



2009 Research Grant Program Winning Abstract

Novel Role for IL5 in Sepsis

By Jeffrey Gold

Sepsis, defined as the systemic inflammatory response to infection affects over 700,000 people/year in the United States with nearly 250,000 deaths. This results in a net cost of \$17 billion/year, is the leading cause of death in ICU and the 10th leading cause of death overall. Despite recent advances and adequate antimicrobial therapy, mortality remains in excess of 25%.

Inflammation in the early stages of severe sepsis and septic shock is controlled primarily by the innate immune response. Activation of innate immune effector cells including neutrophils (PMNs) and monocytes/macrophages is essential for control of bacterial pathogens as well as production of the majority of pro and anti-inflammatory cytokines observed in sepsis. The central role for these cells in the host response is supported by an increase in mortality with severe neutropenia and the improved outcomes with either administration of PMN derived granule products or stimulation/recruitment of PMNs and monocytes through the use of recombinant GM-CSF. Eosinophils are another granulocyte subset with in vitro antibacterial activities. Sepsis is associated with a relative eosinopenia with the degree of eosinopenia associated with severity of disease. This suggests a potential beneficial role for Eosinophils in the host response to sepsis.

We recently published that transgenic mice which constitutively express the Eosinophil maturation factor IL-5 and have a corresponding profound Eosinophilia have improved survival and bacterial clearance in polymicrobial sepsis induced by Cecal Ligation and Puncture (CLP) (Yousefi and Gold et al, Nat Med 2008). This effect was specifically attributed to Eosinophils, based on improved survival with adoptive transfer of Eosinophils. A potent antibacterial role for Eosinophils further supported both by increased Eosinophils mediated bacterial killing in vitro and in a defined in vivo bacterial challenge model. Given that Eosinophil transfusion is not a feasible therapeutic intervention for humans, we next explored whether administration of the Eosinophil maturation factor, IL-5, could result in a similar improvement. Although failing to stimulate Eosinophil recruitment, IL-5 did increase PMN recruitment and administration of IL-5 significantly improved survival in CLP even when given up to 4hrs after the onset of sepsis, suggesting this is a potentially important rescue therapy. We confirmed this to be an Eosinophil independent effect as IL-5 overexpressing mice with a congenital loss of Eosinophils were equally protected in our sepsis model. Further, mice deficient in IL-5 had increased mortality and bacterial burden. In an attempt to understand the mechanism of IL-5 protection, we have begun to identify new targets for IL-5 stimulation in the innate immune response. We have now discovered a functional IL-5 receptor on PMNs and monocytes after CLP or LPS stimulation in vitro suggesting other innate immune effector cells are capable of responding to IL-5 stimulation. Finally, in humans with sepsis we demonstrated expression of IL-5 on circulating PMNs and monocytes and increased levels of IL-5 are associated with increased survival suggesting a protective role for IL-5 in sepsis and septic shock.



We hypothesize that IL-5 is essential to the host response in polymicrobial sepsis and administration of IL-5 will improve survival through its action on non-eosinophilic granulocytes.

In the initial experiments, we will define the role for IL-5 in modulating PMN and macrophage function in vitro. Flow cytometry will provide the core of these experiments both for identification of the IL-5 receptor on the respective cell types, but also for assessment of IL-5 stimulation on numerous cellular activities including cytokine productions, using intracellular cytokine staining, and apoptosis, using annexin-V staining. This data will then be used as the basis for determining the mechanism of IL-5 protection in polymicrobial sepsis in vivo using a combination of genetic and pharmacological approaches. Again, flow cytometry will be at the core of these experiments to determine the source of endogenous IL-5 production using intracellular cytokine staining, and to directly associate IL-5 receptor expression with the in vitro activities determined above.

We will then begin to translate these findings into humans. Initial experiments will be focused on confirming expression of IL-5 receptor on human macrophages and PMNs via flow cytometry and whether IL-5 stimulation of these cells produces a similar outcome as observed with the murine cells. This is essential to ascertaining whether our initial data are purely a species specific phenomenon. Further, we will confirm our preliminary data and assess the ability of either plasma IL-5 or expression of IL-5 Receptor on circulating PMNs or monocytes to serve as biomarkers for outcome in human sepsis. These experiments will be centered on using multicolor flow-cytometry in similar manner to our previously reported data with CD80 and CD40 in human sepsis (Nolan et al 2008; Nolan et al 2009).

In conclusion, this proposal will provide important and novel information regarding the role of IL-5 in regulating the innate immune response in sepsis. At the core of this proposal is the identification and functional stimulation of the IL-5 receptor on new cellular subsets, PMNs and macrophages. The funding for BD related products and services will be essential for gathering this information.

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