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Comparison of the composition and opsonic activities of imported and South African-manufactured intravenous and intramuscular immunoglobulin preparations

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We compared the composition and opsonic activities for two common microbial pathogens (*Staphylococcus aureus* and *Streptococcus pyogenes*) of various imported intravenous (IV) (Sandoglobulin, Octagam and Gammagard) and intramuscular (IM) (Beriglobin and Globuman Berna) immunoglobulin (Ig) preparations with those of the corresponding locally manufactured products, Polygam (IV) and Intragam (IM). When tested at equivalent concentrations (1 g/100 ml) the total IgG and IgG subclass concentrations of the various IV and IM preparations were similar. All the test preparations (IV and IM) possessed similar opsonic activity for *S. aureus* and *S. pyogenes*. These findings demonstrate that, in respect of IgG content and protective biological activity, Intragam and Polygam, the locally manufactured IM and IV Ig preparations, respectively, compared extremely favourably with the corresponding imported products.

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Intramuscular (IM) and intravenous (IV) immunoglobulin (Ig) preparations consist of IgG concentrates and are used primarily as prophylactic agents in the substitution therapy of inherited and acquired antibody deficiency states such as agammaglobulinaemia and hypogammaglobulinaemia, including IgG subclass deficiencies with and without deficiency of IgA.[1,2] IV Ig preparations, which contain Fc region-unmodified, functionally intact IgG molecules, are significantly more efficacious than IM preparations in reducing both the frequency and the severity of bacterial infections in antibody-deficient individuals.[3] The clinical application of IV Ig preparations is not, however, limited to IgG replacement therapy of antibody deficiency states. Their usefulness in the treatment of idiopathic thrombocytopenic purpura and Kawasaki disease is well established,[3,4] while beneficial effects of administration of high-dose IV Ig have been reported in several other acute and chronic auto-immune diseases, including pure red cell aplasia, chronic inflammatory demyelinating neuropathy,[3] ophthalmopathy associated with thyroid disease,[3] auto-immune neutropenia,[5] recent-onset diabetes mellitus,[6] systemic lupus erythematosus,[7,8] bullous pemphigoid,[9] myasthenia gravis,[10] polymyositis,[11,12] rheumatoid arthritis[13] (including patients refractory to chemotherapy with first- and second-line drugs),[14] and juvenile chronic arthritis.[15,16] Encouraging results have also been reported in other conditions which may not have an auto-immune cause, such as spontaneous recurrent abortion, intractable childhood epilepsy, myalgic encephalomyelitis, severe steroid-dependent asthma and chronic inflammatory bowel disease (reviewed by Webster[3]). Moreover, IV Ig not only reduces the frequency of bacterial infections in children with symptomatic human immunodeficiency virus (HIV) infection[17] but may also protect against T-cell deletion.[18]

Given the ever-increasing list of human diseases which are apparently responsive to administration of IV Ig, we investigated the Ig composition and opsonic activities of various imported and locally manufactured IV and IM Ig preparations.

Materials and methods

Immunoglobulin preparations

The various IV and IM Ig preparations investigated in this study are listed in Table I. With the exception of Octagam (liquid), all the IV Ig preparations were freeze-dried and reconstituted in sterile physiological saline. In the assays of Ig composition and opsonic activity described below the various Ig preparations (IV and IM) were tested at an identical final concentration of 1 g Ig/100 ml. Pooled serum from blood specimens taken from 12 young, healthy adult human volunteers was decomplemented (56°C/60 min) and used as a reference source for the assays of Ig content and opsonic activity described below.

Immunoglobulin levels

Concentrations of total IgG, IgM and IgA in the various test preparations and pooled control serum were measured using standard laser nephelometric procedures in the Department of Chemical Pathology, Institute for Pathology, University of Pretoria. IgE levels were measured by radio-immunoassay (Pharmacia, Uppsala, Sweden), while levels of the IgG subclasses (IgG_1, IgG_2, IgG_3 and IgG_4) were determined by radial immunodiffusion (The Binding Site Ltd, Birmingham, England). All preparations were assayed at a concentration of 1 g protein/100 ml.

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Table I. IV and IM immunoglobulin preparations

Preparation	Type	Batch No.	Manufacturer/distributor
Gammagard	IV	2807V111AA	Baxter Healthcare, Hyland Division, Glendale, Calif., USA; distributed by SABAX, Johannesburg
Octagam	IV	1512084	Octapharma AG, Glarus, Switzerland; not clinically available in South Africa.
Sandoglobulin	IV	0.345.184.0	Swiss Red Cross; distributed by Sandoz Products, Johannesburg
Polygam	IV	GIV18, GIV21 and GIV25	Natal Blood Transfusion Service, Durban
Beriglobin	IM	017/1B 5	Hoechst AG, Frankfurt, Germany
Globuman Berna	IM	120 010 103	Swiss Serum and Vaccine Institute, Berne, Switzerland; distributed by Swisspharm, Johannesburg
Intragam	IM	GH66, GL99 and GH74	Natal Blood Transfusion Service, Durban

Opsonic activity

For purposes of comparison all the test Ig preparations were used at a fixed concentration of 1 g/100 ml IgG in these assays. The opsonic activity of the various Ig preparations was measured according to the extent of uptake or iodination of bacteria by human neutrophils *in vitro*. *Staphylococcus aureus* (ATCC25923) or *Streptococcus pyogenes* (clinical isolate, obtained from the South African Institute for Medical Research, Johannesburg) were used as the test microbial pathogens in assays of opsonic activity. Neutrophils were prepared from heparinised (5 U preservative-free heparin/ml blood) venous blood taken from healthy, adult human volunteers using standard methods. Briefly, the blood was centrifuged on Ficoll-Hypaque gradients, followed by sedimentation of erythrocytes in the neutrophil/erythrocyte pellet with 3% gelatin and removal of residual erythrocytes in the neutrophil-enriched supernatant by treatment with 0,83% NH_4Cl. Neutrophils were resuspended to 1 x 10^7/ml in Hanks's balanced salt solution (HBSS) (Highveld Biological, Johannesburg).

Uptake of opsonised *S. aureus* by neutrophils was measured by a modification of the method of Peterson *et al.*[19] *S. aureus* micro-organisms were radiolabelled during overnight culture in 10 ml nutrient broth containing 5 µCi radiolabelled amino acids (L-amino acid mixture [$^{14}C(U)$], specific activity 55 mCi per milliatom of carbon, Du Pont-NEN Research Products, Boston, Mass, USA). The bacteria were then washed and resuspended to a concentration of 2 x 10^8 colony-forming units (CFU)/ml. In assays of opsonic activity neutrophils (4 x 10^6) were co-incubated with *S. aureus* at a ratio of 1 neutrophil to 50 CFU for 60 minutes (predetermined in preliminary experiments) at 37°C in a final volume of 1 ml HBSS containing the different Ig preparations or pooled serum (10%). The neutrophil bacteria mixtures were rotated on a turntable throughout the 60-minute incubation period, after which the neutrophils and cell-associated *S. aureus* were separated from non-adherent bacteria by differential centrifugation (1 000 rpm/5 min). The cell-associated bacteria were released by treatment of the neutrophil pellets with 0,4 ml 1% Triton X-100/0,1M NaOH and the radioactivity was measured in a liquid scintillation spectrometer.

S. pyogenes could not be used in this assay because of the tendency of these micro-organisms to form aggregates, thus complicating the separation of neutrophil-associated and non-adherent bacteria. The opsonic activity of the test preparations for *S. pyogenes* was therefore measured by an alternative assay, viz. myeloperoxidase-mediated (MPO) iodination.[20]

MPO iodination

Using this assay, reaction mixtures contained neutrophils (4 x 10^6) and *S. pyogenes* micro-organisms at a ratio of 1:50, 0,6 µCi of a ^{125}I-labelled solution of sodium iodide (Du Pont-NEN), and the various Ig preparations or heat-inactivated pooled normal serum (10%) in a final volume of 1 ml HBSS. After 60 minutes' incubation at 37°C the reactions were terminated by the addition of 10% trichloroacetic acid (TCA), and after three washes with TCA the amount of cell-associated ^{125}I was determined in a solid-state gamma counter.

Expression and statistical analysis of results

The results for each series of experiments are expressed as mean values ± the standard error (SE).

Results

Immunoglobulin levels

These are set out in Tables II and III. Intragam, the locally manufactured IM product, compared favourably with the two imported IM preparations (Beriglobin and Globuman Berna) in respect of content of total IgG and IgG subclasses (Table II), relative proportions of the IgG subclasses (Table III), and levels of contaminating IgA, IgE and IgM. Of the four IV preparations tested, the total IgG and IgG subclass levels of Polygam (batches GIV 18, 21 and 25) were comparable with those of Sandoglobulin, Octagam and Gammagard. Levels of contaminating IgA, IgE and IgM were minimal in all the test IV preparations.

Opsonic activity

These data are shown in Figs 1 and 2 for opsonisation of *S. aureus* and *S. pyogenes*, respectively. All the test

Table II. Immunoglobulin composition of the test preparations

	Total IgG	IgG_1	IgG_2	IgG_3	IgG_4	IgA	IgM	IgE
Immunoglobulin source								
Pooled serum	11,9	10,6	5,7	1,3	0,77	1,88	1,65	55
IV Ig preparations								
Sandoglobulin	10,9	6,61	3,93	0,46	0,25	0,18	<0,04	9,1
Octagam	10,7	5,43	4,24	0,42	0,12	<0,07	<0,04	2,0
Gammagard	10,7	5,87	2,28	0,56	0,05	<0,07	<0,04	<1
Polygam GIV 25	9,6	6,01	3,34	0,53	0,13	0,09	<0,04	36,6
Polygam GIV 18	8,5	4,93	2,93	0,44	0,15	0,15	<0,04	36,1
Polygam GIV 21	10,2	5,43	2,79	0,53	0,19	0,17	<0,04	43,0
IM Ig preparations								
Globuman Berna	9,2	5,39	2,79	0,51	0,19	0,08	0,08	26,0
Beriglobin	10,3	6,01	3,21	0,53	0,25	0,12	0,07	61,4
Intragam GH 74	9,1	4,93	3,49	0,39	0,28	0,16	<0,04	42,7
Intragam GH 66	8,8	4,93	3,06	0,58	0,25	0,15	<0,04	57,1
Intragam GL 99	9,9	6,86	3,34	0,61	0,31	0,24	0,05	60,8

The various IV and IM Ig preparations were tested at a final concentration of 1 g protein/100 ml. Levels of IgG, IgA, IgM and IgG subclasses are expressed in g/l, while those of IgE are expressed as IU/ml.

Table III. Relative proportions of IgG subclasses in the various test preparations

	Proportion (%)			
	IgG_1	IgG_2	IgG_3	IgG_4
Pooled serum	58	31	7	4
Intravenous preparations				
Sandoglobulin	59	35	4	2
Octagam	53	42	4	1
Gammagard	67	26	6	1
Polygam GIV 25	60	33	5	1
Polygam GIV 18	58	35	5	2
Polygam GIV 21	61	31	6	2
Intramuscular preparations				
Globuman Berna	61	31	6	2
Beriglobin	60	32	5	3
Intragam GH 74	54	38	4	3
Intragam GH 66	56	35	7	3
Intragam GL 99	62	30	5	3

preparations (IV and IM) possessed comparable opsonic activity for *S. aureus*, with Gammagard being slightly better than the others. The various IM preparations possessed comparable opsonic activity for *S. pyogenes*, although some differences were evident with the IV preparations. Octagam and Gammagard were the most impressive of the IV preparations in respect of opsonisation of *S. pyogenes*, while Sandoglobulin and Polygam possessed similar activity. The data for opsonisation of *S. pyogenes* by pooled normal serum are shown in the legend to Fig. 2. The relatively unimpressive activity of pooled normal serum is an artefact caused by the oxidant-scavenging activity of low-molecular-weight anti-oxidants such as vitamin C and uric acid,[21] which are present in serum but not in the Ig preparations.

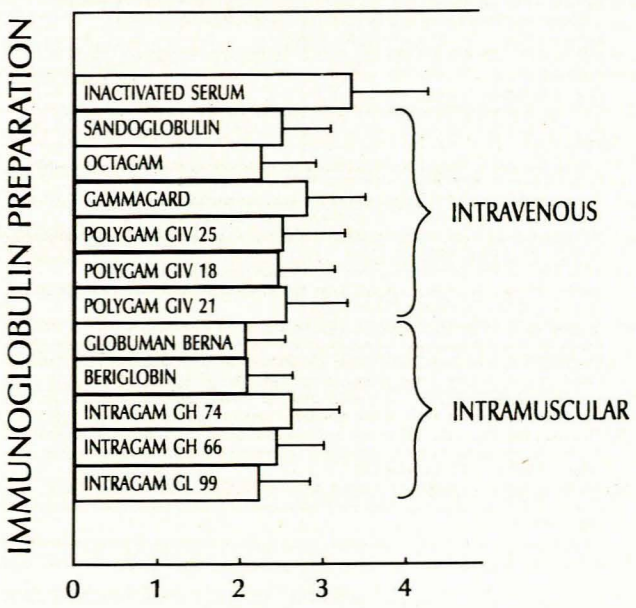

Fig. 1. Uptake of radiolabelled *S. aureus* by human neutrophils (counts per minute x 10^3) in the presence of 10% heat-inactivated pooled normal serum or the various test IV and IM Ig preparations. Data are expressed as mean ±SE of 3 different experiments using neutrophils from 3 different donors.

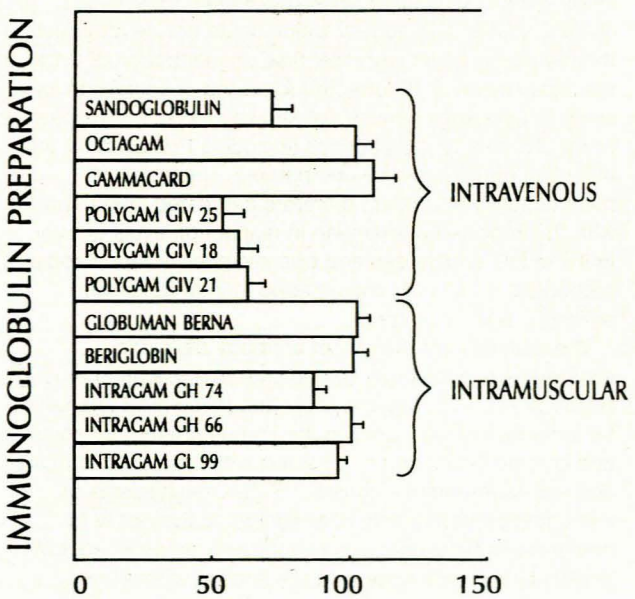

Fig. 2. MPO-mediated iodination of *S. pyogenes* (counts per minute x 10^3) by human neutrophils in the presence of the various test IV and IM Ig preparations. The value for inactivated serum was 17 ± 4 cpm x 10^3. In the corresponding Ig-free control systems the iodination of *S. pyogenes* was 617 ± 135 cpm. Data are expressed as the mean values ± SEMs of 3 different experiments.

Discussion

In this study we compared the Ig concentrations and opsonic activities of the currently available (with the exception of Octagam) imported and locally manufactured IM and IV Ig preparations. Of the three IM preparations tested, the locally manufactured product Intragam compared extremely favourably with the two imported products (Beriglobin and Globuman Berna). All three of these IM products contained high levels of IgG, with the various IgG subclasses being present in proportions which correspond to those of normal serum globulin. There was minor, but similar, contamination by IgA, IgM and IgE. The three IM preparations possessed similar levels of opsonic activity for the microbial pathogens *S. aureus* and *S. pyogenes.* In a previous study[22] communicated in 1983, we reported that the locally manufactured IM Ig product performed less impressively in these assays than Beriglobin. However, the data presented here demonstrate that Intragam is the equal of Beriglobin and Globuman Berna in respect of Ig content and opsonic activity.

Equivalent concentrations of IgG were also present in the various IV preparations, with the IgG subclasses again present in representative proportions. When tested at identical concentrations (1 g/100 ml), all the test IV preparations were found to possess similar opsonic activity for *S. aureus*, while that of Sandoglobulin and Polygam was less than Gammagard and Octagam for *S. pyogenes.* However, the iodination assay used for measuring opsonisation of *S. pyogenes* is prone to false-negative results if oxidant-scavenging activity is present in the assay system. Additional studies (data not shown) were performed to investigate the oxidant-scavenging potential of the various IV Ig preparations, using a cell-free oxidant-generating system. We observed that Polygam, and to a lesser extent Sandoglobulin and Octagam, possessed oxidant-scavenging activity which could not be attributed to the stabilising agent (sucrose; final concentration of 0,2% in the assay system). If corrected for in the opsonisation assay using *S. pyogenes* (results not shown), the opsonic activities of the different IV preparations (including Polygam) for this microbial pathogen were comparable. The locally manufactured IV product therefore compared favourably with the imported preparation in respect of levels of total IgG and IgG subclasses and opsonic activity for microbial pathogens commonly encountered in individuals with antibody deficiency states.

The replacement therapy of antibody deficiency syndromes is a relatively limited clinical application of IV Ig preparations, and it seems likely that these products will become increasingly used in the immunotherapy of acute and chronic inflammatory diseases with both auto-immune and non-auto-immune causes.[4-8,23] The mechanisms of immunosuppressive/anti-inflammatory activity of IV Ig preparations have not been conclusively established, but may include interference with the binding of immune complexes,[24] modulation of the production of pro-inflammatory cytokines,[25] suppression of immune responses by anti-idiotypic antibodies[26] or neutralisation of microbial superantigens which have been implicated in both the pathogenesis of auto-immune diseases[27] and the activation-induced destruction of $CD4^+$ T lymphocytes in HIV-infected individuals.[28]

In conclusion, the locally manufactured IM and IV Ig preparations Intragam and Polygam compare impressively with the corresponding imported products, and are likely to command increasing recognition.

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