Efficacy of locally delivered polyclonal immunoglobulin against *Pseudomonas aeruginosa* peritonitis in a murine model

N.A. Barekzi,¹ K.A. Poelstra,¹ A. Felts,¹ I.A. Rojas,¹
J.B. Slunt,² D.W. Grainger²

¹. Anthony G. Gristina Institute for Biomedical Research, Herndon VA USA
². Gamma-A Technologies, Inc., Herndon VA USA

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Abstract

Infectious peritonitis results from bacterial contamination of the abdominal cavity. Conventional antibiotic treatment is complicated both by the emergence of antibiotic-resistant bacteria and by increased patient populations intrinsically at risk for nosocomial infections. To complement antibiotic therapies, the efficacy of direct, locally applied pooled human immunoglobulin G (IgG) was assessed in a murine model (strains CF-1, CD-1 and CFW) of peritonitis caused by intraperitoneal inoculations of $10^6$ or $10^7$ CFU Pseudomonas aeruginosa (strains IFO-3455, M-2, and MSRI-7072). Various doses of IgG (0.005-10mg/mouse) administered intraperitoneally simultaneous with local bacterial challenge significantly increased survival in a dose-dependent manner. Local intraperitoneal application of 10 mg IgG increased animal survival independent of either the P. aeruginosa or murine strains used. A local dose of 10 mg IgG administered up to 6 hours prophylactically or at the time of bacterial challenge resulted in 100% survival. Therapeutic 10 mg IgG treatment given up to 12 hours post-infection also significantly increased survival. Human IgG administered to the mouse peritoneal cavity was rapidly detected systemically in serum. Additionally, administered IgG in peritoneal lavage fluid samples actively opsonized and decreased bacterial burden via phagocytosis at 2 and 4 hours post-bacterial challenge. Tissue microbial quantification studies showed that 1.0 mg of locally applied IgG significantly reduced the bacterial burden in the liver, peritoneal cavity and blood and correlated with reduced levels of interleukin-6 in serum.

Peritonitis is often caused by ulcers, appendicitis, diverticulitis, ileus (bowel obstruction), gunshot or stab wounds, and disturbances during abdominal surgical procedures, allowing the escape of indigenous bowel bacteria into the peritoneal cavity. Nosocomial peritonitis is caused by exogenous pathogenic bacteria, including Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermidis that gain access to the abdominal cavity during prolonged surgical procedures, or via a port of entry such as that created for continuous ambulatory peritoneal dialysis (CAPD). These pathogens cause nosocomial peritonitis at even higher rates in immunocompromised and geriatric populations when compared to
Polyclonal IgG against P. aeruginosa in murine peritonitis

typical patients,\textsuperscript{44} resulting in a significant, growing medical problem impacting both patient mortality and rising healthcare costs.\textsuperscript{38}

The current treatment regimen for peritonitis relies on the use of intravenous antibiotics: penicillin, third- and fourth-generation cephalosporins, or quinolones.\textsuperscript{3,24,28,33,45} Selection of antibiotics is complicated by uncertainties surrounding the identification of infecting pathogens in a mixed contaminating flora and a documented lack of correlation between \textit{in vitro} antibiotic studies of pathogen susceptibility and antibiotic efficacy in clinical settings.\textsuperscript{13,14,24} However, initial antibiotic therapy for severe intra-abdominal infection fails in 20-40% of all cases, leading to additional antibiotic use.\textsuperscript{34}

Antibiotic resistance occurs at a significant rate\textsuperscript{33} among intra-abdominal infections, and this condition is frequently associated with clinical failure.\textsuperscript{9} Increasing emergence of antibiotic resistant bacteria coupled with increasing immunocompromised and elderly patient populations are significant incentives prompting development of new anti-infective therapies. Among many therapeutic approaches, the use of systemic intravenous immunoglobulins (IVIG) has shown promising but inconsistent results in preventing \textit{P. aeruginosa} and other bacterial infections.\textsuperscript{4,5,7,20,25,26,29,42,43} Early studies reported therapeutic benefit against CAPD-associated peritonitis by using pooled human IgG added directly to dialysate fluid.\textsuperscript{17,25,26} No other local applications of immunoglobulins to treat peritonitis are known, although a recent publication supports local use of injected IVIG subcutaneously in treating \textit{P. aeruginosa} burn infection.\textsuperscript{10}

This study explores the feasibility of using locally delivered pooled human IgG directly to the peritoneal cavity as a potential therapeutic complement or alternative to the antibiotic treatment of peritonitis. IgG delivered to a contaminated tissue site immediately opsonizes invading bacteria, promoting subsequent pathogen agglutination and, stimulated by cytokines and chemotactic factors, killing by invading macrophages and neutrophils.\textsuperscript{11,22,23} Major advantages of locally delivered polyclonal IgG include its application in controlled dosage formulations directly to infected sites and its ability to clear infection independently of antibiotic resistance mechanisms.
The aim of this study was to determine the prophylactic efficacy of locally applied, pooled human IgG against intra-abdominal challenges of different *P. aeruginosa* strains. Both *in vitro* and murine *in vivo* data support the use of pooled polyclonal IgG to neutralize *P. aeruginosa* in the host peritoneal cavity, preventing the systemic spread of bacteria as well as sepsis and mortality.

**Materials and Methods**

**Animals.** Female CF-1, CD-1 and CFW mice (22-24 g) were purchased from Charles River Laboratories (Raleigh, NC). All animals were acclimated for seven days, given food and water *ad libitum* and kept on a twelve hour light-dark cycle. The Gristina Institute’s animal care and use committee (IACUC) approved all of the animal procedures in this study.

**Bacteria.** *Pseudomonas aeruginosa* strains (IFO-3455, obtained from Dr. A. S. Kreger (27); M-2, obtained from Dr. I. A. Holder (30); and MSRI-7072, a local hospital clinical isolate) were grown for 18 hours in 20 ml of trypticase soy broth at 37°C while agitated at 150rpm in a benchtop incubator shaker. Cultured bacteria were twice sedimented by centrifugation at 7649 *x* g for 10 minutes, washed, and diluted in saline to obtain a concentrated bacteriae suspension. Serial bacterial dilutions were plated on Trypticase soy agar (TSA) and colonies were counted after 24 hours incubation at 37°C to determine initial colony forming units (CFU) per ml. In parallel, the optical absorbance of these dilutions was measured with a Beckman DB-GT grating spectrophotometer (λ=650nm, visible filter). Standard curves plotting optical absorbance versus CFU concentrations were then constructed. Typically, bacterial suspension absorbance ranges of 0.46-0.9 resulted in ~10^9 CFU/ml. Heat-killed *P. aeruginosa* M-2 was produced by incubating these bacterial cultures at 56°C for 3 hours and plating 100 µl of the 10^7 CFU/ml stock solution on TSA to confirm non-viability.
**Murine peritoneal infection model.** The peritonitis model involved injecting mice with either live or heat-killed *P. aeruginosa* in 500 µl (IFO-3455, LD₉₀ = 10⁷ CFU; M-2, LD₉₀ = 10⁷ CFU; MSRI-7072, LD₅₀ = 10⁷ CFU) intra-abdominally by using a syringe with a 30G needle. The infectious challenge was followed immediately by a separate 500 µl co-localized abdominal injection of IgG (therapy) or either human serum albumin (HSA, Lot# 66H9306, Sigma, St. Louis, MO), 0.2 M glycine or 5% dextrose as placebo treatments. Mortality studies involved the intra-abdominal injection of *P. aeruginosa* where animal survival was assessed over a 10-day period post-challenge and survival outcomes in the treatment and control groups were compared.

**Immunoglobulin therapy.** Commercially pooled human IgG (Lot# 2620M039A, Gammagard®, Baxter International Inc., Deerfield, IL) was diluted in 5% dextrose (recommended by the manufacturer) to obtain the varying IgG concentrations used in these trials. An anti-human IgG ELISA¹ was used to determine polyclonal human IgG titers against three different *P. aeruginosa* strains. Titer numbers express the inverse log dilution of IgG concentration at 50% ELISA optical absorbance (450nm) from the inflection mid-point on each IgG-bacteria binding curve. Higher titer numbers reflect increased IgG binding to each bacteria strain. A second ELISA using a capture mouse anti-human IgG and detection peroxidase-conjugated anti-human IgG (Jackson Immuno Research Laboratories, Inc.; products 209-005-088 and 209-035-088) was used to detect human IgG (optical absorbance at 450nm) in mice serum and peritoneal lavage as described below.

**Quantitative Microbiology.** At various times post-infection, mice were anesthetized with Metofane® (Mallinckrodt Veterinary, Inc., Mundelein, IL) and blood withdrawn via cardiac puncture. Following euthanization (cervical dislocation), a saline lavage of the peritoneal cavity was performed using 3 or 5 ml of sterile saline and lavage fluid (~2-4 ml) was collected. Livers were excised, weighed in 10 ml saline and homogenized (Omni-International GLH Homogenizer, Marietta, GA). Blood, peritoneal lavage fluid, and homogenized livers were serially diluted and plated on TSA and bacterial colonies were enumerated after 24 hours incubation at 37°C.
Serum IL-6 and human IgG assay. Serum was separated from the blood (obtained via cardiac puncture) using a benchtop HN-SII centrifuge (10 min. at 3000rpm, IEC, Needham Heights, MA) and assayed with a commercial ELISA (Pharmingen, Inc., San Diego, CA, \(\lambda=450\) nm) to determine the levels of interleukin-6 (IL-6) and human IgG. The detection range for the IL-6 assay was between 15 - 2000 pg/ml, and for the human IgG between 5 - 5000 ng/ml. Standard curves were constructed from known amounts of murine IL-6 contained in the ELISA kit and from commercially pooled human IgG (Lot\# 2620M039A, Gammagard®️, Baxter International Inc., Deerfield, IL), respectively. Murine serum IL-6 and human IgG levels were determined by comparing the experimental absorbance values from serum or peritoneal lavage to standard curves.

In vitro opsonophagocytic assay. Murine peritoneal lavage fluid, collected 2 hours after bacterial challenge and human IgG treatment, was assayed to determine the opsonizing activity of the applied IgG. Fixed volumes of peritoneal fluid (2 ml in test tubes) were incubated in vitro at 37°C and agitated at 150 RPM. The bacterial burden in peritoneal lavage fluid was assayed immediately upon collection and after 2 hours of incubation by plating 100 \(\mu\)l of serially diluted peritoneal fluid on TSA. Colonies were enumerated after 24 hours incubation at 37°C.

Statistical analysis. Data in this study are expressed as the mean \(\pm\) the standard error of the mean (SEM). Student T tests were used to compare the control and therapy groups of the bacterial burden enumeration studies while Z tests and analysis of variance (=ANOVA) tests were used to compare mortality. All probabilities of less than 5% were considered significant. Datum outliers, defined as any datum outside of the range of the mean \(\pm\) 2 times the standard deviation, were excluded.
Results

**Polyclonal human IgG titer determination against Pseudomonas aeruginosa strains.** Titers of commercial pooled human IgG were determined against three strains of *P. aeruginosa* by using a published ELISA method.\(^{18}\) Titers of 355, 501, and 398 were calculated for this IgG lot against *P. aeruginosa* IFO-3455, M-2, and MSRI-7072, respectively. These titers represent a significant IgG binding activity against the pathogens.

**Local intraperitoneal delivery of IgG.** Various doses of locally delivered IgG were tested against a lethal dose of *P. aeruginosa* (IFO-3455, \(10^7\) CFU) in four separate experiments with CF-1 mice to determine the dose benefit range. Survival of IgG-treated groups increased from the control dose of 0.005 mg and higher in a dose-dependent manner. As shown in Figure 1, the highest percentage of survival resulted from the highest concentration of IgG (96% with 10.0 mg) delivered directly to the peritoneal cavity. A step wise threshold of IgG efficacy is observed over a narrow therapeutic dose range beginning at approximately 0.5 mg IgG per mouse. All IgG doses applied intraperitoneally that were given higher than this produced significant improvements in mouse survival (ANOVA with Tukey’s test, p<0.008 comparing survival with doses of 0.5 and 10mg). An optimal efficacious dose of 10 mg IgG per 22-24 g mouse (strains CF-1, CD-1 and CFW) was chosen for the survival studies to provide the most consistent results in lower numbers of mice with less variance and greater reliability.

![Dose-response curve for locally applied intra-abdominal IgG against *P. aeruginosa* IFO-3455: CF-1 mouse survival (n=10-25/group) at day 10 post-challenge with \(10^7\) CFU i.p. injected simultaneous with a separate injected dose of IgG (0.005, 0.05, 0.2, 0.5, 1.0, 5.0 or 10 mg per animal). IgG therapy increased survival in a dose dependent-manner. Data represent the mean survival of IgG-treated mice from four different experiments. Differences between 0.5mg/animal and 10mg/animal IgG doses is statistically significant (ANOVA with Tukey’s test, p<0.008).](image-url)
Mortality studies were conducted with CF-1, CD-1 and CFW mice to determine the efficacy of locally delivered IgG on bacterial challenges in different mouse strains. The results in Figure 2 show the 10-day survival of mice challenged with IFO-3455 and given either a single local 10mg IgG dose or a placebo (5% dextrose) treatment. Statistical differences were assessed by using an ANOVA with Tukey’s test.

The 96% survival of IgG-treated CF-1 mice is significantly higher than the 23% survival of the placebo-treated group (p<0.001). The 80% survival of IgG-treated CD-1 mice is significantly higher than the 10% survival of the placebo-treated group (p<0.001). The IgG-treated CFW mice showed reduced but still significantly improved percentage of survival over the ten-day period compared to the placebo-treated group (p<0.001).

CF-1 murine mortality studies were conducted by using single 10-mg local IgG treatments against lethal doses of three different P. aeruginosa strains (IFO-3455, M-2 and MSRI-7072) to determine whether protection imparted by locally delivered IgG was dependent on bacterial strain. The results presented in Figure 3 show bacterial strain-dependent survival with or without local IgG protection. Mice challenged with a lethal dose inoculum of the IFO-3455 strain and treated with a single local 10 mg IgG dose exhibited a 90% survival, which was significantly higher than the observed 20% survival.
of the placebo-treated group (z test, p<0.01). Figure 3 also shows that 100% of the mice injected with a lethal dose of the M-2 strain survived with a single local 10-mg IgG treatment, whereas the placebo-treated group survival rate was only 6% (p<0.001). Furthermore, mice inoculated with the clinical MSRI-7072 strain and treated with a single local 10 mg IgG dose exhibited 100% survival, a value significantly higher than the 50% survival seen in the placebo-treated group (p<0.05). Figure 3 also shows that the control experiment with $10^7$ CFU heat-killed \textit{P. aeruginosa} (strain M-2) inoculum with or without IgG treatment produced 100% survival (n = 6 mice), whereas, without IgG treatment, live M-2 at the same inoculum dose produced little survival. In addition, control experiments with single local 10-mg HSA doses produced no significant differences between 5% dextrose-treated and HSA-treated control groups in either the mortality studies or the tissue bacterial quantification (data not shown).

\begin{figure}
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\caption{Pathogen strain influence on mouse survival (day 10) in a peritonitis model using local IgG administration. Three strains of \textit{Pseudomonas aeruginosa} were each separately injected intraperitoneally (dose=$10^7$ CFU) simultaneously with separate, single injections of 10 mg pooled human IgG, or placebo (5% dextrose, n=10/group). IgG treatment significantly increased survival compared to placebo treatment (z test, p<0.05)}
\end{figure}

**Efficacy of local IgG application pre- and post-challenge.** To investigate the prophylactic and therapeutic properties of locally applied IgG, 10 mg IgG doses were delivered intraperitoneally in CF-1 mice one, three or six hours before, at the time of, and one, three, six, twelve or eighteen hours following bacterial challenge (IFO-3455, $10^7$ CFU). Figure 4 shows results for these studies. IgG administered one, three or six hours prior to bacterial challenge and simultaneously with bacterial challenge produced 100% survival (p<0.05 compared to placebo-treated group). Mice treated with locally injected IgG one, three, six and twelve hours post bacterial challenge exhibited significantly higher survival compared to the placebo-treated group (p<0.05) while mice treated at eighteen hours post-challenge showed no significant differences in survival.
Systemic and local IgG in vivo distribution over time. Serum and peritoneal lavage fluid were collected from groups of CF-1 mice treated with 10-mg IgG and euthanized at 0, 2, 3, 6, 9, 12, 24, 36 and 48 hours and every 24 hours thereafter up to day 7 after intraperitoneal challenge with IFO-3455 to compare systemic and local distributions of human IgG. Placebo (5% dextrose)-treated mice were only analyzed at 0, 2 and 3 hours and no human IgG was detectable (data not shown). As shown in Figure 5, the amounts of intraperitoneally resident human IgG decline sharply by between 2 and 3 hours post-administration (half-life ~2.5 hours) and decrease constantly over time. Simultaneously, human IgG levels in serum increase as peritoneal IgG decreases, spiking to almost 3 mg/ml at 9 hours and decreasing thereafter. Human IgG is detectable rapidly in serum after intraperitoneal administration and remains detectable by ELISA methods in both serum and peritoneal lavage for up to 7 days post challenge.

Quantification of bacteria in systemic tissues and IL-6 levels in serum. Tissue samples were collected from groups of CF-1 mice treated with 1.0 mg IgG or placebo (0.2 M glycine) 6, 24 or 72 hours after intraperitoneal challenge with IFO-3455 in order to compare the bacterial burdens in the liver, peritoneal cavity and blood. A lower, non-lethal $10^6$ CFU challenge was used to ensure animal survival up to the 72 hour time point. Liver, blood and peritoneal lavage samples from placebo-treated control mice and
local IgG-treated mice were homogenized, serially diluted and plated, and the values for log CFU per tissue were compared. The results (Figure 6) show that IgG-treated mice have significantly reduced numbers of bacteria in the liver, blood and peritoneal lavage six hours post-challenge compared to the bacterial burden in control mice (p<0.05).

Figure 5:
ELISA detection of human IgG levels in murine serum (closed diamonds) and peritoneal lavage fluid (open triangles) post bacterial-challenge after intraperitoneal (i.p.) injection of IgG and i.p. lethal injections of Pseudomonas aeruginosa (strain IFO-3455, 10⁷ CFU, n=3-4/time point in each group) over 7 days. Human IgG is detectable rapidly in mouse serum after i.p. administration and remains detectable by ELISA methods in both serum and mouse peritoneal lavage for up to 7 days. Inset: Human IgG levels in mouse serum and peritoneal lavage in the first 12 hours post-administration.

Figure 6:
Bacterial burden at various tissue sites assessed at 6 hours following intraperitoneal (i.p.) injection of 1.0 mg IgG against 10⁶ CFU Pseudomonas aeruginosa (strain IFO-3455) i.p. Mice (n=10) peritoneal lavage (PL), liver homogenate, and blood analysis yielded CFU values that show IgG treatment significantly decreased bacterial burden compared to placebo (0.2 M glycine) treatment after 6 hours in all samples (t test, p<0.05). The PL and blood bar graphs represent the log₁₀ CFU/ml and the liver bar graph shows the log₁₀ CFU/g liver.
Bacteria were not present in the liver, peritoneal lavage or blood of IgG-treated mice by 24 hours post challenge. Additionally, ELISA was used to determine the murine serum levels of the inflammatory cytokine, IL-6. Figure 7 shows that IgG-treated mice had significantly lower levels of IL-6 at six hours post-challenge compared to the control groups (p<0.05). The low IL-6 levels at 24 and 72 hours post-bacterial challenge were comparable to normal circulating murine IL-6 levels and correlated with the low bacterial burden found in the peritoneal cavity, liver and blood (Figure 6).

**Figure 7:** Serum IL-6 levels following intraperitoneal (i.p.) injection of 1.0 mg IgG against a non-lethal i.p. dose (10⁶ CFU) of *P. aeruginosa* (strain IFO-3455). Serum IL-6 levels of CF-1 mice (n=5-25) were determined using an ELISA. IgG treatment decreased IL-6 levels significantly compared to control (p = 0.04) at six hours post bacterial challenge. Saline + glycine placebo treatment without a bacterial challenge was used to determine normal background IL-6 levels in CF-1 mice. Saline + 10 mg IgG treatment without a bacterial challenge shows that IL-6 levels resulting from IgG treatment alone are not significantly different from normal background IL-6 levels (t test, p<0.05).

*In vitro opsonophagocytic assay.* Murine peritoneal lavage fluid was assayed *in vitro* 2 hours post-challenge with 10⁷ CFU *P. aeruginosa* IFO-3455 to determine the opsonizing influence of applied human IgG. Peritoneal lavage fluid of mice treated with 10 mg IgG had significantly reduced levels of bacteria compared to placebo- (5% dextrose) treated mice both immediately after lavage and 2 hours later (Figure 8). The presence of human IgG facilitated the clearance of bacteria from lavage fluid whereas control-treated lavage exhibited bacterial growth during this incubation period.
Figure 8:
*In vitro opsonophagocytic assay using mouse peritoneal lavage shows enhanced bacterial clearance with IgG. Murine peritoneal lavage fluid was assayed in vitro 2 hours after intraperitoneal (i.p.) dosing with human IgG and i.p. challenge with $10^7$ CFU P. aeruginosa (strain IFO-3455). Peritoneal lavage fluid of mice treated with 10 mg IgG significantly reduced levels of bacteria compared to placebo- (5% dextrose) treated mice both immediately after peritoneal lavage and 2 hours later (t test, p<0.05). The presence of human IgG facilitated clearance of bacteria from lavage fluid while control-treated lavage exhibited bacterial growth during the incubation.

Discussion

Local delivery of IgG directly to tissue and wound surfaces represents a potential alternative strategy against infections that is both independent of antibiotic resistance and complementary to current antibiotic treatment regimens. In this study, locally delivered IgG has been assessed in a murine peritonitis model to determine its efficacy alone against P. aeruginosa. This common nosocomial pathogen is responsible for 5-10% of CAPD-related and 24% of acute community-acquired perforating appendicitis infections, and it is a pathogen of particular clinical concern due to its increasingly frequent antibiotic-resistant forms that are emerging during treatment with broad spectrum antibiotics, its late complications, and its high morbidity. In 1986, Lamperi and co-workers reported that the local application of pooled human IgG (SRK-Ig [Swiss Red Cross]; pooled IgG from volunteers) as an intra-abdominal dialysate lavage treatment was beneficial against certain forms of peritonitis.  

**Note:** The diagram shows the log CFU remaining in peritoneal lavage at 2 hours and 4 hours after bacterial challenge.
The current study shows that locally delivered pooled human IgG significantly increases the survival of all IgG-treated groups in a dose-dependent manner against different challenges of multiple *P. aeruginosa* strains and in different strains of mice compared to controls.

Recent studies linking the inhibition of *P. aeruginosa* motility and associated virulence to human pooled polyclonal IgG and its titers *in vitro* support a specific IgG mechanism that confers protection.\(^{37}\) Since all *P. aeruginosa* strains used here are flagellate pathogens, and since commercial human polyclonal IgG is known to significantly hinder both flagellar pathogen motility *in vitro*\(^{37}\) and infection *in vivo*,\(^{10}\) the observed efficacy of IgG against infection is attributed to these immunospecific modes of action. Treatment with HSA failed to either improve survival or decrease bacterial burden over placebo treatment, demonstrating that local IgG efficacy is due to specific polyclonal IgG antibody interactions with *P. aeruginosa* and not due to non-specific protein effects. The ELISA-based high IgG titers against the three *P. aeruginosa* strains are consistent with the observed reduction of burden and enhanced survival.

The observed success of this commercial IVIG preparation in enhancing prophylactic survival indicates that specific hyperimmune\(^{15,16}\) and monoclonal\(^{1,35}\) sera produced against gram-negative exo- and endotoxins may not be required for prophylactic efficacy. The observed decline of IgG therapeutic efficacy post-infection suggests that these alternative sera may prove useful for improving titers or efficacy for this late therapeutic condition.\(^{32}\) Higher survival rates of IgG groups against the lethal IFO-3455 strain in outbred cohorts of CF-1, CD-1 and CFW mice (Figure 2) together with the significantly increased survival of IgG-treated CF-1 mice against the M-2, MSRI-7072 and IFO-3455 pathogen strains (Figure 3), show that IgG efficacy is not strain dependent in either bacteria or mice. Differences observed in the survival of the three different mouse strains against IFO-3455 challenge (Figure 2) are not readily explained. All strains are outbred genetically, supporting some statistical variance in their immune responses. Otherwise, all strains are white albino breeds, with CD-1 and CFW strains originating overseas (e.g., Switzerland).
Abundant peritoneal macrophages and opsonins, including IgG and complement, are major endogenous constituents of the host’s immune defense against peritoneal infection.\textsuperscript{20,22,23} Macrophages and neutrophils are chemotactically attracted to bacterial endotoxins and are signalled by cytokines. Therefore, the prophylactic presence of specific IgG pools should benefit the host against \textit{P. aeruginosa} infections and peritonitis in general. Measurable IgG titers reflect extensive and rapid IgG binding to \textit{P. aeruginosa} epitopes, limiting \textit{P. aeruginosa} motility, sterically hindering peritoneal epithelial attachment, and enhancing phagocytic clearance. The data in Figure 8 support IgG-enhanced killing in peritoneal lavage isolates as a result of increased opsonic activity and bacterial opsonization by peritoneally applied exogenous human IgG. Locally administered IgG alone confers on the mouse the ability to survive infection by otherwise lethal bacterial challenges from the three \textit{P. aeruginosa} strains.

Preventative (prophylactic) antibiotics are most effective against infection when therapeutic tissue concentrations are present at the time of bacterial contamination; antibiotic effectiveness is lost when administered three hours after tissue pathogen contamination.\textsuperscript{41} In this study, locally applied IgG was most beneficial as a prophylaxis when given prior to and simultaneously with bacterial challenge (Figure 4). This effect coincides with the detected rapid clearance of intraperitoneally administered IgG into mouse systemic circulation. That is, protection against infection appears to be a combination of IgG-mediated effects both locally and systemically. Figure 5 shows that locally delivered IgG is taken up systemically within 3 hours of injection into the peritoneal cavity. This result is consistent with extensive perfusion of the peritoneum and the use of intraperitoneal injection as an established method for giving systemic anesthetics to mice. Hence, a significant fraction of human IgG given locally is rapidly systemically available. Nonetheless, data from Figure 8 show that the fraction of human IgG still present in the peritoneal cavity maintains a substantial capability to facilitate bacterial clearance. The proliferation of bacteria from the site of initial abdominal infection leads to the infection of other organs, the over-production of endotoxins, the induction of cytokine cascades, the progression to septic shock, and sepsis.\textsuperscript{40} Increases in circulating levels of inflammatory cytokines, including tumor necrosis factor-\textit{\textalpha},
interferon-γ, IL-8 and IL-6\textsuperscript{2,4,21,31,47} are clinical indicators of peritonitis.\textsuperscript{40} Reduced circulating IL-6 correlates with decreased host microbial load. Low levels of systemic bacteria detected at 6 hours (Figure 6) and decreased IL-6 levels (Figure 7) in locally IgG-treated groups compared to placebo-treated control groups are consistent with both local and systemic IgG opsonophagocytic activity. Opsonophagocytic data (Figure 8) support continued bacterial clearance in peritoneal lavage fluid containing human IgG, while the bacterial burden increases in this lavage without exogenous IgG. Human IgG is still detectable peritoneally for up to 7 days, with more substantial amounts circulating in blood (Figure 5). Extrapolation of the detected peritoneal human IgG bacterial clearance activity (Figure 8) to longer times in the presence of the remaining peritoneal human IgG (Figure 5) supports possibly prolonged local opsonophagocytic reduction of host bacterial burden along with systemic IgG protection to confer survival.

The data indicate that locally delivered IgG, applied most beneficially as a prophylactic measure, lowers the incidence and severity of infection by reducing the acute bacterial burden and systemically inhibiting sepsis. Because peritonitis is considered a compartmentalized inflammatory process, with much more significant cytokine production locally versus systemically, it has been suggested that anticytokine therapies would be most effectively directed locally at the peritoneal cavity.\textsuperscript{40} The use of locally administered, pooled human IgG is also complementary to current antibiotic therapies. Combined IgG-antibiotic treatments are a potentially useful extension of therapy against infection. Additionally, this strategy is an option for combating bacteria that are resistant or may develop resistance to antibiotics\textsuperscript{6,12,17,19,43} since IgG functions independently of resistance mechanisms. As a potential clinical prophylactic, pooled human IgG might be applied prior to closure during abdominal surgery as a topical lavage, as a treatment in CAPD dialysate fluid by using targeted delivery vehicles or controlled release strategies (e.g., microspheres, gels, or coatings). Tailored, optimization of IgG local dose and delivery kinetics is an anti-infective strategy that is entirely different from the use of IVIG. Such an alternative approach could suit a variety of infectious complications and clinical needs beyond the scope of peritonitis. Such approaches offer new possibilities for decreasing the risks of post-surgical infection, associated morbidity and for lowering mortality rates.
References


