Archives of Disease in Childhood, 1989, **64**, 530–534

Opsonic activity in serum from septic infants treated with intravenous immunoglobulin

L MARÓDI, Á KALMÁR, AND I SZABÓ

Department of Paediatrics, University Medical School of Debrecen, Hungary

SUMMARY Thirteen infants with staphylococcal sepsis and reduced opsonic activity received infusions of acid treated immunoglobulin together with antibiotics. Opsonic activity (using *Staphylococcus aureus* (type 42D) as the test organism), haemolytic activity of complement, and concentrations of complement C3 and IgG were measured in serum prepared before and after three days of treatment with immunoglobulin at a dose of 250–300 mg/kg/day. There was increased ingestion of *S aureus* by normal human granulocytes in the presence of fresh serum prepared after infusion of immunoglobulin and significantly increased opsonic activity of heat inactivated serum after treatment with immunoglobulin. The haemolytic activity of complement and concentrations of complement C3 were not influenced, and serum concentrations of IgG increased as the result of receiving a total of 800–900 mg/kg immunoglobulin over a period of three days. This study shows that administration of acid treated IgG to septic infants leads to functionally increased opsonisation.

Intravenous immunoglobulin is the treatment of choice for agammaglobulinaemia or hypogammaglobulinaemia. In patients with normal concentrations of gammaglobulin, immunoglobulins exert an immunomodulatory function.[1 2] High doses of immunoglobulins produce rapid increases in the platelet counts in patients with idiopathic thrombocytopenic purpura.[1 3] Intravenous immunoglobulins alone or with antiviral drugs may improve the efficacy of treatment of life threatening viral diseases, apparently by replacement of selectively missing or exhausted antibodies.[2] The combined use of intravenous immunoglobulins and antibiotics is effective in the treatment of neonatal sepsis, particularly in premature infants.[4 5]

Intravenous immunoglobulins contribute to antibacterial host defense by opsonising micro-organisms. Binding of specific antibodies of the IgG class, with or without subsequent binding of complement to the surface of bacteria, facilitates phagocytosis by granulocytes or mononuclear phagocytes.[6–8] In vitro studies have shown that intravenous immunoglobulin preparations that contain intact 7S IgG could mediate reasonable opsonic activity towards a number of bacterial pathogens and could enhance phagocytosis of these micro-organisms by granulocytes.[9] Addition of such a preparation to serum from neonates resulted in increased phagocytosis of group B streptococcus type III in the presence of adult granulocytes.[10]

In this study we report increased opsonisation using *S aureus* as the test organism in young infants receiving intravenous immunoglobulin for the treatment of established staphylococcal infection.

Patients and methods

Selected clinical and laboratory data of the 13 infants studied are shown in table 1. All these infants had septicaemia or other severe bacterial infections (meningitis, ventriculitis, pneumonia) caused by *S aureus* either singly or in combination with another bacterial pathogen. Congenital malformations in cases 4, 8, 9, and 12 could have been predisposing factors for the development of the severe infection. Case 13 developed a paraoesophageal abscess after swallowing a drawing pin that stuck in the oesophagus. When these patients presented with severe symptoms or septicaemia, or both, informed consent was obtained from the parents and conventional treatment with antibiotics was supplemented with intravenous immunoglobulin 250–300 mg/kg/day for three consecutive days. When immunoglobulin treatment was started, all the patients had already been receiving antibiotics for more than two days. During the immunoglobulin

Table 1 *Details of patients treated with immunoglobulin*

Case No	*Birth weight (g)*	*Gestational age (weeks)*	*Age of patient*	*Sex*	*Clinical diagnosis*	*Polymorphonuclear leucocytes ($\times 10^9$/l)*	*Source of bacteria*	*Bacteria cultured*
1	3500	41	2 Days	Female	Meconium aspiration, septicaemia	4·0	Blood	*S aureus, Klebsiella* species
2	3300	37	3 Days	Female	Septicaemia	10·0	Blood	*S aureus*
3	2800	35	5 Days	Male	Intracranial haemorrhage, septicaemia	6·0	Blood	*S aureus, Klebsiella* species
4	3000	37	7 Days	Female	Meningocele, meningitis	7·6	Cerebrospinal fluid	*S aureus*
5	3600	38	9 Days	Male	Umbilical sepsis, septicaemia	10·5	Blood	*S aureus*
6	3450	38	3 Weeks	Female	Pneumonia, septicaemia	9·8	Blood	*S aureus*
7	2500	33	4 Weeks	Male	Pneumonia	10·4	Pus	*S aureus*
8	3900	40	6 Weeks	Male	Hypoplastic lungs, pneumonia	4·6	Pus	*S aureus, Pseudomonas aeruginosa*
9	3200	40	8 Weeks	Female	Hypoplastic cricoid, septicaemia	14·0	Blood	*S aureus*
10	2450	40	8 Weeks	Female	Ventriculitis, septicaemia	8·0	Blood, cerebrospinal fluid	*S aureus, Pseudomonas aeruginosa*
11	3200	37	6 Months	Female	Mastoiditis, septicaemia	14·6	Blood	*S aureus*
12	3400	39	8 Months	Male	Hydrocephalus, shunt, septicaemia	12·6	Blood, cerebrospinal fluid	*S aureus*
13	2150	35	9 Months	Female	Paraoesophageal abscess, septicaemia	13·8	Blood, pus	*S aureus*

Table 2 *Details of control patients*

Case No	*Birth weight (g)*	*Gestational age (weeks)*	*Age of patient*	*Sex*	*Clinical diagnosis*	*Polymorphonuclear leucocytes ($\times 10^9$/l)*	*Source of bacteria*	*Bacteria cultured*
1	2800	36	2 Days	Female	Infantile respiratory distress syndrome, pneumonia, septicaemia	11·0	Blood	*S aureus, Escherichia coli*
2	2650	36	2 Days	Female	Oesophageal atresia, pneumonia	10·4	Pus	*S aureus*
3	2100	33	3 Days	Female	Meconium aspiration, respiratory distress syndrome, septicaemia	11·5	Blood	*S aureus, Klebsiella* species
4	2100	34	5 Days	Male	Aspiration pneumonia, septicaemia	9·4	Blood	*S aureus, Pseudomonas aeruginosa*
5	2700	40	6 Days	Female	Thrombophlebitis, septicaemia	6·1	Blood	*S aureus, Klebsiella species*
6	2200	31	3 Weeks	Female	Umbilical sepsis, septicaemia	8·2	Blood	*S aureus, Staphylococcus epidermidis*
7	3000	38	5 Weeks	Male	Pneumonia, diffuse intravascular coagulation	7·0	Pus	*S aureus*
8	2900	38	10 Weeks	Male	Otitis medis, septicaemia	3·1	Blood	*S aureus, Pseudomonas aeruginosa*
9	3200	37	5 Months	Female	Pneumonia, septicaemia	6·2	Blood	*S aureus*
10	3100	40	5 Months	Male	Retropharyngeal abscess	13·6	Pus	*S aureus*
11	2100	34	9 Months	Female	Osteomyelitis of tibia, septicaemia	17·2	Blood	*S aureus*
12	3550	40	10 Months	Female	Osteomyelitis of humerus	13·4	Pus	*S aureus*
13	3000	40	12 Months	Female	Pneumonia	7·6	Pus	*S aureus*

treatment none of the patients received blood or other blood products.

Table 2 shows the clinical and laboratory data of 13 infants who were reviewed retrospectively and acted as a historical control group. The criteria for selecting a patient as a control were one or more cultures of *S aureus* from blood or pus together with documented signs and symptoms consistent with septicaemia or other severe bacterial infection. Five patients were less than 2 weeks old, and the rest between 3 weeks and 12 months. There were seven patients with septicaemia, three with pneumonia, two with osteomyelitis (one with septicaemia), and one with a retropharyngeal abscess.

Blood samples were taken twice during the period of immunoglobulin treatment: before the first dose

of acid treated (pH 4) immunoglobulin was given, and on the day after the three days of treatment. The first of the two daily doses of antibiotics was always given after blood had been collected. For the control experiments blood was obtained from age matched healthy infants. Serum was prepared by clotting the blood for one hour at room temperature followed by centrifugation for 20 minutes at 1200 *g*, and aliquots were stored at −30°C. Inactivated serum was prepared by heating serum for 30 minutes at 56°C.

Granulocyte enriched cell suspensions were prepared from the blood of healthy adult donors by Dextran sedimentation of the erythrocytes as previously described, and suspended in Hanks's balanced salt solution with 0·1% gelatine to a final concentration of 10^7 cells/ml.[11 12]

S aureus type 42D was cultured overnight at 37°C in nutrient broth (Oxoid), harvested by centrifugation at 1500 *g* for 10 minutes, washed twice with phosphate buffered saline, and resuspended in gelatine and Hanks's balanced salt solution to a concentration of about 10^7 bacteria/ml.[7]

Titration of complement by the classical pathway was performed according to the method of Kabat and Mayer.[13] Serum concentrations of complement C3 and IgG were measured by radial immunodiffusion using specific antisera (Human, Budapest). Serum samples prepared before and after infusion of intravenous immunoglobulin were tested individually from each patient.

The opsonic activity of serum was defined as its capacity to promote ingestion of bacteria by granulocytes. One hundred microlitres of a suspension of granulocytes (concentration 10^7/ml) were incubated with an equal volume of a suspension of 10^7 bacteria/ml in various concentrations of serum, and mixed by tumbling (4 rpm) at 37°C. After 60 minutes of phagocytosis, 50 µl aliquots of the mixture were added to 450 µl of ice cold Hanks's balanced salt solution, and cells were pelleted by centrifugation for 6 minutes at 75 *g*. The number of viable extracellular bacteria was determined by microbiological assay.[7] The opsonic activity of serum before and after infusion of intravenous immunoglobulin was determined individually from each infant. The viability of granulocytes after 60 minutes of phagocytosis remained higher than 96% (checked by trypan blue exclusion).

Phagocytosis of *S aureus* by normal human granulocytes at a concentration of 5×10^6/ml at 1:1 bacterium:cell ratio in the presence of fresh or heat inactivated sera from patients was measured before and after a three day treatment with acid treated intravenous immunoglobulin. Serum samples from age matched healthy infants were used in control experiments. Percentages of phagocytosed bacteria were determined by colony counts after 60 minutes incubation (table 3).

An acid treated intravenous immunoglobulin concentrate (Sandoz AG) prepared at pH 4 was administered to the patients by slow infusion in a dose of 250–300 mg/kg daily for three days.

Statistical analysis of the data was carried out by standard methods.[14] The significance of differences was assessed by the unpaired *t* test.

Results

Intravenous immunoglobulin treatment was well tolerated without side effects. All except case 1 recovered from the acute bacterial infection; she died of meconium aspiration followed by septicaemia. Cases 12 and 13 developed hydrocephalus and oesophageal stenosis, respectively. The rest of the treated patients recovered without complications. No clinical signs or symptoms of septic shock were observed.

In the control group of 13 infants (including five neonates) with septicaemia or other severe *S aureus* infections who were given only conventional antibiotic treatment, three patients (cases 1, 3, and 7) died, and one (case 2) recovered with complications. The others recovered from the bacterial infection without complications. Days in hospital, duration of fever, and dosage of antibiotics were all about the same in the two groups. There were no significant differences in time from the onset of symptoms of infection to administration of antimicrobial drugs between study patients and controls (mean (SD) number of days, 2·9 (1·8) compared with 3·2 (2·0).

Before treatment with immunoglobulin was introduced patients' serum opsonised *S aureus* less effectively than serum from age matched controls. After three days of treatment with immunoglobulin, opsonisation of *S aureus* by both fresh and heat inactivated serum increased, and was similar to that of the control values for all serum concentrations tested (table 3). Mean differences, between opsonic values before and after infusion were higher when heat inactivated serum samples were used as a source of opsonins than when fresh serum samples were used (table 3 and 4). This might be explained by the combined effect of complement and immunoglobulins in opsonising *S aureus* in fresh serum. In heat inactivated serum, however, the activity of complement is abolished and opsonisation of bacteria is related mainly to the concentration and functional activity of IgG. Differences between opsonic values in fresh and heat inactivated serum taken before and after intravenous infusion of immunoglobulin were significant (table 4).

Table 3 *Phagocytosis promoting activity of serum from 13 infants with severe sepsis treated with immunoglobulin. Figures are mean (SD) of 13 experiments*

	Concentration of serum (%)	*Percentage phagocytosed bacteria in the presence of patients' sera*		*Control sera*
		Before treatment	*After treatment*	
Incubation of granulocytes and bacteria with:				
Fresh serum	10	72·9 (14·3)	96·8 (6·0)	94·8 (2·9)
	5	72·0 (12·9)	86·7 (10·7)	92·3 (12·5)
	1	58·9 (13·6)	74·7 (9·8)	78·4 (10·7)
Heat inactivated serum	10	22·8 (10·2)	63·5 (14·3)	59·4 (10·4)
	5	21·8 (13·8)	58·1 (12·8)	58·5 (9·2)
	1	7·9 (6·7)	38·2 (12·3)	32·9 (9·9)

Table 4 *Statistical evaluation of the effect of treatment with intravenous immunoglobulin on opsonic activity of serum from 13 infants with severe sepsis*

Concentration (%)	*Mean differences (%)*	*SE of mean differences*	*Confidence intervals (%)*	*p Value*
Fresh serum:				
10	23·9	4·02	15·1 to 32·6	<0·001
5	14·7	4·64	4·6 to 24·8	<0·01
1	15·8	4·65	5·6 to 25·9	<0·01
Heat inactivated serum:				
10	40·8	4·89	30·1 to 51·4	<0·001
5	36·3	5·21	24·9 to 47·7	<0·001
1	30·3	4·09	21·4 to 39·2	<0·001

Table 5 *In vitro growth of S aureus in the presence of heat inactivated sera from 13 infants with severe sepsis treated with immunoglobulin*

Time of serum preparation	*Concentration of serum (%)*	*Mean (SD)% of viable bacteria*
Before treatment:	10	138 (19·2)
	5	130 (18·5)
	1	131 (20·1)
After treatment:	10	129 (17·6)
	5	132 (18·2)
	1	121 (14·8)

The bactericidal activity of serum from the study patients was measured by the in vitro growth of *S aureus* in various concentrations of heat inactivated sera (table 5). Incubation of bacteria without granulocytes in heat inactivated serum showed an increase in the number of micro-organisms, which indicated that there was no bactericidal activity in the samples tested. The number of viable bacteria after 60 minutes' incubation was about the same in the serum samples taken before and after treatment with immunoglobulin (table 5).

Haemolytic activity of the classical complement pathway was not influenced by immunoglobulin treatment (table 6). The same was true for concentrations of complement C3, whereas the serum concentration of IgG was significantly increased by the total of 800–900 mg immunoglobulin given over the three day period.

Discussion

S aureus and other staphylococci cause a diverse group of infections. *S aureus* accounts for more than 10% of nosocomial infections and causes 20% of infections in paediatric wards.[15] Polymorphonuclear neutrophil leucocytes play a key part in the host

Table 6 *Haemolytic activity of complement and concentrations of complement C3 and IgG in serum from infants with severe sepsis treated with immunoglobulin. Figures are mean (SD)*

	Before treatment	*After treatment*	*p Value*
Complement haemolytic activity (U/ml)	37·50 (5·10)	36·20 (3·90)	NS
Complement C3 (g/l)	0·80 (0·15)	0·86 (0·18)	NS
IgG (g/l)	7·70 (1·90)	12·80 (1·80)	<0·01

defence against staphylococci, yet without opsonins they inhibit *S aureus* only to a limited extent.[16] Complement clearly augments inhibition in vitro, but alone it is not impressive.[17] Although the role of specific antibodies in protection against infection with *S aureus* is not completely understood, it is known that IgG is essential to inhibit *S aureus* even at a concentration of 0·25 g/l.[16] Defects in antibody mediated opsonisation can occur as primary deficiencies of immunoglobulin synthesis or can be a consequence of sytemic infection caused by consumption of specific antibodies by the infecting organism.

In this study we found that both IgG mediated and IgG and complement mediated opsonic activity against *S aureus* could be improved in infants with *S aureus* septicaemia by treatment with intravenous immunoglobulin; the infused IgG resulted in functionally increased opsonisation. The presence of specific antibodies in the polyvalent IgG preparations against common bacterial pathogens may explain this. Indeed, the IgG preparations are fractionated from a pool of several thousand blood donations, so that antibodies to endemic pathogens are concentrated and rare antibodies are diluted.

The higher rate of ingestion of *S aureus* by normal granulocytes in fresh serum compared with heat inactivated serum suggested that opsonisation was optimal when complement was functional. These results further emphasise the contributory effect of complement in the opsonisation of micro-organisms. The significant improvement of the opsonic activity of heat inactivated serum after infusion of immunoglobulin could be explained by the action of heat stable opsonins, specific IgG antibodies directed against the given pathogen.

The growth of bacteria in various concentrations of heat inactivated serum taken before treatment was comparable with that in serum taken after treatment with immunoglobulin. This excludes the possibility of improved opsonisation of *S aureus* by the antibiotics that were given at the same time as the immunoglobulin to all the patients.

It is important to point out that the haemolytic activity of complement and concentrations of complement C3 were not significantly influenced by treatment with immunoglobulin. This suggests that administration of high dose immunoglobulin did not result in impairment of complement mediated effector function in septicaemic infants.

These results indicate that treatment with intravenous immunoglobulin may be useful in modifying disease caused by *S aureus* in infants. Further evaluation will require a prospective, controlled study of the use of intravenous immunoglobulin in septicaemic infants in order to determine the real clinical importance of this treatment in the outcome of severe staphylococcal disease. It is necessary to identify groups of infants with severe sepsis in whom treatment with intravenous immunoglobulin might be useful.

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Correspondence to Dr L Marodi, Department of Paediatrics, University Medical School of Debrecen, H-4012 Debrecen, POB:32, Hungary.

Accepted 18 September 1988