Interaction of intravenous immunoglobulines (IVIG) with Fcγ-receptors: activation and inhibition of phagocyte functions

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Chapter 2
Human intravenous immunoglobulin preparations degranulate human neutrophils in vitro


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Abstract

Intravenous immunoglobulin (IVIG) preparations have biological effects in vivo that are not fully understood. Possible effects include the property to stimulate Fcγ-receptors on various cell types. To study whether IVIG may interact with neutrophils we developed an in vitro system, in which neutrophils, in whole blood or purified were incubated with IVIG and assessed for degranulation by measuring the release of elastase and lactoferrin in culture medium.

All IVIG preparations tested induced degranulation of neutrophils when incubated for 2 hours at therapeutically relevant concentrations. In studies with blocking antibodies against FcγR, this degranulation was shown to be dependent on FcγRII, whereas FcγRIII had no effect. Experiments with purified neutrophils as well as binding experiments with labelled IVIG preparations, indicated that neutrophil degranulation resulted from a direct interaction of IVIG with neutrophils. Using gel-filtration fractions, it was found that polymeric and dimeric IgG present in IVIG was mainly responsible for the degranulation. We suggest that degranulation of neutrophils may contribute to the (side) effects of IVIG-treatment in vivo.

Introduction

Administration of intravenous γ-globulin (IVIG) is a treatment of choice for primary immunodeficiency disorders associated with hypo-/agammaglobulinemia. In addition, IVIG is increasingly used as a therapy for a number of secondary immunodeficiencies and (auto)-immune diseases.1-5 Though IVIG preparations are said to have a wide range of biological effects in vivo, their mechanism of action in the latter group of diseases is not fully understood. One of the mechanisms proposed is that IVIG inhibit Fc-mediated destruction of antibody-sensitized cells such as platelets by binding to Fcγ receptors on mononuclear phagocytes.6 However, also other cells than mononuclear phagocytes carry Fcγ receptors, and interaction of IVIG with these cells may also induce biological effects.
Under normal conditions neutrophils express two types of receptors for the Fc region of IgG, FcγRIIa and FcγRIIib, whereas upon stimulation with interferon-γ they also express FcγRII. Triggering of these receptors, for example upon binding of IgG-complexes consisting of more that one IgG molecule, leads to activation of neutrophils and stimulates the respiratory burst, phagocytosis, and degranulation. To examine whether neutrophil activation may play a role in therapeutical and/or side effects in patients receiving IVIG, we developed an in vitro system, in which neutrophils either in whole blood or purified were incubated with IVIG and assessed for degranulation. Furthermore, the contribution of FcγRII or FcγRIII to the observed phenomena was observed. Our results indicate that commercially available IVIG is capable of stimulating neutrophils by interacting with their Fcγ receptors.

MATERIAL AND METHODS

Immunoglobulin preparations

Human gammaglobulin for intravenous use was obtained from our institute (Immunoglobulin I.V., 6% (w/v); Lot no. 960215), and used in most experiments. Other commercial freeze-dried preparations evaluated were Gammagard® (5%; Lot no. 94119AB21B, Baxter Healthcare Corp., Glendale, CA) and Sandoglobulin® (6%; Lot no. 4.364.446.0, Sandoz Pharma Ltd, Basel, Switzerland). An IgG preparation for intramuscular administration (Immunoglobulin I.M., 16% w/v; 921223-034) was obtained from our institute.

Initial experiments with the freeze-dried preparations were performed immediately after reconstitution; the preparations were stored at 4°C for later experiments.

ELISA for elastase

Elastase levels were assessed with a sandwich-type ELISA modified from a radioimmunoassay procedure described previously. Briefly, affinity-purified polyclonal rabbit anti-human elastase was diluted in 0.1 M carbonate buffer, pH 9.6, at a final concentration of 1.5 μg/ml, and incubated overnight at room temperature in ELISA plates (Maxisorb; Nunc, Roshilde, Denmark). After washing with PBS-0.02% (w/v) Tween-20 (PT), residual binding sites were blocked by incubation with PBS containing 2% (v/v) cow milk for 30 minutes at room temperature. The plates were washed 5 times with PT. Supernatants of whole blood cultures were appropriately diluted in PBS-0.1% (w/v) Tween-20-0.2% (w/v) gelatine (PTG), and incubated for 1 hour at room temperature. Thereafter, the plates were washed 5 times with PT and incubated for 60 minutes at room temperature with biotinylated IgG fraction of polyclonal rabbit-anti-human-elastase in PTG supplemented with 0.1% (v/v) bovine/rabbit serum. After 5 washes with PT, the wells were incubated with 1:1000 diluted streptavidin-horseradish peroxidase (Amersham Life Sciences; Buckinghamshire, UK) in PTG for 30 minutes at room tempera-
ture. Finally, after 5 washes the plates were developed with tetramethyl-benzidine (TMB, Sigma Chemical Co. St. Louis, MO). At a concentration of 100 µg/ml in 0.11 sodium acetate, pH 5.5, containing 0.003% (v/v) H₂O₂. Substrate conversion was stopped by adding 1 volume of 2 M H₂SO₄ and absorbance at 450 nm was determined with a Titertek Multiscan (Flow Laboratories, Mc Lean, VA).

A serial dilution of human neutrophil elastase purified from sputum (Elastin Products Co.; Pacific, MO) was used as a standard. The amount of elastase in samples was expressed as ng/ml

**ELISA for lactoferrin**

Levels of lactoferrin in whole blood cultures were assessed by a sandwich-type ELISA similar to that for elastase except that plates (polysorb; Nunc) were coated with 1 µg/ml affinity purified rabbit-anti-human-lactoferrin in 0.1 M carbonate buffer, pH 9.6, and that bovine-anti-human-lactoferrin conjugated with peroxidase were used as detecting antibodies. Human lactoferrin was used as a standard, the amount of lactoferrin was expressed as ng/ml. Lactoferrin as well as the anti-lactoferrin antibodies were kindly provided by Dr. J.H. Nuijens, Pharming BV, Leiden, The Netherlands.

**Antibodies**

mAb AT10 directed against FcγRII¹³ (CD32, IgG1) was a generous gift from Prof. dr. J.v.d.Winkel. CLB-FcR/gran-1 (5D2, IgG2a) was produced in our own institute.¹⁴ Monoclonal antibodies against TNF-α (αTNF/5, αTNF/7) and IL-8 (αIL-8/1) have been described before.¹⁵,¹⁶ F(ab')₂ fragments were prepared by digestion with 2% (w/w) pepsin at pH 3.5 for 16 hours at 37°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis revealed that the preparation did not contain detectable intact antibodies or Fc-fragments.

**Blood samples**

Venous blood was collected from healthy volunteers by venapuncture in vacutainer tubes containing heparin at a final concentration of 15 U/ml (Becton Dickinson, Rutherford, NJ). Whole blood was cultured in 96-well micotiter plates (Nunc, Roskilde, Denmark) at a final volume of 0.2 ml/well in a humidified atmosphere of 5% CO₂ at 37 °C. When indicated, blood was diluted in endotoxin-free Iscove's Modified Dulbeccos Medium (IMDM, Biowhitaker, Verviers, Belgium) supplemented with 0.1 % (v/v) endotoxin-free fetal calf serum (FCS; Biowhithaker), 100 IU/ml (w/v) penicillin and 100 µg/ml (w/v) streptomycin, further referred to as culture medium. Except when indicated otherwise, whole blood was incubated for 2 hours in culture medium. For cell activation, varying concentrations of IVIG, TNF-α (kindly provided by Dr. A. Creasey, Chiron Corp., Emeryville, CA) or lipo-oligosaccharide (LOS; kindly provided by Dr. J. Poolman, RIVM, the Netherlands) were added to the culture medium. To harvest the supernatants, the plates were centrifuged at 2000 rpm for 1 minute at room temperature,
and the cell free supernatants were transferred into microtiter-plates and stored at -20°C until further analysis.

**Isolation of human neutrophils**

Neutrophilic granulocytes were purified from peripheral blood anticoagulated with 0.4 % (w/v) trisodium citrate, as described before. Briefly, mononuclear cells and platelets were removed by density centrifugation over isotonic Percoll with a specific gravity of 1.078 g/ml, at room temperature. The pellet (granulocytes and erythrocytes) was lysed for 10 minutes in ice-cold isotonic NH₄Cl solution (155 mmol/l NH₄Cl, 10 mmol/l KHCO₃, 0.1 mmol/l EDTA, pH 7.4). After centrifugation, the granulocytes were washed twice at room temperature, and resuspended in culture medium containing 5% FCS at a concentration of approximately 5x10⁶ cells/ml.

**Flowcytometric analysis**

Expression of surface markers was assessed with different mAbs diluted at appropriate concentrations in incubation medium (10 mM HEPES, 150 mM NaCl, 5 mM KCl, 1.5 mM CaCl₂, 2 mM MgCl₂, pH 7.4) supplemented with 0.01 % (w/v) NaN₃. Incubation medium was kept on melting ice during the experiment and was used to wash the cells. The expression of the surface marker CD66e on the neutrophil surface was used as an indicator of degranulation, CD15 was used as an overall neutrophil marker. PE-conjugated mouse monoclonal anti-CD66e and fluorescein-(FITC)-conjugated anti-CD15 were obtained from our institute (CLB, Dept. of Immune Reagents).

**Degranulation of neutrophils**

Whole blood at a 1 to 10 dilution was incubated with IVIG (10 mg/ml), TNF-α (50 ng/ml) or culture medium alone as a control, for 2 hours at 37°C. Thereafter, the cells were washed and incubated with PE-conjugated anti-CD66e mAb for 20 minutes. Subsequently, erythrocytes were lysed after 20 minutes using FACS lysing solution, according to manufacturer's instructions (Becton Dickinson). The cells were resuspended in incubation medium supplemented with 0.1% (w/v) bovine serum albumin (BSA) and kept in the dark at 4°C until analysis.

**Detection of cell-bound IVIG**

IVIG was biotinylated according to standard procedures and used to detect binding of IVIG to neutrophilic granulocytes. Whole blood was washed 4 times to remove plasma proteins, diluted 10 times in incubation medium, and incubated with biotinylated IVIG (12 μg/ml) for 20 minutes at 4°C. The cells were then washed and incubated with phycoerytrin-labelled streptavidin (Becton Dickinson) for 30 minutes at 4°C.
To assess the contribution of FcγRII and/or FRγRIII to the binding of IVIG by neutrophils, whole blood was preincubated for 30 minutes with F(ab')2 or F(ab) fragments of mAbs against FcγRII, FcγRIII or a combination of both antibodies. Binding of labelled IVIG to cells was then assessed as described before.

Samples were analysed with a FACScan flowcytometer (Becton Dickinson). Apart from characteristic forward-scatter and sideward-scatter patterns, neutrophils were identified on basis of CD15 expression, using a FITC-labelled mAb against CD15.

**Fractionation of IVIG**

IVIG (300 mg) was fractionated by gelfiltration on a Sephacryl S-300 HR (100 cm/500 ml, Pharmacia, Piscataway, NJ) column with PBS as a running buffer. Fractions corresponding to the monomeric dimeric, and polymeric peaks were collected and analysed for actual mono-, di-, and polymer contents by using a calibrated Superose 12 gelfiltration column in a FPLC® system (Pharmacia, Sweden) and a computer program (EzchromChromatography Data system version 6.5) for determining the peak areas of the chromatograms.

**Results**

**Degranulation of neutrophils upon stimulation with IVIG**

Different commercially available IVIG preparations from various manufactures were tested for their capacity to degranulate neutrophils in the whole blood culture system. As shown in Figure 2, all preparations tested induced the release of elastase at low concentrations of IVIG, indicative for degranulation of neutrophils (Figure 2A). Similar results were obtained when the release of lactoferrin was measured (not shown). However, further experiments were done with high doses of IVIG (10 mg/ml), which represent therapeutic concentrations.

Figure 2 Degranulation of neutrophils by different commercially available IVIG preparations in whole blood cultures.
To investigate the mechanism via which IVIG induced degranulation, the preparations were tested in presence of F(ab')$_2$ or F(ab) fragments of anti-FcγRII mAb or anti-FcγRIII. Figure 3 shows that anti-FcγRII partially prevented elastase and lactoferrin release induced by high doses of IVIG, whereas anti-FcγRIII had no effect. Inhibition by a combination of F(ab')$_2$ anti-FcγRII mAb and anti-FcγRIII blocked the release of granule proteins to the same extent as anti-FcγRII alone. Notably, the amount of elastase in the experiment shown in Figure 3 was higher than that shown in Figure 2. This appeared to be due to the fact that the blood used in these experiments was from different donors. These results suggested that the degranulation was mainly induced via triggering of FcγRII.

![Figure 3 Effect of anti-FcγR mAbs on IVIG or TNF-α- induced elastase and lactoferrin release in whole blood cultures.](image)

Whole blood cultures were incubated at 37°C in the presence of 10 mg/ml IVIG with or without F(ab')$_2$ fragments of mAb against FcγRII (5 mg/ml), Fab fragments of FcγRIII (5 mg/ml) or a combination of both mAbs. After 2 hours, degranulation was assessed by measuring elastase (A) or lactoferrin (B) in the supernatant. Cultures incubated with TNF-α (50 ng/ml) or culture medium alone were tested as control. Results represent mean and SEM (error bars) of 8 experiments.

IVIG may induce degranulation of neutrophils via various mechanisms including direct stimulation of neutrophils via binding to FcγR on these cells or indirect stimulation via cytokines released by mononuclear blood cells upon triggering of their FcγR by IVIG. This was investigated in various experiments. First, we incubated purified neutrophils with IVIG and assessed degranulation. Release of elastase and lactoferrin by purified neutrophils was indeed observed upon stimulation with IVIG (Figure 4). Second, the involvement of the cytokines TNF-α or IL-8 in the activation of neutrophils was investigated by examining the effect of F(ab')$_2$ fragments of blocking anti-TNF-α or anti-IL-8 in the whole blood system. Neutralization of either cytokine, however, had no effect on the degranulation of neutrophils by IVIG (Table IV). As a control, degranulation induced by TNF-α was also tested. This cytokine caused a marked release of elastase, which could not be blocked by F(ab')$_2$ fragments of
anti-FcγRII mAb. Together these results indicated that IVIG induced degranulation via a direct effect on neutrophils.

Degranulation was also studied phenotypically by measuring the up-regulation of membrane marker CD66. CD66 resides in the specific granules of resting neutrophils and appears in the membrane upon degranulation. Expression of CD66 was assessed using a mAb against CD66e, of which expression is strongly increased following granulocyte activation. Neutrophils were identified in the whole blood system by double staining with FITC-labelled mAb against CD15. As a control, cells were pre-incubated with an irrelevant isotype-matched antibody. As shown in Figure 5, up-regulation of CD66e was observed in whole blood cultures incubated with IVIG, and was blocked by addition of mAb against FcγRII. Incubation of whole blood cultures with TNF-α also induced up regulation of CD66e, which in contrast to IVIG-induced up regulation, was not inhibited by anti-FcγRII antibodies. Similarly as for elastase or lactoferrin release, blocking antibodies against TNF-α had no effect on IVIG-induced upregulation of CD66e, whereas that by TNF-α was completely blocked.

Figure 4 Effect of anti-FcγR mAbs on IVIG- or TNF-α-induced elastase and lactoferrin release by purified human neutrophils.

Purified human neutrophils (1x10⁵ cells/ml) were incubated at 37°C in the presence of 10 mg/ml IVIG with or without mAb against FcγRII (5 mg/ml), FcγRIII (5 mg/ml) or a combination of both mAbs. After 1 hour, degranulation was assessed by measuring elastase (A) or lactoferrin (B) in the supernatant. Cultures incubated with TNF-α (50 ng/ml) or culture medium alone were tested for control. Results represent mean and SEM (error bars) of 8 experiments.
Figure 5 Upregulation of CD66e on neutrophils in IVIG or TNF-α-stimulated whole blood cultures.

Whole blood was incubated at 37°C with IVIG (A; bold line) or TNF-α (B; bold line) or with culture medium alone (shadowed curves). Addition of mAb against FcγRII (dotted line) resulted in blockade of CD66e expression in IVIG-stimulated whole blood cultures, while this mAb had no effect on TNF-α stimulated cells. Addition of mAb against TNF-α (thin continuous line) did not block the upregulation of CD66e on IVIG-stimulated cells, whereas anti-TNF-α blocked CD66e expression by TNF-α-stimulated blood cells. Neutrophils were distinguished from contaminating cells on basis of their CD15 expression. CD15-negative cells were excluded from the analysis.
Binding of IVIG to neutrophils

The results thus far indicated that IVIG induced degranulation of neutrophils by binding to FcγRII. To further substantiate this, we performed binding experiments. Peripheral blood cells were washed three times and preincubated with aFcγRII and/or aFcγRIII antibodies, followed by addition of biotinylated IVIG. Neutrophils were identified by staining for CD15. As shown in Figure 6, CD15-positive cells bound IVIG. Binding of IVIG to neutrophils was abolished by addition of mAb against FcγRII. Antibodies against FcγRIII had no effect either or not in combination with anti-FcγRII.

**Figure 6 Binding of IVIG to neutrophils**

Peripheral blood cells were incubated with biotinylated IVIG as described in material and methods. Involvement of FcγR was investigated by preincubation of mAb against FcγRII and/or FcγRIII. To detect cell-bound IVIG, cells were incubated with PE-labelled streptavidin (FL 2). FITC-labelled CD15 was used as a neutrophil marker (FL 1), and CD15 negative cells were excluded from the analysis. The figure shows mean FL2 of CD15 positive cells. Results are mean of 4 experiments ± SEM.

Polymeric and dimeric IgG cause degranulation of neutrophils

To identify which of its constituents caused degranulation of neutrophils, IVIG was fractionated. At the time of the actual experiment, the monomeric, dimeric, or polymeric composition was verified by FPLC-analysis (Table IV). Immediately after gel-filtration, IVIG fractions were incubated with whole blood of 3 different donors. Incubation of whole blood cultures with fractions in a 1:1 dilution results in elastase release (Figure 7A). As the total amount of protein in the fractions containing monomeric IgG was about 10 times higher than the dimer or polymer enriched fractions, all fractions tested were titrated in the whole blood cultures to compare release after incubation. Fractions containing polymer and dimeric enriched IgG appeared to be more potent than monomeric IgG in degranulating neutrophils (Figure 7B). To study whether polymeric and dimeric IgG were also more potent in binding to neutrophils compared to monomeric IgG, peak fractions were biotinylated and used in binding experiments as described before. A large percentage of CD15 positive cells bound polymeric and dimeric IgG (mean FL2 = 90±30 and 68±10 respectively (n=3), whereas only a small percentage CD15 positive cells bound monomeric IgG (mean FL2 = 20±3; n=3), compared to cells without addition of IgG (mean FL2 = 8±3; n=3).
To identify which of its constituents caused degranulation of neutrophils, IVIG was fractionated on a S-300 Sepharose column as described in material and methods. At the time of the actual experiment, the monomeric, dimeric, or polymeric composition was verified by FPLC-analysis (table-2). Immediately after gel-filtration on the S-300 column, IVIG fractions were incubated with whole blood of 3 different donors. Figure 6 shows the results of elastase release during a 2 hours-incubation by the fractions. Fraction containing polymer and dimeric enriched IgG appeared to be more potent than monomeric IgG in degranulating neutrophils (see also figure 7). Notably, degranulation of neutrophils by polymeric and dimeric fractions was observed with blood obtained from each donor, whereas monomeric IgG induced a measurable release of elastase in the whole blood system in only 1 of the 3 donors (results with this donor are shown in figure 6 and 7).

Figure 7 Release of elastase by various fractions of IVIG in whole blood cultures.

Table IV Polymer, dimer and monomeric IgG content (%) of total IgG of different fractions separated on a S-300 column as measured by FPLC.

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<thead>
<tr>
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<th>Polymer</th>
<th>dimer</th>
<th>monomer</th>
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<tr>
<td>IVIG</td>
<td>0.4</td>
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<td>91.7</td>
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<td>33.7</td>
<td>8.9</td>
<td>32.3</td>
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<tr>
<td>fraction 30</td>
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<td>32.4</td>
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<td>fraction 50</td>
<td>0.04</td>
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Discussion

The beneficial effect of IVIG administration in idiopathic thrombocytopenia (ITP) has been claimed to be due to the capacity of IVIG to bind to Fcγ receptors. Here we show that IVIG induces neutrophils to degranulate by binding to Fcγ receptors on these cells. Neutrophils mainly express Fcγ receptors type Ila and type IIIb which both have low affinity for monomeric IgG, and a higher affinity for immune-complexes or IgG dimers. The latter are present at significant concentrations in IVIG preparations, and, as we show here, may interact with neutrophils.

To study interaction of IVIG with neutrophils we developed an in vitro system, in which whole blood or purified neutrophils were incubated with IVIG and assessed for degranulation. All IVIG preparations tested in this system appeared to induce a dose-dependent degranulation of neutrophils at concentrations that are therapeutically achieved in vivo. Furthermore, degranulation was observed with blood obtained from various donors. Thus, degranulation of neutrophils by IVIG is not a particular effect of a given IVIG preparation with blood from a particular donor, but rather represents a general feature of IVIG.

IVIG preparations are able to induce the secretion of cytokines by monocytes. Hence, a possible mechanism for the observed degranulation of neutrophils was the secretion of cytokines such as TNF-α or IL-8 by mononuclear blood cells stimulated by IVIG. However, mAb against IL-8 and TNF-α were not able to block IVIG-induced degranulation in the in vitro system, whereas TNF-α-induced degranulation was inhibited at least by the anti-TNFα mAb. We therefore concluded that IVIG did not activate neutrophils via the release of cytokines from activated mononuclear cells. In contrast, degranulation was at least in part prevented by the addition of a blocking antibody against Fcγ receptor type-IIa, whereas a blocking antibody against Fcγ receptor type-IIIb had no effect. Huizinga et al have shown that IgG-mediated activation of neutrophils is initiated by IgG binding to FcγRII resulting in respiratory burst and phagocytosis, whereas the IgG-dependent release of granule proteins can be mediated via either FcγRII or FcγRIII. Hence, our results fit with a mechanism that IVIG by binding to FcγRII induce degranulation of neutrophils. Consistent herewith was that IVIG also induced degranulation when incubated with purified neutrophils, and that labelled IVIG bound to neutrophils, which was reduced with blocking anti-FcγRII. Notably, that the degranulation of neutrophils by IVIG was not always completely inhibited by anti-FcγRII, suggesting involvement of other mechanisms. We are currently investigating the molecular background of these mechanisms and have some evidence that complement is involved.

Polymer and dimer enriched fractions of IVIG on a weight-base appeared to be more potent in inducing degranulation than the monomeric fraction. The degranulation observed after incubation with monomeric IgG was donor-dependent and could be blocked by a mAb against FcγRII. Falk et al demonstrated that anti-neutrophil cytoplasmic antibodies (ANCA) directed against antigens at the surface of neutrophils can induce the respiratory burst and degranulation of normal neutrophils primed with TNF-α. This activation by ANCA is FcγRII-dependent and not mediated by F(ab’)2 fragments alone.28
Hence, the degranulation observed upon stimulation with the monomeric fraction of IVIG may have been caused by ANCA or other antibodies in IVIG directed against antigens on the neutrophil. Taken together, these findings suggest that degranulation of neutrophils by IVIG preparations occurs via at least two different FcγRII-dependent mechanisms: one involving direct cross linking of FcγRII by Fc-parts of poly- or dimeric IgG, another involving binding of monomeric IgG via their antigens on the surface of the neutrophils and subsequently triggering of FcγRII by the Fc-regions of this monomeric IgG. The latter mechanism would suggest that IVIG preparations might contain ANCA or ANCA-like antibodies against neutrophil antigens, which upon binding trigger activation. Tam et al who described a child that developed neutropenia upon treatment with IVIG for Guillain-Barré syndrome illustrate such a mechanism. This IVIG preparation was found to contain high levels of antibodies against the neutrophils of the patient.29

Several cases of neutropenia induced by treatment with IVIG have been reported, indicating that degranulation of neutrophils by IVIG may also occur in vivo.30-32 However other mechanisms have been proposed to explain the neutropenia induced by IVIG treatment, including the presence of aggregates of IgG in the infused IVIG which, like immune-complexes, alter the expression of neutrophil CR3 thereby increasing the adhesion of neutrophils to the endothelium and reducing the number of circulating neutrophils.31 In addition, IVIG-induced complement activation and formation of C5a may result in activation and increased adherence of neutrophils. Which of these mechanisms predominate in vivo is currently not known.

In summary, we show that all IVIG preparations tested induced degranulation of neutrophils in an in vitro blood culture system, which was largely inhibited by mAb against FcγRII. We suggest that degranulation of neutrophils by IVIG not only may contribute to the side effects of this immunotherapy, but also may add to the anti-inflammatory therapeutic effects of IVIG since activation and degranulation of neutrophils in the circulation may impair the migration of neutrophils through the vessel wall and reduces infiltration of neutrophils in inflamed tissues as has been shown for example for TNF-α and IL-8.33,34 We are currently investigating the validity of this concept.

References


