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Modulation of lymphocyte and monocyte activity after intravenous immunoglobulin administration *in vivo*

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SUMMARY

In 12 patients with primary hypogammaglobulinaemia we investigated the *in vivo* effect of one bolus injection (400 mg/kg) of intravenous immunoglobulin (IVIG) on lymphocyte subsets and monocytes in peripheral blood, on plasma levels of soluble factors reflecting monocyte and lymphocyte activity and on lymphocyte proliferation and generation of reactive oxygen species (ROS) from monocytes analysed *in vitro*. Several immunological changes were induced by IVIG infusion. First, there was a significant decrease in $CD4^+/CD8^+$ lymphocyte ratio in peripheral blood, reflecting a significant increase in circulating numbers of $CD8^+$ lymphocytes. Second, although there was no significant change in plasma levels of soluble CD8 antigen, there was a significant decrease in soluble CD8 antigen/$CD8^+$ lymphocyte ratio, suggesting a down-regulation of $CD8^+$ lymphocyte activity. Third, there was a significant increase in plasma levels of neopterin, suggesting *in vivo* activation of monocytes/macrophages. Fourth, the was a down-modulation of mitogen-stimulated lymphocyte proliferation *in vitro*, and this down-regulation was significantly correlated with the increase in plasma neopterin levels. Finally, there was a significant decrease in zymosan-stimulated, but not in phorbol myristate acetate-stimulated, ROS generation from monocytes as evaluated by nitroblue tetrazolium reduction. The ability of IVIG administration *in vivo* to down-modulate lymphocyte proliferation and ROS generation from monocytes in patients with persistent immune activation may be relevant for the clinical effects of IVIG in a variety of immune-mediated disorders.

Keywords intravenous immunoglobulin lymphocyte proliferation reactive oxygen species neopterin soluble CD8 antigen

INTRODUCTION

Intravenous immunoglobulin (IVIG) is increasingly used for treatment of immune-mediated diseases, e.g. immune thrombocytopenic purpura, Kawasaki's syndrome, dermatomyositis and rheumatoid arthritis [1]. Several mechanisms have been proposed to explain the immunomodulating effects of IVIG in these disorders, including anti-idiotypic antibodies and non-specific blockade of Fc receptors [1], and modulation of the cytokine network has also been suggested to play a role [2]. However, the mode of action of IVIG in clinical diseases is still unclear, and various mechanisms may be operative, possibly in part depending on the clinical disorder.

Modulation of monocyte and lymphocyte functions is an important goal of immunomodulating therapy. Several *in vitro* studies with human cells have demonstrated effects of IVIG on both T and B lymphocyte as well as monocyte functions, e.g. decreased lymphocyte proliferation [3,4], altered cytokine production [5] and impaired phagocytosis by monocytes [6]. However, except for measurements of absolute numbers of cell subsets and expression of cell surface markers [7–10], there are to our knowledge no studies in man analysing the *in vivo* effects of IVIG on functional properties of lymphocytes or monocytes.

We have recently reported the effect of IVIG administration *in vivo* on plasma levels of cytokines, soluble cytokine receptors and IL-1 receptor antagonist in patients with primary hypogammaglobulinaemia [2]. In the present study we have further analysed the effect of IVIG infusion *in vivo* on lymphocytes and monocytes in the same patient population. First, we measured the effects of IVIG administration on the absolute numbers of lymphocyte subsets and monocytes in peripheral blood. Second, we measured the effect of IVIG infusion on plasma levels of soluble (s) factors reflecting lymphocyte and monocyte activity. Finally, we examined the effect of IVIG infusion *in vivo* on lymphocyte

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proliferation and generation of reactive oxygen species (ROS) from monocytes analysed *in vitro*.

PATIENTS AND METHODS

Patients

The study population has been described previously [2]. Twelve patients (four males and eight females; median age 39 years, range 20–60 years) under treatment at the Section of Clinical Immunology and Infectious Diseases, Medical Department A, The National Hospital, Oslo, Norway, with the diagnosis of primary hypogammaglobulinaemia based on established criteria [11,12], were included in the study. Ten patients were classified as common variable immunodeficiency and two as congenital hypogammaglobulinaemia as previously described [13,14]. All patients had been treated with subcutaneous self-administered immunoglobulin for a minimum of 15 months and all had serum IgG levels >5·0 g/*l* before the study. No patients had shown any signs of overt infection during the last 3 weeks before blood collection. None was taking antibiotics or immunosuppressive drugs except for one patient who received tetracyclines because of rosacea. At the time of the study serum level of alanine aminotransferase was < 55 U/*l* and serum creatinine level < 100 μmol/*l* in all patients.

Immunoglobulin preparation

Octagam (Octapharma, Vienna, Austria) is a liquid virus-inactivated IVIG preparation (pH 4) produced from Norwegian fresh frozen plasma collected in Norwegian blood banks. The final product is dispensed in sterile water containing 10% maltose (final IgG concentration 50 g/*l*, IgA and IgM < 0·1 g/*l*). Each portion has been tested and found negative for antibodies to HIV type 1 and 2 and hepatitis B and C virus. The endotoxin level in the IVIG preparation was < 10 pg/ml (limulus amoebocyte lysate test). Using the assays described below, we could not detect any amounts of sCD8 antigen or neopterin in the IVIG product used in the study.

Study design

The study was part of an Octagam tolerance study performed at the Section of Clinical Immunology and Infectious Diseases, Medical Department A, Rikshospitalet, Oslo, Norway, as previously described [2]. Briefly, when entering the trial, the patients underwent clinical, haematologic and biochemical investigations. All patients received a single infusion of Octagam (0·4 g/kg) using an infusion set with filter. The patients were clinically followed during infusion and until 6 h post-infusion. Blood samples were taken preinfusion and post-infusion at 1, 3, 20 and 44 h. The study was approved by the Regional Ethical Committee and by the Norwegian Medicines Control Authority (Statens Legemiddelkontroll). Signed informed consent was obtained from each patient.

Blood sampling protocol

Blood for neopterin and sCD8 antigen analyses was drawn into sterile pyrogen-free vacuum blood collection tubes (Sarstedt, Numbrecht, Germany) using heparin (15 U/ml) as anticoagulant. Blood was immediately immersed in melting ice and the tubes were centrifuged within 20 min (400 ***g*** and 4°C for 10 min). Plasma was then transferred to sterile Eppendorf tubes (Treff AG, Degersheim, Switzerland) and further centrifuged at 10 000 ***g*** and 4°C for 5 min to obtain platelet-free plasma. Plasma was stored at −70°C in multiple aliquots until analysis (median storage time 4 weeks, range 1–8 weeks). Samples were frozen and thawed only once.

Isolation of cells

Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by Isopaque–Ficoll (Lymphoprep; Nycomed Pharma AS, Oslo, Norway) gradient centrifugation within 45 min after blood sampling, as previously described [15]. Mononuclear cells were resuspended in RPMI 1640 (GIBCO, Paisley, UK) with 2 mmol/*l* L-glutamine and 25 mmol/*l* HEPES buffer (GIBCO) supplemented with gentamicin (40 μg/ml) (hereafter referred to as medium) and 10% heat-inactivated pooled human AB+ serum. The fraction of monocytes ($CD14^+$) and lymphocyte subsets ($CD2^+$ and $CD19^+$) in the isolated PBMC was determined by immunomagnetic quantification [16]. Isolation of monocytes was performed by plastic adherence (1 h) as previously described [15,17]. The adherent cells routinely contained >90% monocytes as determined by non-specific esterase staining, and viability exceeded 95% by acridine orange (Sigma, St Louis, MO) and ethidium bromide (Sigma) staining. The endotoxin level in the culture medium was <10 pg/ml (limulus amoebocyte lysate test).

Culture conditions

PBMC (10^6 cells/ml, 200 μl/well) and adherent monocytes (3×10^5/ml, 200 μl/well) were cultured at 37°C in a humidified atmosphere containing 5% CO_2 in flat-bottomed 96-well microtitre trays (Costar, Cambridge, UK) in medium and 10% AB+ serum (monocyte cultures used RPMI without HEPES).

Lymphocyte proliferation

PBMC were cultured in triplicates with or without stimulants phytohaemagglutinin (PHA), Murex, Dartford, UK, final concentration 1:150; concanavalin A (Con A), Calbiochem, Los Angeles, CA, final concentration 20 μg/ml; and pokeweed mitogen (PWM), Grand Island Biological Co., New York, NY, final concentration 1:150. Lymphocyte proliferation was assessed by pulsing the cells with 1 μCi ^{3}H-thymidine (Amersham, Little Chalfont, UK) 48 h after seeding. Cultures were harvested 16 h later onto glass filter strips, using an automated multisample harvester (Skatron, Lier, Norway). ^{3}H-thymidine incorporation was determined by liquid scintillation counting. Data are given as ct/min per 10^6 lymphocytes ($CD3^+$ and $CD19^+$ lymphocytes) in PBMC culture.

Determination of reduced nitroblue tetrazolium

After 20 h in culture, adherent monocytes were assayed for nitroblue tetrazolium (NBT) reduction with and without stimulants as previously described [17,18]. Briefly, cells were washed once with prewarmed Hanks' balanced salt solution (HBSS; GIBCO), incubated at 37°C in a humidified atmosphere containing 5% CO_2 with 100 μl/well NBT (Sigma; final concentration 1 mg/ml) and 100 μl/well with stimulants dissolved in RPMI (PMA, Sigma, final concentration 100 ng/ml; and unopsonized zymosan, Sigma, final concentration 250 μg/ml) or RPMI alone. After 45 min the cells were fixed with methanol, dried and reduced NBT dissolved in 2 M KOH (120 μl/well) and dimethyl sulphoxide (DMSO; Sigma; 140 μl/well). Optical density (OD) was read at 630 nm in a Multiskan Multisoft photometer (Labsystem, Helsinki, Finland). NBT reduction was expressed as OD per 10^6 monocytes.

Measurement of sCD8 antigen and neopterin levels in plasma

Plasma levels of sCD8 antigen were determined with a sandwich immunoassay (Cellfree CD8; T Cell Science, Cambridge, MA) according to the manufacturer's description using two MoAbs against different epitopes of the CD8 molecule. Plasma levels of

Table 1. Numbers of $CD2^+$, $CD4^+$, $CD8^+$ and $CD19^+$ lymphocytes, $CD4^+/CD8^+$ lymphocyte ratio and monocytes in peripheral blood after intravenous immunoglobulin (IVIG) infusion *in vivo* in 12 patients with primary hypogammaglobulinaemia*

	Preinfusion	20 h post-infusion	44 h post-infusion
$CD2^+$ lymphocytes ($10^6/l$)	810 (640–1335)	890 (525–1265)	1080 (620–1425)
$CD4^+$ lymphocytes ($10^6/l$)	390 (195–500)	320 (225–555)	360 (230–485)
$CD8^+$ lymphocytes ($10^6/l$)	295 (210–655)	420† (330–745)	550 (230–670)
$CD4^+/CD8^+$ lymphocyte ratio	1·04 (0·62–1·41)	0·76‡ (0·50–1·10)	0·71 (0·54–1·34)
$CD19^+$ lymphocytes ($10^6/l$)	85 (35–110)	60 (40–150)	100 (60–155)
Monocytes ($10^6/l$)	290 (225–375)	285 (200–330)	265 (215–320)

* Data are given as medians and 25th–75th percentiles.
† $P < 0{\cdot}05$ *versus* preinfusion.
‡ $P < 0{\cdot}005$ *versus* preinfusion.

neopterin were determined by a commercially available radioimmunoassay (RIA) method (IMMUtest Neopterin; Henning Berlin GMBH, Berlin, Germany) following the procedure recommended by the manufacturer.

Quantification of lymphocyte subsets and monocytes in peripheral blood

The numbers of $CD2^+$, $CD4^+$, $CD8^+$ and $CD19^+$ lymphocytes in peripheral blood were determined by immunomagnetic quantification, which has been shown to agree well with flow cytometry [19]. The numbers of monocytes were determined by leucocyte differential count using routine hospital methods.

Statistical analysis

For each parameter the post-infusion values were compared with preinfusion value by the non-parametric Wilcoxon matched pairs test. Coefficients of correlation (r) were calculated by the Spearman rank test. The calculations were performed using the STATISTICA (StatSoft, Tulsa, OK) software package. Data are given as medians and 25th to 75th percentiles if not otherwise stated. P values are two-sided and considered significant when $P < 0{\cdot}05$.

RESULTS

Lymphocyte subpopulations and monocytes in peripheral blood after IVIG administration

We first evaluated the effect of IVIG infusion on numbers of different lymphocyte subsets and monocytes in peripheral blood. As can be seen in Table 1, there was a significant increase in numbers of $CD8^+$ lymphocytes (approx. 50% increase) and a significant decrease in $CD4^+/CD8^+$ lymphocyte ratio (approx. 35% decrease) after IVIG infusion. All but one patient had a rise in $CD8^+$ lymphocyte counts and all but two patients had a decline in $CD4^+/CD8^+$ lymphocyte ratio. There was no significant change in circulating numbers of $CD2^+$ lymphocytes, $CD4^+$ lymphocytes or monocytes (Table 1). A marked increase in $CD19^+$ lymphocyte counts was seen in one patient, rising from $10 \times 10^6/l$ preinfusion to 510 and $520 \times 10^6/l$ at 20 h and 44 h post-infusion, respectively. However, such a rise was not seen in any of the other patients (Table 1).

Plasma levels of sCD8 antigen and neopterin after IVIG infusion

To further evaluate the effects of IVIG administration *in vivo* on

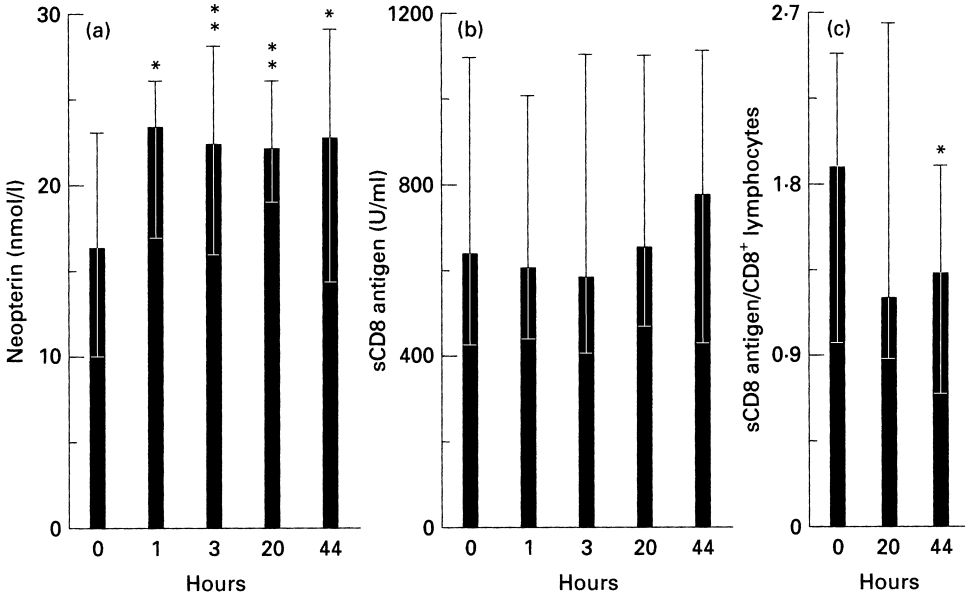

Fig. 1. Plasma levels of neopterin (a), plasma levels of soluble (s) CD8 antigen (b), and ratio of sCD8 antigen concentration to the absolute numbers of $CD8^+$ lymphocytes in peripheral blood (c) before and after intravenous immunoglobulin (IVIG) infusion in 12 patients with primary hypogammaglobulinaemia. Plasma neopterin levels at 1 h post-infusion were only analysed in nine patients. Data are given as medians and 25th–75th percentiles. *$P < 0{\cdot}05$ *versus* preinfusion; **$P < 0{\cdot}01$ *versus* preinfusion.

lymphocytes and monocytes, plasma levels of neopterin and sCD8 antigen were analysed in all patients. Neopterin is a pyrazinopyramidine compound derived from guanosine triphosphate within the biosynthetic pathway of tetrahydrobiopterin [20]. Monocytes/macrophages seem to be the major source of neopterin in humans and increased plasma levels are thought to reflect hyperactivity of these cells [20,21].

As can be seen in Fig. 1, there was a significant rise in plasma neopterin levels after IVG infusion (approx. 40% increase) and this increase was seen in all patients. Such an increase was also found when the neopterin level was expressed as concentration of neopterin per number of monocytes in peripheral blood (data not shown). In contrast, IVIG infusion did not induce any significant changes in levels of sCD8 antigen (Fig. 1). However, we found a significant decrease in the ratio of sCD8 antigen concentration to the absolute numbers of $CD8^+$ lymphocytes (Fig. 1), and this decline was seen in all but four patients.

Effect of IVIG administration in vivo *on lymphocyte proliferation* in vitro

We further examined the effect of IVIG administration *in vivo* by analysing the ability of lymphocytes to proliferate after stimulation *in vitro* preinfusion and 20 h post-infusion. A proliferation stimulation index (SI_{prol}) was calculated by the formula: SI_{prol} = (ct/min in stimulated lymphocyte culture)/(ct/min in medium control lymphocyte cultures). As shown in Fig. 2, there was a significant decrease in the SI_{prol} for all three mitogens tested, with approx. 30%, 30% and 45% decrease in PHA, Con A and PWM, respectively. Such a decrease in SI_{prol}, reflecting a slight increase in unstimulated and a more marked and statistically significant decrease in stimulated lymphocyte proliferation (data not shown), was seen in all but two patients. Interestingly, the maximum decrease in SI_{prol} was correlated with the maximum increase in plasma neopterin levels, although the correlation with the decrease in the SI_{prol} for PWM did not reach statistical significance ($r = -0{\cdot}73$, $P < 0{\cdot}01$; $r = -0{\cdot}62$, $P < 0{\cdot}03$; $r = -0{\cdot}52$, $P = 0{\cdot}08$; PHA, Con A and PWM, respectively).

Three of the patients participating in the study received one additional bolus infusion of IVIG within 6 months after the study, and again we found a similar pattern, with a marked decrease in SI_{prol} for all three mitogens (data not shown).

Effect of IVIG administration in vivo *on monocyte NBT reduction* in vitro

The ability to generate ROS after stimulation is an important biological function of monocytes [22]. In the present study NBT reduction in monocytes, reflecting the generation of ROS in these cells [17,18], was evaluated before and 20 h after IVIG administration in all patients. The NBT reduction was calculated as stimulation index (SI) $OD_{Stimulants}/OD_{Medium}$, and we found a significant decrease in zymosan-stimulated (approx. 35% decrease) NBT reduction after IVIG administration (Fig. 3). This decrease in SI, reflecting a slight increase in unstimulated and a more marked decrease in zymosan-stimulated NBT reduction (data not shown), was seen in all but one patient. There was no significant correlation between this decrease in SI and the maximum increase in plasma neopterin level (data not shown). This decrease in SI for zymosan was also found when three of the patients were given an additional bolus infusion of IVIG within 6 months after the study (data not shown).

In contrast to zymosan stimulation, we could not demonstrate any significant changes in PMA-stimulated NBT reduction after IVIG infusion (Fig. 3).

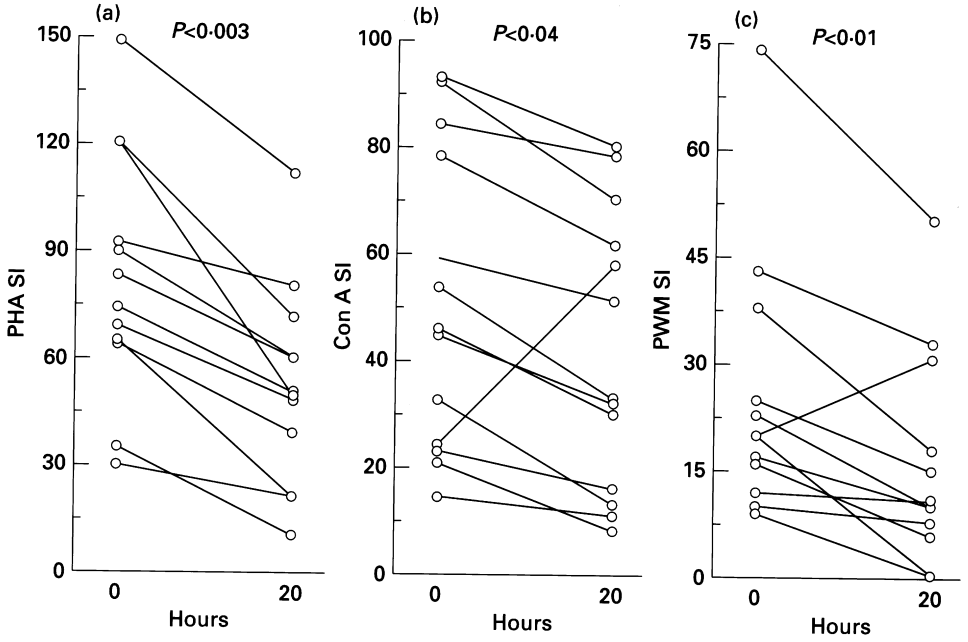

Fig. 2. Lymphocyte proliferation given as stimulation index (ct/min in stimulated peripheral blood mononuclear cell (PBMC) culture/ct/min in medium control PBMC culture) (see Patients and Methods) for phytohaemagglutinin (PHA) (a), concanavalin A (Con A) (b) and pokeweed mitogen (PWM) (c) in PBMC isolated before and 20 h after intravenous immunoglobulin (IVIG) infusion in 12 patients with primary hypogammaglobulinaemia. SI, Stimulation index.

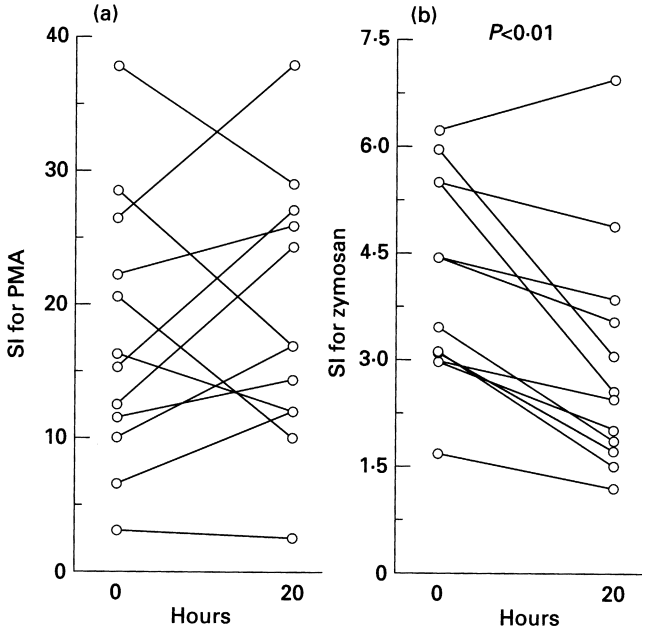

Fig. 3. Nitrublue tetrazolium (NBT) reduction given as stimulation index (SI) (OD (optical density)$_{Stimulants}$/OD$_{Medium}$, see Patients and Methods) for phorbol myristate acetate (PMA) (a) and unopsonized zymosan (b) in monocytes isolated before and 20 h after intravenous immunoglobulin (IVIG) infusion in 12 patients with primary hypogammaglobulinaemia. NBT reduction is expressed as OD per 10^6 monocytes (see Patients and Methods).

DISCUSSION

A major finding of the present study was that IVIG administration *in vivo* down-regulates both proliferation of lymphocytes and generation of ROS from monocytes *in vitro*. This down-modulation of lymphocyte proliferation seems to be correlated with an activation of monocytes/macrophages *in vivo* reflected in raised plasma levels of neopterin after IVIG infusion.

Most studies examining the *in vivo* effect of IVIG have only analysed numerical alterations in lymphocyte subpopulations and not functions of these cells. In accordance with some other studies [7,8], we found that IVIG infusion induced a marked decrease in $CD4^+/CD8^+$ lymphocyte ratio reflecting increased numbers of circulating $CD8^+$ lymphocytes. Notably, however, this rise in $CD8^+$ lymphocyte counts was accompanied by a decrease in sCD8 antigen/$CD8^+$ lymphocyte ratio. Soluble CD8 antigen appears to be released from $CD8^+$ lymphocytes upon activation [23,24]. The decrease in sCD8 antigen/$CD8^+$ lymphocyte ratio may indicate that the amount of sCD8 antigen attributable to individual cells is significantly decreased after IVIG infusion [12,24], and although we did not examine any direct functional activity of $CD8^+$ lymphocytes, the present findings may suggest a down-modulation of $CD8^+$ lymphocyte activity *in vivo* after IVIG administration. In contrast, the rise in plasma neopterin levels suggests *in vivo* activation of monocytes/macrophages. Thus, it is possible that IVIG infusion *in vivo* may have different effects on monocytes/macrophages and $CD8^+$ lymphocytes.

Down-regulation of lymphocyte proliferation *in vitro* in the presence of IVIG has been reported by several authors [3,4,25]. However, the present study analysing the function of lymphocytes and monocytes taken from patients before and after IVIG infusion represents to our knowledge the first report of such antiproliferative effects after IVIG administration *in vivo* in man. There is, however, one report of down-regulated lymphocyte proliferation after IVIG infusion *in vivo* in rats with experimental uveoretinitis [26]. The significant correlation between down-regulated lymphocyte proliferation and raised neopterin levels suggests that increased release of inhibitory factors from monocytes/macrophages might be involved in this phenomenon. Indeed, IVIG has been demonstrated to be a potent inducer of IL-1 receptor antagonist both *in vivo* [2] and *in vitro* [27,28], and cross-linking of Fc receptors in monocytes may increase prostaglandin E_2 synthesis in these cells [29]. Both these factors have inhibitory effects on lymphocyte proliferation [29,30]. In fact, we have previously reported a marked increase in plasma levels of IL-1 receptor antagonist after IVIG infusion in the same patient population [2], although we could not demonstrate any significant correlation between the rise in plasma levels of IL-1 receptor antagonist and the decrease in lymphocyte proliferation (unpublished observations). However, the down-regulation of lymphocyte proliferation after IVIG infusion may also reflect direct suppressive action of IVIG on T cells [3]. *In vitro* studies have suggested that IVIG may directly down-regulate IL-2 synthesis as well as IL-2 receptor expression in T cells, which indeed will impair the proliferative response in these cells [31].

While the significant rise in plasma neopterin level found in the present study might suggest that there is an initial *in vivo* activation of monocytes/macrophages after IVIG administration, the demonstration of an impaired SI for NBT reduction when stimulated with unopsonized zymosan suggests that this *in vivo* activation may render the monocytes less susceptible to further stimulation *in vitro*. It is of interest that no impairment of stimulation was seen when monocytes were stimulated with PMA, possibly reflecting inhibitory effects of IVIG upstream to protein kinase C activation. Such a mechanism for inhibitory effects of IVIG on monocyte activation has also recently been suggested by others [32]. However, the exact mechanism leading to the IVIG-induced impairment of the *in vitro* response will have to be further elucidated.

The results of published studies analysing the immunological effects of IVIG should be interpreted with caution. First, most studies have analysed the effect of IVIG after administration *in vitro*, and the results from such studies may not be relevant to the much more complex *in vivo* situation. The present study, analysing functional properties of cells before and after IVIG administration *in vivo*, represents a novel methodological approach to study correlation between *in vitro* and *in vivo* immunomodulatory effects of IVIG. Second, the effects of IVIG may differ somewhat between different IVIG preparations, possibly associated with the method of preparation [25,33]. Third, the effect of one bolus infusion, as in the present study, may differ somewhat from the effects of repeated IVIG infusions [34]. Finally, most studies analysing the *in vitro* effect of IVIG have been performed on cells from healthy blood donors. However, the immunomodulating effects of IVIG may well differ in different patient groups with variable pre-existing immunological abnormalities [2,28].

The present study was performed in patients with primary hypogammaglobulinaemia, and the well known beneficial effect of repeated IVIG infusion in these patients is primarily caused by increasing the IgG level. The reduction in infectious complications might be expected to have beneficial effects on several immunological functions. Notably, down-modulation of ROS generation from monocytes in patients with primary

hypogammaglobulinaemia, characterized by increased oxidative stress [17,35], may represent an additional beneficial effect of IVIG in these patients. On the other hand, down-regulation of lymphocyte proliferation in patients with primary hypogammaglobulinaemia, characterized by decreased proliferation at baseline [11–13], may represent a possible side effect of IVIG in this group of patients of unknown clinical significance. Importantly, however, in patients with, for example, several autoimmune disorders, both down-regulation of ROS production and down-modulation of lymphocyte proliferation may represent important therapeutic goals.

In conclusion, lymphocyte activation and enhanced ROS generation resulting in increased oxidative stress may play an important pathogenic role in several immune-mediated disorders [1,36]. If the demonstrated refractory state of lymphocytes and monocytes induced by IVIG also exists when these cells are naturally stimulated *in vivo*, it may contribute significantly to the mechanism by which IVIG induces its anti-inflammatory effects. Thus, the ability of IVIG administration *in vivo* to down-modulate lymphocyte proliferation and ROS generation from monocytes in patients with persistent immune activation, in the absence of many of the known side effects of other immunosuppressive drugs, provides further support for the potential use of IVIG in a variety of immune-mediated disorders.

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REFERENCES

1 Dwyer JM. Manipulating the immune system with immune globulin. N Engl J Med 1992; **326**:107–16.
2 Aukrust P, Frøland SS, Liabakk N-B, Müller F, Nordøy I, Haug C, Espevik T. Release of cytokines, soluble cytokine receptors and interleukin-1 receptor antagonist after intravenous immunoglobulin administration *in vivo*. Blood 1994; **84**:2136–43.
3 Amran D, Renz H, Lack G, Bradley K, Gelfand EW. Suppression of cytokine-dependent human T-cell proliferation by intravenous immunoglobulin. Clin Immunol Immunopathol 1994; **73**:180–6.
4 van Schaik IN, Lundkvist I, Vermeulen M, Brand A. Polyvalent immunoglobulin for intravenous use interferes with cell proliferation *in vitro*. J Clin Immunol 1992; **12**:325–34.
5 Andersson U, Björk L, Skansén-Saphir U, Andersson J. Pooled human IgG modulates cytokine production in lymphocytes and monocytes. Immunol Rev 1994; **139**:21–42.
6 Jungi TW, Brcic M, Kurnert P, Spycher MO, Li F, Nydegger UE. Effect of IgG for intravenous use on Fc receptor-mediated phagocytosis by human monocytes. Clin Exp Immunol 1990; **82**:163–9.
7 White WB, Desbonnet CR, Ballow M. Immunoregulatory effects of intravenous immune globulin therapy in common variable immunodeficiency. Am J Med 1987; **83**:431–6.
8 Macey MG, Newland AC. CD4 and CD8 subpopulation changes during high dose intravenous immunoglobulin treatment. Br J Haematol 1990;**76**:513–20.
9 Rigal D, Vermot-Desroches C, Heitz S, Bernaud J, Alfonsi F, Monier JC. Effects of intravenous immunoglobulin (IVIG) on peripheral blood B, NK and T cell subpopulations in women with recurrent spontaneous abortion: specific effects on LFA-1 and CD56 molecules. Clin Immunol Immunopathol 1994; **71**:309–14.
10 Ling Z-D, Yeoh E, Webb BT, Farrell K, Doucette J, Matheson DS. Intravenous immunoglobulin induces interferon-γ and interleukin-6 *in vivo*. J Clin Immunol 1993; **13**:302–9.
11 WHO Scientific Group Report. Primary immunodeficiency. Clin Exp Immunol 1995; **97**(Suppl. 1):1–24.
12 Spickett GP, Misbah SA, Chapel HM. Primary antibody deficiency in adults. Lancet 1991; **337**:281–4.
13 Aukrust P, Frøland SS, Müller F. Raised serum neopterin levels in patient with primary hypogammaglobulinaemia: correlation to other immunological parameters and to clinical and histological features. Clin Exp Immunol 1992; **89**:211–6.
14 Aukrust P, Müller F, Frøland SS. Elevated serum levels of interleukin-4 and interleukin-6 in patients with common variable immunodeficiency are associated with chronic immune activation and low numbers of $CD4^+$ lymphocytes. Clin Immunol Immunopathol 1994; **70**:217–24.
15 Müller F, Rollag H, Gaudernack G, Frøland SS. Impaired *in vitro* survival of monocytes from patients with HIV infection. Clin Exp Immunol 1990; **81**:25–30.
16 Gaudernack G, Lundin KE. Rapid immunomagnetic phenotyping of cells. J Immunogenet 1989; **16**:169–75.
17 Aukrust P, Müller F, Frøland SS. Enhanced generation of reactive oxygen species in monocytes from patients with common variable immunodeficiency. Clin Exp Immunol 1994; **97**:232–8.
18 Müller F, Rollag H, Frøland SS. Nitroblue tetrazolium reduction in monocytes and monocyte-derived macrophages. APMIS 1989; **97**:490–6.
19 Brinchmann JE, Vartdal F, Gaudernack G, Markussen G, Funderud S, Ugelstad J, Thorsby E. Direct immunomagnetic quantification of lymphocyte substes in blood. Clin Exp Immunol 1988; **71**:182–6.
20 Huber C, Batchelor JR, Fuchs D *et al.* Immune-response associated with neopterin production. J Exp Med 1984; **160**:310–6.
21 Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Wachter H. Neopterin as a marker for cell-mediated immunity. Immunol Today 1988; **9**:150–5.
22 Segal AW. The respiratory burst in monocytes and macrophages. In: Zembala M, Asherson G, eds, Human monocytes. London: Academic Press, 1989;89–100.
23 Tomkinson BE, Bown MC, Ip SH, Carrabis S, Sullivan JL. Soluble CD8 during T-cell activation. J Immunol 1989; **142**:2230–6.
24 Agostini C, Pizzolo G, Zambello R *et al.* Shedding of the soluble form of the CD8 complex by $CD8^+$/$HLA\text{-}DR^+$ cells in HIV-1-infected patients. AIDS 1991; **5**:813–9.
25 Klaesson S, Ringden O, Markling L, Remberger M, Lundkvist I. Immune modulatory effects of immunoglobulins on cell-mediated immune response *in vitro*. Scand J Immunol 1993; **38**:477–84.
26 Saoudi A, Hurez V, de Kozak Y *et al.* Human immunoglobulin preparations for intravenous use prevent experimental autoimmune uveoretinitis. Int Immunol 1993; **5**:1559–67.
27 Arend WP, Leung DYM. IgG induction of IL-1 receptor antagonist by human monocytes. Immunol Rev 1994; **139**:71–78.
28 de Souza VR, Carreno M-P, Kaveri SV *et al.* Selective induction of interleukin-1 receptor antagonist and interleukin-8 in human monocytes by normal polyspecific IgG (intravenous immunoglobulin). Eur J Immunol 1995; **25**:1267–73.
29 Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. Immunol Today 1991; **12**:349–52.
30 Dinarello CA, Thompson RC. Blocking IL-1: interleukin-1 receptor antagonist *in vivo* and *in vitro*. Immunol Today 1991; **12**:404–10.
31 Andersson UG, Björk L, Skansén-Saphir U, Andersson JP. Down-regulation of cytokine production and interleukin-2 receptor expression by pooled human IgG. Immunology 1993; **79**:211–6.
32 Abe Y, Horiuchi A, Miyake M, Kimura S. Anti-cytokine nature of human immunoglobulin: one possible mechanism of the clinical effects of intravenous immunoglobulin therapy. Immunol Rev 1994; **139**:6–14.
33 Farber CM, Crusiaux A, Schandené L, van Vooren JP, Goldman M,

Dupont E, Tasiaux N. Tumor necrosis factor and intravenous gammaglobulins in common variable immunodeficiency. Clin Immunol Immunopathol 1994; **72**:233–6.

34 Achiron A, Margalit R, Hershkoviz R, Markovits D, Reshef T, Melamed E, Cohen IR, Lider O. Intravenous immunoglobulin treatment of experimental T cell-mediated autoimmune disease. J Clin Invest 1994; **93**:600–5.

35 Aukrust P, Svardal AM, Müller F, Lunden B, Berge RK, Frøland SS. Decreased levels of total and reduced glutathione in $CD4^+$ lymphocytes in common variable immunodeficiency are associated with activation of the tumor necrosis factor α system: possible immunopathogenic role of oxidative stress. Blood 1995; **86**:1383–91.

36 Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease: where are we now? J Lab Clin Med 1992; **119**:598–620.