Locally delivered polyclonal antibodies potentiate the efficacy of a systemic antibiotic against infections

K.A. Poelstra,¹ N.A. Barekzi,¹ A.G. Felts,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

submitted
Abstract

Antibiotic treatment of clinical infections is complicated both by the increasing emergence of antibiotic-resistant pathogens and increased patient populations intrinsically at risk for nosocomial infections. Combination therapies comprising multiple intravenous antibiotics alone, or in tandem with either intravenous immunoglobulins or local antibiotics, have all been used to improve efficacy against clinical infections. We now report that pooled human immunoglobulins applied locally to sites of infection in vivo substantially improve the anti-microbial benefits of a clinically important intravenous antibiotic – ceftazidime – against both E.coli-induced peritonitis and Klebsiella-induced burn wound infection. Synergistic improvements in host survival, bacterial burden, and sepsis indicators are observed with this unique treatment combination. Because immunotherapy functions independently of antibiotic resistance mechanisms, local delivery of polyclonal or monoclonal antimicrobial antibodies together with clinically routine intravenous antibiotics exploit diverse, complementary antimicrobial properties to confer improved protection against infection and extend the efficacy of front-line antibiotics.

Current clinical standards of care often utilize, either prophylactically or therapeutically, systemic antibiotics to manage and control the threat of infection in many indications. Antibiotic resistant pathogens are an increasingly problematic cause of hospital-based infections. A wide variety of pathogens now demonstrate clinical resistance to antibiotics of choice including methicillin resistant strains that account for 80% of all Staphylococcus aureus clinical infections, and vancomycin resistant enterococci. The continuously increasing prevalence of antibiotic resistant bacteria has greatly elevated concern that front-line antibiotics will become ineffective in managing clinical infections. Recently, clinical reports of vancomycin-methicillin resistant Staphylococcus aureus infection provide evidence that vancomycin’s clinical utility as the last antibiotic of choice is also threatened. Proof that selective pressure from increasing vancomycin use promotes even more rapid vancomycin resistance has prompted renewed attention directed both to understanding mechanisms of antibiotic resistance, as well as to developing alternative antimicrobial methods. Although new de novo
antibiotic synthesis is a logical, compelling choice, few new, original synthetic antibiotics are in clinical phase trials. Furthermore, many prospective antibiotic candidates represent iterations on long-standing drug structure-function paradigms susceptible to resistance mechanisms.

Commercial pooled polyclonal human immunoglobulins (IgG) represent a broad-spectrum antimicrobial approach currently administered by intravenous infusion to millions of patients annually (IVIG). These exogenous polyclonal antibodies supplement host humoral immunity through both specific and non-specific opsonization and neutralization reactions against many ubiquitous, clinically relevant pathogens, ultimately leading to microbial phagocytic clearance. Because antibodies facilitate antimicrobial mechanisms distinct from those of antibiotics, they do not engender antibiotic resistance. Moreover, the appearance of pathogen antibiotic resistance does not alter bacterial susceptibility to opsonization and phagocytic neutralization. However, therapeutic benefit of IVIG alone in various scenarios has not been compelling. By contrast, combination therapy comprising both systemic antibiotics and systemic polyclonal IVIG has demonstrated benefit against sepsis in newborns, high-risk neonates, and ventilated ICU patients. Additionally, combination therapy has also been reported to reduce post-operative infection rates leading to sepsis after surgery for colorectal cancer and has been applied as an adjunct therapy to systemic antibiotics against chronic sinus disease in children. Clinical problems with antibiotic-induced release of pathogen toxins in septic patients have been addressed using antibodies administered systemically against these toxins. Most recently, systemic monoclonal antibody infusion has also been used successfully in cystic fibrosis patients infected with therapy resistant Pseudomonas aeruginosa, and systemic monoclonal antibody infusion has emerged as the treatment of choice against respiratory syncytial virus (RSV). Systemic antimicrobial antibodies, therefore, have shown to be complementary to antibiotics in preventing and facilitating clearance of infection. Combination therapies represent the clinical capability to exploit pathogen susceptibility to multiple antimicrobial agents that individually no longer have acceptable clinical efficacy.
Improved, often additive clinical benefits observed for simultaneously co-administered antibiotics, or antibiotic and antibody combination therapies can be attributed to several phenomena. First, pathogen antibiotic resistance is never complete. While minimum inhibitory concentrations for an antibiotic against a given antibiotic resistant pathogen can be significantly increased, they remain finite and microcidal in vitro at elevated doses. Clinically, however, these doses may be toxic, precluding use. Given the limited genomic and metabolic capacity of many pathogens, genetic acquisition of resistance against a specific antibiotic may compromise further broad-spectrum multiple drug resistance. Secondly, antibiotic/antibody mixed infusions provide simultaneous microcidal, antimicrobial and anti-toxin efficacy important in neutralizing both pathogen growth and toxin release from viable and antibiotic-lysed pathogens.

We report here observation of enhanced efficacy for ceftazidime, a commonly used systemic antibiotic, in combination with locally applied polyclonal antibodies as a new treatment strategy against infection. Ceftazidime is a third-generation cephalosporin and first-line antibiotic against *Klebsiella pneumoniae*, *Serratia marcescens*, *Escherichia coli* and *Proteus mirabilis* infections. However, emergence of ceftazidime-resistant *Klebsiella* and *Escherichia coli* strains in hospital-acquired infection scenarios warrants assessment of other strategies. Two different murine infection models (outbred CF-1 mice) detailed previously were used to study the efficacy of systemic antibiotic/local antibody combination therapy over each monotherapy, respectively.

In the first *in vivo* infection model, full-thickness burn wounds created on the backs of anesthetized mice were immediately challenged with *Klebsiella pneumoniae* (strain 2270, $10^2$ CFU) directly into the wound site via a subcutaneous (s.c.) injection, producing a consistently lethal infection. The second *in vivo* infection model uses a direct intra-abdominal challenge with *Escherichia coli* (strain KI08ACH7, $10^6$ CFU) to consistently produce a lethal peritonitis. Each infection model was subject to combination therapies using prophylactic, intravenous ceftazidime (tail vein infusion) and/or pooled human polyclonal antibodies either systemically infused (IVIG) or locally delivered to the site of infection.
Methods

**Animals and Animal Care:** Outbred female Crl-CF-1 mice (22-24 g) were obtained from Charles River Laboratories (Wilmington, MA) and housed five per cage in a biosafety level 2 facility with a 12 hour light/dark cycle. Standard mouse chow and water were provided *ad libitum*. All animals were maintained according to the *Guide for the Care and Use of Laboratory Animals* and all protocols were approved by the Gristina Institute Animal Care and Use Committee.

**Bacteria:** *Klebsiella pneumoniae* 2270 and *Escherichia coli* K108ACH7 (donated by Dr. Ian Holder, Shriners Burn Institute, Cincinnati, OH and Dr. J. Curtis Nickel, Queens University, Kingston Ontario, Canada, respectively) were grown for 18 hours in 20 ml trypticase soy broth at 37°C while agitated at 150 RPM using a benchtop incubator shaker. Cultured bacteria were twice sedimented by centrifugation at 7649 × g for 10 minutes, washed and diluted in saline to obtain a concentrated bacteria suspension. Serial bacterial dilutions were plated on trypticase soy agar (TSA) and colonies were counted to determine initial colony forming units (CFU) per ml after 24 hours incubation at 37°C. In parallel, optical absorbance (λ=650 nm) of these bacterial dilutions was measured (Beckman DB-GT grating spectrophotometer) and standard curves plotting optical absorbance versus CFU/ml concentrations were then constructed for each organism. Optical absorbance values of 1.16 for *K. pneumoniae* and 1.05 for *E. coli* resulted in ~10⁹ CFU/ml.

**Local and systemic antimicrobial therapies:** Mice were treated locally (sub-eschar, s.c., intravenously, i.v., or intraperitoneally, i.p.) with commercially pooled human intravenous immunoglobulin (Lot# 2620M039A, Gammagard® S/D, Baxter Healthcare Corporation, Glendale, CA) and/or i.v. with sub-optimal doses of ceftazidime (Lot# 8ZP0340, Fortaz®, Glaxo Wellcome Inc., Research Triangle Park, NC). Gammagard® S/D was supplied as a freeze-dried preparation, and reconstituted with supplied diluent (Sterile Water for Injection, USP) to 10wt% protein/ml (98% pure IgG) at an approximate pH of 6.8. Manufacturer specifications indicate that this reconstitution provides an iso-osmolar
solution comprising dextrose and saline. Ceftazidime, supplied as a lyophilized powder, was reconstituted to the optimal human equivalent clinical dose (200 or 22.7 mg/kg for pediatric burn wounds\textsuperscript{35} and adult peritonitis,\textsuperscript{51} respectively) in sterile distilled water. Stock IgG solutions were diluted in 5% dextrose (recommended by the manufacturer) and ceftazidime was diluted in sterile water to obtain the desired working concentrations. Injections of sterile diluents (0.1 ml for s.c. and i.v. injections, 0.5 ml for i.p. injections) served as local and systemic control treatments in all experiments.

**Animal Models:** A previously described full thickness murine burn wound infection model was used.\textsuperscript{35} Briefly, mice were shaved and then anesthetized by methoxyflurane inhalation (Metofane\textsuperscript{®}, Schering Plough, Union, NJ) and a heat-resistant plastic board with a 1.0 (25 mm) by 1.5 inch (38 mm) window was pressed firmly against the shaved dorsum. Ethanol (200 proof; 0.50 ml) was spread evenly over the window opening, ignited, and allowed to burn for 10 seconds. The procedure produced a non-lethal full thickness burn wound over 10-15% of the body surface. Immediately after the burn, mice were given 0.5 ml of sterile saline intraperitoneally as fluid replacement therapy and acetaminophen (0.25 mg/ml; Children’s Tylenol suspension liquid) in drinking water as a post-burn analgesic. A lethal dose of *K. pneumoniae* (LD\textsubscript{100}=10\textsuperscript{2} CFU/0.1 ml) and local immunoglobulin treatment (10 mg/0.1 ml) were independently injected s.c. under the burn site immediately following the burn. Intravenous sub-optimal single doses of ceftazidime (44\textmu g/0.1 ml; 2 mg/kg) were infused by tail vein injection either alone (monotherapy) or followed by immunoglobulin treatment (combination therapy).

In the peritonitis model,\textsuperscript{43} mice were injected intraperitoneally (i.p.) with a lethal dose inoculum dose of *E. coli* (LD\textsubscript{90}=10\textsuperscript{6} CFU/0.5 ml) followed immediately by immunoglobulin treatment (1, 5, or 10 mg/0.5 ml i.p. or 10mg/0.1ml i.v.) and/or sub-optimal i.v. ceftazidime tail vein infusion (25 or 50 \textmu g/0.1 ml). Animal survival in both models was assessed for ten days thereafter.
Quantitative Microbiology: Approximately one ml of blood was collected via cardiac puncture into heparinized tubes (40 units; Sigma Chemical Co., St. Louis, MO) from each anesthetized mouse either 24 hours (burn wound model) or 12 hours (peritonitis model) post bacterial challenge. Immediately following blood collection each anesthetized mouse was euthanized by cervical dislocation. Blood was serially diluted in sterile saline and plated on trypticase soy agar (TSA) plates for enumeration of bacteria. In the peritonitis model, each peritoneal cavity was lavaged with 5 ml sterile saline. Lavage was serially diluted and plated on TSA plates for enumeration of bacteria. Resulting colony counts are expressed as log CFU/g blood or lavage, respectively. Post-euthanasia in the burn wound model, the burned eschar was surgically removed and homogenized in 10 ml sterile saline. In both models, livers were excised post-mortem and placed in 10 ml sterile saline. All tissue samples were weighed prior to homogenization (Omni-International GLH Homogenizer, Marietta, GA). Homogenate fluids were then serially diluted in sterile saline and plated on TSA plates for bacterial cultures and enumeration. Resulting colony counts are expressed as log CFU/g tissue.

Quantitation of Systemic Interleukin-6 (IL-6): Immediately following serial dilution and plating of the blood samples, serum was separated by centrifugation at 3,000 rpm for 10 minutes at room temperature, collected and assayed with an ELISA specifically designed to detect IL-6. Optical density was measured at 405 nm (SLT Spectra Reader, Tecan Company, Durham, NC). The detection range for the assay was 15 - 2000 pg/ml. The concentration of IL-6 present in each sample is reported as pg/ml serum.

Statistical Analysis: Data are expressed as the mean ± standard error of the mean (SEM). Student’s t tests were used to compare the control and therapy groups of the bacterial burden enumeration and IL-6 studies while ANOVA with Tukey’s tests were used to compare rates of mortality. All probabilities less than 5% (p < 0.05) were considered significant. Data outliers, defined as any datum outside the range of the mean ± two times the standard deviation, were excluded.
Results

**Antibiotic/antibody therapy benefits in burn wound infection.**

In untreated murine burn wounds, low levels of *K. pneumoniae* challenge (10² CFU s.c.) consistently killed 100% of burned and 80% of unburned mice (Figure 1a). Significantly, previous work has shown that other relevant burn wound pathogens required substantially higher inocula (e.g., *Pseudomonas aeruginosa*, 10⁴ CFU) to produce consistent lethal infections in this model,⁴⁴ and that locally applied polyclonal IgG monotherapy protected ~90% of the animals from *P. aeruginosa* lethal infection. However, Figure 1a shows that local polyclonal antibody monotherapy, or sub-optimal i.v. ceftazidime (44 µg/animal, 2.0 mg/kg) monotherapy with *Klebsiella*-infected burn wounds each produced low survival (0% for local IgG, 50% for i.v. ceftazidime). However, the combination of local antibody treatment with systemic ceftazidime combination produced 90% survival, a significant 40% synergistic benefit (p<0.05).

Quantification of tissue bacterial burdens in the burn infection model at 24 hours post-infection exhibits similar trends (Figure 1b). In the harvested burned eschar, *K. pneumoniae* colony counts in dextrose-treated controls (placebo) and in cohorts treated with local s.c. antibody monotherapy increased dramatically (>6 log order increase over challenge inoculum dose) by 24 hours after bacterial challenge. Pathogen colonization of eschar tissue after systemic infusion of a sub-optimal dose of ceftazidime alone also was significantly increased (>2 log order increase over challenge inoculum dose). In contrast, combining local antibody delivery and systemic sub-optimal antibiotic treatment at the time of bacterial challenge decreases bacterial levels in this tissue by 0.5 log CFU/g tissue compared to the initial inoculum (Figure 1b).

While this strain of *K. pneumoniae* exhibited high virulence due to the presence of an extracellular polysaccharide capsule,⁴⁶,⁴⁷ a 24-hour incubation period in the host is still too brief for the infection to become fully systemized. No colonies were detected in blood samples from all groups after 24 hours (data not shown). Colonies were detected in liver homogenates, however, exhibiting similar, higher bacterial counts for placebo
and both monotherapy-treated mice cohorts after 24h. These counts will increase to lethal levels in the following 24h period, resulting in the notable increase in mortality over time (Figure 1a). By contrast, livers of all mice treated with both locally delivered antibodies and systemic sub-optimal antibiotics exhibited average pathogen levels below the $10^2$ CFU/g detection limit, conferring protection consistent with survival benefits observed for this therapy group.

**Figure 1:**
(a) Host survival over time for systemic sub-optimal antibiotic dose (44µg ceftazidime via tail vein infusion) or locally applied polyclonal antibody monotherapy (sub-eschar, s.c., injection) compared to various combination therapies in a murine burn wound infection model using a lethal dose ($10^2$ CFU) of K. pneumoniae as the pathogen inoculum. Mice were left unburned or burned and then lethally challenged following established protocols. Combination therapy of systemic ceftazidime and locally injected polyclonal antibodies s.c. into the wound confers synergistic survival benefit over either ceftazidime or locally applied IgG alone. (Significance: ANOVA and Tukey’s test, n=10; *p<0.05 compared to all other data).

(b) Bacterial tissue burden assayed 24 hours post-burn and post-challenge with $10^6$ CFU of K. pneumoniae following mono- or combination antimicrobial therapy. Combination therapy of systemic ceftazidime and locally injected polyclonal antibodies provides substantial reduction in eschar bacterial burden over either ceftazidime or locally applied IgG monotherapies (Student’s t-test, *p<0.01 versus all harvested eschar groups).
Cytokine interleukin-6 (IL-6), a clinical indicator of systemic inflammatory acute phase reactions, was detected in serum of all groups post-infection. Both systemic antibiotic monotherapy and combination therapy groups had significantly reduced IL-6 levels over that observed for placebo or local antibody monotherapy groups (Figure 1c). No significant difference in IL-6 reduction was observed for the combination treatment group compared to the antibiotic monotherapy group. Reduced systemic IL-6 is consistent with the absence of detectable systemic bacteria in blood and low liver bacterial counts observed for both of these treatments.43,44

Figure 1 (continued):

(c) Levels of interleukin-6 (IL-6) in serum assayed 24 hours post-burn and post-challenge with $10^2$ CFU of K. pneumoniae following mono- or combination antimicrobial therapy. Combination therapy of systemic ceftazidime and locally injected polyclonal antibodies provides substantial reduction in circulating IL-6 over either ceftazidime or locally applied IgG alone (Student’s t-test, *p<0.003 versus placebo and IgG s.c. treatment, **p<0.05 versus all treatments).
Antibiotic/antibody combination therapy benefits in peritonitis.

In the second model, intra-abdominal injection of a clinically isolated *E. coli* (KI08ACH7, $10^6$ CFU) produced consistent lethality in untreated mice (Figure 2a). This *E. coli* isolate is not fully antibiotic resistant, proving sensitive to ampicillin, cefazolin, nitrofurantoin, gentamicin and trimethoprim-sulfamethoxazole (personal communication, Dr. J. Curtis Nickel, Queens University, Kingston Ontario, Canada). Analogous to the burn infection model survival synergy shown in Figure 1a, synergistic survival benefits were also observed in this peritonitis model for combinations of sub-optimal i.v. ceftazidime (50µg/animal, 2.3mg/kg) with intraperitoneally administered polyclonal antibodies (Figure 2a).

**Figure 2(a)**

(a) Murine survival over time for systemic sub-optimal antibiotic dose (50µg ceftazidime via tail vein infusion) or locally applied polyclonal antibody monotherapy (intraperitoneal injection, i.p.) compared to various combination therapies in a murine peritonitis infection model challenged with a lethal dose ($1x10^6$ CFU) of *E. coli*, following established protocols. Combination therapy of systemic ceftazidime with 10mg locally (i.p.) injected polyclonal antibodies confers synergistic survival benefit over either systemic ceftazidime, locally applied IgG (i.p.) monotherapy, or the additive benefit of both monotherapies considered together. Additionally, survival benefits for combinations of systemic ceftazidime plus either 1mg or 5mg IgG i.p. were nearly identical to that for combination therapy of systemic ceftazidime and 10mg IgG intravenously.

All of these survival benefits were comparable to the additive benefit provided by systemic ceftazidime and 10mg IgG i.p. monotherapies considered together (significance: ANOVA and Tukey’s test, n=5-20; p<0.001 for each combination antibiotic/IgG therapy versus each monotherapy, and p<0.001 for each monotherapy compared to placebo).
In combination with sub-optimal 50 µg i.v. ceftazidime doses, two different, reduced doses of locally applied antibodies (1 and 5mg IgG intraperitoneally (i.p.)) conferred survival equivalent to that observed for a substantially higher dose (10mg i.v.) of systemically infused IgG (conventional IVIG). Overall survival for local antibody monotherapy is 40% against this pathogen, substantially less effective than previously observed for the same local IgG monotherapy in the same model against several different virulent strains of *P. aeruginosa*.43 Local antibody monotherapy proved slightly less effective than systemic antibiotic monotherapy that confers 50% survival with a single prophylactic sub-optimal dose of i.v. ceftazidime alone. Combination therapy comprising systemic antibiotic and locally delivered IgG produced a synergistic 95% survival (Figure 2a), compared to each monotherapy, a benefit significantly different than all other treatment groups (ANOVA, p<0.05).

Enhanced efficacy of locally delivered IgG i.p. over systemic IgG is more apparent when both are compared in combination with a further reduced sub-optimal 25µg ceftazidime systemic infusion and slightly reduced *E. coli* challenge (7x10⁵ CFU, Figure 2b) in this peritonitis model. Addition of any antibody therapy – systemic or local – to ceftazidime i.v. treatment improves survival over ceftazidime monotherapy. Combination therapy involving local IgG applications even at this low systemic antibiotic dose produced higher survival than systemic antibiotics combined with systemic antibody infusion at two different doses (1 and 10mg IVIG). The lower local 1mg IgG dose i.p. in combination therapy conferred equivalent survival to that observed for a log higher dose (10mg) of systemic IgG with the same antibiotic infusion (Figure 2b).

Bacterial burdens found in tissues harvested from the peritonitis model at 12 hours post-infection support these trends in survival (Figure 2c). Enumeration of pathogens from peritoneal lavage, liver and blood post-treatment indicates that systemic antibiotic/local antibody therapy significantly and synergistically reduces viable bacteria in the host compared to either monotherapy. Local IgG monotherapy i.p. has little efficacy in reducing pathogen burden in all sites examined, while sub-optimal i.v. ceftazidime monotherapy reduced bacterial burdens substantially and consistently at all sites.
Local IgG potentiates efficacy of systemic antibiotic

**Figure 2 (b)**

Murine survival over time for a lower systemic sub-optimal antibiotic dose (25µg i.v. ceftazidime) in combination with various locally or systemically applied polyclonal antibody doses (1 and 10mg IgG i.p. or i.v.) compared to placebo and combination therapies in a murine peritonitis infection model using a lethal dose (7x10⁵ CFU) of E. coli as the pathogen inoculum. Combination therapy of sub-optimal systemic ceftazidime and locally (i.p.) injected polyclonal antibodies (1 or 10mg IgG) confers improved survival benefit over either systemic ceftazidime monotherapy or combination of systemic ceftazidime with corresponding 1 or 10 mg systemic IgG dose i.v., respectively. Additionally, survival benefit for combination therapy comprising sub-optimal systemic ceftazidime plus 1mg IgG i.p. was equivalent to that for combination therapy of systemic ceftazidime and 10mg IgG i.v. (significance: ANOVA and Tukey’s test, n=10; p<0.001 for each combination antibiotic/IgG therapy versus each monotherapy, p<0.001 for ceftazidime monotherapy compared to placebo, and p<0.001 for all combination therapies using local IgG dosing (i.p.) versus combination therapy using systemic IgG (i.v.));

**Figure 2 (c)**

Tissue and blood bacterial burden assayed 12 hours post-challenge with 5x10⁷ CFU of E. coli and mono- or combination antimicrobial therapy. Combination therapy comprising a sub-optimal systemic ceftazidime dose (25 : g i.v.) with locally injected polyclonal antibodies i.p. provides substantial reduction in bacterial burden in all sites assayed over either ceftazidime or locally applied IgG monotherapy (significance: Student’s t-test, *p<0.05 comparing ceftazidime versus placebo and IgG monotherapy; **p<0.05 comparing combination therapy versus all treatments);
Together, the combined therapy would be predicted to show a benefit similar to ceftazidime alone. However, data shown in Figure 2c indicate that a bacterial reduction benefit substantially greater than simple additivity is produced by the combination of ceftazidime i.v. and IgG i.p., consistent with the survival synergy noted in Figure 2a.

Analogous to treatment effects seen in Figure 1c for the burn infection, levels of circulating IL-6 detected in serum of both antibody monotherapy and placebo treatment groups for peritonitis are dramatically increased over IL-6 quantified for antibiotic monotherapy or combination antibiotic/antibody treatment. (Figure 2d). Combination therapy reduces IL-6 to near-baseline detection limits and is significantly reduced compared to i.v. ceftazidime monotherapy. Serum IL-6 levels after combination therapy show a significant and synergistic reduction compared to placebo or either monotherapy, consistent with low bacterial burden (Figure 2c) and survival synergy (Figure 2a and b).

![Figure 2(d)](image)

**Figure 2 (continued):**

(d) Levels of interleukin-6 (IL-6) in serum assayed 12 hours post-challenge with $1\times10^6$ CFU of E. coli following mono- or combination antimicrobial therapy. Combination therapy comprising systemic ceftazidime with locally injected polyclonal antibodies provides substantial reduction in circulating IL-6 over placebo, ceftazidime or locally applied IgG monotherapies (Student's t-test, *p<0.05 comparing ceftazidime versus placebo and IgG monotherapy, **p<0.05 comparing combination therapy versus all treatments).
Discussion

Combining locally applied polyclonal antibodies with sub-optimal systemic i.v. antibiotic prophylaxis produces synergistic survival benefits over that observed for each separate monotherapy. Significantly, mortality resulting from administration of sub-optimal i.v. antibiotic doses was consistently improved by low level, local co-administration of IgG. Survival benefits from combination therapy in both infection models using two different pathogens directly correlate with observed synergistic reductions in both tissue bacterial burdens at several sites, and systemic levels for a cytokine indicator of sepsis (IL-6). The data suggest that prevention of systemization of the infection generally enhances survival, and that such prevention is significantly enhanced using combination therapy over monotherapies or double systemic infusion therapy of both antibiotic and IgG. The interesting question surrounds the mechanism for this effect. Systemic clearance of intraperitoneally administered IgG is known to be rapid (~3 hrs) while human immunoglobulins injected s.c. into murine full thickness burn wounds are first detectable by ELISA in mouse serum after 3 hours (data not shown). Once present in murine systemic circulation, however, human IgG is detectable beyond seven days. Hence, the synergy observed in reducing infection mortality and morbidity in these models could be attributable to the same, unelucidated yet therapeutically beneficial systemic interactions reported between systemic antibiotics and IVIG. Improved, synergistic benefit observed for the combination of systemic antibiotic/local antibody therapy over the efficacy of combination systemic IgG therapy also supports a distinct, locally enhanced contribution from doses of exogenous polyclonal IgG at the sites of infection. Local pathogen opsonization by IgG in the peritoneal cavity can occur immediately, reducing clearance of IgG to systemic circulation. Abundant, endogenous peritoneal macrophages could conceivably become rapidly activated by IgG opsonization of the bacteria as well as by pathogen proliferation, clearing bacteria in early stages of contamination locally prior to systemic spread of infection. Opsonization would not only serve to promote local phagocytic clearance but also hinder or delay rapid pathogen proliferation kinetics necessary to achieve systemization of the infection. Because the burn wound is severely immunocompromised, avascular and often necrotic, clearance of
local infection would rely on local, extravascular transport of fresh IgG, other immunocomponents (e.g., complement), and cellular elements including macrophages, monocytes and neutrophils from the unburned surrounding tissue. Direct injection of IgG into the immune-depleted site overcomes transport limitations for this important component. Lastly, increased capability for IgG binding and neutralization of toxins locally limits septicemia and systemic infection, as supported by IL-6 results.

Sub-optimal systemic antibiotic monotherapy has shown reduced efficacy against pathogens, but in conjunction or in combination with other antibiotics, synergistic effects have been previously observed. Sub-optimal antibiotic doses were chosen in this study to permit discrimination of therapeutic benefits attributable to IgG in each infection model. Specifically, sub-optimal prophylactic antibiotic doses for each infection model were calculated from published human equivalent full clinical i.v. ceftazidime doses relevant to treating pediatric burn wounds or adult peritonitis. The full equivalent antibiotic monotherapy doses in mice (440 or 500 µg/animal i.v., respectively) produced 75% survival in the burn wound model and 100% survival in the peritonitis model, regardless of initial bacterial challenge between $10^2$ and $10^6$ CFU (data not shown). Ceftazidime reduction to 10% of the full dose constitutes a legitimate regimen to determine antimicrobial synergy and produced consistent infection mortality while permitting significant influence of local IgG administration.

Observed anti-infective benefits using combinations of antibiotics and antibodies might be general to many other infections and could have important clinical implications for both reducing selective pressure involved with producing antibiotic resistance, improving the performance and extending the clinical lifetime of current front-line antibiotics facing resistance, as well as in treating antibiotic resistant infection. Combination antibiotic/antibody therapies might improve patient health, provide better clinical control of antibiotic resistant pathogens and reduce costs by reducing dose and simplifying delivery requirements for antimicrobial antibodies administered locally and directly to the site of infection in tandem with routine systemic antibiotics. Importantly, reduction of systemic antibiotic levels by sub-optimal dosing mimics a possible future clinical
scenario in which selective pressures encouraging ceftazidime resistance are decreased while optimizing therapeutic benefit against infection using exogenous, locally delivered antibodies\textsuperscript{11}.
References


