Clin. exp. Immunol. (1989) **76**, 8–12

Opsonic activity of a new intravenous immunoglobulin preparation: Pentaglobin compared with Sandoglobulin

N. D. GARBETT, C. S. MUNRO & P. J. COLE *Host Defence Unit, Department of Thoracic Medicine, National Heart and Lung Institute, London*

(Accepted for publication 20 December 1988)

SUMMARY

Standard preparations of immunoglobulin for intravenous use consist predominantly of IgG (> 95%). We have compared the ability of a standard preparation (Sandoglobulin) with that of a new preparation (Pentaglobin, containing 12% IgM and 12% IgA) to improve the opsonic activity of antibody-deficient human sera *in vitro*. Panhypogammaglobulinaemic sera were poorly opsonic for five of six organisms tested, particularly *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pneumoniae*, but opsonized *Staphylococcus aureus* almost normally. Both immunoglobulin preparations significantly improved the opsonic activity of the antibody-deficient sera for most organisms. The major difference between the two preparations was the ability of Pentaglobin to supply opsonins for *P. aeruginosa*, *E. coli* and *Klebsiella pneumoniae*, while Sandoglobulin was significantly more potent in opsonins for *Haemophilus influenzae*. Pentaglobin demonstrates significant *in vitro* opsonic activity, particularly for enterobacteria (coliforms) and *P. aeruginosa*. Its content of IgM antibodies appears to confer special properties on Pentaglobin not seen with standard preparations of immunoglobulin for intravenous use. Its place in clinical practice remains to be determined but it may have a possible role in augmenting host defence mechanisms in Gram-negative septicaemia.

Keywords hypogammaglobulinaemia intravenous immunoglobulin opsonization Gram-negative septicaemia

INTRODUCTION

Regular administration of intravenous immunoglobulin preparations is now established as replacement therapy for panhypogammaglobulinaemia. There are several other clinical situations where the humoral immune response of the host is impaired and where administration of parenteral immune therapy may be useful (Alving & Finlayson, 1979): in patients with multiple trauma, or undergoing major surgery or having sustained extensive burns, immunosuppressed patients as defined by more conventional criteria (eg, therapy with immunosuppressive drugs) and patients with apparently normal immunity presenting with overwhelming infection.

Gram-negative aerobic bacilli are the leading cause of fatal nosocomial infections (McGowan, 1985). Pre-eminent among these pathogens in *Pseudomonas aeruginosa*, whose ability to cause life-threatening infections in compromised patients is well documented (Cross *et al.*, 1983). Mortality rates for nosocomial bacteraemic episodes have remained unchanged for the past three decades, indicating the limited impact of antibiotic therapy (Bodey *et al.*, 1983). To reduce mortality and morbidity in these patients enhancement of their defence mechanisms is probably required in addition to anti-microbial chemotherapy.

Normal human immunoglobulin is prepared from pooled human plasma by various fractionation procedures and then modified to obtain a preparation suitable for intravenous use. Standard preparations contain predominantly immunoglobulin (Ig) G, antibodies of the IgM and IgA classes being lost during fractionation. Sandoglobulin is such a preparation, consisting of 96% IgG, 3·6% IgM and 0·4% IgA. Pentaglobin is a new preparation containing significant amounts of IgM (12%) and IgA (12%) but less IgG (76%).

Of the immunoglobulin classes, only IgG can directly promote phagocytosis. It does so by interacting with Fc receptors on the phagocyte membrane, either alone or in the presence of C3b (Silverstein, Steinman & Cohn, 1977). IgM can also prepare particles for ingestion by phagocytic leucocytes but, unlike IgG, it requires the participation of serum complement. Binding of IgM to a particle in the presence of serum initiates the fixation of complement to the surface of that particle which can then interact with C3b receptors on the leucocyte membrane (Silverstein, Steinman & Cohn, 1977). IgM is the major component of the primary antibody response, is produced before IgG and it is the most effective immunoglobulin class at fixing complement (Roitt, 1984).

Correspondence: Professor Peter Cole, Host Defence Unit, Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK.

We have used an *in vitro* neutrophil chemiluminescence assay which we have described previously (Williams *et al.*, 1980), to determine the ability of Pentaglobin to reconstitute panhypogammaglobulinaemic human sera with opsonins for a range of bacteria. We have also compared this ability with that of Sandoglobulin which we have previously shown to improve such impaired opsonic activity (Munro, Stanley & Cole, 1985).

MATERIALS AND METHODS

Sera

Sera were obtained from 12 healthy subjects and pooled as a standard. Sera were also obtained from five patients with idiopathic adult onset panhypogammaglobulinaemia who had not received replacement therapy for at least 4 weeks. Total IgG, IgA and C3 were measured by standard techniques.

Immunoglobulin preparations

The intravenous immunoglobulin preparations tested were Sandoglobulin (pH 4 and pepsin-treated; Sandoz Products Ltd) and Pentaglobin (beta-propiolactone-treated; Biotest UK Ltd).

Bacteria

Bacteria used were single clinical isolates of *Haemophilus influenzae* (non-capsulated), *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus-aureus* and *Pseudomonas aeruginosa* (non-mucoid). These organisms were grown in broth overnight, then centrifuged and washed twice in PBS. The final suspensions were adjusted with a nephelometer to a standard concentration of approximately 1×10^9 colony forming units/ml.

Neutrophil preparation

Neutrophils were obtained from healthy donors by adding 20 ml of blood to 6 ml of 6% Dextran in saline and 200 iu heparin. After sedimentation at room temperature for 45 min, the plasma was layered onto Ficoll-Paque (Pharmacia) separation medium and centrifuged at 500 *g* for 40 min at 18°C. Residual erythrocytes were lysed by resuspension of the pellet in 0·8% ammonium chloride buffer, pH 7·2. The remaining cells were washed twice (100 *g* for 10 min at 4°C) and finally suspended in colourless medium 199 (Flow Labs) at a concentration of 2×10^6 neutrophils/ml. The cell population was >95% pure and variability was >99% as determined by trypan blue exclusion.

Opsonization of bacteria

One-hundred microlitres of the bacterial suspension were incubated for 30 min at 37°C in 1 ml of phosphate-buffered saline (PBS) containing 10% pooled normal serum or panhypogammaglobulinaemic serum, either with or without added immunoglobulin solution at a final concentration of 10 mg IgG/ml of serum. PBS, heat-inactivated serum (56°C for 30 min) and immunoglobulin solution were used alone as controls. The suspension of opsonized bacteria was centrifuged at 3000 rpm for 10 min at 4°C and the supernatant discarded. Immediately prior to the assay, the pellet was resuspended in 900 μl of warm (37°C), colourless Hanks' solution (GIBCO) buffered to pH 7·4 by 10 mM HEPES containing 10^{-5}M luminol (Sigma). All opsonizations were performed in triplicate.

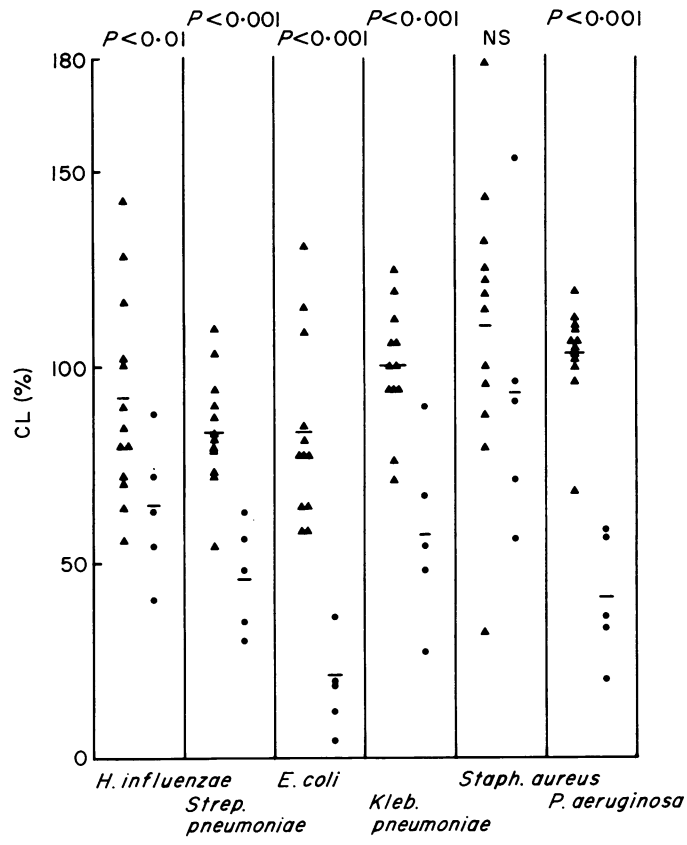

Fig. 1. Chemiluminescence (CL) stimulated (as percentage of pooled serum standard) by bacteria opsonized by 12 normal (▲) and 5 panhypogammaglobulinaemic (●) sera. Mean value for each group is indicated by a horizontal bar.

Chemiluminescence assay

Five-hundred microlitres of the neutrophil suspension were warmed to 37°C for 1h and added to the suspension of opsonized bacteria in the medium containing luminol. Chemiluminescence emitted was measured by an LKB 1250 luminometer. The final ratio of bacteria to neutrophils was 100:1.

Statistical analysis

The peak chemiluminescence measured in each test was expressed as a percentage of the peak chemiluminescence produced when the bacteria were opsonized by pooled normal serum. Each result was the mean of three experiments. Statistical analyses were by Student's unpaired and paired *t*-tests.

RESULTS

The immunoglobulin levels of all panhypogammaglobulinaemic sera were well below the normal range (IgG <3·5 g/l, IgM <0·4 g/l and IgA <0·1 g/l). The immunoglobulin levels in the pooled serum were normal (IgG 10·2 g/l, IgM 1·37 g/l, and IgA 1·90 g/l). The level of C3 was normal in all sera. When normal but heat-inactivated serum or either of the two immunoglobulin preparations were used as the sole source of opsonins, the chemiluminescence produced was similar to that from the PBS control. Figure 1 shows the chemiluminescence produced when target bacteria were opsonized by normal and by panhypogammaglobulinaemic sera. With normal sera the range of opsonic activity was wide for all organisms except *P. aeruginosa*,

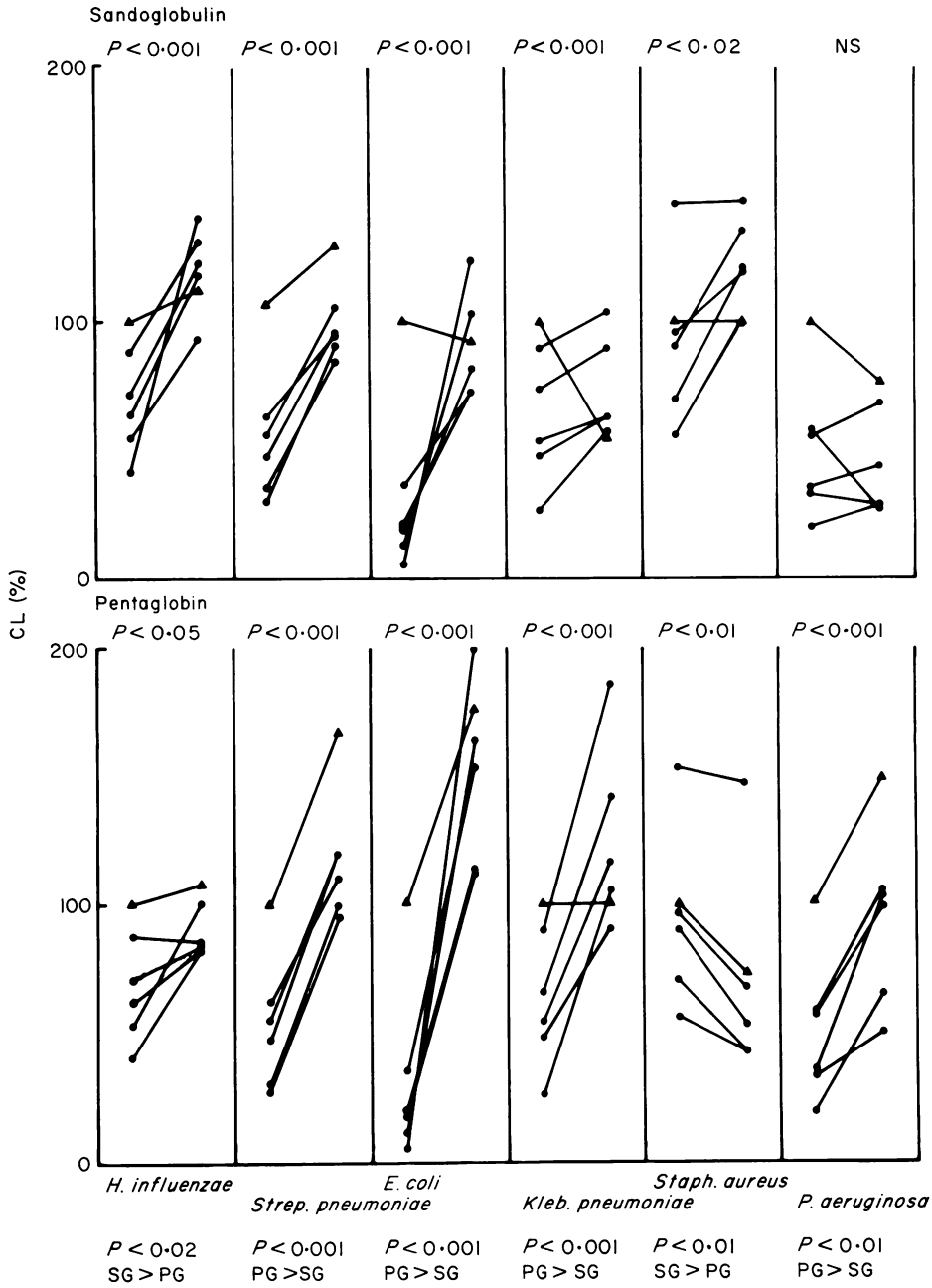

Fig. 2. Chemiluminescence (CL) stimulated (as percentage of pooled serum standard) by bacteria opsonized by panhypogammaglobulinaemic (●—●) and pooled normal (▲—▲) sera before and after adding immunoglobulin preparations. SG is Sandoglobulin and PG is Pentaglobin.

ranging from 50–150% of that obtained using pooled sera. For *P. aeruginosa* the range was 68–119% and, as with *E. coli*, there was no overlap with that obtained using panhypogammaglobulinaemic sera. The opsonic activity of panhypogammaglobulinaemic sera was reduced for all organisms except *S. aureus* which was not significantly different from normal. The greatest reductions were seen with *E. coli* and *P. aeruginosa* while opsonic activity for *H. influenzae* was only moderately impaired.

The results of adding immunoglobulin to panhypogammaglobulinaemic or pooled normal serum are shown in Fig. 2. When Pentaglobin was added there was in every case an improvement in the opsonic properties of the panhypogammaglobulinaemic sera for all bacteria except *S. aureus*. Significant improvement was also observed with Sandoglobulin for all bacteria except *P. aeruginosa* but was generally of lesser degree than that seen with Pentaglobin. The most obvious differences between the two preparations were seen with *P. aeruginosa*, when Sandoglobulin was unable to improve the opsonic activity of the test sera while Pentaglobin made a significant difference ($P < 0{\cdot}001$), and with *H. influenzae*, when Sandoglobulin demonstrated significantly better activity than Pentaglobin

($P<0{\cdot}02$). A significant difference ($P<0{\cdot}001$) in favour of Pentaglobin was also observed for *E. coli*, *K. pneumoniae* and *S. pneumoniae* but this was of a lesser degree. While Sandoglobulin managed to improve the opsonization of *S. aureus*, the addition of Pentaglobin actually reduced the chemiluminescence obtained.

Addition of the immunoglobin preparations to pooled normal serum resulted in similar effects on the opsonization of most bacteria but was generally of a lesser degree and in some cases the direction of change was actually opposite to that seen with the test sera, implying an impairment of opsonic activity.

DISCUSSION

The present study compares the opsonic activity of a standard intravenous immunoglobulin preparation, Sandoglobulin, with a new preparation, Pentaglobin, for a number of common bacterial pathogens. The major differences between these two preparations are their immunoglobulin class composition and the method used to modify them for intravenous use. Sandoglobulin contains predominantly IgG and is pH 4- and pepsin-treated, while Pentaglobin contains significant proportions of IgM and IgA and is betapropiolactone-treated. It has previously been shown that different methods of preparation can alter the functional characteristics of immunoglobulins for intravenous use (Romer, 1982). Measurement of opsonic characteristics quantifies one of the most important functions of immunoglobulins *in vivo* and may therefore be regarded as a way of directly assessing the functional activity of an immunoglobulin preparation (van Furth, Leijh & Klein, 1984). The method used in this study to compare the ability of the immunoglobulin preparations to provide opsonins was the measurement of luminol-enhanced neutrophil chemiluminescence when the membrane of such cells was stimulated by suitably opsonized bacteria (Williams *et al.*, 1980); this has previously been shown to be useful in the assessment of serum opsonic activity (Stevens & Young, 1977; Munro, Stanley & Cole, 1985).

Under the conditions of the present study, it was interesting that Sandoglobulin enhanced, whereas Pentaglobin suppressed, the opsonization of *S. aureus*. This could be due to an imbalance of different isotypes in the case of Pentaglobin and might be of relevance *in vivo* in some patients. In general, the opsonic activity of Pentaglobin was similar to that of Sandoglobulin but in the case of *P. aeruginosa*, *K. pneumoniae* and *E. coli*, Pentaglobin appeared to have much greater activity; in keeping with an additional content of (IgM) opsonizing antibodies. The reciprocal antibody titres, measured by passive haemagglutination in our test batch of Pentaglobin, were high for these three organisms at 1:1280, 1:640, and 1:320 respectively. These figures are considerably higher than those published for Sandoglobulin, which are 1:20, 1:2 and 1:16 respectively. Conversely, the opsonic activity of Sandoglobulin was superior in the case of *H. influenzae*, probably reflecting increased amounts of IgG opsonizing antibody for this organism. Sandoglobulin suppressed the phagocyte response to all Gram-negative bacteria (except *H. influenzae*) opsonized in pooled normal human serum, while Pentaglobin enhanced the response in these cases (except *K. pneumoniae*). The sugar residues present in Sandoglobulin might block the bacterial-phagocyte interaction and this possibility could be explored by dialysing the preparation to remove sugar residues. Alternatively, anti-idiotypic antibodies contained in the intravenous immunoglobulin preparations may have a similar effect.

Fresh normal serum is effective in promoting the phagocytosis and intracellular killing of *Pseudomonas* by neutrophils, virtually all of the opsonic activity being due to 'natural' antibodies of the IgM class (Young & Armstrong, 1972). Immunity to *P. aeruginosa* has been found to correlate with the presence of antibody directed against lipopolysaccharide (LPS) and exotoxin A (Pollack & Young, 1979). There is substantial evidence that the main functional role of antibodies against *P. aeruginosa* is opsonization for ingestion by neutrophils. Recovery from septicaemia is associated with an increase in both IgM and IgG specific antibodies and several studies have demonstrated a direct correlation between titre of opsonizing antibody and prognosis (Young, 1974; Weinstein & Young, 1976; Pollack & Young, 1979; Pollack *et al.*, 1983). There is also much evidence that IgM antibodies are the primary opsonins in this process (McCall *et al.*, 1973; Young, 1973; Pollack *et al.*, 1983).

Patients with severe panhypogammaglobulinaemia characteristically are little troubled by Gram-negative septicaemia and their frequent infections are usually prevented by intravenous immunoglobulin preparations such as Sandoglobulin. Hence, a role for IgM antibody is probably only critical when severe Gram-negative septicaemia occurs in the context of severe neutropenia.

Despite multiple antibiotic regimens and extensive support procedures in our modern intensive care units, there is still a significant mortality from sepsis due to bacteria such as *P. aeruginosa*, *E. coli* and *S. aureus* (Bodey *et al.*, 1983). There have been experimental and clinical attempts to augment circulating antibody to prevent or ameliorate Gram-negative sepsis. Interest in both active (Jones, Roe & Gupta, 1979) and passive immunization procedures has been re-awakened in recent years and various studies have demonstrated significant reductions in mortality and morbidity using hyperimmune serum directed against the core glycolipid region of LPS (Ziegler et al, 1982; Baumgartner *et al.*, 1985). In the past there have been significant problems limiting the use of normal human immunoglobulin in the management of Gram-negative sepsis. Results using standard preparations in the prophylaxis and treatment of infections have not been consistent and many reasons, including inadequate doses, low levels of specific antibodies, lack of IgM containing preparations (Pollack *et al.*, 1983), and reduced functional activity of the immunoglobulins due to alteration during manufacture, have been given for this (Pollack, 1983; Collins & Roby, 1984; Stiehm, 1987; Yap, 1987). With the development of new intravenous immunoglobulin preparations, their true role in the prevention and treatment of infections will begin to be answered. A recent study by Pennington & Small (1987), using neutropenic guinea pigs, demonstrated a significant reduction in mortality from *Pseudomonas aeruginosa* infection by combining passive immune therapy (intravenous hyperimmune IgG antibody to *P. aeruginosa*) with intravenous antibiotics.

The present study indicates that Pentaglobin, a new intravenous immunoglobulin preparation containing a significant quantity of IgM, possesses excellent opsonic activity *in vitro* against the common bacterial pathogens causing Gram-negative septicaemia, especially *P. aeruginosa* and *E. coli*. It can be expected that preparations with good functional characteristics

in vitro will also have good functional activity *in vivo* (van Furth, Leijh & Klein, 1984). Therefore, we suggest that there is a sound case to pursue clinical trials of Pentaglobin in severely ill patients with Gram-negative septicaemia, although these will need to be carefully controlled as it is difficult to prove the effect of new treatment in this difficult clinical area.

ACKNOWLEDGMENTS

We are grateful to Biotest UK Ltd for providing us with Pentaglobin and for supporting this work. We thank also Howard Todd for bacteriological advice and assistance.

REFERENCES

ALVING, B.M. & FINLAYSON, J.S. (eds). (1979) *Immunoglobulins: characteristics and uses of intravenous preparations* US Dept. of Health & Human Services Publication no. 80-9005. Washington DC.

BAUMGARTNER, J.D., MCCUTCHAN, J.A., MELL G.V., *et al* (1985) Prevention of Gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. *Lancet*, **ii**, 59.

BODEY, G.P., BOLIVAR, R., FAINSTEIN, V. & JADEJA, L. (1983) Infections caused by *Pseudomonas aeruginosa*. *Rev. Infect. Dis.* **5**, 279.

COLLINS, M.S. & ROBY, R.E. (1984) Protective activity of an intravenous immune globulin (human) enriched in antibody against lipopolysaccaride antigens of *Pseudomonas aeruginosa*. *Am. J. Med.* **76**, 168.

CROSS, A.J.R., ALLEN, J., BURKE, J., *et al.* (1983) Nosocomial infections due to *Pseudomonas aeruginosa*: review of recent trends. *Rev. Infect. Dis.* (Suppl) **5**, S837.

JONES, R.J., ROE, E.A. & GUPTA, I.J. (1979) Controlled trials of a polyvalent pseudomonas vaccine in burns. *Lancet*, **ii**, 977.

MCCALL, C., BARTLETT, L., QUALLIOTINE-MANN, D., DE-CHATELET, L. & COOPER, R. (1973) Isolated IgM deficiency and defective opsonic activity against *Pseudomonas*. *Clin. Res.* **21**, 607.

MCGOWAN, J.E., JR. (1985) Changing aetiology of nosocomial bacteraemia and fungaemia and other hospital acquired infections. *Rev. Infect. Dis.* (Suppl) **7**, S357.

MUNRO, C.S., STANLEY, P.J. & COLE, P.J. (1985) Assessment of biological activity of immunoglobulin preparations by using opsonized microorganisms to stimulate neutrophil chemiluminescence. *Clin. exp. Immunol.* **61**, 183.

PENNINGTON, J.E. & SMALL, G.J. (1987) Passive immune therapy for experimental *Pseudomonas aeruginosa* pneumonia in the neutropenic host. *J. Infect. Dis.* **155**, 973.

POLLACK, M. & YOUNG, L. (1979) Protective activity of antibodies to exotoxin A and lipopolysaccharides at the onset of *Pseudomonas aeruginosa* septicaemia in man. *J. clin. Invest.* **63**, 276.

POLLACK M., HUANG A.I., PRESCOTT R.K., *et al.* (1983) Enhanced survival in *Pseudomonas aeruginosa* septicaemia associated with high levels of circulating antibody to *Escherichia coli* endotoxin core. *J. Clin. Invest.* **72**, 1874.

POLLACK, M. (1983) Antibody activity against *Pseudomonas aeruginosa* in immune globulins prepared for intravenous use in humans. *J. Infect. Dis.* **147**, 1090.

ROITT, I.M. (1984) *Essential Immunology* Blackwell Scientific Publications, Oxford.

ROMER, J., SPATH, P.J., SKVARIL, F. & NYDEGGER, U.E. (1982) Characterisation of various immunoglobulin preparations for intravenous application II. Complement activation and binding to Staphylococcus Protein A. *Vox Sang.* **42**, 74.

SILVERSTEIN, S.C., STEINMAN, R.M. & COHN, Z.A. (1977) Endocytosis. *Ann. Rev. Biochem.* **46**, 669.

STEVENS, P. & YOUNG, L.S. (1977) Quantitative granulocyte chemiluminescence in the rapid detection of impaired opsonisation of *Escherichia coli*. *Infect. Immun.* **16**, 796.

STIEHM, E.R. (moderator). (1987) Intravenous immunoglobulins as therapeutic agents. *Ann. Intern. Med.* **107**, 367.

VAN FURTH, R., LEIJH, P.C.J. & KLEIN, F. (1984) Correlation between opsonic activity for various microorganisms and composition of gammaglobulin preparations for intravenous use. *J. Infect. Dis.* **149**, 511.

WEINSTEIN, R.J. & YOUNG, L.S. (1976) Neutrophil function in Gram-negative rod bacteraemia: the interaction between phagocytic cells, infecting organisms, and humoral factors. *J. clin. Invest.* **58**, 190.

WILLIAMS, A.J., HASTINGS, M.J.G., EASMON, C.S.F. & COLE, P.J. (1980) Factors affecting the *in vitro* assessment of opsonisation: a study of the kinetics of opsonisation using the technique of phagocytic chemiluminescence. *Immunology*, **41**, 903.

YAP, P.L. (1987) The use of intravenous immunoglobulin for the treatment of infections: an overview. *J. Infect.* **15** (Suppl 1), 21.

YOUNG, L.S. & ARMSTRONG, D. (1972) Human immunity to *Pseudomonas aeruginosa*. I. In vitro interaction of bacteria, polymorphonuclear leucocytes, and serum factors. *J. Infect. Dis.* **126**, 257.

YOUNG, L.S. (1973) Role of antibody in infections due to *Pseudomonas aeruginosa*. *J. Infect. Dis.* (Suppl) **130**, S111.

YOUNG, L.S. (1974) Opsonising antibodies and prognosis in *Pseudomonas aeruginosa* septicaemia. *Clin. Res.* **22**, 187A.

ZIEGLER, E.J., MCCUTCHAN, J.A., FIERER, J., *et al.* (1982) Treatment of Gram-negative bacteraemia and shock with human antiserum to a mutant *Escherichia coli*. *N. Engl. J. Med.* **307**, 1225.