Innate Immune Response Induced in Gnotobiotic Piglets by a Mixed Culture of Commensal Bacteria

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Abstract
Our laboratory has developed a recombined porcine-derived mixed bacterial culture (RPCF) isolated from the ceca of a healthy, pathogen-free pig and have maintained it at steady state in a continuous-flow chemostat. The culture has been shown to protect neonatal and weaned pigs from infection and disease caused by Salmonella and E. coli. However, the mechanism of action of the protection from pathogens observed with the RPCF culture remains unclear. In the present study, 40 piglets were delivered by caesarian section and reared under gnotobiotic conditions. Piglets were either given RPCF within 1 hr after birth or were given sterile media. At times 0, 8, 24, 48, and 72 hr post-birth, piglets were euthanized and samples of spleen taken. Splenic cells from individual piglets were isolated and cultured with or without concanavalin A (conA). Splenic cells from RPCF-treated piglets had increased levels of IL-1β, IFN-γ, IL-18, and IL-10 at 8 hr after birth compared to control piglets as measured by porcine cytokine ELISA. The increased levels of cytokines produced by RPCF-treated piglet splenocytes then declined over time, returning to levels observed in control pigs, or in some instances, below control levels. These results suggest that RPCF may act as a modulator for certain aspects of innate immune development.

Introduction
Our laboratory has developed a recombined, porcine-derived continuous-flow culture of mixed commensal bacteria (Gram-positive obligate anaerobes), designated as RPCF. RPCF has eliminated multiple strains of Escherichia coli and Salmonella from chemostats during in vitro challenge (1). When piglets are orally dosed with RPCF within 24 h of birth, the treated piglets have reduced colonization, shedding, and disease from E. coli and Salmonella following laboratory challenge with virulent strains of these organisms (2,3). Furthermore, under field trial conditions, treatment of piglets at birth decreased mortality and medication costs associated with enterotoxigenic strains of E. coli (4). The mechanism of protection is unknown, but the authors speculate that it could be exclusion of pathogens by competition for attachment sites, receptors, or nutrition; production of bactericidal compounds such as bacteriocins; modulation and enhancement of immune function; or all of the above. It is a known fact that commensal bacteria play an important role in the development of innate immune responses. The objective of the present study was to determine if RPCF could affect innate immune response in gnotobiotic piglets.

Materials and Methods
Forty piglets were delivered by caesarian section and reared under gnotobiotic conditions. Piglets were either given RPCF within 1 h after birth or were given sterile media. At times 0, 8, 24, 48, and 72 h post-birth, 4 piglets from each group (total of 8 per time frame) were euthanized and samples of spleen taken. Splenic cells from RPCF-treated piglets were isolated and cultured with or without concanavalin A (ConA) and analyzed by porcine cytokine ELISA for concentrations of IL-1β, IFN-γ, IL-4, IL-10, and IL-18.

Results and Conclusions
Splenic cells from RPCF-treated piglets had increased levels of IL-1β, IFN-γ, IL-10, and IL-18 at 8 h after birth compared to control piglets. The increased levels of cytokines produced by RPCF-treated splenocytes then declined over time, returning to levels observed in control pigs, or in some instances, below control levels. We conclude that RPCF can affect immune function in neonates and speculate that early innate responses observed herein may positively impact acquired immune responses later.

Discussion
These results suggest that RPCF may act as a modulator for certain aspects of innate immune function. If so,
RPCF could be used to “prime the pump” of the immune system which could induce earlier development of the innate system in neonates. In neonatal mammals, it appears that the immune system becomes “tolerant” of the commensal flora as it is exposed to them, eventually leading to a “non-response” to these bacterial species (5,6,7,8). Although there is a response to these organisms, this response is not detrimental to the host, keeping the normal flora relegated to their normal niche within or on the host. In this way, the host does not continue to respond to the normal flora in a costly way, saving a robust response for pathogens the host encounters. Failure of the immune system to tolerate the commensal flora can lead to inflammatory disease in the host (5). Indeed, in swine, Lactobacillus species have been shown to enhance the immune response of pigs to an E. coli challenge, inducing T cell differentiation and enhanced cytokine expression (9). IL-1β, IFN-γ and IL-18 are considered pro-inflammatory cytokines, aiding in the production of an immune response to non-self antigens. These cytokines would also be considered to be indicators of an innate immune response, the first line of defense of the host, leading to the production of the acquired immune response. It is unclear, however, if the production of these cytokines and the subsequent induction of the innate immune response are responsible for the protective effects of the RPCF culture, or if this response is part of the tolerance process. The induction of IL-10 production would indicate an attempt to modulate the response to the microflora, and perhaps an indication of the beginning of the tolerance process. Further studies would need to be specifically targeted to the tolerance process to flesh out the response, perhaps covering longer periods of time after birth.

References