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Human neutrophil Fc receptor-mediated adhesion under flow: a hollow fibre model of intravascular arrest

C. D'ARRIGO, J. J. CANDAL-COUTO, M. GREER, D. J. VEALE* & J. M. WOOF *Departments of Pathology and *Medicine, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK*

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SUMMARY

Human polymorphonuclear cells (PMN) were found to adhere to a novel model of blood vessel wall-associated IgG. The internal surfaces of cellulose acetate hollow fibres, of comparable internal diameter to small blood vessels, were coated with normal serum human IgG, heat-aggregated IgG (HAIgG), laminin or fibrinogen. Under conditions of flow mimicking those in a small vessel, PMN were found to adhere markedly only to immunoglobulin-coated fibres. Arrest on HAIgG was inhibited by excess soluble IgG but not by bovine serum albumin (BSA), demonstrating that the adhesion was IgG-specific and presumably mediated by FcγR on the PMN surface. Pre-adsorption of serum components onto HAIgG-coated fibres enhanced PMN arrest, due most probably to fixation of complement components by immobilized HAIgG, resulting in additional potential to entrap PMN via complement receptors such as CR3. Treatment of PMN with the regulatory neuropeptide substance P also enhanced adhesion to HAIgG-coated fibres and caused increased surface expression of FcγRI, FcγRII and FcγRIII. A mouse cell line derived from L cells, hR4C6, stably transfected with human FcγRII, was found to adhere under flow to HAIgG-coated fibres, whilst untransfected parent L cells did not. This adhesion was similarly inhibited by excess soluble IgG, confirming the capability of FcγR to mediate cell arrest. The study strongly suggests that FcγR may play an important role in intravascular PMN arrest and we speculate that in inflammatory diseases PMN may adhere via FcγR to immobilized immunoglobulin on the vascular endothelium, with subsequent degranulation and tissue damage.

Keywords neutrophils adhesion Fc receptors IgG aggregates

INTRODUCTION

Chronic inflammatory disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are frequently associated with elevated levels of circulating immune complexes (IC). Due, in some cases, to defects in normal complement function, these IC may not be effectively cleared, resulting in IC deposition in vessel walls and tissues [1–4]. Deposited IC may then engage blood polymorphonuclear cells (PMN) and trigger their degranulation, resulting in vasculitis and progressive tissue damage [5]. Soluble IC have been demonstrated to enhance adherence of PMN to cultured vascular endothelial cells *in vitro* in a static assay [6], where it was suggested that the increased adherence might be mediated by the interaction of IC with Fcγ receptors (FcγR) on the PMN surface. Although the contribution of numerous adhesion molecules, particularly intercellular adhesion molecules (ICAM), selectins and integrins, to PMN–endothelial cell interaction has been the subject of considerable research [7–9], the role played by FcγR in PMN arrest has not yet been thoroughly investigated. In the present study, we therefore assessed the ability of FcγR to mediate adhesion of PMN to a novel model of vessel wall-associated IgG. Many assays measure adhesion in the absence of flow, but this does not allow clear discrimination between molecules involved in arrest and those maintaining cell contacts after arrest. Hence, in order to assess PMN arrest, our assays were conducted under conditions of flow designed to mimic those in a small blood vessel. The adhesion of a mouse cell line stably transfected with a human FcγRII cDNA was also investigated.

The influence of the nervous system on joint inflammation in IC disease has been the subject of much recent investigation. Regulatory neuropeptides and substance P (SP) in particular, are now believed to play important roles in the inflammatory response in the human joint associated with arthritis [10]. As SP

Correspondence: Dr C. D'Arrigo, Department of Pathology, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK.

has been shown to activate PMN [11], we therefore sought to investigate whether SP might influence FcγR expression and PMN arrest in our model.

MATERIALS AND METHODS

Isolation of PMN

PMN were isolated from heparinized blood taken from healthy volunteers by sedimentation of erythrocytes in 6% dextran T70 (Pharmacia, Uppsala, Sweden) at 37°C for 30 min, followed by leucocyte separation on a discontinuous density gradient of Lymphoprep (ρ = 1·077 g/cm^3; Nycomed, Birmingham, UK) over Ficoll–Hypaque (ρ = 1·119 g/cm^3), centrifuged at 700 ***g*** for 20 min. The PMN were washed in PBS, before being counted and analysed on a Coulter counter (Coulter Electronics, Luton, UK) and resuspended at 10^6 cells/ml in PBS. Contamination with lymphocytes was typically less than 1%.

Culture of L cells and hR4C6

Untransfected mouse L cells and hR4C6 (L cells stably transfected with a human FcγRIIA cDNA [12]) were cultured in minimum essential medium with Earle's salts supplemented with 2 mM glutamine, 1% vitamin solution, 1 mM sodium pyruvate, 50 U/ml penicillin, 50 μg/ml streptomycin, 1% non-essential amino acids and 10% fetal calf serum (FCS; Life Technologies, Paisley, UK). For the adhesion experiments, cells were detached using 2 mM EDTA, washed and resuspended at 10^6 cells/ml in PBS containing 0·1% bovine serum albumin (BSA).

Adhesion assay under flow

The model used to study adhesion under flow is a modification of a previously described system (D'Arrigo & Charon, submitted for publication) using cellulose acetate hollow fibres of 200 μm internal diameter taken from an unused artificial dialyser cartridge (Duo-Flux CD; Medical Incorporated, Miami Lakes, FL) (Fig. 1). The ends of each fibre were secured using superglue inside two 1-cm lengths of glass capillary (1·3 mm external and 0·5 mm internal diameter), which themselves were secured in holes drilled in opposite walls of a 6-cm Petri dish (Nunclon; Life Technologies). The hollow fibre was connected to a flow-delivery system incorporating a gas-tight 0·5-ml syringe with a Teflon plunger (SGE, Burke Analytical, Glasgow, UK) and a digital syringe pump (Treonic IP 3; Vickers Medical, Sidcup, UK) with a microprocessor-controlled syringe driver providing positive displacement. The assay was conducted inside an insulated hot air cabinet at 37°C. In each experiment 40 μl of cell suspension were introduced into a reservoir at one end of a fresh fibre immediately before the flow was started. The fibre was then visualized under a phase contrast microscope with the eyepiece port connected to a low level light video camera with a shutter speed of 1/1000 (Panasonic model WV-CL700/B) in turn connected to a VHS video recorder (Sony, model SLV-425) and a colour monitor (Sony Trinitron, M14410). The overall magnification was × 750. Each experiment was run for 6 min at a constant flow velocity within the fibre of 0·1 cm/s, after which the number of cells adhered in 10 random fields (a representative sample of the 82 fields in each fibre) were counted. The connecting tubing was thoroughly flushed with PBS between experiments, which were performed in duplicate and repeated three times using cells from different donors, unless otherwise stated.

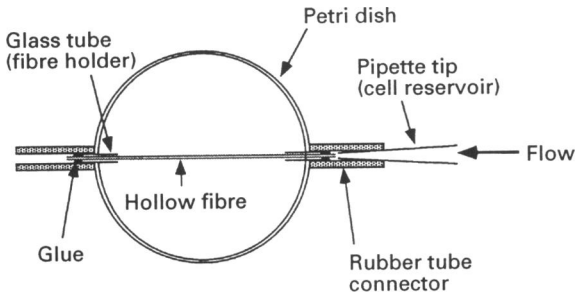

Fig. 1. A diagrammatic representation of the flow model.

Rheology of the system

We can assume that the fluid used in this model is Newtonian and that cell–cell interactions are negligible. At the flow velocity used, the Reynold's number (*Re*) of the hollow fibre is Re_{hf} = 0·28 ($Re = \nu\, 2r\, \rho/\eta$, where ν = mean flow velocity and r = radius of the fibre), assuming a viscosity of the medium η = 0·007 poise and density of the medium ρ = 1 g/cm^3. This *Re* value is well below the critical value for turbulent flow in round pipes (2300), and we can assume that flow is of Poiseuille type (laminar). Since the *Re* is well below 1, steady Poiseuille flow is established within 1 diameter (200 μm) from the entrance of the hollow fibre, which is negligible in relation to the fibre length (1/250).

To estimate the influence of a stationary adhering PMN on the type of flow, we calculated the *Re* for an adhering PMN (Re_{PMN}). In this system Re_{PMN} = 0·005, assuming a PMN diameter of 10 μm and a flow velocity at 10 μm from the wall of 0·038 cm/s. This *Re* is well below the required value for initiation of turbulence (0·5).

The wall shear stress is s_w = 0·28 dyne/cm^2 ($s_w = 4\,\nu\,\eta/r$), and the wall shear rate is τ_w = 40 s^{-1} ($\tau_w = 4\,\nu/r$). A PMN close to the wall, but not interacting with it, would travel at a velocity of 195 μm/s. In *ex vivo* experiments, PMN adhesion to the endothelium has been observed in vessels between 30 and 200 μm in diameter with flow velocities between 0·04 and 0·36 cm/s [13]. Plasma viscosity at 37°C is approximately 0·0115 poise. We can calculate that in a venule of 200 μm in diameter, s_w is between 0·18 and 1·6 dyne/cm^2.

Coating of lumen wall of fibres

After mounting in the Petri dish, the interior of each fibre was incubated at room temperature and saturated humidity for 1–2 h with a solution in PBS of one of the following: 1 mg/ml mouse laminin (Gibco-BRL, Life Technologies), 1·75 mg/ml fibrinogen (Sigma, Poole, UK), 6 mg/ml human IgG (Sigma), heat-aggregated human IgG, or 0·1% BSA (Sigma). Although exact amounts of protein adsorbed were not quantified, these incubation conditions have been shown in preliminary experiments to be sufficient to achieve maximal adhesion in each case. The lumen was then flushed with PBS before the adhesion assay. For some experiments, a second round of adsorption with 50% freshly prepared human serum in PBS was performed for a further 15 min followed by flushing with PBS. Heat-aggregated IgG was prepared by heating a solution of 6 mg/ml

IgG in PBS at 63°C for 20 min, followed by centrifugation at 1000 *g* for 20 min to remove gross aggregates [14].

Inhibition of adhesion by soluble IgG or BSA

In some experiments, a solution of human IgG in PBS was added to the PMN suspension to give a final concentration of 3 mg/ml (0·02 mM) and 10^6 cells/ml before introduction into the adhesion assay. Alternatively, the PMN suspension was supplemented with BSA in PBS to give a final concentration of 1 mg/ml (0·015 mM).

Substance P treatment

SP (Sigma) was dissolved in PBS at 10 μg/ml and stored at −20°C until use. Appropriately diluted SP in PBS was added to the cell suspension immediately before the adhesion assay, giving final concentrations of 740 nM and 7 nM.

Flow cytometric analysis

To assess FcγRII expression in purified PMN, L cells and hR4C6, cells were suspended at 10^7/ml in a 1:5000 dilution in Hanks' buffered salt solution + 0·1% BSA (HBSS/BSA) of anti-FcγRII MoAb IV.3 ascites or in HBSS/BSA alone (negative control) and incubated for 1 h at 4°C. Following washing, cells were incubated in a 1:500 dilution of an anti-mouse IgG–FITC conjugate (Sigma) in HBSS/BSA for 1 h at 4°C. Cells were washed and fixed in 1% paraformaldehyde before analysis on a FACScan flow cytometer.

To compare FcγR expression on purified PMN with that in whole blood, fresh heparinized blood was divided into two portions. One of these was subjected to the isolation procedures described earlier and the PMN obtained were treated as above for flow cytometric analysis, except that the primary antibodies used were 1:1000 dilutions of supernatants of MoAbs against FcγRI (22.2), FcγRII (IV.3) and FcγRIII (3G8) [15]. The second portion of blood, in 50-μl aliquots (containing around 10^7 leucocytes), was cooled to 4°C for 30 min. After incubation steps with primary and secondary antibodies as above, the cells were washed and treated with 1 ml FACS lysing solution (Becton Dickinson, Oxford, UK) for 20 min at room temperature to lyse erythrocytes. After washing, the leucocyte pellet was resuspended in 0·5 ml 1% paraformaldehyde before the FACScan analysis. When acquired by the flow cytometer, a live gate of 2000 cells was drawn around the PMN population as assessed by forward and side scatter parameters.

The effect of SP on FcγR expression was assessed by addition of SP to purified PMN at 10^7 cells/ml, to give a final SP concentration of 7 nM, 740 nM and 7 μM. The cells were incubated at 37°C for 1 min, then placed on ice. Without washing, the primary antibody was added and subsequent assay performed as above.

RESULTS

Effect of purification on PMN FcγR expression

FACS analysis revealed that PMN in whole blood express very low levels of FcγRI but much higher levels of FcγRII and FcγRIII, as demonstrated by mean fluorescence intensities of staining with FcγR class-specific antibodies (Table 1). We found that the procedures used to isolate PMN from blood appeared to slightly up-regulate FcγRI expression on PMN but, as this receptor class is only expressed at very low levels, the actual increase in receptor number was presumably very small. More importantly, PMN purification did not appear to affect markedly expression of the major species FcγRII and FcγRIII (Table 1) and isolated PMN were therefore used in adhesion assays.

PMN adhesion

The adhesion after 6 min under flow of PMN to immobilized substrata is shown in Fig. 2. The observed adhesion to laminin (496 ± 103 cells/cm^2) and fibrinogen (319 ± 98 cells/cm^2) was much lower than that to IgG (10817 ± 1962 cells/cm^2; $P < 0.001$) or heat-aggregated IgG (HAIgG) (10101 ± 515 cells/cm^2; $P < 0.001$). No significant difference was observed between adhesion to IgG and HAIgG ($P = 0.68$). No adhesion was seen to BSA which is commonly used as a non-specific blocker in adhesion assays.

In order to demonstrate the specificity of the adhesion to HAIgG we added soluble IgG at 3 mg/ml to the PMN suspension before the adhesion assay. A mean inhibition of adhesion of 86·7 ± 4·1% (paired analysis) was seen (Fig. 2), implicating FcγR on PMN in the observed adhesion to immunoglobulin-coated fibres. In constrast, BSA at 1 mg/ml was unable to inhibit the interaction.

The additional adsorption of serum components to an HAIgG-coated fibre before the flow assay resulted in an increased level of PMN adhesion (14952 ± 1911 cells/cm^2; $P = 0.006$ in paired experiments) (Fig. 2). However, similar pre-treatment produced no significant change in adhesion to laminin- or IgG-coated fibres ($P = 0.82$ and 0·88, respectively).

Table 1. Expression of FcγR on polymorphonuclear cells (PMN) and percentage changes upon isolation and treatment with substance P (SP)

	PMN in whole blood (mean fluorescence intensity)	Purified PMN (%)	+SP 7 nM (%)	+SP 740 nM (%)	+SP 7 μM (%)
FcγRI	4·2	+22	+15	+91	+129
FCγRII	51·1	+2	+5	+5	+65
FcγRIII	145·5	+5	−17	+7	+20

The mean fluorescence intensities are given for a typical experiment. The percentage change in expression levels upon PMN isolation was calculated from two experiments (one in triplicate, the other in duplicate). For SP treatment, the mean values of two experiments performed in triplicate are given.

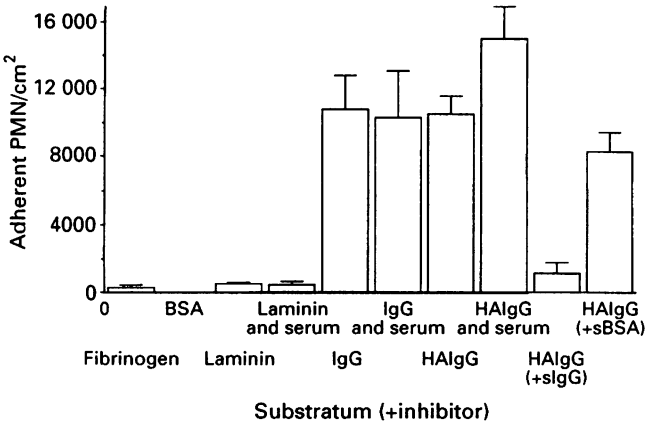

Fig. 2. Polymorphonuclear cell (PMN) adhesion to coated fibres under flow. Hollow fibres were coated as described with fibrinogen ($n=3$), bovine serum albumin (BSA) ($n=6$), laminin ($n=4$), IgG ($n=4$) and heat-aggregated IgG (HAIgG) ($n=6$). Some fibres were additionally coated with 50% fresh serum (laminin and serum, $n=3$; IgG and serum, $n=3$; HAIgG and serum, $n=3$). PMN (4×10^4 in 40 μl) were inoculated into fibres and perfused at a flow velocity of 0·1 cm/s. In some experiments, 3 mg/ml soluble IgG (+ sIgG, $n=3$) or 1 mg/ml soluble BSA (+ sBSA, $n=3$) were mixed with PMN and immediately inoculated into HAIgG-coated fibres. After perfusion for 6 min, adherent cells were quantified by visual count. All experiments were performed in duplicate, values represent the mean and error bars the s.e.m.

L cell and hR4C6 adhesion

To investigate further the ability of FcγR to mediate cell adhesion, we turned to a mouse L cell line, hR4C6, stably transfected with a human FcγRIIA cDNA. FACS analysis revealed reactivity of the hR4C6 cells with an anti-human FcγRII MoAb (mean fluorescence less background = 8·6), verifying the surface expression of FcγRII and the suitability of the cell line for investigation of adhesion via FcγRII in isolation from other FcγR types (Fig. 3). The parent untransfected L cells were not recognized by the anti-FcγRII MoAb and thus served as a good negative control (mean fluorescence less background = 0). PMN may express slightly higher levels of FcγRII than hR4C6 as indicated by their higher mean fluorescence (less background = 13·7) in the assay.

In the adhesion assay under flow the hR4C6 cells were found to adhere to the HAIgG fibres (1700 ± 455 cells/cm^2) whilst the untransfected L cells did not ($P < 0.001$) (Fig. 4). Untransfected L cells were nevertheless able to adhere under

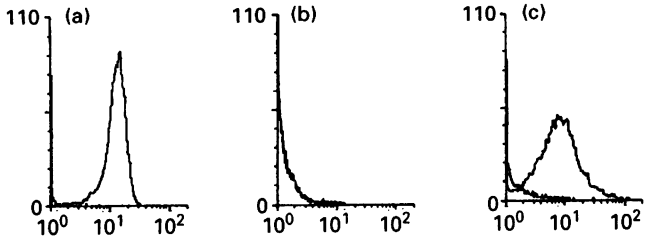

Fig. 3. FACS analysis of reactivity on anti-FcγRII MoAb IV.3. Profiles for cells incubated with and without (negative control) IV.3 are shown in each panel. Ordinate, cell number; abscissa, fluorescence intensity. (a) Polymorphonuclear cells (PMN). (b) Untransfected L cells. (c) hR4C6 transfectants.

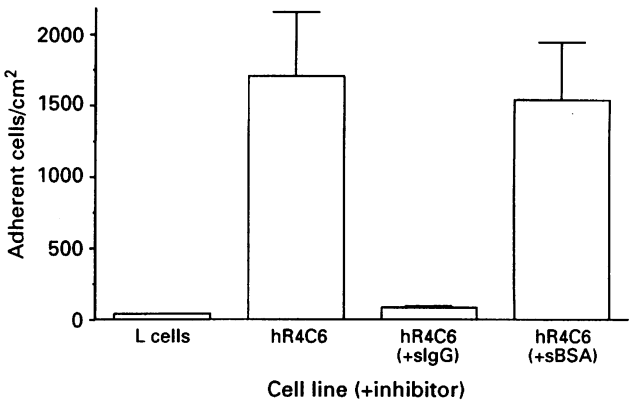

Fig. 4. Adhesion of untransfected L cells and hR4C6 cells to heat-aggregated IgG (HAIgG) and inhibition with soluble IgG. A suspension of untransfected L cells ($n=3$) or FcγRII-transfected hR4C6 cells ($n=3$) was inoculated into HAIgG-coated fibres and perfused at 0·1 cm/s. In some experiments, hR4C6 cells were inoculated into fibres with 3 mg/ml soluble IgG (+ sIgG, $n=3$) or 1 mg/ml soluble bovine serum albumin (BSA) (+ sBSA, $n=3$). After perfusion for 6 min, adherent cells were quantified by visual count. All experiments were performed in duplicate, values represent the mean and error bars the s.e.m.

flow to laminin (1817 ± 97 cells/cm^2, results not shown), indicating that their non-adherence to HAIgG fibres was not due to an inherent inability to adhere under flow. The number of hR4C6 cells adhering/cm^2 to HAIgG fibres was about five-fold less than that seen with PMN under similar conditions.

Soluble IgG at 3 mg/ml was shown to be able to inhibit competitively adhesion to hR4C6 (mean inhibition = 94 ± 1·9%), but BSA at 1 mg/ml was unable to do so (Fig. 4).

Effect of SP on FcγR expression

When purified PMN were treated with different concentrations

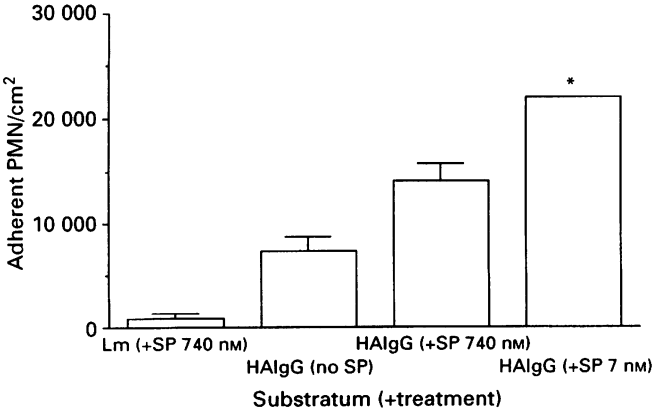

Fig. 5. Effect of substance P on polymorphonuclear cell (PMN) adhesion. Hollow fibres were coated with laminin or heat-aggregated IgG (HAIgG). A suspension of PMN was stimulated with substance P at final concentrations of 7 nM (+ SP 7 nM) and 740 nM (+ SP 740 nM) or with buffer only (no SP), immediately inoculated into the fibres and perfused at 0·1 cm/s. After 6 min, adherent cells were quantified by visual count. Experiments were performed in duplicate and repeated three times using cells from different donors in matched experiments, except for SP 7 nM (*) which was only repeated twice. Values represent the mean and error bars the s.e.m.

of SP, an increased expression of all three classes of FcγR was seen (Table 1). While the apparent expression of FcγRI doubled when PMN were exposed to 740 nM SP and continued to increase at 7 μM, because of the very low level of receptors, the actual increase in receptor number was presumably very small. FcγRII and FcγRIII showed little change with 740 nM SP, but at the higher concentration of 7 μM SP there was an apparent substantial rise in surface receptor number.

With low SP concentration (7 nM) surface expression of FcγRIII dropped. It is possible that at low SP concentration shedding of FcγRIII is greater than the increase of its expression.

Effect of SP on PMN adhesion

When SP was added to the PMN suspension before introduction into the fibre, an increase in adhesion to HAIgG was observed (Fig. 5). In paired experiments in the absence of SP, 7294 ± 1380 cells adhered per cm^2, whilst following treatment with 740 nM SP 14 009 ± 1637 cells attached per cm^2 of the fibre ($P = 0{\cdot}07$). With SP at 7 nM an even more marked enhancement was seen (22 072 cells/cm^2, results from two experiments only). In contrast, the response to laminin after SP treatment did not differ from that observed without SP stimulation ($P = 0{\cdot}47$).

DISCUSSION

The hollow fibre model of PMN arrest used here provides a valuable *in vitro* assessment of adhesion under flow. In this model the flow is laminar and the behaviour of the suspended particles is predictable according to Poiseuille's law (D'Arrigo & Charon, submitted for publication). The geometry, flow velocity and PMN concentration are more closely matched to conditions within a small blood vessel than in alternative adhesion assays. Indeed, a number of other assays are performed in the absence of flow. *Ex vivo* studies of PMN adhesion to the endothelium are usually confined to venules, such as post-capillary venules, which are of smaller calibre than the vessels used in our work. This tendency may be related to difficulties in visualizing leucocytes in whole blood when in larger vessels and should not be interpreted as indicating that PMN adhesion occurs exclusively in post-capillary venules. Indeed, *ex vivo* PMN adhesion to the vessel wall has been observed also in larger vessels (200 μm in diameter) of size equivalent to those of the hollow fibres we used [13]. Our assay system, although lacking certain characteristics of flow in blood vessels such as the presence of erythrocytes and the irregularity of wall surface and cross-section, generates reproducible results and readily allows assessment of different adhesion molecules in isolation. This simple model system has allowed an investigation into the potential of immobilized aggregates of IgG to mediate PMN arrest. The heat-aggregated IgG used serves as a model for the immune complexes seen in certain rheumatic diseases, although HAIgG and immune complexes may differ somewhat in size and adhesive properties. However, HAIgG is a convenient substrate and a reasonable model, particularly where the antigen is IgG itself as in rheumatoid arthritis.

Both IgG and HAIgG coated onto hollow fibres were found to mediate PMN arrest. In fact, the numbers of PMN arrested on such fibres, about 10% of those introduced, greatly exceeded those retained on any other adhesive substratum tested, including known adhesion molecules laminin and fibrinogen. These findings are in agreement with an earlier report in which both HAIgG and antibody–antigen complexes were found to increase adherence of PMN in the absence of flow to human endothelial cells and to serum-coated plastic [6].

The arrest on the HAIgG substratum was shown to be an IgG-specific response, since the addition of soluble IgG was able to competitively inhibit the interaction. This implies that PMN adhesion to fixed HAIgG is mediated by FcγR present on the PMN surface. Other studies have also postulated a role for FcγR–IC interactions in PMN adhesion [16,17].

Enhanced PMN adhesion was seen upon adsorption of serum components to fibres already coated with HAIgG. This might be explained by fixation of complement components within the serum by HAIgG aggregates on the fibre surface, resulting in the additional potential to entrap PMN via complement receptors such as CR3. The lack of similar enhancement of adhesion to fibres coated with native IgG may be explained by the inability of unaggregated IgG to fix complement [18]. Although we have as yet no direct evidence for complement involvement, current investigations into the effects of heat inactivation of the serum should more rigorously demonstrate any role played by complement components in the observed enhancement of adhesion. We do, however, note that co-operation between CR3 and FcγRIII function on PMN has been demonstrated, resulting, at least in part, from their physical association at the plasma membrane [19]. The enhancement of PMN adhesion seen here, if mediated by engagement of both receptor types, is consistent with such co-operation.

An earlier study reported that adhesion of PMN to porcine endothelium enhanced by IgG aggregates was inhibited by 20% serum [17], a result which may at first appear to be contrary to the results presented here. However, in this case the serum was present throughout the assay, suggesting that the inhibition might be due to the high concentrations of soluble IgG in 20% serum (2–3 mg/ml).

Human PMN constitutively express all three classes of FcγR, FcγRI (CD64) FcγRII (CD32) and FcγRIII (CD16). FcγRI is present at very low number (< 1000/cell) on unstimulated PMN, but its expression is increased up to 20-fold upon PMN stimulation [15]. FcγRII is a 40-kD transmembrane protein capable of triggering antibody-dependent cell-mediated cytotoxicity, phagocytosis, respiratory burst and degranulation. In contrast, FcγRIII is anchored to the PMN membrane by a glycophosphatidylinositol linkage and is capable of mediating degranulation and possibly also phagocytosis and superoxide release [20]. In order to investigate the contribution made by FcγRII alone to HAIgG-mediated cell arrest, we turned to a cell line, hR4C6, stably transfected with a human FcγRIIA cDNA, which encodes an FcγRII isoform (FcγRIIa) present on PMN. The presence of FcγRII on the surface of hR4C6 was confirmed by reactivity with the anti-FcγRII MoAb IV.3, though at slightly lower levels than on PMN.

The finding that hR4C6 cells and not the untransfected parent L cells are arrested by immobilized HAIgG in an IgG-specific manner confirms the capability of FcγR to mediate cell arrest. Furthermore, it indicates that FcγRII is capable of mediating such adhesion in isolation from other FcγR classes. The number of hR4C6 cells arresting is approximately a fifth of the number of PMN found to arrest under similar

conditions. This may suggest that it is the high levels of FcγRIII expressed on PMN ($1-2 \times 10^5$ per cell compared with $3-6 \times 10^4$ FcγRII per PMN [15]) which play the major role in their arrest described here. It is also possible that FcγRII and FcγRIII behave synergistically in this function, in a manner similar to their reported co-operation in PMN triggering [21,22]. However, these differences between L cells and PMN may simply result from a lower expression of FcγRII by L cells and/or altered distribution within the laminar flow of cells of differing size and buoyancy. With regard to this point we estimate from forward scatter data in FACS analysis that the mean volume of L cells is about 40% greater than that of PMN, but around 25% smaller than that of monocytes. Since the diameter ranges of PMN are 10–12 μm and those of monocytes are 12–15 μm, it is likely that the L cells have a mean diameter just greater than 12 μm.

A number of observations have suggested a role for the nervous system in inflammatory joint disease. For example, RA is very frequently symmetrical and paralysed limbs are often unaffected when RA develops after hemiplegia [10,23]. Regulatory peptides such as SP, which play a role in homeostasis of the normal joint, are believed to have a particular involvement in this inflammation [24]. SP is frequently found at high levels in synovial fluid [25]. However, in a hemiplegic RA patient, SP has been found instead to be sequestered in the nerve terminals in the paralysed limb unaffected by the disease [10]. In our assay, treatment of PMN with SP appears to enhance adhesion to HAIgG under flow. Possible explanations for this observation include modulation of activation, shedding or surface expression of FcγR. Our experiments suggest that an up-regulation of FcγR on PMN following SP treatment may, at least in part, be responsible for the increased adhesion, but we cannot rule out a role for shedding or indeed that the effect is due to a general activation of PMN by SP. Regardless of the mechanism, one might speculate that elevated SP release at inflamed joints could enhance local PMN arrest with subsequent PMN activation and degranulation, culminating in further tissue damage.

The study presented here strongly suggests that FcγR may play a much more important role in the intravascular arrest of PMN than previously thought. We can speculate that in inflammatory diseases PMN adhere to IC immobilized on the vascular endothelium, with subsequent degranulation resulting ultimately in vasculitis. IC may become preferentially immobilized at these sites in a number of ways. In certain inflammatory conditions, antibodies directed against determinants on endothelial cells may develop and become localized on the endothelial membrane. Alternatively, endothelial cells, under certain conditions such as injury [26] or viral infection [27], may express FcγR themselves, which could entrap IC of any antigen specificity. In order to define further this potential means of PMN intravascular arrest and investigate each FcγR class in isolation, we plan future experiments using additional FcγR transfectants.

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