Introduction

Background: ARDS is a life threatening condition where fluid fills lung alveoli causing respiratory failure. ARDS is triggered by trauma, sepsis, pneumonia, or inhalation of harmful substances. ARDS indiscriminately victimizes 150,000 Americans annually. Patients will generally present with increasing shortness of breath, a rapid respiration rate, and low blood pressure. Within hours, ARDS patients will have to be paralyzed, intubated and treated with mechanical ventilation. ARDS kills more people annually than AIDS and breast cancer combined with a mortality rate of ~42%.

Inflammation contributes to ARDS: John E. Repine M.D. pioneered the discovery that neutrophils may damage the alveolar capillary barrier and cause ARDS. ARDS is characterized by inflammatory cell infiltration.1 David Wagner PhD., described that CD40 T cells play a major role in inflammation in autoimmune diseases such as systemic lupus erythematosus and type 1 diabetes. T cells that are CD40+ can give rise to pathogenic effector T cells.2 These inflammatory T-Cells, specifically the CD4 T-Cell and the CD40 T-Cell, have caustic effects when they mistakenly attack the pancreas in patients with type 1 diabetes. The role of CD40 T cells in ARDS has not been studied.

Hypothesis: Our Hypothesis was that CD40 T cells contribute to ARDS. We tested our premise by comparing the levels of CD4 and CD40 T cells in the lungs and other organs between control rats and ARDS resistant/tolerant rats after exposure to hyperoxia for 52 hours which produces ARDS.

Materials and Methods

Inducing ARDS

The control and tolerant rats were placed in a hyperbaric chamber at sea level pressure with a 100% oxygen pressure for 52 hours inducing ARDS.

Tissue Collection

The chamber was depressurized and the rats were anesthetized with an abdominal pentobarbital injection. Blood is drawn from the heart using a heparinized syringe. The spleen, pancreas, thymus, and lung tissue were also collected and placed in PBS. Lung tissue was placed in Collagenase solution then incubated for 4 hours.

Tissue Processing

Tissues were homogenized and filtered than used in staining. Peripheral blood leukocytes were isolated on Ficoll gradients then used in staining, with fluorochrome labeled antibodies to CD4 (eBioscience), CD40 (Webb-Waring), and CD3(BD Bioscience) antibodies. Flow cytometry using a MACSQuant (Miltenyi) measured the target T cell populations.

Results

CD4 and CD40 levels in control and tolerant blood pre and post hyperoxia exposure

CD4+CD40+ cell levels

Fig.1 T cells were isolated from the lungs of control rats and tolerant rats. Cells were stained with anti CD4, anti CD40, and anti CD3. CD3 cells were gated for CD40+ and CD4+ both in the control rats as well as the tolerant rats. 72.4% of the cells from the tolerant rat were CD4+CD40+ while only 45.6% of the cells from the tolerant rats were CD4+CD40+. This demonstrates that significantly fewer CD40+ and CD4+ T cells are activated in the tolerant rats than in the control rats.

CD4 and CD40 in tolerant and control blood pre and post hyperoxia exposure

Fig.2 Blood samples were collected from control and tolerant rats pre and post hyperoxia exposure. T cells were isolated from the blood of control rats and tolerant rats. Cells were stained with anti CD4, anti CD40, and anti CD3. CD3 cells were gated for CD40+ and CD4+ both in the control rats as well as the tolerant rats. Comparison of T-Cell levels between the pre and post hyperoxia blood samples showed that in both control and tolerant there was a decline in the number of CD40+ cells while there was an increase in the number of CD4+ cells. The control rats had a 10% greater decrease in CD40 cells than the tolerant rats and had a 12% higher increase in the number of CD4+ cells than the tolerant rats.

Summary

We found that total CD40/CD4 T cell populations were similar in both rat strains. However, the control rats had higher levels of CD40/CD40+ T cells. The blood samples prior to hyperoxia showed higher CD40/CD40 T cell populations in both control and tolerant rats compared to their levels after. We found that although the total number of CD40/CD40 cell population remains similar before and after hyperoxia, the CD40/CD40+ population present in the blood before hyperoxia decreases after treatment. At the same time the CD40/CD40 population increases after hyperoxia treatment (Fig 2).

Conclusions

CD40/CD4 T cells may contribute to the inflammatory response in ARDS but more studies need to be done:

• Rat tissue samples could be studied without hyperoxia treatment to have a control for ARDS induced rats.

• Studying more rats would make statistical evidence possible.

• Pretreating the rats with a CD40 blocking peptide then study the CD40/CD4 T cell levels would determine whether CD40 is critical in ARDS.

• Hyperoxia exposure to T cell deficient rats to determine if they are resistant.

• Possible consideration of using a mouse model as it would offer more tools, strains, and antibodies.

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References
