Clin Exp Immunol 1995; **101**:507–514

A systematic study of neutrophil degranulation and respiratory burst *in vitro* by defined immune complexes

W. ZHANG, J. VOICE & P. J. LACHMANN *Molecular Immunopathology Unit, MRC Centre, Cambridge, UK*

(Accepted for publication 26 May 1995)

SUMMARY

Defined immune complexes (IC) were used to compare the effect of antibodies of different classes and subclasses on neutrophil respiratory burst and degranulation. IC were made from 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP) conjugated to bovine serum albumin (BSA) and chimaeric mouse–human anti-NIP monoclonal antibodies including IgA2, IgE and all four IgG subclasses. The activation of neutrophils by IC depended on antibody class and subclass, on antigen epitope density, on antigen : antibody ratio and on the medium used. The ability to generate the respiratory burst showed a different pattern to the ability to give rise to degranulation. Compared with other IC, IgA2 IC provided the strongest stimulus for neutrophil activation. IgG1 IC, IgG2 IC and IgG4 IC activated neutrophils moderately or weakly. IgG3 IC were unable to stimulate the respiratory burst, but could cause strong degranulation. IgE IC could hardly cause any neutrophil response. Neutrophil degranulation in response to IgG3 IC in serum-free medium or heat-inactivated serum was fast, and it quickly reached maximum. Degranulation caused by IgA IC was relatively slow, but gradually increased during incubation. The activity of IgG1 IC, IgG2 IC and IgG4 IC generated a respiratory burst increased with antibody excess and decreased with antigen excess. The activity of IgA2 IC, however, was not affected by change of antigen and antibody ratio. A specific role of serum, possibly due to complement, was found in enhancing degranulation, both temporally and quantitatively, by IgA2 IC.

Keywords immune complexes neutrophils respiratory burst degranulation

INTRODUCTION

Immune complexes (IC) are constantly formed and cleared in the human body as a normal part of antibody formation. Nevertheless, IC can accumulate in the body under pathological conditions and can be deposited at certain sites, causing inflammatory reactions and tissue damage [1].

It is well known that neutrophils can be activated by IC or aggregated immunoglobulins. However, little information is available about the effect of an individual immunoglobulin of a certain class or subclass on the activation of neutrophils. The difficulty lies in making comparable IC of different classes. Several methods have been tried to solve this problem, such as using heat or chemically aggregated myeloma immunoglobulins, using complexes formed by cross-linking $F(ab')_2$ fragments of anti-IgG MoAb to human IgG subclasses or using immunoglobulin-coated Sepharose beads or microtitre plates [2–6]. However, the extent to which these artificial complexes can represent natural IC is questionable. The 'packing' of immunoglobulin molecules in IC depends critically on the epitope distribution in the antigen, and this cannot be imitated by artificially aggregated immunoglobulins.

The direct binding of IC to neutrophils can occur either through neutrophil Fc receptors or complement receptors. Some Fc receptors (FcγRII, FcγRIII) may directly associate with complement receptors (CR1, CR3) on the cell membrane [7–10]. However, most neutrophil functional tests have been done in serum-free medium, which neglects the influence of other serum proteins, such as complement, in the activation of neutrophils by IC.

We have used a well defined IC system to study neutrophil activation in the presence or absence of complement. The IC was made from 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP) conjugated to bovine serum albumin (BSA) and chimaeric anti-NIP MoAbs with the same mouse variable regions and different human constant regions [11]. The data show that neutrophil activation by IC varies according to the antibody class or subclass, the antigen epitope densities as well as the antigen and antibody ratio. Activation can be also influenced by complement.

Correspondence: W. Zhang, Molecular Immunopathology Unit, MRC Centre, Hills Road, Cambridge CB2 2QH, UK.

MATERIALS AND METHODS

NIP–BSA

NIP-caproate-*o*-succinimide (10 mg/ml; Cambridge Research Biochemicals, Northwich, UK) was mixed with an equal volume of BSA from 1 mg/ml to 10 mg/ml in 50 mM carbonate-bicarbonate buffer containing 0·5 M NaCl pH 9·6 at 4°C overnight. NIP-substituted BSA was separated from free NIP by gel filtration. The molar ratio of NIP to BSA was 8·9–1, 12·9–1, 15·6–1 and 24·6–1 as determined by measurement of optical density (OD) at 430 nm for NIP with a molar extinction coefficient of 4900. BSA concentration was measured by the Folin method.

Anti-NIP antibodies

The chimaeric anti-NIP MoAbs were originally raised by Brüggemann *et al.* [11] and were produced by several cell lines (European Collection of Animal Cell Cultures, Salisbury, UK). They were IgG1 (THG1–24), IgG2 (JW183/5/1), IgG3 (TH3-MP-2–19–3-8), IgG4 (JW184/2/1), IgA2 (JW393/A) and IgE (JW8/5/13). The cells were grown in Dulbecco's modified Eagle's medium (Sigma, Poole, UK) containing 10% heat-inactivated fetal calf serum (FCS; Sigma, Poole, UK) until saturated. The supernatants were affinity purified through a NIP-caproate-*o*-succinimide cross-linked AH-Sepharose (Pharmacia, St Albans, UK) column. The purity of the antibodies was checked by ELISA using NIP–BSA-coated plates and alkaline phosphatase-conjugated goat anti-human IgG (γ-chain-specific) (Sigma), goat anti-human IgA (α-chain-specific) (Sigma) and goat anti-human IgE (ϵ-chain-specific) (Sigma) as detection antibodies. The purity of the IgG subclasses was checked by MoAbs against IgG1, IgG2, IgG3 and IgG4 (Sigma) followed by alkaline phosphatase-conjugated goat anti-mouse IgG antibody (Fc-specific) (Sigma).

Affinity of anti-NIP antibodies

The affinity of anti-NIP antibodies was measured by Farr assay as described previously [12].

Antigen and antibody ratio

The antigen and antibody ratio at equivalence was determined by the precipitin method. NIP–BSA was serially diluted × 4/5. Anti-NIP antibodies with trace amounts of ^{125}I-labelled antibody was used as a 200 μg/ml constant concentration. NIP–BSA (40 μl) and 40 μl of anti-NIP antibodies were mixed. The mixtures were left at 4°C overnight and centrifuged for 15 min. The supernatant was aspirated and the precipitate counted. The highest point of IC precipitation occurred at the equivalence ratio for the antigen and the antibody.

Serum

AB-positive normal human serum (NHS) was obtained from healthy donors from the blood bank. The classical complement pathway activity of NHS was determined using the 'Mayer' technique [13]. Two-hundred microlitres of the 500-fold-diluted serum gave 50% lysis of 100 μl 1% sheep erythrocytes (E) coated with anti-sheep E antibody. The alternative complement pathway activity of NHS was determined using the method of Platts-Mills [14]. Two-hundred microlitres of the 28-fold diluted serum gave 50% lysis of 40 μl 1% rabbit E. Heat-inactivated normal human serum (HINHS) was made from NHS treated at 56°C for 35 min. It did not have any detectable complement activity.

Solubility and size of IC

Anti-NIP antibodies (20 μg) and 15·6 NIP–BSA at equivalence, containing trace amounts of ^{125}I-labelled NIP–BSA, were incubated in 150 μl Hanks' balanced saline solution (HBSS; containing 1 mM $CaCl_2$ and 1·5 mM $MgCl_2$), HBSS containing 16% HINHS or 16% or 50% NHS at 37°C for 1 h. They were then centrifuged in a bench centrifuge for 10 min. The pellets were washed once with PBS and counted. The supernatant was loaded onto a 5–25% sucrose gradient and centrifuged at 27 000 rev/min using a TST 28.38/17 swinging bucket rotor (Kontron Instruments, Watford, UK) for 16 h. The fractions were collected and counted.

Isolation of human neutrophils

Neutrophils were isolated from buffy coat from healthy donors by Dextran T500 (Pharmacia) sedimentation followed by density centrifugation using discontinuous Percoll gradients (Pharmacia). After the final wash, neutrophils were resuspended in HBSS. They were normally >95% pure and >99% viable when tested by trypan blue exclusion.

Hydrogen peroxide production

The respiratory burst was measured by hydrogen peroxide production according to the method of Pick [15] with some modifications. IC (25 μl), which were preformed at 37°C for 3 h in HBSS containing 2% BSA, 20% HINHS or 20% NHS with 1 mM sodium azide, were added to 75 μl of a neutrophil suspension containing 4×10^5 cells, 2 μl horseradish peroxidase (HRP; 1000 U/ml; Sigma), 2 μl of 1% phenol red (Sigma) and 1 mM sodium azide in 96-well plates. Hydrogen peroxide with a molar extinction coefficient of 81 M^{-1} cm^{-1} at 230 nm was diluted in 2% BSA, 20% HINHS and 20% NHS containing 1 mM sodium azide to make a standard curve. After incubation at 37°C, 25 μl of 0·4 M NaOH were added to each well. OD was read at 595 nm against the wells in which neutrophils were incubated in the absence of IC.

Degranulation assay

IC at equivalence were formed in HBSS containing 3% BSA, 50% HINHS or 50% NHS at 37°C for 3 h. A neutrophil suspension (100 μl) containing 7·5 μg/ml cytochalasin B (Sigma) at 4×10^6 cells/ml was added to 96-well plates and incubated at 37°C for 10 min. Then 50 μl of IC were added. After incubation at 37°C, the supernatant was taken out and used for the degranulation assay.

β-glucuronidase was used as an indication of primary granule release. The method was similar to that described before [16] but adapted for microtitre plates. In brief, 77 μl of 0·04 M sodium acetate pH 4·5 and 23 μl of 0·01 M phenolphthalein glucuronic acid (Sigma) were added to 96-well plates. Then 25 μl of neutrophil supernatant were added. The standard was 25 μl of phenolphthalein (Sigma) which was double diluted from 250 μg/ml in 1% BSA, 16% HINHS or 16% NHS. After incubation at 37°C in a moist box for 18 h, the plates were centrifuged and 90 μl of supernatant were transferred into another set of 96-well plates containing 90 μl of 0·4 M glycine pH 10·5. OD was read at 550 nm.

Lactoferrin was used as an indication of secondary granule

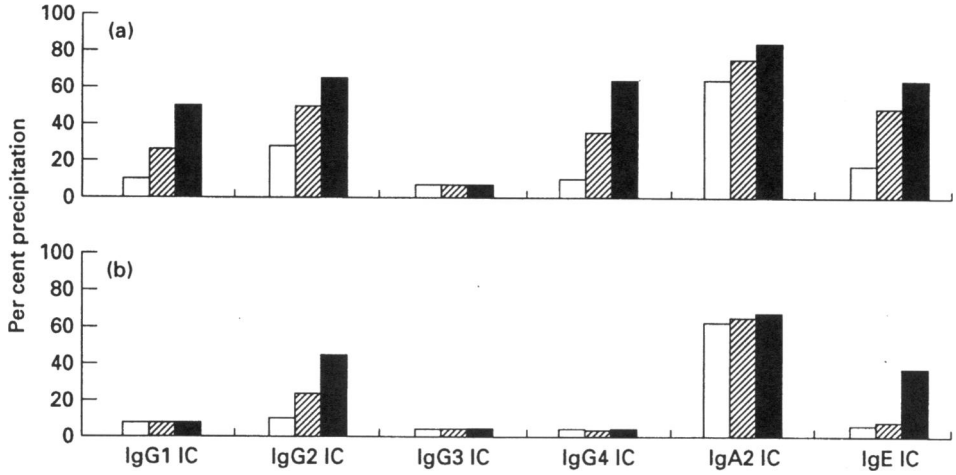

Fig. 1. Antigen epitope density and immune complex (IC) solubility. IC were formed at equivalence (a) or three times antigen excess (b) using ^{125}I-labelled antibodies as tracers. Antibodies (40 μl; 100 μg/ml) in PBS were mixed with 40 μl 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP)–bovine serum albumin (BSA) at 4°C overnight. Then the mixture was centrifuged and the percentage precipitation was calculated from the counts of the pellets divided by the total counts offered. □, 8·9 NIP–BSA; ▨, 12·9 NIP–BSA; ■, 24·6 NIP–BSA.

release. It was quantified by sandwich ELISA. The IgG fraction of rabbit anti-lactoferrin serum (Dako Ltd, High Wycombe, UK) was further affinity-purified through a lactoferrin (Calbiochem, Nottingham, UK)-coupled CL-4B column. Ninety-six-well plates were coated with anti-lactoferrin antibody. The bound lactoferrin was detected by alkaline phosphatase-conjugated anti-lactoferrin antibody. The standard curve was determined using lactoferrin (Calbiochem).

RESULTS

Anti-NIP antibodies

Purified anti-NIP antibodies showed the same band patterns on SDS–PAGE under either non-reducing or reducing conditions as reported before [11]. All of them reacted with anti-mouse IgG (Fab specific) antibody and each of them reacted in ELISA only with the appropriate antibody which was restricted to a specific immunoglobulin class or subclass (data not shown). No cross-reactions were found between them. The affinity constants of the antibodies in their reaction with NIP were $2{\cdot}1 \times 10^7\,M^{-1}$ (IgG1), $2{\cdot}5 \times 10^7\,M^{-1}$ (IgG2), $2{\cdot}4 \times 10^7\,M^{-1}$ (IgG3), $2{\cdot}6 \times 10^7\,M^{-1}$ (IgG4) and $2{\cdot}7 \times 10^7\,M^{-1}$ (IgA2).

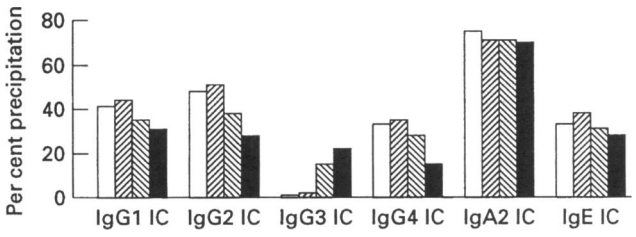

Fig. 2. Precipitation of immune complexes (IC) in different media. IC were formed at equivalence using ^{125}I-labelled 15·6 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP)–bovine serum albumin (BSA) as a tracer and incubated at 37°C for 1 h in different media (see Materials and Methods). The percentage precipitation was calculated from the counts of the pellets divided by the total counts offered. □, HBSS; ▨, 16% heat-inactivated normal human serum (HINHS); ▧, 16% NHS; ■, 50% NHS.

IC size and solubility

The solubility of IC at equivalence varied according to the epitope density, the antibody class and subclass as well as the medium used. All IC formed at high epitope density were less soluble than those formed at low epitope density (Fig. 1). IC were generally more soluble in three-fold antigen excess than at equivalence. However, the solubility of IgA2 IC was less affected by antigen excess (Fig. 1).

When 15·6 NIP–BSA was used at equivalence, most IgA2 IC were insoluble in HBSS, whereas IgG3 IC were very soluble (Fig. 2). Most IC formed in 16% HINHS had solubility similar to those formed in HBSS. When IC were formed in 16% or 50% NHS, they generally became more soluble, presumably due to the complement-mediated prevention of immune precipitation and solubilization of IC [17,18]. The solubility of IgA2 IC was least affected by NHS, as has also been observed by others [19]. IgG3 IC were an interesting exception. They were less soluble in NHS than in HBSS.

The supernatants of IC made with 15·6 NIP–BSA were analysed by centrifugation through a 5–25% sucrose gradient. Supernantants from IgA2 IC had the radiolabelled NIP–BSA at the same peak position as free NIP–BSA, indicating that most IgA2 IC had precipitated (Fig. 3). The soluble IC formed by the other antibodies in HBSS and 16% HINHS were all of similar size with a peak position around fraction 33, with only one fraction variation, corresponding to a sedimentation coefficient about 10S. When NHS was used, some curves shifted to a lower fraction position indicating increase of IC size, presumably due to deposition of complement components on IC. However, IgG3 IC, although they fix C well, did not have any obvious curve shift.

Respiratory burst

The production of hydrogen peroxide as a consequence of neutrophil stimulation by IC varied according to antibody

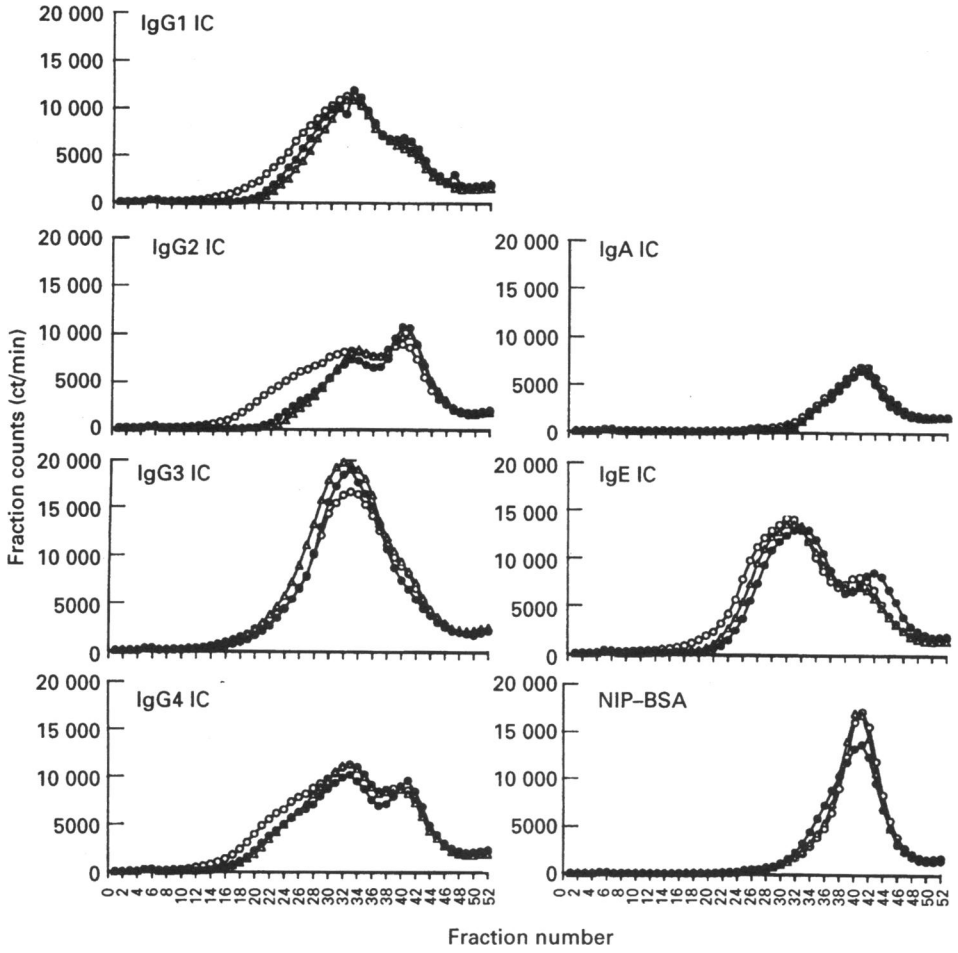

Fig. 3. Sucrose gradient (5–25%) centrifugation of soluble immune complexes (IC) from the supernatant of anti-5-iodo-4-hydroxy-3-nitrophenacetyl (NIP) antibody and 15·6 NIP–bovine serum albumin (BSA). Each IC had the same amount of ^{125}I-labelled 15·6 NIP–BSA as a tracer. Fractions of equal volume were collected and numbered from 1 (bottom of the centrifuge tube, 25% sucrose) to 52 (top of the centrifuge tube, 5% sucrose). ●, Incubated in HBSS; Δ, incubated in 16% heat-inactivated normal human serum (HINHS); ○, incubated in 16% NHS.

class and subclass as well as antigen epitope density. Of all the IC tested at equivalence, IgA2 IC stimulated neutrophils the most. The differences were greatest when the antigen had a low epitope density of 8·9 NIP–BSA (Fig. 4). When 24·6 NIP–BSA, the antigen with high epitope density, was used, IgG1 IC, IgG2 IC, IgG4 IC but not IgG3 IC caused a neutrophil respiratory burst. The responses to IgG2 IC and IgG4 IC were rather strong, whereas those to IgG1 IC were weaker. When 8·9 NIP–BSA was used, the activities of IgG1 IC, IgG2 IC and IgG4 IC were much decreased, whereas the activity of IgA2 IC remained at the same level (Fig. 4). IgE IC did not stimulate neutrophils in any media with either high epitope density or low epitope density antigens. No stimulation was observed when neutrophils were incubated with free antibody and free antigen (data not shown). IC formed in 20% NHS did not have obvious differences in the activity to those IC formed in 2% BSA or 20% HINHS. Higher concentration of NHS was not used, as the sensitivity of the assay decreased while the serum concentration increased.

Since the antigen/antibody ratio also affects the activity of IC, neutrophil stimulation was tested with high epitope density (24·6 NIP–BSA) IC made with three times antigen excess or three times antibody excess. Compared with IC at equivalence, the activity of IgG1 IC and IgG4 IC in antigen excess decreased by 65–75% and the activity of IgG2 IC decreased by about 35%. On the other hand, in antibody excess the activity of IgG1 IC, IgG2 IC and IgG4 IC increased at least two-fold (Fig. 5). The activity of IgA2 IC did not change greatly in either antibody excess or antigen excess. IgG3 IC and IgE IC did not cause a neutrophil respiratory burst in either antigen excess or antibody excess. When 8·9 NIP–BSA was used, IgG1 IC, IgG2 IC and IgG4 IC gave a slight increase of activity in three times antibody excess; whereas IgA2 IC kept the same level of activity as at equivalence, even at four times antigen or antibody excess (data not shown).

Degranulation

The primary (azurophilic) granule release and the secondary (specific) granule release showed similar dose response and time course patterns in neutrophil degranulation. Only the secondary granule release results are shown here.

The dose response profiles of neutrophil degranulation

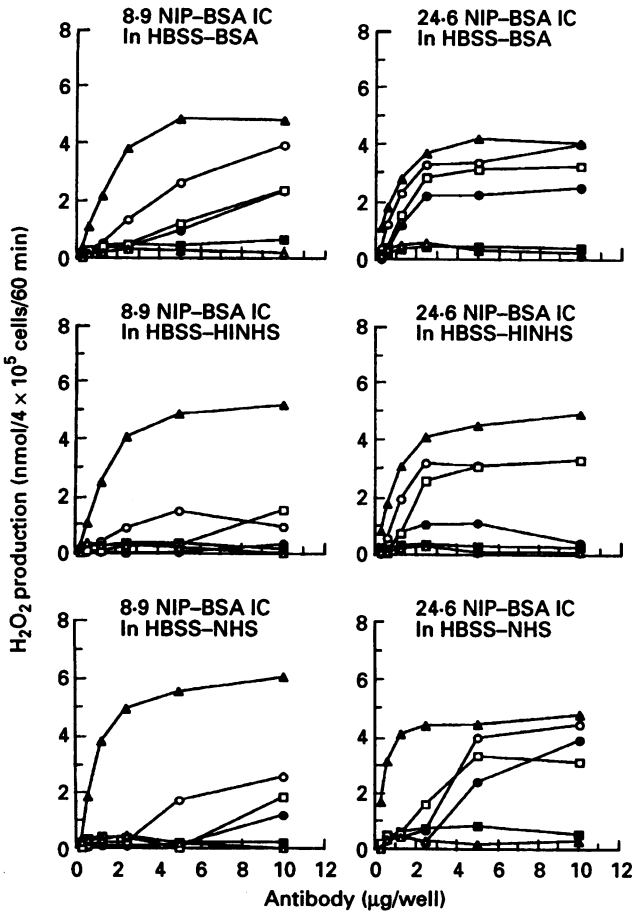

Fig. 4. The dose response of neutrophil respiratory burst induced by immune complexes (IC) with different antigen epitope density and different immunoglobulin class and subclass. IC were preformed at equivalence with 8·9 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP)–bovine serum albumin (BSA) or 24·6 NIP–BSA in the indicated media at 37°C for 3 h. Neutrophils (4 × 10^5) were then incubated with IC in 96-well plates at 37°C for 1 h and the hydrogen peroxide concentration in each well was measured (see Materials and Methods). Samples were tested in duplicates. HINHS, Heat-inactivated normal human serum. ●, IgG1 IC; ○, IgG2 IC; ■, IgG3 IC; □, IgG4 IC; ▲, IgA2 IC; △, IgE IC.

differed with the antibody class and subclass of the IC (Fig. 6). After neutrophils were incubated with preformed IC at 37°C for 60 min, IgA2 IC again proved to be the best stimulus, especially in the presence of serum. All IgG IC could activate neutrophils moderately or weakly. IgG3 IC, which did not stimulate neutrophils to undergo a respiratory burst, caused the highest level of degranulation of the IgG subclasses. IgG4 IC, which were quite strong in inducing neutrophil respiratory burst, were weak in inducing neutrophil degranulation. IC with 24·6 NIP–BSA as the antigen in general gave a higher activity than the IC with 8·9 NIP–BSA as the antigen, although with IgG1 IC the reverse was found. IgA2 IC, IgG2 IC and IgG4 IC formed in 50% NHS were more active than those formed in 3% BSA or 50% HINHS. The activities of IgG1 IC and IgG3 IC, even though they are strong classical complement pathway activators, were not substantially different when formed either in the presence or in the absence of complement. Neutrophils did not give a significant degranulation response to IgE IC. Free antibodies and free NIP–BSA did not cause neutrophil degranulation (data not shown).

A time course of neutrophil degranulation in response to IC was tested. The speed of degranulation differed according to antibody class and subclass (Fig. 7). IgG3 IC and IgG1 8·9 NIP–BSA IC triggered neutrophils to undergo rapid degranulation in HBSS containing BSA or HINHS. Maximal degranulation was reached within the first 15 min. However, the degranulation caused by IgA2 IC, IgG2 IC and IgG4 IC was rather slow, but steadily increased over a 120-min incubation time. Although the level of neutrophil degranulation in response to IgA2 IC was initially less than the response to IgG3 IC, it eventually exceeded the degranulation caused by IgG3 IC, with IgA2 IC becoming the strongest stimulus after longer periods of incubation. The neutrophil response to IgA2 IC formed in the presence of NHS was at least twice as fast as their response to IgA2 IC formed in the absence of NHS. However, NHS seemed to slow the neutrophil response to IgG3 IC. Neutrophils did not show any response to IgE IC over the 180-min incubation.

DISCUSSION

IgA constitutes about 20% of the immunoglobulin pool in human serum and it is the most abundant immunoglobulin in

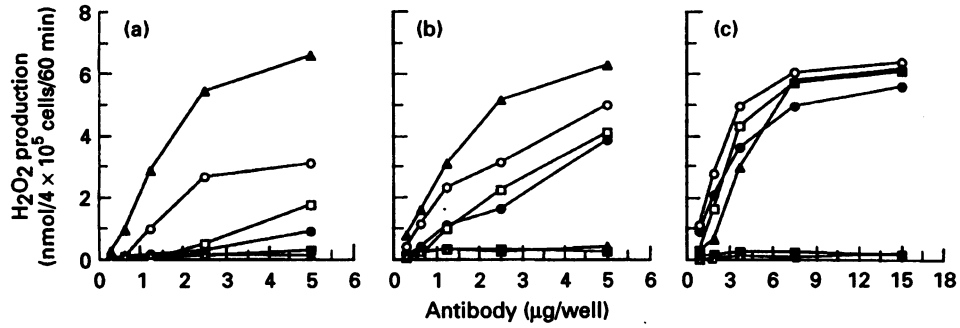

Fig. 5. The effect of antigen:antibody ratio on neutrophil respiratory burst. Antibodies with different immunoglobulin class and subclass were incubated with 24·6 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP)–bovine serum albumin (BSA) at equivalence (b), three times antigen excess (a), or three times antibody excess (c) in 2% BSA at 37°C for 3 h. They were then incubated with 4 × 10^5 neutrophils at 37°C for 1 h and the hydrogen peroxide concentration in each well was measured (see Materials and Methods). Samples were tested in duplicates. ●, IgG1 IC; ○, IgG2 IC; ■, IgG3 IC; □, IgG4 IC; ▲, IgA2 IC; △, IgE IC.

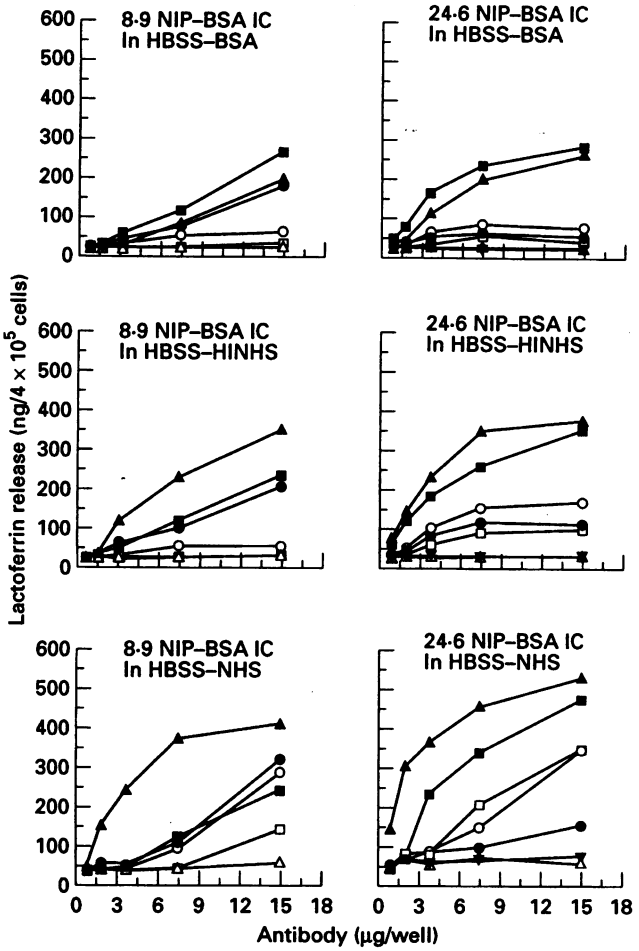

Fig. 6. The dose response of neutrophil degranulation induced by immune complexes (IC) with different antigen epitope density and different immunoglobulin class and subclass. IC were preformed at equivalence with 8·9 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP)–bovine serum albumin (BSA) or 24·6 NIP–BSA in the indicated media at 37°C for 3 h. Neutrophils (4×10^5) were then incubated with IC in 96-well plates at 37°C for 1 h and the lactoferrin concentration from the supernatant in each well was measured (see Materials and Methods). Samples were tested in duplicates. ●, IgG1 IC; ○, IgG2 IC; ■, IgG3 IC; □, IgG4 IC; ▲, IgA2 IC; △, IgE IC; ▼, medium. HINHS, Heat-inactivated normal human serum.

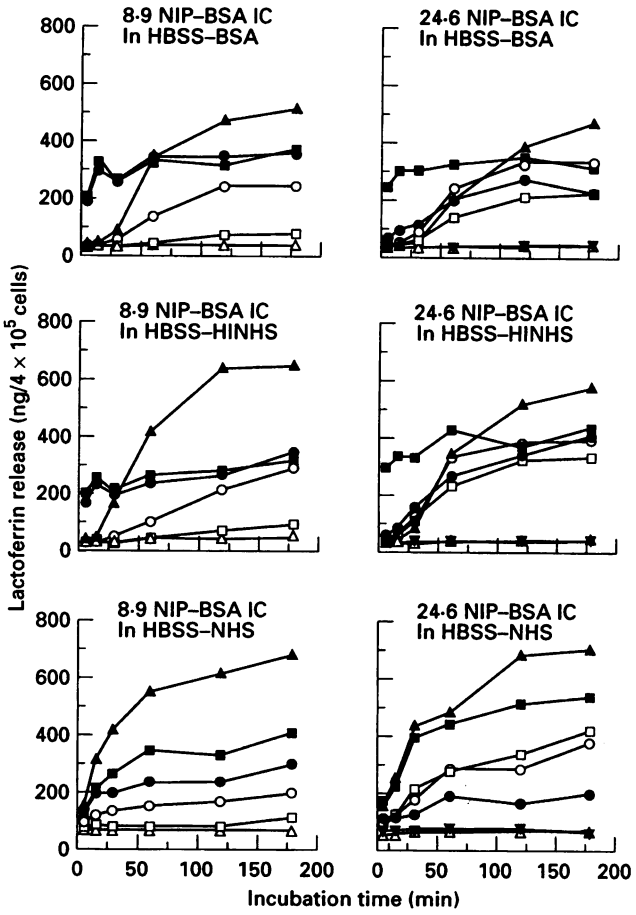

Fig. 7. The time course of neutrophil degranulation. Antibodies with different immunoglobulin class and subclass were incubated with 8·9 5-iodo-4-hydroxy-3-nitrophenacetyl (NIP)–bovine serum albumin (BSA) or 24·6 NIP–BSA at equivalence at 37°C for 3 h in the indicated media. Then IC with 10 μg/well antibody concentration was incubated with 4×10^5 neutrophils at 37°C from 5–180 min. The released lactoferrin in the supernatant was measured (see Materials and Methods). Samples were tested in duplicates. ●, IgG1 IC; ○, IgG2 IC; ■, IgG3 IC; □, IgG4 IC; ▲, IgA2 IC; △, IgE IC; ▼, medium. HINHS, Heat-inactivated normal human serum.

secretions. It has a faster turnover rate than other immunoglobulins. The role of IgA in the inflammatory response is controversial. It used to be thought that IgA had anti-inflammatory functions [20–22]. It has also been reported that IgA IC do not stimulate a rat neutrophil respiratory burst or lysosomal enzyme release [23]. However, some reports showed that aggregated IgA or IgA-opsonized bacteria or beads are able to stimulate human neutrophils to degranulate, phagocytose and induce a respiratory burst [2,5,6,24]. It has also been reported that IgA is able to induce eosinophil degranulation and to mediate the killing of schistosomula by eosinophils [25,26]. In our study, the results showed that IgA2 IC were the best stimulus for inducing neutrophil degranulation and respiratory burst. We have not measured the ability of IgA1 IC in activation of neutrophils since there is no IgA1 anti-NIP antibody available to us at present. We expect that IgA1 IC will have similar effects, since the binding of FcαR to both IgA subclasses is equal [27,28].

Freshly isolated neutrophils express two kinds of Fc receptors for IgG, FcγRII and FcγRIII. FcγRII is capable of mediating both degranulation and respiratory burst [29–32]. FcγRIII is capable of mediating only degranulation and not respiratory burst [29,30,33] and neutrophils do not generate hydrogen peroxide by anti-FcγRIII MoAb which is coated on solid surface [34]. The affinity of Fcγ receptors to IgG differs according to the immunoglobulin subclass. FcγRIII has relatively high affinity to IgG3 and FcγRII can have relatively high or very low affinity to IgG3 [35,36]. Although both FcγRII and FcγRIII can bind IC or immunoglobulin-coated particles, the binding of neutrophils to small IC is FcγRIII-dependent [29]. A preliminary result in our laboratory showed that the binding of neutrophils to our IgG3 IC was through FcγRIII rather than FcγRII, which could be due either to a very low affinity of

FcγRII to this particular antibody or to the exceptional solubility (indicating the IC were small) of the IC. In this study we have shown that IgG3 IC do not induce a neutrophil respiratory burst, although they provide a strong stimulus for inducing neutrophil degranulation. Our explanation for this phenomenon is that the binding of neutrophils to the IgG3 IC is through FcγRIII and this receptor does not mediate respiratory burst. However, FcγRIII functions are rather controversial. It has been observed that neutrophils can generate O_2^- by cross-linking their FcγRIII [37]. IC-induced neutrophil respiratory burst can be inhibited by anti-FcγRIII antibodies [31]. In addition, it has also been reported that Sepharose beads and sheep erythrocytes coated with IgG3 can induce neutrophil respiratory burst [38,39]. We will investigate further why our IgG3 IC did not cause a respiratory burst.

Neutrophils express neither a high-affinity IgE receptor (FcεRI) nor a low-affinity IgE receptor (FcεRII). However, an IgE binding protein (Mac-2) expressed on neutrophils has recently been reported [40]. The authors have observed an induced respiratory burst using anti-IgE antibody cross-linking IgE which is prebound on the neutrophils. It is difficult to judge whether the activation is caused by IgE or by anti-IgE antibody if the Fc part of anti-IgE antibody has not been removed. In our study, we were unable to detect any respiratory burst or degranulation when neutrophils were incubated with IgE IC.

It has been reported that C3b and C3bi alone are unable to stimulate production of hydrogen peroxide and degranulation [41,42]. Deposition of C3 on IC can slightly increase the neutrophil respiratory burst and degranulation [42,43]. However, no increased generation of hydrogen peroxide or even decreased release of myeloperoxidase caused by C3 deposition on IC has been observed [44]. In our study, NHS did not obviously enhance neutrophil respiratory burst and degranulation for IgG1 and IgG3 IC, which were good complement activators [45]. NHS, however, enhanced neutrophil degranulation caused by IgA2 IC. At present, we do not know if this enhancement is caused by complement. It has been reported that the expression of both FcαR and CR3 are up-regulated but FcγRII remains unchanged when neutrophils are exposed to zymosan-activated serum (a source of C5a) [46]. It would be interesting to investigate if there are any associations between complement receptors and Fcα receptor.

Our results showed that different IC had a different kinetic process for neutrophil degranulation. IgG3 IC caused quick degranulation which only increased slightly during longer incubation, whereas IgA2 IC caused slow degranulation which increased greatly during incubation. The kinetic difference may be due to different signal transduction pathways. The level of Fc receptor expression and recycling during neutrophil incubation with IC may also play a part in the kinetics of neutrophil activation.

ACKNOWLEDGMENTS

This work was partly supported by the British Heart Foundation (BHF Grant no. 90/96). J. V. is in receipt of a Medical Research Council studentship. We would like to thank Dr Marianne Brüggemann for providing anti-NIP antibody cell lines and Dr Pat Clissold, Dr Anne Wilson and Dr Tim Farries for very helpful discussions. Mrs Sarah Coppendale corrected the English.

REFERENCES

1 Schifferli JA, Taylor RP. Physiological and pathological aspects of circulating immne complexes. Kidney Int 1989; **35**:993–1003.
2 Henson PM, Johnson HB, Spiegelberg HL. The release of granule enzymes from human neutrophils stimulated by aggregated immunoglobulins of different classes and subclasses. J Immunol 1972; **109**:1182–92.
3 Huizinga TWJ, Kerst M, Nuyens JH *et al.* Binding characteristics of dimeric IgG subclass complexes to human neutrophils. J Immunol 1989; **142**:2359–64.
4 Yazdanbakhsh M, Eckmann CM, Roos D. Characterization of the interaction of human eosinophils and neutrophils with opsonized particles. J Immunol 1985; **135**:1378–84.
5 Stewart WW, Kerr MA. The specificity of the human neutrophil IgA receptor (FcαR) determined by measurement of chemiluminescence induced by serum or secretory IgA1 and IgA2. Immunology 1990; **71**:328–34.
6 Albrechtsen M, Yeaman GR, Kerr MA. Characterization of the IgA receptor from human polymorphonuclear leucocytes. Immunology 1988; **64**:201–5.
7 Jack RM, Fearon DT. Altered surface distribution of both C3b receptors and Fc receptors on neutrophils induced by anti-C3b receptor or aggregated IgG. J Immunol 1984; **132**:3028–33.
8 Graham IL, Lefkowith JB, Anderson DC *et al.* Immune complex-stimulated neutrophil LTB_4 production is dependent on β_2 integrins. J Cell Biol 1993; **120**:1509–17.
9 Zhou MJ, Todd III RF, van de Winkel JGJ *et al.* Cocapping of the leukoadhesin molecules complement receptor type 3 and lymphocyte function-associated antigen-1 with Fcγ receptor III on human neutrophils. J Immunol 1993; **150**:3030–41.
10 Sehgal G, Zhang K, Todd III RF *et al.* Lectin-like inhibition of immune complex receptor-mediated stimulation of neutrophils. J Immunol 1993; **150**:4571–80.
11 Brüggemann M, Williams GT, Bindon CI *et al.* Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. J Exp Med 1987; **166**:1351–61.
12 Zhang W, Lachmann PJ. Glycosylation of IgA is required for optimal activation of the alternative complement pathway by immune complexes. Immunology 1994; **81**:137–41.
13 Harrison RA, Lachmann PJ. Complement technology. In: Weir DM, Herzenberg LA, Blackwell CL, Herzenberg A, eds. Handbook of experimental immunology, Vol. 1. Oxford: Blackwell Scientific Publications, 1986: 39·25–6.
14 Platts-Mills TAE, Ishizaka K. Activation of the alternate pathway of human complement by rabbit cells. J Immunol 1974; **113**:348–58.
15 Pick E. Microassays for superoxide and hydrogen peroxide production and nitroblue tetrazolium reduction using an enzyme immunoassay microplate reader. Methods Enzymol 1986; **132**: 407–21.
16 Wright DG. Human neutrophil degranulation. Methods Enzymol 1988; **162**:538–51.
17 Schifferli JA, Bartolotti SR, Peters DK. Inhibition of immune precipitation by complement. Clin Exp Immunol 1980; **42**:387–94.
18 Czop J, Nussenzweig V. Studies on the mechanism of solubilization of immune precipitation by serum. J Exp Med 1976; **143**:615–30.
19 Johnson A, Harkin S, Steward MW *et al.* The effects of immunoglobulin isotype and antibody affinity on complement-mediated inhibition of immune precipitation and solubilization. Mol Immunol 1987; **24**:1211–7.
20 Russel MW, Reinholdt J, Kilian M. Anti-inflammatory activity of human IgA antibodies and their Fabα fragments: inhibition of IgG-mediated complement activition. Eur J Immunol 1989; **19**:2243–9.
21 van Epps DE, Williams RC. Suppression of leukocyte chemotaxis by human IgA myeloma components. J Exp Med 1976; **144**:1227–42.
22 Saito K, Kato C, Katsuragi H *et al.* IgA-mediated inhibition of

human leukocyte function by interference with Fcγ and C3b receptors. Immunology 1991; **74**:99–106.

23 Warren JS, Kunkel SL, Johnson KJ *et al. In vitro* activation of rat neutrophils and alveolar macrophages with IgA and IgG immune complexes. Am J Pathol 1987; **129**:578–88.

24 Gorter A, Hiemstra PS, Leijh PCJ *et al.* IgA- and secretory IgA-opsonized *S. aureus* induce a respiratory burst and phagocytosis by polymorphonuclear leucocytes. Immunology 1987; **61**:303–9.

25 Dunne DW, Richardson BA, Jones FM *et al.* The use of mouse/human chimaeric antibodies to investigate the roles of different antibody isotypes, including IgA2, in the killing of *Schistosoma mansoni* schistosomula by eosinophils. Parasite Immunol 1993; **15**:181–5.

26 Abu-Ghazaleh RI, Fujisawa T, Mestecky J *et al.* IgA-induced eosinophil degranulation. J Immunol 1989; **142**:2393–400.

27 Kerr MA. The structure and function of human IgA. Biochem J 1990; **271**:285–96.

28 Mazengera RL, Kerr MA. The specificity of the IgA receptor purified from human neutrophils. Biochem J 1990; **272**:159–65.

29 Huizinga TWJ, van Kemenade F, Koenderman L *et al.* The 40-kDa Fcγ receptor (FcRII) on human neutrophils is essential for the IgG-induced respiratory burst and IgG-induced phagocytosis. J Immunol 1989; **142**:2365–9.

30 Tosi MF, Berger M. Functional differences between the 40 kDa and 50–70 kDa IgG Fc receptors on human neutrophils revealed by elastase treatment and antireceptor antibodies. J Immunol 1988; **141**:2097–103.

31 Crockett-Torabi E, Fantone JC. Soluble and insoluble immune complexes activate human neutrophil NADPH oxidase by distinct Fcγ receptor-specific mechanisms. J Immunol 1990; **145**:3026–32.

32 Willis HE, Browder B, Feister AJ *et al.* Monoclonal antibody to human IgG Fc receptors. J Immunol 1988; **140**:234–9.

33 Huizinga TWJ, Dolman KM, van der Linden NJM *et al.* Phosphatidylinositol-linked FcRIII mediates exocytosis of neutrophil granule proteins, but does not mediate initiation of the respiratory burst. J Immunol 1990; **144**:1432–7.

34 Zhou MJ, Brown EJ. CR3 (Mac-1, $\alpha_M\beta_2$, CD11β/CD18) and FcγRIII cooperate in generation of a neutrophil respiratory burst: requirement for FcγRII and tyrosine phosphorylation. J Cell Biol 1994; **125**:1407–16.

35 Stengelin S, Stamenkovic I, Seed B. Isolation of cDNAs for two distinct human Fc receptors by ligand affinity cloning. EMBO J 1988; **7**:1053–9.

36 Huizinga TWJ, Roos D, von dem Borne AEGKr. Neutrophil Fc-γ receptors: a two-way bridge in the immune system. Blood 1990; **75**:1211–4.

37 Walker BAM, Hagenlocker BE, Stubbs EB *et al.* Signal transduction events and FcγR engagement in human neutrophils stimulated with immune complexes. J Immunol 1991; **146**:735–41.

38 Aase A, Michaelsen TE. Opsonophagocytic activity induced by chimeric antibodies of the four human IgG subclasses with or without help from complement. Scand J Immunol 1994; **39**:581–7.

39 Yazdanbakhsh M, Eckmann CM, Roos D. Characterization of the interaction of human eosinophils and neutrophils with opsonized particles. J Immunol 1985; **135**:1378–84.

40 Truong MJ, Gruart V, Kusnierz JP *et al.* Human neutrophils express immunoglobulin E (IgE)-binding proteins (Mac-2/εBP) of the S-type lactin family: role in IgE-dependent activation. J Exp Med 1993; **177**:243–8.

41 Newman SL, Johnston RB. Role of binding through C3b and IgG in polymorphonuclear neutrophil function: studies with trypsin-generated C3b. J Immunol 1979; **123**:1839–46.

42 Lucisano YM, Mantovani B. The role of complement in the stimulation of lysosomal enzyme release by polymorphonuclear leucocytes induced by immune complexes of IgG and IgM. Immunology 1988; **65**:171–5.

43 Wright SD, Silverstein SC. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. J Exp Med 1983; **158**:2016–23.

44 Chatham WW, Blackburn WD. Fixation of C3 to IgG attenuates neutrophil HOCl generation and collagenase activation. J Immunol 1993; **151**:949–58.

45 Valim YML, Lachmann PJ. The effect of antibody isotype and antigenic epitope density on the complement-fixing activity of immune complexes: a systematic study using chimaeric anti-NIP antibodies with human Fc regions. Clin Exp Immunol 1991; **84**:1–8.

46 Hostoffer RW, Krukovets I, Berger M. Increased FcαR expression and IgA-mediated function on neutrophils induced by chemoattractants. J Immunol 1993; **150**:4532–40.