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The effect of intravenous immunoglobulin on the in vitro function of human neutrophils

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Abstract: Three commercially available preparations of human immunoglobulin for intravenous use (IVIgG), namely Gammune N, Sandoglobulin and Intraglobin F, were tested for their ability to modulate human neutrophil function in vitro IVIgG consistently stimulated the neutrophil respiratory burst at concentrations of 0 5 to 1 mg/ml, concentrations readily achieved in vivo by moderate-dose therapy Superoxide (O_2^-) release was increased by 3 5–4 5 nmol per 5 × 10^5 cells at these concentrations of IVIgG, and H_2O_2 production increased in a dose-dependent fashion up to 8 mg/ml IVIgG Luminol-dependent chemiluminescence (CL) was also directly stimulated by IVIgG In addition, the effects of both soluble and particulate stimulators (*N*-formyl-methionyl-leucyl-phenylalanine, phorbol myristate acetate and opsonized zymosan) on the neutrophil respiratory burst were enhanced by IVIgG In a filter assay of neutrophil migration, using a modified Boyden chamber, no consistent effect on neutrophil locomotion or chemotaxis could be demonstrated The effect of IVIgG on neutrophil metabolism may contribute to its beneficial therapeutic effect in severe, life-threatening infections

Key words: Neutrophil, Intravenous immunoglobulin, Superoxide, Hydrogen peroxide, Chemiluminescence, Chemotaxis

Introduction

The use of intravenous immunoglobulin (IVIgG) is established in the treatment of idiopathic thrombocytopenic purpura (ITP), it may be utilized as first-line therapy in children and as second-line therapy in adults who are refractory to steroids (Imbach et al., 1981; Newland, 1987). It is also effective in the treatment of alloimmune neonatal neutropenia (Barundun et al., 1982) and post-transfusion purpura (Mueller-Eckhardt et al., 1983). Regular infusion of IVIgG in primary hypogammaglobulinemia and related disorders may, in fact, be superior to conventional replacement regimens using intramuscular IgG (McClelland and Yap, 1987). It is of value in the treatment of infants with AIDS (Calvelli and Rubinstein, 1986), however, its place in the treatment of severe infections in the immuno-compromised host has not yet been clearly defined (Yap, 1987)

The mechanism by which IVIgG decreases cell destruction in the immune cytopenias has been a subject of speculation (Imbach, 1983, Salama et al., 1983) Jungi et al. (1986) demonstrated that IVIgG preparations could interact with Fc receptors of hu-

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Abbreviations IVIgG, normal human immunoglobulin for intravenous use, CL, chemiluminescence, ITP, idiopathic thrombocytopenic purpura, AIDS, acquired immunodeficiency syndrome, HBSS, Hank's balanced salt solution, OZ, opsonized zymosan, PMA, phorbol myristate acetate, FMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine, SOD, superoxide dismutase, DCFH-DA, 2′,7′-dichlorofluorescin diacetate, DCFH, 2′,7′-dichlorofluorescin, DCF, 2′,7′-dichlorofluorescein, PBS, phosphate-buffered saline, cpm, counts per minute, BSA, bovine serum albumin, HSA, human serum albumin, IV, intravenous, IM, intramuscular

man monocytes and macrophages to inhibit the phagocytosis of IgG-sensitized erythrocytes High-dose IVIgG may also modulate the immune response by reducing the lymphocyte CD4 CD8 ratio and by reducing B-cell immunoglobulin synthesis (Tsubakio et al., 1983, Dammacco et al., 1986) Since the neutrophil is central to the acute inflammatory response and in defence against most bacterial pathogens, it is important to know whether neutrophil functions are in any way modulated by IVIgG in the concentrations achieved by high-dose therapy In the present study we set out to examine the effect of three commercially available IVIgG preparations on neutrophil function in vitro, i e, superoxide ion (O_2^-) release, H_2O_2 production, chemiluminescence (CL) and chemotaxis

Materials and Methods

Intravenous immunoglobulin

Three IVIgG preparations were used in this study Intraglobin F (Biotest Pharma Gmbh, Dreieich, F R G , lot 412087), Sandoglobulin (Sandoz Pharmaceuticals Corp., East Haven, NJ, lot 7 369.157 0) and Gamimune N (Cutter Biological, Berkeley, CA, lot 40C13A). Sandoglobulin is prepared by the method developed by the Swiss Red Cross, Cohn effluent III IgG from pooled human plasma is treated at low pH (4- 4.5) in the presence of a very low concentration of porcine pepsin Gamimune is made by essentially the same method except that pepsin is omitted. In contrast, Intraglobin F is chemically modified, Cohn effluent III IgG is treated with β-propiolactone These preparations all contain >98% IgG, Gamimune N contains negligible amounts of aggregates, dimers and fragments Sandoglobulin contains <1% aggregates, 9–10% dimers and no detectable fragments Intraglobin F contains no detectable aggregates, 3–4% dimers and 9–10% fragments (Lundblad et al., 1987)

Intraglobin F and Gamimune N are supplied as solutions of 50 mg protein/ml. For convenience these were diluted to 40 mg/ml with Hank's balanced salt solution (HBSS), pH 7.4, so that when further diluted 1/10 under experimental conditions a final concentration of 4 mg/ml was obtained Sandoglobulin was supplied freeze-dried, and was reconstituted in HBSS at a concentration of 40 mg/ml Doubling dilutions were made in HBSS for dose-response experiments Concentrations of IVIgG used in vitro ranged from 0 25 mg/ml to 8 mg/ml. High-dose IVIgG therapy is known to achieve serum concentrations of the order of 8 mg/ml (Pirofsky, 1987)

Each IVIgG contains carrier sugar as a stabilizer and it has been shown previously that both maltose and glucose inhibit the stimulatory effect of IVIgG on neutrophils, measured by O_2^- release (Koch et al., 1984) Therefore, in all experiments the equivalent concentration of the sugar was added to control tubes which did not contain IVIgG. Intraglobin F, 40 mg/ml, contained glucose 18 mg/ml in excess of glucose in HBSS Sandoglobulin, 40 mg/ml, contained sucrose 67 mg/ml. Gamimune N, 40 mg/ml, contained maltose 80 mg/ml The control sugar concentrations are referred to below as 'carrier-sugar'.

pH effect of IVIgG

When added to HBSS Intraglobin F at 8 mg and 4 mg/ml reduced the pH by 0.1 and 0 05 respectively. Sandoglobulin reduced the pH by 0 02 and 0 01 In contrast, Gamimune N reduced the pH by 0 8 at 8 mg/ml and by 0 3 at 4 mg/ml. Because of this effect, the highest final concentration of Gamimune N used experimentally was 4 mg/ml A maximum drop in pH from 7 4 to 7 1 was considered acceptable in the assay systems of PMN function

Cell stimulants

Particulate and soluble stimulants of the neutrophil respiratory burst were employed to determine whether the stimulated response was modified by the presence of IVIgG Opsonized zymosan (OZ) was used as the particulate stimulant. Zymosan (Sigma Chemical Co., St Louis, MO) was boiled in normal saline for 1 h, washed once in HBSS and resuspended in HBSS at 50 mg/ml as a stock suspension which was stored at 4°C Zymosan stock was opsonized by incubation with an equal volume of fresh normal human serum at 37°C for 30 min

After washing twice in HBSS it was resuspended in HBSS at 10 mg/ml and stored at 4°C. OZ was used within one week of preparation Phorbol myristate acetate (PMA, LC Services Corp., Woburn, MA) and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, Sigma Chemical Co., St. Louis, MO) were the soluble stimulants employed

Chemoattractant

Zymosan-activated serum (ZAS) was the standard chemoattractant. Fresh human serum was incubated with washed Zymosan (5 mg Zymosan to 1 ml of serum) at 37°C for 30 min The Zymosan was spun down and the serum pipetted off. ZAS was aliquotted and stored at −70°C. A 1/7 dilution of ZAS was used in all experiments

Use of cell stimulants in respiratory burst assays

OZ was added to the cells to a final concentration of 1 mg OZ per 5×10^5 cells PMA was used at a final concentration of 10 ng/ml and FMLP at 10^{-6} M. In each case stock solution was diluted in HBSS to an appropriate dilution and 0.1 ml of this dilution was added to 0 9 ml of the cell reaction mixture. The modifying effect of IVIgG on the stimulation of the respiratory burst was also examined by adding 0.1 ml of the appropriate dilution of IVIgG immediately before the stimulant.

Separation of neutrophils from blood

Human blood was taken from healthy volunteers and anticoagulated with preservative-free heparin (Elkins-Sinn Inc , Cherry Hill, NJ) at 10 U/ml of blood Neutrophils were separated by buoyant density centrifugation over Ficoll/Hypaque according to the method of Ferrante and Thong (1980). Seven ml of blood were layered over 4 ml of Neutrophil Isolation Medium (Los Alamos Diagnostics, Los Alamos, NM) in a 15 ml tube and centrifuged at 400 × *g* for 30 min. After discarding the plasma and mononuclear cell/platelet layers, the neutrophil layer was carefully separated using a Pasteur pipette and immediately diluted in HBSS. The neutrophils together with the contaminating erythrocytes were spun down at 500 × *g* for 5 min, and the erythrocytes were lysed by 20 s exposure to distilled H_2O, two cycles. Leukocytes were then washed once in HBSS, resuspended in HBSS and kept on ice until tested for respiratory burst activity or chemotaxis. The final cell preparation contained 88–99% neutrophils (mean, 95 5%) and viability was greater than 99% by trypan blue exclusion.

Superoxide release assay

Release of superoxide ion (O_2^-) was measured by the superoxide dismutase (SOD) inhibitable reduction of cytochrome *c* as described by McCord and Fridovich (1969) The test was performed in 5 ml polypropylene tubes (Sarstedt, Numbrecht, W Germany). Neutrophils were suspended in HBSS to a concentration of 1×10^6 cells/ml; 0.5 ml of cell suspension was added to each tube together with 1 mg/ml of ferricytochrome *c* (Sigma Type III, Sigma Chemical Co., St. Louis, MO). Control tubes contained 0.023 mg/ml of SOD (Diagnostic Data, Mountainview, CA). Volumes were adjusted to 1 ml with HBSS. Tubes were incubated in a shaking water bath for 20 min at 37°C and the reaction was stopped in the tubes without SOD by adding 0.023 mg of SOD in 100 μl. The volumes in all tubes were then made up to 1.8 ml with HBSS and the cells were spun down at 1500 × *g* for 5 min at 4°C Finally, the reduction of cytochrome *c* was measured by reading the absorbance of the supernatant at 550 nm in a spectrophotometer, taking the SOD-containing supernatant as the reference. The difference between the −SOD and +SOD tubes was calculated. Using an extinction coefficient of 18 5 cm^{-1} mM^{-1} for cytochrome *c* (reduced-oxidized) at 550 nm, the difference in absorbance was multiplied by 97 to obtain nm O_2^-/ml (Margoliash and Frohwirt, 1959). Addition of IVIgG and stimulants was carried out as described above.

Intracellular H_2O_2 production

The assay used was that originally described by Keston and Brandt (1965) and adapted for flow cytometry by Bass et al. (1983). It depends on the use of the nonfluorescent hydrophobic compound 2′,7′-dichlorofluorescin diacetate (DCFH-DA) which penetrates the cell membrane and is trapped intracellularly by hydrolytic deacetylation to 2′,7′-di-

chlorofluorescin (DCFH) This compound is readily oxidized by H_2O_2, in the presence of peroxidase, to the fluorescent compound 2',7'-dichlorofluorescein (DCF). The conversion to DCF can be measured by flow cytometry, thus giving a quantitative assessment of H_2O_2 production within the cells Freshly isolated neutrophils were suspended in PBS containing 0 1% gelatin, pH 7 4, at 5 5 × 10^5 cells/ml, DCFH-DA (Molecular Probes, Eugene, OR) was added to give a concentration of 20 μM and the cells were incubated at 37°C for 15 min To each 5 ml polystyrene tube was added 0 45 ml of the DCFH-DA labelled cell suspension and after addition of 0 05 ml of IVIgG and/or cell stimulant the tubes were incubated at 37°C for 45 min in a water bath. Three thousand cells were examined in a flow cytometer (EPICS-541, EPICS Division, Coulter Electronics, Hialeah, FL) equipped with an argon laser set at an excitation wavelength of 488 nm Green fluorescent emission (510–550 nm) was recorded Fluorescing neutrophils were separated from other contaminating cell types by electronic gating Cell fluorescence was expressed as the mean channel number after correction for the gain setting of the photomultiplier tube Dose responses to IVIgG preparations were done with single samples In experiments to examine the modulating influence of IVIgG on the effect of cell stimulants, samples were run in triplicate

Chemiluminescence assay

The Luminol-amplified chemiluminescent response (CL) of neutrophils was assayed essentially as described by Robinson and Penny (1982) Freshly isolated neutrophils were suspended in HBSS at 2 × 10^5 cells/ml 0 5 ml aliquots of the cell suspension were added to glass scintillation vials, followed by 0 05 ml of a 10^{-3} M stock solution of 5-amino-2,3-dihydro-1,4-phthalazinedione (Luminol, Eastman Kodak, Rochester, NY) and HBBS so that the final reaction volume was 1 ml IVIgG and/or stimulants were added and the vials were immediately placed in a liquid scintillation counter (Beckman LS 5801, Beckman Instruments, Fullertom, CA) set for single-photon counting Chemiluminescence was measured at ambient temperature (23°C). Samples were recycled for up to 1 h and CL was recorded as peak counts per minute (cpm). All CL experiments were performed using single samples because of the need to recycle vials at short time intervals to measure the peak CL response

Chemotaxis assay

This was performed by measuring the distance of cell migration through a 5 μm-pore-size nitrocellulose filter in a modified Boyden chamber Chemoattractant (ZAS) or HBSS was pipetted into the lower chambers. The filters (Schleicher & Schuell, Keene, NH) were saturated in HBSS containing 0.1% BSA and clamped between the lower and upper chambers Neutrophils suspended in HBSS with 0 1% BSA (1 5 × 10^6 cells/ml) were dispensed into the upper chambers and cell migration was allowed to proceed at 37°C for 50 min, after which the filters were stained with haematoxylin The distance of cell migration was measured by the leading-front method of Zigmond and Hirsch (1972) using a standard microscope micrometer A reading was made in five different fields of each filter and the mean distance migrated was calculated in micrometers All assays were run in duplicate. The effect of IVIgG on chemokinesis or ZAS-induced chemotaxis was examined by adding IVIgG or carrier-sugar to the cell suspension immediately before dispensing cells into the chambers

Statistical analysis

The significance of differences between test groups and controls was calculated using the one-tailed *t* test of Student

Results

Effect of IVIgG on O_2^- release and H_2O_2 generation

The effect of the three IVIgG preparations on O_2^- release by human neutrophils in vitro was tested over a range of concentrations from 0 13 mg/ml to 8 mg/ml The results are summarized in Table I Stimulation at levels of statistical significance was seen in the concentration range 1–2 mg/ml. Sandoglobu-

lin had the greatest stimulatory effect, and the maximum increment of 4.7 nm was seen at a dose of 1 mg/ml. Intraglobin F was inhibitory at the highest concentration used (8 mg/ml)

To exclude the possibility that this stimulation of O_2^- release was simply due to protein, human serum albumin (HSA) was added to neutrophils at concentrations of 2 and 4 mg/ml and O_2^- release was compared with control cells to which no HSA was added HSA inhibited O_2^- release at these concentrations

In contrast to the effect on O_2^- release, Intraglobin F and Sandoglobulin resulted in increased H_2O_2 production by neutrophils in a dose-dependent fashion up to the highest concentration (8 mg/ml) Peak stimulation by Gammune N was seen at 1 mg/ml (Fig. 1.)

Further experiments were performed to determine whether IVIgG had any enhancing or additive effect on stimulated O_2^- release and H_2O_2 production when OZ, PMA and FMLP were used a stimulants. The stimulants were added to the cells immediately after the IVIgG (or control sugar solution). The results of these experiments are summarized in Table II Sandoglobulin enhanced O_2^- release in the presence of all three stimulants, whereas Gammune N enhanced only FMLP stimulation and Intraglobin significantly decreased PMA stimulation.

All three IVIgG preparations enhanced the stimulatory effects on H_2O_2 production, with the single exception that Gammune N had no additional effect in the presence of PMA.

TABLE I

Effect of IVIgG on O_2^- release from human neutrophils

IVIgG (mg/ml)	ΔO_2^- release (nmol)[a]		
	Intraglobin F	Sandoglobulin	Gammune N
0 13	1 13	NT	0 44
0 25	0 49	2 45	2 25
0 5	1 46	3 14	2 30
1	1 67[c]	4 70[c]	2 06[c]
2	2 55[c]	3 58[c]	3 43
4	1 03	3 53[c]	2 79
8	−2 16	4 31[c]	NT[b]

[a] ΔO_2^- release is the increase in O_2^- release in nanomoles per 5 × 10^5 cells above controls containing carrier-sugar in place of IVIgG

[b] NT, not tested

[c] $p < 0.05$

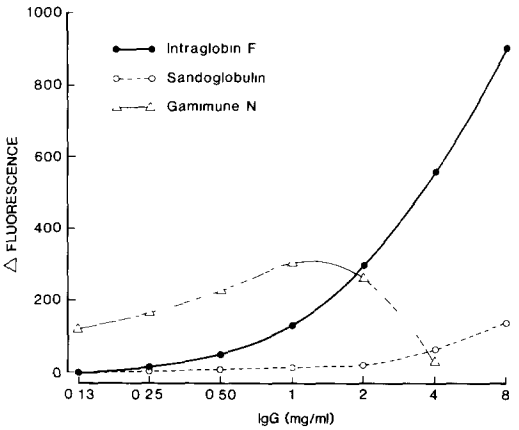

Fig 1 Effect of IVIgG on H_2O_2 production The increase in intracellular H_2O_2 is measured by the net increase in green fluorescence (Δfluorescence) expressed as the increase in mean channel number above controls The maximum channel number, after correction for gain setting, was 2560 The assay was modified from Bass et al (1983) In this dose-response experiment, because of strict time limitations in performing the assay on freshly prepared cells, single samples were assayed

Effect of IVIgG on neutrophil chemiluminescence

Both Intraglobin F and Gamimune N stimulated Luminol-amplified chemiluminescence at concentrations in the region of 0.5–1 mg/ml Stimulation indices (SI = peak stimulated cpm · peak control cpm) were in the range 1.5 to 2 3. Sandoglobulin, on the other hand, stimulated only at the highest dose (8 mg/ml), SI 2 7.

Assays were carried out to determine the effect of the presence of IVIgG on CL stimulated by OZ particles. IVIgG (or control sugar solution) was added to the cells immediately before the OZ. Intraglobin had no additive effect and was inhibitory at 4 and 8 mg/ml Sandoglobulin and Gamimune N both showed a dose-dependent enhancing effect, maximal at 1 mg/ml. The results using Gamimune N are

TABLE II

Effect of IVIgG on stimulated O_2^- release and H_2O_2 production by human neutrophils

IVIgG	Stimulant[b]	ΔO$_2$ (nmol)[a]	p	ΔFluorescence[a]	p
Intraglobin F, 4 mg/ml	OZ	−1 03	NS[c]	1277	0 005
	PMA	−8 58	0 048	660	0 006
	FMLP	1 27	NS[c]	623	0 005
Sandoglobulin, 4 mg/ml	OZ	8 53	0 009	187	0 007
	PMA	9 51	0 01	227	0 003
	FMLP	4 56	0 01	228	0 002
Gammune N, 1 mg/ml	OZ	1 96	NS[c]	347	0 0002
	PMA	2 99	NS[c]	−10	NS[c]
	FMLP	7 74	0 002	197	0 0003

[a] ΔO_2^- is the increase in O_2^- release in nanomoles per 5 × 10^5 cells, ΔFluorescence is the net increase in green fluorescence expressed as the increase in mean channel number above controls containing appropriate carrier-sugar in place of IVIgG

[b] OZ, 1 mg/ml, PMA, 10 ng/ml, FMLP, 10^{-6} M

[c] NS = not significant, $p > 0\,05$

shown in Table III, and the effect of Sandoglobulin is shown in Fig. 2

Effect of IVIgG on neutrophil locomotion and chemotaxis

In this part of the study only three concentrations of IVIgG were utilized: 0.25, 1 and 4 mg/ml When the lower chamber contained only HBSS, migration of cells through the filter was significantly increased only by Intraglobin 0.25 mg/ml and Gammune N 1 mg/ml ZAS-induced directed migration of neutro-

TABLE III

Enhancing effect of Gammune N on neutrophil chemiluminescence response to OZ 0 2 mg/ml

Gammune N (mg/ml)	ΔCL[a] (cpm × 10^{-6})	Stimulation index[b]
0 25	20 4	1 71
0 5	36 7	2 52
1	36 5	2 81
2	27 2	2 16
4	4 95	1 22
8	−14 4[c]	0 47

[a] ΔCL, increase in peak chemiluminescence response above controls containing carrier-maltose in place of IVIgG

[b] Stimulation index, ratio of peak CL responses with and without IVIgG

[c] Depressed response probably accounted for by fall in pH at highest concentrations of Gammune N

TABLE IV

Effect of IVIgG on neutrophil locomotion and ZAS-induced directed migration

	IVIgG (mg/ml)	ΔCell migration (μm)[a]	
		HBSS lower chamber	ZAS in lower chamber
Intraglobulin	0 25	12 4[b]	6 1
	1	12 2	− 9 4
	4	3 8	−19 9
Sandoglobulin	0 25	− 1 3	− 12 2
	1	−10 3	−13 41
	4	6 3	− 1 3
Gammune	0 25	7 5	17 9
	1	24 8[b]	46 4
	4	27 5	13 2[c]

[a] ΔCell migration, increase in distance travelled compared to control cells with carrier-sugar in place of IVIgG (A negative value indicates an actual decrease in distance travelled compared to control cells)

[b] $p < 0\,05$

[c] Cells migrated through filters Figure is increase in mean cells per HPF

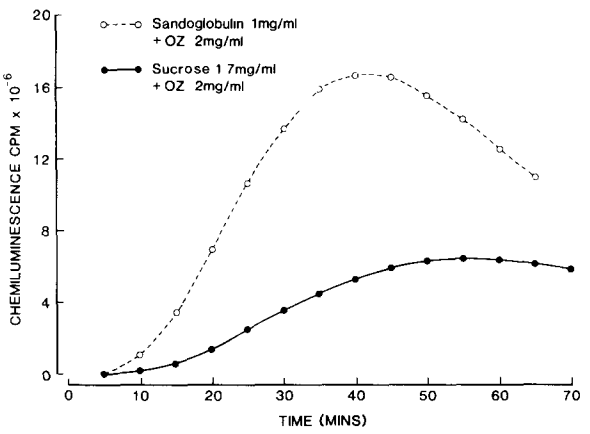

Fig 2 Enhancing effect of Sandoglobulin 0 2 mg/ml on neutrophil chemiluminescence response to opsonized zymosan Controls contained sucrose 1 7 mg/ml in place of IVIgG In order to recycle vials as rapidly as possible in the counter, single samples were assayed

phils was decreased by most concentrations of Intraglobin and Sandoglobulin, and in the presence of Gamimune N migration was increased, these differences did not reach levels of statistical significance (Table IV).

Discussion

Various mechanisms have been proposed to explain how IVIgG reduces cell destruction in the immune cytopenias (Imbach, 1983). The most popular theory is that IVIgG interacts with Fc receptors of mononuclear phagocytes, thereby inhibiting the phagocytosis of cells (red cells, platelets or neutrophils) opsonized by autoantibody (or alloantibody). There is in vitro experimental evidence to support this concept (Jungi et al., 1986). An equally attractive hypothesis is that infused immunoglobulin binds to red blood cells which are then preferentially sequestered by macrophages; support for this mechanism is provided by the clinical finding that the injection of anti-Rho(D) is effective in treating ITP (Salama et al., 1983, Baglin et al., 1986). In addition to the effect of macrophage blockade, IVIgG may modulate the immune response by altering the proportions of T-cell subsets or by non-specific inhibition of B-cell responsiveness (Tsubiakio et al., 1983; Dammacco et al., 1986; Stohl, 1986).

The beneficial effect of IgG replacement therapy in hypogammaglobulinemia is presumably due largely to passive immunization with preformed specific antibodies; such antibodies against a wide range of bacterial and viral pathogens can readily be demonstrated in human IgG for both IV and IM use (Yap, 1987). However, experimental and clinical evidence indicates that IVIgG may interact with macrophage Fc receptors and, in other unspecified ways, modulate the immune response, as already discussed This raises the possibility that it may also influence neutrophil function. Koch et al. (1984) showed that IVIgG can stimulate neutrophil O_2^- release and chemiluminescence in vitro; the present study was designed to further investigate this effect. The neutrophil respiratory burst was examined using three assays; O_2^- release, H_2O_2 production and Luminol-dependent chemiluminescence (CL). The CL response of activated neutrophils correlates well with metabolic activation (Allen et al , 1972), but there has been considerable controversy as to what is the chief oxidizing species measured in Luminol-dependent CL. De Chatelet and Shirley (1981) have argued that it is largely due to HOCl which diffuses extracellularly and reacts with Luminol to form aminophthalate ions and light generation.

In all three types of assay our results indicated that IVIgG can directly stimulate the neutrophil respiratory burst. The maximum increase in O_2^- release was of the order of 3.5–4.55 nmol (per 5×10^5 cells) and this was achieved by concentrations of 0.5–1 mg/ml IVIgG, concentrations which are readily achieved in moderate-dose IVIgG therapy. High-dose therapy can achieve serum concentrations of the order of 8 mg/ml (Pirofsky, 1987). In the case of H_2O_2 production, the stimulatory effect on H_2O_2 was more clearly dose-dependent, and for two IVIgG preparations it appeared that the maximal H_2O_2 response was not reached in the range of concentrations used. The stimulatory effects of OZ, PMA and FMLP were enhanced by IVIgG and this enhancing effect was more clearly seen in the H_2O_2 assay.

CL responses also indicated that IVIgG can directly stimulate neutrophils, as well as being able to enhance the metabolic response following ingestion of OZ.

There are few published reports on the effects of IVIgG on neutrophils, either in vivo or in vitro. Koch et al (1984), using a blood leukocyte preparation consisting of both neutrophils and mononuclear cells, demonstrated that the neutrophil respiratory burst was stimulated in vitro by IVIgG. The maximal increases in O_2^- release, 5 nmol per 10^6 cells, was quite consistent with our own findings. Redd et al (1988) reported that human IVIgG reduced the severity of neutropenia in newborn rats after intrapulmonic injection of group B streptococci This was thought to be due to increased release of neutrophils from the marrow and egress from the blood into infected tissue IVIgG did not affect the production of neutrophils in vitro or in vivo

Of the three IVIgG preparations used in the present study, Intraglobin F (Biotest) has the highest content of dimers and aggregates (Lundblad et al., 1987). If the observed stimulation of neutrophils was initiated by the interaction of aggregated IgG with Fc receptors, we would have expected Intraglobin F to give consistently higher levels of stimulation This was not observed Monomeric IgG is known to attach to Fc receptors of human monocytes (Kurlander, 1980) and, in the same way, must be able to bind to Fc receptors of neutrophils. The stimulating effect of IVIgG on neutrophil metabolism could not be reproduced by another serum protein (HSA), suggesting that Fc receptor–monomeric IgG interaction may be the initiating event

There is no doubt that the benefit of IVIgG therapy in hypogammaglobulinemia, and in severe infection in the immunocompromised host, is primarily due to the passive provision of preformed antibodies. However, the findings of this study suggest that host defence may benefit secondarily from the direct stimulation of neutrophil metabolism by IVIgG

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