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Depletion of natural $CD4^+CD25^+$ T regulatory cells with anti-CD25 antibody does not change the course of *Pseudomonas aeruginosa*-induced acute lung infection in mice

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Abstract

Pseudomonas aeruginosa is a common cause of lung infection in immune compromised individuals. Studies in humans and mice have demonstrated that *P. aeruginosa* lung infection is associated with a predominant Th2 immune response, whereas Th1 responses are accompanied by a better pulmonary outcome. Regulatory T cells (Tregs) are a subpopulation of T cells with unique immunologic characteristics that suppress effector T cell functions. Whether Tregs contribute to *P. aeruginosa*-induced host responses has not been studied previously. We found that *P. aeruginosa* lung infection induced an increase in natural Treg cells ($CD4^+CD25^+FOXP3^+$ T cells) in the spleen of mice. To investigate a role of natural $CD4^+CD25^+$ Tregs in the host response to *P. aeruginosa* lung infection *in vivo*, anti-CD25 Ab was used to deplete endogenous $CD4^+CD25^+$ Tregs. Anti-CD25 treatment depleted 90% of $CD4^+CD25^+FOXP3^+$ cells. Surprisingly, no differences of *P. aeruginosa*-induced NF-κB activation and cytokine/chemokine production (IL-1β, TNF, IL-6, IL-10, RANTES or MIP-2) were observed between anti-CD25-treated and isotype control Ab-treated animals. Similarly, no differences in lung histology and airway neutrophil infiltration were observed between anti-CD25 and control Ab-treated animals. Furthermore, no difference in survival outcome was found between anti-CD25 and control Ab-treated animals. These data demonstrate that although *P. aeruginosa* lung infection causes an increase of Tregs, the endogenous natural $CD4^+CD25^+$ Treg cells do not contribute significantly to the host response to this bacterium.

Keywords: Inflammation; Innate immunity; Lung infection; *Pseudomonas aeruginosa*; Regulatory T cells

Introduction

Pseudomonas aeruginosa is a major opportunistic pathogen in immune compromised patients (FitzSimmons, 1993). Once *P. aeruginosa* colonizes the lung, it is extremely difficult to completely eradicate this bacterium from the body, likely due to the inadequate host

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responses. *P. aeruginosa* appear to initiate a unique pattern of immune responses that is distinct from those induced by other bacteria (Cripps et al., 1995; Heeckeren et al., 1997; Kipnis et al., 2006; Skerrett et al., 2004). The unique features of the immune system in the lung (Holt et al., 2008) and the specific pathogenic mechanism of *P. aeruginosa* (Govan and Deretic, 1996) contribute to the pattern of immune responses during *P. aeruginosa* lung infection. Major efforts have been made in dissecting the complexity of cell types involved, cytokines/chemokines produced, and signaling pathways activated by *P. aeruginosa* lung infection.

Studies in human pulmonary cells and peripheral blood demonstrated that *P. aeruginosa* infection is associated with a predominant Th2 immune response, whereas Th1 responses are accompanied by a better pulmonary outcome (Brazova et al., 2005; Hartl et al., 2006; Moser et al., 2000). Mouse models of chronic *P. aeruginosa* infection showed that Th1-reacting mice had a better disease outcome with an enhanced bacterial clearance, lesser lung inflammation, and better survival compared with Th2-reacting mice (Moser et al., 1999, 2002). Accordingly, T cell-regulated immune responses have been associated with *P. aeruginosa* lung infection.

Naturally occurring $CD4^+CD25^+$ regulatory T cells (Tregs) arise during the normal process of thymic development and constitute 5–10% of blood $CD4^+$ T cells (Ziegler, 2006). The transcription factor forkhead box P3 (FoxP3) has been shown to be essential for Treg development and function (Ziegler, 2006). These cells constitutively express CD25 and inhibit inflammatory responses through suppressive activities against T effector cells, dendritic cells, neutrophils and other cells (Ziegler, 2006). $CD4^+CD25^+$ Tregs have been shown to express a full spectrum of toll-like receptors (TLR) and respond to microbial products (Caramalho et al., 2003; LaRosa et al., 2007; Liu and Zhao, 2007; Sutmuller et al., 2006; Wang, 2006). Evidence is emerging that Tregs can control immune responses to microbial pathogens including virus, parasites, bacteria and fungi (Belkaid, 2007; Demengeot et al., 2006; Mills, 2004; Suvas and Rouse, 2006). However, the outcome of pathogen infection modulated by Tregs remains controversial. Studies have showed a protective role, no effect or a detrimental role of Tregs during microbial infection.

The protective role of Tregs during microbial infection is likely due to the inhibition of the damaging effects associated with excessive anti-pathogen immune responses. For example, Th1-cell responses were enhanced and the severity of T cell-mediated lesions in the cornea of virus-infected mice was increased if mice were depleted of $CD4^+CD25^+$ Tregs (Suvas et al., 2004). Similarly, the absence of $CD4^+CD25^+$ regulatory T cells is associated with increased pathology in *Helicobacter pylori*-infected mice (Raghavan et al., 2003).

No major effects of Tregs on the course of microbial infection have also been reported. In a mouse model of *Helicobacter* infection, depletion of $CD25^+$ Treg prior to and during infection did not affect either bacterial colonization or the severity of gastritis (Kaparakis et al., 2006). Similarly, depletion of endogenous Treg did not affect the bacterial burden or survival of mice following mycobacterial lung infection (Quinn et al., 2006) or polymicrobial sepsis (Scumpia et al., 2006).

The detrimental effect of Tregs in some models is likely mediated by the inhibition of protective immune responses to infection. Accordingly, removal of Tregs leads to improved host defense. In a mouse model of *Helicobacter pilori* infection, depletion of endogenous $CD4^+CD25^+$ T cells lead to heightened host responses and more efficient bacterial clearance (Rad et al., 2006). In humans, the number of Tregs increases in the blood or at the site of infection in active tuberculosis patients. The frequency of Tregs correlates inversely with specific immunity to *Mycobacterium tuberculosis*, suggesting that expansion of Tregs during infection likely plays a role in depressed T cell response to this bacterium in these patients (Chen et al., 2007; Ribeiro-Rodrigues et al., 2006).

The mechanisms underlying the controversial biological roles of Tregs during microbial infection remain unclear. It appears that a role of Tregs in infection varies depending upon the type of infection and specific cell type and tissues involved. The role of Tregs in *P. aeruginosa* lung infection has not been reported previously.

In light of the important role of T cell-regulated immune responses in *P. aeruginosa* infection, we examined whether Tregs are involved in the host response to this bacteria in lung infection *in vivo*. We found that *P. aeruginosa* lung infection in mice caused an increase of the natural Tregs in the spleen. Surprisingly, depletion of endogenous Tregs had no effect on bacterial clearance, neutrophil infiltration, cytokine production or animal survival.

Materials and methods

Mice, antibodies and reagents

Male C57/BL6 mice (8–12 weeks old) were purchased from Charles River Laboratories (Wilmington, MA). Mice were intranasally infected *P. aeruginosa* strain 8821 (1×10^9 CFU/mouse) or strain PAK (1×10^7 or 1×10^8 CFU/mouse). The protocols were approved by the University Committee on Laboratory Animals, Dalhousie University, in accordance with the guidelines of the Canadian Council on Animal Care.