Local application of human IgG to prevent biomaterial-centered bacterial infection
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2000

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Download date: 17-12-2018
Summary
More than a century ago, medical doctors and researchers began isolating and applying sera from healthy donors to treat patients suffering from various infectious diseases. Early in this century, antibody infusion was the standard treatment for pneumococcal pneumonia and meningitis until antibiotics replaced them in the 1940’s. The interest in antibody therapy then faded away, but with increases in immunocompromised individuals (e.g., HIV, elderly and obese patients and low birth-weight neonates) and antibiotic resistant pathogens, renewed attention and research efforts are focussed on antibody therapy. After approval of intravenous immunoglobulin products (IVIG) for human use by the FDA in 1981 clinical trials demonstrated large benefits of IgG therapy for neonates and immunocompromised patients to overcome lethal bacterial and viral infections. The blood-derived IgG product became safer after solvent/detergent treatment in addition to Cohn’s original cold-ethanol fractionation process and highly purified hyperimmune globulins are now routinely manufactured, directed specifically against virulent pathogens. Pooled human polyclonal IgG could become a significant therapeutic in the approaching post-antibiotic era, because IVIG is an ‘up-to-date’ pooled human immunoglobulin product comprising thousands of specific opsonic antibodies that can improve clearance of bacteria, viruses and toxins by the host immune system. This could provide clinicians with an important therapeutic in the battle against antibiotic resistant organisms.

However, systemic, prophylactic IgG treatment is expensive, requiring large quantities of IVIG to be administered over time to achieve sufficient tissue levels to potentiate the immune response against invading pathogens. Direct, local application of IgG could overcome distribution and diffusion barriers from systemic application, reduce the required dosages per treatment and risks of adverse side effects and decrease the costs associated with IgG treatment. These were the main reasons, as described in Chapter 1, to investigate the efficacy of locally applied, pooled human IgG to reduce or prevent bacterial infection.
Chapter 2 describes the direct effect of pooled human IgG on the in vitro adherence of Pseudomonas aeruginosa IFO3455 in a parallel plate flow chamber. Adherence of IgG to this bacterium and pre-adsorption of IgG to the glass surface both effectively reduced initial pathogen deposition rates and subsequent surface colonization and growth. Bacterial surface characteristics changed in the presence of opsonized IgG as measured by zeta-potentials, water contact angles and X-ray photoelectron spectroscopy. Water contact angles dropped from 120 degrees to 64 degrees after IgG opsonization, while XPS elemental ratio changes for nitrogen, oxygen and phosphorus each compared to carbon were consistent with the adsorption of antibodies (e.g., amide groups) to the bacterial cell wall surfaces.

Based on results from three P. aeruginosa strains (IFO3455, M2 and MSRI7072), Chapter 3 describes the prophylactic protection of local IgG against peritonitis in a murine model. Host survival was significantly increased in a dose-dependent matter following local intraperitoneal application of IgG and lethal bacterial challenges: 100% survival up to 6 hours prophylactically or at the time of bacterial challenge with 10mg of IgG. Therapeutic 10mg IgG treatment administered up to 12 hours post-challenge also significantly increased host survival. Tissue bacterial burden was significantly reduced in the liver, peritoneal lavage and the blood after IgG treatment, correlating with reduced serum IL-6 levels as a marker for sepsis.

To study the efficacy of local IgG to prevent biomaterial-centered infection, a new spinal implant infection model in rabbits was initially developed and described in Chapter 4. The incidence of postoperative spinal implant infection in adult spinal surgery is up to 8% due to the length of the procedure, the amount of tissue damage and the creation of dead space necessary for the implantation of spinal stabilizing systems -- the highest rates in postoperative wound infection. In the rabbit spinal implant infection model, a virulent methicillin resistant Staphylococcus aureus strain (ATCC33593) was used to inoculate three non-contiguous spinal implant sites with stainless steel, threaded Kirschner-wires ('K-wire', orthopaedic osteosynthesis material) after partial laminectomy to create the dangerous ‘dead-space’ defects. Inoculation of the sites with $5 \times 10^2$ CFU
appeared to cause a consistent, biomaterial-centered infection in all challenged sites, while cross-contamination and systemic spread of the infections were prevented. Control procedures lacking K-wire implantation required substantial higher inocula (10⁴ CFU MRSA) to establish an infection, proving the local immune compromising effect of the biomaterial implant.

The spinal implant infection model in rabbits described in Chapter 5 was then used to study the efficacy of IgG, locally applied to the site of spinal implantation and bacterial challenge to prevent postoperative biomaterial-centered infection. Multiple aqueous lavages of isotonic saline were compared to the same procedure using 1 wt% pooled human IgG applied directly to the surgical implantation sites. Since three sites could be used in each rabbit, the total number of animals necessary for this study could be reduced by two-third, while IgG lavaged sites and saline-lavaged controls were present in one animal. Visually observed clinical signs of infection were supported by bacterial enumeration from multiple biopsied tissue and bone sites post-mortem, 7- and 28 days post-surgery. Clinical signs of postoperative infection were significantly reduced in the IgG-lavaged sites, while bacterial enumeration also exhibited statistically significant reductions in the soft tissues and bone, and on the stainless steel implants using IgG lavage compared with saline. After 28 days, complete healing of the surgical wounds was seen in all sites.

In the battle against infection, combination therapies comprising multiple intravenous antibiotics alone or in tandem with either intravenous immunoglobulins or local antibiotics have all been investigated and used in clinical scenarios. In Chapter 6, the potentiation of systemic antibiotics by locally applied IgG is described, using two different in vivo murine models. The anti-microbial efficacy of ceftazidime (third generation cephalosporin) against both E. coli induced peritonitis and K. pneumoniae induced burn-wound infection was significantly improved in combination with locally applied IgG. Synergistic improvements in animal survival by reduced sepsis indicators and bacterial burden post-mortem were observed with this treatment combination. Because the additional immunotherapy functions independently of antibiotic resistance
mechanisms, local delivery of polyclonal or monoclonal antibodies together with routine intravenous antibiotics could confer, also in the clinical situation, improved protection against infection and enhance the efficacy of front-line antibiotics.

**Chapter 7** describes the experiments with a newly developed abdominal implant infection model in mice to study the efficacy of local IgG in a hydrogel carrier to prevent polymer biomaterial-centered infection with MRSA, and *P. aeruginosa* strains IFO3455 and M2. During open abdominal surgery, a polypropylene mesh implant (1x1cm.) was introduced into the abdominal cavity of the mice and challenged with different amounts of bacteria. Subsequently, 0.5ml of hydrogel with or without 10mg IgG was used to cover the implant. *In vivo* IgG antibody release from the hydrogel was rapid to surrounding tissues, nearing a 100% *in vitro* after 48 hours in PBS. Although released IgG alone or in combination with the systemic antibiotics cefazolin or vancomycin did not enhance survival in mice challenged with MSRA (100% mortality after 72 hours), significantly improved survival was observed in cohorts of mice against both gram-negative *P. aeruginosa* strains with IgG monotherapy released from the hydrogel.

The results of these studies are discussed in the perspective of current antibiotic-resistance problems and poly- and monoclonal antibody developments in **Chapter 8**, the general discussion of this thesis.

In summary, this thesis describes different studies investigating the efficacy of local application of pooled human IgG against bacterial, biomaterial-centered infection. Although local application of IgG appeared to be more efficacious than systemic IVIG administration in *in vivo* studies and could be administered in lower dosages to achieve those improved benefits, complete prevention of bacterial infection with single prophylactic applications of local IgG was not possible. Clearance of encapsulated microorganisms requires large amounts of specific antibodies for successful opsonization. These quantities are only available in highly specific hyper-immune globulins or monoclonal antibody cocktails, but unfortunately not in pooled human immunoglobulin products. However, once combined with standard intravenous antibiotic
therapy, even synergistically improved anti-infection results were achieved in some cases with combinations of antibiotics and the local application of pooled human IgG.

The combination of systemic antibiotics with local IgG could provide clinicians with an alternative prophylactic therapeutic treatment against antibiotic-resistant organisms. By combining these antibiotics additionally with locally applied hyperimmune sera or monoclonal antibody cocktails against specific bacteria for high-risk individuals, the required dosages can be reduced while achieving maximum protection of the host against disease-causing organisms at the site of surgical implantation. Immediate postoperative infection and probably also latent biomaterial-centered infections can be reduced if contaminating bacteria can be prevented from compromising initial tissue integration. This will hopefully result in the achievement of the ultimate goal of this research: an increased quality of life for many patients receiving artificial joints and other implanted biomaterials that restore both form and function as well as newly designed tissue engineered scaffold implants.

Because the use of implanted devices will only increase, the magnitude of the associated infection problem must be addressed with solutions that overcome antibiotic resistance. Exploitation of natural immune mechanisms is an important alternative to conventional therapies with the direct application of antibodies as an attractive addition to this mechanism.