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Neutrophils from the synovial fluid of patients with rheumatoid arthritis express the high affinity immunoglobulin G receptor, FcγRI (CD64): role of immune complexes and cytokines in induction of receptor expression

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SUMMARY

Neutrophils isolated from the synovial fluid of 16/24 patients with rheumatoid arthritis expressed FcγRI (CD64), the high-affinity receptor for monomeric immunoglobulin G (IgG), on their cell surface. Receptor expression ranged from 17% to 168% of the level of expression obtained after incubation of control blood neutrophils with 100 U/ml interferon-γ (IFN-γ) for 24 hr *in vitro*. Similarly, mRNA for FcγRI was detected in synovial fluid neutrophils from 12/15 patients and transcript levels ranged from 5% to 200% of the values obtained after treatment of blood neutrophils with IFN-γ for 4 hr *in vitro*. No surface expression nor mRNA were detected in freshly isolated blood neutrophils from either patients or from healthy controls. Addition of cell-free synovial fluid to control blood neutrophils induced both mRNA and surface expression of FcγRI to levels that were comparable to those achieved after addition of IFN-γ. Neither soluble nor insoluble immune complexes appeared to be involved in induction of FcγRI expression in spite of the ability of these complexes to induce protein biosynthesis. Synovial fluid-induced expression of FcγRI was partially blocked by incubation with neutralizing IFN-γ antibodies, whilst neutralizing interleukin (IL)-6 antibodies had little effect. Levels of IFN-γ measured within these synovial fluids ranged from 0 to 2·7 U/ml, well within the range known to induce neutrophil FcγRI expression. These data thus indicate that gene expression in synovial fluid neutrophils is selectively activated as the cells enter the diseased joint. Furthermore, these data indicate that induced expression of FcγRI may alter the ability of infiltrating neutrophils to respond to IgG-containing immune complexes present in these joints.

INTRODUCTION

During active phases of rheumatoid arthritis, diseased joints become heavily infiltrated with white blood cells, with neutrophils comprising 80–90% of the total population.[1,2] Much evidence in the literature now indicates that these infiltrating neutrophils have been both primed and activated *in vivo* and that they have secreted both reactive oxygen metabolites and granule enzymes.[3–8] Rheumatoid synovial fluid contains a variety of cytokines[9,10] that may serve to prime the infiltrating neutrophils whilst immune complexes within the fluid can cause neutrophil activation.[11–14] At least two types of immunoglobulin G (IgG)-containing immune complexes are present in rheumatoid synovial fluid, that are capable of activating neutrophils, and these differ markedly in their mode of action.[11,15,16] Insoluble immune complexes activate the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase of both primed and unprimed neutrophils with near equal efficacy, but the majority of reactive oxygen metabolites that are generated are not released from the neutrophil (i.e. these complexes are largely phagocytosed).[12] On the other hand, soluble immune complexes are incapable of activating the NADPH oxidase in unprimed neutrophils, but they stimulate a rapid and extensive *release* of reactive oxygen metabolites and granule enzymes from neutrophils that have been primed either *in vivo* or *in vitro*.[15] The signal transduction systems activated by these soluble and insoluble immune complexes show some clear distinctions.[11,16] Because the soluble immune complexes activate the *secretion* of potentially tissue damaging species into the joint and because the population of synovial fluid neutrophils has been primed, immune complex activation is likely to be of great importance in disease pathology. These IgG-containing immune complexes will thus activate neutrophils via their Fcγ receptors.

Neutrophils can possess up to three types of receptor on their cell surface that can recognize the Fc region of IgG.[1,17–19]

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Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; FITC, fluorescein isothiocyanate; LPS, lipopolysaccharide; HSA, human serum albumin; CTP, cytidine triphosphate.

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FcγRI is a heavily glycosylated 72 000 MW transmembrane protein which binds monomeric IgG with high affinity. It is not normally present on the surface of blood neutrophils, but it can be expressed following exposure to interferon-γ (IFN-γ) for 18–24 hr *in vitro*, with expression requiring activation of both transcription and translation.[20–22] It has also been reported to be present on the surface of blood neutrophils of patients undergoing granulocyte colony-stimulating factor (G-CSF) therapy or γ-interferon therapy, those with streptococcal pharyngitis or those with leukocyte adhesion deficiency.[23–27] The function of FcγRI on neutrophils is not clearly defined, but its increased expression (following *in vitro* exposure to IFN-γ) coincides with enhanced antibody-dependent cellular cytotoxicity.[21] Blood neutrophils constitutively express FcγRII and FcγRIIIb. FcγRII is a 40 000 MW transmembrane glycoprotein that binds monomeric IgG with very low affinity, but binds well to dimers, trimers and aggregated IgG.[17–19] It is present on the neutrophil cell surface at about $1–2 \times 10^4$ molecules per cell. FcγRIIIb is a 50 000–70 000 MW glycosylated protein that binds IgG aggregates with low affinity. It is the most abundant Fcγ receptor on the neutrophil cell surface and is attached to the membrane via an easily cleaved glycosyl-phosphatidylinositol link.[28–30] Thus, this receptor is readily shed from the cell surface, but may be replaced via the translocation of pre-formed pools from sub-cellular organelles to the plasma membrane.[31]

In this report, we show that neutrophils isolated from the synovial fluid (but not the blood) of patients with rheumatoid arthritis express FcγRI, in addition to FcγRII and FcγRIIIb. Expression of this former receptor was detected by measuring cell surface levels and presence of mRNA. Addition of cell-free synovial fluid isolated from these patients could also induce FcγRI expression in control, blood neutrophils. Soluble and insoluble immune complexes present within these fluids played an insignificant role in induction of expression of this receptor, and the stimulating effects of synovial fluid were abrogated by addition of anti-IFN-γ antibody. These observations thus have important implications for understanding the molecular pathology of rheumatoid arthritis for the following reasons: (a), gene expression is selectively activated in synovial fluid neutrophils; (b), this activation of gene expression is likely to occur as the cells enter the diseased joint; (c), the population of synovial fluid neutrophils is likely to have been exposed for >18–24 hr to factors that activate gene expression; (d), expression of FcγRI by synovial fluid neutrophils will affect the ability of these cells to respond to IgG-containing immune complexes within diseased joints.

MATERIALS AND METHODS

Materials

Dextran T500 and ficoll–hypaque were from Pharmacia (Uppsala, Sweden), RPMI-1640 medium was from Flow Laboratories (Rickmansworth, UK) whilst the random labelling kit and IFN-γ were from Boehringer (Lewes, Sussex, UK). Zetaprobe was from Bio-Rad (Hemel Hempstead, Herts, UK) and [^{32}P]CTP was from ICN. The monoclonal antibodies IV3, 197 and 322 were from Mederex (Annandale, NJ) whilst Leu11b and fluorescein isothiocyanate (FITC)-labelled goat-(anti-mouse) immunoglobulin were from Becton Dickinson (Oxford, UK). Human serum albumin (HSA) and rabbit anti-HSA antibodies were from Sigma (Poole, UK). Sheep anti-human IFN-γ antibody (H90) and goat anti-human interleukin (IL)-6 antibody (GM150/BM) were from NIBSC (Potters Bar, UK). Pansorbin was from Calbiochem (Nottingham, UK). All other reagents were of the highest purity available.

Patients

A total of 24 patients with rheumatoid arthritis attending the rheumatology clinics of the Royal Liverpool University Hospital were studied. All patients were receiving non-steroidal anti-inflammatory drugs but none had received steroids within the previous 6 months. Local Ethical Committee approval was granted for this study.

Isolation of neutrophils

Neutrophils were isolated from the venous blood of healthy controls and patients by a combined dextran/ficoll–hypaque technique, as described previously.[32] Contaminating erythrocytes were removed by hypotonic lysis. Neutrophils were isolated from synovial fluid aspirated from knee joints, as described previously.[7,8] The synovial fluid was centrifuged at 1000 ***g*** for 5 min to pellet the cells which were then washed three times in phosphate-buffered saline (PBS: 10 mM potassium phosphate, 0·9% NaCl, pH 7·4). The neutrophils were then purified on Ficoll–Hypaque, followed by hypotonic lysis to remove any contaminating erythrocytes. After purification, cells were suspended in RPMI-1640 medium and counted using a Fuchs–Rosenthal haemocytometer slide. Purity and viability were routinely assessed and found to be >97% and >95%, respectively, as assessed by Wright's staining and Trypan blue exclusion.

Preparation of synovial fluid

Synovial fluid, aspirated from patients with knee effusions was collected into heparinized tubes. The fluid was then centrifuged at 1000 ***g*** for 5 min to remove the cells, which were then purified as described above. The cell-free supernatants (checked microscopically to assess for acellularity) were either used immediately or else were stored in aliquots at −80°.

Cell incubations

Neutrophils, suspended in RPMI-1640 medium, were incubated at 1×10^7 cells/ml at 37° with gentle agitation in the presence and absence of IFN-γ (100 U/ml) or cell-free synovial fluid (10% final volume). At time intervals up to 24 hr, aliquots were removed for assessment of receptor expression or for isolation of RNA. Where indicated, suspensions were also incubated with anti-IFN-γ antibody (1 : 1000 dilution, sufficient to neutralize 1000 U/ml γ-interferon) and anti-IL-6 antibody (at 1 : 500, sufficient to neutralize 15 ng/ml IL-6).

Receptor expression by fluorescence-activated cell sorting (FACS) analysis

The monoclonal antibodies used were IV3 (anti-CD32, FcγRII) and Leu 11b (anti-CD16, FcγRIIIb). Expression of FcγRI (the high affinity receptor for monomeric IgG) was measured using the anti-CD64 monoclonal antibodies 322 and 197. For immunostaining, isolated neutrophils were suspended in PBS/1% bovine serum albumin (BSA) (globulin-free)/0·1% sodium azide, pH 7·2, and receptor expression was measured using a standard indirect immunofluorescence technique using FITC-labelled goat-(anti-mouse) immunoglobulin as a second layer.[7,33] Both first and second layer antibodies were added at saturating concentrations, and in all experiments non-immune

mouse IgG of the appropriate isotype was included as a class-specific first layer control. Stained cells were fixed in 1% paraformaldehyde in PBS and analysed using a Becton Dickinson (Oxford, UK) Ortho Diagnostics Cytron analyser. Fluorescence distributions represent a total of 5000 gated events (cells), with the mean fluorescence proportional to the number of specific antigenic sites per cell.

The number of binding sites for anti-FcγRI antibodies was determined using the Quantum Simply Cellular Microbeads kit from Sigma. In all experiments, the number of binding sites for anti-FcγRI antibodies (equivalent to the number of FcγRI molecules on the cell surface) on blood and synovial fluid neutrophils, or on synovial fluid treated neutrophils, is expressed as a percentage of the number of binding sites induced by treatment of control blood neutrophils with 100 U/ml IFN-γ for 24 hr at 37°, which was taken as 100%.

Immune complexes

Synthetic soluble and insoluble immune complexes were prepared from HSA and anti-HSA antibodies, as described previously.[16] Soluble immune complexes were prepared at 180 μg/ml antigen and 125 μg/ml antibody, which was five times the concentration of antigen required to form insoluble immune complexes. They were added to neutrophil suspensions at 10% (v/v). Insoluble immune complexes were removed from cell-free synovial fluid by centrifugation at 11 600 *g* for 2 min in a microfuge.[11] The supernatant, still containing soluble immune complexes was either added to neutrophil suspensions or else incubated with Pansorbin (Calbiochem, Nottingham, UK) (150 μl Pansorbin/ml synovial fluid) for 1 hr at 4° to remove these soluble complexes. After this incubation, the Pansorbin was removed by centrifugation at 13 000 *g* for 2 min. The supernatant was tested for the presence of soluble and insoluble immune complexes by its ability to activate luminol chemiluminescence in primed and unprimed neutrophil suspensions, as described by Robinson *et al.*[11]

RNA extraction

RNA was isolated from control blood neutrophils that had been incubated at 37° for periods of up to 24 hr in the absence or presence of IFN-γ or cell-free synovial fluid. RNA was also extracted from blood neutrophils (patient or control) and synovial fluid neutrophils immediately after isolation. Cell pellets were suspended in 4 M guanidinium isothiocyanate,[34] 5% β-mercaptoethanol, 50 mM ethylenediaminetetra-acetic acid (EDTA), 50 mM Tris, pH 7·0 and lysed by drawing through a 23-gauge needle to shear chromosomal DNA. The suspension was then layered onto a CsCl/EDTA gradient (5·7 M CsCl, 50 mM EDTA, density = 1·3995 ± 0·001) and centrifuged at 100 000 *g* for 16 hr at 14°. The RNA pellet was then suspended in diethylpyrocarbonate (DEPC)-treated water and precipitated overnight with 0·3 M sodium acetate and 2·5 vol ethanol at −80°. The recovered RNA was then quantified by UV spectroscopy (typically 2 μg RNA recovered/10^7 neutrophils) and stored in aliquots at −80° as ethanol precipitates.

Northern blot analyses

Aliquots of RNA (10 μg) were electrophoresed on 1·2% agarose gels (containing 1% (v/v) formaldehyde, 20 mM 3-*N*-morpholinopropane sulphonic acid, 1 mM EDTA, 5 mM sodium acetate, pH 8·0) for 16 hr at 5 mA. The gels were then stained in ethidium bromide and RNA visualized by UV illumination. The RNA was then transferred by capillary blotting onto Zetaprobe GT nylon membrane in 20 × SSC (3 M NaCl, 0·3 M sodium citrate, pH 7·0) The filter was then baked at 80° for 30 min and stored at room temperature wrapped in Saranwrap until use. The filters were probed with the following cDNA clones: FcγRI (a kind gift from Dr B. Seed, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA); β-actin (ATCC 65 128 (Rockville, MD)). The cDNA inserts were excised using appropriate restriction endonucleases and isolated from vector DNA by electrophoresis in low melting point agarose. Then 50–100 ng of each insert was radiolabelled using a random-primed labelling system using 25 μCi [^{32}P]CTP. Labelling proceeded for 20 hr at 16° and unincorporated CTP was removed from the labelled cDNA using Nuctrap columns (Stratagene, Cambridge, UK)). The amount of radioactivity incorporated was typically $>10^8$ c.p.m./μg DNA. The filters were pre-hybridized for 30 min at 65° in 0·25 M $Na_2HPO_4 \cdot 7H_2O$/7% sodium dodecyl sulphate (SDS), pH 7·2, and then hybridized with the probe for 16–20 hr in the same buffer at 65°. The filters were washed in 20 mM $Na_2HPO_4 \cdot 7H_2O$/5% SDS, pH 7·2 for 2 × 30 min at 65°, followed by 2 × 10 min washes in 20 mM $Na_2HPO_4 \cdot 7H_2O$/0·1% SDS at the same temperature. The filters were blotted dry, wrapped in Saranwrap and exposed to X-ray film (Fuji RX; Fuji, London, UK) for 24–48 hr. After radiography, the probes were removed by heating the filters to 95° in 0·1% SSC containing 0·5% SDS for 2 × 20 min. After checking for removal of the probe by autoradiography, the filters were then re-probed. Hybridization signals were quantified by densitometry of autoradiographs; films were digitized and analysed using Image software.

Measurement of IFN-γ

Levels of IFN-γ in synovial fluid were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) (Genzyme, West Malling, UK), as described in the manufacturer's instructions. Samples were diluted 1 : 1 with PBS prior to analysis and some were also 'spiked' with known amounts of IFN-γ prior to analysis; the fluids did not contain factors that interfered with IFN-γ detection and quantification.

RESULTS

Expression of FcγRI by blood and synovial fluid neutrophils

Neutrophils were purified from the blood and synovial fluid of patients with rheumatoid arthritis and expression of FcγRI, II and IIIb was determined by FACS analysis. Blood neutrophils from rheumatoid patients expressed surface levels of FcγRII and FcγRIIIb that were comparable to those expressed on the surface of blood neutrophils isolated from healthy controls (Fig. 1a, b). There were some variations, however, in expression of FcγRII and FcγRIIIb in the synovial fluid neutrophils. For example, in some patients, expression of FcγRIIIb was decreased in the synovial fluid neutrophils compared to the levels expressed on blood cells, presumably indicating this receptor had been shed as a consequence of cell activation. Median fluorescence values for control blood, patient blood and synovial fluid neutrophils were $1{\cdot}4 \times 10^5$ (interquartile range (IQR) $1{\cdot}3$–$2{\cdot}0 \times 10^5$, $1{\cdot}0 \times 10^5$ (IQR $0{\cdot}8$–$1{\cdot}5 \times 10^5$) and $0{\cdot}8 \times 10^5$ (IQR $0{\cdot}7$–$1{\cdot}0 \times 10^5$, $P > 0{\cdot}005$ compared with control or patient blood), respectively. Also, in some instances FcγRII expression was apparently decreased,

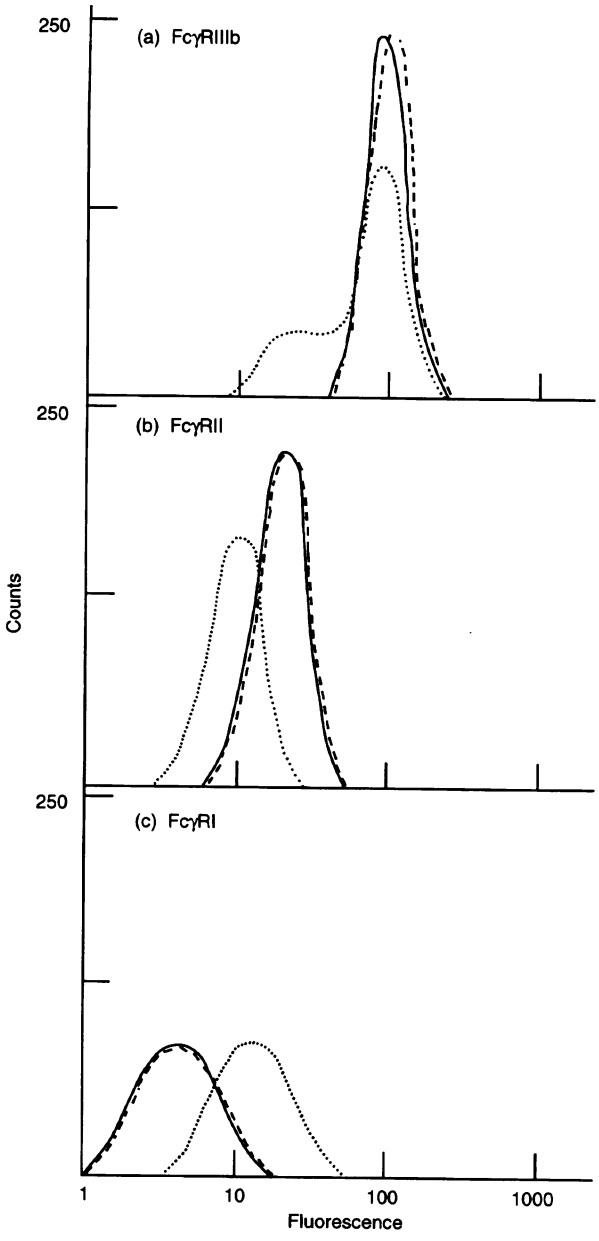

Figure 1. Fcγ receptor expression in blood and synovial fluid neutrophils. Neutrophils were isolated from the blood and synovial fluid of patients with rheumatoid arthritis and from the blood of healthy controls ($n=24$). Surface expression of FcγRIIIb (a), FcγRII (b) and FcγRI (c) was then measured by FACS analysis. The traces shown are representative of those obtained from the blood (—) and synovial fluid (.....) of a typical patient and the blood (----) of healthy controls.

possibly because this receptor was bound to ligands normally present in synovial fluid and this ligand-binding prevented binding to the monoclonal antibody used for immunostaining. For example, in control and patient blood neutrophils 80% (IQR 80–98) and 75% (IQR 61–93), respectively, of the total cell population in different samples stained for FcγRII, whilst in synovial fluid neutrophils only 16% of the cells (IQR 11–48, P0·005) stained positive for this receptor: in the remaining cells, expression was low and overlapped with the isotype control antibody.

Blood neutrophils from either healthy controls or patients with rheumatoid arthritis did not express FcγRI on their cell surface. However, in the majority of patients analysed (16/24) this receptor was detected on the surface of synovial fluid neutrophils (Fig. 1c). There was considerable variation amongst these 16 patients with values ranging between 17 and 168% of the maximal value observed (100%) after treatment of control blood neutrophils with IFN-γ for 24 hr. In the other patients, expression of this receptor could not be detected above the binding observed with non-immune, control antibodies.

mRNA levels of FcγRI in blood and synovial fluid neutrophils

Previous work has shown that induction of FcγRI expression on the cell surface of neutrophils following IFN-γ treatment is preceded by increases in mRNA for this gene product. Therefore, mRNA levels for FcγRI were probed in northern transfers of neutrophil RNA isolated from both the blood and synovial fluid of rheumatoid patients, and also from control blood neutrophils incubated for 4 hr with IFN-γ. Transcripts for FcγRI could not be detected in either control (untreated) blood neutrophils or in neutrophils isolated from the blood of patients (Fig. 2a). However, treatment of blood neutrophils

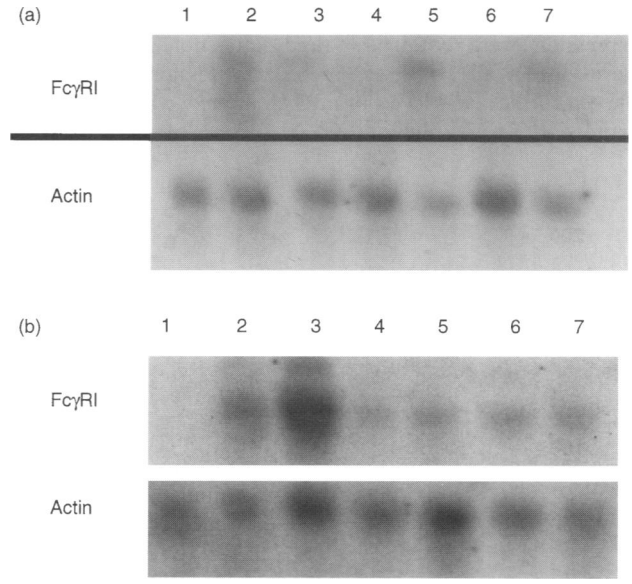

Figure 2. Expression of mRNA for FcγRI in blood and synovial fluid neutrophils. Neutrophils were isolated from the blood of healthy controls and from the blood and synovial fluid of patients with rheumatoid arthritis. The control blood neutrophils were incubated for 4 hr in the absence and presence of 100 U/ml IFN-γ prior to RNA extraction, whereas RNA was extracted from patient neutrophils immediately after isolation. RNA was then probed for FcγRI as follows: In (a) lane 1, control blood neutrophils; lane 2, control blood neutrophils treated with IFN-γ; lane 3, synovial fluid neutrophils from patient 1; lane 4, blood neutrophils from patient 1; lane 5, synovial fluid neutrophils from patient 2; lane 6, blood neutrophils from patient 2; lane 7, synovial fluid neutrophils from patient 3. In (b) lane 1, control blood neutrophils; lane 2, control blood neutrophils treated with IFN-γ; lanes 3–7, synovial fluid neutrophils from five different patients. After probing for FcγRI, the filters were stripped and probed for actin mRNA.

with IFN-γ resulted in a marked increase in mRNA levels for FcγRI. In synovial fluid neutrophils from 12/15 rheumatoid patients, mRNA for FcγRI could be detected (Fig. 2b) and levels of this transcript ranged from 5 to 200% of the levels that could be induced by IFN-γ treatment of control, blood neutrophils (taken as 100%).

Correlation between surface expression of FcγRI and mRNA levels

A separate series of experiments were then performed in which cell surface expression and mRNA levels of FcγRI were measured in the same preparations of synovial fluid neutrophils. For both types of measurement, values were compared with those obtained after treatment of control, blood neutrophils with 100 U/ml γ-interferon at 37°, for 24 hr prior to measurement of surface expression and for 4 hr prior to extraction and measurement of mRNA. No correlation between mRNA levels and surface expression in these samples was observed (Fig. 3). The time course of expression of mRNA and surface receptor following IFN-γ treatment was then measured in order to explore this lack of correlation further. mRNA for FcγRI was detectable within 1–2 hr after addition of IFN-γ, peaked by 4 hr and was only about 30% of this maximal value by 24 hr[22] (and data not shown). In contrast, surface expression could not be detected up to 4 hr after addition of IFN-γ, but by 18–24 hr maximal levels of surface expression were obtained[20] (and data not shown). Thus, elevations in mRNA levels precede expression of FcγRI on the cell surface, and levels of mRNA are low when cell surface expression is at its highest.

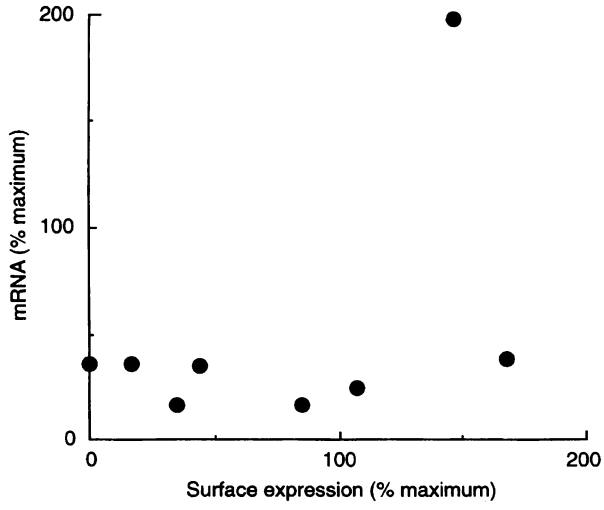

Figure 3. Surface expression and mRNA for FcγRI in synovial fluid neutrophils. Neutrophils were isolated from the synovial fluid of eight patients with rheumatoid arthritis. Surface expression of FcγRI was measured by FACS and expressed as a percentage of the maximal expression obtained after treatment of control blood neutrophils with 100 U/ml IFN-γ for 24 hr (100%). Transcripts for FcγRI were measured in northern blots and levels expressed as a percentage of the maximal values obtained after treatment of control blood neutrophils with 100 U/ml IFN-γ for 4 hr (100%), after correction for the actin signal.

Stimulation of FcγRI expression by cell-free synovial fluid

Figure 1 shows that whereas synovial fluid neutrophils from patients with rheumatoid arthritis clearly express FcγRI, neutrophils isolated from the bloodstream of rheumatoid patients do not. This observation would suggest therefore that expression of this receptor is activated when the neutrophils are recruited into the diseased joints. It was therefore necessary to determine if factors present within rheumatoid synovial fluid were capable of inducing expression of FcγRI on blood neutrophils. Therefore, neutrophils isolated from control blood were incubated with cell-free synovial fluids from three separate patients and expression of FcγRI mRNA was measured after 4 hr incubation, whilst surface expression of this receptor was measured after 24 hr incubation of the same neutrophil preparations. Addition of cell-free synovial fluid to blood neutrophils was capable of inducing increases in mRNA cell surface expression (Fig. 4a) and of FcγRI (Fig. 4b) in blood neutrophils. Maximal levels of mRNA that were induced following incubation with synovial fluid under these conditions were 20% ($\pm$3%, $n=3$) of the maximal values obtained after incubation with IFN-γ. Similarly, incubation of blood neutrophils with synovial fluid for 24 hr induced expression of FcγRI on the cell surface to values that were 68% ($\pm$19%, $n=3$) of

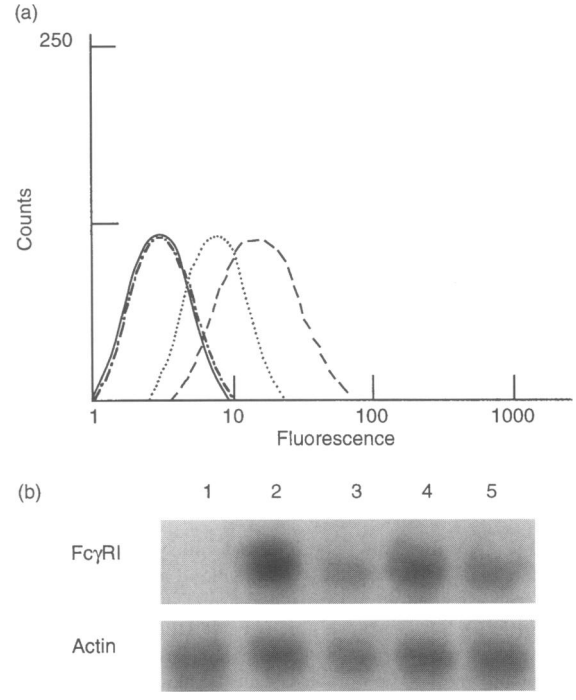

Figure 4. Synovial fluid-induced expression of FcγRI mRNA. In (a) neutrophils were incubated for 24 hr in the absence (—) or presence of 100 U/ml IFN-γ (----) or 10% (v/v) synovial fluid (.....) from a patient with rheumatoid arthritis. After incubation, expression of FcγRI was determined by FACS. Trace (·--·) shows FcγRI expression of freshly isolated control neutrophils. Similar results were obtained in 2 other experiments using synovial fluid from different patients. In (b) neutrophils were isolated from the blood of healthy controls and divided into aliquots. These were then incubated in the absence (lane 1) and presence of 100 U/ml IFN-γ (lane 2) or 10% (v/v) cell free synovial fluid from three patients with rheumatoid arthritis (lanes 3–5). After isolation of RNA and probing for transcripts for FcγRI, the filters were stripped and probed for actin mRNA.

the maximal values obtained following treatment of neutrophils under identical conditions with IFN-γ.

Role of cytokines and immune complexes in synovial fluid-induced FcγRI expression

Rheumatoid synovial fluid contains both soluble and insoluble IgG-containing immune complexes that can activate the respiratory burst in neutrophils.[11] These two types of immune complex can also stimulate *de novo* protein biosynthesis in neutrophils (F. White, J.A. Quayle and S.W. Edwards, unpublished data). Therefore, we measured the effects of synthetic immune complexes on the induction of expresson of neutrophil FcγRI. Neutrophils were therefore incubated for 24 hr at 37° in the presence and absence of a range of concentrations of either soluble or insoluble immune complexes and then FACS analysis was used to detect FcγRI expression. Whilst IFN-γ could induce FcγRI expression under these conditions, neither type of immune complex could induce expression of this receptor (data not shown). We then sequentially removed insoluble and the soluble immune complexes from synovial fluid and determined the effects on induction of receptor expression. However, depletion of either insoluble or soluble immune complexes had no effect on the level of expression of FcγRI induced by the synovial fluid (data not shown). These experiments taken together thus indicate that immune complexes play little, if any, role in the induction of FcγRI expression in neutrophils.

Previous work has indicated that IFN-γ[20] and IL-6[35] may play a role in induction of FcγRI expression in macrophages. We therefore immuno-depleted synovial fluids of these cytokines and then determined their effects on induction of FcγRI expression. Anti- IFN-γ antibody completely blocked the ability of 100 U/ml IFN-γ to induce FcγRI expression (Fig. 5a) indicating that under these experimental conditions the activity of this cytokine is blocked. When added to neutrophil suspensions stimulated with synovial fluid, this antibody decreased induction of FcγRI in 52% of the cells (±15%, $n=3$), as shown in Fig. 5b. However, in parallel experiments, anti-IL-6 antibody was ineffective in blocking FcγRI expression induced by synovial fluid (data not shown).

IFN-γ levels in synovial fluids

Measurements of IFN-γ levels in 10 synovial fluid samples used in these studies using an ELISA assay, revealed that levels ranged from 0 to 133 pg/ml (2·7 U/ml). The mean value was 0·72±0·9 U/ml (33·2±45 pg/ml).

DISCUSSION

For many years it was believed that mature neutrophils were terminally differentiated end cells with little or no requirement or capacity for *de novo* biosynthesis. The fact that neutrophils isolated from the bloodstream had a relatively short lifespan appeared to support this idea. This short lifespan, now known to be a result of bloodstream neutrophils constitutively undergoing apoptosis,[36,37] and supposed biosynthetic incapacity, thus precluded the possibility that these cells could respond to pro-inflammatory signals by changing their cellular makeup via activated gene expression. However, it is now appreciated that neutrophils can selectively undergo enhanced gene

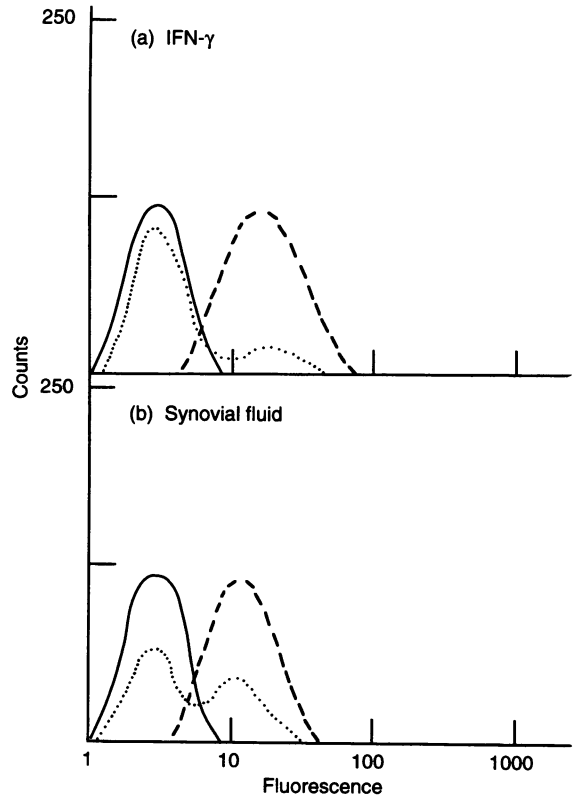

Figure 5. Effect of anti- IFN-γ antibodies on synovial fluid induced FcγRI expression. In (a) neutrophils were incubated for 24 hr in the absence (—) or presence (– – –) of 100U/ IFN-γ or 100 U/ml IFN-γ plus anti- IFN-γ antibody (.....), as described in Materials and Methods. In (b) neutrophils were incubated for 24 hr in the absence (—) or presence (----) of 10% (v/v) synovial fluid or 10% (v/v) synovial fluid plus anti- IFN-γ antibodies (.....). Similar results were obtained in two other separate experiments.

expression during inflammatory challenge[38,39] and so it has been necessary to identify the up-regulated gene products. To date, it has been shown that in response to certain cytokines, lipopolysaccharide (LPS) or other pro-inflammatory signals, neutrophils can actively express several plasma membrane receptors, some components of the NADPH oxidase and several secondary cytokines.[40–44] Thus, inflammatory neutrophils will have quite different biochemical properties to those isolated from the bloodstream. We show here that neutrophils isolated from the synovial fluid of most patients with rheumatoid arthritis express FcγRI on their cell surface and also express mRNA for this receptor. This observation has many implications for the understanding of the molecular pathology of rheumatoid arthritis, and in particular, for elucidating the function of infiltrating neutrophils in this disease.

Transcripts for FcγRI were not present in blood neutrophils from patients with rheumatoid arthritis and surface expression was not detected on these cells. Thus, in this respect, the patient blood neutrophils were not different to those isolated from control, healthy blood. However, in the majority of samples analysed, the synovial fluid neutrophils possessed both surface expression and mRNA for FcγRI. In most cases, surface expression and mRNA levels were comparable to those that could be maximally induced by treatment of control

blood neutrophils with IFN-γ *in vitro*. These observations suggest that in these patients, activation of expression of this gene occurs not in the circulation, but as the cells are recruited into the diseased joint. Indeed, cell-free synovial fluid from these patients could induce increases in mRNA and surface expression of FcγRI in control blood neutrophils. It has been shown that IFN-γ[20,21] and G-CSF,[23] but not granulocyte–macrophage colony-stimulating factor (GM-CSF) (data not shown) can induce this expression in neutrophils. Whilst there is some evidence that both these colony-stimulating factors can be produced in diseased joints, only relatively low levels of IFN-γ have been detected.[45–51] In synovial fluids from rheumatoid arthritis, values of 2–4 U/ml have been reported, but these may be 10-fold higher in osteoarthritis[10,52] Our measurements agree closely with these and are well within the range of IFN-γ levels (>0·4 U/ml) capable of inducing neutrophil FcγRI expression.[53]

It has been proposed that synovial fluid IL-6 can play a role in induction of FcγRI expression on macrophages.[35] In our experiments, immuno-depletion of synovial fluids of IL-6 had little effect on their ability to induce FcγRI expression, whereas IFN-γ appeared to play a major role. Furthermore, these fluids contain soluble and insoluble immune complexes both of which can stimulate a respiratory burst[11] and protein biosynthesis (F. White, J. A. Quayle and S. W. Edwards, unpublished data) in neutrophils. However, synthetic immune complexes failed to induce expression and depletion of these complexes from synovial fluid did little to abrogate receptor induction. These experiments thus point away from immune complexes as the stimuli triggering receptor expression and point towards IFN-γ.

An important observation from these studies is that gene expression is selectively activated in synovial fluid neutrophils. We could not find evidence for increases in mRNA levels for either FcγRII or FcγRIII in synovial fluid neutrophils (data not shown) and likewise no changes in actin mRNA levels were observed. We have previously shown that synovial fluid neutrophils express mRNA for interleukin-1β[33] and thus it is likely that other gene products are up-regulated as neutrophils enter diseased joints.

We found little correlation between levels of mRNA and surface expression of FcγRI in several synovial fluid neutrophil preparations. However, the time courses of changes in mRNA levels and surface expression after treatment with IFN-γ are kinetically distinct. mRNA levels were highest at 4 hr when surface expression was undetectable, whilst surface expression was highest when mRNA levels were declining. The effects of IFN-γ on the levels of neutrophil FcγRI expression that can be induced are also variable in different donors,[53] and a direct correlation between levels of mRNA induction and subsequent cell surface expression has not been established. Thus, a correlation between surface expression and mRNA levels in the population of synovial fluid neutrophils, which are recruited into the joint at different times, would be difficult to detect.

In summary, synovial fluid neutrophils express FcγRI on their cell surface which results from activation of gene expression. Because IgG-containing immune complexes are the major activators of neutrophils within synovial fluid that result in secretion of reactive oxygen metabolites and granule enzymes, increased expression of FcγRI by synovial fluid neutrophils is likely to alter their responsiveness to these complexes. However, the precise function of FcγRI on neutrophils is not yet established and its role in cell activation following the binding of soluble and insoluble immune complexes must be determined.

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