Chapter 7

Treatment of gram-negative and gram-positive abdominal implant infections using locally delivered polyclonal antibodies

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Abstract

Virulent methicillin resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa strains produced lethal implant-associated infections in a mouse open abdominal implant surgery model. These infections were treated with prophylactic combinations of systemic antibiotics and pooled polyclonal human antibodies (IgG) locally delivered from a controlled-release hydrogel carrier applied directly onto a polymer mesh abdominal implant prior to surgical closing. In vitro antibody release from the hydrogel matrix was rapid, nearing completion after 48 hours. Human antibodies released intraperitoneally from this matrix in vivo were detected in mouse serum after 3 hours and persisted beyond 7 days. Released antibodies showed little to no benefit against MRSA infections either alone (100% animal mortality after 3 days) or in combination with systemic cefazolin or vancomycin antibiotic infusions. Controlled release IgG monotherapy produced significant survival benefit in gram-negative infections from two different strains of P. aeruginosa. A role for locally delivered antibodies against implant-associated gram-negative infection is suggested, utilizing humoral protection mechanisms that both circumvent increasingly antibiotic resistant pathogens and potentiate local host immune responses.

Infection is a common complication associated with the surgical implantation of foreign materials.\textsuperscript{1-6} Several types of biomaterials that are commonly used to gain therapeutic access to the peritoneal cavity (e.g., peritoneal dialysis catheters) are associated with high infection rates.\textsuperscript{6-8} Other biomaterials used to ensure proper tissue repair or wound closure after abdominal surgery also have significant infection statistics.\textsuperscript{9} Despite routine clinical use of prophylactic antibiotics, a major complication of abdominal implant surgery is infection caused by normal flora or ubiquitous gram-positive and gram-negative bacteria and anaerobic organisms of enteric origin seeding from the surgical site into surrounding tissues.\textsuperscript{1,4,10,11} Postoperative infections lead to costly, prolonged hospital stays, additional intravenous antibiotic administration, surgical interventions and patient discomfort.\textsuperscript{12} Geriatric, immunocompromised, diabetic and obese patients all have greater risks of infections\textsuperscript{13,14} that contribute to increasing patient mortality and rising healthcare costs.\textsuperscript{12}
The intrinsic ability of the host to eliminate contaminating bacteria inside the surgical wound is significantly reduced once pathogens colonize biomaterials. This is reflected in the increased incidence of infections associated with implanted foreign bodies.\(^{2,5,15-17}\) Many pathogenic bacteria require adhesion-dependent colonization to effectively establish infection in surrounding tissues and the formation of a surface-adherent and protective ‘biofilm’ that characterizes mature device-related infections. Once established, biofilms are difficult to eradicate, despite use of antibiotics with proven efficacy for killing pathogens in standard \textit{in vitro} susceptibility tests.\(^{5,18-20}\) In addition, the emergence of various methicillin- and vancomycin-resistant bacteria together with a large and increasing immunocompromised patient population both add significantly to the surgical infection problem.\(^{20-22}\) Failure of most common clinical treatments to overcome implant-centered infection often requires removal of the device in order to eradicate the therapy-resistant infection.\(^{1, 2, 5, 11}\)

In addition to clinically standard systemic prophylactic antibiotics, other treatment regimes have been used to prevent postoperative, implant-associated infection. Correction of malnutrition and normalizing albumin levels in blood assist the patient’s immune system to battle contaminating bacteria after abdominal surgery. Other therapeutic approaches as alternatives to antibiotics that promote bacterial-resistance include systemic supplementation with intravenous, broad-spectrum immunoglobulins (IVIG). The addition of exogenous opsonic antibodies has been used clinically for decades to fight infection from viruses and pathogenic bacteria.\(^{23-26}\) In animal experiments, IVIG has also been reported to synergize with β-lactam antibiotics since IVIG contains anti-lactamase antibodies against such antibiotic resistant pathogens.\(^{27}\) Due to promising but inconsistent clinical results with IVIG in preventing bacterial infection,\(^{28-36}\) systemic antibody therapy is currently indicated in relatively few clinical infection situations, whereas antibiotic monotherapy remains the clinical treatment of choice.
Nevertheless, the combination of increasingly widespread antibiotic resistance, emergence of new pathogens and increases in immunocompromised patient populations leave many antimicrobial drugs less effective than ever before. The increased reliance on biomaterial implants and their propensity to promote biofilm-forming infections\textsuperscript{1,5} also call for the development of new anti-infective strategies. Reducing bacterial adhesion to biomaterial implant surfaces immediately during implantation\textsuperscript{37-40} decreases the risk for biofilm formation while improving host tissue integration of the implant. Improved tissue integration and wound healing, uncomplicated by postoperative wound infection, could also be important in preventing latent infections caused by hematogenous spread of bacteria from distant infectious foci, (e.g., after dental restoration procedures, total joint or heart valve replacement, or after penetrating traumata) that colonize biomaterials even years after initial implantation. Neutralization of contaminating organisms intra-operatively during the initial 4-6h “decisive period” prior to bacterial colonization\textsuperscript{41,42} could reduce postoperative, biomaterial-centered infection from virulent, antibiotic-resistant bacteria.

While currently not indicated or approved as a systemic, prophylactic antimicrobial, use of systemic IVIG may be appropriate in some indications to achieve this goal.\textsuperscript{23,26} However, human IgG is currently scarce and continues to be costly. Additionally, systemic administration of large quantities of IgG is associated with adverse effects, including finite risks of blood-borne pathogen infection, and renal insufficiency and failure.\textsuperscript{43} Since infectious complications leading to septic shock frequently systemize initially from local device-centered infections (e.g., i.v. catheters, urinary tract (urological catheter) infections (UTI), orthopaedic implants), local anti-infective therapy applied directly to sites of infection as an alternative or supplement to systemic antibiotic prophylaxis could prove very useful. Infection incidence might be reduced by locally potentiating the body’s own intrinsic ability to neutralize contaminating pathogens prior to colonization, prevent biofilm formation and the systemic spread of pathogens leading to sepsis, ultimately allowing a reduction in systemic antibiotic use and thereby slowing the growth of antibiotic resistance.\textsuperscript{44-46} In particular, local humoral immunopotentiation sidesteps antibiotic resistance mechanisms because humoral immunity functions by a
distinctly different mechanism. Since bacterial antibiotic resistance does not alter bacterial susceptibility for opsonization and phagocytosis, local immunotherapy using IgG should function just as effectively against resistant pathogens. While IVIG has demonstrated limited clinical appeal, shifting the use of immunotherapy from systemic treatment to local application provides several advantages for humoral immunopotentiation at sites of high infection risk. This local approach could be effective using lower, highly selective antibody doses while exploiting sophisticated delivery strategies to reduce infection incidence, severity, and antibiotic resistance.

This research aimed primarily to investigate the efficacy of pooled human polyclonal antibodies delivered locally from a controlled-release hydrogel carrier to prevent biomaterial-centered infection in a murine open abdominal surgery model. Different therapeutic scenarios after challenge with either gram-positive (methicillin resistant Staphylococcus aureus) or gram-negative (Pseudomonas aeruginosa) bacteria utilized polyclonal human antibodies in both the absence and presence of clinically relevant systemic antibiotic prophylaxis.

**Materials and Methods**

**Animals:** After approval of all experiments by the Institutional Animal Care and Use Committee (IACUC), female CF-1 mice were purchased (30-33g) from Charles River Laboratories (Raleigh, NC). All animals were housed for at least 7 days in a 12 hour light-dark cycle, and given food and water *ad libitum* before use.

**Bacterial species and preparation of inocula:** Methicillin resistant *Staphylococcus aureus* (MRSA, ATCC33593), *Pseudomonas aeruginosa* strains IFO 3455 and M2 were grown in Tryptic soy broth (TSB, BBL® USP, Becton Dickinson, Cockeysville, MD, Lot H8DFLS) for 18h at 37°C while agitated at 150 rpm. Cultured bacteria were twice sedimented by centrifugation at 7649 x g for 10min, washed, and diluted into sterile saline to obtain a concentrated bacterial suspension. Inoculum concentrations
producing reliable infections were determined from LD\textsubscript{90-100} values from preliminary dose-response experiments using each pathogen in the presence of the implant. Inoculum doses of $1 \times 10^8$ Colony Forming Units (CFU) for MRSA, $1 \times 10^5$ CFU for IFO3455 and $1 \times 10^4$ CFU for M2 per 500 µl application were adjusted using a standard spectrophotometric assay\textsuperscript{49} and confirmed by direct bacterial enumeration on Tryptic soy agar (TSA, Difco - Becton Dickinson, Sparks, MD, Lot 128882XA).

**Hydrogel delivery vehicles and saline lavage preparation.** Carboxymethylcellulose (CMC, Sigma Chemical product C-4888, St.Louis, MO, lot 125H0899) was mixed with sterile, de-ionized water and boiled to create a clear, transparent, homogeneous 5wt% gel. This vehicle was cooled and used as the controlled-release carrier for antibody delivery to the peritoneal cavity. Lyophilized, solid pooled polyclonal human immunoglobulin G (IgG, Baxter Gammagard S/D, Deerfield IL, Lot 98F03AB11) was weighed and mixed gently into the CMC gel using a syringe-syringe mixing/exchange method. This provided a homogenous 2wt% IgG formulation (20mg IgG/ml CMC-gel) after solid antibody dissolution into the CMC gel-carrier. This IgG-gel was stored a maximum of several hours in sterile syringes (3ml) with 18 gauze (G) needles for application in the animal implant model. As a control, empty CMC gel was drawn into identical syringes with 18G needles. Sterile isotonic saline was drawn into sterile syringes with 26G needles to serve as a surgical lavage solution.

**In vitro IgG binding titer determination:** An anti-human IgG ELISA\textsuperscript{22,49,51} was used to determine polyclonal human IgG binding titers against MRSA strain ATCC33593 and *P. aeruginosa* strains IFO3455 and M2. For the gram positive MRSA binding assay only, the ELISA was modified by using 3% rabbit serum as a blocking agent.\textsuperscript{51} Titer numbers express the inverse log dilution of IgG concentration at 50% ELISA optical absorbance (450nm) from the inflection mid-point on each IgG-bacterial binding curve.\textsuperscript{49} A higher titer number reflects higher IgG binding to the bacterium strain.
**In vitro IgG hydrogel release:** Samples of the CMC-IgG-gel (weight ~1.0g, 10wt% IgG in CMC-gel) were loaded into sterile gauze pouches and suspended in 200ml of PBS (pH 7.0) release medium supplemented with 0.05% sodium azide at 37°C on a rotary shaker at 100rpm. Aliquots (1ml) of PBS were assayed for IgG at regular intervals up to 48h using both UV spectroscopy ($\lambda_{280nm}$) and a BCA protein assay (BCA-200 kit, Pierce, Rockford IL).49

**Murine abdominal implant surgical procedure:** Mice were anesthetized with an intramuscular cocktail of ketamine (1.9mg = 60mg/kg) and xylazine (0.6mg = 20mg/kg) in a total injection volume of 50µl. Mice were then temporarily strapped on their backs to a surgery table, the exposed abdominal area was prepared with povidone-iodine and sterile cloth was used to cover each mouse, leaving an open oval area to perform surgery (~2.5cm max. diam.). The abdominal skin was cut longitudinally along the midline (~1.5cm), after which the exposed peritoneum was lifted using forceps and cut identically. Small stainless steel retractors were used to keep the wound open. The exposed intestines were lavaged twice with 0.5ml sterile saline and dried separately using sterile gauze. A square polypropylene mesh implant (1x1cm, 105µm pores, Spectrum Medical Industries, Inc. Los Angeles, CA), previously sterilized in ethanol (70%) for 15 minutes and dried in ambient air, was subsequently placed inside the abdominal cavity and challenged with the inoculum dose of one of the three bacterial strains in 500µl of saline immediately delivered directly onto the implant. After 60 seconds, either blank- (empty) or IgG-containing CMC-gel (500µl containing 10mg of IgG49) was applied by syringe directly onto the implant and the wound was then closed in layers by suturing. The animals were kept in a cage heated by warm water under a warm light until fully recovered from anaesthesia (after ~3h) and Tylenol® was added to their drinking water as a post-surgical analgesic. Aseptic precautions were rigorously observed during the entire procedure. To standardize the surgical trauma, the same surgeons performed all operations.
A second ELISA,\textsuperscript{49} using a capture mouse anti-human IgG and detection peroxidase-conjugated anti-human IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) was used to detect human IgG (optical absorbance at 450nm) in sampled mouse serum and peritoneal lavages. For these analyses, serum was separated from murine blood obtained via cardiac puncture after anaesthesia with Metofane\textsuperscript{®} (Mallinkrodt Veterinary, Inc., Mundelein, IL) at different time points post-surgery, using a benchtop HN-SII centrifuge (10 min. at 3000rpm, IEC, Needham Heights, MA). Following euthanization by cervical dislocation, the peritoneal cavity was immediately exposed and lavaged using 5ml of sterile saline. Lavage fluid (2-4ml) was then collected and the ELISA described above was used to determine human IgG levels in both samples (detection range between 5 - 5000 ng/ml). Human IgG levels in mice were determined by comparing the experimental absorbance values from serum or peritoneal lavage to an IgG standard curve constructed from known amounts of IgG from the same lot of IgG in buffer.

**Combination therapies using systemic antibiotics:** Clinically relevant antibiotics were systemically infused in combination therapy with locally applied IgG. Cefazolin, (Sigma Chemical, St.Louis, MO, product C-5020, lot 115H1078) a first generation cephalosporin and common prophylactic antibiotic in abdominal surgery,\textsuperscript{52} or vancomycin (Sigma Chemical, product V-2002, lot 38H14001), the only current clinical choice against methicillin resistant pathogens, were administered 30min prior to surgery in volumes of 100\(\mu\)l via tail-vein infusion using a 30G needle. Single doses were increased step-wise in successive dose-response infection experiments from 0\(\mu\)g to 400\(\mu\)g of each antibiotic in sterile H\(_2\)O per 30-33g mouse, eventually reaching the clinical equivalent dose of systemically infused antibiotics in a human patient (1g/70kg patient). Sterile H\(_2\)O was systemically infused via the tail vain in placebo treated mice as a negative control.
Results

**Polyclonal IgG binding titer determination:** *In vitro* binding titers of polyclonal IgG determined using a published ELISA method\(^6\) were 1778, 423 and 489 for this IgG lot against MRSA, *P. aeruginosa* IFO3455 and M2, respectively. These values represent significant IgG binding activity against these different pathogens, representative of opsonization.

**In vitro IgG hydrogel release:** Figure 1 shows kinetics for 10 wt% IgG release from CMC gel into buffer at 37°C over two time formats. Nearly 90% of the loaded IgG is released by 9 hours. The inset release graph plots the square root of time release and shows that this early portion of release is linear, consistent with IgG diffusion-limited release kinetics in the water-swollen gel matrix.

### Figure 1:
Kinetics for 10 wt% IgG release from the CMC controlled-release carrier into PBS in vitro at 37°C. Complete release was observed after ~30h. **INSET:** Square root of time release for IgG from the gel carrier, showing linear, diffusion-limited release kinetics from the water-swollen gel.
Complete antibody release is observed after ~30h and comparable IgG levels maintain detectable up to day 16 (data not shown). These release properties indicate that CMC-gel delivery of antibody to the implant site can be rapid, limited only by outward diffusive transport of IgG in the high water-content gel.

In vivo IgG biodistribution over time: Serum and peritoneal lavage fluids collected from groups of mice treated with 10mg of IgG in the CMC-gel carrier and euthanized at various time points up to day 7 were used to compare systemic and local biodistributions of human IgG. Placebo (empty CMC-gel)-treated mice were analyzed only at 0h and no human IgG was detectable in any sample using this assay (data not shown). Figure 2 shows both the amounts of human IgG detected intraperitoneally and systemically over time in IgG-CMC-gel treated mice. Levels of human IgG detected from peritoneal lavage begin at 0.8mg/ml at 0h and decline thereafter until 3h post-administration. Consistent with in vitro release kinetics in Figure 1, CMC-gel rapidly releases most of its IgG in vivo with a spike observed 6-8h post-gel placement.

After day 2, only small quantities of human IgG are recovered from the abdominal cavity. Simultaneously, human IgG levels in serum (Figure 2) are first detectable at the first 3h post-gel placement time point (when the animals have completely recovered). A rapid increase in serum human IgG follows, spiking to almost 1.25mg/ml after 12h, and remaining constant at 0.85mg/ml for up to 4 days post-gel placement. Human IgG remains detectable by ELISA methods thereafter in both serum and peritoneal lavage for up to 7 days, reflecting prolonged bioavailability after gel release.

Local IgG administration against MRSA peritonitis: CMC-gel released IgG (10mg) was delivered against lethal challenges of MRSA (10⁸ CFU) in 4 different abdominal implant infection experimental cohorts. Mouse survival with 10mg of IgG released from CMC-gel was not enhanced when compared to control mice treated with placebo (blank CMC-gel). Complete mortality was observed after day 4 in all cohorts with IgG monotherapy (data not shown).
Local IgG and systemic vancomycin against MRSA

Figure 2:
ELISA detection of human IgG levels in mouse serum (●) and peritoneal lavage fluid (△) post bacterial challenge with *P. aeruginosa* (IFO3455, 10⁵ CFU, n=3-4 mice per time point) and 10mg IgG treatment in CMC hydrogel carrier over 7 days. Human IgG was detectable in mouse serum after 3h, and remained present over the duration of the experiment.
INSET: Human IgG levels in mouse serum and peritoneal lavage fluid in the first 12 hours post-surgery.

In a second set of experiments, mice were administered single systemic i.v. doses of cefazolin (increasing from 0.4μg to 400μg/mouse), ultimately approximating the clinical prophylactic dose in humans. No dose improved infection survival (data not shown), consistent with *in vitro* evidence indicating cefazolin resistance for this bacterial strain. Addition of 10mg of IgG in CMC-gel to 400μg systemic cefazolin prophylaxis i.v. resulted in improved survival to 25% for the combination therapy (shown in Figure 3), compared to 100% mortality in the three control groups (blank CMC-gel/systemic H₂O, CMC-IgG gel/systemic H₂O, and blank CMC-gel/systemic antibiotic). Although low, this survival enhancement was statistically significant against all other therapeutic scenarios using an ANOVA over 10 days (p<0.05).
Prophylactic vancomycin was also infused systemically in a third set of MRSA-infected abdominal surgical experiments. Mice were administered increasing systemic i.v. vancomycin doses up to 400μg/mouse 30min prior to surgery, approximating the relevant clinical prophylactic dose in humans. The highest dose (400μg/mouse) of vancomycin conferred 100% survival (Figure 4), whereas the lower i.v. doses did not enhance survival as compared to control untreated mice (data not shown). An intermediate, sub-optimal dose of vancomycin (200 μg) was then chosen for a further experiment to assess combination effects of additional, locally delivered human IgG on mouse survival in this model. Combination therapy (200 μg vancomycin i.v. plus 10 mg of IgG delivered locally from the CMC-gel) only moderately improved survival to 25% after Day 6, a result no better than the survival percentage already observed in the 200 μg vancomycin monotherapy group after day 5 (Figure 4). Additionally, lower doses of vancomycin (40 μg) in combination with 10 mg of IgG delivered intraperitoneally from the CMC-gel did not enhance survival compared to 40 μg of vancomycin i.v. alone (data not shown).
Local IgG and systemic vancomycin against MRSA

Figure 4: Mouse survival after MRSA challenge and treatment with increasing prophylactic doses of vancomycin administered via tail vein injection, and blank CMC-gel delivered to the peritoneal cavity onto the implant surface. Survival experiments using a sub-optimal dose of vancomycin (200 µg/mouse) and local delivery of IgG via the CMC-gel carrier to implant surfaces showed no survival improvements over 200 µg vancomycin + blank CMC-gel (both 25% survival).

Survival studies were conducted analogously in this surgical implant infection model using lethal challenges of two virulent strains of gram-negative P. aeruginosa: IFO3455 (10⁵ CFU in 500 µl) or M2 (10⁴ CFU in 500 µl) administered directly onto the implant. Results in Figure 5 show the 10-day survival curves for mice treated with 10 mg of IgG in 500 µl CMC-gel versus placebo-treated mice (blank CMC-gel) as monotherapy (i.e., no antibiotics).

Significantly improved survival was achieved against IFO3455 infection using local IgG delivery without use of any antibiotics, producing 70% survival benefit while all control mice died by day 3 (ANOVA, p<0.05). Improved survival benefits were achieved using the same treatment and the more virulent P. aeruginosa strain M2.⁴⁹ The M2 bacterial challenge produced 80% mortality in the placebo-treated mice while 100% survival was observed in mice cohorts treated with a single prophylactic dose of 10 mg IgG delivered to the implant from the CMC controlled-release hydrogel (Figure 5). These results are
statistically significant over the 10-day study period (ANOVA, p<0.05). Based on the significant efficacy of locally delivered IgG in enhancing survival in these gram-negative implant-centered infections, further studies with systemic antibiotics were discontinued.

**Figure 5:**
Challenges of *P. aeruginosa* M2 ($10^4$ CFU) or IFO3455 ($10^5$ CFU) caused lethal infections in 80 or 100 percent of the control-mice, respectively. Local delivery of human IgG from the CMC-gel conferred significantly improved survival benefits against both pathogens, up to 100% survival after 10 days.

**Discussion**

A 2wt% pooled polyclonal human IgG formulation released from a hydrogel controlled release carrier was used to improve the survival of mice after abdominal implant surgery and subsequent challenge with either gram-positive and gram-negative pathogens. Mice received a polypropylene mesh implant and were challenged with lethal doses of MRSA or *P. aeruginosa* during surgery. Both pathogens were chosen because of their
frequency in postoperative and nosocomial (i.e., CAPD) abdominal implant infections and the difficulty encountered in successfully treating clinical, biomaterial-centered infections. Human polyclonal IgG in a CMC controlled-release gel carrier was released directly to the surgical wound and implant site in the peritoneal cavity.

Previous work has shown that human polyclonal IgG i.p. injection was effective in protecting mice against lethal closed abscess peritonitis induced by several *Pseudomonads*. In this study, however, similar mice and IgG doses as described before were applied and the resulting survival benefits were shown to be independent of either mouse strain or *P. aeruginosa* strain. Timing of the local application of polyclonal IgG was important to optimize survival benefit, with prophylactic antibody applications producing the highest survival. Several significant differences distinguish the current study and this recently reported peritonitis model: (1) the murine infection models differ significantly, with earlier work using a closed abscess infection, and the current study focusing on open surgical implant infection, and (2) the distinctly different delivery modes of antibodies. Previous work used i.p. injection, known to provide a convenient route to rapidly systemize antibodies, while this current delivery method utilizes a controlled release matrix that delays systemization of IgG and sustains local delivery to the implant-centered infection. The impact of these differences lies in the kinetics and resulting bioavailability of the IgG doses to fight bolus lethal bacterial inocula, and in the increased trauma and host compromise suffered through the surgical procedure and the presence of the implant.

Comparable surgical implantation and bacterial challenge experiments were performed by Ward and coworkers using rabbits which, prior to surgery, were primed with killed *P. aeruginosa* to induce high systemic circulating levels of specific IgG in serum to combat inocula of the same species. Their results demonstrated the failure of the peritoneal defense mechanisms to clear the infection in the presence of a biomaterial device even with these primed animals and high systemic levels of IgG. Our results, however, show remarkable improvement in animal survival using a local presence of pooled polyclonal IgG in the abdominal implant site in gram-negative *P. aeruginosa*
infections. This difference could be due to three possible factors: 1) immediate IgG-mediated reduction of biomaterial colonization by *P. aeruginosa* in the initial period after surgery,\(^{37}\) 2) lack of distribution and diffusion barriers for IgG between the highly perfused peritoneum and systemic circulation, providing rapid biodistribution of protective antibodies, and 3) substantial non-specific opsonization benefit for gram-negative bacteria due to the wide variety of antibodies naturally occurring in pooled polyclonal human IgG.\(^{53,54}\)

Human polyclonal antibodies demonstrate sufficient binding titers *in vitro* against infective pathogens. Since antibody release from the gel *in vitro* was rapid and diffusion limited, nearing 100% release within 30h (Figure 1), and human antibodies released *in vivo* from the gel in the peritoneal cavity were detected in mouse serum after 3h (Figure 2), exogenous human IgG is presumed to become rapidly available to opsonize bacteria at the implant site. Nevertheless, initial amounts of IgG assayed in the peritoneal cavity (0, 2, and 3h time points) were lower than expected (0.8mg/ml in ~4ml of lavage fluid). This is attributed to the lavage technique, which cannot account for IgG remaining within the gel carrier at the implant site and does not sufficiently affect dissolution of residual gel with associated IgG. Complete recovery of the animals and dissolution of the CMC-gel carrier allowed for the sustained release of the remaining human IgG to mice systemic circulation, shown as the serum spike after 12h (~1.25mg/ml serum).

While the addition of high dose systemic antibiotics in both pathogenic infections significantly improved survival, only the human equivalent dose of vancomycin provided complete protection against gram-positive infection. The pronounced differences observed between the efficacy of polyclonal IgG against the gram-positive and gram-negative challenges may be attributed to the differences in antibody opsonization efficiency and reactivity due to presence of protein A on the surface of the gram-positive MRSA. Protein A can non-specifically “reverse bind” IgG thereby effectively preventing it from opsonizing the bacteria for phagocytic clearance.\(^{37,51}\) Another factor may be the dramatic changes in surface characteristics of the gram-negative *P. aeruginosa* in the presence of IgG. These surface changes have been attributed to substantial adsorptive
interactions of non-specific human IgG with these bacteria that can lead to non-specific IgG mediated killing.\textsuperscript{37,53,54} This effect is additive to the specific opsonization occurring against \textit{P. aeruginosa} and has not been reported for gram-positive organisms such as \textit{S. aureus}, in which only pathogen-specific antibodies are capable of enhancing humoral immune-mediated clearance.\textsuperscript{53,54}

Comparisons of the \textit{P. aeruginosa} inoculum doses used to produce 80-100\% mortality in CF-1 mice in the presence of a surgically implanted biomaterial with earlier experiments involving the same bacteria and mouse strain but without biomaterial implantation or open abdominal surgery\textsuperscript{49} are interesting. The LD\textsubscript{90-100} doses used previously in a closed abscess peritonitis infection model\textsuperscript{49} (10\textsuperscript{7} CFU for both \textit{P. aeruginosa} strains injected directly into the peritoneal cavity) were a log-order higher than inoculum needed to cause 100\% mortality after open abdominal surgery without the polypropylene mesh implant (i.e. 10\textsuperscript{5} CFU of \textit{P. aeruginosa} IFO 3455, data not shown). Additionally, only 10\textsuperscript{5} CFU (IFO 3455) and 10\textsuperscript{4} CFU (M-2) killed 80-100\% of mice in the surgical infection model if the biomaterial was implanted. This comparison provides further evidence of the compromising influence of both open surgical procedures and biomaterial implants on the host’s immune response to effectively fight contaminating organisms inside a surgical wound.\textsuperscript{1,4,11}

Results for this abdominal surgical infection model confirm the increased propensity for infectious complications associated with surgically implanted biomaterial devices. Biomaterial-centered infection with both gram-positive MRSA and gram-negative \textit{P. aeruginosa} strains with abdominal polymer implants consistently produced host mortality if no therapeutic was prophylactically applied. Moreover, methicillin resistant \textit{Staphylococcus aureus}-induced biomaterial-centered infection was demonstrated to be more refractory to (1) sub-optimal monotherapy using systemic clinically relevant antibiotics (2) local polyclonal antibody monotherapy, and (3) combinations of these two treatments.
The local, controlled delivery of antibodies from a matrix or carrier directly to implant sites prone to colonization shows therapeutic potential. Pooled human IgG could be delivered locally to the peritoneal cavity or other sites of bacterial contamination or infection (e.g., appendicitis, diverticulitis, primary anastomosis surgery) using a biocompatible hydrogel vehicle capable of rapid release of bioactive antibodies to enhance host survival after contamination with relevant gram-negative organisms. We suggest, therefore, a role for locally applied IgG as a supplement to systemic antibiotic prophylaxis to potentiate local host immune responses. Antibodies function to clear pathogens via mechanisms that circumvent further antibiotic resistance, a role that complements the strengths and eliminates some of the weaknesses of current antibiotic prophylaxis. Ultimate success against infection depends on antibody-mediated neutralization of pathogens, the pharmacodynamics (e.g., release rates, clearance, bioavailability, biodistribution) of delivered antibody, required doses against various infections, and the virulence of clinically relevant pathogens.
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