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Functional Capacity of Fcγ Receptor III (CD16) on Human Neutrophils

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Abstract
Receptors for the Fc region of immunoglobulin G (IgG) are a structurally diverse group of molecules. Within the three FcγR families (FcγRI, FcγRII and FcγRIII), the presence of distinct genes and alternative splicing variants leads to a variety of receptor isoforms that are most strikingly different in the transmembrane and intracellular regions. An obvious example of structural variation in the transmembrane and cytoplasmic domains is observed in the FcγRIII family. FcγRIIIB, which is nearly identical to FcγRIIIA in the extracellular domains, lacks both transmembrane and cytoplasmic protein domains and is anchored to the cell through a glycosyl phosphatidylinositol anchor. Analysis of FcγRIII function presents a considerable challenge in understanding the role of different FcγR receptors in polymorphonuclear neutrophil (PMN) function. While one hypothesis for the role of FcγRIII in FcγR-dependent PMN effector functions is that FcγRIII serves as a binding molecule which focuses the IgG ligand for more efficient recognition and intracellular signaling by FcγRII, recent observations from a number of laboratories suggest that FcγRIII on PMN can transduce signals across the membrane independent of ligand-dependent engagement of FcγRII. We will review these data and present recent data which suggest that the role of FcγRIII extends beyond direct initiation of functions to a more complex role of synergistic receptor interactions. These findings will be reviewed in the context of the experimental approaches that thave been used to examine the roles of FcγRII and FcγRIII on PMN function.

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Introduction

Three distinct families of human receptors for the Fc region of immunoglobulin G (IgG) (FcγRI, FcγRII, and FcγRIII) have been identified based on analysis of monoclonal antibody (mAb) reactivity, cellular distribution, and cDNA sequences [1, 2]. Within these three FcγR families, the presence of distinct genes and alternative splicing variants leads to a variety of receptor isoforms that are most strikingly different in the transmembrane and intracellular regions. Given the number of effector functions which can be elicited by ligation of FcγR – the binding and internalization of IgG-coated particles and IgG-containing soluble immune complexes, antibody-dependent cellular cytotoxicity (ADCC), induction of the respiratory burst, and release of granule proteins by phagocytes [2, 3] – recent work has been directed towards an examination of what role individual structural isoforms of FcγR have in initiating these effector functions [4]. A striking example of structural variation in the transmembrane and cytoplasmic domains is observed in the FcγRIII family. FcγRIIIB, which is nearly identical to FcγRIIIA in the extracellular domains [5], lacks both transmembrane and cytoplasmic protein domains and is anchored to the cell through a glycosyl phosphatidylinositol (GPI) anchor [5–9]. All available evidence suggests that the expression of the FcγRIII genes is highly regulated in a cell-specific manner with the GPI-linked FcγRIII isoform expressed exclusively on polymorphonuclear neutrophils (PMNs) [5–12]. Analysis of FcγRIII function presents a considerable challenge in understanding the role of different Fcγ receptors in PMN function.

Members of each of the three families of receptors for the Fc region of IgG may be expressed on the surface of human PMNs (fig. 1) [2, 3]. While FcγRI expression on PMNs can be induced by stimulation with interferon-γ (IFN-γ), it is not constitutively expressed in the resting state and does not contribute to FcγR function on nonstimulated PMNs [13]. Both FcγRII, the product of the FcγRIIA gene, and $FcγRIII_{PMN}$, the product of the FcγRIIIB gene, are expressed on resting PMNs. FcγRII is a transmembrane protein and can elicit a number of different cell functions [2, 13]. Although some cytokines can modestly alter its level of expression after in vitro culture [14], quantitative FcγRII expression on PMNs is quite stable both within given individuals over time and across normal populations [8, 15, 16]. The heterogeneity in quantitative FcγRII expression seen

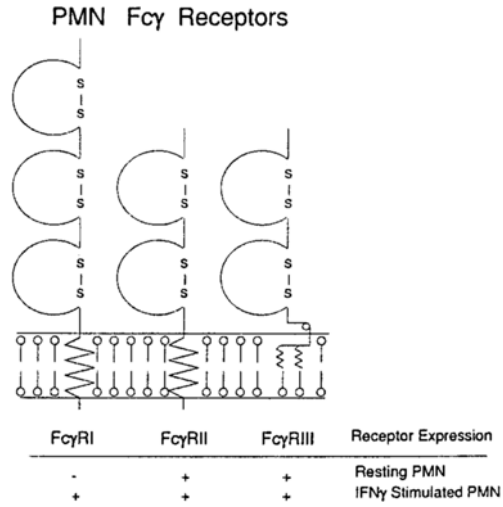

Fig. 1. Schematic representation of the three families of human Fcγ receptors expressed on either resting PMN or IFNγ stimulated PMN. FcγRI, expressed on IFNγ-stimulated PMN, and FcγRII are transmembrane proteins. FcγRIII are expressed on PMN is a GPI-linked protein. These FcγR receptors are members of the immunoglobulin superfamily and the characteristic extracellular intrachain disulfide-linked domains are illustrated [75].

in platelets does not appear to be reflected in PMNs [17]. Quantitative FcγRIII expression on resting PMN is also quite stable [8, 16], but in contrast to FcγRII, it may be shed with cell activation [7, 18]. This property, coupled with the GPI anchor of FcγRIII and the lack of an obvious link with the cytoplasm, has stimulated a great deal of interest in this receptor and its function on the PMN.

Perhaps the most prevalent hypothesis for the role of FcγRIII in FcγR-dependent PMN effector functions is that FcγRIII serves as a binding molecule which focuses the IgG ligand for more efficient recognition and intracellular signaling by FcγRII [1, 6, 19, 20]. The appeal of this hypothesis is supported by the higher density of FcγRIII on the cell surface compared to FcγRII [19, 21, 22] and the potential for a greater lateral mobility of the GPI-linked FcγRIII within the plane of the membrane [23]. However, recent observations suggest that FcγRIII on PMN can transduce signals across the membrane independent of ligand-dependent engagement of FcγRII (see below). Further analysis suggests that the role of FcγRIII extends beyond direct initiation of functions to a more complex role of synergistic receptor interactions. We will review a number of approaches used to examine the roles of FcγRII and FcγRIII on PMN function and the conclusions supported, or implied, by the experimental data.

Approaches to the Function of Individual Receptor Isoforms

A number of different experimental approaches have been used in an attempt to define the individual contributions of FcγRII and FcγRIII to PMN functions. Each of these approaches provides an important perspective, but each also has certain caveats which must be kept in mind.

Use of Anti-Fcγ Receptor mAb to Block Function Induced by Multivalent IgG Ligand

In this paradigm, PMNs are incubated with an anti-FcγR mAb which blocks the ligand-binding site of one receptor and then the PMNs are stimulated through the other receptor with a multivalent IgG ligand. Of course, depending on the isotype of the mAb, the Fc piece of the intact IgG mAb may engage the other FcγR and necessitate the use of Fab fragments. Interpretation of the results must consider whether complete blockade of ligand binding has been achieved experimentally and whether a multimeric IgG ligand may be able to engage some of the 'blocked' receptors [24]. Furthermore, interpretation of results requires careful definition of the conceptual model of receptor function. For example, the distinction between altered function due to decreased binding and altered function due to a block of receptor-initiated signal transduction may be difficult to make.

Direct Stimulation by Anti-Fcγ Receptor mAb

Ligation of a single receptor species by anti-Fcγ receptor mAb can be used to mimic engagement of receptors by native ligand. Some degree of receptor cross-linking is required [25], and usually $F(ab')_2$ fragments of an anti-mouse reagent or avidin in a biotin-avidin system are used to achieve sufficient cross-linking. As mentioned above, the Fc piece of certain intact IgG mAbs may engage the other FcγR and Fab or $F(ab')_2$ fragments are often required to obtain interpretable results. In addition, the ability of any given mAb to induce effector functions may be epitope dependent, thereby reflecting the properties of the mAb rather than the intrinsic properties of the receptor.

Fcγ Receptor Deficiencies

Although neither spontaneous nor experimental deficiences of FcγRII have been defined, several models of FcγRIII deficiency on PMNs have been studied. Experimentally, relative $Fc\gamma RIII_{PMN}$ deficiency has been induced by cleavage of the GPI anchor with PI-PLC [5–10] and by elastase treatment of intact PMNs [26]. While theoretically attractive, interpretation of results has been confounded by incomplete removal of the receptor and the potential for cell activation during the enzymatic cleavage. Paroxysmal noctural hemoglobinuria (PNH), an acquired disease characterized by clonally affected cells which have an altered capacity for GPI anchor biosynthesis [23, 27], presents an alternative approach, but PNH PMNs are not completely deficient in $Fc\gamma RIII_{PMN}$ [10]. Several individuals lacking FcγRIIIB expression, presumably on a genetic basis, have been described [28–30], but at least one person expresses FcγRI to varying degrees on her PMNs (Edberg, Kimberly, Clark and Goldstein, unpublished observations).

Receptor Polymorphisms

Analysis of differences in effector functions in normal donors, homozygous for various alleles of FcγR, provides a potentially powerful tool for understanding the biological role of the different receptors. Since the two characterized FcγR allelic polymorphisms – the FcγRIIIB NA system and the FcγRIIA high responder/low responder (HR/LR) system – are the results of a few nucleotide changes [1, 2], normal populations with defined phenotypes represent, in effect, nature's own experiments in site-directed mutagenesis within which a number of technical experimental issues can be avoided. Of course, one cannot be certain a priori that any given polymorphism will be 'insightful' with regard to the function of individual FcγR isoforms, and identification of new informative polymorphisms is a difficult undertaking.

Functional Capacities of FcγRIII on Neutrophils

Early Signaling Events

In order for $Fc\gamma RIII_{PMN}$ to initiate FcγR-dependent effector functions, this GPI-linked receptor must have the ability to transduce signals to the cytoplasm of the PMN. As an initial approach, we have examined the ability of $Fc\gamma RIII_{PMN}$ to induce an intracellular Ca^{2+} flux and membrane depolarization [31]. In resting PMNs oligovalent, but not univalent, ligation of $Fc\gamma RIII_{PMN}$ leads to a rapid increase in $[Ca^{2+}]_i$ and membrane depolarization. These responses are independent of ligand-mediated engagement of FcγRII since anti-FcγRIII mAb $F(ab')_2$ or Fab fragments cross-linked with anti-mouse $F(ab')_2$ fragments [31] and biotinylated Fab fragments cross-linked with streptavidin (Ory, personal commun.) lead to an increase in $[Ca^{2+}]_i$. The FcγRIII-induced increase in $[Ca^{2+}]_i$ is derived from intracellular Ca^{2+} stores. Addition of EGTA to the extracellular medium does not influence the increase in $[Ca^{2+}]_i$, while the intracellular Ca^{2+} chelator, BAPTA, completely abolishes the rise in $[Ca^{2+}]_i$ induced by receptor cross-linking. These data indicate that $Fc\gamma RIII_{PMN}$ may be more than a focusing molecule for FcγRII and that it has the potential to be an active participant in integrated cell processes.

Intracellular Events

To explore the capacity of FcγRIII to generate intracellular signals beyond Ca^{2+} fluxing, we analyzed the ability of anti-Fcγ receptor mAb to induce actin filament assembly in PMN [32]. Shifts in actin from its globular

monomeric form to its filamentous form are a prerequisite for motile behaviors such as phagocytosis, secretion, and locomotion, and often accompany priming of the PMN for enhanced responses to other stimuli. Cross-linking of either FcγRII or $FcγRIII_{PMN}$ with mAb IV.3 Fab or mAb 3G8 Fab, respectively, results in actin polymerization. Both the kinetics and magnitude of the F-actin polymerization response are similar to that observed with aggregates of human IgG [32, 33]. Together these results demonstrate that the GPI-linked FcγRIII can induce intracellular events that are relevant to FcγR-mediated effector functions.

Integrated Cell Functions

A number of different experimental approaches have been taken to determine the contribution of FcγRII and FcγRIII in FcγR-mediated PMN effector functions. Using multivalent IgG ligand and specific anti-FcγR mAb, several studies have concluded that FcγRII contributes to the triggering of internalization of antibody-opsonized erythrocytes, the respiratory burst induced by certain probes, and the release of granule proteins [2, 16, 19, 26, 34, 35]. Likewise, blockade of FcγRIII with specific mAb has been found to diminish the ability of the PMN to internalize antibody-opsonized erythrocytes, to partially inhibit the induction of a respiratory burst by insoluble immune complexes and to inhibit degranulation [16, 19, 34–36]. These experiments with anti-FcγR mAb clearly show that both FcγRII and FcγRIII are involved in phagocytosis, the respiratory burst, and degranulation by the PMN upon engagement with multivalent ligand. However, based on these data, it is difficult to distinguish between receptor contributions based on a role in transmembrane signaling as opposed to a role in the quantitative binding of the stimulus.

In an attempt to resolve this ambiguity, a number of investigators have used anti-FcγR mAb to ligate receptors and investigate the ability of either FcγRII or FcγRIII alone to initiate integrated cell functions. Soluble anti-FcγRII mAbs IV.3 and KuFc79, followed by cross-linking with anti-mouse $F(ab')_2$, are able to induce both degranulation and a respiratory burst in PMNs [19, 36–38]. Similarly, soluble anti-FcγRIII mAb CLBFcRgran1 can induce degranulation after cross-linking, but this mAb apparently does not initiate a respiratory burst in PMNs [34]. In contrast, cross-linking of the anti-FcγRIII mAb 3G8 can induce a respiratory burst in PMNs [35]. These two divergent observations suggest that the properties of the individual mAb and/or cross-linking reagent and the spatial orientation of cross-linking may be important variables in receptor function.

ADCC activity varies among different cell types and depends, in part, on the nature of the target cell. In a clever approach to the role of individual Fcγ receptors in ADCC, murine B-cell hybridomas expressing specific anti-Fcγ receptor surface IgG have been used as ADCC targets [13]. Resting PMNs can weakly lyse anti-FcγRII mAb IV.3-bearing tumor targets, while anti-FcγRIII-bearing tumor targets are not lysed [9, 39, 40]. When the PMNs are activated with IFNγ, the ability to lyse tumor targets via FcγRII but not FcγRIII is increased without changing the level of surface expression of FcγRII or FcγRIII [39]. Similarly, dinitrophenyl (DNP)-coated tumor cells are not lysed by anti-FcγRIII/anti-DNP heteroantibody and PMNs [41]. While these results indicate that $FcγRIII_{PMN}$ cannot initiate lysis of tumor targets, it can mediate ADCC of erythrocyte (E) targets [22]. MAb-coated erythrocytes prepared with either DNP and opsonized with anti-DNP/anti-FcγR heteroantibodies or non-derivatized erythrocytes coated with anti-E/anti-FcγR mAb, are lysed

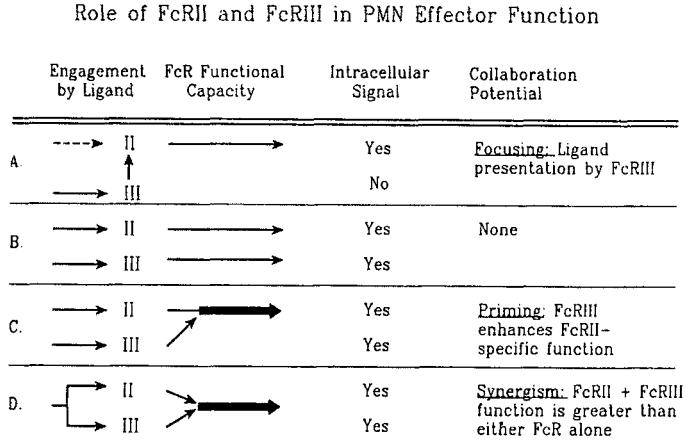

Fig. 2. Proposed models of the role of FcγRII and FcγRIII in the induction of PMN Fcγ receptor-mediated effector functions. In model A, the initial engagement of ligand by FcγRIII is favored due to the higher density of this receptor relative to FcγRII. The FcγRIII-bound ligand is then capable of engaging FcγRII which triggers a functional response. In model B, ligand independently binds to and initiates a functional response through both FcγRII and FcγRIII. In model C, initial engagement of FcγRIII leads to subsequent enhancement of the functional response via FcγRII (receptor priming). In model D, engagement by both FcγRII and FcγRIII leads to an enhanced functional response (synergism). In models C and D, the thickness of the arrow representing FcγR functional capacity is proportional to the magnitude of the response.

by both FcγRII and $FcγRIII_{PMN}$ [22, 41, 42]. The conclusion that $FcγRIII_{PMN}$ can mediate ADCC for some targets is supported by the observation that $F(ab')_2$ fragments of the anti-FcγRIII mAb B73.1 were able to block ADCC of erythrocytes mediated by PMN [43]. Taken together, these results suggest that $FcγRIII_{PMN}$ can initiate a lytic process but that the lytic mechanisms induced by FcγRII and $FcγRIII_{PMN}$ may be distinct.

The concept that quantitative, and perhaps qualitative, differences exist between functions initiated by FcγRII and $FcγRIII_{PMN}$ is supported by analysis of receptor-mediated phagocytosis. With anti-E/anti-FcγR heteroantibodies as the opsonin, FcγRII but not $FcγRIII_{PMN}$ supports internalization of opsonized erythrocytes [20]. Additional data, however, suggest that the quantitative level of mAb presented on the erythrocyte is critical in determining whether $FcγRIII_{PMN}$ is phagocytic. We have developed a biotin-avidin system as an alternative technique for coating erythrocytes with anti-FcγR-specific mAb (Edberg JC and Kimberly RP: Receptor specific probes for the study of Fcγ receptor spe-

cific function. J Immunol Methods 1992; in press). In this system, the level of mAb on the target erythrocyte surface can be titrated and quantitated by flow cytometry. We have found that FcγRIII$_{PMN}$ is phagocytic when engaged by the anti-FcγRIII mAb 3G8, although the quantitative level of internalization is less than that observed with FcγRII when target erythrocytes are opsonized with equal amounts of anti-FcγR Fab. Whether the differences in FcγRIII$_{PMN}$ phagocytosis, observed with the heteroantibody and E-biotin/avidin techniques, reflect differences in presentation of the mAb to the PMN, differences in opsonization density or other factors remain to be determined. These results, however, coupled with data from many different laboratories, demonstrate that the GPI-linked FcγRIII expressed on PMN can initiate integrated cell functions independent of ligand-mediated interactions with FcγRII.

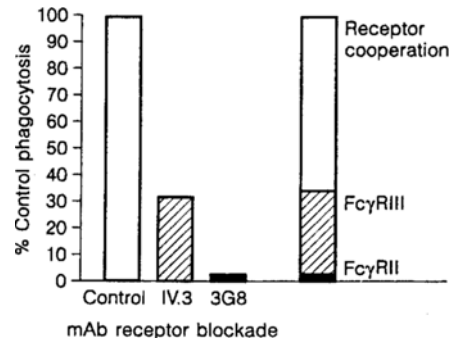

Fig. 3. The percent control of EA phagocytosis is shown for control (no blockade, □), FcγRII blockade induced by mAb IV. 3 Fab (▨), and FcγRIII blockade induced by mAb 3G8 Fab (■). When the percent control phagocytosis for the anti-FcγRII and anti-FcγRIII blockade data are summed, only 38% control is achieved. This is suggestive of receptor cooperation (synergism) when both PMN and FcγR are engaged by multimeric ligand [16].

Receptors Working in Concert (Fig. 2)

Three observations suggest that even the more sophisticated model of FcγRIII$_{PMN}$ function which includes both focusing of ligand for FcγRII and independent initiation of some PMN functions may be incomplete. First, in some experimental paradigms, the sum of the percent blockade of function resulting from each anti-receptor mAb alone is greater that 100% (fig. 3) [16, 19]. While data are often not available to determine whether this is due to changes in the ability to the stimulus to bind to PMN, this observation is consistent with a synergic interaction between FcγRII and FcγRIII$_{PMN}$. More compelling, perhaps, are the observations with the FcγRIII$_{PMN}$-dependent ingestion of erythrocytes coated with Concanavalin A (E-ConA) [44, 45]. This property is not contingent on FcγRIII$_{PMN}$ serving as a binding molecule [46] since maximal binding of the E-ConA is still observed after blockade of internalization with anti-receptor mAb 3G8 [44, 45]. Similarly, this property may not be contingent on FcγRIII$_{PMN}$ serving as an exclusive mediator of phagocytosis since the maximal phagocytic capacity of FcγRIII$_{PMN}$ defined by 3G8 Fab-coated erythrocytes is significantly less than that for E-ConA [44, 45] (unpublished observations). These observations imply that some collaborative or synergistic interaction, other than simple binding of E-ConA by FcγRIII$_{PMN}$ and occurring between FcγRIII$_{PMN}$ and other cell surface molecules, may be important. Finally, work by Brown et al. [47] and Graham et al. [48] have implicated an interaction between PMN Fcγ receptors and CDIIb/CD18. Although the precise mechanisms for each of these observations have not been defined, the suggestion of interactions between cell surface molecules, on a

physical basis (beyond 'focusing' of binding) and/or on an intracellular biochemical basis, is clear.

With this perspective, we have devised two experimental approaches to define interactions between FcγRII and $Fc\gamma RIII_{PMN}$: in the first, $Fc\gamma RIII_{PMN}$ is cross-linked with $F(ab')_2$ fragments of the anti-receptor mAb 3G8 and then FcγRII is probed in a receptor-specific fasion using erythrocytes coated with Fab fragments of the anti-FcγRII mAb IV.3, and in the second, FcγRII and $Fc\gamma RIII_{PMN}$ are engaged individually or simultaneously with erythrocyte-bound Fab fragments of anti-receptor mAbs under careful quantitative conditions such that the total number of receptors engaged in each condition remains constant. Although the first paradigm is called 'priming' of FcγRII function by $Fc\gamma RIII_{PMN}$ (engagement of the two receptor species separated in time) and the second is called 'synergism' (both receptor species engaged simultaneously), these distinctions may be primarily semantic. The principle concept is the premise that engagement of the two receptors may lead to quantitatively and perhaps qualitatively distinct cell functions in relation to engagement of either receptor alone. The relationship of these engagements in time and in orientation on the cell surface may be important additional variables.

To explore receptor priming, we have used soluble anti-FcγRIII mAb 3G8 $F(ab')_2$ to cross-link two $Fc\gamma RIII_{PMN}$ molecules and erythrocytes coated with either IV.3 heteroantibodies or biotinylated IV.3 Fab-coated erythrocytes to determine the amount of FcγRII specific phagocytosis. When the PMN are briefly incubated with soluble mAb 3G8 $F(ab')_2$ followed by the addition of IV.3 Fab-coated erythrocytes (E-IV.3), an increase in the amount of internalization of the E-IV.3 is observed [132]. This increase in internalization ranges from 104 to 179% of the control E-IV.3 phagocytic index [32]. Despite the fact that mAb 3G8 $F(ab')_2$ delivers a submaximal stimulus for transmembrane signaling (unpublished observations), these data indicate that $Fc\gamma RIII_{PMN}$ can prime FcγRII for enhanced phagocytic function.

To probe PMN FcγR for functional synergism, we modified the E-biotin/avidin (E_{BA}) phagocytic system to generate erythrocytes with both anti-FcγRII and anti-FcγRIII mAbs bound to their surface. E_{BA} were prepared with equivalent total amounts of either: (1) mAb IV.3 Fab alone; (2) mAb 3G8 Fab alone, or (3) both mAb IV.3 Fab and mAb 3G8 Fab (unpublished observations). These three different mAb-coated erythrocyte species showed comparable attachment indices yet large differences in phagocytic indices. For example, with the opsonization density for both mAbs titered down so that the phagocytic response of the E_{BA}–3G8 Fab was negligible, the phagocytic index for E_{BA}-(IV.3 + 3G8) was found to be 200 ± 20% of the E_{BA}-IV.3 alone. Since the binding of both the E_{BA}-(IV.3 + 3G8) and the E_{BA}-IV.3 to the PMN was identical, the enhancement in phagocytosis must reflect receptor synergism. Although currently undefined, the basis for this synergism presumably involves an interaction between the signaling pathways stimulated by both FcγRII and $Fc\gamma RIII_{PMN}$.

Allelic Polymorphisms: An Alternative Approach to the Study of Receptor Interactions

The occurrence of synergism prompts several interesting considerations. First, unless the capacity to mediate synergism is the distinct and unique function of $Fc\gamma RIII_{PMN}$, the general model that each Fcγ receptor isoform may perform a distinct function may be too restrictive, and a more interactive framework

may be appropriate [49]. In that context, ligation of individual receptor species may demonstrate the functional potential of a given receptor but may not provide the full insight into the balanced contribution of that receptor. Indeed, from the perspective of the intact PMN, simultaneous engagement of both FcγRII and $Fc\gamma RIII_{PMN}$ with multimeric human IgG may be the most important physiological stimulus. Accordingly, allelic receptor polymorphisms provide a unique opportunity to study this interactive system since one can provide multimeric ligand to an unperturbed cell within which a single variable has been manipulated by nature.

To date, two FcγR allelic polymorphisms have been described [2]. The NA polymorphism is expressed in the FcγRIIIB gene product only and can be detected on PMNs but not on NK cells or monocyte/macrophages [5, 8, 11, 12, 50–52]. The HR/LR polymorphism is expressed in the FcγRIIA gene product and can be detected on PMN, monocytes and platelets [53–56]. Functional studies using PMN from NA-phenotyped donors has shown that NA1 homozygous individuals have a higher level of quantitative phagocytosis of IgG-coated erythrocytes than do NA2 homozygous individuals [16], (unpublished observations). This difference between the homozygous donor populations is evident with either human or rabbit IgG as the opsonin [16] and is independent of the HR/LR phenotype of the donor. Current evidence suggests that the NA phagocytic difference is not due to a difference in the binding of target erythrocytes or in the affinity of the ligand-binding site measured by the binding of IgG aggregates [16]. Furthermore, the NA phagocytic difference can be detected with E-ConA which ligate FcγRIII in a carbohydrate-lectin manner independent of the ability of FcγRIII to bind ligand [16]. These functional data support an important role for $Fc\gamma RIII_{PMN}$ in FcγR-mediated phagocytosis and independently predict that $Fc\gamma RIII_{PMN}$ is involved in the initiation of effector functions beyond recognition of ligand.

Biochemical Mechanisms of Synergism

The ability of $Fc\gamma RIII_{PMN}$, with its lack of an obvious mechanism to couple with signal-transducing G proteins and the cytoskeleton, to initiate cell functions prompts several interesting questions about the mechanism of signal transduction by GPI-anchored receptors in general and about the signaling pathways used by FcγRII and $Fc\gamma RIII_{PMN}$ in PMNs in particular. Transmembrane signaling and stimulation of cell proliferation have been demonstrated upon cross-linking of several GPI-linked proteins including Thy-1 and T-cell-activating protein [57]. In some systems there is an apparent requirement for the T-cell receptor (TCR) complex, but since shared binding of antigen by the TCR is not required for this activation process, a non-ligand-dependent interaction between Thy-1 (or T-cell-activating protein) and some shared component(s) of the TCR complex may be important [58]. CD45, which is found in association with both Thy-1 and the TCR, may serve as this component [59]. Obviously in PMNs, the TCR is not expressed, and other collaborative molecules forming a multi-molecular receptor complex have not yet been identified. Precedent for such a multi-molecular receptor complex, however, is found with FcγRIII on NK cells and monocyte/macrophages. Cell surface expression of this receptor isoform requires the co-expression of an accessory chain [60–63], and it has become clear that several members of a family of signal-transducing chains including the ζ chain of the TCR and the γ chain of the high affinity receptor for IgE can associate with $Fc\gamma RIII_{NK}$ [64–67]. Whether

$Fc\gamma RIII_{PMN}$ signals through a multimolecular signal-transducing receptor (similar to the TCR/CD45/Thy-l and $Fc\gamma RIII_{NK}/\gamma/\zeta$ complexes) or through alternative mechanisms [68–70] remains to be determined.

Although $Fc\gamma RIII_{PMN}$ signaling is not dependent on the bridging of ligand binding sites between FcγRII and $Fc\gamma RIII_{PMN}$ by multivalent ligand, the formal possibility that FcγRII interacts with $Fc\gamma RIII_{PMN}$, perhaps through ligand-induced alterations in extracellular domains, and serves as the signal-transducing molecule must be considered. Several lines of evidence, however, argue that FcγRII and $Fc\gamma RIII_{PMN}$ utilize distinct signal transduction pathways. First, the FcγRII induced respiratory burst is pertussis toxin (PT), [36, 38] sensitive, while the $Fc\gamma RIII_{PMN}$ induced respiratory burst is PT insensitive [36]. The Ca^{2+} flux observed upon cross-linking of $Fc\gamma RIII_{PMN}$ is also PT insensitive [31]. Second, the early phase of the FcγRII-induced actin polymerization is independent of intracellular Ca^{2+} since the intracellular calcium chelator, BAPTA, does not alter this actin response while the late phase of this response is only partially sensitive to BAPTA [32]. In contrast, both the early and late phases of FcγRIII-induced actin polymerization are dependent on intracellular Ca^{2+} fluxes and are markedly decreased by BAPTA [32].

Additional observations suggest that multiple pathways may be important and may be engaged by a single receptor species. For example, FcγRII-induced granule release is only partially PT sensitive [35, 38]. Furthermore, Fcγ receptors in activated PMNs appear to use some pathways distinct from those used in the resting state [71]. Taken together, these observations argue against FcγRII and $Fc\gamma RIII_{PMN}$ obligatorily using the same signal activating complex and suggest that multiple signaling pathways may be available in order to generate a complex biological response.

Conclusions

$Fc\gamma RIII_{PMN}$ serves a number of roles: a binding molecule which promotes the engagement of ligand, a signal-transducing molecule which can initiate integrated cell functions, and a signal-transducing molecule which works synergistically with FcγRII to enhance cell functions. The biochemical mechanisms underlying these actions remain, in large part, undefined, but their understanding may set important precedents in Fcγ-receptor biology. Their understanding may also provide insight into the larger questions of selective advantage of a GPI-anchored Fcγ receptor on PMNs. The several individuals described without $Fc\gamma RIII_{PMN}$ indicate that $Fc\gamma RIII_{PMN}$ is not essential under all circumstances. Similar non-lethal receptor deficiencies have been described for FcγRI in 4 related healthy individuals [72]. However, as illustrated by healthy β_2-microglobulin-deficient transgenic mice, elements of the immune system may be critical only in the face of certain challenges [73, 74]. The participation of $Fc\gamma RIII_{PMN}$ in handling certain types of bacteria clearly indicates that in some settings $Fc\gamma RIII_{PMN}$ may be centrally important in host defense [44].

Acknowledgements

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