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In-vitro myoglobin clearance by a novel sorbent system.

Kuntsevich VI, Feinfeld DA, Audia PF, Young W, Capponi V, Markella M, Winchester JF; Artif Cells Blood Substit Immobil Biotechnol. 2009; 37(1):45-7.

Hemoadsorption improves long-term survival after sepsis in the rat.

Peng ZY, Wang H, Carter MJ, DiLeo M, Kellum JA; Crit Care Med. 2008 Mar;36(12 suppl):A1.

Effects of hemoadsorption on cytokine removal and short-term survival in septic rats.

Peng, ZY, Carter MJ, Kellum JA; Crit Care Med. 2008 Mar;36(5):1573-77.

The potential application of sorbents in peritoneal dialysis

Winchester JF, Amerling R, Harbord N, Capponi V, Ronco C.; Contrib Nephrol. 2006;150:336-43.

Extracorporeal strategies for the removal of middle molecules

Winchester JF, Audia PF; Semin Dial. 2006 Mar-Apr;19(2):110-4.

Novel Changes in Beta-2-Microglobulin in Dialysis Patients

Winchester JF, Clinical Chemistry. 2005;51:1089-1090

Absence of NF- B Activation by a New Polystyrene-Type Adsorbent Designed for Hemoperfusion

Elisa Menegatti, Claudio Ronco, James F. Winchester, Antonella Dragonetti, Debora Di Simone, Annalisa Davit, Giulio Mengozzi, Giorgio Marietti, Giuseppina Loduca, Morteza Mansouri, Gian Piero Sancipriano, Luigi M. Sena, Dario Roccatello, Blood Purification 2005, Vol. 23, No. 1

Hemoadsorption removes tumor necrosis factor, interleukin-6, and interleukin-10, reduces nuclear factor-kappaB DNA binding, and improves short-term survival in lethal endotoxemia.

Kellum JA, Song M, Venkataraman R.; Crit Care Med. 2004 Mar;32(3):801-5.

Cytokine Removal with a Novel Adsorbent Polymer

Mingchen Song, James Winchester, Robert L. Albright, Vincent J. Capponi, Michael D. Choquette, John A. Kellum, Blood Purification 2004, Vol. 22, No. 5.

Hemoadsorption to Improve Organ Recovery from Brain-Dead Organ Donors: A Novel Therapy for a Novel Indication

Ramesh Venkataraman, Mingchen Song, Rachel Lynas, John A. Kellum, Blood Purification 2004, Vol. 22, No. 1.

Sorbents in Acute Renal Failure and End-Stage Renal Disease: Middle Molecule and Cytokine Removal

James F. Winchester, Jeffrey Silberzweig, Claudio Ronco, Viktoria Kuntsevich, Daniel Levine, Tom Parker, John A. Kellum, Jamie A. Salsberg, Peter Quartararo, Nathan W. Levin, Blood Purification 2004, Vol. 22, No. 1

Sorbents in Acute Renal Failure and the Systemic Inflammatory Response Syndrome

Winchester JF, Kellum JA, Ronco C, Brady JA, Quartararo P, Salsberg J, Levin NW. Blood Purification, 2003;21:79-84.

Beta-2 Microglobulin and ESRD: An In-Depth Review

Winchester JF, Salsberg J. Advances in Renal Replacement Therapy, 2003 Oct;10(4): 279-309.

Middle molecules and small-molecular-weight proteins in ESRD: properties and strategies for their removal

Clark WR, Winchester JF. *Advances in Renal Replacement Therapy*, 2003 Oct; 10(4):270-8.

Effect of a novel adsorbent on cytokine responsiveness to uremic plasma.

Morena MD, Guo D, Balakrishnan VS, Brady JA, Winchester JF, Jaber BL. *Kidney International* 2003 Mar; 63(3): 1150-4.

Removal of Middle Molecules with Sorbents

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Extracorporeal Removal of Toxic Substances, in Critical Care Toxicology

JF Winchester (in press)

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JF Winchester; editors Horl W, Koch KM, Lindsay RM, Ronco C, Winchester JF (Editor in Chief). Kluwer Academic Publishers, Dordrecht, Boston (in press)

Bleeding Disorders in Renal Failure, in Replacement of Renal Function by Dialysis (5th Edition)

JF Winchester; editors Horl W, Koch KM, Lindsay RM, Ronco C, Winchester JF (Editor in Chief). Kluwer Academic Publishers, Dordrecht, Boston (in press)

Peritoneal Dialysis Program Management, in Replacement of Renal Function by Dialysis (5th Edition)

JF Winchester; editors Horl W, Koch KM, Lindsay RM, Ronco C, Winchester JF (Editor in Chief). Kluwer Academic Publishers, Dordrecht, Boston (in press)

Dialysis and Hemoperfusion in Poisoning, in Therapy in Nephrology and Hypertension

JF Winchester; editors HR Brady, CS Wilcox. WB Saunders Co, Philadelphia 2003:947-953

Special Issue: Sorbent-Based Extracorporeal Blood Treatments

SR Ash and JF Winchester (Guest Editors). *Advances in Renal Replacement Therapy*, 2002; Vol 9, No 1.

Sorbent Hemoperfusion in End Stage Renal Disease: An In-Depth Review

JF Winchester, C Ronco. *Advances in Renal Replacement Therapy*, 2002; 9:19-25.

The Next Step From High Flux Dialysis: Application Of Sorbent Technology

James F. Winchester, Claudio Ronco, James A. Brady, Larry D Cowgill, Jamie Salsberg, Eric Yousha, Mike Choquette, Robert Albright, Jonathan Clemmer, Vadim Davankov, Maria Tsyurupa, Ludmila Pavlova, Mikhail Pavlov, Gerald Cohen, Walter Horl, Frank Gotch, Nathan Levin. *Blood Purification*, 2002; 20: 81-86.

Sorbents in Extracorporeal Blood Therapy

SR Ash, JF Winchester. *Advances in Renal Replacement Therapy*, 2002; 9:1-2.

Effect of the Betasorb(TM) Perfusion Column On The Bioreactivity Of Uremic Plasma

Marion D. Morena, Daqing Guo, V. S. Balakrishnan, James A. Brady, James F. Winchester, Bertrand L. Jaber. *Abstr ASAIO Journal* 2002; 48: 178.

Sorbent Augmented Dialysis Systems, in Contributions to Nephrology

James F. Winchester, Ronco C, Salsberg J, Yousha E, Brady JA, Cowgill LD, Choquette M, Albright R, Clemmer J, Davankov V, Tsyurupa M, Pavlova L, Pavlov M, Cohen G, Horl W, Gotch F, Levin NW; eds Ronco C, LaGreca G. 2002;137: 181-188.

Hemoadsorption improves long-term survival after sepsis in the rat.

Peng ZY, Wang H, Carter MJ, DiLeo M, Kellum JA; Crit Care Med. 2008 Mar; 36(12 suppl):A1.

Introduction: The over-production of inflammatory mediators is associated with the multi-organ failure and death in sepsis. We have previously shown that hemoadsorption could remove inflammatory cytokines and improve short-term survival in rats using a lethal model of cecal ligation and puncture. With the current study, we sought to determine if this treatment can also be effective to improve the long-term survival in a less lethal model. **Hypothesis:** Hemoadsorption could eliminate cytokines and improve long-term survival after sepsis in the rat. **Methods:** 18 h after cecal ligation and puncture (CLP), ligating 25% of the cecum and using two punctures, we randomized 46 adult Sprague Dawley rats to receive hemoadsorption (HA) or sham HA for four hours. HA was performed using a venous-venous circuit with a cartridge containing 1g of CytoSorb beads (MedaSorb Technologies, Princeton NJ) using a blood flow of 1-1.5 ml/min. We measured plasma by ELISA and analyzed changes using ANOVA for repeated measures. Survival time was assessed for one week and analyzed by Kaplan-Meier and overall survival in each group was compared using Fisher's exact. **Results:** Baseline concentrations of IL-6 were similar between HA and sham groups (385.69 pg/ml vs 336.16 pg/ml). IL-6 continued to increase in the sham-treated animals with time, while the increase in IL-6 was significantly inhibited in the HA group (219.41 pg/ml vs 522.66 pg/ml after treatment; 259.70 pg/ml vs 677.40 pg/ml after day 3, $p < 0.05$). The survival time was also significantly longer in the HA group (5.65 days vs 4.52 days, $p < 0.05$). The seven-day survival rate was 52% vs 28%, (hazard ratio: 0.50, 0.17-0.96, $p < 0.05$) in favor of HA. **Conclusions:** Hemoadsorption begun 18 hours after onset of experimental sepsis in the rat resulted in reduced plasma IL-6 concentrations and improved the long-term survival

Effects of hemoadsorption on cytokine removal and short-term survival in septic rats.

Peng, ZY, Carter MJ, Kellum JA; Crit Care Med. 2008 Mar; 36(5):1573-77.

OBJECTIVE: A broad-spectrum immune-regulating therapy could be beneficial in the treatment of sepsis. Our previous studies have shown that a hemoadsorption device (CytoSorb) removes both pro- and anti-inflammatory cytokines and improves survival in experimental endotoxemia. We sought to determine whether hemoadsorption can also be effective in the treatment of sepsis. **DESIGN:** Randomized controlled laboratory experiment. **SETTING:** University laboratory. **INTERVENTIONS:** Rats were subjected to cecal ligation and puncture (CLP) and 20 hrs later were randomized to receive either hemoadsorption or sham treatment using an arterial-venous circuit. Hemoadsorption was accomplished using a cartridge containing Cytosorb beads. Blood was drawn for cytokine measurements and mean arterial pressure (MAP) was continuously monitored. Cytokines were measured via multiplex bead immunoassays. Survival time was observed for 9 hours after the intervention and assessed by Kaplan-Meier statistics. The overall survival in each group was compared using Fisher's exact test. Finally, we used a Cox proportional-hazards model to examine the effects of cytokine removal on survival time. **MEASUREMENTS AND MAIN RESULTS:** Baseline plasma cytokine concentrations and MAP were similar between hemoadsorption and sham-treated groups. However, the concentrations of tumor necrosis factor, interleukin (IL)-1 β , IL-6, and IL-10 were significantly lower after hemoadsorption compared to the sham group. Six hours after treatment ended, IL-6 and IL-10 concentrations were still lower in hemoadsorption group. MAP was significantly better in hemoadsorption compared to sham-treated animals ($p < .05$). Finally, mean survival time was significantly longer (720 vs. 381 min, $p < .05$, Mann-Whitney test), and overall survival was significantly better (11/17 vs. 2/16, $p < .01$) with hemoadsorption compared to sham. Combined reduction in both IL-6 and IL-10 was associated with a significantly decreased risk of death (hazard ratio, .11, $p = .005$). **CONCLUSION:** Hemoadsorption reduced circulating cytokines, improved MAP, and resulted in better short-term survival in CLP-induced septic rats.

Hemoadsorption removes tumor necrosis factor, interleukin-6, and interleukin-10, reduces nuclear factor-kappaB DNA binding, and improves short-term survival in lethal endotoxemia.

Kellum JA, Song M, Venkataraman R.; Crit Care Med. 2004 Mar; 32(3):801-5.

OBJECTIVES: Previous studies have shown that inflammatory mediators can be removed from the circulation with hemofiltration and that adsorption plays an important role. Because adsorptive capacity of hollow-fiber dialyzers is limited, we sought to determine whether hemoadsorption using high surface area beads would result in greater mediator removal and improved survival in experimental sepsis. **DESIGN:** Randomized controlled laboratory experiment. **SETTING:** University laboratory. **SUBJECTS:** Sixty-six adult Sprague-Dawley rats. **INTERVENTIONS:** We conducted two ex vivo and two in vivo experiments. For in vivo experiments, we administered Escherichia coli endotoxin (20 mg/kg) by intravenous infusion and then randomized each animal

to receive either hemoadsorption or a sham circuit for 4 hrs. Hemoadsorption was performed for 4 hrs using an arterial-venous circuit and a CytoSorb cartridge containing 10 g of polystyrene divinyl benzene copolymer beads with a biocompatible polyvinylpyrrolidone coating. Survival time was measured to a maximum of 12 hrs. In a separate set of experiments, we studied 12 animals using the same protocol except that we killed all animals at 4 hrs and removed standardized sections of liver for analysis of nuclear factor-kappaB DNA binding. MEASUREMENTS AND MAIN RESULTS: Mean survival time among hemoadsorption-treated animals was 629+/-114 vs. 518+/-120 mins for sham-treated animals ($p < .01$). Overall survival (defined at 12 hrs) was also significantly better in the hemoadsorption group, seven of 20 vs. one of 20 ($p < .05$). Plasma interleukin-6 and interleukin-10 concentrations and liver nuclear factor-kappaB DNA binding were significantly reduced by hemoadsorption. Ex vivo experiments showed no endotoxin adsorption but strengthened our in vivo observations by showing rapid adsorption of tumor necrosis factor, interleukin-6, and interleukin-10. CONCLUSIONS: Hemoadsorption was associated with reduced inflammation and improved survival in this murine model of septic shock.

Cytokine Removal with a Novel Adsorbent Polymer

Mingchen Song, James Winchester, Robert L. Albright, Vincent J. Capponi, Michael D. Choquette, John A. Kellum, Blood Purification 2004, Vol. 22, No. 5.

BACKGROUND/AIMS: We sought to characterize a novel adsorbent polymer in terms of cytokine removal. METHODS: We challenged 50 rats with lipopolysaccharide to obtain cytokine-rich blood and circulated this through cartridges containing polymer. In separate experiments, cell-free supernatants were passed through cartridges containing polymer. We measured tumor necrosis factor alpha, interleukin 10 and interleukin 6 concentrations under a variety of conditions to evaluate adsorption kinetics. RESULTS: All three cytokines were rapidly removed from the blood with less than 50% of the initial concentrations present after 1 h of circulation through the cartridge. There was no significant difference in the effect across a range of blood flows and Ca²⁺ concentrations. Adsorption was decreased somewhat by extremely low temperature (4 degrees C). CONCLUSION: The adsorbent polymer removes cytokines with high efficiency, and binding is relatively unaffected by a variety of physical conditions.

Sorbents in Acute Renal Failure and the Systemic Inflammatory Response Syndrome

Winchester JF, Kellum JA, Ronco C, Brady JA, Quartararo P, Salsberg J, Levin NW. Blood Purification, 2003;21: 79-84.

Renal replacement therapy in acute renal failure is currently focused on the use of modifications of dialysis (continuous arteriovenous hemofiltration and hemodiafiltration) to remove middle molecular weight toxins, consisting of small proteins, and cytokines involved in the systemic inflammatory response syndrome (SIRS). Conventional high-flux dialyzers are not efficient at removing these molecules, prompting the investigation of sorbents to augment or replace dialysis. Sorbents have been developed to modulate SIRS by targeting cytokines such as IL-1, IL-6, IL-10, IL-18 and TNF, among others. Extensive pre-clinical studies are underway to demonstrate the clinical utility and safety of either adding sorbent hemoadsorption devices to hemodialysis, or the use of such devices alone in SIRS, sepsis, acute renal failure, cardiopulmonary bypass and end-stage renal disease.

In-vitro myoglobin clearance by a novel sorbent system.

Kuntsevich VI, Feinfeld DA, Audia PF, Young W, Capponi V, Markella M, Winchester JF; Artif Cells Blood Substit Immobil Biotechnol. 2009; 37(1):45-7.

Rhabdomyolysis may lead to acute kidney injury following deposition of myoglobin in renal tubules. Although high-flux dialysis membranes may remove a substantial amount of myoglobin from plasma, this may still not be sufficient to prevent renal damage. We tested a new polymer sorbent, X-Sorb, in vitro to determine its potential to clear myoglobin from solutions. Normal saline or human serum in which myoglobin was dissolved was perfused by a peristaltic pump through a column packed with the sorbent. After a 4-hour perfusion, the myoglobin level in normal saline fell from 200,000 ng/ml to virtually undetectable (<780 ng/ml). Perfusion through the sorbent was then found to lower concentrations of dissolved myoglobin in 3 different 110-ml samples of human serum consistently by > 90% over 4 hours. X-Sorb appears to be an effective sorbent for myoglobin and warrants a trial in vivo to determine whether it is equally effective and safe.

Non-Company External Articles of Interest

Note: This JAMA paper is important because it demonstrates, in a randomized controlled prospective trial, the benefit of blood purification as a strategy to treat sepsis, albeit in a highly selective population.

Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial

Cruz, DN, Antonelli M, Fumagalli R, Foltran F, Brienza N, Donati A, et al. JAMA 2009 Jun 17; 301(23):2445-52.

Polymyxin B fiber column is a medical device designed to reduce blood endotoxin levels in sepsis. Gram-negative-induced abdominal sepsis is likely associated with high circulating endotoxin. Reducing circulating endotoxin levels with polymyxin B hemoperfusion could potentially improve patient clinical outcomes. **OBJECTIVE:** To determine whether polymyxin B hemoperfusion added to conventional medical therapy improves clinical outcomes (mean arterial pressure [MAP], vasopressor requirement, oxygenation, organ dysfunction) and mortality compared with conventional therapy alone. **DESIGN, SETTING, AND PATIENTS:** A prospective, multicenter, randomized controlled trial (Early Use of Polymyxin B Hemoperfusion in Abdominal Sepsis [EUPHAS]) conducted at 10 Italian tertiary care intensive care units between December 2004 and December 2007. Sixty-four patients were enrolled with severe sepsis or septic shock who underwent emergency surgery for intra-abdominal infection. **INTERVENTION:** Patients were randomized to either conventional therapy (n=30) or conventional therapy plus 2 sessions of polymyxin B hemoperfusion (n=34). **MAIN OUTCOME MEASURES:** Primary outcome was change in MAP and vasopressor requirement, and secondary outcomes were PaO₂/FIO₂ (fraction of inspired oxygen) ratio, change in organ dysfunction measured using Sequential Organ Failure Assessment (SOFA) scores, and 28-day mortality. **RESULTS:** MAP increased (76 to 84 mm Hg; P = .001) and vasopressor requirement decreased (inotropic score, 29.9 to 6.8; P < .001) at 72 hours in the polymyxin B group but not in the conventional therapy group (MAP, 74 to 77 mm Hg; P = .37; inotropic score, 28.6 to 22.4; P = .14). The PaO₂/FIO₂ ratio increased slightly (235 to 264; P = .049) in the polymyxin B group but not in the conventional therapy group (217 to 228; P = .79). SOFA scores improved in the polymyxin B group but not in the conventional therapy group (change in SOFA, -3.4 vs -0.1; P < .001), and 28-day mortality was 32% (11/34 patients) in the polymyxin B group and 53% (16/30 patients) in the conventional therapy group (unadjusted hazard ratio [HR], 0.43; 95% confidence interval [CI], 0.20-0.94; adjusted HR, 0.36; 95% CI, 0.16-0.80). **CONCLUSION:** In this preliminary study, polymyxin B hemoperfusion added to conventional therapy significantly improved hemodynamics and organ dysfunction and reduced 28-day mortality in a targeted population with severe sepsis and/or septic shock from intra-abdominal gram-negative infections.

Note: The following NEJM paper is important because it documents the direct role of non-infectious cytokine storm in causing severe sepsis/systemic inflammatory response syndrome in 6 otherwise healthy young men. This is in the absence of an infection or endotoxin.

Cytokine storm in a phase I trial of the anti-CD28 monoclonal antibody TGN1412

Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, Panoskaltzis N. NEJM 2006 Sep 7; 355(10):1018-28

Six healthy young male volunteers at a contract research organization were enrolled in the first phase 1 clinical trial of TGN1412, a novel superagonist anti-CD28 monoclonal antibody that directly stimulates T cells. Within 90 minutes after receiving a single intravenous dose of the drug, all six volunteers had a systemic inflammatory response characterized by a rapid induction of proinflammatory cytokines and accompanied by headache, myalgias, nausea, diarrhea, erythema, vasodilatation, and hypotension. Within 12 to 16 hours after infusion, they became critically ill, with pulmonary infiltrates and lung injury, renal failure, and disseminated intravascular coagulation. Severe and unexpected depletion of lymphocytes and monocytes occurred within 24 hours after infusion. All six patients were transferred to the care of the authors at an intensive care unit at a public hospital, where they received intensive cardiopulmonary support (including dialysis), high-dose methylprednisolone, and an anti-interleukin-2 receptor antagonist antibody. Prolonged cardiovascular shock and acute respiratory distress syndrome developed in two patients, who required intensive organ support for 8 and 16 days. Despite evidence of the multiple cytokine-release syndrome, all six patients survived. Documentation of the clinical course occurring over the 30 days after infusion offers insight into the systemic inflammatory response syndrome in the absence of contaminating pathogens, endotoxin, or underlying disease.

Blood purification for hypercytokinemia.

Nakada T, Hirasawa H, Oda S, Shiga H, Matsuda K. *Transf Apher Science* 2006; 35:253-264

Blood purification has been steadily improved in the field of critical care, supported by advances in related biomedical technologies as well as efforts to develop better operating procedures. As it has become clear that hypercytokinemia plays a key role in the pathophysiology of critical pathological conditions, use of various blood purification techniques to control hypercytokinemia has been investigated. Answers to questions concerning the optimal cytokine-removing device (dialyzer/ hemofilter/adsorber) as well as operating procedures and conditions of such devices in particular clinical conditions have been obtained in the course of such investigations. The recent success in real-time monitoring of cytokine levels in clinical practice to assess the extent of cytokine network activation may improve the precision and efficacy of blood purification in the treatment of hypercytokinemia. In addition, the recently documented effects of genetic factors on hypercytokinemia suggest that the introduction of tailor-made medicine considering the differences in genetic background among individual patients may improve the efficacy of blood purification as a countermeasure to hypercytokinemia.

Clinical review: extracorporeal blood purification in severe sepsis

Venkataraman R, Subramanian S, Kellum JA

Sepsis and septic shock are the leading causes of acute renal failure, multiple organ system dysfunction, and death in the intensive care unit. The pathogenesis of sepsis is complex and comprises a mosaic of interconnected pathways. Several attempts to improve patient outcomes by targeting specific components of this network have been unsuccessful. For these reasons, the ideal immunomodulating strategy would be one that restores immunologic stability rather than blindly inhibiting or stimulating one or another component of this complex network. Hence, the recent focus of immunomodulatory therapy in sepsis has shifted to nonspecific methods of influencing the entire inflammatory response without suppressing it. Here, we discuss the various modalities of extracorporeal blood purification, the existing evidence, and future prospects.

Endotoxin and cytokine removal in sepsis.

Tetta C, Bellomo R, Inquaqiato, Wratten ML, Ronco C. *Ther Apher* 2002 Apr; 6(2): 109-15.

Sepsis, the leading cause of mortality in intensive care units, is a complex series of interrelated effects caused by the overproduction of multiple mediators and their unrestrained biological activity. Both proinflammatory and antiinflammatory mediators participate in the high complexity of sepsis and explain the failure of specific therapies to improve survival. Continuous extracorporeal therapies have been proposed as therapeutic options and as tools for blood purification in sepsis. Along these lines and in order to achieve higher clearances and mass removal rates, we studied the effects of plasmafiltration coupled with adsorption and provided in vitro and in vivo evidence that adsorption of multiple cytokines, activated complement components, and lipid mediators such as the platelet-activating factor occurs. We also showed that such treatment may lead to improved survival in a rabbit model of sepsis and to improved hemodynamics, reduced norepinephrine dose, and restoration of near-to-normal responsiveness of blood leukocytes to endotoxin in humans. It is anticipated that treatment of plasma, as a modular device to conventional hemofiltration, may pave the way to innovative approaches in the extracorporeal treatment of septic patients.

Blood purification in sepsis: a reasonable scientific hypothesis or pipe dream?

Bellomo, R. *Crit Care Resuscitation* 2001; 3:202-5.

Hemofiltration-absorption systems for the treatment of experimental sepsis: Is it possible to remove the "evil humors" responsible for septic shock?

Opal, SM. *Crit Care Med* 2000 May; 28(5):1681-1682

Immunosuppression in sepsis

Lyn-Kew K, Standiford TJ. *Curr Pharm Des* 2008; 14(19):1870-81

The often fatal sepsis syndrome is characterized by the systemic release of inflammatory mediators, which is regulated and counterbalanced by the coordinated expression of anti-inflammatory molecules. The magnitude of sepsis-induced tissue injury and subsequent risk of infectious complications is dictated by the balance between the expression of pro- and anti-inflammatory mediators. As our understanding of the pathophysiology of sepsis continues to evolve, we have gained a greater appreciation for the profound effects that sepsis and similar states of overwhelming stress have on host innate and adaptive immunity. Impaired leukocyte function in sepsis has important clinical consequences, as high mortality rates have been observed in patients who display evidence of sepsis-induced immune dysregulation. Functional defects in leukocytes isolated from patients with sepsis include diminished expression of important cell surface molecules, dysregulated cytokine production, alterations in antigen-presenting ability, and accelerated apoptosis. In this article, we review the current literature supporting the notion that dysregulation of host immunity occurs during sepsis syndrome, and describe novel therapeutic interventions directed at augmenting host immunity during sepsis.

The inflammatory balance in human sepsis

Adrie C, Pinsky MR. *Intensive Care Med* 2000; 26:364-75.

Cytokine signaling-regulation of the immune response in normal and critically ill states.

Oberholzer, A; Oberholzer, C; Moldawer, LL. *Crit Care Med* 2000; 28(Suppl):N3-N12

Cytokines are produced during the activation of innate and acquired immunity, and are the principal means for intercellular communication of a microbial invasion. Cytokines serve to initiate the inflammatory response and to define the magnitude and the nature of the acquired immune response. The response of critically ill patients to their injury and/or invading pathogens is dependent, in large part, on the pattern of cytokines which are produced. The immunologic response of critically ill patients can vary from a strongly proinflammatory response, characterized by increased production of tumor necrosis factor-[alpha], interleukin (IL)-1, interferon (IFN)-[gamma], and IL-12 to one predominantly of anergy, characterized by increased production of TH2 cytokines, like IL-10 and to IL-4. Therapeutic efforts to modify the host immune response in critical illness will require a more thorough understanding of the cytokine milieu and the factors that determine their production.

The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome (Review)

Wang H, Ma S. *American J of Emer Med* 2008;26:711-715

Multiple organ dysfunction syndrome (MODS) is a major cause of morbidity and mortality in intensive care units. It is being encountered frequently in critically ill patients owing to advancements in organ-specific supportive technologies to survive the acute phase of severe sepsis and shock. It is now believed that MODS is the result of an inappropriate generalized inflammatory response of the host to a variety of acute insults. The pathologic mechanisms of MODS were reviewed, and factors determining the sequence and severity of organ dysfunction were discussed in depth. In the early phase of MODS, circulating cytokines cause universal endothelium injury in organs. In the later phase of MODS, overexpression of inflammatory mediators in the interstitial space of various organs is considered a main mechanism of parenchyma injury. The difference in constitutive expression and the upregulation of adhesion molecules in vascular beds and the density and potency of intrinsic inflammatory cells in different organs are the key factors determining the sequence and severity of organ dysfunction. By activating the intrinsic inflammatory cell in a distant organ, organ dysfunctions are linked in a positive feedback loop through circulating inflammatory mediators. Antagonists targeted at adhesion molecules may alleviate the severity of endothelial damage. And nonsteroidal anti-inflammatory drugs or steroids administered judiciously in the early phase of MODS may retard the progress of multiple organ failure.

Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia

De Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, et al. *Nature Medicine* 2006 Oct; 12(10): 1203-7.

Avian influenza A (H5N1) viruses cause severe disease in humans, but the basis for their virulence remains unclear. In vitro and animal studies indicate that high and disseminated viral replication is important for disease pathogenesis. Laboratory experiments suggest that virus-induced cytokine dysregulation may contribute to disease severity. To assess the relevance of these findings for human disease, we performed virological and immunological studies in 18 individuals with H5N1 and 8 individuals infected with human influenza virus subtypes. Influenza H5N1 infection in humans is characterized by high pharyngeal virus loads and frequent detection of viral RNA in rectum and blood. Viral RNA in blood was present only in fatal H5N1 cases and was associated with higher pharyngeal viral loads. We observed low peripheral blood T-lymphocyte counts and high chemokine and cytokine levels in H5N1-infected individuals, particularly in those who died, and these correlated with pharyngeal viral loads. Genetic characterization of H5N1 viruses revealed mutations in the viral polymerase complex associated with mammalian adaptation and virulence. Our observations indicate that high viral load, and the resulting intense inflammatory responses, are central to influenza H5N1 pathogenesis. The focus of clinical management should be on preventing this intense cytokine response, by early diagnosis and effective antiviral treatment

Abberant innate immune response in lethal infection of macaques with the 1918 influenza virus

Kobasa D, Jones SM, Shinya K, Kash JC, Copps J, Ebihara H, et al. *Nature* 2007 Jan 18; 445:319-323.

The 1918 influenza pandemic was unusually severe, resulting in about 50 million deaths worldwide. The 1918 virus is also highly pathogenic in mice, and studies have identified a multigenic origin of this virulent phenotype in mice. However, these initial characterizations of the 1918 virus did not address the question of its pathogenic potential in primates. Here we demonstrate that the 1918 virus caused a highly pathogenic respiratory infection in a cynomolgus macaque model that culminated in acute respiratory distress and a fatal outcome. Furthermore, infected animals mounted an immune response, characterized by dysregulation of the antiviral response, that was insufficient for protection, indicating that atypical host innate immune responses may contribute to lethality. The ability of influenza viruses to modulate host immune responses, such as that demonstrated for the avian H5N1 influenza viruses, may be a feature shared by the virulent influenza viruses.

Pathology of fatal human infection associated with avian influenza A H5N1 virus

To KF, Chan PK, Chan KF, Lee WK, et al. *J Med Virol* 2001 Mar; 63(3):242-6.

Eighteen cases of human influenza A H5N1 infection were identified in Hong Kong from May to December 1997. Two of the six fatal cases had undergone a full post-mortem which showed reactive hemophagocytic syndrome as the most prominent feature. Other findings included organizing diffuse alveolar damage with interstitial fibrosis, extensive hepatic central lobular necrosis, acute renal tubular necrosis and lymphoid depletion. Elevation of soluble interleukin-2 receptor, interleukin-6 and interferon-gamma was demonstrated in both patients, whereas secondary bacterial pneumonia was not observed. Virus detection using isolation, reverse transcription-polymerase chain reaction and immunostaining were all negative. It is postulated that in fatal human infections with this avian subtype, initial virus replication in the respiratory tract triggers hypercytokinemia complicated by the reactive hemophagocytic syndrome. These findings suggest that the pathogenesis of influenza A H5N1 infection might be different from that of the usual human subtypes H1-H3.

Cytokine storm in avian influenza (Review)

Us D. *Mikrobiyol Bul* 2008 Apr; 42(2):365-80.

The most dramatic example of defining the pathogenicity of influenza virus A/H5N1 strains is the higher fatality rate of avian influenza epidemic (>50%) occurred in Southeast Asia in 1997 comparing to the pandemic caused by influenza virus A/H1N1 in 1918 (5-10%) which was recorded as the most destructive pandemic in the world. When considering the fatal/total case numbers (208/340) reported by World Health Organization in respect of December 14th, 2007, the mortality rate has now reached to 61 percent. Recent studies have shown that the high fatality rate of avian influenza virus infections is a consequence of an overactive inflammatory response and the severity of infection is closely related with virus-induced cytokine dysregulation. The most important feature of A/H5N1 immunopathogenesis is the appearance of hypercytokinemia ("cytokine storm") which is characterized by the extreme (exaggerated) production and secretion of large numbers and excessive levels of pro-inflammatory cytokines. This phenomenon is blamed on the emergence of lethal clinical symptoms such as extensive pulmonary oedema, acute bronchopneumoniae, alveolar haemorrhage, reactive haemophagocytosis, and acute respiratory distress syndrome, associated with necrosis and tissue destruction. Numerous in vitro, in vivo and clinical studies have pointed out that A/H5N1 viruses are very strong inducers of various cytokines and

chemokines [Tumor Necrosis Factor (TNF)-alpha, Interferon (IFN)-gamma, IFN-alpha/beta, Interleukin (IL)-6, IL-1, MIP-1 (Macrophage Inflammatory Protein), MIG (Monokine Induced by IFN-gamma), IP-10 (Interferon-gamma-Inducible Protein), MCP-1 (Monocyte Chemoattractant Protein), RANTES (Regulated on Activation Normal T-cell Expressed and Secreted), IL-8], in both humans and animals. The privileged cells of cytokine storm are macrophages and CD8+ T-lymphocytes, while the primary contributor cytokines are TNF-alpha, IL-6 and IFN-gamma. It has been detected that, mutations of some viral genes (NS1, PB2, HA and NA) are responsible for the cytokine storm, by increasing the viral replication rate, expanding the tissue tropism, facilitating the systemic invasion and emerging of resistance against the host antiviral response. It has been shown that Glu92 and Ala149 mutations, and carboxyl-terminal ESEV/EPEV motif of NS1 protein have been implicated as determinants of virulence for A/H5N1 strains. In addition, Lys627 mutation in PB2 protein, polybasic aminoacid mutations in the cleavage region of hemagglutinin (HA) polyprotein, and glycosylation and sialylation mutations in HA and neuraminidase (NA) proteins were found to enhance the immune-mediated pathology of highly virulent A/H5N1 strains. In this review article, the immunopathogenesis of influenza infection and the mechanisms of cytokine storm caused by influenza A/H5N1 viruses have been discussed under the light of recent literature.

A probable role for IFN-gamma in the development of a lung immunopathology in SARS

Theron, M, Huang K-J, Chen Y-W, Liu, C-C, Lei H-Y Cytokine 2005 Oct 7; 32(1):30-38.

Recent work carried out in our laboratory showed the existence of a cytokine storm in SARS patients, dominated by Th1-type mediators. We thus hypothesized that IFN-gamma may play a major role in the pathology by triggering immune-mediated alveolar damage. As we assessed or re-assessed some effects of IFN-gamma on a number of human lung epithelial and fibroblast cell lines, chosen for their wide use in the literature, we found that alveolar epithelial cells were more sensitive to IFN-gamma, in terms of proliferation inhibition and enhancement of Fas-mediated apoptosis. While similar effects were obtained on fibroblasts, concentrations of IFN-gamma 4--8-fold greater were required. In addition, both epithelial and fibroblastic cell lines were able to secrete large quantities of T cell-targeting chemokines, similar to the ones detected in SARS patients. Based on the clinical data collected previously, the available literature and our in vitro experimentation, we propose that IFN-gamma may be responsible for acute lung injury in the late phase of the SARS pathology.

Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells.

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Fatal human respiratory disease associated with influenza A subtype H5N1 has been documented in Hong Kong, and more recently in Vietnam, Thailand and Cambodia. We previously demonstrated that patients with H5N1 disease had unusually high serum levels of IP-10 (interferon-gamma-inducible protein-10). Furthermore, when compared with human influenza virus subtype H1N1, the H5N1 viruses in 1997 (A/Hong Kong/483/97) (H5N1/97) were more potent inducers of pro-inflammatory cytokines (e.g. tumor necrosis factor- α) and chemokines (e.g. IP-10) from primary human macrophages in vitro, which suggests that cytokines dysregulation may play a role in pathogenesis of H5N1 disease. Since respiratory epithelial cells are the primary target cell for replication of influenza viruses, it is pertinent to investigate the cytokine induction profile of H5N1 viruses in these cells. METHODS: We used quantitative RT-PCR and ELISA to compare the profile of cytokine and chemokine gene expression induced by H5N1 viruses A/HK/483/97 (H5N1/97), A/Vietnam/1194/04 and A/Vietnam/3046/04 (both H5N1/04) with that of human H1N1 virus in human primary alveolar and bronchial epithelial cells in vitro. RESULTS: We demonstrated that in comparison to human H1N1 viruses, H5N1/97 and H5N1/04 viruses were more potent inducers of IP-10, interferon beta, RANTES (regulated on activation, normal T cell expressed and secreted) and interleukin 6 (IL-6) in primary human alveolar and bronchial epithelial cells in vitro. Recent H5N1 viruses from Vietnam (H5N1/04) appeared to be even more potent at inducing IP-10 than H5N1/97 virus. CONCLUSION: The H5N1/97 and H5N1/04 subtype influenza A viruses are more potent inducers of proinflammatory cytokines and chemokines in primary human respiratory epithelial cells than subtype H1N1 virus. We suggest that this hyper-induction of cytokines may be relevant to the pathogenesis of human H5N1 disease.