Effect of Diabetes Mellitus on Endotoxin-Induced Lung Injury

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Objective: To examine the effects of diabetes mellitus on lipopolysaccharide (LPS)–induced pulmonary edema and alveolar neutrophil recruitment and activation.

Hypothesis: Zucker diabetic fatty rats are resistant to the effects of intratracheal LPS on the extravasation of plasma proteins into the lungs.

Design: Zucker diabetic fatty (ZDF) rats (genotype fa/fa) were used as a model of diabetes mellitus, while their normoglycemic heterozygous littermates served as controls. Lipopolysaccharide (Escherichia coli 0111: B4; 100-200 µg) or vehicle (0.25 mL of isotonic sodium chloride solution) was instilled into the airways of ZDF and control rats. Four hours later, pulmonary microvascular dysfunction was assessed by measuring the extravasation of Evans blue dye into the lung. Lipopolysaccharide-induced neutrophil recruitment was assessed by counting the number of neutrophils within the bronchoalveolar lavage fluid and measuring their expression of CD11b/CD18 by fluorescence-activated cell analysis sorting.

Results: The LPS (200 µg) induced a 32% increase in Evans blue dye extravasation into the lungs of controls (P = .008) but had no such effect in diabetic animals. Pulmonary extravasation of Evans blue dye in controls was greater than that of ZDF rats both at baseline (P = .002) and in response to 200 µg of LPS (P<.001). The LPS up-regulated neutrophil CD11b/CD18 expression in diabetic and nondiabetic groups and induced a greater than 50-fold increase in the number of neutrophils within the airways of both control and diabetic groups (P<.001).

Conclusion: Despite the recruitment of a large number of neutrophils into the lung, the LPS-induced change in pulmonary microvascular permeability in diabetic animals is substantially less than that of nondiabetic controls.

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MATERIALS AND METHODS

ANIMAL MODEL OF TYPE 2 DIABETES MELLITUS

Male ZDF rats (21-26 weeks of age; 400-600 g) were obtained from a colony at the Dallas Veterans Affairs Medical Center, Dallas, Tex. These animals possess a mutation of the leptin receptor that is phenotypically expressed as obesity, hypertriglyceridemia, and hyperglycemia by 7 to 9 weeks of age; as such, they have been commonly used as an animal model of type 2 diabetes mellitus. Controls consisted of age-matched, lean, normoglycemic male heterozygotic rats.

These experiments were reviewed and approved by the Subcommittee on the Care and Use of Animals in Research at the University of Texas Southwestern Medical School and the Dallas Veterans Affairs Medical Center.

EXPERIMENTAL PROTOCOL

The ZDF and control animals were anesthetized with pentobarbital sodium (40 mg/kg of lean body weight, intraperitoneally) and the trachea was exposed through a small cervical incision. Escherichia coli LPS (100 or 200 μg in 250 μL of sterile isotonic sodium chloride solution; E coli serotype 0111:B4; Sigma-Aldrich Corp, St Louis, Mo) or an equal volume of sterile isotonic sodium chloride solution was injected rapidly into the trachea during inspiration by means of a 27-gauge needle. The wound was then closed and the animals were allowed to recover. This method induces an acute lung injury manifested by increased epithelial and microvascular permeability, neutrophil sequestration, and interstitial edema. Pulmonary microvascular leak (as assessed by Evans blue dye [EBD] extravasation) and neutrophil activation (CD11b/CD18 expression) and sequestration within the lung were assessed 4 hours after the instillation of LPS into the airways. This time point was chosen on the basis of preliminary experiments demonstrating maximal extravasation of plasma proteins into the lung at 4 to 8 hours after exposure to endotoxin. By 24 to 48 hours after injury, the microvascular leak is significantly attenuated, with concentrations of dye within the lungs being no different from that in controls (unpublished data, 1998).

MEASUREMENT OF ACUTE LUNG INJURY

Evans blue dye has been used to quantitate protein extravasation during acute inflammatory events. Previous studies have demonstrated that, after intravenous infusion, more than 99% of the dye exists bound to plasma proteins. This method has been shown to compare favorably with the use of radiolabeled albumin as a marker of plasma protein extravasation and, hence, microvascular “leakiness” during acute inflammatory events.

Three hours after the administration of LPS or isotonic sodium chloride solution, EBD (20 mg/kg) was infused into the femoral vein of the ZDF and control animals (n = 3-8 per group). Sixty minutes later, the heart and lungs were excised and the pulmonary vasculature was flushed free of blood by gently infusing sterile saline into the beating right ventricle. The left lower lobe of the lung was then excised, weighed, and placed in 5 mL of formamide. The tissue was homogenized and incubated in formamide for 18 hours at 37°C. The tissue suspension was then centrifuged at 12 000 rpm for 20 minutes, after which the supernatant was collected and its optical density determined spectrophotometrically (OD620). The amount of dye contained within the sample was extrapolated by means of a standard curve and expressed as milligrams of dye per gram of wet lung weight.

MEASUREMENT OF NEUTROPHIL ACTIVATION AND RECRUITMENT INTO THE LUNG

Quantitation of Neutrophils Within the Airways

In a separate set of experiments, bronchoalveolar lavage (BAL) was performed 4 hours after exposure to LPS (200 μg) or an equivalent volume of sterile isotonic sodium chloride solution. In these experiments, 7 mL of sterile isotonic sodium chloride solution was instilled into the right lung 3 times. The lavage fluid was collected and pooled, and the total cell count was obtained with an automated cell counter. The number of neutrophils present within the lavage fluid was determined by light microscopy after Wright staining.

Quantitation of Neutrophil CD11b/CD18 Expression

An aliquot of BAL fluid was collected to quantitate the expression of the adhesion glycoproteins CD11b and CD18 on neutrophils. In this experiment, neutrophils isolated from the BAL fluid were stained with fluorescein isothiocyanate–conjugated mouse anti–rat monoclonal antibodies against CD11b and CD18 (Pharmigen Inc, San Diego, Calif). An isotopic IgG was used as a control. Cell staining was quantitated by fluorescence-activated cell analysis sorting (FACScan; Beckton-Dickinson Co, San Jose, Calif) and expressed as the percentage of cells stained by the fluorescein isothiocyanate–labeled antibodies. A minimum of 10 000 cells were examined per sample.

STATISTICAL ANALYSIS

All data are expressed as mean ± SEM and analyzed by analysis of variance with a Fisher post hoc test. A P value of less than .05 was considered a statistically significant difference between groups.

or 200 μg of LPS is shown in Figure 1. In the absence of LPS, the amount of EBD within the lungs of diabetic animals was about 33% less than that of nondiabetic controls (P = .002). The injection of LPS into the trachea of the control animals resulted in a dose-dependent increase in the extravasation of EBD into the lungs, with a 32% increase for the 200-μg dose (P = .008). In contrast, the injection of even 200 μg of LPS into the airways of the ZDF rats had no significant effect on the concentration of EBD within the lungs when compared with that of ZDF animals exposed to isotonic sodium chloride solution alone. The concentration of EBD within the lungs of control animals exposed to 200 μg of LPS was more than twice that of similarly treated ZDF animals (P<.001).
Figure 1. The concentration of Evans blue dye (EBD) within the lungs of obese diabetic rats (black bars; n = 4-8 per group) or lean normoglycemic heterozygotic rats (gray bars; n = 4-8 per group) 4 hours after exposure to vehicle (sterile isotonic sodium chloride solution) or lipopolysaccharide (LPS) administered intratracheally. Asterisk indicates P < .005 vs similarly treated heterozygotes; dagger, P = .008 vs isotonic sodium chloride solution–treated heterozygotes.

Figure 2. The neutrophil count within the airways of obese diabetic rats or lean normoglycemic heterozygotic rats exposed to sterile isotonic sodium chloride solution or lipopolysaccharide (LPS) administered intratracheally. The gray bars represent animals exposed to sterile isotonic sodium chloride solution or lipopolysaccharide (n = 3 per group). The black bars represent animals exposed to 200 µg of lipopolysaccharide (n = 3 per group). Asterisk indicates P < .001 vs isotonic sodium chloride solution–treated animals of a similar phenotype. PMN indicates polymorphonuclear neutrophil.

Figure 3. The effect of intratracheal lipopolysaccharide (200 µg) on the expression of the neutrophil adhesion glycoprotein CD11b. The gray bars represent animals exposed to sterile isotonic sodium chloride solution (n = 3 per group). The black bars represent animals exposed to 200 µg of lipopolysaccharide (n = 3 per group). Asterisk indicates P < .001 vs isotonic sodium chloride solution–treated animals of a similar phenotype. PMN indicates polymorphonuclear neutrophil.

The frequency of type 2 diabetes mellitus in the population in general, and in hospitalized patients in particular, makes it imperative that clinicians have a better understanding of the effects of diabetes on the local and systemic inflammatory response to injury, proinflammatory mediators, and infection. The purpose of this study was to examine the effect of diabetes mellitus on the pulmonary microvascular response to intratracheal endotoxin exposure. The results of this study suggest that the pulmonary microvasculature of diabetic animals is significantly less permeable to plasma proteins both at baseline and in response to intratracheal LPS than that of normoglycemic controls. This difference in microvascular protein extravasation occurs despite similar degrees of neutrophil adhesion molecule expression and neutrophil sequestration within the lungs of the diabetic and nondiabetic groups.

The intratracheal instillation of LPS into rats is a commonly used model of acute inflammatory lung injury, and, as such, it has been used to investigate the pathophysiological characteristics of acute respiratory distress syndrome. The experimental model is associated with hypoxemia, impaired surfactant production, neutrophil sequestration within the lungs, and pulmonary edema with parenchymal consolidation. The increased microvascular and epithelial permeability associated with
Diabetes mellitus is one of the most common chronic diseases in the United States, being present in nearly 20% of hospitalized patients. Despite this nearly unparalleled prevalence, little is known regarding the effect of diabetes on the pulmonary microvascular response to injury. The present study utilizes a commonly employed animal model of type 2 diabetes mellitus to examine its effect on the pulmonary microvascular response to endotoxin. These experiments suggest that the pulmonary microvasculature of diabetic animals is significantly less responsive to intratracheal endotoxin than that of non-diabetic controls, despite similar degrees of neutrophil activation and sequestration within the airways. A similar phenomenon in patients with diabetes mellitus may impair the ability of diabetic patients to clear infectious challenges from the lungs. Conversely, this blunted microvascular responsiveness may limit the pulmonary edema that characterizes many systemic inflammatory states.

In summary, these data suggest that the pulmonary microvascular response to intratracheal endotoxin is severely blunted in diabetic rats when compared with that of normoglycemic controls. This occurs despite similar degrees of neutrophil CD11b/CD18 expression and pulmonary neutrophil sequestration within the lungs. These studies support the contention that chronic illnesses such as diabetes mellitus may significantly alter the responsiveness of an organ to acute inflammatory stimuli. This assumes particular importance given the frequent incidence of chronic illnesses among critically ill patients.

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REFERENCES

Daniel L. Treber, MD, Galveston, Tex: I would like to thank the Society for the honor of being able to discuss this very excellent paper. It is relevant to what has preceded here at this meeting, especially Dr Chaudry’s presidential address as well as the symposium we had this morning, because we point out the importance of a correct animal model to look at sepsis, and this model and this study have several features about them that make them especially clinically relevant. First of all, it is a 2-hit model, since you had some injury, not necessarily morphological injury, but certainly influx of polymorphonuclear cells with saline alone, and then secondly, of course, the second hit being the sepsis insult, and then thirdly, we have what is commonly seen in the clinical situation, an underlying disease process. And I have several questions that I would like to propose.

First of all, because the model is so important, I would like to ask a question related to the saline-alone group. As I calculate these data from the presentation—and I would like again to thank the group for having given me the manuscript well ahead of the time of this meeting—I know that in our institution and in many others, they use about 40 to 50 mL of saline as they are lavaging patients who are on ventilators, and that is exactly the amount if you extrapolated the size of a rat to the size of a human being, his 0.25 mL of saline as a control would be what a normal therapist would be giving to a patient, and yet he is seeing some rather interesting changes in microvascular permeability to albumin. I would like to know if he had data from unstimulated animals for the albumin leakage to be able to quantitate.

And then the other aspect of this study is that this disease entity, the diabetic situation, as well as the injury to the lung, are also systemic responses that occur, and so important in a model of sepsis that we are indeed looking at a resuscitated animal. Perhaps it may not be possible to resuscitate to an end point, but whether or not this group has used resuscitation techniques and whether or not there are cardiovascular changes that were seen in the 2 groups of animals, at least from a systemic standpoint, might have been similar.

The authors have in a very elegant manner described morphological changes and some of the microvascular changes that were seen in the preparation, but were there any physiological changes? Mainly when we are dealing with ARDS [acute respiratory distress syndrome] in sepsis, we like to look at PF ratios [PaO2/FiO2]. Whether or not there was indeed a fall in PaO2 is a major question.

Now, getting to the diabetic model, the major lesion appears to be the fact that these cells can migrate across the microvascular and don’t appear to be able, but they aren’t being activated for some reason. I know that cell signaling nowadays by many individuals is thought to be the result of the selectin family of molecules. These contain carbohydrate materials whether or not this diabetic animal has a defect in its selectins. Of course, the other aspect of this is the damage that normally is seen with changes in permeability due to the release of oxygen free radi-
We are still in the process of examining that. Line and in response to injury, than the nondiabetic animals, phospholipids, which then causes a relative deficiency of arachidonic acid, which leads to an altered composition of membrane baddics. The diabetic animals do have altered fatty acid metabo-

And regarding the cardiovascular status of these animals, we have not measured cardiac output or blood pressure or any other hemodynamic variables. Intratracheal endotoxin is not associated with a severe hemodynamic compromise as IV administra-

With regard to problems with selectins in diabetic animals, I am not aware of any defects in these animals, although mem-

And with regard to the oxidative burst in these neutro-

We spoke about relevancy. Throwing endotoxin down an animal's lung is not how our patients get challenged, so let's start getting relevant and throw bacteria, which is a replicat-

And second of all, you mentioned oxygen radicals. Do you have any data or have you measured the capability of these cells to respond to any trigger, either membrane associated with FMLP [N-formyl-methionyl-leucyl-phenylalanine] or PMA [phorbol-

And I wonder if you have any evidence or data to support that. These animals are young enough that they probably wouldn't show structural changes on morphology as far as thick-

And regarding the cardiovascular status of these animals, we have not measured cardiac output or blood pressure or any other hemodynamic variables. Intratracheal endotoxin is not associ-

That is a very good question, and I have looked at some studies showing those differences. Talking to some of the pul-

No, we have not done that. If I understand you correctly from your preliminary data where you used basically a hydrostatic force, mechanical force, and showed decreased leak, and now in this series where you show that you do get neutro-

E. Patchen Dellinger, MD, Seattle: Are there any data sug-

Ronald V. Maier, MD, Seattle, Wash: If I understand you correctly from your preliminary data where you used basically a hydrostatic force, mechanical force, and showed decreased leak, and now in this series where you show that you do get neutro-

Cathy L. White Owen, MD, Cleveland, Ohio: Since you are using an isolated perfusion model, is there any possibility that you would consider looking at a cell-free system to try and sort out whether this is a PMN [neutrophil]–related issue vs a lung-related issue, and have you considered doing studies where you used diabetic lungs with normal neutrophils and normal lungs with diabetic neutrophils?

With regard to a more relevant model, such as bacteria in-

Dr Wright: No, we have not done that.