Effects of igM-enriched solution on polymorphonuclear neutrophil function, bacterial clearance, and lung histology in endotoxemia

Axel R. Heller, Sebastian N. Stehr, Lilla Knels, Christian Weissflog, Johannes Schober, Dirk Haufe, Amelie Lupp, Thea Koch

Angaben zur Veröffentlichung / Publication details:

Heller, Axel R., Sebastian N. Stehr, Lilla Knels, Christian Weissflog, Johannes Schober, Dirk Haufe, Amelie Lupp, and Thea Koch. 2008. "Effects of igM-enriched solution on polymorphonuclear neutrophil function, bacterial clearance, and lung histology in endotoxemia." *Shock* 29 (2): 167–72. https://doi.org/10.1097/shk.0b013e318067df15.

EFFECTS OF IGM-ENRICHED SOLUTION ON POLYMORPHONUCLEAR NEUTROPHIL FUNCTION, BACTERIAL CLEARANCE, AND LUNG HISTOLOGY IN ENDOTOXEMIA

Sebastian N. Stehr,* Lilla Knels,* Christian Weissflog,* Johannes Schober,* Dirk Haufe,* Amelie Lupp,† Thea Koch,* and Axel R. Heller*

**Department of Anaesthesiology and Intensive Care Medicine, University Hospital Carl Gustav Carus, Dresden; and †Institute of Pharmacology and Toxicology, Medical Faculty of the Friedrich-Schiller-University Jena, Germany*

ABSTRACT—Immunological interventions in endotoxemia and sepsis have been tested in experimental and clinical studies. Our group evaluated the effects of an immunoglobulin (Ig)M-enriched solution in an established model of Gram-negative bacteraemia. Ten New Zealand White rabbits (2 – 3 kg) were randomized to a treatment or control group. In both groups, LPS was infused at a rate of 40 mg kg^{-1} h^{-1}. Immunoglobulin M–enriched solution (Pentaglobin; 2 mL kg^{-1} h^{-1}) was applied in the intervention group 15 min after beginning LPS infusion. 1×10^8 colony forming units of Escherichia coli were injected 30 min after LPS infusion was commenced. Baseline hemodynamic and respiratory parameters, blood *E. coli* concentration (30 min before and 1, 15, 30, 60, 90, 120, and 180 min after *E. coli* injection), polymorphonuclear neutrophil oxidative burst activity, and phagocytosis dead space (both 30 min before and 1, 15, 60, 120, and 180 min postinjection) were measured. *Ex vivo* phagocytosis activity was measured in a separate experiment and evaluated by electron microscopy. Diffuse alveolar damage (DAD) was measured. Organ colonization (kidney, lung, liver, spleen) was assessed in aseptic organ samples. Hemodynamic parameters did not differ between the two groups. Bacterial blood clearance was not influenced by application of IgM-enriched solution. Liver and spleen colonization was significantly reduced in the IgM group. Immunoglobulin M–enriched solution reduced *in vitro* residual phagocytosis capacity at 30, 90, and 180 min and improved respiratory burst at 180 min. Correspondingly, *ex vivo* phagocytosis activity as documented by electron microscopy was increased in the IgM group.The sum of all weighted DAD scores (except overdistension) was significantly better in the IgM group (23 ± 5 vs. 30 ± 8). Immunoglobulin M–enriched solution significantly improved six of seven DAD score parameters and reduced liver and spleen *E. coli* count. Residual phagocytosis capacity was significantly decreased in the IgM group, whereas burst activity was increased, pointing to an increased *in vivo* phagocytosis efficiency. Short-term IgM-enriched solution intervention had an especially beneficial effect on LPS-induced pulmonary histological changes.

KEYWORDS—IgM-enriched solution, respiratory burst, phagocytosis, neutrophils, bacterial clearance, bacterial killing, *E. coli*, lung histology

INTRODUCTION

Despite extensive research, the mortality rate of patients with sepsis is still high. Systemic sepsis is a predisposing factor for pulmonary inflammatory pathology (1), and acute respiratory distress syndrome is therefore commonly encountered in patients with sepsis (2). The inflammatory cascade leading to pulmonary damage in Gram-negative sepsis is initiated by bacterial endotoxin (LPS) (3). Antibiotic treatment does not influence existing bacterial endotoxin load at the time of application. In some cases, antibiotic pathogen disintegration may even aggravate endotoxin release from bacteria (4).

Our group has examined the effects of treatment strategies such as class G immunoglobulin application (5), NO synthase inhibition (6), and low-dose hydrocortisone treatment (7) on bacterial clearance and host defense.

Pentaglobin is an immunoglobulin (Ig)M-enriched solution consisting of 38 g L^{-1} IgG, 6 g L^{-1} IgM, and 6 g L^{-1} IgA, and also toxic-binding and neutralizing antibodies to various Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas* sp., and *Klebsiella* sp (8, 9). Immunoglobulins neutralize endotoxins by scavenging LPS, stimulating opsonizing, and reducing proinflammatory mediators (10). Recent clinical trials showed that the endotoxin-neutralizing effects of IgM are superior to those of IgG and improve sepsis outcome (11). Lower procalcitonin plasma levels were found in patients treated with IgM-enriched solutions (12). In a study comparing the outcome of intensive care unit patients with abdominal sepsis treated with Pentaglobin in addition to antibiotics versus sole antibiotic therapy plus 5% albumin, the authors describe a 20% reduction in the mortality rate that was not significant, probably due to a small sample size (13).

In this study, we evaluated the effects of IgM-enriched solution in an established model of endotoxemia. We hypothesized that administration of a substitution dose (nonhigh dose) of IgM-enriched solution would improve bactericidal activity without untoward effects on tissue integrity of secondary

Address reprint requests to Sebastian N. Stehr, Klinik für Anaesthesiologie und Intensivtherapie, Universitätsklinikum Carl Gustav Carus, Fetscherstrasse 74, D-01309 Dresden, Germany. E-mail: sebastian.stehr@gmx.de.

This study was supported by the Research Fund of the Medical Faculty Carl Gustav Carus Dresden (MedDrive), by Biotest AG, Dreieich, Germany, and institutional funding by the Department of Anesthesiology, University of Dresden, Germany.

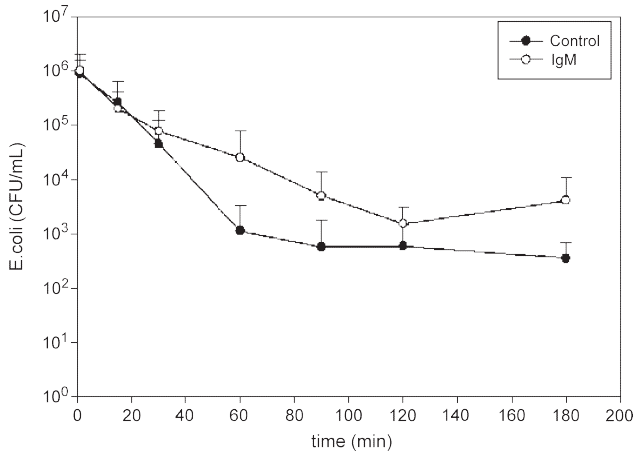

FIG. 1. ***Escherichia coli*** **blood clearance (mean ± SD) of controls and animals receiving IgM-enriched solution after injection of 10^8 CFU *E. coli*.**

hyperinflammatory tissue damage. Systemic *E. coli* elimination kinetics, bacterial tissue distribution in the lung, liver, spleen, kidney, residual granulocyte phagocytosis activity and respiratory burst activity, and lung histology were assessed with a special focus on early pulmonary pathological changes.

MATERIALS AND METHODS

Animal model

The experiments were performed in agreement with the local government's commission for animal protection (AZ 24-9168.11-1-2004-10). Care and handling of the animals were in accordance with the National Institutes of Health guidelines.

Ten New Zealand rabbits weighing 2 to 3 kg were anesthetized with 50 mg kg^{-1} ketamine (Parke Davis, Freiburg, Germany) and 4 mg kg^{-1} xylazine (Bayer, Leverkusen, Germany) and were anticoagulated with heparin-sodium (1,000 IE kg^{-1}) injected into an ear vein catheter as described previously (6). Briefly, the animals were placed in a supine position on a temperature-controlled (37°C) operating table. After tracheotomy and intubation, the rabbits were mechanically ventilated with an Fio_2 of 0.3 (tidal volume, 30 mL; frequency, 30 min^{-1}; peak airway pressure of 20 cmH_2O, then adapted to keep normal blood gas analysis values) via respirator (Cato, Dräger, Lübeck, Germany) during the entire observation period. A polyvinyl chloride catheter (ID, 1.4 mm) was inserted into the left carotid artery for measurements of the arterial blood pressure and for collection of arterial blood samples. Anesthesia was maintained by a continuous infusion of ketamine (5–10 mg kg^{-1} h^{-1}) and xylazine (0.5 – 1.5 mg kg^{-1} h^{-1}). In addition to the basal fluid requirement of 3 to 4 mL kg^{-1} h^{-1}, blood loss from sampling was replaced by isovolemic injection of normal saline.

Monitoring

After instrumentation of the rabbits, arterial pressure, rectal temperature, and electrocardiogram were continuously monitored via Statham strain gauge transducers connected to an SC 6000P recorder (Siemens, Munich, Germany). To ensure adequate mechanical ventilation, blood samples were drawn intermittently for measurements of O_2 saturation, pH, partial oxygen pressure, and partial carbon dioxide pressure (Radiometer ABL 505; Copenhagen, Denmark). Furthermore, hemoglobin values and hematocrit, as well as differential blood counts and serum lactate levels were obtained. The polymorphonuclear neutrophil (PMN) burst and residual phagocytosis activity were determined according to the experimental protocol.

Bacterial inoculum

An encapsulated, serum-resistant, nonhemolytic strain of *E. coli* (O111) with a smooth LPS phenotype was titrated to a density of 10^8 colony-forming units (CFU) mL^{-1} and frozen in aliquots at −20°C until use. The dose was chosen according to previous experiments (5–7).

Immunoglobulin M

An IgM-enriched solution (Pentaglobin) was a kind gift of the manufacturer (Biotest AG, Dreieich, Germany). One milliliter of solution contains 50 mg plasma proteins more than 95% of these are immunoglobulins (ca 6 mg IgM, ca 6 mg IgA, and ca 38 mg IgG). Immunoglobulin G subclasses are distributed into IgG1, 63%; IgG2, 26%; IgG3, 4%; and IgG4, 7%. The dosage was chosen according to a low-substitution dose as specified by the manufacturer. A plasma fraction that mainly contains IgM and IgA is used in production of IgM-enriched solution. Octanoic acid and β-propiolactone treatment are important virus inactivation methods (14). Therefore, the risk of viral disease transmission is negligible, and no case of viral transmission has been reported up to date.

Experimental protocol

Each experiment lasted 240 min. The first 30 min were used for stabilization after tracheotomy etc. (t_{-60} − t_{-30}). After this 30-min period, blood samples were collected for baseline measurements (t_{-30}), and a 40-mg^{-1} kg^{-1} h LPS infusion (Sigma, Deisenhofen, Germany) was started for the complete duration of the experiments. At t_{-15}, animals were randomly assigned to either the IgM-enriched solution or control group, receiving a 2-mL kg^{-1} h^{-1} IgM-enriched solution infusion or a 2-mL kg^{-1} h^{-1} NaCl 0.9% (n = 5 per group), respectively. At t_0, a standardized amount of *E. coli* (10^8 CFU suspended in 1 mL of tryptic soy broth) was injected into the ear vein catheter of all animals.

For analysis of blood gases, leukocyte counts, hematocrit, and hemoglobin concentrations and bacterial blood clearance arterial blood was aseptically drawn at t_{-30}, t_0, t_1, t_{15}, t_{30}, t_{90}, and t_{180}. Polymorphonuclear neutrophil oxidative burst and residual phagocytosis activity were determined from arterial blood at t_{-30} and at t_1, t_{15}, t_{30}, t_{90}, and t_{180}. At the end of the experiment (after 240 min), the animals were killed with an overdose of ketamine and xylazine. Subsequently, tissue samples of liver, spleen, kidney, and the left lung were removed under aseptic conditions for bacterial cultures. The right lung was fixed by intrabronchial application of buffered 4% formaldehyde with a perfusion pressure of 20 cm H_2O for 20 min and then continuously fixed by immersion in the same solution for 7 days. One sample from a central (next to the main bronchus) and two from a peripheral-dependent and nondependent region were embedded in paraffin, stained with hematoxylin-eosin, and cut in slices for morphometric and histological analysis according to routine histological procedures.

Quantitative microbiology

Blood and tissue samples were immediately assayed in duplicate. Blood samples were serially diluted in sterile saline. One hundred microliters each of whole blood and of blood in various states of dilution were plated onto cysteine lactose electrolyte–deficient agar plates according to Sandys (15). Aseptically collected organs, liver, spleen, lung, and kidney were weighed, and 1 g of each organ was homogenized in 1 mL of sterile saline. Serial dilutions of tissue suspension (100 mL) were plated onto cysteine lactose electrolyte–deficient agar

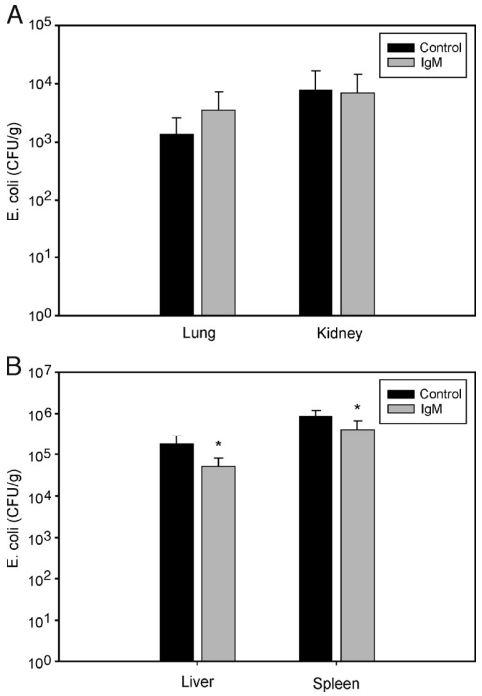

FIG. 2. ***Escherichia coli*** **organ culture counts (mean ± SD; *$P < 0.05$) 3 h after injection of 10^8 CFU *E. coli* in controls and after IgM-enriched solution in lung and kidney (A) and liver and spleen (B).**

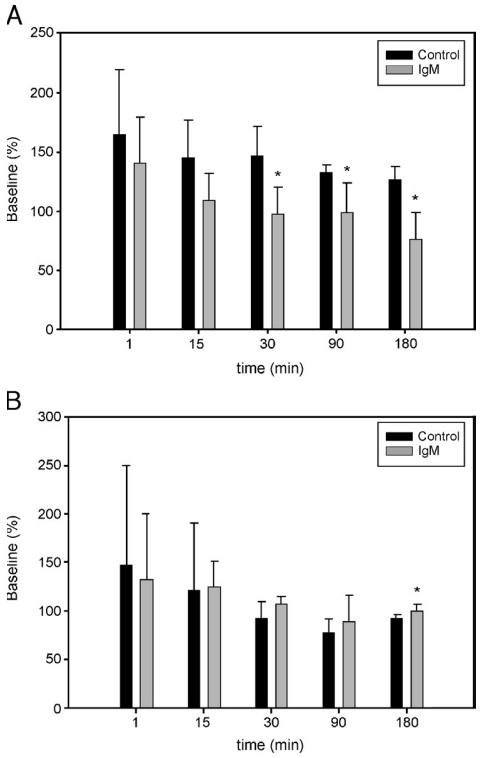

FIG. 3. **Polymorphonuclear neutrophil residual phagocytosis (A, mean ± SD, *$P < 0.05$) in percent of baseline measurements (t_{-30} min) of FITC-marked *E. coli* after *in vivo* bolus administration of unlabeled *E. coli*.** Polymorphonuclear neutrophil burst activity (B, mean ± SD; *$P < 0.05$) in percentage of baseline measurements (t_{-30} min) in controls and after IgM-enriched solution.

plates. After incubation of the cultures at 37°C for 24 h, respective colonies of *E. coli* were counted. The final bacterial concentration was calculated as numbers of CFU milliliter of blood, or as CFU per gram of tissue.

PMN burst activity

The extent of intracellular radical oxygen species production was determined using samples of freshly drawn heparinized blood. The oxidative burst of PMN was determined by means of a test kit (Bursttest; Orpegen Pharma, Heidelberg, Germany). Single-cell analysis was performed by laser flow cytometry (FACScan/Lysis II; Becton Dickinson, Heidelberg, Germany). The method of the quantitative assay for monitoring the oxidative burst has been described in detail in a previous study (6). In a brief outline, two assays (100 mL) of each sample were incubated (10 min, 37°C) with either phosphate-buffered saline (PBS; negative control) or with opsonized *E. coli*, a potent stimulator for cellular production of reactive oxygen intermediates. After an oxidization step (10 min, 37°C) in which the nonfluorescent substrate dihydrorhodamine 123 was taken up by the phagocytes and converted into a green fluorescent compound (rhodamine 123) through respiratory burst metabolites, the whole blood is lysed and fixed. This oxidation process is highly specific for the radical oxygen species. To guarantee that no cell debris or bacteria interfere with the measurements, DNA staining was performed with propidiumiodide (red fluorescence, FL-3). Ten thousand cells of each sample were measured with the laser flow cytometer using blue-green excitation light (488-nm argon laser). The gain setting of the photomultiplier was adjusted to register all intensities of cellular fluorescence within the scale of 256-channel resolution (four decades). Neutrophils were identified by their characteristic size to granularity ratio in the forward versus sideward scatter plot. Only the selected cell population was considered for further analysis using WinMDI 2.0 (J. Trotter, Scripps Research Institute, La Jolla, Calif). During data acquisition, a live "gate" was set in the red fluorescent dot plot on those events that had at least the same DNA content as the rabbit diploid cells. Only the mean intracellular rhodamine 123 fluorescence of these cells, representing PMN burst activity, was analyzed in mean channel fluorescence.

PMN phagocytosis dead space

A test kit (Phagotest; Orpegen) was used to evaluate the residual *ex vivo* phagocytic activity of PMN in whole blood after animals had received a bolus load of unlabeled *E. coli* as described in the experimental protocol at t_0. Thus, the obtained *ex vivo* values represent the idle PMN phagocyte capacity (PMN dead space) in our model, which is an inverse measure of the *in vivo* PMN phagocyte activity. Single-cell analysis was performed by flow cytometry. The quantitative method of assaying phagocytic activity has been previously described in detail (7). Four assays of each sample (2 × 100 μL) were incubated with fluoresceine-isothiocyanate (FITC)–marked opsonized *E. coli* for 10 min either at 0 (negative control) or at 37°C to allow phagocytosis of bacteria. To exclude extracellular bacteria from measurement, two assays were quenched with a staining solution. Further steps such as lysis, fixation, DNA staining, and data acquisition by flow cytometer were performed in the same manner as in the burst assay. The PMN residual phagocytosis capacity was determined by the intracellular content of FITC-marked *E. coli*, expressed as mean channel fluorescence per cell.

Histopathological analysis–diffuse alveolar damage score

All further examinations of histological samples were performed using digitalized photomicrographs of three nonoverlapping fields for each hematoxylin-eosin stained specimen of the right lung (DM RB; Leica, Wetzlar, Germany). Images were digitalized and processed by means of a computer-based system and imaging-analyzing software (AnalySIS, version 3.1; Soft Imaging System, Munster, Germany). Diffuse alveolar damage (DAD) was quantified systematically by an investigator (L.K.) blinded to the experimental protocol and the therapy groups using a DAD scoring system.

The pathologic features of the lung in adult respiratory distress syndrome are characterized under the term "DAD" (16). The histopathological characteristics of our scoring system were adapted from the literature (17) and modified to include a weighting system similar to that proposed by Broccard et al. (18). This modification was performed to improve the quantification of the pathologic features according to their extension. We restricted the numerous features described in the literature to seven basic ones, which are most representative for the early exudative phase of DAD, namely, alveolar edema, interstitial edema, hemorrhage, inflammatory infiltration, epithelial destruction, microatelectasis, and overdistension. The severity of the feature characteristic scored as follows: 0, normal appearance; 1, slight effect; 2, middle effect; and 3, severe effect. Furthermore, we described the percentage of involvement in each field of view as follows: 0, lack of involvement of the feature (0%); 1, 1% to 25%; 2, 26% to 50%; 3, 51% to 75%; 4, 76% to 99%; and 5, total involvement of the field (100%). To

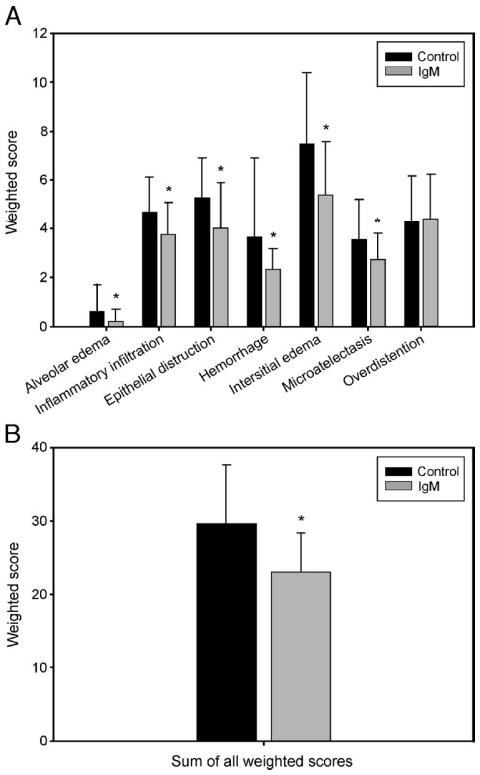

FIG. 4. **A, Diffuse alveolar damage score of controls and animals after IgM-enriched solution after injection of 10^8 CFU *E. coli*.** B, Sum of all weighted DAD scores of controls and animals after IgM-enriched solution after injection of 10^8 CFU *E. coli* (*$P < 0.05$).

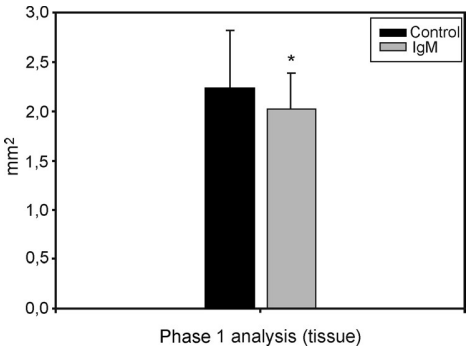

FIG. 5. **Histological phase analysis of lung slices in controls and animals after IgM-enriched solution (*$P < 0.05$).** Phase 1 represents tissue and edema (nonaerated) as opposed to aerated areas in histological slices.

calculate a weighted histological score, the severity was multiplied by the percentage of involvement.

Morphometric analysis

For morphometric analysis, digitized photomicrographs were binarized (AnalySIS, version 3.1, Soft Imaging System), with black portions representing parenchyma, edema, or infiltration (nonaerated), and white portions representing aerated areas.

Sample preparation for ultrastructural studies of bacterial phagocytosis

After approval by the local ethics committee and obtaining informed consent, heparinized venous blood was collected from healthy volunteers. Morphological studies were performed separately on populations of neutrophils (PMN) and peripheral blood mononuclear cells. Polymorphonuclear neutrophils were isolated from whole blood that was incubated for 45 min with an equal volume of dextran 6% (Gentran 70, Baxter, Lessines, Belgium) to allow red blood cell sedimentation, followed by density gradient centrifugation over Biocoll separation solution (density, 1.077 g mL^{-1}, Biochrom AG, Berlin, Germany). After washing in PBS, the remaining erythrocytes were eliminated by hypotonic lysis for 30 s, and PMNs were resuspended in Dulbecco modified Eagle medium (Biochrom) supplemented with 10% fetal calf serum, yielding a concentration of 3×10^6 cells mL^{-1}. Cell preparations resulted in a population purity of greater than 95% as determined with an automated hematology analyzer (Gen-S, Beckman Coulter, Krefeld, Germany). Before and after *in vitro* cultivation, viability of both PMN was greater than 98% in all experiments as assessed by Trypan blue exclusion.

Cells were incubated for 15 min at 37°C with either IgM-enriched solution or PBS, then 1.2×10^8 CFU mL^{-1} *E. coli* (representing a bacteria to phagocyte ratio of ~40:1) were added, and the samples were incubated for 30 min in a rotating incubator.

For transmission electron microscopy, cell pellets were resuspended in 100 mL 2% fibrinogen (Sigma) and incubated with 40 mL thrombin (100 U mL^{-1}, Sigma) until clot formation was reached. The fibrin clots, including the cells, were fixed in 0.1 M sodium cacodylate buffer (pH 7.4) containing 2.5% glutaraldehyde overnight at 4°C. After clots were postfixed with 1% osmium tetroxide (pH 7.4) in 0.1 M cacodylate buffer for 1 h at 4°C, dehydration was performed with ethanol and ethanol-Epon. Thereafter, clots were Epon-embedded overnight at 4°C, and Epon-filled flat embedding molds were subsequently polymerized for 48 h at 60°C. Ultrathin sections (60 – 80 nm) were mounted on nickel grids and contrasted with lead citrate and uranyl acetate. Ultrastructural studies were carried out using an EM 906 electron microscope (Carl Zeiss, Oberkochen, Germany). All buffers, fixatives, and embedding materials for electron microscopy were purchased from Serva (Heidelberg, Germany).

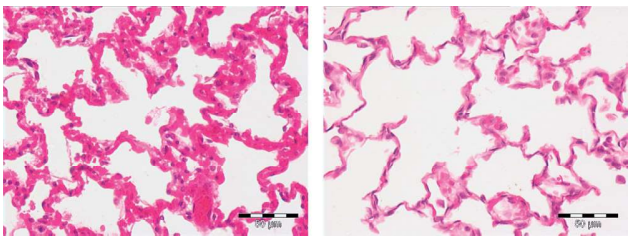

FIG. 6. **Representative lung histology from a peripheral-dependent area stained with hematoxylin-eosin (control, left; IgM-enriched solution, right).**

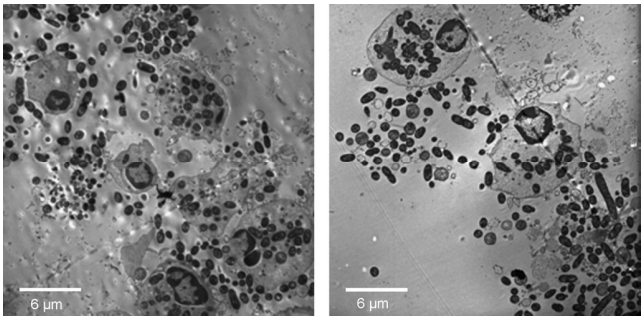

FIG. 7. **Representative electron microscopy of PMN without (left) or with (right) preincubation with IgM-enriched solution.**

Statistical analysis

Data are presented as arithmetic mean ± standard deviation. Single measurements between the groups were compared with unpaired two-sided Student *t* test. Between-group differences after repeated measurements were tested by general linear model statistics according to a 2-way ANOVA. Significance was accepted at $P < 0.05$. Statistical analysis was performed with SPSS software for MS Windows (release 10.0.7; SPSS Inc., Chicago, Ill).

RESULTS

Baseline hemodynamic and blood gas

Baseline measurements of MAP were 69 ± 4 (control) vs. 69 ± 8 mmHg (IgM) and did not significantly differ among the groups. Blood gas, differential blood counts, and serum lactate levels were comparable at baseline and did not differ during the time course of the experiment (data not shown).

Bacterial blood clearance and organ culture

Bacterial clearance was not significantly altered by the intervention with IgM (Fig. 1).

Bacterial count in the respective organ cultures also did not significantly differ between the groups for kidney and lung samples (Fig. 2A). In spleen and liver organ samples (Fig. 2B), CFU counts per gram were significantly reduced in the IgM group ($P < 0.05$).

Phagocytosis/burst activity

At 30, 90, and 180 min, residual PMN phagocytosis activity, as indicated by additional uptake of FITC-marked *E. coli*, was significant lower in the IgM group as compared with the control group (Fig. 3A). Comparison of PMN burst activity revealed a significant augmentation in the IgM group at 180 min (Fig. 3B).

DAD score

For all DAD parameters, IgM intervention led to a significant improvement in comparison to the control group except for overdistension (Fig. 4A; $P < 0.05$). The sum of all weighted scores was also significantly better in the IgM group (23.2 ± 5.2 vs. 29.7 ± 8.0; Fig. 4B; $P < 0.05$).

Phase analysis

Phase analysis showed a significant increase of the tissue phase in the control group in comparison to the IgM group

(2.246 ± 0.57 vs 2.031 ± 0.35 mm^2; Fig. 5; $P < 0.05$). A representative histological slice can be seen in Figure 6.

Ultrastructural studies of bacterial phagocytosis

Electron microscopy analysis of human PMN after incubation with or without IgM-enriched solution revealed an increase in intracellular *E. coli*, as seen in the representative slice in Figure 7.

DISCUSSION

During sepsis and endotoxemia, both proinflammatory and anti-inflammatory cascade systems are initiated at the same time, resulting in a mixed antagonistic response syndrome (19). Overstimulated neutrophils have been identified as a key mechanism for the development of tissue injury in the course of sepsis. However, therapeutic blockade of inflammatory cascade systems, for example, neutrophil oxygen radical generation and consequently reduction of collateral oxidative tissue injury, may, on the opposite, impede organism host defense (20). Use of immunostimulating therapies on the other hand may *vice versa* induce additional tissue injury. Well-directed immunomodulation can be one possible way to solve the problem of augmenting host defense and at the same time limiting self-destructing hyperinflammation. The main goal of this study was to describe the short-term effects of a low-dose substitution dose of an IgM-enriched solution in a rabbit endotoxemia model.

We can document a striking pulmonary protective effect of IgM. Using this model, our group has previously examined the short-term effects of IgG (5), *N*-nitro-l-arginine methyl ester (6), and low-dose hydrocortisone (7). As for the past series, the *E. coli* dose we chose was based on controls where this concentration showed reproducible elimination kinetics and organ distribution without resulting in hemodynamic changes. Although allowing excellent detection of *in vivo* effects on bacterial clearance, this model is based on endotoxemia, a situation seen only in few clinical infections.

In this set of experiments, IgM-enriched solution did not significantly affect bacterial blood elimination kinetics (Fig. 1). Clearance of bacteria seems to follow a biphasic time course, as seen in previous studies with this model. A rapid phase described by an exponential decrease of CFUs within the first minute is followed by a progressively slower clearance. One would expect that bacterial clearance from the blood in animals receiving IgM-enriched solution would be higher because of the opsonization function of IgM, which should facilitate phagocytosis, subsequent engulfment, and disintegration of bacteria, as previously shown by our group with IgG (5). In this current study, we applied a low substitution dose of IgM to avoid excessive immune stimulation and subsequent collateral organ damage. Our results are in line with previous work on IgM effects. In experiments using human serum, Walpen et al. (21) studied the effects of IgM-enriched solution on classical and alternative complement pathway activation in addition to antibactericidal activity. Their experiments documented a specific IgM inhibition on the classical complement pathway without impairment of antibactericidal defense (21). In a study comparing Pentaglobin and i.v. IgG, neither negatively affected *in vitro* phagocytosis of *E. coli* by human granulocytes (22).

Although no significant difference in circulating *E coli* CFU was seen, the examination of IgM effects on organ *E. coli* concentrations at the end of our experiments showed that IgM significantly reduced liver and spleen *E. col*i counts, indicating an improved reticuloendothelial system clearance effect of IgM-enriched solution (Fig. 2). This is especially important in light of reticuloendothelial depression, seen as a main factor in the development of septic events (23) and as a contributor to organ failure (24). No effects on lung and kidney colonization can be distinguished between the IgM and control groups. The cause for this observation may be found in the100-fold lower CFU counts in both organs as compared with liver and spleen ranging within the normal bactericidal organ capacity, whereas killing capacity in liver and spleen was exhausted, and, thus, protective IgM effects were uncovered. The cause for lower bacterial counts in lung and kidney as such have already been reported in previous experiments (7) and were attributed to the prepulmonary immunologic filter function of the liver.

We further assessed PMN phagocytosis and burst activity at different time points during the experiment. Immunoglobulin M–enriched solution improved respiratory burst at 180 min (Fig. 3B) and enhanced *in vivo* phagocytosis as depicted in electron microscopy (Fig. 7), being associated with idle PMN phagocytosis capacity in the control group. This was further quantified by the reduced ability of neutrophil *ex vivo* phagocytosis of FITC-labeled *E. coli* at 30, 90, and 180 min (Fig. 3A). These data are well in line with *in vitro* experiments performed with human PMN and IgM-enriched solutions that have proven to boost *E. coli* phagocytosis activity (25).

A strong reactivity of IgM-enriched solution has been previously shown for multiple important pathogens, including *Klebsiella pneumonia* (26). In a rat acute respiratory distress model, Lachmann et al. (27) found that *K. pneumonia* translocation from the lung into the systemic circulation was reduced after IgM application, signifying a protective IgM effect on the alveolar-capillary barrier. These findings are confirmed by our study. As mentioned above, IgM-enriched solutions have a modulating function on the classical complement pathway (21), and complement pathways are known to play a major role in pulmonary damage (28–30). In our experiments, animals treated with IgM-enriched solution had significantly reduced diffuse alveolar damage, especially concerning alveolar edema, interstitial edema, hemorrhage, inflammatory infiltration, epithelial destruction, and microatelectasis (Figs. 4–6). In the present setting, changes in overdistension score would not have been expected because ventilator inspiratory peak airway pressures in all animals were limited to 20 cm H_2O.

In conclusion, we found that IgM-enriched solution significantly improved six of seven DAD score parameters and enhanced reticuloendothelial system *E. coli* clearance without altering systemic *E. coli* blood clearance. Residual phagocytosis activity was significantly decreased in the IgM group, a finding supported by electron microscopic observations of increased phagocytosis and increased burst activity,

pointing to increased *in vivo* phagocytosis efficiency. This augmentation of host defense was not associated with collateral tissue damage, but had an especially beneficial effect on LPS-induced pulmonary damage.

ACKNOWLEDGMENT

The authors thank Sabine Böttcher (Department of Anesthesiology and Intensive Care Medicine) for excellent technical assistance.

REFERENCES

1. Niederman MS, Fein AM: Sepsis syndrome, the adult respiratory distress syndrome, and nosocomial pneumonia. A common clinical sequence. *Clin Chest Med* 11:633–656, 1990.
2. Repine JE: Scientific perspectives on adult respiratory distress syndrome. *Lancet* 339:466–469, 1992.
3. Cannon JG, Tompkins RG, Gelfand JA, Michie HR, Stanford GG, van der Meer JW, Endres S, Lonnemann G, Corsetti J, Chernow B: Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 161:79–84, 1990.
4. Vianna RC, Gomes RN, Bozza FA, Amancio RT, Bozza PT, David CM, Castro-Faria-Neto HC: Antibiotic treatment in a murine model of sepsis: impact on cytokines and endotoxin release. *Shock* 21:115–120, 2004.
5. Koch T, Heller S, Weber K, Heller A, Urbaschek R: [Effects of human i.v. Immunglobulin on bacterial clearance and granulocyte function in endotoxemia]. *Anasthesiol Intensivmed Notfallmed Schmerzther* 32:420–425, 1997.
6. Stehr SN, Weber S, Heller SC, Weikel J, Hubler M, Koch T, Heller AR: *N*(omega)-nitro-l-arginine methyl ester effects on neutrophil function and bacterial clearance. *Shock* 22:180–185, 2004.
7. Heller AR, Heller SC, Borkenstein A, Stehr SN, Koch T: Modulation of host defense by hydrocortisone in stress doses during endotoxemia. *Intensive Care Med* 29:1456–1463, 2003.
8. Stephan W, Dichtelmuller H, Schedel I: [Properties and efficacy of a human immunoglobulin M preparation for intravenous administration]. *Arzneimittelforschung* 35:933–936, 1985.
9. Dichtelmuller H, Stephan W: [The effect of immunoglobulin M-enriched intravenous immunoglobulins against bacterial infections and on the neutralization of bacterial toxins]. *Arzneimittelforschung* 37:1273–1276, 1987.
10. McCuskey RS, Nishida J, McDonnell D, Baker GL, Urbaschek R, Urbaschek B: Effect of immunoglobulin G on the hepatic microvascular inflammatory response during sepsis. *Shock* 5:28–33, 1996.
11. Kress HG, Scheidewig C, Schmidt H, Silber R: Reduced incidence of postoperative infection after intravenous administration of an immunoglobulin A- and immunoglobulin M-enriched preparation in anergic patients undergoing cardiac surgery. *Crit Care Med* 27:1281–1287, 1999.
12. Tugrul S, Ozcan PE, Akinci O, Seyhun Y, Cagatay A, Cakar N, Esen F: The effects of IgM-enriched immunoglobulin preparations in patients with severe sepsis [ISRCTN28863830]. *Crit Care* 6:357–362, 2002.
13. Rodriguez A, Rello J, Neira J, Maskin B, Ceraso D, Vasta L, Palizas F: Effects of high-dose of intravenous immunoglobulin and antibiotics on survival for severe sepsis undergoing surgery. *Shock* 23:298–304, 2005.
14. Haque KL: Immuno-modulation in neonatal sepsis: intravenous immunoglobulin therapy in the prevention and treatment of neonatal sepsis: is the answer, 'Yes', 'No', or 'Don't know'? *Haematologica Reports* 2:38–41, 2006.
15. Sandys GH: A new method of preventing swarming of *Proteus* sp. with a description of a new medium suitable for use in routine laboratory practice. *J Med Lab Technol* 17:224–233, 1960.
16. Katzenstein AL, Bloor CM, Leibow AA: Diffuse alveolar damage—the role of oxygen, shock, and related factors. A review. *Am J Pathol* 85:209–228, 1976.
17. Ichikado K, Suga M, Gushima Y, Johkoh T, Iyonaga K, Yokoyama T, Honda O, Shigeto Y, Tomiguchi S, Takahashi M, et al.: Hyperoxia-induced diffuse alveolar damage in pigs: correlation between thin-section CT and histopathologic findings. *Radiology* 216:531–538, 2000.
18. Broccard AF, Shapiro RS, Schmitz LL, Ravenscraft SA, Marini JJ: Influence of prone position on the extent and distribution of lung injury in a high tidal volume oleic acid model of acute respiratory distress syndrome. *Crit Care Med* 25:16–27, 1997.
19. Bone RC: Sir Isaac Newton, sepsis, SIRS, and CARS. *Crit Care Med* 24:1125–1128, 1996.
20. Heller AR, Groth G, Heller SC, Breitkreutz R, Nebe T, Quintel M, Koch T: *N*-acetylcysteine reduces respiratory burst but augments neutrophil phagocytosis in intensive care unit patients. *Crit Care Med* 29:272–276, 2001.
21. Walpen AJ, Laumonier T, Aebi C, Mohacsi PJ, Rieben R: Immunoglobulin M-enriched intravenous immunoglobulin inhibits classical pathway complement activation, but not bactericidal activity of human serum. *Xenotransplantation* 11:141–148, 2004.
22. Rieben R, Roos A, Muizert Y, Tinguely C, Gerritsen AF, Daha MR: Immunoglobulin M-enriched human intravenous immunoglobulin prevents complement activation in vitro and in vivo in a rat model of acute inflammation. *Blood* 93:942–951, 1999.
23. Koch T, Duncker HP, Axt R, Schiefer HG, van Ackern K, Neuhof H: Effects of hemorrhage, hypoxia, and intravascular coagulation on bacterial clearance and translocation. *Crit Care Med* 21:1758–1764, 1993.
24. Loegering DJ: Humoral factor depletion and reticuloendothelial depression during hemorrhagic shock. *Am J Physiol* 232:H283-H287, 1977.
25. Tinguely C, Schaller M, Nydegger UE: Mononuclear cells ingest *E. coli* opsonized by investigational intravenous immunoglobulin preparations in the absence of complement more efficiently than polymorphonuclear phagocytes. *Transfus Apher Sci* 25:43–50, 2001.
26. Lissner R, Struff WG, Autenrieth IB, Woodcock BG, Karch H: Efficacy and potential clinical applications of Pentaglobin, an IgM-enriched immunoglobulin concentrate suitable for intravenous infusion. *Eur J Surg Suppl* (584):17–25, 1999.
27. Lachmann RA, van Kaam AH, Haitsma JJ, Verbrugge SJ, Delreu F, Lachmann B: Immunoglobulin M–enriched intravenous polyclonal immunoglobulins reduce bacteremia following *Klebsiella pneumoniae* infection in an acute respiratory distress syndrome rat model. *Exp Lung Res* 30:251–260, 2004.
28. Sarma VJ, Huber-Lang M, Ward PA: Complement in lung disease. *Autoimmunity* 39:387–394, 2006.
29. Heller A, Kunz M, Samakas A, Haase M, Kirschfink M, Koch T: The complement regulators C1 inhibitor and soluble complement receptor 1 attenuate acute lung injury in rabbits. *Shock* 13:285–290, 2000.
30. Heller AR, Fiedler F, Braun P, Stehr SN, Bodeker H, Koch T: Clusterin protects the lung from leukocyte-induced injury. *Shock* 20:166–170, 2003.