Functional polymorphism of IgG FcRII (CD32) on human neutrophils

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SUMMARY

In this study we demonstrate that FcγRII on polymorphonuclear cells (PMN) (i) mediates binding of immune complexes, and (ii) is polymorphic, similar to the polymorphism described for monocytes and platelets. This was demonstrated in an adherence assay of PMN to activated human umbilical vein endothelial cells (HUVEC). Precoating of activated HUVEC with a mouse IgG1 monoclonal antibody (mAb) ENA1, which is highly reactive with activated HUVEC, caused an enhanced adhesion in 11 of 15 experiments using PMN from different donors. Enhanced adhesion of the PMN corresponded with expression of a high-responder (HR) FcγRII on monocytes isolated from the same donor, as identified by anti-CD3-induced T-cell proliferation. These data led to the conclusion that FcγRII on PMN is polymorphic. This conclusion was, furthermore, supported by immunofluorescence (IF) studies using a new mAb 41H16, which selectively reacts with the HR allotype of FcγRII.

Receptors for the Fc part of IgG (FcγR) play an important role in immunity by providing a bridge between cellular and humoral branches of the immune system. Three classes of FcγR are currently recognized in man, based upon a set of diverse criteria. These include molecular size, affinity and specificity for ligands, tissue distribution, and reactivity with specific mAb. FcγRI (CD64), a 72,000 molecular weight (MW) receptor, interacts with human (h) IgG with high affinity. The second class, FcγRII, has an apparent MW of 40,000 and interacts only weakly with hIgG. In contrast to the former, this receptor cross-reacts with murine (m) IgG1 in immune complex form. Several isoforms of this class of receptors have been described, all belonging to the CD32 cluster. The third class, FcγRIII (CD16), also interacts only with IgG in immune complex form and can be expressed either as a transmembrane protein (FcγRIII-TM) or is GPI-linked (FcγRIII-PI) (reviewed by Anderson & Looney, 1986; Ravetch & Anderson, 1990).

A functional polymorphism has been reported for FcγRII based on interaction with mIgG1 immune complexes. Monocytes of different human individuals can either interact well (high-responders, HR) or weakly (low-responders, LR) with mIgG1 in different assays. These include support of mIgG1 anti-CD3-induced T-cell mitogenesis (Tax *et al.*, 1983), non-specific anti-CD3-induced cytotoxicity (Leeuwenberg, Lems & Capel, 1987), ADCC (Van de Winkel *et al.*, 1989a), EA-mIgG1 rosetting (Van de Winkel *et al.*, 1987), and support of mIgG1-induced TNF-α release (Debets *et al.*, 1990). The two types of FcγRII differ, furthermore, in iso-electric focusing pattern, both on monocytes and platelets (Anderson *et al.*, 1987, Looney *et al.*, 1988).

Human neutrophils express constitutively only FcγRII and FcγRIII-PI. Since FcγRIII-PI is expressed in a seven- to 10-fold excess (Huizinga *et al.*, 1989a), this receptor is believed to be primarily involved in binding of immune complexes. FcγRII, on the other hand, is essential for triggering in phagocytosis and superoxide generation (Huizinga *et al.*, 1989b).

We studied the capacity of FcγRII on neutrophils to mediate interaction with mIgG1 in immune complexes, by studying the FcγR-mediated adhesion of neutrophils to endothelial cells. We have previously described an Fc-receptor-mediated adhesion of neutrophils to activated endothelial cells, coated with intact mAb ENA1, reactive with the endothelial leucocyte adhesion molecule 1 (ELAM-1) (Leeuwenberg, Jeunhomme & Buurman, 1990a). This phenomenon was further investigated in this study.

Human umbilical vein endothelial cells (HUVEC) were obtained by collagenase treatment of the umbilical vein, and cultured as described elsewhere (Leeuwenberg *et al.*, 1990a). For the adhesion assay ELAM-1 expressing activated HUVEC were

Abbreviations: ELAM-1, endothelial leucocyte adhesion molecule-1; FcγR, receptor(s) for the Fc part of IgG; h, human; HR, high-responder; HUVEC, human umbilical vein endothelial cells; IF, immunofluorescence; IL, interleukin; LR, low-responder; m, murine; mAb monoclonal antibody; PBL, peripheral blood lymphocytes; PI, phosphatidyl-inositol; PMN, polymorphonuclear cells; r, recombinant; TNF-α, tumour necrosis factor-alpha.

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Table 1. Comparison of mIgG1-mediated adhesion of PMN to activated HUVEC with anti-CD3 induced proliferation of PBL from the same donors

	% relative adherence*				
	Intact mAb		$F(ab')_2$		
Donor	ENA1	ENA2	ENA1	ENA2	Proliferation with WT31†
1	200	134	85	80	10,000 (33)‡
2	133	108	80	73	8000 (5)
3	164	114	NT	80	3600 (3)
4	130	110	NT	55	10,000 (3)
5	122	NT	70	65	4000 (4)
6	129	NT	55	NT	4300 (3)
7	179	NT	NT	39	5400 (3)
8	121	NT	70	NT	11,000 (11)
9	132	109	55	45	3200 (8)
10	147	NT	68	53	6300 (3)
11	130	NT	76	69	1300 (4)
12	92	80	NT	78	300 (1)
13	90	62	NT	48	80 (1)
14	80	65	76	65	30 (1)
15	90	60	NT	56	200 (1)

* HUVEC were grown to confluency during 48 hr in fibronectin-coated 96-well plates and activated with 10 U/ml rIL-1α (kindly provided by Dr S. Gillis, Immunex, Seattle, WA). The adhesion assay and the washings were carried out with RPMI/1%FCS. The wells were rinsed twice before addition of appropriate mAb (10 μg/ml) and then ^{51}Cr-labelled PMN ($2{\cdot}5 \times 10^5$ per well) were added. After a 3-min centrifugation and incubation for 15 min at 37°, wells were washed thrice to remove non-adherent cells. The remaining adherent cells were then solubilized (10% acetic acid) and the radioactivity was measured in a gamma-counter. Percentage adherence was calculated according to the formula: % = (c.p.m. harvested)/(c.p.m. added) × 100%. The results were presented as percent relative adhesion = % adherence (experiment in the presence of mAb)/% adhesion of PMN to activated HUVEC (= control in the absence of mAb) × 100%. The absolute percentage of adherent PMN to activated HUVEC varied between different experiments, and ranged from 15% to 35%. All tests were carried out in quadruplicate.

† The capacity of the anti-CD3 mAb WT31 (mIgG1, 50 ng/ml) and OKT3 (mIgG2a, ATCC, Rockville, MD) (data not shown) to induce T-cell mitogenesis was determined by measurement of [^{3}H]thymidine incorporation (c.p.m.), as described before (Tax *et al.*, 1983). Proliferation was assessed in triplicate.

‡ Stimulation index is indicated between brackets = c.p.m. in the presence of WT31/c.p.m. in the absence of WT31.

NT, not tested.

preincubated as indicated in the experiments with our mIgG1 anti-ELAM-1 mAb ENA1 or ENA2 (Leeuwenberg *et al.*, 1990b), or their $F(ab')_2$ fragments.

The adhesion of ^{51}Cr-labelled polymorphonuclear cells (PMN) of different donors to these HUVEC was evaluated and compared with adhesion to activated HUVEC pretreated with intact as well as with $F(ab')_2$ fragments of mIgG1 mAb ENA1 and ENA2. The results of 15 experiments are shown in Table 1. $F(ab')_2$ fragments of mAb ENA1 and mAb ENA2 reduced adhesion of PMN to activated endothelial cells. The inhibitory effect of $F(ab')_2$ fragments of mAb ENA2 was stronger than of mAb ENA1. Intact mAb ENA1 and ENA2, however, enhanced the adhesion in 11 experiments. Concerning enhancement, intact mAb ENA1 had a stronger effect than intact mAb ENA2. In experiments using PMN of donors 12, 13 14 and 15, however, no enhancement was observed. In these experiments intact mAb ENA1 and ENA2 inhibited the adhesion of PMN. The absence of an enhanced adhesion of PMN by both mAb suggests an FcγR-dependent process. An FcγR expressed on PMN, effectively interacting with mouse IgG1 (mAb ENA1 and mAb ENA2 are both mIgG1), is FcγRII. It is conceivable that a functional polymorphism of FcγRII on PMN, similar to that described for FcγRII on monocytes, exists.

We further explored this, and in order to distinguish between HR and LR individuals the accessory function of monocytes was tested in a proliferation assay of peripheral blood lymphocytes (PBL), using mAb WT31 (mIgG1) as T-cell mitogen. PBL were isolated from blood from the same donors as used for isolation of PMN. OKT3 (mIgG2a) was used as positive control, and induced proliferation of T cells in all donors evaluated (data not shown). In Table 1 the results of the proliferation experiments are shown. PBL from all donors, except from donors 12, 13, 14 and 15, proliferated upon stimulation with WT31, and represent the so called HR. Apparently, monocytes of donors 12, 13, 14 and 15 lack a functional FcγR for mIgG1. These individuals are LR. Complete concordance was observed with the data of the Fc–FcγR-mediated adhesion, as shown in Table 1.

The expression of FcγRI, FcγRII and FcγRIII on PMN and monocytes, obtained from HR as well as LR individuals, was determined using mAb specifically reactive with the different FcγR classes. The results are shown in Table 2. FcγRI was either absent or very weakly expressed on PMN as shown by using

Table 2. Flow microfluorimetry analysis of anti-FcγR mAb on PMN of HR and LR*

	HR		LR	
mAb	Monocytes	PMN	Monocytes	PMN
Anti-FcγRI				
32·2	13† (43)‡	0 (0)	11 (36)	0 (0)
Anti-FcγRII				
IV.3	21 (56)	19 (66)	20 (61)	19 (63)
41H16§	18 (47)	16 (52)	7 (9)	10 (15)
Anti-FcγRIII				
3G8	0 (0)	19 (69)	1 (4)	33 (87)
CLBFcRgran1	NT	33 (94)	NT	46 (89)

* The expression of different FcγR on PMN was assayed by indirect immunofluorescence. PMN or PBL were incubated with appropriate mAb in the presence of azide (0·05%), washed and stained with FITC-labelled $F(ab')_2$ fraction of goat antiserum specific for mouse IgG (Jackson, Westgrove, PA). The fluorescence was determined with a FACS IV cell sorter (Becton-Dickinson, Sunny Valley, CA). The monocyte fraction was set on the basis of forward scatter and right angle scatter.

† Channel number of mean fluorescent intensity minus background.

‡ Percentage of cells with mean fluorescent intensity.

§ Incubation with mAb 41H16 was carried out in the presence of 25% human serum in order to prevent non-specific binding of 41H16 (mIgG2a) with FcγRI.

mAb 32.2. All PMN were positive with mAb IV.3 which recognizes both allotypic forms of FcγRII (Anderson & Looney, 1986). A different pattern, however, was observed with mAb 41H16. PMN isolated from a HR stained positive, whereas LR-PMN stained to a significantly lesser degree. These results are in concordance with the reactivity profile of mAb 41H16 on monocytes, which is known to react exclusively with FcγRII of HR type, and not with LR-FcγRII (Gosselin *et al.*, 1990). FcγRIII was most abundantly expressed on all PMN, as established by using mAb 3G8 (Fleit, Wright & Unkeless, 1982) and CLBFcRgran1 (Werner *et al.*, 1986).

In this report we analysed the binding of mIgG1 immune complexes by human neutrophils in an adhesion assay. Adhesion of neutrophils to endothelial cells sensitized with mIgG1 mAb was heterogeneous. Neutrophils of most individuals (73%) bound well to these complexes, as shown by enhanced adhesion, in contrast to PMN from the rest (27%). This process was Fc-dependent since $F(ab')_2$ fragments of these mAb inhibited adhesion. Although it is generally assumed that FcγRIII-PI, by means of its large excess in number, is the predominant receptor mediating binding of immune complexes to neutrophils (Huizinga *et al.*, 1989a), our data show that mIgG1-immune complexes are bound efficiently via FcγRII on PMN from human HR individuals. The involvement of FcγRII on PMN was deduced from the mIgG1 adhesion assay, as well as from experiments using mAb 41H16. Furthermore, the data corresponded exactly with those of monocytes of the same donors. mAb 41H16 interacts only with the HR form of FcγRII, as has recently been shown using transfectants of both HR and LR allotypes (Warmerdam *et al.*, 1990). The two types of FcγRII differ only in two amino acids: a glutamine (HR) instead of tryptophan (LR), and an arginine (HR) instead of histidine (LR) (Warmerdam *et al.*, 1990). These differences appear to have an essential influence on the affinity of this receptor for both murine and human IgG. The affinity of FcγRII, however, can be enhanced by treatment of monocytes with proteolytic enzymes such as trypsin and pronase, resulting in an enhanced functional response, e.g. ADCC (Van de Winkel *et al.*, 1989b) or the secretion of TNF-α (Debets *et al.*, 1990). These data suggest that FcγRII may have an enhanced biological function in inflammatory areas, where proteases are abundantly present. It is conceivable that also the effector functions of PMN FcγRII, such as phagocytosis and superoxide production, are affected by proteases. The functional activity of LR, however, is always found to be lower than that of HR (Debets *et al.*, 1990).

The biological meanings of the differences between HR and LR forms of FcγRII remain unclear so far, since FcγRI-deficient individuals of HR as well as LR types revealed no signs of disease or serological abnormalities (Ceuppens *et al.*, 1988). Interestingly, however, a difference in clearing of T cells coated with a mIgG1 mAb was recently observed between HR and LR individuals, which might be relevant for clinical therapy (Lee *et al.*, 1989).

In conclusion, in this report we established that FcγRII is functionally and antigenically polymorphic on human neutrophils. Additional studies, however, are essential to reveal the basis of this neutrophil dimorphism in detail.

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