Meconium Is a Potent Activator of Complement in Human Serum and in Piglets

ALBERT CASTELLHEIM, PAAL H. H. LINDENSKOV, ANNE PHARO, MICHAEL FUNG,
OLA D. SAUGSTAD, AND TOM E. MOLLNES

Department of Pediatric Research [A.C., P.H.H.L., O.D.S.], Rikshospitalet University Hospital, Oslo 0027, Norway; Institute of Immunology [A.C., A.P., T.E.M.], Rikshospitalet University Hospital, Oslo 0027, Norway; and Tanox Inc. [M.F.], Houston, Texas, U.S.A.

ABSTRACT

Meconium aspiration syndrome (MAS) is a clinical condition in the newborn infant with a significant morbidity and mortality. The complex pathophysiology of MAS, leading to both pulmonary and systemic complications, is characterized by an incompletely understood inflammatory reaction. Treatment is symptomatic, mainly limited to airway cleaning and ventilatory support. In this study, we show for the first time that meconium is a potent activator of complement, a key mediator of inflammation. *In vitro*, meconium activated the alternative complement pathway in human umbilical cord serum as judged by a substantial increase in the alternative pathway convertase C3bBbP. The activation proceeded through C3 (C3bc) and the terminal C5-9 pathway (terminal SC5b-9 complement complex), whereas the classical and lectin pathways were not activated (C1rs-C1-inhibitor complexes and C4bc). The lipid fraction, containing, *e.g.* free fatty acids, and the water fraction, containing, *e.g.* bile acids, contributed equally to the complement activation. A blocking antibody to factor D (alternative pathway) completely inhibited the meconium-induced complement activation, whereas blocking antibodies to mannose-binding lectin (lectin pathway) and C2 (classical and lectin pathway) had no effect. *In vivo*,

meconium induced systemic complement activation in a piglet model of MAS, paralleling the increase in lung dysfunction. In conclusion, meconium is a potent activator of the complement system both *in vitro* and *in vivo*. Complement may be important in the pathogenesis of MAS, and specific complement inhibition might be a possible treatment approach in MAS. (*Pediatr Res* 55: 310–318, 2004)

Abbreviations

MAS, meconium aspiration syndrome
MSAF, meconium-stained amniotic fluid
SIRS, systemic inflammatory response syndrome
HSA, human serum albumin
mAb, monoclonal antibody
MBL, mannose-binding lectin;
EIA, enzyme immunoassay
TCC, terminal SC5b-9 complement complex
LPS, lipopolysaccharide
AU, arbitrary units.
Fio₂, fractional inspired oxygen concentration
OI, oxygenation index

The aspiration of meconium-stained amniotic fluid (MSAF) in the newborn can lead to meconium aspiration syndrome (MAS), which is a clinical syndrome with significant morbidity and mortality (1). MAS might be defined as respiratory distress in an infant who is born through MSAF and whose symptoms cannot be otherwise explained (2). The pathophysiology of lung injury in MAS is complex and includes both mechanical obstruction by meconium and pronounced inflammatory responses (3). It has been postulated that other conditions, including intrauterine infection and chronic and acute asphyxia,

rather than inhaled meconium, may explain the primary pathology of MAS (4). The pathophysiology of MAS (hypoxemia, pulmonary inflammation, and disturbed pulmonary vasoregulation) bears similarities with acute respiratory distress syndrome (5), the latter frequently being a manifestation of the systemic inflammatory response syndrome (SIRS) in the presence or absence of bacterial infection (6, 7).

Lung inflammation by itself can lead to systemic inflammation (8). Leakage of meconium content through the alveolar wall to the lung capillaries may bring substances from meconium in direct contact with blood, leading to systemic inflammation. Furthermore, lung rupture and meconium embolism in chronic intrauterine meconium aspiration has been described (9), which may be an additional mechanism for meconium exposure to blood. Antenatal hypoxia and MAS may result in muscularization in the distal pulmonary vasculature, pulmonary hypertension, and persistent fetal circulation (10) associ-

Received May 15, 2003; accepted August 4, 2003.

Correspondence: Albert Castellheim, M.D., Ph.D., Department of Pediatric Research, Rikshospitalet, 0027 Oslo, Norway; e-mail: albert.castellheim@klinmed.uio.no

Supported financially by Letten Saugstad Fund, The Research Council of Norway, The Norwegian Foundation for Health and Rehabilitation, and Research Council of Rikshospitalet.

DOI: 10.1203/01.PDR.0000100902.76021.8E

ated with more therapeutic difficulties. MAS prevention and management is a subject for current discussion (11, 12). Early steroids have been used as an attempt to control inflammation in MAS (13), however, without significant success to establish this as a therapeutic regimen. Meconium is a potent inhibitor of surfactant, and surfactant replacement therapy has been associated with beneficial effects (14), whereas surfactant lavage has been controversial (15). Severe MAS is a frequent indication for ventilatory support (16) and extracorporeal membrane oxygenation in critical cases (17). However, because the pathophysiology of MAS is poorly understood, appropriate treatment options are still limited. We hypothesized that meconium might activate the complement system, an important mediator of inflammation, and show in the present study that meconium is a potent activator of the alternative pathway of complement in umbilical cord serum and induces a systemic complement activation in experimental MAS.

METHODS

Sera and Reagents

Adult and cord serum were prepared and pooled at 4°C. Adult serum was obtained from five healthy individuals, and cord serum was obtained from seven placentae immediately after delivery (healthy mothers and newborns). Zymosan A (Z-4250) and low molecular weight dextran sulfate (average molecular weight, 5000; D-7037) were purchased from Sigma (St. Louis, MO, U.S.A.), and human serum albumin (HSA; 200 mg/mL, Vnr. 478172) was purchased from Octapharma (Vienna, Austria). C1 inhibitor (Berinert) was a gift from Aventis (Marburg, Germany).

Antibodies

Mouse monoclonal antibodies (mAbs) inhibiting C2 (clone 175-62, IgG1) and factor D (clone 166-32, IgG1) and an isotype-matched control mAb (clone G3-519, anti HIV1 gp120, IgG1) were produced under identical conditions in one of the co-authors' laboratory (M.F.) (18). Anti-mannose-binding lectin (MBL; mAb HYB 131-01) was purchased from Antibody Shop (Copenhagen, Denmark).

Enzyme Immunoassays

Human complement activation products were measured in enzyme immunoassays (EIAs) based on mAbs to activation products of the different pathways. Assay of C1rs-C1-inhibitor complexes (C1rs-C1-inhibitor) was used to determine the degree of activation of the classical pathway as described previously (19). Activation of C4, indicating classical and/or lectin pathway activation, was detected by C4bc as described previously (20). Alternative pathway activation was measured by C3bBbP formation (21), and activation of C3, common for all initial pathways, was detected by C3bc formation (22). TCC (terminal SC5b-9 complement complex) was quantified principally as described previously (23).

Porcine TCC was quantified in EIA using the mAb aE11, which cross-reacts with pig (24), as capture antibody diluted in PBS and incubated in microtiter plates (COSTAR EIA plate 3590;

Corning Inc., Corning, NY, U.S.A.) overnight (50 μ L/well). As standard, Zymosan-activated human serum was made by incubation of 10 mg of Zymosan (Sigma) per milliliter of a serum pool ($n = 10$) for 1 h at 37°C. The standard was defined to contain 1000 arbitrary units (AU)/mL and applied as a standard curve diluted 2-fold in six steps with 2.5 AU/mL as the highest standard. The porcine plasma samples were diluted 1:4. Both standard and samples were diluted in PBS containing 0.2% azide, 0.2% Tween 20, and 20 mM of ethylenediaminetetraacetic acid and incubated for 60 min at 4°C (50 μ L/well). Detection antibody was mAb anti-human C6 cross-reacting with pig (Quidel, San Diego, CA, U.S.A.), which was biotinylated, diluted in PBS containing 0.2% Tween 20, and incubated for 60 min at 37°C (50 μ L/well). Streptavidin-horseradish peroxidase (RPN1231V; Amersham Biosciences Europe GmbH, Freiburg, Germany), diluted 1:400, was then added and incubated for 60 min at 37°C (50 μ L/well). Finally, substrate solution was added (100 μ L/well): 0.003% H₂O₂ in 0.33 mM of ABTS (Roche Diagnostics GmbH, Mannheim, Germany) in 0.1 M of Na-acetate buffer (pH 4.0), incubated at room temperature for 15 min before stop solution (2 N of H₂SO₄) was added (100 μ L/well). Between each incubation, the plates were washed three times with PBS containing 0.1% Tween 20. Optical density was read at 405 nm using 490 nm as reference (MRX microplate reader; Dynex Technologies, Denkendorf, Germany).

Lipopolysaccharide and Colony-Forming Units

Lipopolysaccharide (LPS) was quantified using the *Limulus* Amebocyte Lysate Assay (LAL QCL-1000, 50-648U; Bio-Whittaker/Cambrex Bio Science, Walkersville, MD, U.S.A.). Bacteria were detected as colony-forming units (CFU) in the routine laboratory for Medical Microbiology, Rikshospitalet University Hospital.

Meconium

Meconium was prepared according to a previously described method (25). Briefly, samples of meconium from 55 healthy newborns was collected from diapers with a wooden-spatula and stored in 50 mL of polypropylene centrifuge tubes (Corning Inc., Acton, MA, U.S.A.) at -20°C. The samples were thawed, pooled, and processed by a hand blender (Bosch MSM 4001/01, Nazarje, Slovenia) in physiologic saline until the mixture was homogeneous. The batch was then freeze-dried and irradiated to secure sterility (γ -rays, 32 kGy; Institute for Energy Technology, Kjeller, Norway). For the piglet experiments, the preparation was reconstituted with saline to a weight concentration of 135 mg/mL and frozen in aliquots at -20°C until the day of the experiment. For the *in vitro* experiments, the preparation was further diluted with sterile PBS to a concentration of 100 mg/mL.

Fractionation of Meconium. Meconium can be separated into a water and a lipid fraction. Bile acids are found in the water fraction, and free fatty acids (FFA) are found in the lipid fraction. Meconium was fractionated as described previously (26). There are unequal amounts of these two fractions in whole meconium. In our preparation, the weight of lipid fraction was 14% of the original weight of the whole meco-

nium, and that of water fraction was 63%. To compare the relative contribution of each fraction, we used equivalent concentrations of meconium and its fractions, suggesting that a concentration of 14 mg/mL of lipid fraction and 63 mg/mL of water fraction were equivalent to 100 mg/mL of whole meconium.

In vitro Experimental Model

Adult or cord serum was incubated with meconium or equivalent amounts of fractions thereof for 1 h at 37°C. Inhibition experiments were performed with preincubation of the inhibitors and controls with serum for 5 min at 37°C. Then meconium was added and incubated for 1 h as described above. In experiments in which meconium was mixed with HSA before incubation in serum, preincubation was made with different concentrations of HSA during different time periods before serum incubation. Complement activation was always stopped at the end of the incubation period by adding ethylenediaminetetraacetic acid to a final concentration of 20 mM.

In vivo Piglet Model of MAS

To examine whether meconium activated complement *in vivo*, we performed a pilot study according to a previously described model (25). Briefly, the piglets were anesthetized, tracheotomized, and connected to a respirator. Surgery to gain vascular access for infusions, blood samples, and invasive monitoring was then performed. Lung function parameters were registered by the respirator. Fractional inspired oxygen concentration (F_{IO_2}) and end-tidal CO_2 were continuously monitored separately. After surgery, the piglets were first stabilized and then subjected to a hypoxemia period in a gas mixture of 8% O_2 in N_2 until base excess reached -20 mM. Two test piglets received meconium (4 mL/kg of a preparation containing 135 mg/mL) *via* the endotracheal tube, and bronchoalveolar lavage was performed after 5 min. One control piglet received the same volume of physiologic saline. The piglets were reoxygenated immediately after tracheal instillations and observed for 5 h after reoxygenation. Blood samples were drawn immediately after surgery and then at the following time points after reoxygenation: 10 min, 30 min, 1 h, and hourly thereafter until the end of the experiment. Oxygenation index (OI; mean airway pressure $\times F_{IO_2} \times 100/P_{AO_2}$) was calculated to assess the severity of lung injury.

Experiments using animals were done with the permission of the national authorities (accreditation number of the laboratory is 27, and accreditation number of the study is 7/2002).

RESULTS

Bacterial culture and LPS. The meconium batch used was sterile because no bacterial growth could be detected. The LPS content was 3 ng/mg of meconium.

Meconium-induced complement activation in cord serum. Meconium induced a dose-dependent complement activation in cord serum as detected by increase in TCC from baseline 6 (5–8) AU/mL to 91 (73–117) AU/mL at 10 mg/mL (median and range of three experiments), whereas HSA had no effect (Fig. 1).

Figure 1. Meconium-induced complement activation in cord serum. Incubation of meconium (○) in cord serum for 60 min at 37°C activated complement substantially, as detected by a dose-dependent increase in the soluble TCC. In contrast, HSA (●) had no effect on complement activation. Median and range of three separate experiments are shown. ■, baseline (spontaneous serum activation at 60 min).

Comparison of cord and adult serum. Because complement components are generally lower in cord serum than in adult serum, we compared complement activation in these two using zymosan, a yeast cell preparation, which is a potent activator of the alternative complement pathway. Zymosan-induced complement activation in adult serum was almost four times stronger than that in cord serum (Fig. 2, *left*). Maximum TCC formation by zymosan incubated in adult serum is defined as 1000 AU/mL and is used as standard in the TCC assay. Thus, these incubation experiments revealed full activation of serum by zymosan. The lower degree of activation in cord serum is consistent with the lower concentration of complement components in this serum. Meconium induced a degree of complement activation in cord serum (TCC = 91 AU/mL) that was more than half of that induced in adult serum (TCC = 141 AU/mL), indicating the potent complement activation property of meconium (Fig. 2, *right*). Increasing the concentrations of meconium to 20 and 40 mg/mL did not lead to further increase in complement activation in either cord or adult serum.

Figure 2. Comparison of complement activation in cord and adult serum. The maximum level of TCC formed by zymosan (*left*) in cord serum (Δ) was approximately 25% of that achieved in adult serum ($\blacktriangle$), consistent with the lower amount of complement components in the former. Similarly, meconium (*right*) activated complement to a greater extent in adult serum ($\bullet$) than in cord serum ($\circ$), although to a substantial degree also in the latter. One representative of three experiments is shown.

Meconium fractions-induced complement activation in cord serum. The lipid and the water fractions induced equal complement activation, as judged by TCC formation, and reconstitution of the fractions restored the complement activation potential of whole meconium (Fig. 3). Activation of C3 as detected by C3bc showed virtually identical results.

Preincubation of meconium with HSA. We have previously suggested that albumin has a beneficial effect by neutralizing FFA in meconium (26). The effect of HSA on meconium-induced complement activation therefore was investigated. Preincubation of meconium with increasing doses of HSA for 5, 15, and 30 min before incubation in cord serum for 1 h had no inhibitory effect on complement activation (Fig. 4).

Effect of C1 inhibitor and dextran sulfate on meconium-induced complement activation. Preincubation of C1 inhibitor in increasing doses up to four times the physiologic concentration (4 U/mL) only modestly reduced meconium-induced complement activation (Fig. 5). Low molecular weight dextran sulfate completely abolished meconium-induced complement activation at 1.25 mg/mL, either combined with exogenously added C1 inhibitor or alone (Fig. 5).

Meconium and its fractions activate the alternative complement pathway. Activation products from the three initial activation pathways of complement were measured after incubation of whole meconium in cord serum. C1rs-C1-inhibitor complexes as indicator of the classical pathway activation did not increase; C4bc as indicator of the classical and/or lectin

pathway activation hardly changed; whereas a substantial increase in C3bBbP, the alternative pathway convertase, was observed (Fig. 6). Identical results were obtained for the lipid and water fractions of meconium (data not shown).

Inhibition of the alternative pathway abolishes meconium-induced complement activation. Meconium-induced complement activation was completely abolished using the mAb anti-D (12.5 μ g/mL), blocking the alternative pathway, whereas no effect was seen using a blocking mAb anti-C2, which inhibits the classical and lectin pathways, or a blocking mAb anti-MBL, which inhibits the lectin pathway (Fig. 7).

Meconium-induced complement activation in vivo. An established piglet model of MAS was used for a pilot *in vivo* study. Two piglets (P1 and P2) received meconium instillation in the lungs and therapeutic lavage, and one control received saline (P3). OI and TCC increased in both test piglets but not in the control (Fig. 8). Test piglet P1 had a rapid increase in both OI and TCC and died after 3 h, whereas test piglet P2 had a similar but later onset of increased OI and less pronounced TCC increase and died immediately before the end of the experiment (5 h). The control piglet survived in a good clinical condition with increase in neither OI nor TCC formation.

DISCUSSION

In this study, we show for the first time that meconium activates complement. The *in vitro* data indicate that meco-

Figure 3. Effect of meconium fractions on complement activation. Whole meconium (○) was fractionated into the lipid fraction (●) and the water fraction (△), and the effects of the fractions on TCC formation was examined by incubation in serum for 60 min at 37°C. The concentration of whole meconium is indicated on the x axis, and the fractions were added in equivalent concentrations compared with whole meconium. Reconstituted meconium (▲) was obtained by mixing the lipid and water fractions in the same ratio as in the original meconium. The lipid and the water fractions contributed equally to the TCC formation, and reconstitution restored the activity of whole meconium. One representative of three experiments is shown. ■, baseline (spontaneous serum activation at 60 min).

nium is a potent activator of the alternative complement pathway, and the *in vivo* data lend support to a systemic complement activation taking place during experimental MAS.

Meconium is composed of water up to 80%. Other components include gastrointestinal secretions, bile, bile acids, mucus, pancreatic juice, cellular debris, swallowed amniotic fluid, lanugo, vernix caseosa, and blood (1). It has been suggested that the lipid fraction, containing, *e.g.* FFA, and the water fraction, containing, *e.g.* bile acids, may have different adverse effects on tissue homeostasis. Albumin mixed with meconium attenuated pulmonary dysfunction in a piglet model of MAS (26) probably as a result of neutralization of FFA and/or other

Figure 4. Effect of preincubation of meconium with albumin. Increasing concentrations of HSA (○) added to meconium 15 min before incubation in serum for 60 min at 37°C did not inhibit formation of TCC. Median and range of three separate experiments are shown. ■, baseline (spontaneous serum activation at 60 min).

constituents in meconium. In fact, we found that the lipid and water fractions activated complement to the same extent, indicating that there are several complement-activating components in meconium. To approach the pathophysiologic and clinical condition of MAS, we compared the relative complement activation potential of the two fractions in concentrations equivalent to their presence in whole meconium. Thus, per weight, the lipid fraction was substantially more potent in activating complement than the water fraction. Mixing meconium with albumin did not reduce the complement activation, suggesting that a possible beneficial effect of albumin is not mediated *via* complement inhibition.

Activation of the immune system during inflammation is complex with release of numerous cytokines (27) and chemokines (28). Furthermore, various lipid mediators are formed when phospholipase A₂ metabolizes the membrane phospholipids of inflammatory cells to produce platelet-activating factor and arachidonic acid, which is further metabolized to a number of prostaglandins and leukotrienes with potent proinflammatory effects (29). Although the pathophysiology of the inflammatory reaction in MAS is poorly understood, there is some evidence that cytokines (30) and in particular lipid

Figure 5. Effect of C1 inhibitor and dextran sulfate on meconium-induced complement activation. Only a modest decrease in TCC formation was observed for C1 inhibitor (○) when added in amounts up to four times the physiologic concentration (4 U/mL). Low molecular weight dextran sulfate (DEX; ●) abolished TCC formation dose-dependently, and dextran sulfate in fixed concentration (1.25 mg/mL) potentiated the effect of C1 inhibitor markedly (△). HSA (▲) was used as a control. One representative of three experiments is shown. ■, baseline (spontaneous serum activation at 60 min).

metabolites (31) are produced in experimental MAS and may be associated with the tissue damage.

Complement is part of the host defense system and a key mediator and initiator of inflammation and inflammatory tissue damage. Uncontrolled and systemic activation of complement might have deleterious effects on the host (32, 33). To protect against invading organisms, complement is activated by microbes and microbial components. Complement is in fact activated by any foreign surface considered as "nonself" by the system (34). Principally, the only fully complement compatible

Figure 6. Initial complement pathway activation by meconium. Meconium incubated in cord serum for 60 min at 37°C induced a marked increase in the alternative pathway C3bBbP convertase (△). In contrast, no and virtually no increases were seen for the classical pathway C1rs-C1-inhibitor complexes (○) and classical and lectin pathway C4bc (●), respectively. Median and range of three separate experiments are shown, and times increase from baseline is indicated.

surface is the autologous endothelial cell lining. Thus, it is not surprising that complement is activated by meconium, which contains innumerable substances from the gastrointestinal tract, including cellular debris, FFA, bile acids, and lanugo. The meconium that we used did not contain live bacteria. Furthermore, the LPS content was in the low nanomolar range, which is several logs below the amount required to induce complement activation, although sufficient to cause some cytokine production. The lack of live bacteria and the LPS concentration below the complement-activating range, however, does not fully exclude other bacterial components as possible complement-activating candidates.

The participation of complement in lung injury has been demonstrated in an experimental model of systemic complement activation (35). Furthermore, complement and neutrophils mediate the lung injury of acid aspiration in rodents, and complement inhibition reduces both local and systemic organ injury associated with this condition (36–39). Acid aspiration

Figure 7. Inhibition of meconium-induced complement activation by anti-complement mAbs. Meconium was incubated in cord serum for 60 min at 37°C in the presence of inhibitory mAbs, all IgG1 subclass, to C2 (classical/lectin pathway; Δ), MBL (lectin pathway; $\blacktriangle$), factor D (alternative pathway; $\circ$), or the isotype control antibody G3-519 ($\bullet$). Anti-factor D completely prevented TCC formation, whereas the other antibodies had no effect. One representative of three experiments is shown.

is a potentially serious complication in trauma and surgical patients and is the leading cause of acute respiratory distress syndrome after elective surgery. There are similarities between the pneumonitis associated with acid aspiration and that of MAS, because MAS is also associated with a profound inflammatory response, with alveolar and parenchymal inflammation and edema, protein leakage into airways, mediator release, and surfactant dysfunction, which occurs within hours of exposure to meconium (2). The possibility of an acid-induced activation of complement by meconium thus should be considered. We therefore measured pH in the meconium batch used and found it to be 7.0. Thus, it is unlikely that an acidic component *per se* could explain the complement activation.

Anti-factor D mAb, a specific inhibitor of the alternative pathway, completely abolished the meconium-induced com-

Figure 8. *In vivo* activation of complement in a porcine model of MAS. An established piglet model of MAS was used for a pilot *in vivo* study. Two piglets (P1 and P2) received meconium, and one control (P3) received saline. OI (left) and TCC (right) increased in both test piglets but not in the control. Test piglet P1 had a rapid increase in both OI and TCC and died after 3 h, whereas test piglet P2 died immediately before the end of the experiment (5 h) and had a later and less pronounced increase in TCC. Indications on x axes (time): A, after surgery; B, induction of hypoxia and reoxygenation; C, 10 min after reoxygenation; D, 30 min after reoxygenation; E, 1 h after reoxygenation; F, 5 h after reoxygenation.

plement activation. This suggests an alternative pathway mechanism of activation, although it should be emphasized that the alternative pathway may be activated as an amplification by both classical and lectin pathway activation. However, two lines of evidence indicate that the last two pathways are not activated by meconium. First, activation products from the classical and lectin pathway (C1rs-C1-inhibitor complexes and C4bc) were not increased, in contrast to the alternative pathway convertase C3bBbP. Second, blocking of C2, a common component of the classical and lectin pathways, as well as blocking of MBL did not inhibit meconium-induced complement activation. C1 inhibitor is a well-known inhibitor of the classical (C1rs) and the lectin pathways of complement (40), as well as of other cascade systems (41). We observed a minor but reproducible inhibitory effect of high concentrations of C1 inhibitor on meconium-induced complement activation. This may be explained by the recently described inhibitory effect of C1 inhibitor on the alternative complement pathway (42).

Glycosaminoglycans (e.g. heparin, heparan sulfate, dermatan sulfate, chondroitin sulfates, dextran sulfate), possessing highly sulfated structural domains, might regulate the complement system at different steps (43), including potentiation of the effect of C1 inhibitor (44) and interaction with factor H of alternative pathway activation (45), although the latter effect has been questioned (46). Synthetic low molecular weight dextran sulfate has been demonstrated to inhibit complement-mediated hyperacute xenotransplant rejection (45), which is largely classical pathway mediated, and the effect of C1 inhibitor *in vivo* was demonstrated to be transient (47). In our study, we found that meconium-induced complement activation was efficiently reduced by dextran sulfate, whereas exogenous C1 inhibitor together with dextran sulfate had virtually no additional effect. These data indicate that if the effect of dextran

sulfate in our system is mediated *via* C1 inhibitor, then the endogenously present C1 inhibitor is sufficient and exogenous supply of C1 inhibitor may not be indicated.

The importance of inflammation in meconium-induced lung injury is well established (48). Systemic corticosteroid therapy has been used as a nonspecific global anti-inflammatory treatment regimen in MAS (2), emphasizing the idea that MAS is a systemic disease and could be considered as a SIRS (49). Studies on the systemic inflammatory reaction in MAS are largely missing. Our *in vivo* data showing substantial systemic complement activation are in support of a MAS-associated SIRS.

The therapeutic approaches in MAS have mainly been symptomatic and focused on local treatment of the lung injury (1–3). In serious cases, there is a need for ventilatory support, and in the case of critically ill newborns, institution of extracorporeal membrane oxygenation is mandatory. Targeting specific complement components might be a reasonable therapeutic option in complement-mediated inflammatory diseases (32). Although the role of complement in the pathogenesis of MAS remains to be established, our data indicate that specific complement inhibition therapy might be a possible future treatment approach in MAS. With respect to the agents that we tested in the present study, it should be noted that C1 inhibitor had minimal effect used alone and that serious side effects such as thromboembolic complications of high-dose C1 inhibitor recently have been described (50). Dextran sulfate efficiently reduced complement activation but may through other mechanisms induce adverse side effects such as disturbances in coagulation by C1 inhibitor-mediated inactivation of factor XIa (44), as well as disturbed primary and secondary hemostasis (51) and thrombocytopenia (47). We therefore suggest that specific inhibition of complement activation, *e.g.* by blocking the alternative pathway by mAb anti-factor D, may be an appropriate therapeutic approach of meconium-induced complement activation in severe MAS.

CONCLUSION

In summary, the present study shows for the first time that meconium is a potent activator of the complement system both *in vitro* and *in vivo*, suggesting that complement may be important in the pathogenesis of MAS.

Acknowledgments. We thank Anne-Randi Alvestad, Institute for Nutrition Research, University of Oslo, for kindly providing the lipid and water fractions of meconium, and Roger Ødegård for professional assistance in performing the animal studies.

REFERENCES

1. Fuloria M, Wiswell TE 1999 Resuscitation of the meconium-stained infant and prevention of meconium aspiration syndrome. *J Perinatol* 19:234–241
2. Cleary GM, Wiswell TE 1998 Meconium-stained amniotic fluid and the meconium aspiration syndrome. An update. *Pediatr Clin North Am* 45:511–529
3. Wiswell TE, Fuloria M 1998 The meconium aspiration syndrome: the saga continues. *Indian Pediatr* 35:1059–1062
4. Ghidini A, Spong CY 2001 Severe meconium aspiration syndrome is not caused by aspiration of meconium. *Am J Obstet Gynecol* 185:931–938
5. Soukka H, Rautanen M, Halkola L, Kero P, Kaapa P 1997 Meconium aspiration induces ARDS-like pulmonary response in lungs of ten-week-old pigs. *Pediatr Pulmonol* 23:205–211
6. Fry DE 2000 Sepsis syndrome. *Am Surg* 66:126–132
7. Cheng IW, Ware LB, Greene KE, Nuckton TJ, Eisner MD, Matthay MA 2003 Prognostic value of surfactant proteins A and D in patients with acute lung injury. *Crit Care Med* 31:20–27
8. Slutsky AS, Tremblay LN 1998 Multiple system organ failure. Is mechanical ventilation a contributing factor? *Am J Respir Crit Care Med* 157:1721–1725
9. Kearney MS 1999 Chronic intrauterine meconium aspiration causes fetal lung infarcts, lung rupture, and meconium embolism. *Pediatr Dev Pathol* 2:544–551
10. Katz VL, Bowes WA Jr 1992 Meconium aspiration syndrome: reflections on a murky subject. *Am J Obstet Gynecol* 166:171–183
11. Srinivasan HB, Vidyasagar D 1999 Meconium aspiration syndrome: current concepts and management. *Compr Ther* 25:82–89
12. Klingner MC, Kruse J 1999 Meconium aspiration syndrome: pathophysiology and prevention. *J Am Board Fam Pract* 12:450–466
13. Yeh TF, Srinivasan G, Harris V, Pildes RS 1977 Hydrocortisone therapy in meconium aspiration syndrome: a controlled study. *J Pediatr* 90:140–143
14. McCabe AJ, Wilcox DT, Holm BA, Glick PL 2000 Surfactant—a review for pediatric surgeons. *J Pediatr Surg* 35:1687–1700
15. Kattwinkel J 2002 Surfactant lavage for meconium aspiration: a word of caution. *Pediatrics* 109:1167–1168
16. Vidyasagar D, Harris V, Pildes RS 1975 Assisted ventilation in infants with meconium aspiration syndrome. *Pediatrics* 56:208–213
17. Gill BS, Neville HL, Khan AM, Cox CS Jr, Lally KP 2002 Delayed institution of extracorporeal membrane oxygenation is associated with increased mortality rate and prolonged hospital stay. *J Pediatr Surg* 37:7–10
18. Fung M, Loubser PG, Undar A, Mueller M, Sun C, Sun WN, Vaughn WK, Fraser CD Jr 2001 Inhibition of complement, neutrophil, and platelet activation by an anti-factor D monoclonal antibody in simulated cardiopulmonary bypass circuits. *J Thorac Cardiovasc Surg* 122:113–122
19. Fure H, Nielsen EW, Hack CE, Mollnes TE 1997 A neopeptide-based enzyme immunoassay for quantification of C1-inhibitor in complex with C1r and C1s. *Scand J Immunol* 46:553–557
20. Wolbink GJ, Bollen J, Baars JW, ten Berge RJ, Swaak AJ, Paardekooper J, Hack CE 1993 Application of a monoclonal antibody against a neopeptide on activated C4 in an ELISA for the quantification of complement activation via the classical pathway. *J Immunol Methods* 163:67–76
21. Mollnes TE, Brekke OL, Fung M, Fure H, Christiansen D, Bergseth G, Videm V, Lappégard KT, Kohl J, Lambris JD 2002 Essential role of the C5a receptor in E coli-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 100:1869–1877
22. Garred P, Mollnes TE, Lea T 1988 Quantification in enzyme-linked immunosorbent assay of a C3 neopeptide expressed on activated human complement factor C3. *Scand J Immunol* 27:329–335
23. Mollnes TE, Lea T, Frøland SS, Harboe M 1985 Quantification of the terminal complement complex in human plasma by an enzyme-linked immunosorbent assay based on monoclonal antibodies against a neoantigen of the complex. *Scand J Immunol* 22:197–202
24. Mollnes TE, Lea T, Harboe M, Tschopp J 1985 Monoclonal antibodies recognizing a neoantigen of poly(C9) detect the human terminal complement complex in tissue and plasma. *Scand J Immunol* 22:183–195
25. Tølløfsrud PA, Solås AB, Saugstad OD 2001 Newborn piglets with meconium aspiration resuscitated with room air or 100% oxygen. *Pediatr Res* 50:423–429
26. Tølløfsrud PA, Medbø S, Solås AB, Drevon CA, Saugstad OD 2002 Albumin mixed with meconium attenuates pulmonary dysfunction in a newborn piglet model with meconium aspiration. *Pediatr Res* 52:545–553
27. Oberholzer A, Oberholzer C, Moldawer LL 2000 Cytokine signaling—regulation of the immune response in normal and critically ill states. *Crit Care Med* 28:N3–N12
28. Baggiolini M, Loetscher P 2000 Chemokines in inflammation and immunity. *Immunol Today* 21:418–420
29. Bulger EM, Maier RV 2000 Lipid mediators in the pathophysiology of critical illness. *Crit Care Med* 28:N27–N36
30. Khan AM, Elidemir O, Epstein CE, Lally KP, Xue H, Blackburn M, Larsen GL, Colasurdo GN 2002 Meconium aspiration produces airway hyperresponsiveness and eosinophilic inflammation in a murine model. *Am J Physiol* 283:L785–L790
31. Korhonen K, Soukka H, Halkola L, Peuravuori H, Aho H, Pulkki K, Kero P, Kaapa PO 2003 Meconium induces only localized inflammatory lung injury in piglets. *Pediatr Res* 54:192–197
32. Mollnes TE, Song WC, Lambris JD 2002 Complement in inflammatory tissue damage and disease. *Trends Immunol* 23:61–64
33. Bhole D, Stahl GL 2003 Therapeutic potential of targeting the complement cascade in critical care medicine. *Crit Care Med* 31:S97–S104
34. Atkinson JP, Oglesby TJ, White D, Adams EA, Liszewski MK 1991 Separation of self from non-self in the complement system: a role for membrane cofactor protein and decay accelerating factor. *Clin Exp Immunol* 86 Suppl 1:27–30
35. Ward PA 1994 Oxygen radicals, cytokines, adhesion molecules, and lung injury. *Environ Health Perspect* 102(suppl 10):13–16
36. Nishizawa H, Yamada H, Miyazaki H, Ohara M, Kaneko K, Yamakawa T, Wiener-Kronish J, Kudoh I 1996 Soluble complement receptor type 1 inhibited the systemic organ injury caused by acid instillation into a lung. *Anesthesiology* 85:1120–1128
37. Kyriakides C, Austen W Jr, Wang Y, Favuzza J, Kobzik L, Moore FD Jr, Hechtman HB 1999 Membrane attack complex of complement and neutrophils mediate the injury of acid aspiration. *J Appl Physiol* 87:2357–2361

38. Kyriakides C, Wang Y, Austen WG Jr, Favuzza J, Kobzik L, Moore FD Jr, Hechtman HB 2001 Sialyl Lewis(x) hybridized complement receptor type 1 moderates acid aspiration injury. *Am J Physiol* 281:L1494–L1499
39. Kyriakides C, Austen WG Jr, Wang Y, Favuzza J, Kobzik L, Moore FD Jr, Hechtman HB 2001 Mast cells mediate complement activation after acid aspiration. *Shock* 16:21–24
40. Petersen SV, Thiel S, Jensen L, Vorup-Jensen T, Koch C, Jensenius JC 2000 Control of the classical and the MBL pathway of complement activation. *Mol Immunol* 37:803–811
41. Caliezi C, Wuillemin WA, Zeerleder S, Redondo M, Eisele B, Hack CE 2000 C1-Esterase inhibitor: an anti-inflammatory agent and its potential use in the treatment of diseases other than hereditary angioedema. *Pharmacol Rev* 52:91–112
42. Jiang H, Wagner E, Zhang H, Frank MM 2001 Complement 1 inhibitor is a regulator of the alternative complement pathway. *J Exp Med* 194:1609–1616
43. Edens RE, Linhardt RJ, Weiler JM 1993 Heparin is not just an anticoagulant anymore: six and one-half decades of studies on the ability of heparin to regulate complement activity. In: Cruse JM, Lewis RE (eds) *Complement Today*. Karger, Basel, pp 96–120
44. Wuillemin WA, te Velthuis H, Lubbers YT, de Ruig CP, Eldering E, Hack CE 1997 Potentiation of C1 inhibitor by glycosaminoglycans: dextran sulfate species are effective inhibitors of in vitro complement activation in plasma. *J Immunol* 159:1953–1960
45. Fiorante P, Banz Y, Mohacs PJ, Kappeler A, Wuillemin WA, Macchiarini P, Roos A, Daha MR, Schaffner T, Haeblerli A, Mazmanian GM, Rieben R 2001 Low molecular weight dextran sulfate prevents complement activation and delays hyperacute rejection in pig-to-human xenotransplantation models. *Xenotransplantation* 8:24–35
46. Koistinen V 1993 Effects of sulphated polyanions on functions of complement factor H. *Mol Immunol* 30:113–118
47. Bos IG, van Mierlo GJ, Bleeker WK, Rigter GM, te Velthuis H, Dickneite G, Hack CE 2001 The potentiation of human C1-inhibitor by dextran sulphate is transient in vivo: studies in a rat model. *Int Immunopharmacol* 1:1583–1595
48. Wu JM, Yeh TF, Wang JY, Wang JN, Lin YJ, Hsieh WS, Lin CH 1999 The role of pulmonary inflammation in the development of pulmonary hypertension in newborn with meconium aspiration syndrome (MAS). *Pediatr Pulmonol Suppl* 18:205–208
49. Oberholzer A, Oberholzer C, Moldawer LL 2001 Sepsis syndromes: understanding the role of innate and acquired immunity. *Shock* 16:83–96
50. Horstick G, Berg O, Heimann A, Gotze O, Loos M, Hafner G, Bierbach B, Petersen S, Bhakdi S, Darius H, Horstick M, Meyer J, Kempinski O 2001 Application of C1-esterase inhibitor during reperfusion of ischemic myocardium: dose-related beneficial versus detrimental effects. *Circulation* 104:3125–3131
51. Zeerleder S, Mauron T, Lammle B, Wuillemin WA 2002 Effect of low-molecular weight dextran sulfate on coagulation and platelet function tests. *Thromb Res* 105:441–446