NOTE *Parasitology*

Expression of regulatory dendritic cell-related cytokines in cattle experimentally infected with *Trypanosoma evansi*

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ABSTRACT. *Trypanosoma evansi* causes wasting disease in many livestock. *T. evansi* infection gives rise to inflammatory immune responses, which contribute to the development of inflammation-associated tissue injury. We previously reported that regulatory dendritic cells (DCs), which act as potential regulators of inflammation, were activated in infected mice and transfer of regulatory DCs to infected mice prolonged their survival. However, the kinetics of regulatory DCs in cattle, which are natural hosts of *T. evansi*, remained unclear. In this study, we report that the expressions of CCL8 and IL-10, which promote the development of regulatory DCs, were up-regulated in cattle experimentally infected with *T. evansi*. This finding is potentially useful for studying the control strategy of *T. evansi* infection in cattle.
KEY WORDS: cattle, CCL8, IL-10, regulatory DC, *Trypanosoma evansi*

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Trypanosoma evansi (*T. evansi*) is a widely distributed species of trypanosome that causes a disease called surra in most livestock [5, 17]. Surra is a wasting disease, and the clinical symptoms include fever, anemia and weight loss. *T. evansi* is spread by mechanical transmission of infected blood through biting flies, such as horse fly (*Tabanus*) and stable fly (*Stomoxys* spp.) [2, 18]. In recent years, the damage of surra has been increasing, because of expansion of the biting fly's range as well as transportation of infected livestock. The well-known immune evasion systems of trypanosome make it difficult to develop an effective vaccine [20]. Therefore, the elucidation of surra immune pathology in livestock is required to establish the treatment protocol and construct disease-tolerant livestock.

Effective elimination of pathogens requires the induction of inflammatory immune responses. Inflammatory cytokines, such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), play major roles in the control of trypanosome infections [3, 7–9, 15]. However, the production of excessive inflammatory cytokines or chemokines was already identified as a key player in trypanosome infection-associated pathogenicity [1, 6, 10, 13]. The cellular and molecular components that govern this delicate balance are necessary to understand the pathology of *T. evansi* infection. We have previously reported that regulatory dendritic cells (DCs), which secret a high dose of IL-10 and activate regulatory T cells [19, 22], were induced in mice experimentally infected with *T. evansi* and controlled excessive inflammation [11]. In this study, we reveal that the phenomenon also occurs in cattle, the natural hosts of *T. evansi*.

In this study, four Holstein cattle (*Bos taurus*, 8–9-month-old males) were used for experimental infection. All cattle were obtained from the Experiment Farm, Field Science Center for Northern Biosphere, Hokkaido University (Sapporo, Japan) and reared in the experimental animal facility of the Graduate School of Veterinary Medicine, Hokkaido University. This study was conducted in strict accordance with the recommendations set out in the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. The protocol for the animal experiment was approved by the Hokkaido University Animal Care and Use Committee (Approval number: 1028 and 11-0060). *T. evansi* strains L2 and L3 [12], isolated from water buffalo in Philippines, were subcutaneously injected into the cattle's cervical region. Two cattle were infected with strain L2. The others were infected with strain L3. The pathogenicity in mice and cattle was different between strains L2 and L3, and strain L2 had higher pathogenicity than strain L3 [12]. One day and just before the injection of trypanosome to cattle, we collected blood from the cattle and confirmed that those cattle were not infected with trypanosome. The number of trypanosome in blood was estimated by quantitative real-time polymerase chain reaction (PCR) as described by Konnai *et al*. [4]. Extraction of total RNA from the peripheral blood mononuclear cells (PBMCs) and quantification of the cytokines by real-time PCR were performed as described by Mekata *et al.* [11]. We set the mean cytokine expression of

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Table 1. Primers used for cytokine gene quantification in this study

Target gene	Primer name	Nucleotide sequence
CCL8	Bos-CCL8-F	5′-GGG ATT CTG TGT CTG CTG CT-3′
	Bos-CCL8-R	5′-GCA GGT GAT TGG GGT AGA AA-3′
IL-10	Bos-IL10-F	5′-TGT TGA CCC AGT CTC TGC TG-3′
	Bos-IL10-R	5′-GGC ATC ACC TCT TCC AGG TA-3′
Glucuronidase β	Bos-Gusb-F	5′-CAG ATG CCA TTG AAG GGT TT-3′
	Bos-Gusb-R	5′-TTT GGT CCA GAA CCA CAT GA-3′
Heat shock protein 90	Bos-Hsp90-F	5′-GCC AAG TCT GGC ACT AAA GC-3′
	Bos-Hsp90-R	5′-AGG CAG AGT AGA AGC CCA CA-3′

one day and just before the injection of trypanosome as the reference of relative ratio. The primers used for quantification of cytokines in this study are listed in Table 1.

In order to clarify the immunobiological aspects, we used two strains of *T. evansi* that differ in virulence in mice and cattle. In cattle infected with strains L2 and L3, the parasite was initially detected at 4–5 days post-infection, and parasitemic waves were observed at 4–5-day intervals (Fig. 1). The mRNA expression of IL-10 was up-regulated in 5–7 days post-infection (Fig. 2a). The expression of IL-10 in cattle infected with strain L2 decreased after 8 days post-infection. However, IL-10 expression increase was sustained for one month in strain L3. Although the maximum increase in IL-10 expression in *T. evansi*-infected mice was approximately 80-fold [11], the maximum up-regulation in infected cattle was only 3 to 6-fold. The difference in gene expression increase may be a result of the difference in infection sensitivity between mice and cattle. The expression of CCL8 was strongly up-regulated in 5–6 days post-infection and down-regulated in the late phase (Fig. 2b). The expression pattern of CCL8 during the early phase of infection was similar to that of *T. evansi*-infected mice [11]. CCL8 and IL-10 are known to promote the development of and increase in regulatory DCs in mice [16, 19]. Therefore, the increase in CCL8 and IL-10 production in cattle indicates the induction and development of regulatory DCs. However, the detailed function of regulatory DCs in cattle remains unclear. We need to confirm that regulatory DCs in cattle have a similar function and character as those in mice.

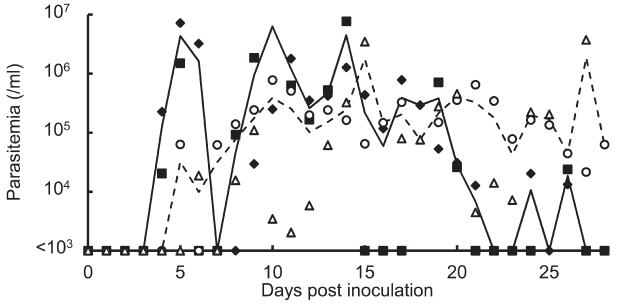

Fig. 1. The changes in parasitemia in four experimentally infected cattle. Cattle were subcutaneously inoculated with 2.0×10^7 *T. evansi* strain L2 or L3 [12]. The black (box and diamond) and white (triangle and circle) shapes indicate the parasitemia changes of each cattle infected with *T. evansi* strain L2 (high virulence) and L3 (low virulence), respectively. The black and dotted lines indicate the mean parasitemia of strains L2 and L3, respectively.

In contrast to trypanosomiasis in mice, the immunobiological aspects of *T. evansi* infection in livestock have hardly been documented. The pathogenicity of *T. evansi* infection widely differs among the animal species. The symptoms of *T. evansi*-infected camels and horses become worse than

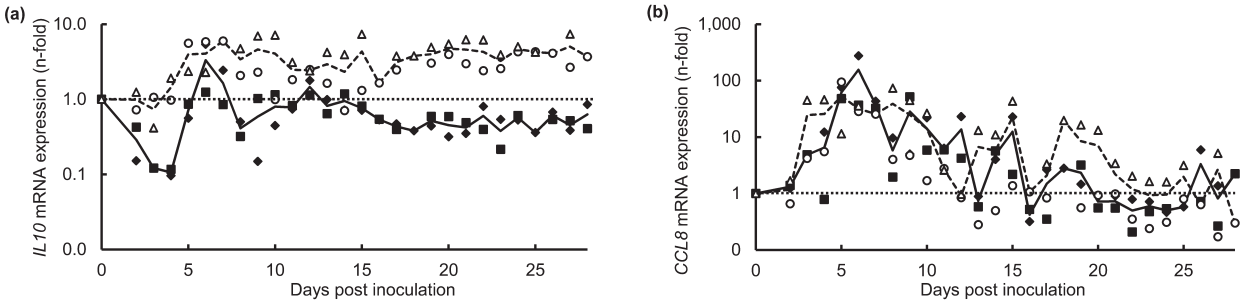

Fig. 2. The expression levels of CCL8 and IL-10 in PBMCs of *T. evansi*-infected cattle. The quantitative PCR results for the transcripts of IL-10 (a) and CCL8 (b) in PBMCs of *T. evansi*-infected cattle during 28 days post-infection are shown. Relative values of cytokine expression were calculated as the ratio divided by average quantity of cytokine expression in one day and just before *T. evansi* infections. The black (box and diamond) and white (triangle and circle) shapes indicate the amount of transcript of each cattle infected with *T. evansi* strain L2 (high virulence) and L3 (low virulence), respectively. The black and dotted lines indicate the mean transcripts of strains L2 and L3, respectively.

those of cattle or water buffalo [21]. In Philippines, water buffalo is the major livestock, and the damage of surra is mainly from cattle and water buffalo. These animals are closely related and have small differences in the cytokine structure [14]. Clarifying the immune responses against trypanosome infection in cattle also assists in understanding of immune response in water buffalo. Many factors are involved in the sensitivity of trypanosome infections. Excessive inflammation control is an important factor to resist trypanosome virulence [1, 6, 10, 13]. In this study, we revealed that CCL8 and IL-10 expression was increased with *T. evansi* infection in cattle. The result did not directly prove the existence of regulatory DCs in cattle, because of the unavailability of monoclonal antibodies of CD11c and CD45RB in cattle. However, this research will be helpful for developing a therapeutic agent or constructing a *T. evansi*-tolerant livestock strain.

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