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FcγRIII Mediates Neutrophil Recruitment to Immune Complexes: A Mechanism for Neutrophil Accumulation in Immune-Mediated Inflammation

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Summary

Neutrophil accumulation is a hallmark of immune complex-mediated inflammatory disorders. Current models of neutrophil recruitment envision the capture of circulating neutrophils by activated endothelial cells. We now demonstrate that immobilized immune complexes alone support the rapid attachment of neutrophils, under physiologic flow conditions. Initial cell tethering requires the low-affinity Fcγ receptor IIIB (FcγRIIIB), and the β_2 integrins are additionally required for the subsequent shear-resistant adhesion. The attachment function of FcγRIIIB may be facilitated by its observed presentation on neutrophil microvilli. In vivo, in a model of acute antiglomerular basement membrane nephritis in which immune complexes are accessible to circulating neutrophils, FcγRIII-deficient mice had a significant reduction in neutrophil recruitment. Thus, the interaction of immune complexes with FcγRIII may mediate early neutrophil recruitment in immune complex-mediated inflammation.

Introduction

There is compelling evidence for a primary pathogenic role of immune complexes (ICs) in many systemic immunologic diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis, and certain forms of vasculitis and glomerulonephritis. IC deposition is associated with the accumulation of neutrophils, which play an important role in the pathogenesis of immune-mediated disease. The interaction of neutrophils with ICs leads to phagocytosis, generation of a respiratory burst, degranulation, and leukotriene production. Neutrophils interact with ICs through Fcγ receptors which bind the Fc domain of immunoglobulins (IgG), and the β_2 integrins (CD11/CD18), particularly Mac-1 (CR3, CD11b/CD18), cooperate with the Fcγ receptors to sustain neutrophil adhesion (Jones et al., 1998). Human neutrophils constitutively express the low-affinity FcγRs, Fcγ receptor IIA (Fcγ RIIA) (CD32) and the glycophosphatidyl inositol (GPI)-linked Fcγ receptor IIIB (FcγRIIIB) (CD16) (Jones and Brown, 1996; van de Winkel and Capel, 1993), both of which bind IgG ICs. The high-affinity activating receptor FcγRI, which binds monomeric IgG, is induced on neutrophils stimulated with IFNγ (Buckle and Hogg, 1989; Hoffmeyer et al., 1997). The importance of FcγRs in promoting IC-mediated inflammation is suggested by studies in FcγR-deficient mice. For example, Fcγ chain-deficient mice had an attenuation of lupus and proliferative glomerulonephritis, and FcγRIII-deficient and Fcγ chain-deficient mice do not develop an Arthus reaction (Hazenbos et al., 1996; Ravetch and Clynes, 1998).

Neutrophil recruitment is an early consequence of IC-mediated inflammation (Abbas et al., 1997). The current model of antibody-dependent neutrophil recruitment is the upregulation of endothelial leukocyte adhesion receptors by cytokines released either from endothelial cells to which antibody is bound and/or by other cells within the vessel wall (Abbas et al., 1997; Carvalho et al., 1996). Members of the selectin family are known to support the initial leukocyte rolling on activated endothelium, and the subsequent firm adhesion is triggered by vessel-associated signals (e.g., chemokines) that upregulate the adhesivity of β_2 integrins on neutrophils for ICAM-1 on endothelial cells (Carlos and Harlan, 1994). Subsequent adhesion of recruited neutrophils to ICs and activated complement amplifies neutrophil activation (Abbas et al., 1997).

In this study, we considered the possibility that FcγR interactions with ICs alone can serve to tether peripheral blood neutrophils under physiologic flow and represent a potential alternate mechanism to selectin-mediated neutrophil recruitment at sites of IC deposition. Previous in vitro studies have shown that neutrophils adhere to ICs under static conditions (Jones and Brown, 1996) and under very low, nonphysiological levels of fluid shear stress (0.28 dynes/cm^2) (D'Arrigo et al., 1995) and that Fcγ RIIA plays a dominant role in this process (D'Arrigo et al., 1995; Graham et al., 1993; Jones and Brown, 1996). However, these studies do not address whether the IC-FcγR interaction can support neutrophil tethering under physiologic flow conditions found in postcapillary venules (Heisig, 1968). Here, we demonstrate that such an interaction does occur in vitro, that FcγRIIIB and not FcγRIIA mediates the initial capture, and that the β_2 integrins mediate the subsequent shear-resistant adhesion. The new function of FcγRIIIB as a tethering receptor is revealed only in a physiological environment containing defined fluid shear stress, since this receptor plays only a minor role in neutrophil adhesion to ICs under static conditions. As is the case for other previously described tethering receptors, we observed FcγRIIIB on the microvilli of neutrophils which may represent the principal sites of contact with substrates under flow. The biological relevance of our findings in vitro was explored in vivo in a model of acute, antiglomerular basement membrane (GBM) glomerulonephritis in which ICs are formed in situ in the GBM and are accessible to circulating neutrophils through open endothelial fenestrae. We show that FcγRIII-deficient mice had a 75%–83% reduction in glomerular neutrophil accumula-

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tion in this model. Similar analysis in Fcγ chain-deficient mice, which in addition to lacking FcγRIII also lack FcγRI, revealed no further decrease in glomerular neutrophil accumulation. Together, these data suggest that FcγRIII interaction with ICs plays a significant role in rapid neutrophil recruitment in IC-mediated inflammation.

Results

Human Peripheral Blood Neutrophils Attach to Immune Complexes under Physiological Flow

Two types of ICs deposit in tissue and have been implicated in the pathogenesis of disease: insoluble ICs (iICs) (precipitates formed at equivalence), which are large latticed precipitates, and smaller soluble ICs (sICs), which are formed in circulation or in situ (Cochrane et al., 1959; Cream et al., 1971; Haakenstad et al., 1982; Joselow et al., 1985; Joselow and Mannik, 1984; Scherzer and Ward, 1978). We generated iICs at equivalence and sICs at eight times antigen excess, using BSA and a polyclonal rabbit antibody to BSA. iICs or sICs were immobilized on glass coverslips, mounted in an in vitro flow chamber, and human peripheral blood neutrophils were perfused across the ICs at different levels of fluid shear stress. Flowing neutrophils readily attached and arrested on iICs at physiologically relevant fluid shear stress (2.0 dynes/cm^2). Neutrophil attachment to sICs occurred at 1.5 dynes/cm^2 (refer to Figure 2B). Neutrophils did not attach to BSA (Figure 1A) under identical conditions, nor did they attach to immobilized human serum prepared to produce complement C3bi or other physiologically relevant substrates, such as fibronectin (data not shown). A comparison of the shear dependence of neutrophil accumulation on iICs and P-selectin, a member of the selectin family of leukocyte adhesion receptors, revealed that neutrophils accumulated on iICs as efficiently as on P-selectin (Figure 1A). However, there were qualitative differences between IC and P-selectin adhesion mechanisms. As previously described (Lawrence and Springer, 1991), neutrophils rolled but did not develop firm adhesion on P-selectin, whereas, on iICs, neutrophils did not roll but arrested and spread after initial attachment (Figure 1B). Spreading occurred within 29.8 $\pm$ 1.8 and 23.6 $\pm$ 1.7 s of initial attachment to iICs and sICs, respectively.

The Low-Affinity FcγRIIIB on Neutrophils Is Required for Neutrophil Tethering to Immune Complexes

The role of FcγRs in neutrophil recruitment to ICs under physiological flow was determined using blocking monoclonal antibodies (mAb) and Fcγ chain-deficient neutrophils. Functional blocking Fab fragments of a mAb to FcγRIII (Mab 3G8) blocked human neutrophil attachment to both iICs and sICs by >95% (Figures 2A and 2B). In contrast, human neutrophils treated with functional blocking Fab fragments of a mAb to FcγRII (Mab IV.3) attached to iIC and sIC with the same efficiency as neutrophils treated with a control antibody against the MHC complex (W6/32) (Figures 2A and 2B). To further address the role of FcγRIII, neutrophils from Fcγ chain deficient-mice were examined. These cells had a complete defect in attachment to iICs under flow (Figure 2C). Interestingly, the critical requirement for FcγRIII in attachment to ICs was apparent only under flow conditions. In a static adhesion assay, treatment of human neutrophils with the functional blocking antibody to FcγRIII (3G8) used in the flow assays was only partially effective (<25%) in preventing adhesion to ICs (data not shown) (Graham et al., 1993; Kusunoki et al., 1994).

The β_2 Integrins Are Required for Shear-Resistant Neutrophil Adhesion to ICs

Neutrophils from patients with leukocyte adhesion deficiency syndrome I (LADI), a disease resulting from a congenital deficiency in β_2 integrins, and Mac-1-deficient murine neutrophils are compromised in IC-stimulated adhesion under static conditions (Graham et al., 1993; Tang et al., 1997). To determine the role of the β_2 integrins in neutrophil interaction with ICs under physiological flow, we utilized neutrophils isolated from two LADI patients with severe and moderate phenotypes and mice deficient in all β_2 integrins, generated by gene targeting (Scharffetter-Kochanek et al., 1998). Initial leukocyte attachment to ICs was not affected in neutrophils isolated from two LADI patients (Figure 3A) or from mice deficient in all β_2 integrins (data not shown). To determine whether LADI neutrophils had a defect in maintaining shear-resistant adhesion to iIC, we used an assay in which cells were allowed to adhere to ICs under static conditions and were then exposed to increasing levels of shear stress to create a detachment force (Figure 3B). Under these conditions, 70%–100% of normal neutrophils remained adherent and spread at shear stress levels up to 30 dynes/cm^2. On the other hand, LADI neutrophils were unable to sustain adhesion at high shear stress. Therefore, the β_2 integrins are not essential for the initial neutrophil attachment to ICs but are required for sustained shear-resistant adhesion and spreading.

FcγRIIIB and Mac-1 Are Sufficient for Optimal Cell Attachment to ICs under Flow

To determine whether FcγRs and β_2 integrins are sufficient for cell capture to ICs under flow, we transfected K562 cells (a human myeloid leukemia cell line with endogenous FcγRIIA) with the relevant receptors. K562 cells or K562 cells stably expressing Mac-1 (K562/Mac-1) were transiently transfected with human FcγRIIIB or FcγRIIA cDNA. A representative FACS analysis of FcγRIIIB/Mac-1-transfected cells is shown (Figure 4A). Similar numbers of FcγRIIIB-transfected K562 cells (Figure 4B) and FcγRIIA-transfected K562 cells (data not shown) attached to ICs under flow conditions. However, accumulation of K562 cells expressing both FcγRIIIB and Mac-1 was significantly higher than K562 cells transfected with FcγRIIIB alone. The enhanced binding to ICs was blocked with an antibody to FcγRIII (Figure 4B). A similar result was obtained with stable transfectants of a sister β_2 integrin, p150,95 (CD11c/CD18) (data not shown). In contrast, K562/Mac-1 cells transfected with FcγRIIA did not show increased binding to ICs compared to FcγRIIA-transfected K562 cells (data not shown). Surface expression of FcγRIIA in FcγRIIA-transfected K562/Mac-1 cells was similar to that of FcγRIIIB in transfected K562/Mac-1 cells (data not shown). Thus,

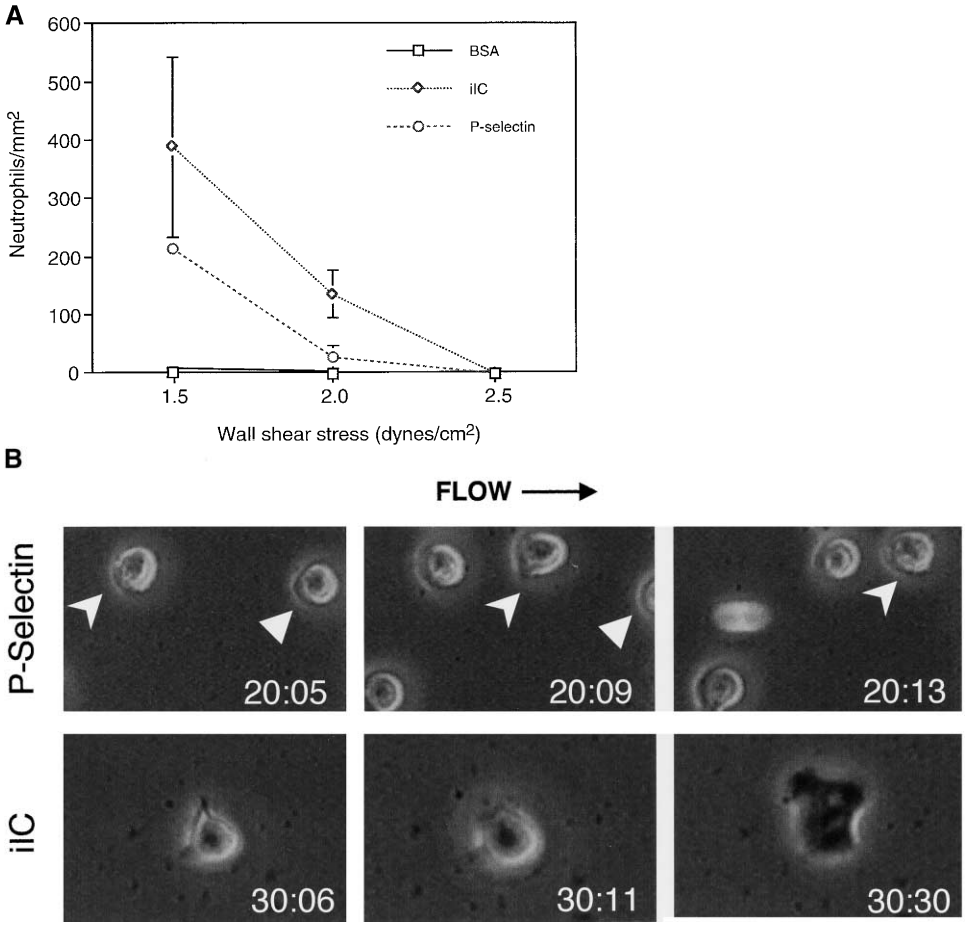


Figure 1. Attachment of Neutrophils to Immune Complexes or P-Selectin under Flow

(A) Insoluble iIC or BSA (5 μg) or purified soluble P-selectin (1 μg) was adsorbed to glass coverslips. Isolated human neutrophils were perfused at varying wall shear stresses into the flow chamber apparatus at 37°C. After 1 min, four fields were analyzed for the number of attached cells. Neutrophils did not adhere to BSA under the shear conditions examined but interacted with iIC beginning at 2.0 dynes/cm^2. Neutrophils also accumulated on P-selectin at 2.0 dynes/cm^2 ($n = 4$).

(B) Representative fields of neutrophils interacting with iIC or P-selectin at 2.0 dynes/cm^2 are shown. The direction of flow is indicated by an arrow. Rolling neutrophils (followed with arrowheads) were observed on P-selectin, whereas adherent, phase-dark cells were observed on iICs over a similar time period.

differences in receptor density cannot account for the observed difference in the ability of Mac-1 to enhance attachment of cells expressing FcγRIIIB versus FcγRIIA to ICs. Therefore, both FcγRIIIB and the β_2 integrins are required for optimal cell attachment to ICs under flow. The requirement for β_2 integrins in the initial attachment of transfected K562 cells under flow is reminiscent of results obtained under static conditions in human monocytes (Graham et al., 1989) and murine macrophages (A.C. and T.N.M., unpublished data), wherein Mac-1 was required both for initial FcγR-mediated cell attachment as well as the subsequent sustained adhesion to ICs. However, this situation is different from our observed results in neutrophils where the β_2 integrins were not required for initial attachment to ICs under flow (Figure 3A). We speculate that the requirement for the functional cooperation of FcγRIIIB with the β_2 integrins may be substituted by an additional surface receptor on neutrophils. Indeed, there is precedence for FcγRs associating with other proteins: FcγRIIA has been recently shown to associate with the gpIb-IX-V complex on platelets (Falati et al., 1999; Sullam et al., 1998).

FcγRIIIB Is Present on the Microvilli

Unattached leukocytes are characterized by numerous cell surface microvilli. The topographic localization of receptors on microvilli may be a property of tethering receptors in general (Breuhl et al., 1997; Butcher and Picker, 1996; Moore et al., 1995; von Andrian et al., 1995). To obtain mechanistic insight into the attachment role of FcγRIII to ICs, we analyzed the surface distribution of FcγRIII by scanning electron microscopy, using immunogold-labeled antibody. In addition, we analyzed the spatial localization of L-selectin, a well-described tethering receptor, and Mac-1. In both human neutrophils (Figures 5A and 5B) and transfected K562 cells (Figures 5D and 5E), 50% of FcγRIIIB was expressed on the microvilli and 50% on the cell body. L-selectin was

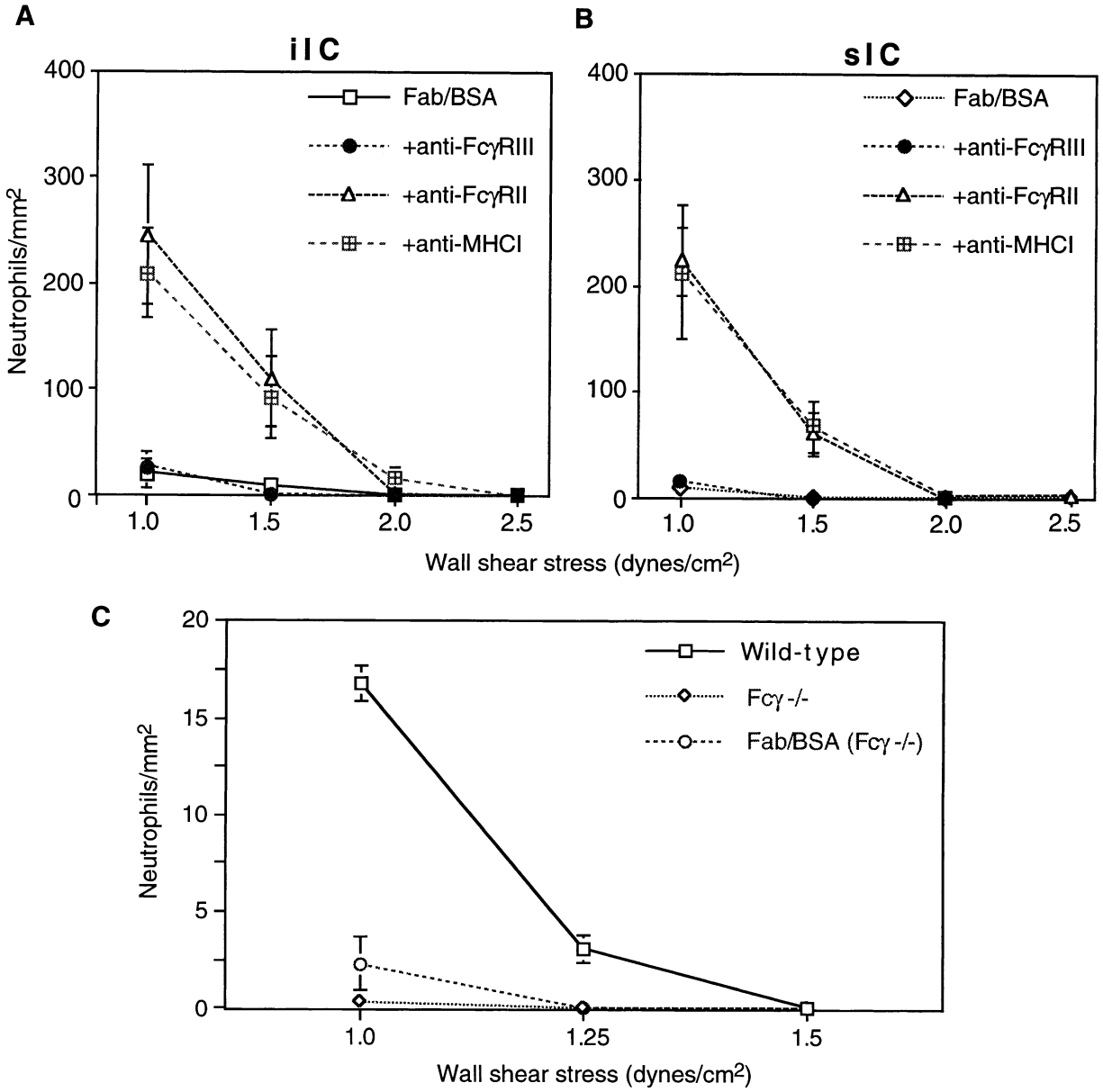


Figure 2. Role of FcγRs in Neutrophil-Immune Complex Interactions under Flow

(A and B) The role of FcγRs in neutrophil attachment to iIC (A) and sIC (B) was examined. Neutrophils pretreated with monoclonal antibodies to FcγRIII (Mab 3G8), FcγRII (Mab IV.3), or MHC complex (W6/32) were perfused into the flow chamber apparatus at various shear rates and the numbers of adherent cells determined. Control antibody W6/32 to the MHC complex caused a slight reduction in binding to iIC as did antibody to FcγRII compared to untreated neutrophils (see Figure 1A). These two antibodies had no effect on neutrophil binding to sIC. Antibody to FcγRIII completely blocked attachment of neutrophils to both iIC and sIC. Minimal binding of untreated neutrophils to Fab/BSA-coated coverslips was observed; n = 6 and 5 independent experiments for iIC and sIC, respectively.

(C) Bone marrow-derived neutrophils from wild-type and Fcγ chain-deficient mice ($Fc\gamma^{-/-}$) were examined for attachment to iIC and Fab/BSA under flow. Normal murine neutrophils attached to iIC, while there was no attachment of $Fc\gamma^{-/-}$ neutrophils to iIC above that seen for Fab/BSA; n = 2 independent experiments with two mice of each genotype per experiment.

present at greater than 90% on the microvilli of neutrophils (Figure 5C), as previously shown (Erlandsen et al., 1993; von Andrian et al., 1995), suggesting that our procedure for detecting microvillus localization of receptors was optimized. Mac-1 was predominantly on the cell body of neutrophils (data not shown), as previously reported (Berlin et al., 1995; Erlandsen et al., 1993). Thus, topographic presentation of FcγRIII on the microvilli may facilitate contact of the cell with ICs, whereas those on the cell body may cooperate with Mac-1 to promote firm adhesion.

FcγRIII Is Required for Neutrophil Accumulation following Anti-Glomerular Basement Membrane Nephritis

Wild-type, FcγRIII-deficient, or Fcγ chain-deficient mice were subjected to acute, passive, heterologous antiglomerular basement membrane nephritis. Nephritis is in-

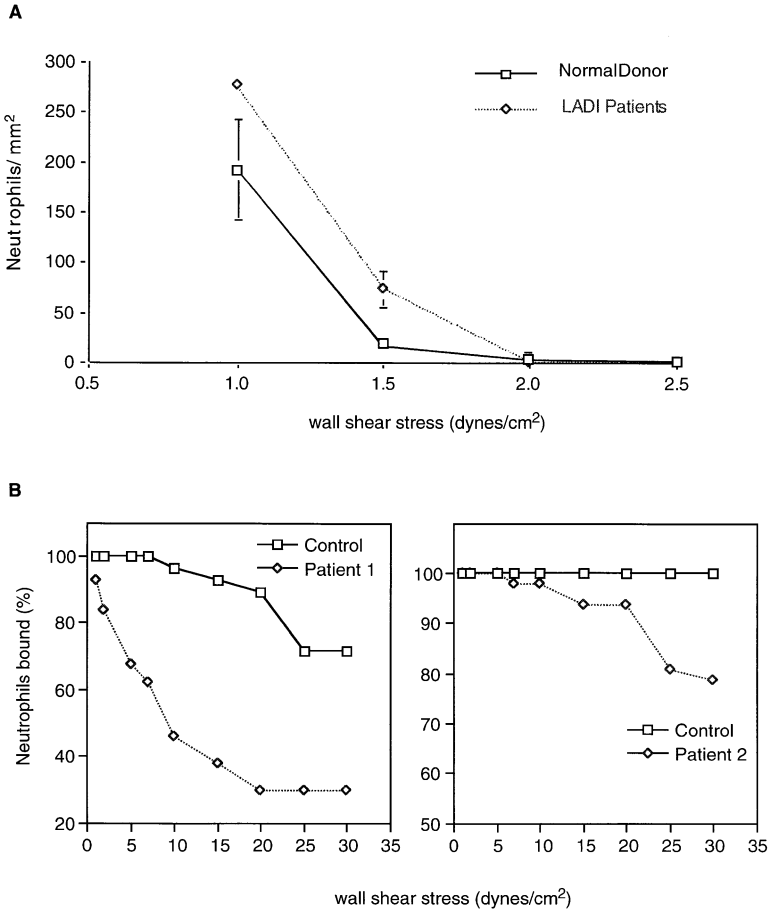


Figure 3. Role of β_2 Integrins in Attachment and Firm Adhesion to ICs under Flow

(A) Neutrophils from normal donors or LADI patients were assayed for interaction with iIC under shear. Attachment of normal and LADI neutrophils to iICs were similar at all wall shear stresses examined. Data are the average of two normal donors and two LADI patients.

(B) To assess the strength of neutrophil adhesion to iIC, neutrophils were incubated with iIC under static conditions, and, after 5 min of contact, controlled flow was applied in staged increments to create a detachment force. The number of neutrophils that remained bound after 1 min at each shear stress point are expressed as the percentage of neutrophils that had initially bound. Data from two normal donors (control) and two patients are shown separately.

duced by the intravenous injection of anti-GBM antisera, which results in the rapid formation of immobilized GBM-anti-GBM ICs in the glomerulus and neutrophil accumulation. On the other hand, an intraperitoneal injection of the antisera does not result in significant neutrophil accumulation (S.K. and T.N.M., unpublished data), suggesting that IC formation in the vessel wall is required for this process. Neutrophil influx peaked at 2 hr after intravenous anti-GBM injection in wild-type mice and returned to baseline levels by 18 hr (Figure 6A). The kinetics of neutrophil accumulation was similar to that previously published by this laboratory (Mayadas et al., 1996; Tang et al., 1997). FcγRIII-deficient mice showed a 75%–83% reduction in glomerular neutrophil accumulation 2 hr and 8 hr after anti-GBM injection (Figure 6A). Indeed, at the 8 hr time point, the number of neutrophils present in the glomeruli of nephritic FcγRIII-deficient mice was similar to that seen in the those of untreated animals (0 hr time point). Total peripheral blood neutrophil counts were similar in wild-type and FcγRIII-deficient mice at the 2 hr time point and were elevated in FcγRIII-deficient mice compared to wild-type mice at the 8 hr time point (Figure 6B). Thus, despite the abundance of circulating neutrophils in FcγRIII-deficient mice, their recruitment to ICs in the glomerulus is severely affected. Both groups of mice had comparable rabbit anti-GBM IgG deposition and glomerular complement C3 deposition, although the latter was low to moderate in both genotypes (Figure 6C).

Tissue injury in acute nephritis is measured by proteinuria. The proximate causes of injury that produce proteinuria include glomerular neutrophil accumulation, glomerular complement deposition, and antibody reactivity with the renal epithelium (Mayadas et al., 1999; Rennke et al., 1981). Proteinuria was minimal at all time points tested ($<55.9 \pm 22.0$ μg albumin/mg creatinine). Despite this, a 2- and 4-fold decrease in proteinuria in FcγRIII-deficient compared to wild-type mice was detected at the 2 and 8 hr time points, respectively (data not shown); however, this was not statistically significant. The absence of significant proteinuria in either the FcγRIII-deficient or wild-type mice may reflect the relatively low amount of glomerular complement C3 deposition observed in both sets of animals, which has been shown in studies using C3-deficient mice to be critical for the development of proteinuria (Mayadas et al., 1999). On the other hand, it could reflect a relative absence of antibodies to the epithelium in our anti-GBM antibody preparation.

To determine the role of FcγRI in the residual glomerular neutrophil accumulation observed in the FcγRIII-deficient mice at the 2 hr time point, we subjected Fcγ chain-deficient mice to anti-GBM nephritis at this time point, since neutrophils from these mice lack both FcγRI and FcγRIII. The reduction in glomerular neutrophil accumulation did not exceed that observed in FcγRIII-deficient mice (Figure 7). Together, these data suggest that FcγRIII is essential for IC-mediated glomerular neutrophil accumulation in vivo with no additional role for FcγRI in this process.

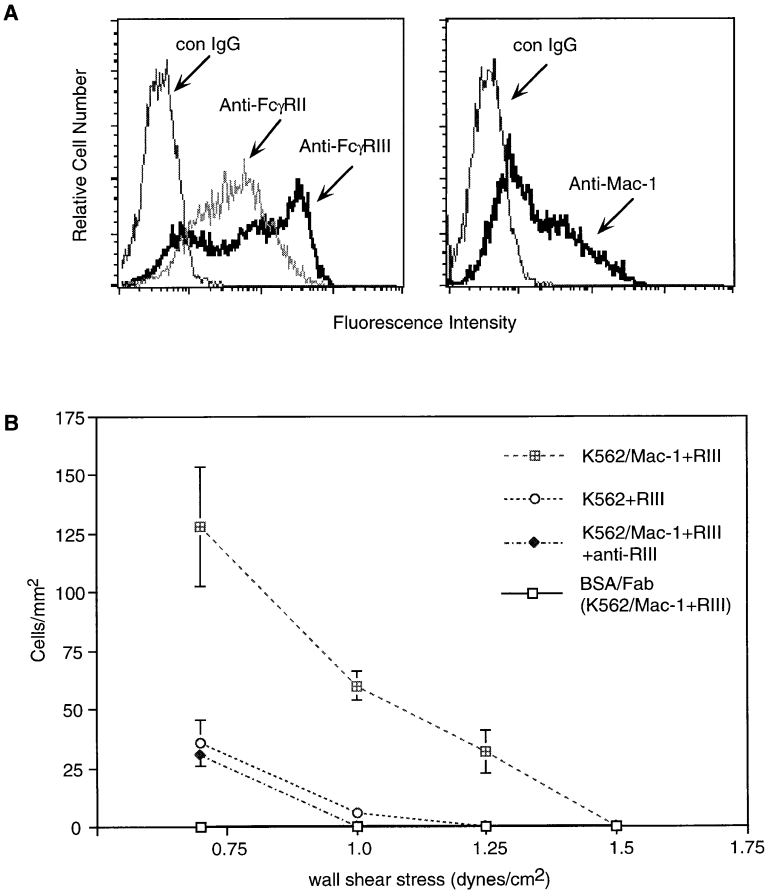


Figure 4. Expression of Both FcγRIIIB and Mac-1 in K562 Cells Is Required for Optimal Cell Attachment to Insoluble Immune Complexes under Physiological Flow

K562 cells (K562) or K562 cells stably expressing human Mac-1 (K562/Mac-1) were transiently transfected with cDNA for human FcγRIIIB(+RIII). (A) Representative FACS profiles are shown for K562/Mac-1 cells transiently transfected with FcγRIIIB and stained with Mab to FcγRIII (3G8), FcγRII (IV.3) (left panel), or Mac-1 (M1/70) (right panel). Positive expression for FcγRIIA is observed in K562/Mac-1 + RIII cells, since K562 cells endogenously express FcγRII. (B) At 48 hr after transfection, the interaction of these cells with iIC under conditions of flow was studied. FcγRIIIB-expressing cells attached to ICs only at low shear stress, while expression of both FcγRIIIB and Mac-1 resulted in significant cell binding to iIC. Antibody to FcγRIII (Mab 3G8) blocked the enhanced attachment of K562/Mac-1 + RIII cells; the remaining adhesion is most likely due to IC interaction with FcγRIIA endogenously expressed by K562 cells. n = 3 independent experiments.

Discussion

The current paradigm of neutrophil recruitment at sites of IC deposition envisions a multistep process of selectin-mediated leukocyte rolling on the endothelium and local, vessel-associated activating signals leading to firm adhesion. It is assumed that the selectin-selectin ligand interactions are uniquely specialized for initiating contact and mediating rolling of leukocytes under flow. It is also widely accepted that an activated endothelium is required for initiating the adhesion cascade (Carlos and Harlan, 1994). Our studies suggest the existence of a selectin-independent mechanism of neutrophil recruitment and arrest at sites of IC deposition and demonstrate a new function for FcγRIII in this process both in vitro and in vivo.

Our in vitro studies suggest that ICs alone can support rapid attachment and arrest of peripheral blood human neutrophils under physiologically relevant fluid shear stress, suggesting that an activated endothelium and

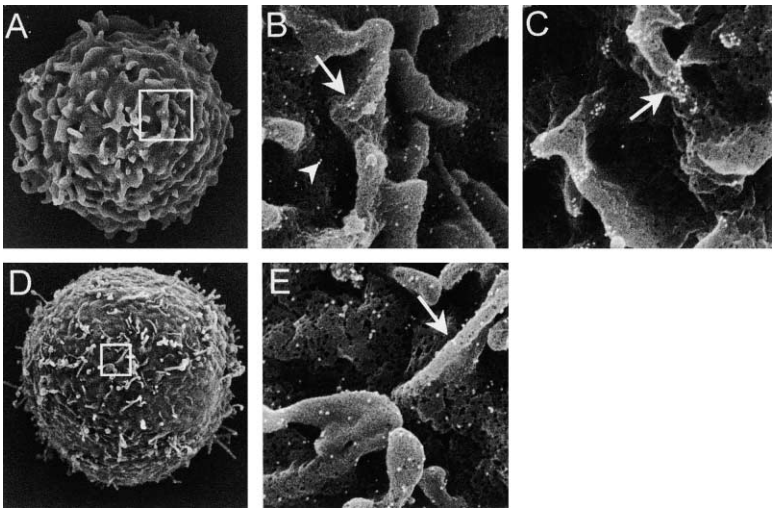
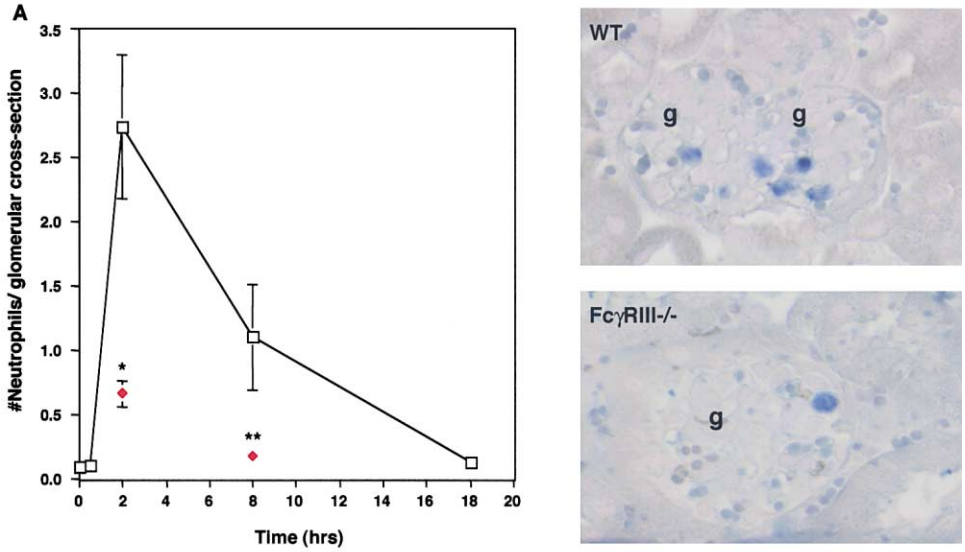


Figure 5. Immunolocalization of FcγRIII and L-Selectin on Human Neutrophils and K562 Cells

Low-magnification LVSEM backscatter electron image showing membrane ruffles and microvilli on the cell surface of a human neutrophil (A) and a K562 cell transfected with FcγRIIIB (D). High-magnification image of immunostaining for FcγRIIIB on neutrophils (B) and transfected K562 cells (E). High-magnification image of neutrophils stained for L-selectin (C). Immunogold particles on the surface projections (arrow) (i.e., microvilli and ruffles) and on the cell body (arrowhead) are indicated.



B

	WT (2hr)	FcγRIII-/- (2hr)	WT (8hr)	FcγRIII-/- (8hr)
% neutrophils	45±5	48±3	69±3	90±7#
Total number of Neutrophils X $10^6/\mu l$	4.3±0.8	3.3±0.6	8.6±1.4	11.9±3.4

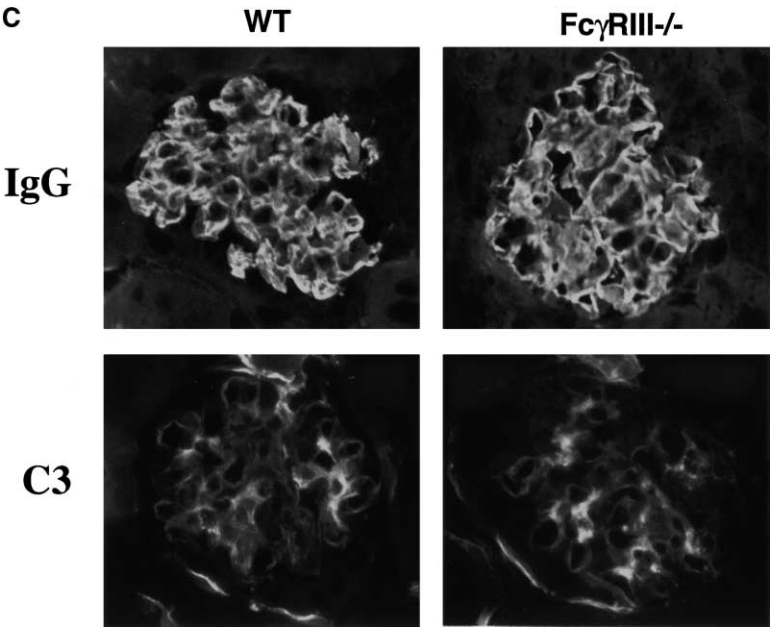


Figure 6. Glomerular Neutrophil Accumulation in FcγRIII-Deficient Mice

FcγRIII-deficient mice and wild-type counterparts were injected intravenously with rabbit anti-mouse GBM antisera, and both kidneys were harvested at the indicated time points. (A) The number of neutrophils per glomerular cross-section in wild-type (square) and FcγRIII-deficient (diamond) mice were assessed on dichloroacetate esterase stained sections. n = 9 and n = 6 for each group at the 2 and 8 hr time point, respectively, and n = 3 for the other time points. The number of neutrophils per glomerular cross-section in the FcγRIII-deficient mice was significantly decreased compared to wild-type mice. Data ± SEM. *, $p < 0.0005$; **, $p < 0.05$. Representative glomerular cross-section with neutrophils (stained blue) from a wild-type and mutant (*FcγRIII*$^{-/-}$) mouse is shown on the right. Individual glomeruli are indicated with "g." (B) The percentage as well as the total number of peripheral blood neutrophils in wild-type and mutant mice was determined. #, $p < 0.05$. (C) Immunohistochemical analysis of complement C3 and rabbit anti-mouse IgG in kidneys from the animals shown in (A) was undertaken. FcγRIII-deficient and wild-type mice had comparable glomerular rabbit IgG deposition (IgG) and complement C3 deposition (C3). Representative sections are shown.

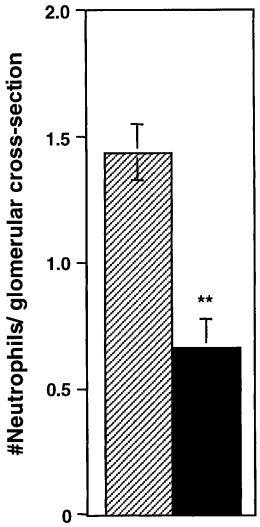


Figure 7. Glomerular Neutrophil Accumulation in Fcγ Chain-Deficient Mice

Wild-type (n = 4; hatched bar) and Fcγ chain-deficient mice (n = 4; black bar) were injected with anti-GBM antisera, and their kidneys were harvested 2 hr later. Glomerular neutrophil accumulation was assessed as described in Figure 6. Fcγ chain-deficient mice had a significant decrease in neutrophil accumulation compared to wild-type mice. **, $p < 0.05$.

vessel-associated activating factors such as chemokines are not essential for neutrophil recruitment at sites of IC deposition. There is correlative in vivo evidence that ICs may be a nidus for recruitment of flowing blood neutrophils. Analyses of experimental models of IC-mediated glomerulonephritis or vasculitis, as well as histologic findings in human glomerulonephritides, show that only ICs accessible to blood elements but not those deposited in the extravascular compartments lead to neutrophil accumulation (Couser, 1985, 1993; Fries et al., 1988; Fujigaki et al., 1997; Salant et al., 1985). ICs in the glomerular basement membrane can be accessed by neutrophils through open endothelial fenestrae in the glomerulus. Alternatively, in tissues where the endothelium is continuous, neutrophils may be recruited to ICs bound to human endothelial FcγRIIA (Groger et al., 1996, 1999; Pan et al., 1998) or deposited between endothelial cells under conditions of vascular permeability (Cochrane and Hawkins, 1968). Our proposed mechanism of IC-stimulated neutrophil recruitment may have relevance not only for IC-mediated inflammation but also for the physiologic clearance of ICs from tissue. This has been shown to occur via neutrophil recruitment, IC clearance, the return of the neutrophils into the circulation, and the restoration of the functional and structural integrity of the tissue (Feintzeig et al., 1986; Fries et al., 1988). Indeed, immune-mediated diseases may occur when the physiologic clearance of tissue deposited and/or circulating ICs is defective or overwhelmed.

FcγRIIIB was essential for the attachment of human peripheral blood neutrophils to ICs under physiological flow. In contrast, FcγRIIA played no detectable role in this process. This is noteworthy, since most previous IC-related neutrophil functions, including neutrophil adhesion to ICs under static conditions, phagocytosis, and antibody-dependent cellular cytotoxicity (ADCC), have been attributed largely to FcγRIIA (Unkeless et al., 1995). On the other hand, FcγRIIIB has been shown to trigger a Ca^{2+} transient (Vossebeld et al., 1995) and a respiratory burst (Zhou and Brown, 1994), but there are conflicting reports of its role in other neutrophil functions (Unkeless et al., 1995; van de Winkel and Capel, 1993). Our studies attribute an IC-stimulated neutrophil function solely to FcγRIII. The critical role of FcγRIIIB in tethering neutrophils to ICs was revealed only under physiologic flow. This situation has striking similarity to the selectins whose function in mediating leukocyte rolling was revealed only under physiologic shear stress (Abbassi et al., 1993; Lawrence and Springer, 1991). Thus, like the selectins, a novel tethering function of FcγRIIIB was revealed under a physiologically relevant environment containing defined fluid shear stress.

The tethering function of FcγRIIIB may be regulated by the functional availability of the receptor on the cell surface. L-selectin as well as other well-described tethering receptors have been shown to be present on the cell surface microvilli of resting neutrophils, which are thought to represent the principal sites of initial contact with the endothelium under flow. Like L-selectin, FcγRIIIB is present on microvillus projections, which may facilitate the initiation of cell contact with ICs under flow. Interestingly, electron micrographs of leukocytes accumulated in the glomerular capillary wall show leukocyte microvilli penetrating the open endothelial fenestrae and in direct contact with the ICs deposited in the subendothelial matrix (Fries et al., 1988), suggesting that the microvillus localization of FcγRIII may be functionally relevant in IC-mediated inflammation. However, unlike L-selectin, which is almost exclusively on microvilli, FcγRIIIB is present both on the microvilli and the cell body. The pool of FcγRIII on the cell body may be important in cooperating with Mac-1, found predominantly on the cell body, to sustain adhesion. Receptor shedding may help limit the availibility of tethering receptors and thus limit neutrophil recruitment. Like L-selectin, which is shed upon neutrophil activation (Kishimoto et al., 1989), FcγRIIIB is shed upon stimulation with f-Met-Leu-Phe (Huizinga et al., 1988; Lamour et al., 1993; Tosi and Zakem, 1992), which we propose may serve to regulate IC-stimulated neutrophil recruitment.

The importance of FcγRIII in neutrophil recruitment in the context of IC-mediated inflammation was revealed in a model of acute, anti-GBM nephritis in which the intravenous injection of anti-GBM antibody elicits rapid glomerular neutrophil accumulation. Previous studies using knockout mice have shown that neutrophil recruitment in this model does not require complement C3 or the endothelial leukocyte adhesion receptors P-selectin, ICAM-1 (Mayadas et al., 1996; Tang et al., 1997), or E-selectin (T.N.M., unpublished data). In contrast, FcγRIII was critical for glomerular neutrophil accumulation. It may be argued that the observed role of FcγRIII in neutrophil recruitment may be due not to FcγRIII on neutrophils but on other cell types in the kidney. Murine kidneys do have resident macrophages (Hume and Gordon, 1983), a source of FcγRIII, and, in vitro, human mesangial cells can be induced to express functional FcγRIIIA after stimulation with a combination of IFNγ and LPS

(Radeke et al., 1994). However, immunohistological analysis of murine renal tissue revealed FcγRII/III expression only following chronic, proliferative anti-GBM nephritis and not after acute anti-GBM nephritis (S.K. and T.N.M., unpublished data). Thus, it is most likely that the deficiency of FcγRIII on neutrophils is principally responsible for the observed phenotype in FcγRIII-deficient mice. The remaining glomerular neutrophil recruitment in these mice at the 2 hr time point indicates that some other adhesion receptor(s) come into play at this time point. Candidates include $\alpha_4\beta_1$ or L-selectin on neutrophils which mediate neutrophil rolling on activated endothelium (Johnston and Kubes, 1999; Luscinskas and Gimbrone, 1996). The function of FcγRIIA relative to FcγRIII in neutrophil accumulation could not be assessed in vivo because, unlike human neutrophils, murine neutrophils express the inhibitory receptor FcγRIIB and not the activating receptor FcγRIIA.

We demonstrate here that the β_2 integrins are not required for initial neutrophil attachment to ICs but are required for shear-resistant adhesion to ICs under flow. Adhesion to ICs occurs in the absence of exogenous activating stimuli and most likely requires FcγR-mediated Mac-1 activation (Jones et al., 1998). Although our studies in transfected K562 cells suggest that the β_2 integrins may also promote cell attachment to ICs, their contribution to this process may be redundant in human neutrophils. The in vivo relevance of the observed role for the β_2 integrins in sustaining shear-resistant neutrophil adhesion under flow has been suggested in our previous study in Mac-1-deficient mice subjected to an acute model of anti-GBM nephritis. We found that Mac-1 was not necessary for initial neutrophil recruitment but was required for sustained neutrophil accumulation in the glomerulus (Tang et al., 1997).

In conclusion, our data suggest that FcγRIII-IC interactions within the vessel wall may be an early step in neutrophil recruitment in IC-mediated inflammation, which is of pathogenic significance in several systemic immunological disorders. This mechanism of recruitment would not require either endothelial cell or neutrophil activation and would thus likely preceed any endothelial activation-dependent mechanisms of leukocyte recruitment envisioned at sites of IC deposition. In addition to neutrophils, FcγRIII and the β_2 integrins are expressed on several other leukocyte subsets involved in immune-mediated inflammation, including monocytes, NK cells, and γδ T cells (Gessner et al., 1998). Discerning the potential role of these receptors in the recruitment of these cells to sites of IC deposition may lead to further insights into the pathogenesis of acute and chronic IC-mediated inflammatory disorders.

Experimental Procedures

Recombinant DNA

The FcγRIIA cDNA in the Rc-CMV expression vector (Invitrogen) was provided by Dr. J. van de Winkel (University Medical Center, Utrecht, Netherlands). The FcγRIIIB cDNA was a gift from Dr. B. Seed (Massachusetts General Hospital, Boston, MA) and the HindIII-FspI fragment was cloned into the CMV expression vector pCR3.1 (Invitrogen).

Cell Culture and Transfection

K562 cells, K562/CR3 cells, and K562/p150,95 cells were provided by Drs. Lloyd Klickstein and Timothy Springer and were cultured in RPMI/10% FCS. For transfection assays, 4×10^6 cells were resuspended in 450 μl RPMI/10% FCS and mixed with 20 μg plasmid DNA, and electroporation was performed using Biorad equipment set at 960 μF/250 v. Cells were then washed, resuspended in RPMI/10% FCS, and used in the flow assay 48 hr after transfection. Transfection efficiency was 30%–40% as monitored by FACs analysis of the expression of surface receptors.

Monoclonal Antibodies

Antibodies to FcγRIII [3G8 F(ab′)$_2$] and FcγRII (IV.3-Fab) were purchased from Medarex. An F(ab′)$_2$ fragment of an antibody to the MHC complex (W6/32) was generated as previously described (Luscinskas et al., 1996). Monoclonal antibody to Mac-1 (M1/70) and mouse low-affinity FcγRIII/II (2.4G2) were purchased from PharMingen. Murine antibody to L-selectin was LAM1-5 (IgG1) (Spertini et al., 1991). Colloidal gold-conjugated secondary monoclonal antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc.

Flow Cytometry

K562 cells were washed with PBS/1% FCS and incubated with fluorescently tagged primary antibodies for 30 min on ice, washed twice with PBS, and fixed with 1% formaldehyde. An isotype-matched antibody was included as a control. Expression was then analyzed using a Becton-Dickinson FACScan.

Neutrophil Isolation

Anti-coagulated whole blood was collected from normal donors and from two patients who clinically had mild and severe forms of LADI with 50% and 12% of normal expression level of the b2 integrin, respectively (T.N.M., unpublished data). Human neutrophils were isolated as previously described (Bevilacqua et al., 1987) and resuspended in RPMI + 0.1% BSA. Murine neutrophils were isolated as follows. Bone marrow was harvested from the femur and tibias of mice and subjected to RBC lysis as previously described (Lowell et al., 1996). Neutrophils represented 60%–70% of the total cell population, as identified by morphology on Wright-Giemsa-stained cytospins of the samples.

Adhesion Assays under Flow

The parallel plate flow chamber used in this study has been described in detail (Luscinskas et al., 1994). Coverslips coated with substrates were incorporated into a parallel plate flow chamber, and peripheral blood human neutrophils (10^6/ml) or murine neutrophils (10^6/ml) in RPMI (Biowhittaker) + 0.1% BSA (35% solution purchased from ICN) were infused at a shear stress of 0.60–2.0 dynes/cm^2, which is within the range measured in postcapillary venules and shown to support selectin-mediated rolling (Heisig, 1968; Lawrence and Springer, 1991). In antibody blocking experiments, cells were pretreated with antibody for 12 min at room temperature and then diluted with perfusion medium to 10^6 cells/ml. After 1 min of continuous flow to allow the cells to equilibrate, the number of adherent neutrophils in four random fields visualized for 10 s per field were determined and averaged.

Substrate-Coated Coverslips

ICs at equivalence (iIC) and at eight times antigen excess (sIC) were formed using BSA and a polyclonal antibody to BSA (Sigma Chemical Company). iICs were washed in PBS and then resuspended in PBS, using a 25 G needle, and sICs were centrifuged to remove insoluble complexes. Acetone-cleaned glass coverslips were incubated with 5 μg of iIC and sIC in a circumscribed 5 mm diameter circle within the 25 mm diameter glass coverslips for 2 hr at room temperature. Additional coverslips were coated with 1 μg of soluble P-selectin (gift from Genetics Institute) as described (Goetz et al., 1996). The coverslips were rinsed in one direction just before use in the flow chamber.

Scanning Electron Microscopy

Cells were labeled on ice with primary mAb (20 μg/ml), washed, stained with colloidal gold-conjugated secondary mAb, and fixed as previously described (Erlandsen et al., 1993). Mab M1/70 was used to detect Mac-1, 3G8 was used to detect FcγRIII, and LAM-

1-5 was used to detect L-selectin. Ultrastructural analysis of antigen distribution in samples was performed using a Hitachi S-900 LVSEM equipped with an Autrata-type YAG backscatter detector. The number of gold particles on the cell body and microvilli was quantitated as described by Erlandsen et al. (1993).

Mice

Age-matched wild-type and Fcγ chain-deficient mice of the C57BL/6 strain (Takai et al., 1994) were purchased from Taconic (*Fcer1g* knockout mice). Wild-type and CD18-deficient mice of the 129Sv/C57Bl6 mixed background and their age-matched wild-type counterparts were kindly provided by Dr. Arthur Beaudet (Scharffetter-Kochanek et al., 1998) and were housed in a virus antibody-free facility at Harvard Medical School. Age-matched wild-type and FcγRIII-deficient mice (mice deficient for the ligand binding α chain of FcγRIII) (Hazenbos et al., 1996) of the C57BL/6 strain were purchased from Jackson Laboratory. Commerically purchased mice were housed in a virus antibody-free facility at Longwood Medical Research Center, Brigham and Women's Hospital.

Induction of Acute Anti-GBM Nephritis

Rabbit anti-mouse glomerular basement membrane antisera was prepared by immunizing rabbits with mouse GBM, essentially as described by Schrijver et al. (1990). Awake male mice, 9–10 weeks old, were given a tail vein injection of 300 μl of antisera. This dose was chosen since it gave significant glomerular neutrophil influx in the early heterologous phase. This dose did not lead to glomerular thrombosis and necrosis and, used in the absence of Complete Freunds Adjuvant/Rabbit IgG, was associated with an insignificant autologous phase (S.S. and T.N.M., unpublished data). At different time points, animals were anesthetized with 2.5% avertin (15 μl/g body weight), peripheral blood was sampled from the retroorbital plexus into tubes containing a final concentration of 5 mM EDTA, and mice were euthanized by CO_2 inhalation. Both kidneys were harvested, halved, and snap frozen in optimum cooling temperature (OCT) embedding medium (Miles, Elkhart, Indiana, USA).

Histochemical Analysis of Renal Tissue

Sections (4 μm) were cut from OCT-embedded kidneys. Immunofluorescence microscopy was performed using standard immunofluorescent techniques with the following primary antibodies: FITC-conjugated goat F(ab')$_2$ fragment to mouse complement C3 (ICN Pharmaceuticals, Inc., Aurora, Ohio, USA) at four dilutions from l:100 to 1:800; FITC-conjugated goat anti-rabbit IgG (to detect heterologous rabbit IgG in the glomerulus) (Pel-Freeze Biologicals) at four dilutions from 1:400 to 1:3200. For a semiquantitative assessment of the glomerular deposition of heterologous (rabbit) antibody or complement C3 deposition, a semiquantitative scoring of staining intensity from 0 to 4+ was given, and the end point positive titer for detection of staining assessed in a blinded protocol was determined as previously described (Rosenkranz et al., 1999).

Glomerular PMN infiltration was assessed by the chloroacetate esterase reaction which was perfomed essentially as previously reported (Yam et al., 1971). For each animal, glomerular neutrophil counts in 100 glomeruli/two kidney sections were made, and the number of neutrophils per glomerular cross-section was determined.

Determination of Urinary Albumin and Creatinine Concentration

To determine albuminuria, a double-sandwich ELISA was performed as previously described (Mayadas et al., 1996). Creatinine was quantitated spectrophotometrically using a commerically available kit (Sigma Chemical Co.). To standardize urine albumin excretion for glomerular filtration rate, proteinuria was expressed as μg of urinary albumin per mg of urinary creatinine.

Peripheral Blood Leukocyte Counts

Anticoagulated peripheral blood was subjected to RBC lysis, and total leukocyte counts were determined by Coulter count as previously described (Coxon et al., 1996). Blood smears were prepared, stained with Wright-Giemsa, and a differential neutrophil count was performed. The total peripheral blood neutrophil count for each animal was determined by multiplying the total leukocyte count with percent neutrophils determined on the blood smear.

Statistical Analysis

Data are expressed as the mean $\pm$ SEM. Statistical comparison of means was performed by two-tailed unpaired Student's t test.

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References

Abbas, A.K., Lichtman, A.H., and Pober, J.S. (1997). Immune-mediated tissue injury and disease. In Cellular and Molecular Immunology (Philadelphia: W.B. Saunders), pp. 423–438.

Abbassi, O., Kishimoto, T.K., McIntire, L.V., Anderson, D.C., and Smith, C.W. (1993). E-selectin supports neutrophil rolling in vitro under conditions of flow. J. Clin. Invest. *92*, 2719–2730.

Berlin, C., Bargatze, R.F., Campbell, J.J., von Andrian, U.H., Szabo, M.C., Hasslen, S.R., Nelson, R.D., Berg, E.L., Erlandsen, S.L., and Butcher, E.C. (1995). α4 integrins mediate lymphocyte attachment and rolling under physiologic flow. Cell *80*, 413–422.

Bevilacqua, M.P., Pober, J.S., Wheeler, M.E., Cotran, R.S., and Gimbrone, M.A., Jr. (1987). Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. J. Clin. Invest. *76*, 2003–2011.

Breuhl, R.E., Moore, K.L., Lorant, D.E., Borregard, N., Zimmerman, G.A., McEver, R.P., and Bainton, D.F. (1997). Leukocyte activation induces surface redistribution of P-selectin glycoprotein ligand-1. J. Leukoc. Biol. *61*, 489–499.

Buckle, A.M., and Hogg, N. (1989). The effect of IFN-gamma and colony-stimulating factors on the expression of neutrophil cell membrane receptors. J. Immunol. *143*, 2295–2301.

Butcher, E.C., and Picker, L.J. (1996). Lymphocyte homing and homeostasis. Science *272*, 60–66.

Carlos, T.M., and Harlan, J.M. (1994). Leukocyte-endothelial adhesion molecules. Blood *84*, 2068–2101.

Carvalho, D., Savage, C.O.S., Black, C.M., and Pearson, J.D. (1996). IgG anti-endothelial cell autoantibodies from Scleroderma patients induce leukocyte adhesion to human vascular endothelial cells in vitro. J. Clin. Invest. *97*, 111–119.

Cochrane, C.G., and Hawkins, D. (1968). Studies on circulating immune complexes: Factors governing the ability of circulating complexes to localize in blood vessel. J. Exp. Med. *127*, 137–154.

Cochrane, C.G., Weigle, W.O., and Dixon, F.J. (1959). The role of polymorphonuclear leukocytes in the initiation and cessation of the Arthus vasculitis. J. Exp. Med. *110*, 481–483.

Couser, W.G. (1985). Mechanisms of glomerular injury in immune complex disease. Kidney Int. *28*, 569–583.

Couser, W.G. (1993). Pathogenesis of glomerulonephritis. Kidney Int. *44*, S19–26.

Coxon, A., Rieu, P., Barkalow, F.J., Askari, S., von-Andrian, U.H., Arnaout, M.A., and Mayadas, T.N. (1996). A novel role for the β2 integrin, CD11b/CD18, in neutrophil apoptosis: A homeostatic mechanism in inflammation. Immunity *5*, 653–666.

Cream, J.J., Bryceson, A.D.M., and Ryder, G. (1971). Disappearance of immunoglobulin and complement from the arthus reaction and its relevance to studies of vasculitis in man. Br. J. Dermatol. *84,* 106–109.

D'Arrigo, C., Candal-Couto, J.J., Greer, M., Veale, D.J., and Woof, J.M. (1995). Human neutrophil Fc receptor-mediated adhesion under flow: A hollow fibre model of intravascular arrest. Clin. Exp. Immunol. *100*, 173–179.

Erlandsen, S.L., Hasslen, S.R., and Nelson, R.D. (1993). Detection and spatial distribution of the β2 integrin (Mac-1) and L-selectin (LECAM-1) adherence receptors on human neutrophils by high-resolution field emission SEM. J. Histochem. Cytochem. *41*, 327–333.

Falati, S., Edmead, C.E., and Poole, A.W. (1999). Glycoprotein Ib-V-IX, a receptor for von Willebrand factor, couples physically and functionally to the Fc receptor gamma-chain, Fyn, and Lyn to activate human platelets. Blood *94*, 1648–1656.

Feintzeig, I.D., Dittmer, J.E., Cybulsky, A.V., and Salant, D.J. (1986). Antibody, antigen, and glomerular capillary wall charge interactions: Influence of antigen location on in situ immune complex formation. Kidney Int. *29*, 649–657.

Fries, J.W.U., Mendrick, D.L., and Rennke, H.G. (1988). Determinants of immune complex-mediated glomerulonephritis. Kidney Int. *34*, 333–345.

Fujigaki, Y., Nagase, M., Kojima, K., Yamamoto, T., and Hishida, A. (1997). Glomerular handling of immune complex in the acute phase of active in situ immune complex glomerulonephritis employing cationized ferritin in rats. Ultrastructural localization of immune complex, complements and inflammatory cells. Virchows Arch. *431*, 53–61.

Gessner, J.E., Heiken, H., Tamm, A., and Schmidt, R.E. (1998). The IgG Fc receptor family. Ann. Hematol. *76*, 231–248.

Goetz, D.J., Ding, H., Atkinson, W.J., Vachino, G., Camphausen, R.T., Cumming, D.A., and Luscinskas, F.W. (1996). A human colon carcinoma cell line exhibits adhesive interactions with P-selectin under fluid flow via a PSGL-1 independent mechanism. Am. J. Path. *149*, 1661–1673.

Graham, I.L., Gresham, H.D., and Brown, E.J. (1989). An immobile subset of plasma membrane CD11b/CD18 (Mac-1) is involved in phagocytosis of targets recognized by multiple receptors. J. Immunol. *142*, 2352–2358.

Graham, I.L., Lefkowith, J.B., Anderson, D.C., and Brown, E.J. (1993). Immune complex-stimulated neutrophil LTB4 production is dependent on β2 integrins. J. Cell Biol. *120*, 1509–1517.

Groger, M., Sarmay, G., Fiebiger, E., Wolff, K., and Petzelbaur, P. (1996). Dermal microvascular endothelial cells express CD32 receptors in vivo and in vitro. J. Immunol. *156,* 1549–1556.

Groger, M., Fischer, G.F., Wolff, K., and Petzelbauer, P. (1999). Immune complexes from vasculitis patients bind to endothelial Fc receptors independent of the allelic polymorphism of FcgammaRIIa. J. Invest. Dermatol. *113*, 56–60.

Haakenstad, A.O., Striker, G.E., and Mannik, M. (1982). The disappearance kinetics and glomerular deposition of small-latticed soluble immune complexes. Immunology *47*, 407–414.

Hazenbos, W.L.W., Gessner, J.E., Hofhuis, F.M.A., Kuipers, H., Meyer, D., Heijnen, A.F.M., Schmidt, R.E., Sandor, M., Capel, P.J.A., Daeron, M., et al. (1996). Impaired IgG-dependent anaphylaxis and arthus reaction in FcgRIII (CD16) deficient mice. Immunity *5,* 181–188.

Heisig, N. (1968). Functional analysis of the microcirculation in the exocrine pancreas. Adv. Microcirc. *1*, 89–94.

Hoffmeyer, F., Witte, K., and Schmidt, R.E. (1997). The high-affinity Fc gamma RI on PMN: Regulation of expression and signal transduction. Immunology *92*, 544–552.

Huizinga, T.W., van der Schoot, C.E., Jost, C., Klaassen, R., Kleijer, M., von dem Borne, A.E., Roos, D., and Tetteroo, P.A. (1988). The PI-linked receptor FcRIII is released on stimulation of neutrophils. Nature *333,* 667–669.

Hume, D.A., and Gordon, S. (1983). Mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80. Identification of resident macrophages in renal medullary and cortical interstitium and the juxtaglomerular complex. J. Exp. Med. *157*, 1704–1709.

Johnston, B., and Kubes, P. (1999). The alpha4-integrin: An alternative pathway for neutrophil recruitment? Immunol. Today. *20*, 545–550.

Jones, S.L., and Brown, E.J. (1996). Functional cooperation between Fcγ receptors and complement receptors in phagocytes. In Human IgG Fc Receptors, J.G.J. van de Winkel and P.J.A. Capel, eds (Austin, TX: R.G. Landes Co.), pp. 149–163.

Jones, S.L., Knaus, U.G., Bokoch, G.M., and Brown, E.J. (1998). Two signaling mechanisms for activation of alphaM beta2 avidity in polymorphonuclear neutrophils. J. Biol. Chem. *273*, 10556–10566.

Joselow, S.A., and Mannik, M. (1984). Localization of preformed, circulating immune complexes in murine skin. J. Invest. Dermatol. *82*, 335–340.

Joselow, S.A., Gown, A., and Mannik, M. (1985). Cutaneous deposition of immune complexes in chronic serum sickness of mice induced with cationized or unaltered antigen. J. Invest. Dermatol. *85*, 559–563.

Kishimoto, T.K., Jutila, M.A., Berg, E.L., and Butcher, E.C. (1989). Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. Science *245*, 1238–1241.

Kusunoki, T., Tsuruta, S., Higashi, H., Hosoi, S., Hata, D., Sugie, K., Mayumi, M., and Mikawa, H. (1994). Involvement of CD11b/CD18 in enhanced neutrophil adhesion by Fcγ receptor stimulation. J. Leukoc. Biol. *55*, 735–742.

Lamour, A., Soubrane, C., Ichen, M., Pennec, Y.L., Khayat, D., and Youinou, P. (1993). Fc-gamma receptor III shedding by polymorphonuclear cells in primary Sjogren's syndrome. Eur. J. Clin. Invest. *23*, 97–101.

Lawrence, M.B., and Springer, T.A. (1991). Leukocytes roll on a selectin at physiologic flow rates: Distinction from and prerequisite for adhesion through integrins. Cell *65*, 859–873.

Lowell, C.A., Fumagalli, L., and Berton, G. (1996). Deficiency of src family kinases p59/61hck and p58c-fgr results in defective adhesion-dependent neutrophil functions. J. Cell Biol. *133*, 895–910.

Luscinskas, F.W., and Gimbrone, M.A. (1996). Endothelial-dependent mechanisms in chronic inflammatory leukocyte recruitment. Annu. Rev. Med. *47*, 413–421.

Luscinskas, F.W., Kansas, G.S., Ding, H., Pizcueta, P., Schleiffenbaum, B.E., Tedder, T.F., and Gimbrone, M.A. (1994). Monocyte rolling, arrest and spreading on IL-4-activated vascular endothelium under flow is mediated via sequential action of L-selectin, β1-integrins, and β2 integrins. J. Cell Biol. *125*, 1417–1427.

Luscinskas, F.W., Ding, H., Tan, P., Cumming, D., Tedder, T.F., and Gerritsen, M.E. (1996). L- and P-selectins, but not CD49d (VLA4) integrins, mediate monocyte initial attachment to TNF-activated vascular endothelium in vitro. J. Immunol. *156*, 326–335.

Mayadas, T.N., Mendrick, D.L., Brady, H.R., Tang, T., Papayianni, A., Assmann, K.J.M., Wagner, D.D., Hynes, R.O., and Cotran, R.S. (1996). Acute passive anti-glomerular basement membrane nephritis in P-selectin-deficient mice. Kidney Int. *49*, 1342–1349.

Mayadas, T.N., Rosenkranz, A., and Cotran, R.S. (1999). Glomerular inflammation: Use of genetically deficient mice to elucidate the roles of leukocyte adhesion molecules and Fcg-receptors in vivo. Curr. Opin. Nephrol. Hyperten. *8,* 293–298.

Moore, K.L., Patel, K.D., Breuhl, R.E., Fugang, L., Johnson, D.L., Lichenstein, H.S., Cummings, R.D., Bainton, D.F., and McEver, R.P. (1995). P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. J. Cell Biol. *128*, 661–671.

Pan, L.F., Kreisle, R.A., and Shi, Y.D. (1998). Detection of Fcgamma receptors on human endothelial cells stimulated with cytokines tumour necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma). Clin. Exp. Immunol. *112*, 533–538.

Radeke, H.H., Gessner, J.E., Uciechowski, P., Magert, H.J., Schmidt, R.E., and Resch, K. (1994). Intrinsic human glomerular mesangial cells can express receptors for IgG complexes (hFc gamma RIII-A) and the associated Fc epsilon RI gamma-chain. J. Immunol. *153*, 1281–1292.

Ravetch, J.V., and Clynes, R.A. (1998). Divergent roles for Fc receptors and complement in vivo. Annu. Rev. Immunol. *16*, 421–432.

Rennke, H.G., Olson, J.L., and Venkatachalam, M.A. (1981). Glomerular filtration of macromolecules: Normal mechanisms and the pathogenesis of proteinuria. Contrib. Nephrol. *24*, 30–41.

Rosenkranz, A.R., Mendrick, D.L., Cotran, R.S., and Mayadas, T.N. (1999). P-selectin deficiency exacerbates experimental glomerulonephritis: A protective role for endothelial P-selectin in inflammation. J. Clin. Invest. *103*, 649–659.

Salant, D.J., Adler, S., Darby, C., Capparell, N.J., Groggel, G.C., Feintzeig, I.D., Rennke, H.G., and Dittmer, J.E. (1985). Influence of antigen distribution on the mediation of immunological glomerular injury. Kidney Int. *27*, 938–950.

Scharffetter-Kochanek, K., Lu, H., Norman, K., van Nood, N., Munoz, F., Grabbe, S., McArthur, M., Lorenzo, I., Kaplan, S., Ley, K., et al. (1998). Spontaneous skin ulceration and defective T-cell function in CD18 null mice. J. Exp. Med. *188*, 119–131.

Scherzer, H., and Ward, P.A. (1978). Lung injury produced by immune complexes of varying composition. J. Immunol. *121*, 947–952.

Schrijver, G., Bogman, J.J.T., Assmann, K.J.M., de Waal, R.M.W., Robben, H.C.M., van Gasteren, H., and Koene, R.A.P. (1990). Anti-GBM nephritis in the mouse: Role of granulocytes in the heterologous phase. Kidney Int. *38*, 86–95.

Spertini, O., Kansas, G.S., Reimann, K.A., Mackay, C.R., and Tedder, T.F. (1991). Function and evolutionary conservation of distinct epitopes on the leukocyte adhesion molecule-1 (TQ-1, Leu-8) that regulate leukocyte migration. J. Immunol. *147*, 942–949.

Sullam, P.M., Hyun, W.C., Szollosi, J., Dong, J.F., Foss, W.M., and Lopez, J.A. (1998). Physical proximity and functional interplay of the glycoprotein Ib-IX-V complex and the Fc receptor FcgammaRIIA on the platelet plasma membrane. J. Biol. Chem. *273,* 5331–5336.

Takai, T., Li, M., Sylvestre, D., Clynes, R., and Ravetch, J.V. (1994). FcR gamma chain deletion results in pleiotrophic effector cell defects. Cell *76*, 519–529.

Tang, T., Rosenkranz, A., Assmann, K.J.M., Goodman, M.J., Gutierrez-Ramos, J.-C., Carroll, M.C., Cotran, R.S., and Mayadas, T.N. (1997). A role for Mac-1 (CD11b/CD18) in immune complex-stimulated neutrophil function in vivo: Mac-1 deficiency abrogates sustained Fcγ receptor-dependent neutrophil adhesion and complement-dependent proteinuria in acute glomerulonephritis. J. Exp. Med. *186*, 1853–1863.

Tosi, M.F., and Zakem, H. (1992). Surface expression of Fc gamma receptor III (CD16) on chemoattractant-stimulated neutrophils is determined by both surface shedding and translocation from intracellular storage compartments. J. Clin. Invest. *90*, 462–470.

Unkeless, J.C., Shen, Z., Lin, C.-W., and DeBeus, E. (1995). Function of human FcγRIIA and FcγRIIIB. Sem. Immunol. *7,* 37–44.

van de Winkel, J.G.J., and Capel, P.J.A. (1993). Human IgG Fc receptor heterogeneity: Molecular aspects and clinical implications. Immunol. Today. *14*, 215–221.

von Andrian, U.H., Hasslen, S.R., Nelson, R.D., Erlandsen, S.L., and Butcher, E.C. (1995). A central role for microvillous receptor presentation in leukocyte adhesion under flow. Cell *82*, 989–999.

Vossebeld, P.J., Kessler, J., von dem Borne, A.E., Roos, D., and Verhoeven, A.J. (1995). Heterotypic Fc gamma R clusters evoke a synergistic Ca2+ response in human neutrophils. J. Biol. Chem. *270,* 10671–10679.

Yam, L.T., Li, C.Y., and Crosby, W.H. (1971). Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol. *55*, 283–290.

Zhou, M.J., and Brown, E.J. (1994). CR3 (Mac-1, αmβ2, CD11b/CD18) and Fc(gamma)RIII cooperate in generation of a neutrophil respiratory burst: Requirement for Fc(gamma)RII and tyrosine phosphorylation. J. Cell Biol. *125*, 1407–1416.