

Absolute Requirement of CD11/CD18 Adhesion Molecules, FcRII, and the Phosphatidylinositol-Linked FcRIII for Monoclonal Antibody-Mediated Neutrophil Antihuman Tumor Cytotoxicity

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We have previously shown that 3F8, a murine IgG₃ monoclonal antibody (MoAb) specific for the ganglioside G_{D2}, mediates tumor cell kill in vitro and in vivo. We now describe receptor requirements of polymorphonuclear leukocytes (PMN) in 3F8-mediated cytotoxicity (ADCC) of human G_{D2}(+) melanoma and neuroblastoma cell lines. PMN from a child with leukocyte adhesion deficiency (LAD) were devoid of CD11/CD18 adhesion molecules and mounted no detectable ADCC. MoAb to CD11b, CD11c, and CD18 each efficiently blocked ADCC by normal PMN. In contrast, a panel of different MoAbs to CD11a had no significant inhibitory effect on ADCC, a finding consistent with the low-to-absent expression of the CD11a ligand, intercellular adhesion molecule-1, on the target cells. Granulocyte-macrophage colony-stimulating factor (GM-CSF) significantly increased the expression of CD11b, CD11c, and CD18 on normal PMN, decreased the expression of Fc receptors (FcR), and enhanced ADCC by normal but not by LAD PMN. MoAbs to FcRII and FcRIII each

efficiently blocked ADCC; anti-FcRI MoAb had no effect. Flow cytometry using anti-FcRII MoAb versus anti-FcRIII MoAb did not show cross competition, suggesting that inhibition of ADCC was not a steric effect resulting from FcRII proximity to FcRIII. PMN deficient in FcRIII (obtained from patients with paroxysmal nocturnal hemoglobinuria) and PMN depleted of FcRIII by treatment with elastase or phosphatidylinositol (PI)-specific phospholipase C produced low ADCC, supporting a role for the PI-linked FcRIII. Thus, optimal ADCC using human PMN, human solid tumor cells, and a clinically active MoAb (conditions that contrast with the heterologous antibodies and nonhuman or nonneoplastic targets used in most models of PMN ADCC) required CD11b, CD11c, FcRII, and the PI-linked FcRIII. Furthermore, in this clinically relevant system, GM-CSF enhancement of antitumor PMN ADCC correlated with increased expression of CD11/CD18 molecules. © 1992 by The American Society of Hematology.

3F8 IS A MURINE IgG₃ monoclonal antibody (MoAb) that is well-suited for targeted immunotherapy of cancer because (1) its ganglioside G_{D2} target antigen is highly restricted to neuroectodermal tissues and is genetically stable^{1,2}; (2) it has excellent tumor localization in patients³; and (3) in vitro, it mediates human tumor cell destruction by human complement and by human lymphocytes, cultured monocytes, and polymorphonuclear leukocytes (PMN).^{4,7} This constellation of features, which may account for regressions of G_{D2}(+) tumors in patients treated with 3F8,⁸ has been documented in very few MoAbs. For example, most studies of antibody-dependent cellular cytotoxicity (ADCC) use mononuclear cells as effectors, in part because few MoAbs (clinically applicable or otherwise) are known to mediate ADCC of human tumor cells by PMN.^{6,9-11} Yet, in vivo, PMN are the predominant circulating leukocyte and are activated and increased in number by cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF).^{12,13} Studies showing

PMN ADCC of human tumor cells mediated by heterologous antibodies are also few in number.¹⁴⁻¹⁸ Other studies of antitumor PMN ADCC have used heterologous antibodies and nonhuman, usually lymphohematopoietic, targets.¹⁹⁻²³ The cytotoxic mechanisms in these model systems may differ from those operative in PMN ADCC of human solid tumor cells that not only are large in size, and hence may not be readily phagocytosed by PMN, but are also relatively resistant to reactive oxygen species.^{24,25}

The current study focused on receptor requirements of 3F8-mediated PMN ADCC of G_{D2}(+) human melanoma and neuroblastoma cells. Although CD11/CD18 molecules mediate a wide range of adhesion-dependent activities of PMN,²⁶ no report to date has described the involvement of this complex in PMN ADCC of human tumor cells. The same holds for Fc receptors (FcR), despite the well-established necessity of FcR in ADCC. Of the three reported types of FcR, the low-affinity FcRII and FcRIII predominate on resting PMN.²⁷ While FcRII has a well-documented role in many PMN activities, the importance of the more abundant FcRIII, including in ADCC, is uncertain, given its linkage to PMN via a phosphatidylinositol (PI) moiety.²⁸ This form of linkage, in contrast to the polypeptide anchor of FcRII, lacks a transmembrane connection to the cytoplasm and may limit the capacity of FcRIII to transmit intracellular signals for PMN function. We now demonstrate a critical role in a clinically relevant model of PMN ADCC, not only for FcRII and all subunits of the CD11/CD18 complex, except CD11a, but also for the PI-linked FcRIII.

MATERIALS AND METHODS

MoAbs. Purified 3F8 (anti-G_{D2}; IgG₃) was prepared in our laboratory as described,² and was used at a final concentration of 2 µg/ml, unless otherwise stated. AI10 (anti-decay accelerating factor [DAF])²⁹ was the gift of Dr M.E. Medof (Case Western Reserve University, Cleveland, OH). 197 (anti-FcRI; IgG_{2a})³⁰ prepared by hollow-fiber tubing, was the gift of Dr M.W. Fanger

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(Dartmouth Medical Center, Hanover, NH). Purified R3.1 (anti-CD11a; IgG₁),³¹ R15.7 (anti-CD18; IgG₁),³¹ and R1.1 (anti-intercellular adhesion molecule [ICAM]-1)³² were gifts of Dr R. Rothlein (Boehringer Ingelheim Pharmaceuticals, Inc, Ridgefield, CT). Mo1 (anti-CD11b; IgG_{2a}),³³ as ascites, was the gift of Dr R.F. Todd (University of Michigan, Ann Arbor, MI). Purified IB4 (anti-CD18; IgG_{2a})³⁴ was the gift of Dr S.D. Wright (Rockefeller University, New York, NY). Purified LeuM5 (anti-CD11c; IgG_{2b})³⁵ was obtained from Becton Dickinson Laboratories (Mountain View, CA) and 3.9 (anti-CD11c; IgG₁),³⁶ as ascites, was obtained from Bioproducts for Science (Indianapolis, IN). G25.2 (IgG₁)³⁷ and 2F12 (IgG₁)³⁸ (both anti-CD11a, both ascites) were gifts, respectively, of Drs A.M. Krensky (Stanford University, Palo Alto, CA) and J. Ritz (Dana-Farber Cancer Center, Boston, MA). Purified OKM-1 (IgG_{2b})³⁹ and OKM-10 (IgG_{2b})³⁹ (both anti-CD11b) were gifts of Dr P. Rao (Ortho Diagnostics, Raritan, NJ). LM2/1 (anti-CD11b; IgG₁)⁴⁰ and TS1/18 (anti-CD18; IgG₁)⁴¹ were gifts of Dr T. Springer (Harvard University, Boston, MA). The following were obtained as hybridomas from the American Type Culture Collection (ATCC; Rockville, MD): IV.3 (anti-FcRII; IgG_{2b}),⁴² TS1/22 (anti-CD11a; IgG₁),⁴¹ and BBM.1 (anti-β₂-microglobulin; IgG_{2b}).⁴³ 3G8 (anti-FcRIII; IgG₁)⁴⁴ was kindly provided as a hybridoma by Dr J. Unkeless (Mt Sinai Medical School, New York, NY). Hybridomas were grown as ascites in BALB/c mice, and partially purified by precipitation in 45% ammonium sulfate at 0°C, followed by dialysis against phosphate-buffered saline.

Cell lines. LAN-1 and IMR-6 (both neuroblastoma) were gifts, respectively, of Dr R. Seeger (University of Southern California, Los Angeles, CA) and Dr R.K. Liao (McMaster University, Ontario, Canada). SKMel-1 (melanoma) was obtained from the ATCC. Cell lines were propagated in RPMI 1640 (GIBCO Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated calf serum (HyClone Laboratories, Logan, UT), 2 mmol/L glutamine, 100 U/mL penicillin G, and 100 μg/L streptomycin, but ⁵¹Cr release assays were performed using RPMI 1640 supplemented with 0.5% human serum albumin.

Effector cells. PMN were isolated from heparinized peripheral blood by Ficoll-Paque gradients (Pharmacia Fine Chemicals, Piscataway, NJ), followed by 3% dextran separation and hypotonic red blood cell lysis. PMN comprised more than 95% of the resulting cell population by Wright-Giemsa stain. Normal PMN were obtained from healthy laboratory personnel. PMN were also obtained from a 1-year-old girl diagnosed with leukocyte adhesion deficiency (LAD) by Dr Paul J. Leibson of the Mayo Clinic, and referred to Memorial Sloan-Kettering Cancer Center for a bone marrow transplantation procedure to correct life-threatening leukocyte abnormalities. PMN from two patients with paroxysmal nocturnal hemoglobinuria (PNH), diagnosed by Drs Hugo Castro-Malaspina, Lillian Reich, and Wendell F. Rosse, were also used in selected assays.

Reagents. PI-specific phospholipase C (PI-PLC; from culture supernatants of *Bacillus subtilis* transfected with the PI-PLC gene from *B. thuringiensis*) was generously provided by Dr Martin Low (Columbia University, New York, NY). Elastase was from Elastin Products Co (Owensville, MI). Recombinant human GM-CSF was a gift from Genetics Institute (Cambridge, MA).

Chromium release assay. Target tumor cells were labeled with sodium ⁵¹Cr chromate (Amersham, Arlington Heights, IL) at 100 μCi/10⁶ cells at 37°C for 1 hour. After the cells were washed, loosely bound ⁵¹Cr was leaked for 1 hour at 37°C. After further washing, 5 × 10³ target cells/well were admixed with effector cells, MoAb, and/or GM-CSF in 96-well polystyrene round-bottom plates (Sarstedt, Germany) to a final volume of 250 μL/well. The

plates were incubated at 37°C for 4 hours and then centrifuged at 400g for 5 minutes; the released ⁵¹Cr in supernatant was counted in a γ-counter (Packard Instrument, Downers Grove, IL). Percentage of specific release was calculated using the formula 100% × (experimental cpm – background cpm)/(5% sodium dodecyl sulfate [SDS] cpm – background cpm), where cpm are counts per minute of ⁵¹Cr released. Total release was assessed by lysis with 5% SDS (Sigma, St Louis, MO), and background release was measured in the absence of granulocytes. The background was 10% to 25% for LAN-1 and IMR-6 and 7% to 16% for SKMel-1.

Immunofluorescence and flow cytometry. Purified PMN were incubated for 20 minutes at 4°C with saturating concentrations of test or control MoAb, washed, reacted with a 1:25 dilution of 1 mg/mL of fluorescein-labeled goat antimouse (IgG and IgM) antibody (Tago, Burlingame, CA) for 20 minutes at 4°C, then washed again, fixed in 0.5% paraformaldehyde, and analyzed on a FACScan (Becton Dickinson).

Statistical analysis. When assessing changes in PMN phenotypes after treatment with enzymes or with GM-CSF, parallel control studies showed standard deviations (SD) from the mean of ±2% to ±10% for all antigens, except FcRII, for which the SD from the mean was ±12% to ±15%. *P* values were derived using a Student's *t*-test for paired differences to compare untreated versus treated aliquots of PMN. Examination of the data showed no extreme values that would positively influence this statistic.

RESULTS

Requirement for CD11/CD18 adhesion molecules. Experiments reported elsewhere²⁵ showing no lysis of “innocent bystander” G_{D2}(-) tumor cell targets suggested a requirement for physical attachment between effector and target cells in 3F8-mediated PMN ADCC, which is a nonphagocytic process (Munn D.H., Kushner B.H., unpublished observations, April 1990). In the current study, results using LAD PMN established the importance of CD11/CD18 molecules in this system. LAD PMN, which were confirmed by immunophenotyping to be devoid of the entire CD11/CD18 complex, mounted no detectable ADCC (Table 1). The addition of GM-CSF did not alter this result, even though in parallel assays GM-CSF enhanced ADCC by normal PMN, as previously observed,⁶ and increased the expression of CD11/CD18 on normal PMN (see below).

To identify which CD11/CD18 subunits were required for 3F8-mediated PMN ADCC, subunit-specific MoAbs were added to the ADCC assay (Fig 1). MoAbs to CD11b (LM2/1, Mo1), CD11c (LeuM5, 3.9), and CD18 (R15.7, IB4, TS1/18) each produced efficient concentration-

Table 1. LAD Versus Normal PMN in 3F8-Mediated ADCC

Target	PMN	E:T	3F8 (μg/mL)	% Specific Lysis	
				No GM-CSF	+GM-CSF (2 ng/mL)
LAN-1	Normal	25:1	2	52	72
	LAD	25:1	2	0	0
	LAD	100:1	10	0	0
SKMel-1	Normal	100:1	2	70	ND
	LAD	100:1	2	1	1
	LAD	100:1	10	2	1

These results are representative of three similar studies. Abbreviation: ND, not done.

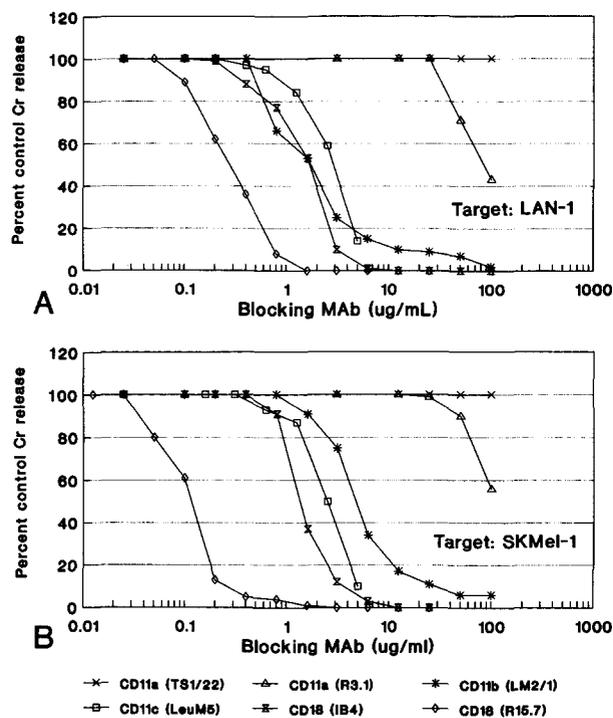


Fig 1. Effects of anti-CD11/CD18 MoAbs on 3F8 (2 $\mu\text{g}/\text{mL}$)-mediated PMN ADCC against (A) neuroblastoma (LAN-1) and (B) melanoma (SKMel-1). MoAbs were added at the initiation of the ADCC assay. The data are from one of six similar experiments. Effects of the anti-CD11a MoAbs 2F12 and G25.2, the anti-CD11b MoAb Mo1, the anti-CD11c MoAb 3.9, and the anti-CD18 MoAb TS1/18 were similar to those shown with TS1/22, LM2/1, LeuM5, and IB4, respectively.

dependent inhibition of ADCC, with complete or near complete abrogation of ADCC at 5 to 10 $\mu\text{g}/\text{mL}$. In contrast, three different anti-CD11a MoAbs (TS1/22, 2F12, G25.2) in concentrations up to 100 $\mu\text{g}/\text{mL}$ had no effect on ADCC. A fourth anti-CD11a MoAb (R3.1) was inhibitory only at concentrations ≥ 25 to 50 $\mu\text{g}/\text{mL}$ when SKMel-1 was the target, and at concentrations ≥ 50 $\mu\text{g}/\text{mL}$ when LAN-1 was the target. This lack of effect of anti-CD11a MoAb may be explained by the low expression on melanoma of ICAM-1,⁴⁵ which is a ligand for CD11a,²⁶ and the

absence of ICAM-1 on LAN-1, which was previously reported⁴⁶ and which we documented using flow cytometry and the anti-ICAM-1 MoAb R1.1 (not shown).

We found variability in the ADCC inhibitory efficiency of different anti-CD11/CD18 MoAb, a phenomenon described with non-ADCC adherence functions of PMN.⁴⁷ Among the anti-CD11/CD18 MoAbs assayed in our system, the anti-CD18 MoAbs were the most efficient inhibitors (R15.7 more than IB4 and TS1/18; Fig 1), and the anti-CD11b MoAbs LM2/1 and Mo1 were more inhibitory than OKM-1 and OKM-10 (not shown). Thus, OKM-1 in concentrations up to 100 $\mu\text{g}/\text{mL}$ produced less than 20% inhibition of ADCC against LAN-1, and inhibited ADCC against SKMel-1 by 50% or more only when present at 12.5 $\mu\text{g}/\text{mL}$ or greater. In comparison to LM2/1 or Mo1, OKM-10 was less inhibitory when LAN-1 was the target, but equally inhibitory when SKMel-1 was the target. The results with OKM-1 and, to a lesser degree, with OKM-10 suggested that nonspecific steric effects did not account for the sensitivity of ADCC to the anti-CD11/CD18 MoAbs. This point was reinforced by the normal ADCC that occurred in the presence of the anti- β -2-microglobulin MoAb BBM.1 in concentrations up to 100 $\mu\text{g}/\text{mL}$ (not shown).

FcR requirements. Blocking MoAbs were used to identify the roles of the different FcR in 3F8-mediated PMN ADCC (Fig 2). The anti-FcRI MoAb 197 in concentrations up to 100 $\mu\text{g}/\text{mL}$ had no effect on ADCC. This result was not unexpected because FcRI is present at very low density on PMN.²⁷ In contrast, the anti-FcRII MoAb IV.3 and the anti-FcRIII MoAb 3G8 each completely abrogated ADCC at 1 $\mu\text{g}/\text{mL}$ or less. Flow cytometry studies with anti-FcRII MoAb versus anti-FcRIII MoAb did not show cross competition (not shown), confirming prior reports of individual anti-FcR MoAb specificity,^{40,48,49} and suggesting that steric factors could not account for the respective ADCC blocking effects of IV.3 and 3G8. Thus, both FcRII and FcRIII appeared to be required for successful ADCC.

Because of evidence that the PI-linked FcRIII of PMN may not be critical for PMN function, including tumor cell kill,⁵⁰⁻⁵² studies were performed to assess directly the require-

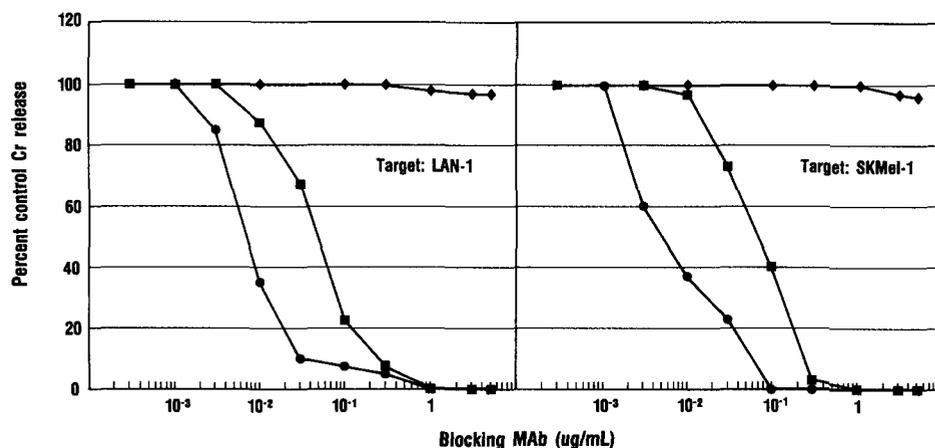


Fig 2. Effects of anti-FcR MoAb on 3F8 (2 $\mu\text{g}/\text{mL}$)-mediated PMN ADCC against $\text{G}_{b2}(+)$ neuroblastoma (LAN-1) and melanoma (SKMel-1). MoAbs were added at the initiation of the ADCC assay. The data are the average of four experiments (standard deviations $\pm 10\%$ or less). (\blacklozenge) 197 (anti-FcRI); (\blacksquare) IV.3 (anti-FcRII); (\bullet) 3G8 (anti-FcRIII).

ment for FcRIII in 3F8-mediated PMN ADCC. Enzyme treatment significantly depleted PMN of FcRIII ($P = .044$), with little effect on the expression of non-PI-linked surface molecules (Table 2). In comparison to normal PMN, the FcRIII-deficient PMN performed poor ADCC in the presence of 3F8 (Table 2). These findings favored an FcRIII requirement for optimal lysis in this system.

Additional support for this conclusion came from studies using PMN from adults with PNH (Table 3). Immunophenotyping confirmed that these PMN, as reported,²⁸ had very low expression of PI-linked surface molecules, including FcRIII and DAF. Similar to findings with PMN enzymatically depleted of FcRIII, PNH PMN were ineffective in ADCC as compared with normal PMN (Table 3).

Effects of GM-CSF. To gain further insight into the roles of FcR and the CD11/CD18 complex in 3F8-mediated PMN ADCC, studies were performed using GM-CSF. This cytokine enhances PMN ADCC^{8,22,23} and has obvious potential clinical relevance. GM-CSF significantly increased CD11b ($P < .001$), CD11c ($P = .007$), and CD18 ($P = .004$) expression and, as expected, enhanced 3F8-mediated PMN ADCC (Table 4). GM-CSF produced a less than significant decrease in both FcRII and FcRIII expression, but did not appreciably change the density of other PMN surface molecules. The inclusion of GM-CSF in the ADCC assay did not alter the relative inhibitory effects of the anti-CD11/CD18 MoAbs, although higher concentrations of these MoAbs were required to inhibit ADCC (not shown). Thus, while anti-CD11a MoAbs had no effect on ADCC by GM-CSF-stimulated PMN, cytotoxicity curves (such as those in Fig 1) using MoAbs to CD11b, CD11c, and CD18 were shifted to the right in the presence of GM-CSF. These results were consistent with the possibility that GM-CSF enhances 3F8-mediated PMN ADCC at least partly via upregulation of CD11b, CD11c, and CD18.

Table 2. Enzyme-Treated PMN: Phenotype and ADCC

	Untreated PMN	Elastase-Treated PMN	PI-PLC-Treated PMN
Phenotype (% cells positive/mean fluorescence)			
MoAb			
IgG ₁ (control)	0.3/7.05	0.9/7.54	1.0/7.98
TS1/22 (CD11a)	99.3/86	99.7/85	99.5/79
OKM-1 (CD11b)	99.7/639	99.7/554	99.7/647
LeuM5 (CD11c)	98.7/64	97.0/55	98.8/66
TS1/18 (CD18)	99.7/193	99.6/199	99.6/188
Al10 (DAF)	76.9/39	54.8/35	13.2/27
IV.3 (FcRII)	99.4/185	99.1/157	99.3/177
3G8 (FcRIII)	97.9/1,038	98.8/246	98.1/241
ADCC (% specific lysis)			
Target			
IMR-6	72	48	28
LAN-1	56	24	16
SKMel-1	37	16	6

PMN were incubated for 1 hour in medium alone, medium plus elastase (10 μ g/mL),⁴⁹ or medium plus PI-PLC (1 U/mL), and were then immunophenotyped and tested in ADCC. Effector:target ratio, 100:1. The data are representative of three experiments.

Table 3. PNH PMN: Phenotype and ADCC

	E:T	Normal PMN	PNH PMN
Phenotype (% positive/mean fluorescence)			
MoAb			
IgG ₁ (control)		2.0/5.7	2.1/5.4
Al10 (DAF)		90.2/25	2.9/5.8
197 (FcRI)		3.8/7	10.3/8.3
IV.3 (FcRII)		99.9/101	99.8/89
3G8 (FcRIII)		99.6/683	98.8/36
ADCC (% specific lysis)			
Target			
IMR-6	100	50	26
	50	32	1
LAN-1	100	50	16
	50	26	1
SKMel-1	100	43	11
	50	25	3

PMN from two adults with PNH were studied in parallel experiments with normal PMN. Representative results are shown here.

Abbreviation: E:T, effector:target ratio.

DISCUSSION

We have defined PMN surface receptors critical for PMN ADCC of human solid tumor cells. In the presence of 3F8, an MoAb that has antitumor activity in patients,⁸ ADCC is dependent on a physical interaction between PMN and G_{D2}(+) human neuroectodermal cells that does not involve phagocytosis but requires most subunits of the CD11/CD18 complex, FcRII, and the PI-linked FcRIII. Several of these features differ from those associated with PMN ADCC in model systems using heterologous antibodies and nonhuman or nonneoplastic target cells.

Table 4. GM-CSF Effects on Phenotype and ADCC

	E:T	Untreated PMN	GM-CSF-Treated PMN
Phenotype (% positive/mean fluorescence)			
MoAb			
IgG ₁ (control)		2.8/7.44	1.6/7.2
TS1/22 (CD11a)		99.5/66	99.8/72
OKM-1 (CD11b)		100/436	100/725
LeuM5 (CD11c)		97.9/49	99.7/78
TS1/18 (CD18)		99.9/132	99.6/223
Al10 (DAF)		99.6/45	99.9/56
197 (FcRI)		13.2/7.8	6.1/9.5
IV.3 (FcRII)		99.8/144	99.1/102
3G8 (FcRIII)		98.9/397	99.1/301
ADCC (% specific lysis)			
Targets			
IMR-6	50	30	54
LAN-1	100	49	79
SKMel-1	100	34	50

PMN were incubated for 2 hours in medium with or without GM-CSF (2 ng/mL), washed, and immunophenotyped or tested in ADCC. In this representative study, GM-CSF upregulated CD11b, CD11c, and CD18 and enhanced 3F8-mediated PMN ADCC.

Abbreviation: E:T, effector:target ratio.

The availability of LAD PMN allowed us to establish the importance of the CD11/CD18 complex in 3F8-mediated PMN ADCC. Inhibition studies using specific MoAbs showed that all subunits of the complex, except CD11a, were critical for normal PMN cytotoxicity with 3F8. Given the results with LAD PMN, the abrogation of 3F8-mediated PMN ADCC produced by MoAbs to CD18, the β subunit common to all three CD11 α subunits, might have been predicted. The same holds for the inhibitory effects of MoAb blockade of CD11b, which is the most abundant of the CD11 α subunits on PMN and has well-established myeloid cell adhesive functions.²⁶ The efficient inhibition produced by MoAbs to CD11c was less expected in view of the relatively low density of CD11c on PMN. Recent studies, however, suggest that CD11c also can function independently as an adhesion molecule.⁵³ Our results suggest that either (1) PMN-target cell attachment via CD11b or CD11c is necessary but not sufficient for 3F8-mediated ADCC to proceed, or (2) in our system, when CD11b or CD11c is blocked, the other becomes nonfunctional. A redundancy of function among the CD11/CD18 molecules has previously been noted, and is supported, for example, by the relative structural homology between CD11b and CD11c.²⁶

No prior report has described the involvement of CD11/CD18 molecules in PMN ADCC of human solid tumor cells (as mediated by MoAbs or by heterologous antibodies). The sole previously reported study of ADCC by LAD PMN targeted herpes simplex virus-infected Chang liver cells.⁵⁴ Findings in that study differed from our own in that defective ADCC was attributed to the absence of CD11a and CD11b, because anti-CD11a (TS1/22) MoAb alone or the combination of anti-CD11b (OKM-1) plus anti-CD18 (TS1/18) MoAbs blocked ADCC by normal PMN (anti-CD11b or anti-CD18 MoAb alone produced insignificant inhibition). The lack of effect of anti-CD11a MoAb on 3F8-mediated PMN ADCC is consistent with the low-to-absent expression of the CD11a ligand ICAM-1 on the melanoma and neuroblastoma targets. The known counter-receptors for CD11b and CD11c include iC3b, fibrinogen, factor X, as well as ICAM-1²⁶; identification of the ligands for CD11b and CD11c in our system awaits further studies.

Our results with GM-CSF are consistent with an important role for CD11/CD18 in 3F8-mediated PMN ADCC, because GM-CSF enhancement of the latter occurred in conjunction with increased expression of CD11b, CD11c, and CD18. Although GM-CSF can affect the respiratory burst of PMN,^{55,56} two lines of evidence suggested to us that GM-CSF may act via CD11/CD18 in our system: (1) We had previously found that GM-CSF enhances 3F8-mediated ADCC by PMN with defective oxidative metabolism (obtained from patients with chronic granulomatous disease).²⁵ (2) The time-course of GM-CSF enhancement of PMN ADCC that we⁶ and others¹⁸ had observed was more consistent with its rapid upregulation of PMN CD11/CD18,^{22,57,58} than with the more delayed onset of its activation of PMN oxidative metabolism.^{55,56}

In addition to its effects on the CD11/CD18 complex, GM-CSF decreased PMN expression of FcR2 and FcR3,

a previously reported phenomenon.^{51,58,59} Our studies cannot rule out the possibility that GM-CSF enhances 3F8-mediated PMN ADCC by non-CD11/CD18 effects, such as increasing the affinity of FcR or mobilizing nonoxidative cytolytic capacities.

Studies of the role of FcR in 3F8-mediated PMN ADCC underscored the importance of physical PMN-target attachment. Thus, anti-FcR2 and anti-FcR3 MoAbs each efficiently inhibited ADCC. Furthermore, PMN enzymatically depleted of FcR3 and PNH PMN, which have low FcR3 expression, were ineffective in ADCC as compared with normal PMN. The results suggest that, as with CD11b and CD11c, the separate involvement of each low-affinity FcR is necessary but not sufficient for optimal 3F8-mediated PMN ADCC. The current report is the first to document a requirement for both FcR2 and FcR3 in PMN ADCC against human (or nonhuman) tumor or nonneoplastic targets. Our findings are noteworthy because, although Fc-FcR interactions are the well-established basis of ADCC, the issue has been clouded by the discovery²⁸ that FcR3 is anchored to the plasma membrane of PMN by a PI moiety and may therefore have a limited capacity for signal transduction (see below).

A requirement for the PI-linked FcR3 in PMN ADCC is evident in some but not all model systems. Thus, PMN lyse murine hybridoma cells bearing antibody directed against FcR2, but not those bearing antibody against FcR3.⁵⁰⁻⁵² In contrast, PMN lyse chick erythrocytes coated with antibody directed against FcR2 or FcR3⁵¹; heteroaggregates of anti-FcR3 and anti-2,4-dinitrophenyl antibodies mediate PMN destruction of 2,4,6-trinitrophenyl-modified RDM4 murine leukemia cells⁶⁰; and salivary PMN, which have very low expression of FcR3, are ineffective in ADCC of herpes simplex virus-infected Chang liver cells.⁶¹ The latter two reports do not include data relating to other FcR, such as FcR2 expression on salivary PMN or the effects on ADCC of anti-FcR2 MoAb.

FcR requirements in PMN ADCC of human tumor cells have not been described before the present report. One attempt to do so was unsuccessful when PMN did not lyse JY human B-lymphoblastoid cells that were precoated with a murine IgG_{2a} MoAb.⁵⁰ This failure illustrates the difficulty in achieving ADCC of human tumor cells by unstimulated PMN^{9,11,14}; it also supports the hypothesis that PMN ADCC of human tumor cells, which are too large for phagocytosis by PMN and are relatively resistant to reactive oxygen species,^{24,25} involves cytotoxic mechanisms different from those in model systems using murine targets.

We have established that FcR3 is one of several PMN receptors required for 3F8-mediated PMN ADCC of human solid tumor (neuroectodermal) cells. It remains to be determined whether the PI-linked FcR3 on PMN is generating an intracellular signal to promote PMN cytotoxicity, or is only serving as a receptor for tight binding to MoAb-coated tumor cells, while signal transduction is effected by FcR2.⁶² Evidence supporting an active role for the PI-linked FcR3 independent of FcR2 includes reports that PMN FcR3 can mediate exocytosis of granule

proteins,⁶³ production of superoxide anion,⁶⁴ and elevation of cytosolic calcium derived from intracellular stores.⁶⁵

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