Function and Heterogeneity of Human Fc Receptors for Immunoglobulin G

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The recognition of foreign antigens by receptors on the surface of cells of the immune system is a fundamental feature of immune defenses. The receptor systems involved include the T cell receptor complex, surface Ig of B cells, class I and II histocompatibility antigens, CD4, CD8, complement receptors, and receptors for the Fc domain of IgG ($Fc_\gamma R$).[1] It is noteworthy that, except for the complement receptors, all of these are membrane-bound glycoproteins that are members of the Ig supergene family (see Williams and Barclay [1] for review). Although the existence of $Fc_\gamma R$ has been appreciated since the late 1960s (2), it was only with the advent of MAb technology that the complexity of the $Fc_\gamma R$ family has become evident. With the cloning of the genes for several murine and human $Fc_\gamma Rs$, it is apparent that the notion of a MAb defining one $Fc_\gamma R$ is simplistic, since several proteins with widely divergent transmembrane and cytoplasmic domains, but conserved extracellular domains, may be recognized by the same MAb reagent. This review will focus on recent progress in the elucidation of the human $Fc_\gamma R$ family; previous reviews cover the older literature and the murine system in more detail (3–6).

The provisional nomenclature discussed at a meeting on Fc receptors and Ig binding factors sponsored by the Federation of American Societies for Experimental Biology in June, 1987 is based primarily on MAb reactivity and secondarily on subclass specificity, and is summarized in Table I.

Structure of murine $Fc_\gamma Rs$

The cloning of murine $Fc_\gamma Rs$ has been accomplished by several groups (7–10). These studies reveal that the murine $Fc_\gamma Rs$ consist of a mature extracellular domain of ~ 180 amino acids, which can be subdivided into two homologous domains most closely related to the Ig constant region C2 domain set (1). Each subdomain contains two cysteine residues that form disulfide bridges (11, 12) with loops from 43 to 45 residues long, in contrast to most other members of the Ig gene superfamily, which have longer loops. Other members of the C2 set are adhesion molecules such as NCAM, myelin-associated protein, CD2, the ϵ chain of CD3, and peptide receptors for PDGF, CSF-1, and IL-6 (13).

There are two genes encoding the low avidity $muFc_\gamma RII$ proteins, $muFc_\gamma RII\alpha$ and $muFc_\gamma RII\beta$, which share 95% homology in the extracellular domains but differ in their transmembrane and cytoplasmic domains. The $muFc_\gamma RII\alpha$ gene is transcribed in macrophages, and has a transmembrane domain remarkable for a charged aspartyl residue in the bilayer leaflet and a 26-residue basic cytoplasmic domain. The protein sequence of the transmembrane domain of the $muFc_\gamma RII\alpha$ is homologous to the transmembrane domain of the IgE-binding α subunit of the high avidity basophil/mast cell $Fc_\epsilon RI$ (14, 15), which suggests that the two receptors may have a similar mechanism of signal transduction. The $muFc_\gamma RII\beta$ gene has two transcripts, $muFc_\gamma RII\beta 1$ and $muFc_\gamma RII\beta 2$, that differ in a 46-amino-acid insertion in the cytoplasmic domain of the $muFc_\gamma RII\beta 1$ protein. The transmembrane domain of the $muFc_\gamma RII\beta$ gene has no charged amino acids. The structure of $muFc_\gamma RI$, the high avidity receptor that binds murine IgG2a and human IgG1, has not yet been determined.

$huFc_\gamma RI$

$HuFc_\gamma RI$, found predominantly on monocytes and macrophages, binds monomeric human IgG with high avidity ($K_a = 1\text{–}3 \times 10^8\ M^{-1}$) and with a subclass specificity of IgG1 > IgG3 > IgG4 ≫ IgG2 (16), in addition to binding murine IgG2a with high avidity. One epitope on murine IgG2a and human IgG1 that binds to $huFc_\gamma RI$ has now been localized to the NH_2-terminal portion of the C_H2 domain by Duncan et al. (17). The sequence of murine IgG2a and human IgG1 from residue 234 of the heavy chain is LLGGP. A single amino acid substitution, which converts the homologous sequence of murine IgG2b from LEGGP to LLGGP, increased the binding of the mutant murine IgG2b to $huFc_\gamma RI$ 100-fold. There may be other epitopes involved in binding of IgG to $huFc_\gamma RI$ and the murine counterpart, $muFc_\gamma RI$, as suggested by inhibition of binding of murine IgG2a immune aggregates to macrophages by aggregated CNBr fragments from both the C_H2 and C_H3 domains of IgG2a (18).

$HuFc_\gamma RI$ was purified by affinity chromatography from monocytes and the U937 monocytic cell line (19). Several MAbs (32.2, FR51, 10.1) (20–22) have been described that immunoprecipitate $huFc_\gamma RI$, but are apparently directed against epitope(s) not directly associated with the binding site of the receptor since they do not efficiently inhibit binding of IgG. Adsorption of $huFc_\gamma RI$ from cell lysates containing $huFc_\gamma RI$ on microtiter wells coated with MAb 32.2 conferred high affinity IgG binding on the wells, confirming the anti-$Fc_\gamma RI$ specificity of MAb 32.2 (23). The protein has an M_r of 72,000 on SDS-PAGE, and is heavily glycosylated. After removal of N-linked carbohydrate of *N*-glycosidase-F a core protein with an M_r of 40,000 was found (21).

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1. *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; $FC_\gamma R$, receptor for the Fc domain of IgG; PIG, phosphatidylinositol glycan; PIPLC, phosphatidyl inositol-specific phospholipase C; PNH, paroxysmal nocturnal hemoglobinuria.

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Table I. $Fc_\gamma R$ Nomenclature

Proposed nomenclature	Previous nomenclature	Cell types bearing receptor	Anti-$Fc_\gamma R$ MAbs
$huFc_\gamma RI$	$Fc_\gamma R_{hi}$, $Fc_\gamma R_{p72}$	Monocyte/macrophage, IFN-γ-stimulated neutrophil	32.2, FR51, 10.1 (20–22)
$huFc_\gamma RII$	$Fc_\gamma R_{p40}$	Monocyte/macrophage neutrophil, eosinophil, platelet, B cell	IV.3 (35)
$huFc_\gamma RIII$	$Fc_\gamma R_{lo}$, $Fc_\gamma R_{p50-70}$	NK cells, neutrophils, eosinophils, macrophages, not on monocytes	3G8, B73.1, leu 11a, b, c (34, 53, 59)

The $huFc_\gamma RI$ is thought to play a role in antibody-dependent cellular cytotoxicity (ADCC) reactions. Graziano and Fanger (24) have demonstrated killing by monocytes of myelomas bearing both anti-$huFc_\gamma RI$ and anti-$huFc_\gamma RII$ MAb. IFN-γ, which in many systems acts to potentiate ADCC reactions and to enhance the oxidative burst (25), increases the expression of $HuFc_\gamma RI$ 8–10-fold (26). IFN-γ treatment of U937 cells potentiates ADCC mediated by murine IgG2a (27), the subclass of mouse IgG that binds most avidly to $huFc_\gamma RI$. In addition, IFN-γ induces expression of $huFc_\gamma RI$ on neutrophils, which usually do not express this receptor (28–30). The IFN-γ-treated neutrophils have increased ADCC potential and mediate ADCC via the induced $huFc_\gamma RI$ (29). Other agents, such as glucocorticoids and retinoic acid (30, 31), and the C5a split product of complement (32), have also been reported to induce $huFc_\gamma RI$ expression.

Of interest is a report by Ceuppens et al. (33) of a Dutch family, four members of which have monocytes that lack $huFc_\gamma RI$ binding sites. Although the monocytes from these individuals do not bind IgG with high avidity, they show no increased susceptibility to infection. This suggests that the $Fc_\gamma R$/complement receptor systems have enough degeneracy in terms of function that absence of the $huFc_\gamma RI$ molecule from the monocyte/macrophage lineage does not seriously compromise immune defenses.

$huFc_\gamma RII$

$HuFc_\gamma RII$, CDw32 (34), is distributed on a wide variety of cells, including monocytes, platelets, neutrophils, B cells, and the K562 cell line. The protein was first isolated by affinity chromatography on IgG Sepharose (19), and subsequently an MAb, IV.3, was isolated that immunoprecipitates a protein of M_r 40,000 (35). MAb IV.3 blocks IgG-mediated aggregation of platelets, which bear only $huFc_\gamma RII$. $HuFc_\gamma RII$ is a low avidity receptor first identified on U937 cells by the binding of aggregated murine IgG2b at low ionic strength (36). The receptor binds human IgG1 with $K_a = \sim 2 \times 10^6\ M^{-1}$. The order of binding activity to recombinant $huFc_\gamma RII$ expressed in COS cells is IgG1 > IgG2 = IgG4 ≫ IgG3 (37).

$HuFc_\gamma RII$ apparently plays only a minor role in the binding of IgG-sensitized erythrocytes to neutrophils, monocytes, or macrophages since MAb IV.3, which inhibits $huFc_\gamma RII$, has only a slight effect on binding of immune aggregates (38, 39). $HuFc_\gamma RII$ will direct ADCC by monocytes and the cytotoxic potential of this receptor is increased in neutrophils by treatment with IFN-γ, although the density of $huFc_\gamma RII$ is not elevated by IFN-γ (24). $HuFc_\gamma RII$ may play a vital role in triggering the oxidative burst, since this response to aggregated IgG is blocked by MAb IV.3 or its $F(ab')_2$ fragment (20). Willis et al. (40) also demonstrate, with a different anti-$huFc_\gamma RII$ MAb, that triggering of the oxidative burst and release of lysosomal enzymes from neutrophils is dependent on crosslinking of the receptors. Neutrophils that have only $huFc_\gamma RII$ on their membrane can be isolated from patients with paroxysmal nocturnal hemoglobinuria (PNH). Yet these neutrophils can mediate a normal oxidative burst in response to stimulation with IgG-coated latex (41), confirming the role of $huFc_\gamma RII$ in triggering oxidative metabolism by neutrophils. Similar results are obtained with neutrophils that have been digested with elastase, which removes most of $huFc_\gamma RIII$ but leaves the $huFc_\gamma RII$ molecule intact. Elastase-treated neutrophils release superoxide in response to aggregated IgG, and the superoxide release is inhibited by preincubation with MAb IV.3 (42).

The monocyte-dependent T cell response to IgG1 anti-CD3 MAbs is blocked by anti-$huFc_\gamma RII$ MAb IV.3 (43) which demonstrates the role of $huFc_\gamma RII$ in crosslinking the anti-CD3 MAb on the T cell plasma membrane. Furthermore, cloned $huFc_\gamma RII$ expressed in mouse L cells will mediate an anti-CD3 dependent T cell response (44). There is allotypic variation in $huFc_\gamma RII$, which has two alleles identified by an isoelectric point polymorphism (45). The polymorphism is reflected in different accessory cell-dependent mitogenic responses of T cells to murine IgG1 anti-CD3 MAbs. However, the T cells of ~ 30% of normal individuals do not proliferate in the presence of monocytes and murine IgG1 anti-CD3 antibody, due to the failure to crosslink the T cell CD3 via the monocyte $huFc_\gamma RII$. The IgG1 anti-CD3 MAbs can trigger T cell mitogenesis in nonresponding individuals when the MAb is coupled to Sepharose (46–48). The percentage of individuals nonresponsive to IgG1 anti-CD3 MAbs correlates well with the isoelectric polymorphism of $huFc_\gamma RII$ (45). The individuals who fail to respond to IgG1 anti-CD3 MAbs apparently fail to do so because the homozygous nonresponder allele of $huFc_\gamma RII$ does not bind murine IgG1 (49). There are also reproducible differences in the amount of $huFc_\gamma RII$ detected on platelets from different individuals. Platelets with higher levels of $huFc_\gamma RII$ are more sensitive to aggregation by IgG complexes (50).

The $huFc_\gamma RII$ cDNA has been cloned (37, 51, 52). The leader sequence of $huFc_\gamma RII$ shares homology with the $muFc_\gamma RII\alpha$ transcript, but the transmembrane domain of $huFc_\gamma RII$ bears homology to $muFc_\gamma RII\beta$. The cytoplasmic domain, which is 76 amino acids long, is composed of neutral and hydrophobic residues and bears no homology to either murine $Fc_\gamma R$. Two mRNA transcripts, of 1.6 and 2.5 kb, are present in lines that express $huFc_\gamma RIII$. The different transcripts may reflect use of different polyadenylation signals.

There may be other $huFc_\gamma RII$ genes yet to be characterized. Daudi, an EBV-transformed B cell line, expresses a low affinity $Fc_\gamma R$ of 40,000 M_r, and synthesizes transcripts that hybridize with $huFc_\gamma RII$ probes. However, the anti-$huFc_\gamma RII$ MAb IV.3 does not bind to Daudi, although it does immunopre-

cipitate the two different allotypes of huFc$_\gamma$RII. Furthermore, Stuart et al. (51) isolated MAbs directed against peptides immediately COOH-terminal to the first cysteine in each disulfide pair in the Ig-like domains of the predicted huFc$_\gamma$RII sequence. Although these sera did bind to U937 and huFc$_\gamma$RII transfectants, they did not react with Daudi. Thus it would appear that Daudi has a huFc$_\gamma$RII that differs from that cloned so far.

huFc$_\gamma$RIII

HuFc$_\gamma$RIII, CD16, is expressed on neutrophils, NK cells, a minor population of T cells, eosinophils, and on tissue macrophages but not on monocytes (38, 53–55). In the granulocyte lineage, huFc$_\gamma$RIII is expressed at the metamyelocyte stage. The HL-60 promyelocytic cell line, which does not express huFc$_\gamma$RIII, can be induced to do so by DMSO or retinoic acid, which induces a more mature granulocytic morphology. The recent cloning and expression of huFc$_\gamma$RIII in COS cells has facilitated a study of receptor specificity. HuFc$_\gamma$RIII binds human IgG1 and IgG3 with a $K_a \sim 5 \times 10^5$ M^{-1} and does not bind human IgG2 and IgG4 (56). These results agree with previous results obtained by Spiegelberg et al. (57) and Gergely et al. (58).

Several lines of evidence suggest that there may be a family of huFc$_\gamma$RIII molecules that have highly conserved extracellular domains but may differ in other domains. There are immunological differences between neutrophil and NK cell antigens that can be demonstrated with anti–Fc$_\gamma$RIII MAbs, and the NA1/NA2 alloantigen system of huFc$_\gamma$RIII is apparently restricted to neutrophils (59). Analysis of SDS-PAGE of huFc$_\gamma$RIII immunoprecipitated from macrophages, neutrophils, and NK cells before and after deglycosylation also illustrates differences between these receptors. Isolation of huFc$_\gamma$RIII from monocytes cultured in vitro for 14 d revealed a protein of M_r 55,000, which was not altered by digestion with *N*-glycanase (39). The neutrophil receptor, by contrast, exhibited a broad band on SDS-PAGE from 50,000–70,000 M_r, which is reduced to a doublet of 28,000–29,000 M_r after deglycosylation. The huFc$_\gamma$RIII molecule on NK cells is also different from the neutrophil receptor, since deglycosylation leads to a complex pattern with peptides of 36,000, 40,000, and 44,000 M_r (60).

The neutrophil huFc$_\gamma$RIII molecule has now been identified as a membrane protein that has a phosphatidylinositol glycan (PIG) membrane anchor (61, 62). The neutrophil huFc$_\gamma$RIII is released from the cells upon treatment with phosphatidyl inositol-specific phospholipase C (PIPLC). Furthermore, neutrophils from patients with PNH, an acquired clonal stem cell disorder characterized by a defect in the biosynthesis of the PIG anchor, show a marked deficiency in huFc$_\gamma$RIII expression. Decay accelerating factor and acetylcholinesterase, two other PIG-anchored proteins, are also deficient in the membranes of patients with PNH. Both the NA1 and NA2 alloantigens of huFc$_\gamma$RIII are released by PIPLC. Results of expression in COS cells on a cDNA encoding huFc$_\gamma$RIII confirm the presence of a PIG anchor (56).

The functional significance of the PIG anchor on neutrophils remains to be determined. Huizinga et al. (62) report that huFc$_\gamma$RIII is released upon stimulation of PMN by FMLP, a chemotactic peptide. This is consistent with a previous report (63) demonstrating a loss of Fc$_\gamma$R expression on neutrophils after stimulation with phorbol esters because both FMLP and immune complexes activate protein kinase C.

There is, however, some question as to whether the huFc$_\gamma$RIII molecule on all cell types has a PIG membrane anchor. Thus far, all the members of the Fc receptor family that have been cloned have had two Ig-like extracellular domains, each of $\sim$ 85 amino acids. The size of the deglycosylated huFc$_\gamma$RIII molecules isolated from NK cells (60) and macrophages (39) is greater than the 26,000–29,000 M_r deglycosylated Fc$_\gamma$RIII molecule reported for neutrophils. Furthermore, while PNH patients have dramatically reduced amounts of huFc$_\gamma$RIII on the neutrophil plasma membrane, the amount of HuFc$_\gamma$RIII on cultured monocytes is normal (62). Thus, it is likely that there are other members of the CD16/huFc$_\gamma$RIII family with different transmembrane and/or cytoplasmic domains.

The activation of NK huFc$_\gamma$RIII results in the expression of IL-2 receptor, the transferrin receptor, and the hormones IFN-γ and TNF (64). IL-2 and huFc$_\gamma$RIII ligands act synergistically to activate NK cells. Thus the synthesis of TNF, barely stimulated by 100 U/ml of IL-2, and only stimulated eightfold by Sepharose B73.1 (an anti–huFc$_\gamma$RIII MAb), is stimulated 50-fold by a combination of combined IL-2 and Sepharose B73.1. Egawa et al. (65) report that anti–huFc$_\gamma$RIII MAb treatment enhances NK cell activity, and similar results are reported by van de Griend et al. (66). These results have important implications for the mechanism of NK cytotoxicity.

HuFc$_\gamma$RIII is present in high concentration on Kuppfer cells in the liver and on macrophages in the red pulp of the spleen, both loci involved in clearance of immune complexes. Blockade of the mononuclear phagocyte system of chimpanzees with either the anti–Fc$_\gamma$RIII MAb 3G8 or its Fab fragment resulted in a dramatic blockade of in vivo clearance of autologous erythrocytes coated with antibody directed against a minor blood group antigen (67). These results suggest that, at least for this type of large immune complex, huFc$_\gamma$RIII on macrophages may be the major receptor involved in clearance. Blockade of huFc$_\gamma$RIII by MAb 3G8 was tested as a therapeutic treatment of chronic immune thrombocytopenic purpura, a disease characterized by anti–platelet antibody (68). Infusion of 3G8 in one reported case had a dramatic short-term effect on platelet levels which rose to normal levels and subsided after 2 wk. Reinfusion of the MAb resulted in a blunted response, possibly due to production of antibodies against the murine IgG.

The huFc$_\gamma$RIII cloned by Simmons and Seed (56) using a eukaryotic expression shuttle vector from a cDNA library constructed from human placenta reveals greatest homology with the muFc$_\gamma$RIIα gene. The predicted protein has a short transmembrane domain followed by only four residues, only one of which is basically charged. The recombinant huFc$_\gamma$RIII expressed in COS cells is immunoreactive with a panel of anti–huFc$_\gamma$RIII MAbs, has an M_r of 50,000–70,000, is reduced in size to 26,000 M_r after *N*-glycanase digestion, and is released from the membrane of transfected cells by digestion with PIPLC, all characteristics of the neutrophil huFc$_\gamma$RIII. There is a conflict, however, between the results of Simmons and Seed (56), who state that the receptor they have isolated is that of NK cells, and Lanier et al. (60) since the latter group reports the NK huFc$_\gamma$RIII has a deglycosylated size of 36–44 kD,

clearly different from the 26–29 kD deglycosylated neutrophil huFc$_\gamma$RIII (39, 60).

Mechanism of signal transduction

As discussed previously, the neutrophil receptor responsible for triggering the oxidative burst is huFc$_\gamma$RII. This receptor may be linked to protein kinase C via inhibitory G proteins, since Feister et al. (69) found that pertussis toxin inhibited the oxidative burst of neutrophils triggered by both the chemotactic peptide FMLP and by cross-linking huFc$_\gamma$RIII via MAb KuFc79. Degranulation triggered by the MAb cross-linking was only partially inhibited by pertussis toxin, in contrast to the total inhibition of degranulation observed under the same conditions for FMLP stimulation. Gresham et al. (70) also find that the phagocytic response of neutrophils mediated by Fc$_\gamma$R is partially linked through G protein. Phagocytosis of EIgG by neutrophils can be stimulated by amphotericin B, by a cytokine from lymphocytes co-cultured with monocytes ingesting EIgG (71), or by phorbol dibutyrate. The stimulation by amphotericin B or cytokine is abrogated by cholera toxin or pertussis toxin, but phorbol dibutyrate stimulation is unaffected by these agents, presumably because the phorbol ester bypasses the steps involving the G proteins.

The respiratory burst of macrophages is activated by phagocytosis of EIgG, but not by EIgMC. Taking advantage of this, Brozna et al. (72) looked for differential phosphorylation of macrophage proteins from cells ingesting EIgG compared with EIgMC, and found a series of proteins more intensely labeled after EIgG stimulation. Some of these proteins also are phosphorylated after phorbol myristate acetate (PMA) stimulation. The activity of protein kinase C in particulate fractions also was dramatically increased within minutes of stimulation by EIgG, consistent with protein kinase C activation.

The role of Ca^{2+} in Fc$_\gamma$R signalling may differ for different cells and systems. Crosslinking of Fc$_\gamma$R of macrophages (73) and neutrophils (74) has been reported to trigger Ca^{2+} mobilization. This may, however, be a secondary phenomenon, since phagocytosis of EIgG is not inhibited after the free Ca^{2+} is buffered to 1–10 nm by EGTA or Quin2 (75).

Phillips and Parker (76, 77) have shown that ternary cross-linking of membrane Ig and Fc$_\gamma$R of murine B lymphocytes inhibits stimulation otherwise found with anti-μ or anti-δ F(ab')$_2$. Murine B cells stimulated by anti-μ Ig or anti-δ F(ab')$_2$ show a transient Ca^{2+} response and generation of inositol triphosphate, and are stimulated to enlarge and express surface Ia. These responses are largely abrogated by intact rabbit anti-μ or anti-δ, but if the Fc$_\gamma$R is blocked by anti-Fc$_\gamma$R MAb 2.4G2, the responses are restored (78). Goroff and Finkelman (79) report that the stimulatory effect on B cell Ia expression of an IgG2b anti-δ MAb was not inhibited by the anti-Fc$_\gamma$R MAb 2.4G2, but the stimulation by the univalent Fab/Fc anti-δ MAb was blocked. Ca^{2+} flux may play a role in activation of NK cells mediated by cross-linking of CD2 and huFc$_\gamma$RIII (80).

It seems only reasonable that proteins other than the Fc$_\gamma$R itself are involved in such complicated cellular behaviors as phagocytosis and ADCC. Monocytes sorted to enrich the Leu M3$^+$ population phagocytose EIgG avidly and lyse EIgG only poorly; the reverse is true of the Leu M3$^-$ population (81). Similar results were observed with different sublines of the U937 cell line after stimulation with PMA. Sublines that express Leu M3 after PMA treatment phagocytose well but lyse EIgG poorly, and vice versa. In each case the receptors (huFc$_\gamma$RI and Fc$_\gamma$RII) are presumably the same, yet the final cellular response is different.

Clinical potential of anti-Fc$_\gamma$R MAbs

A potentially exciting use for anti-Fc$_\gamma$R MAbs is to direct the cytotoxic potential of Fc$_\gamma$R-bearing cells to immunologically defined targets. Two potential problems in the use of anti-tumor antibodies to target ADCC are interference by the high concentrations of IgG found in serum and the capping of antigens triggered by bivalent antibodies. Both difficulties might be bypassed by covalent heteroconjugates between anti-huFc$_\gamma$R MAbs and anti-tumor antibodies. The feasibility of such an approach is shown by experiments in which heteroconjugates were formed between the anti-Fc$_\gamma$RI MAb 32.2 and anti-chicken erythrocyte antibody. These conjugates would direct both monocyte ADCC (82) and ADCC by neutrophils after stimulation with IFN-γ (30), which induces huFc$_\gamma$RI. The observed ADCC was not inhibited by mg/ml concentration of huIgG, in contrast to monocyte ADCC mediated by anti-target antibody, because MAb 32.2 is not directed against an epitope close to the binding of huFc$_\gamma$RI.

A similar approach was used to target NK/K cell ADCC (83). The Fab fragment of the anti-huFc$_\gamma$RIII MAb, 3G8, was coupled to the Fab fragment of an MAb directed against tumor antigens or DNP groups. The conjugates would direct NK/K cell ADCC against cells bearing the appropriate antigen in vitro. The heteroconjugate-directed ADCC was not readily inhibited by cross-linked antibody, and in vivo would neutralize tumor cells in a Winn assay. Activation of the effector cells by IL-2 increased the efficiency of the antineoplastic activity.

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