Comparative Immunology, Microbiology and Infectious Diseases 35 (2012) 233–239

Contents lists available at SciVerse ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Regulatory T cells in cattle and their potential role in bovine paratuberculosis

Paul M. Coussens[a,*], Sue Sipkovsky[a], Brooke Murphy[a], Jon Roussey[b], Christopher J. Colvin[a]

[a] Molecular Pathogenesis Laboratory, Department of Animal Science, Michigan State University, East Lansing, MI 48824, USA
[b] Comparative Medicine and Integrated Biology Program, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

Keywords:
Regulatory T cells
Foxp3
Paratuberculosis
Johne's disease
Bovine leukemia virus

ABSTRACT

The intracellular bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes Johne's disease in wild and domestic ruminants. Johne's disease presents as a chronic enteritis with severe inflammation of intestinal tissues, characterized by widespread infiltration of macrophages, the target cell of MAP. Clinical signs of Johne's disease are typically accompanied by a loss of peripheral CD4+ T cell responses to MAP antigens and an increase in anti-MAP serum IgG levels. Recently, it was proposed that regulatory T cells might develop over the lengthy course of subclinical MAP infection. In the past five years, significant progress in defining bovine regulatory T cells has been made. These studies grew out of observations that IL-10 is produced by PBMCs in response to MAP antigen stimulation and that neutralization of this IL-10 could enhance IFN-γ production from MAP-antigen reactive effector T cells. Depletion studies revealed that MAP responsive cell populations producing IL-10 were largely CD4+ and CD25+, although monocytes have also been shown to produce IL-10 in response to MAP. In addition, evidence for a regulatory population of γδ T cells has also begun to accumulate. We summarize current thinking regarding regulatory T cells in MAP infection and provide data suggesting a potential link between regulatory T cells, bovine leukemia virus, and MAP.

1. Introduction

Infection of ruminants with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) can lead to Johne's disease, a chronic gastrointestinal disease leading to granulomatous enteritis, persistent diarrhea, progressive wasting, and death [1]. Current estimates suggest Johne's disease costs the US dairy industries between $250 million and $1.5 billion each year [2]. Johne's disease has a global distribution and is of considerable concern in cattle, as well as in sheep, farmed red deer, and goats. Vaccines against MAP are available, however these simply limit development of clinical symptoms, but do not prevent infection or shedding. Because they interfere with test and cull control programs, vaccines against MAP are not routinely used in the US [3,4]. Infection of cattle with MAP generally leads to a long subclinical phase of infection, where detection of infected animals can be difficult. Clinical signs of disease typically do not develop until 2–5 years following initial infection.

Typical of most mycobacteria, MAP is an intracellular pathogen that preferentially infects host macrophage cells [5,6]. Once inside these cells, MAP prevents phagosome maturation, thus limiting efficient bacterial destruction and antigen presentation to other immune effector cells, such as T cells [7,8]. We now also know that MAP is very

* Corresponding author at: Department of Animal Science, 1205H Anthony Hall, Michigan State University, East Lansing, MI 48824, USA. Tel.: +1 517 353 3158; fax: +1 517 353 1699.
E-mail address: coussens@msu.edu (P.M. Coussens).

0147-9571/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cimid.2012.01.004

good at preventing spontaneous and induced apoptosis in infected macrophages [9]. Apoptosis would sequester MAP bacteria inside apoptotic bodies that are phagocytosed by other innate immune cells and destroyed in a manner promoting antigen presentation, a process known as efferocytosis. MAP interference with macrophage apoptosis therefore reduces proper antigen presentation and development of effective immune responses. Prevention of macrophage apoptosis may also explain the vast numbers of MAP-containing macrophages that persist in infected intestinal tissues. Infection with MAP also reduces the ability of macrophages to signal T cells following engagement of the CD40 receptor [10]. In particular, the ability of macrophages to produce IL-12 and iNOS in response to CD40 receptor activation is severely reduced following infection with MAP [10].

It is generally accepted that MAP infected cattle initially develop a pro-inflammatory and cytotoxic immune response to MAP antigens, which declines in cattle that progress to clinical disease [5,11,12]. Near the transition from subclinical to clinical disease, MAP infected cattle typically show strong serum antibody reactivity to MAP antigens, with reduced IFNγ production from stimulated T cells. A plausible explanation for reduced effector T cell activity in response to MAP antigens is development of regulatory T cell populations (Tregs) that limit effector T cell responses [11,13,14]. Antigen specific Tregs develop in response to many other infectious diseases, particularly those that, like MAP, have a long subclinical or persistence phase [15–17]. In some cases, effector cells and Tregs appear to establish a balance benefiting both host and pathogen, reducing inflammation and promoting pathogen persistence and transmission [15,18,19].

During the past five years, work on regulatory T cells in cattle has provided us with the tools and reagents to study this diminutive population of specialized cells in various infectious diseases, including Johne's disease. Here, we summarize some of the current thinking regarding bovine regulatory T cells, including both αβTCR+ and γδTCR+ cells. We review recent literature suggesting a role for regulatory T cells in MAP infection and development of Johne's disease, including a possible connection with bovine leukemia virus co-infection. We also discuss potential consequences of regulatory T cell activity on immune responses to MAP infection.

2. Evidence for the existence of bovine Treg cells

Regulatory T cells are now a generally well-recognized critical component of the mammalian immune system. Treg cells derived in the thymus are generally thought to limit autoimmune disorders, such as depression of autoimmune colitis in mouse models. In these studies, the autoimmune depressing cells were shown to constitutively express the surface markers CD4 (CD4+) and CD25 (CD25+; IL-2 receptor) and the forkhead box P3 (Foxp3+) transcription factor [20–22]. Treg cells also express CTLA-4, a co-signaling molecule and CD28 antagonist [23]. In addition to CTLA-4 and Foxp3, Treg cells also express the glucocorticoid induced TNF receptor (GITR), chemokine receptor (CCR8), and surface TNF receptor 2 (TNFR2) [24,25]. Foxp3 expression was once considered highly specific to Treg cells [26–28], but recent literature suggests that this transcription factor is also up-regulated by activated cells to function as a negative regulator of the effector response [29,30]. Because CD4, CD25, GITR, CTLA-4 and perhaps even Foxp3 are expressed by other immune cell types at various times, the combined expression of several of these markers must be used to uniquely define the Treg cell phenotype.

Thymic-derived Treg cells that limit autoimmune reactions exert their regulatory functions via a mainly contact dependent mechanism [24,31]. However, Treg cells that develop in the periphery do not rely on contact to suppress effector T cell functions [32]. Induced Treg cells work through production of anti-inflammatory and immune regulating cytokines, such as TGFβ and IL-10. Though it is unclear how these cells develop, current thought is that co-stimulatory molecules and cytokines play important roles [33–36]. Reduced co-stimulation of T cells by MAP infected macrophages could be one mechanism leading to development of MAP-specific peripheral Treg populations [10].

Induced Treg cells express CD4 and Foxp3 but, in contrast to their thymus-derived counterparts, they can be CD25 negative. Induced Treg cells typically express CD45R0 (a memory cell marker in cattle). Treg cells producing IL-10 are referred to as Tr1 cells, while those producing TGFβ are referred to as T_H3 cells [37–39]. Tr1 cells express the $\alpha_4\beta_1$ peripheral/inflammation homing receptor while Th3 cells typically express the $\alpha_4\beta_7$ mucosal homing receptor [40]. Of interest, PBMCs from cattle infected with MAP tend to secrete IL-10 in response to MAP antigen stimulation and tissues infected with MAP tend to show elevated levels of TGFβ expression [41,42].

Treg cells have been widely recognized in humans and mice [43] and the roles and functions of these cells are becoming clearer. However, studies related to Treg cells in cattle are relatively rare. The first studies reporting presence of regulatory cells in MAP infected cattle were conducted by Chiodini and Davis [44,45]. In these studies, a population of regulatory γδT cells in PBMCs from MAP-infected cattle limited CD4+ T cell proliferative responses to MAP antigen stimulation. Down-regulation of CD4+ T cell responses could be overridden by CD8+ T cells, although the mechanism responsible for this was not identified. In 2002, Valheim et al. noted an inverse relationship between CD25+ cells resident in the lymph node cortex and the number of peripheral cells that responded to MAP antigens by upregulating CD25 expression in vaccinated goats [46]. In other words, as the numbers of CD25+ cells in lymph nodes increased, the peripheral response to MAP antigen stimulation decreased. One explanation put forth for these observations was that CD25+ cells in the lymph node cortex were regulatory cells that limited effector cell responses during MAP antigen stimulation. Unfortunately, reagents were not available at that time to demonstrate that the CD25+ cells in lymph nodes were FoxP3+ and thus likely regulatory T cells.

In 2008, Seo et al. published on development of monoclonal antibodies (MAb) directed against bovine FoxP3 [47]. FoxP3 MAbs were used to characterize Treg cells proliferating in response to stimulation of bovine PBMCs for