contained only the Fab' of the individual mAbs; therefore, binding to FcR was restricted to the specificity of the Ab.

As shown in Table I, ~20–25% of the dual-positive events were E:T cell conjugates at 30 min. However, by 300 min this value was 3% or less, and >90% of dual-positive macrophages contained partial or whole tumor cells. The images in Fig. 1 show conjugates from the 30 min and phagocytosis at the 300 min points.

Phagocytosis was studied after differentiation of MDM in medium supplemented with human recombinant cytokines that have been shown to modulate macrophage function (24, 29, 30). We observed significant FcγRI-mediated phagocytosis (H22×520C9) under all conditions (Fig. 2). M-CSF-treated MDM were consistently the best effector cells for phagocytosis via FcγRI (maximum specific phagocytosis with H22×520C9: M-CSF, 36.8%; GM-CSF, 31.4%; IFN-γ, 23.3%; without cytokine, 24.0%). The level of phagocytosis via the FcαRI (A77×520C9)

### Table I. Analysis of phagocytosis samples by confocal microscopya

| Sample         | Green Cells (%) | Red Cells (%) | Conjugates (%) | Ingested (%) |
|----------------|----------------|--------------|----------------|--------------|
| H22 × 520C9, 30 min | 0.7           | 2.0          | 19.9           | 77.5         |
| H22 × 520C9, 300 min | 1.0           | 2.0          | 3.0            | 94.1         |
| A77 × 520C9, 30 min | 0.0           | 1.5          | 24.2           | 74.2         |
| A77 × 520C9, 300 min | 0.0           | 2.9          | 2.9            | 94.2         |

* MDM cultured with M-CSF were incubated with BsAb (1.0 μg/ml) and SKBR-3 cells for 30 or 300 min. After fixation and flow cytometric analysis of the phagocytosis samples, dual positive cells were sorted, placed onto glass slides, and examined with a laser scanning confocal microscope. Cells were scanned with optical sections of the fluorescence image that were ~1.5 μm thick. A minimum of 100 cells/sample were examined.

![FIGURE 1](image)

**FIGURE 1.** Analysis of phagocytosis via FcαR and FcγRI using confocal microscopy. Confocal microscopy was used to determine the relative proportion of macrophage-tumor cell conjugates to macrophages with internalized tumor cells. Dual-positive cells were sorted after phagocytosis with the two bispecific reagents at 1 μg/ml for 30 or 300 min. The fixed cells were detected in sections ~1.5 μm thick. The images demonstrate conjugate formation, which comprised a significant fraction of the dual-positive cells at the 30 min point, and internalized tumor cells, which represented ~94% of the dual-positive cells after 300-min phagocytosis. See Table I for the exact values.

![FIGURE 2](image)

**FIGURE 2.** Comparison of phagocytosis by MDM treated with different cytokines. Phagocytosis of SKBR-3 cells by FcαRI-directed (A77×520C9) or FcγRI-directed (H22×520C9) BsAb was tested with MDM differentiated in the presence of cytokines. MDM were cultured for 7–10 days in medium without supplemented cytokines (Control) or with M-CSF (2 ng/ml), GM-CSF (10 ng/ml), or IFN-γ (1000 U/ml). The percentage of BsAb-mediated phagocytosis was calculated by subtracting the percentage of phagocytosis in the presence of BsAb from the percentage of phagocytosis in medium. The values represent the mean ± SE from three experiments with different donors. The percentage of phagocytosis without BsAb was 35.2% for controls, 40.6% for IFN-γ, 46.4% for M-CSF, and 38.0% for GM-CSF. ∗, p < 0.05, comparing A77×520C9 vs H22×520C9.
was similar to that observed with FcγRI, except with IFN-γ-treated MDM (maximum specific phagocytosis with A77 × 520C9: M-CSF, 38.7%; GM-CSF, 26.6%; IFN-γ, 9.8%; without cytokine, 21.4%). Using the same IFN-γ-treated MDM, FcγRI-mediated phagocytosis was significantly less than FcγRI-dependent phagocytosis ($p < 0.05$ at 0.1 and 1.0 μg/ml BsAb). Similarly, inhibition of phagocytosis by IFN-γ treatment of MDM has been reported previously for FcγRII and FcγRIII (24, 25).

To further demonstrate the difference in FcγRII- and FcγRI-mediated phagocytosis using IFN-γ-treated MDM, we studied tumor cell survival following phagocytosis. Samples of cells from the phagocytosis assay were taken (just before fixation), washed, and allowed to adhere overnight to microtiter plates in growth medium. The ability of the tumor cells to adhere was the primary requirement for viability, and relative cell survival was assessed by a HER2/neu-specific ELISA. The SKBR-3 cells cultured without MDM served as the 100% survival control, and MDM cultured without SKBR-3 cells was used to determine 0% survival. Fig. 3 illustrates the relative expression of FcγRI and FcγRIII (24, 25).

Our results from the cell survival assay imply greater tumor cell killing (50–60%) than the maximum BsAb-dependent phagocytosis as measured in the flow cytometric assay (35–40%). We postulated that the additional tumor cell killing may be the result of extracellular lysis (ADCC) that occurred during the phagocytosis assay. To test this hypothesis, we examined the ADCC activity of MDM using a $^{51}$Cr release assay (Fig. 4). The FcγRI-dependent lysis of SKBR-3 cells was most efficient using MDM cultured with GM-CSF (maximum specific lysis: A77 × 520C9, 36.2%; H22 × 520C9, 22.0%). Whereas, FcγRII-mediated ADCC was greater with IFN-γ-treated MDM (maximum specific lysis: H22 × 520C9, 34.3%; A77 × 520C9, 14.3%). These differences in FcγRI- and FcγRII-mediated phagocytosis were significant at 0.1 μg/ml BsAb ($p < 0.05$). Essentially no ADCC was observed with MDM not supplemented with cytokines, and MDM cultured in medium containing M-CSF generated relatively low SKBR-3 lysis (maximum specific lysis: A77 × 520C9, 20.0%; H22 × 520C9, 18.0%).

Flow cytometry was used to determine whether changes in FcR expression may account for the differences observed in tumor cytotoxicity by MDM. Previous studies have shown that IFN-γ significantly up-regulated FcγRI on myeloid cells (8–10), while FcγRI levels were relatively unaffected by IFN-γ (20). In contrast, GM-CSF has been shown to increase FcγRI on monocytes (20). Fig. 5 illustrates the relative expression of FcγRI compared with that by MDM cultured without cytokines (averaged from six independent donors). The only statistically significant change was the increase in FcγRI expression with IFN-γ–treated MDM (4.8-fold increase over control, $p < 0.05$). Treatment of MDM with various cytokines did not result in significant differences in FcγRI expression. No effect was demonstrated on FcγRII or CD33 expression, a nontriggering macrophage surface receptor used as a control.

Because FcR surface expression could not account for most of the differences observed in tumoricidal activities of MDM cultured with different cytokines, we hypothesized that the effect may be at the level of association between the signal-transducing γ subunit and FcγRI and FcγRII. To study the level of association between the γ subunit and FcγRI and FcγRII, lysates were prepared from cytokine-differentiated MDM and were analyzed by ELISA. These assays were performed by capturing Fcγ chain complexes with F(ab')$_2$ of Fcγ-specific mAbs or an isotype control, followed by detection of γ subunit with γ-specific rabbit serum (Fig. 6). As expected, the results from three independent MDM preparations...
showed a significant enhancement of total FcγRI γ-chain complexes in IFN-γ-treated MDM. Interestingly, GM-CSF-cultured MDM also had elevated FcγRI γ-chain complexes compared with MDM cultured without exogenous cytokine or with M-CSF (p < 0.05). No significant differences were observed for FcγRIII complexes, although MDM cultured in GM-CSF and IFN-γ had somewhat higher values than control MDM or M-CSF-treated MDM. There was no reactivity when lysates were added to plates coated with irrelevant F(ab′)2 molecules (maximum OD$_{605}$ was <0.06 in all cases). These data imply that most of the differential activities of cytokine cultured MDM are not manifested by changes in total FcγR γ-subunit complex formation. However, the effect of IFN-γ on enhanced FcγRI expression and FcγRII subunit complexes may play a significant role in the FcγRI-mediated tumoricidal activity of IFN-γ-treated MDM.

FIGURE 6. The association of γ-chain with FcγRI and FcγRI in cytokine-treated MDM. Lysates prepared from MDM cultured with cytokines for 5–7 days were incubated at specified concentrations in microtiter wells coated with F(ab′)$_2$ of anti-FcγRI (M22) or anti-FcγRII (A77). The FcγR-associated γ subunit was detected with a γ subunit-specific rabbit serum and alkaline phosphatase-conjugated anti-rabbit IgG probe. The data represent the mean ± SE of lysates processed from three independent MDM preparations for each cytokine condition. *, p < 0.05 comparing γ subunit-FcγR association under different cytokine conditions to that in control cultures.

Discussion

In this study we investigated two cytotoxic mechanisms, phagocytosis and ADCC, mediated by FcγRI and FcγRI on macrophages derived from monocytes in the presence of M-CSF, IFN-γ, or GM-CSF. Although the cytotoxic effector functions of FcγRI have been well studied, relatively little is known regarding the ability of FcγRI to mediate tumor cell killing. To study the activities of individual FcRs, we used chemically linked Fab′ × Fab′ BsAb, which excluded the possibility of interactions with other FcR through Abs. Specifically, we used BsAb A77 × 520C9 and H22 × 520C9, which targeted the HER2/neu-expressing SKBR-3 tumor cells to FcγRI and FcγRII, respectively. These BsAb have been previously described (7, 15), and H22 × 520C9 is currently being investigated in clinical trials for treatment of HER2/neu-expressing malignancies (31, 32). We found significant differences in tumor cell cytotoxicity that were dependent on which FcR was targeted as well as the presence of specific cytokines during the differentiation of monocytes into MDM.

The most striking finding in this study was the lack of significant ADCC or phagocytosis of SKBR-3 cells by the BsAb targeted to the FcγRI when MDM cultured in the presence of IFN-γ were used as effector cells (maximum FcγRI activity, 10% phagocytosis and 14% ADCC). In contrast, the IFN-γ-treated MDM mediated both phagocytosis and ADCC through FcγRI (maximum FcγRI activity, 23% phagocytosis and 34% ADCC). Decreased phagocytic function with IFN-γ-treated MDM have previously been reported with anti-tumor Abs that mediate their activity via FcγRII and FcγRII (22, 24, 25). Interestingly, our results and those reported by Ely et al., which demonstrate enhanced phagocytosis with IFN-γ-treated MDM (26), suggest that the FcγRI is unique from other FcRs. This observation is intriguing, because both FcγRI and FcγRII appear to require association with the FcγR subunit to mediate intracellular signaling (17, 18).

The difference between FcγRI and FcγRII activity using IFN-γ-derived MDM was most pronounced when we examined tumor cell survival following coculture of MDM with tumor cells. Addition of the FcγRI-targeted BsAb to these cultures resulted in 50–60% reduction in SKBR-3 cells, whereas no significant loss of tumor cells was noted (<10%) with the FcγRII BsAb. These results correlated well with the ability of FcγRI on IFN-γ-treated MDM to mediate both phagocytosis and ADCC and the lack of such activity for FcγRII.

We speculated that the variation in FcγRI and FcγRII-mediated activity of cytokine-cultured MDM may have been the result of changes in their surface expression or the ability of γ-chain to functionally associate with the FcR. Recent studies by Launay et al. (33), which demonstrated the presence of FcγRI with and without γ-chain on the surface of monocytes and neutrophils, implicated that receptor activity may be regulated by the magnitude of γ-chain association. However, when we examined the effects of cytokines on MDM FcγR surface expression or total levels of FcγR subunit complexes, we did not find a complete correlation with these parameters and the observed effects of cytokines on tumoricidal function via FcγRI and FcγRII. Consistent with previous reports, IFN-γ treatment of MDM resulted in increased levels of surface FcγRI and FcγRII subunit complexes (8–10). These effects probably contributed to FcγRI-mediated phagocytosis and ADCC with IFN-γ-treated MDM. However, FcγRII-mediated phagocytosis was greater when MDM were generated in the presence of M-CSF and GM-CSF compared to lower FcγRII expression and lower total FcγRI subunit complexes. The fact that FcγRII-mediated phagocytosis with GM-CSF- or M-CSF-cultured MDM was greater than phagocytosis with IFN-γ-treated MDM, supports the
previous finding that IFN-γ treatment reduces the overall phagocytic capacity of macrophages (22, 24, 25). In contrast to FcγRI, no significant change in the total level of surface FcγRI or FcγRIγ subunit complexes were noted among MDM cultured under the influence of different cytokines. Therefore, these parameters were unable to explain the enhanced FcγRI-mediated ADCC with GM-CSF-treated MDM or the low tumoricidal activity of FcγRI using IFN-γ-treated MDM. These data suggest that other molecules and interactions probably contribute significantly to the differential effect of cytokotmes on FcγRI- and FcγRIγ-mediated tumoricidal activity of MDM.

Although IFN-γ treatment has been shown to promote ADCC activity, we found that this did not apply for FcγRI on MDM. We observed <15% FcγRI-dependent ADCC under these conditions. This result was surprising considering our recent findings that demonstrated FcγRI as a potential mediator of ADCC when fresh (untreated) monocytes or PMN were used as effector cells (7). On the other hand, MDM generated in the presence of GM-CSF were efficient at FcγRI-mediated ADCC. In fact, MDM cultured with GM-CSF mediated greater ADCC activity via FcγRI than via FcγRI (p < 0.05), although there was no significant up-regulation of FcγRI on MDM cultured with GM-CSF. Therefore, the positive or negative effects on FcγRI activity induced by cytokine treatment of MDM appear to work downstream from receptor binding. These results further imply that FcγRI and FcγRIγ (and probably other FcγRI) interact with different molecules (in addition to the γ subunit), which uniquely regulate their function.

FcγRI and FcγRIγ both mediated efficient phagocytosis of SKBR-3 cells when MDM were cultured with M-CSF or GM-CSF. Ab-dependent phagocytosis was also demonstrated when MDM were cultured without exogenous cytokines. On the other hand, neither BsAb mediated efficient lysis of the tumor cells with untreated MDM or M-CSF-cultured MDM. The fact that MDM cultured without cytokines were unable to mediate significant ADCC is intriguing, because monocytes (before differentiation into MDM) have been shown to mediate efficient ADCC via both FcγRI and FcγRIγ (7, 15). Previous studies have demonstrated that monocytes rapidly lose ADCC function in culture (30, 34). Therefore, the lack of MDM ADCC activity without addition of cytokines does not imply that macrophages in vivo require cytokines for this function. The data we have presented demonstrate that MDM cultured under the influence of different cytokines result in effector cells with varying capacity to mediate cytotoxic function via FcγRI and FcγRIγ. These results suggest that Ab-dependent cytotoxic activity of human macrophages is dependent on the FcγRI that can be engaged by the anti-tumor Ab and the physical state of the macrophages. Further, the data imply that the use of specific cytokines to regulate macrophage function may enhance or reduce specific cytotoxic mechanisms in a clinical setting.

This study has focused on macrophages; however, in the use of cytokines in combination with Ab treatment for tumor therapy, other effector cell populations that may be recruited for ADCC and phagocytosis need to be considered. For example, the effects of G-CSF and IFN-γ (in vitro and in vivo) on up-regulation of FcγRI levels and cytotoxic activity on granulocytes are well documented (8–10). On the other hand, granulocytes constitutively express FcγRI, which mediates potent ADCC without addition of exogenous cytokines (6, 7). Further, because PMN constitute an abundant effector cell population, they may play an important role in FcγRI-mediated immunotherapy. Having a collective understanding of the important effector cell populations and their different cytotoxic mechanisms will allow for greater exploitation of immunotherapy. Further studies that help optimize the effector functions of FcγRI in the course of immunotherapy will probably lead to more successful protocols for treatment of human cancers.

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