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Short communication

Immunosuppression by T regulatory cells in cows infected with Staphylococcal superantigen

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Our recent study has provided that the *in vitro* SEC-induced proliferation of bovine T cells is preceded by a period of a non-proliferative immunoregulation of T cells that may be associated with cytokine production regulated by type 1 or type 2 T cells. Inversion of $CD4^+$:$CD8^+$ T cell ratio and induction of $CD8^+$ T cells with immunoregulatory activity could increase the probability of intracellular survival of *Staphylococcus aureus* (*S. aureus*). The increase of activated $CD8^+$ ($ACT2^+$ $BoCD8^+$) T cells in cows with mastitis caused by *S. aureus* may be associated with immune-regulatory function in the bovine mammary gland. The difference and similarity between bovine activated $CD8^+$ T cells ($CD8^+CD26^+$) and well-established human $CD4^+CD25^+$ T regulatory (Tr) cells may help to reveal their unique immune regulatory system in the host infected with *S. aureus*.

Key words: bovine mastitis, $CD4^+CD25^+$ T regulatory cells, immunosuppression, *Staphylococcus aureus*, superantigens

Early studies on bovine mononuclear leukocyte subpopulations in peripheral blood (PBL) and mammary gland secretions (MGS) have showed the mean ratios of $CD4^+$:$CD8^+$ T lymphocytes in the PBL and MGS are 1.53 and 0.85, respectively. The lower $CD4^+$:$CD8^+$ T lymphocyte ratios in the MGS were attributable to the presence of activated bovine $CD8^+$ ($ACT2^+$ $BoCD8^+$) T lymphocytes expressing activated molecule 2 ($ACT2^+$). This inversion was more evident in the cows with *Staphylococcus aureus* (*S. aureus*) infection [29,30,31].

We have speculated there were activated $BoCD8^+$ T lymphocytes present in the mammary gland of cows with *S. aureus* infection and these cell types were associated with downregulation of lymphocyte proliferation either stimulated by lectins or *S. aureus* antigen presented by autologous antigen presenting cells. Our previous study demonstrated that hyporesponsiveness of mammary gland lymphocytes, in part, mediated by the activated $BoCD8^+$ lymphocytes and suggested this population enhanced persistent intra-mammary infection by *S. aureus* [30].

Many strains of *S. aureus* associated with bovine mastitis produce staphylococcal enterotoxins (SEs), most frequently type C (SEC) [17]. The SEs and toxic shock syndrome toxin-1 (TSST-1) belong to a family of pyrogen toxins (PTs) produced by streptococcus and staphylococcus species. These PTs are superantigens (SAgs) that interact with conserved segments of the variable β (Vβ) chain of the T cell receptor (TCR) and molecules of the major histocompatibility complex (MHC) class II. SAgs cause MHC class II-dependent oligoclonal activation of large numbers of T cells resulting in proliferation, anergy and apopotosis. Moreover, SAgs may disproportionately affect different T cell subpopulations and reduce the $CD4^+$:$CD8^+$ T cell ratio by inducing $CD8^+$ T cell-mediated suppression of $CD4^+$ T cell proliferation [11].

Suppressor T cells that downregulate the differentiation of T helper (Th) cells or antigen-specific effector cells are recently reemerged as regulatory T (Tr) cells. These Tr cells play a key role in the homeostasis of the peripheral $CD4^+$ T cell pool [1,2] and these Tr cells are required for the inhibition of activation of self-reactive T cells and maintenance of tolerance [22].

Among many candidate regulatory cells, four cell types including $CD4^+CD25^+$, Tr1, Th3, and $CD8^+$ Tr have been focused and we have found T cell subsets uniquely present in bovine PBL stimulated with SAg, which are functionally

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similar to the Tr cells in human and mice.

Recent studies in our research have shown that activated form of $CD8^+$ ($CD8^+CD26^+$) T lymphocyte subpopulation was predominant when bovine mammary gland lymphocytes were stimulated with SEC1 or SEC-bovine toxins [19,20]. These results obtained from bovine mammary gland lymphocytes or PBL lymphocytes stimulated by SAg were dissimilar with those from our another study where $CD4^+CD26^+$ T lymphocytes were significantly high at 7 day *in vitro* culture with concanavalin A (Con A) mitogen.

SAg-activated $CD4^+$ T lymphocyte proliferation may be inhibited by activated $CD8^+$ T lymphocyte populations until 7 day culture with staphylococcal SAg in cows, however this activated $CD4^+$ T lymphocyte proliferation was not inhibited in human or when PBL were stimulated by Con A and pokeweed mitogen (PWM). This result may be attributable to those activated $CD8^+$ T lymphocytes which react as regulatory cells. However, at day 10, $CD4^+$:$CD8^+$ ratios increased as more $CD8^+$ T lymphocyte population decreased than $CD4^+$ T lymphocytes. This phenomenon may be due to residual survived $CD4^+$ T lymphocytes which were converted to Tr cells in the presence of activated $CD8^+$ T lymphocytes and contributed to regaining the immunity.

Although ACT2 molecule was not completely defined yet in bovine species, bovine ACT3 molecule has been determined as human $CD26^+$ orthologue [18]. The $CD8^+$ $CD26^+$ T lymphocytes increased in the presence of SAgs *in vitro* and *in vivo*. This increased population was accompanied by the increase of interleukin (IL)-10, but not by IL-2 or γ-interferon (IFN). The $CD8^+$ T lymphocytes may have an adverse effect on IL-2 which can lead delayed mitogenesis in early stage of *S. aureus* infection in the mammary gland of cows with mastitis [27]. Since human Tr cells from atopic donors are able to suppress Th1 and Th2 cytokine production [5], the increase of IL-10 in SEC treated bovine PBL by $CD8^+CD26^+$ suppressor cells may contribute to this suppression through the inhibition of $CD4^+$ T cell proliferation.

Recent researches have characterized the Tr cells as natural or adaptive forms and there are $CD4^+CD25^+$, Tr1, Th3, and $CD8^+$ Tr cells provoking a shift in the Th1/Th2 paradigm in immunity to infectious diseases [4,25,26]. Among these different T cells having regulatory function, $CD4^+CD25^+$ T cells may control autoimmunity, protective immunity and T-cell mediated inflammatory responses [3,15]. The activities are mainly associated with cell-cell contact basis, but the indirect involvement of cytokines, such as IL-10 or transforming growth factor (TGF)-β could not be excluded [16,23]. It has been also suggested that intranasal or oral administration of antigen may induce Tr cells and these adaptive Tr cells may be promoted by extrinsic regulatory forces, suppressive cytokines, inappropriate presentation of antigen in the absence of costimulation or cell-cell contact with natural Tr cells [6].

Specific antigen with low level of costimulation molecules, CD80 and CD86, may function mainly to maintain Tr cells [6,34]. We have found depressed antigen presenting capabilities of antigen presenting cells (APCs) from bovine mammary gland infected with *S. aureus* [30]. The decreased antigen concentration or exhaustion of antigen-specific effector cells by Tr cells has also been noticed [33]. The expression of MHC class II of APCs could be significantly decreased or blocked by CD95-CD95L interaction by anergic Tr cells in human [13]. And, these anergic Tr cells are induced by the coexpression of TGF-β and TGF-β receptor which are upregulated by TCR stimulation in the presence of APCs [7]. Therefore, this depressed antigen presenting activity may be attributable to the induction of Tr cells which contribute to the immunosuppression by SAgs *in vivo* [14].

$CD8^+CD26^+$ T cells stimulated by SEs may have immune regulatory function against $CD4^+$ T lymphocyte proliferation in cows and this phenomenon has not been shown in humans. When purified bovine $CD4^+$ lymphocytes were stimulated with antigen in the presence of various numbers of $ACT2^+$ $BoCD8^+$ lymphocytes, antigen responsiveness was reduced in a dose-related manner [30]. This suppression induced by $CD8^+$ T cells may act on APCs, which rendering them tolerance to $CD4^+$ T cell activities [9,10]. This distinct characteristic of bovine $CD8^+CD26^+$ T cells are similar to that of human $CD4^+CD25^+$ T cells which convey suppressive activity to conventional $CD4^+$ T cells. However, the activated ($ACT2^+$) $CD8^+$ Tr cells present in bovine mammary gland infected with *S. aureus* have shown suppressive activity against antigen-specific proliferative responses of $CD4^+$ T lymphocyte and these lymphocytes stimulated by SEs may have similar role in the regulation of $CD4^+$ T cell function in cows, which is different from human.

The activated $BoCD8^+$ T cells have apoptotic activity against $CD4^+$ T cells, whereas human $CD4^+CD25^+$ Tr cells are considered to be anergic and relatively resistant to activation-induced cell death (ACID) in the presence of SAg [28]. However, activated $BoCD8^+$ T cells have been found to induce Th2 shift by increasing IL-10, and this is similar to human $CD4^+CD25^+$ Tr cells which suppress the proliferation and IL-2 production of $CD4^+$ responder cells. Tr cells expressing IL-10 mRNA do not produce IL-2 and control T cell homeostasis [32]. IL-10 and TGF-β combined treatment appears to result in the preferential expansion and/or activation of Tr cells that facilitate immunosuppression or induction of tolerance [8]. The increased IL-10 in bovine peripheral blood mononuclear cells (PBMC) treated with SEC may provoke Tr cells which suppress immune responses in the cows with *S. aureus* infection.

Recently, we have found that SEC induced delayed mitogenesis of bovine lymphocytes, which is different phenomenon compared with human lymphocytes. Analysis of cytokine profiles in SEC stimulated bovine PBMC cultures suggested that transcription of Th1-like cytokines, especially

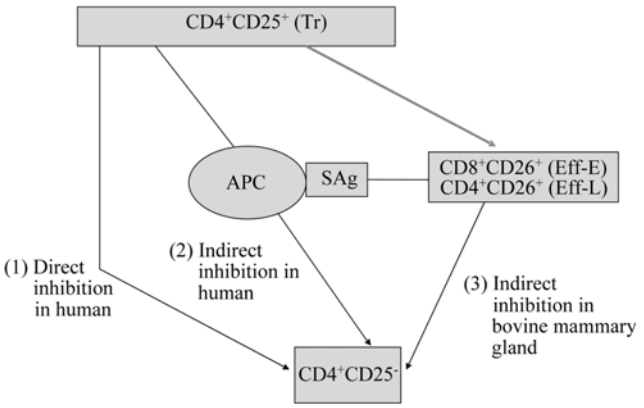

Fig. 1. Putative immunoregulatory mechanism in superantigen (SAg)-induced bovine mammary gland. $CD4^+CD25^-$ Tr cells expressing $CD44^+$, CD45RB, CD45RO, CD62L, CCR4, 5 and 6 inhibit the proliferation of $CD4^+CD25^-$ T cells directly (1) or indirectly (2) in the presence of antigen presenting cells (APCs) stimulated with SAg in human, and through the induction of $CD8^+CD26^+$ and $CD4^+CD26^+$ cells as effector cells at early stage (Eff-E) and later stage (Eff-L) in the bovine mammary gland stimulated with SAg (3). The inhibition is caused by cell-cell contact or IL-10 production. Tr cells and target cells may share the same antigen recognition or homeostatic competition. APCs express CCL17, 22, and have low expression of CD80/86, IL-12 and high expression of IL-10 in SAg-induced mammary gland of cows with *S. aureus* infection.

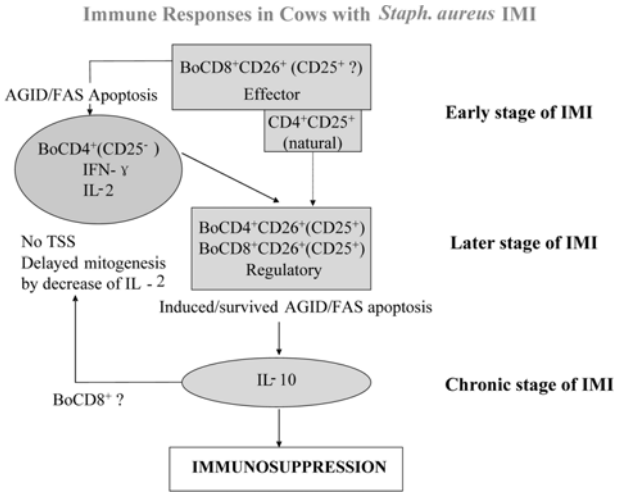

Fig. 2. Immunosuppression in cows with *S. aureus* intramammary infection (IMI). In the early stage of IMI (24-72 hrs post-IMI), $BoCD8^+CD26^+$ effector cells possibly with natural Tr cells ($CD4^+CD25^+$) induce antigen-induced cell death (AGID) or Fas-associated apoptosis (FAA) resulting in no toxic shock syndrome (TSS) and delayed mitogenesis by the decrease of IL-2, which can be produced along with IFN-γ by $BoCD4^+CD25^-$ cells. In later stage of IMI (7-10 days post-IMI), $BoCD4^+CD26^+$ and $BoCD8^+CD26^+$ cells which were induced and survived AGID or FAA may function as regulatory cells. In the chronic stage of IMI (after about two weeks post-IMI), those regulatory cells produce IL-10, which lead immunosuppression in the mammary gland of cows with *S. aureus* IMI.

IL-12, peaked early and was subsequently suppressed, whereas Th2-like cytokine expression, especially IL-4, was sustained over a longer period of time of culture. Altogether, our finding suggests that SEC may induce differentiation of $BoCD8^+$ T cells in a micro-environment biased towards Th2 cytokine profiles [11,25] which may have immunosuppressive activity in cows with *S. aureus* mastitis [30]. The $CD8^+$ T cells have suppressed antigen-specific $CD4^+$ T cell responses in the bovine mammary gland with *S. aureus* infection and these immunosuppressive $CD8^+$ T cells are similar to type 3 T suppressor cells which are affected by IL-10 [12,24].

Recent study has supported the possible presence of several different subsets of Tr cells induced by the chronic stimulation with SAgs [14]. This may partially explain why SAg-induced acute toxic shock syndrome in human is less likely to occur in cows and SAg-induced bovine mastitis is more likely subclinical or chronic status. Suppressor T cells are rebranded as Tr cells which express $CD4^+CD25^+$ [21]. There are candidate Tr cells other than $CD4^+CD25^+$ T cells, Tr1 and Th1/Th2 cells [4]. The other cell types involved in immunosuppression may include NK cells, γδ cells and $CD8^+$ T cells. We should understand more about the regulation of host immune responses by defining the interaction and involvement of these cells in different species of the host.

The difference and similarity between bovine activated $CD8^+$ T cells ($CD8^+CD26^+$) and human $CD4^+CD25^+$ Tr cells may help to establish their unique immune regulatory system in the host. The putative immunoregulatory mechanism in bovine mammary gland of cows with SEC is illustrated in Fig. 1. The immunosuppressive mechanism in cows with *S. aureus* IMI at different stages of infection is illustrated in Fig. 2.

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