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Immunohistochemical analysis of the thymus in newborn and adult yaks (*Bos grunniens*)

Qian Zhang[1], Yan Cui[1]*, Sijiu Yu[2], Junfeng He[1], Meng Wang[1], Yangyang Pan[2], Gengquan Xu[2], Kun Yang[3]

[1]Department of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Gansu Agricultural University, Lanzhou, Gansu, China

[2]Gansu Province Livestock Embryo Engineering Research Center, Gansu Agricultural University, Lanzhou, Gansu, China

[3]Life Science and Engineering College, Northwest Minzu University, Lan Zhou, Gansu, China

Abstract

Introduction. The thymus is the site of development and maturation of functional T lymphocytes and is critically important to the immune system. The purpose of this study was to examine the expression of markers of T lymphocytes, macrophages, dendritic cells, B lymphocytes and plasmocytes in the yak thymus.

Materials and methods. Twenty healthy male yaks were divided into newborn (2–4 weeks old, n = 10) and adult (3–4 years old, n = 10) group. qRT-PCR was used to evaluate the mRNA expression level of the main markers of the studied cell types. Immunohistochemistry was used to detect the distribution of $CD3^+$ T lymphocytes, $CD68^+$ macrophages, $SIRP\alpha^+$ dendritic cells, $CD79\alpha^+$ B lymphocytes, IgA and IgG^+ plasmocytes.

Results. Within the same age group, the mRNA expression of *CD3ε* was highest ($P < 0.05$), followed by that of *CD68*, *SIRPα*, *CD79α*, *IgG* and *IgA*. Furthermore, *CD3ε, CD68*, and *SIRPα* mRNA expression levels were higher in newborn yaks than in the adult ones ($P < 0.05$), whereas those of CD79α, IgA, and IgG were higher in adults ($P < 0.05$). Immunohistochemical results showed localization of $CD3^+$ T lymphocytes in the thymic cortex and medulla. $CD68^+$ macrophages, $SIRP\alpha^+$ dendritic cells, $CD79\alpha^+$ B lymphocytes, IgA^+ and IgG^+ plasmocytes were mainly observed in the cortico-medullary region and medulla. In the same age group, the frequency of $CD3^+$ T lymphocytes was higher than that of $CD68^+$ macrophages and $SIRP\alpha^+$ dendritic cells ($P < 0.05$), followed by those of $CD79\alpha^+$ B lymphocytes and IgA^+ and IgG^+ plasmocytes. No significant difference was observed between B lymphocyte and plasmocyte frequencies in the yak thymus in both age groups ($P > 0.05$). The frequency of $CD3^+$, $CD68^+$ and $SIRP\alpha^+$ cells decreased from newborns to adults ($P < 0.05$). However, the frequencies of $CD79\alpha^+$, IgA^+ and IgG^+ cells increased from newborn to adult yaks ($P < 0.05$).

Conclusions: The thymus of newborn yaks is well-developed, with higher numbers of T lymphocytes, macrophages, and dendritic cells than those in the adult thymus. However, higher frequencies of plasmocytes and B lymphocytes were detected in the adult thymus, suggesting that adults may better resist infections through humoral immunity as this organ undergoes involution. Furthermore, there was no significant difference in the number of IgA and IgG plasmocytes, which differs from what is observed in rodents and humans. This difference might be related to the fact that yaks live in low-oxygen plateaus.

Keywords: newborn yak; thymus; immune cell markers; gene and protein expression; IHC; qRT-PCR

***Correspondence address:** Yan Cui
Faculty of Veterinary Medicine,
Gansu Agricultural University,
Lanzhou, Gansu, China
fax: +86-0931-7631220
e-mail: cuiyan369@sina.com

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Introduction

The thymus is a primary lymphatic organ in which thymocytes differentiate into the T cell population. It is critically important for the immune system, which serves as the body's defense mechanism to provide surveillance and protection against diverse pathogens, tumors, antigens and mediators of tissue damage [1]. In the thymus, bone marrow-derived cells include T-lineage cells as the dominant cell type, as well as lower levels of B cells, macrophages, and dendritic cells [2].

The thymus of newborn bovines [3], equines [4] and humans [5] are known to be well developed, and the distribution characteristics of thymic lymphoid cells, macrophages, dendritic cells, and plasmocytes have been described. In contrast, the thymus of the neonatal mouse is not fully developed, and thymus corpuscles are small and few [6, 7].

Yaks (*Bos grunniens*) live on the Qinghai-Tibetan Plateau, where low oxygen, low temperatures, and high altitudes are the main environmental characteristics [8]. Epidemiological surveys have suggested that plateau yaks are sporadically infected with bacteria and parasites [9, 10]. Therefore, we became interested in the immune cell characteristics of the yak thymus since very little data exists in the literature.

We used a panel of antibodies (CD3, CD68, SIRPα, CD79α, IgA and IgG) to characterize the different immune cells present in the thymus of healthy newborn and adult yaks and assessed the expression levels of immune cell-specific markers at the mRNA level. These results provide a morphological reference for thymic physiological functions and comparative histology.

Material and methods

Animals and tissues collection. The study was approved by the State Forestry Administration, and all procedures were performed in compliance with guidelines for the care and use of laboratory animals adopted by the Ministry of Science and Technology of the People's Republic of China. Newborn yaks (2–4 weeks old, n = 10) and adult yaks (3–4 years old, n = 10) were enrolled in the study. All yaks were considered healthy on the basis of results of a physical examination and serum biochemical analysis. Each yak was euthanized with pentobarbital sodium (200 mg/kg, *i.v.*). The thymus was harvested within 10 min after euthanasia. For mRNA and protein expression analyses, fresh thymic tissue was washed with diethylpyrocarbonate-treated water and stored in liquid nitrogen. For immunohistochemical analyses, small specimens of thymic tissue were fixed in a solution of 4% paraformaldehyde in a phosphate buffer (pH 7.3).

qRT-PCR. Total RNA was isolated from the thymus using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reversely transcribed to single-stranded cDNA using a reverse transcription kit (MBI Fermentas, Burlington, ON, Canada) according to the manufacturer's instructions. The qRT-PCR primers were designed according to the Bos grunniens *CD3ε, CD79α, IgA, IgG, SIRPα, CD68* and *β-actin* gene sequences (GenBank accession numbers: KY911279, KY911280, MG432919, MF099643, MH347358, KY921959 and DQ838049, respectively) using Primer 5 software and synthesized by the Beijing Genomics Institute BGI Company (China). The qRT-PCR primer sequences are presented in Table 1. qRT-PCR was conducted with a Light-Cycler480 thermocycler (Roche, Manheim, Germany) in a 20 μL reaction volume consisting of 1 μL cDNA, 1 μL forward primer, 1 μL reverse primer, 10 μL 2×SYBR Green II PCR mix (TaKaRa, Shiga, Japan), 0.4 μL Rox, and 6.4 μL nuclease-free H_2O. The RT-PCR conditions were as follows: one cycle at 95°C for 3 min, followed by 42 cycles of 95°C for 10 s, 60°C for 15 s and 72°C for 15 s. Four replicates were set for each sample to ensure the accuracy of the relative expression of the target genes in the sample. After amplification, according to the system-generated Ct value, the $2^{-}\Delta\Delta^{Ct}$ method was used with β-actin as an internal standard to normalize the amount and quality of each cDNA.

Immunohistochemistry. The spatial distribution of cells positive for CD3, CD68, SIRPα, CD79α, IgA and IgG in the thymus of yaks was evaluated by the immunohistochemical (IHC) method. Fixed tissue specimens were mounted on microscope slides in a routine manner and exposed to primary antibodies against CD3 (rabbit monoclonal anti- CD3, Abcam, ab16669, 1:200 dilution), SIRPα (rabbit polyclonal anti-SIRPα, Abcam; ab116254, 1:100 dilution), CD68 (rabbit polyclonal anti-CD68, Abbiotec, San Diego, CA, USA; No:252281, 1:100 dilution), CD79α (rabbit monoclonal anti- CD79α, Abcam, ab199001, 1:100 dilution), IgA (rabbit polyclonal anti-IgA, Abcam; ab112630, 1:100 dilution), IgG (rabbit polyclonal anti- IgG, Abcam, Cambridge, UK; ab6692, 1:100 dilution), for 2 h at 37°C in a moist chamber. Biotinylated anti-rabbit secondary antibodies were applied for 10 min. Streptavidin-conjugated peroxidase was then applied to the slides for 10 min. The reaction products were formed using 3,3-diaminobenzidine tetrahydrochloride (DAB). The sections were lightly counterstained with hematoxylin. The negative control for each sample was created by replacing the primary antibody with rabbit serum albumin; all other steps and conditions remained the same.

Cell counting. CD3, CD79α, IgA, IgG, SIRPα and CD68 positive cells were evaluated in the yak thymus manually. In every age group thymus of 10 yaks was assessed with three sections examined in the thymus of every yak, and in

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Table 1. Preimers sequence of target gene and β-*actin*

Genes	Primer names	Primer sequences (5'–3')	Length (bp)	Annealing (°C)
CD3ε	P1 P2	F:GGGCTCATAGTCTGGATTGG R:TGTGTCACTCTGGGCTTGC	156	60
CD79α	P3 P4	F: ACGGCAAGAAGATTCAGCG R:CCAAGGAGGCAATAGGAG	225	60
IgA	P5 P6	F: GGTTCACAGGACCCAGA R: AGCACCTAGTGAAGCCC	227	57
IgG	P7 P8	F: AACCAACACCACAGGAAC R: AGTGTAGTCTCCTATTGCCT	208	60
CD68	P9 P10	F: TGAGAGGAGCAAGTGGGA R: GTGGACATCATCCTGGCTGG	194	56
SIRPα	P11 P12	F: ATCCTGCTGCCCGCTGTA R: AACAGTTGGGCGGCGAG	215	59
β-actin	P13 P14	F: AGGCTGTGCTGTCCCTGTATG R: GCTCGGCTGTGGTGGTAAA	207	60

each section 20 different fields of 2.30×10^4 μm^2 (corresponding approximately to five fields at 1000× magnification) were analyzed in one slide. The mean counts of each positive cell type were expressed as the number of cells in 2.30×10^4 μm^2. The investigator was blinded to the identification information of the animal.

Statistical analysis. All statistical analyses were performed using IBM SPSS (version 21.0; SPSS Inc., Chicago, IL, USA). The relative mRNA levels and positive cell numbers of CD3, CD79α, IgA, IgG, SIRPα and CD68 among the study groups were expressed as the standard error of means. Statistical significance was determined using one-way analysis of variance and was set at $P < 0.05$.

Results

Distribution of T lymphocytes

CD3-positive T lymphocytes were located in the thymic cortex and medulla. Positive immunoreactivity was mainly localized in the cytoplasm of the T lymphocytes. $CD3^+$ positive cells were more prominent in the cortex than in the medulla ($P < 0.05$) (Fig. 1a–f). Furthermore, $CD3^+$- T lymphocytes were more prevalent in newborn yaks than in adults ($P < 0.05$) (Fig. 2a).

Distribution of macrophages

CD68-positive macrophages were particularly common in the cortico-medullary region and the medulla (Fig. 3a–d). Immunoreactivity was localized in the cytoplasm of the macrophages. The population of $CD68^+$ macrophages was higher than that of dendritic cells, B lymphocytes, and plasmocytes in the thymus of both newborn and adult yaks ($P < 0.05$) (Fig. 2b). Additionally, the number of $CD68^+$ macrophages was greater in newborns than adults ($P < 0.05$).

Distribution of dendritic cells

SIRPα-positive dendritic cells with irregular nuclei were observed in the cortico-medullary region and medulla (Fig. 4a– d). Immunoreactivity was localized to the cytoplasm of dendritic cells. In particular, the frequency of $SIRP\alpha^+$ dendritic cells was significantly lower than that of $CD68^+$ macrophages in both newborn and adult yak thymus ($P < 0.05$). Moreover, the number of SIRPα-positive dendritic cells was much higher in newborn yaks than in adult yaks ($P < 0.05$) (Fig. 2b).

Distribution of B lymphocytes

A low frequency of CD79α-positive B cells was detected in the medulla and cortico-medullary areas of the thymus (Fig. 5a–d). Clear immunoreactivity was localized in the cytoplasm of B lymphocytes. The frequency of $CD79\alpha^+$ B lymphocytes was significantly lower than that of CD68-positive macrophages and $SIRP\alpha^+$ dendritic cells in thymus of both newborn and adult yaks ($P < 0.05$). Additionally, the frequency of B lymphocytes in the newborn was higher than that in the adult yak thymus ($P < 0.05$) (Fig. 2b).

Distribution of plasmocytes

Smaller populations of IgA- (Fig. 6a–d) and IgG-positive (Fig. 7a–d) plasmocytes were in the cortico-medullary and medulla of newborn and adult yak thymus. Marked immunoreactivity was observed in the cytoplasm of the plasmocytes. In the same age group,

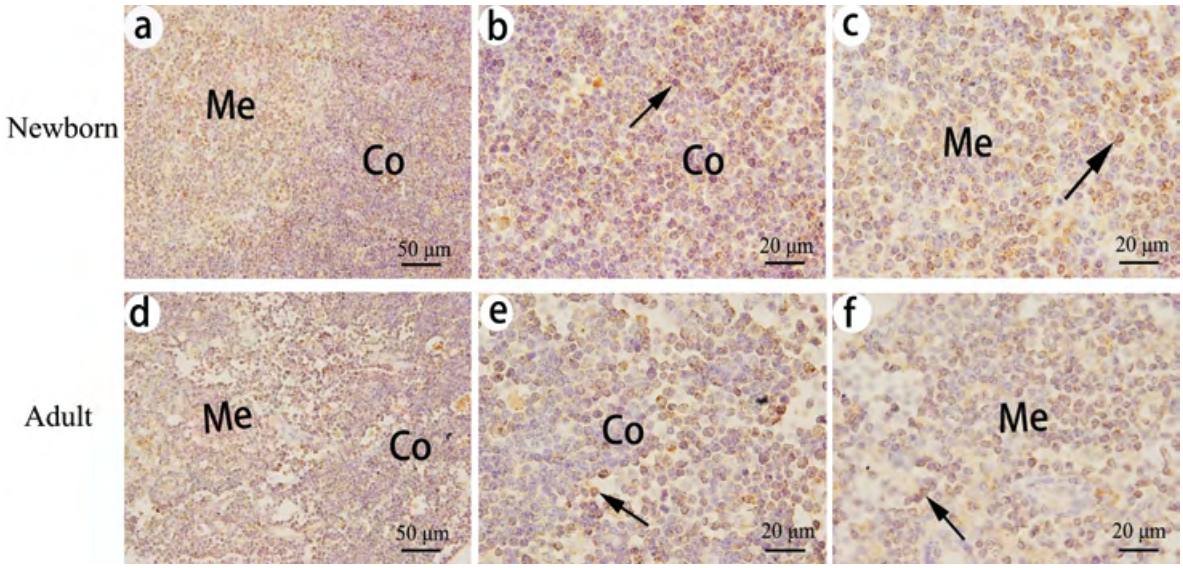

Figure 1. Immunohistochemical localization of CD3-positive T lymphocytes in the thymus of newborn (**a**, **b** and **c**) and adult (**d**, **e** and **f**) yaks. Arrowheads indicate examples of positive cells (brown). Cortex (Co); medulla (Me); Scale bars: 50 μm (400×) for panels a and d; 20 μm (1000×) for panels b, c, e and f.

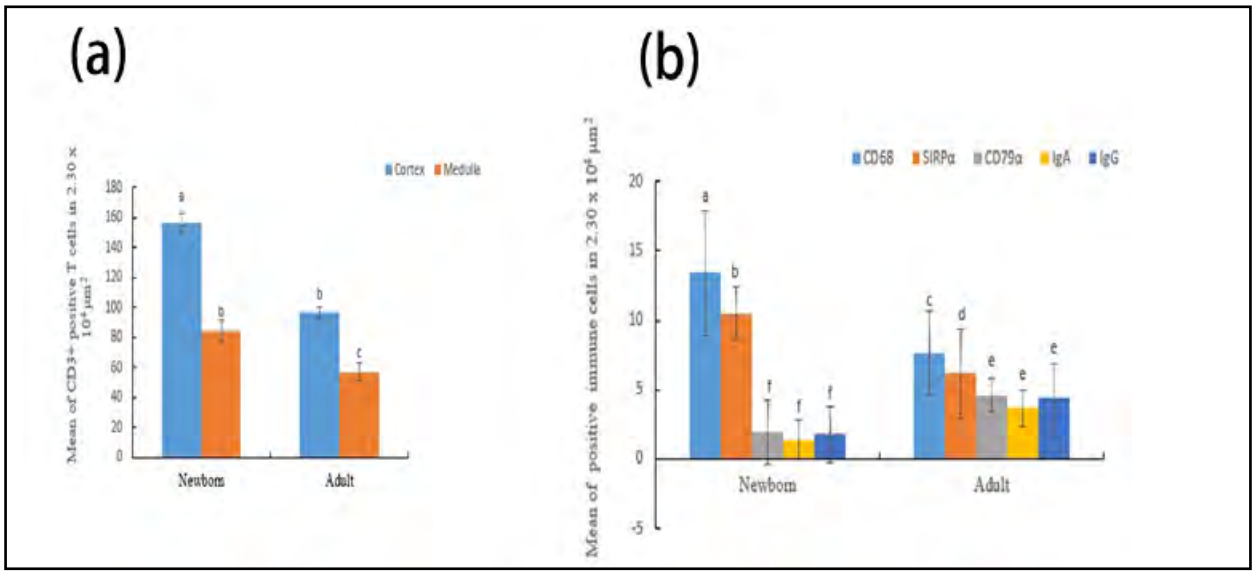

Figure 2. Frequencies of CD3 (**a**), CD68, SIRPα, CD79α, IgA- and IgG -positive cells in the thymus of newborn and adult yaks. Panel (**a**) denotes the number of CD3 positive T lymphocytes in 2.30 × 10^4 μm^2. Panel (b) shows the number of CD68-positive macrophages, SIRPα-positive dendritic cells, CD79α-positive B lymphocytes, IgA- and IgG-positive plasmocytes in 2.30× 10^4 μm^2. Values are mean ± SE of immunoreactive cell number in 2.30 × 10^4 μm^2 (n = 10). Bars with different superscripts denote significantly different values ($P < 0.05$).

the population of IgA^+ and IgG^+ plasmocytes was lower than that of $CD68^+$ macrophages and $SIRP\alpha^+$ dendritic cells ($P < 0.05$). There was no significant difference in the frequency of plasmocytes and B-lymphocytes in the thymus ($P > 0.05$). However, the frequency of IgA^+ and IgG^+ plasmocytes in the adult thymus was higher than that in the newborn yak thymus ($P < 0.05$) (Fig. 2b).

CD3ε, CD68, SIRPα, CD79α, IgA and IgG mRNA expression in the thymus

The mRNA expression of genes encoding *CD3ε, CD68, SIRPα, CD79α, IgA*, and *IgG* differed between the thymus of newborn and adult yaks (Fig. 8). Within the same age group, the expression level of *CD3ε* mRNA was significantly higher than those of CD68 and *SIRPα* mRNA ($P < 0.05$). Addi-

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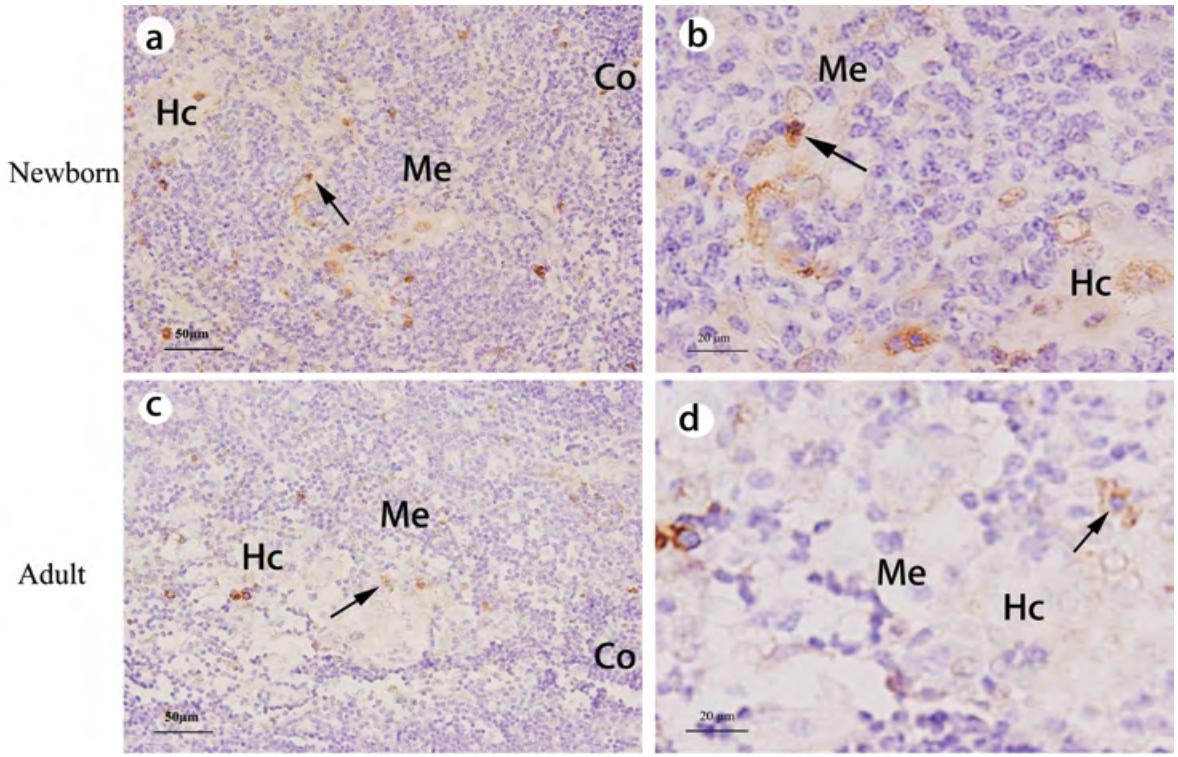

Figure 3. Immunohistochemical localization of CD68-positive macrophages in the thymus of newborn (**a**, **b**) and adult (**c**, **d**) yaks. Arrowheads indicate examples of positive cells (brown). Cortex (Co); medulla (Me); Hassall corpuscles (Hc); Scale bars: 50 μm (400×) for panels a and c; 20 μm (×) for panel b and d.

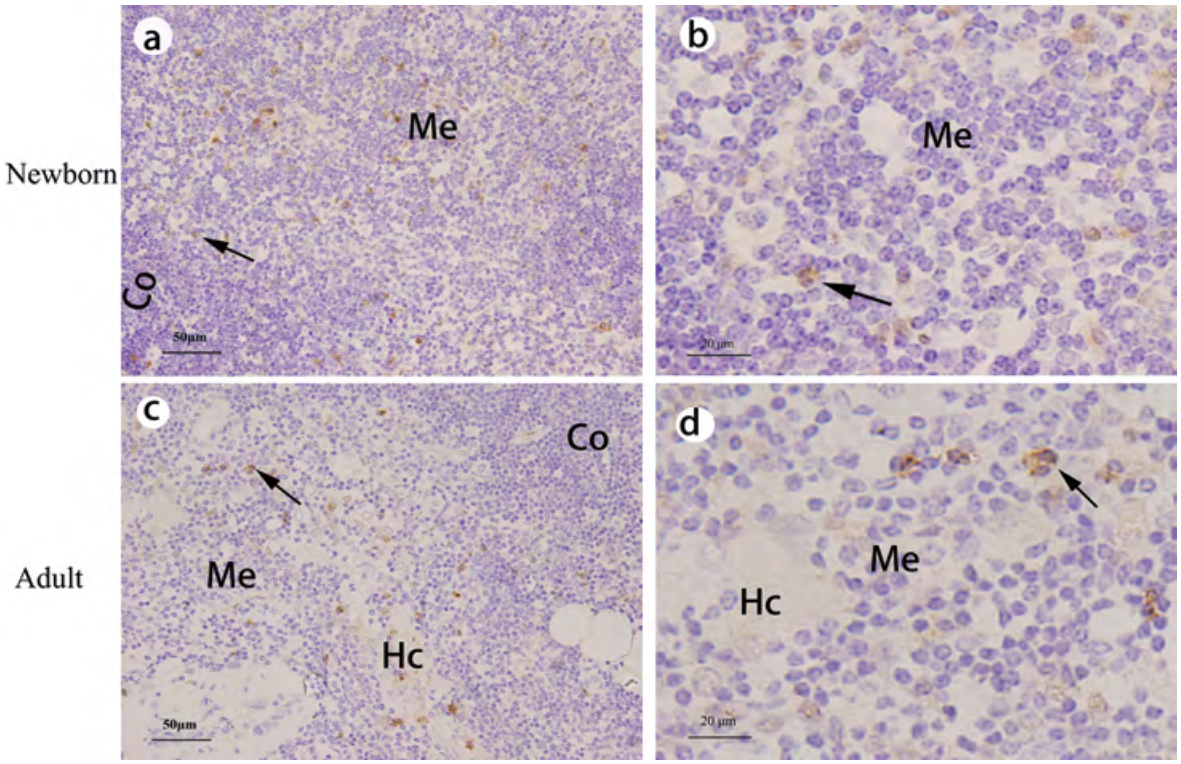

Figure 4. Immunohistochemical localization of SIRPα-positive dendritic cells in the thymus of newborn (**a**, **b**) and adult (**c**, **d**) yaks. Arrowheads indicate examples of positive cells (brown). Cortex (Co); Medulla (Me); Hassall corpuscles (Hc); Scale bar = 50 μm (400×) for panels a and c; 20 μm (1000×) for panels b and d.

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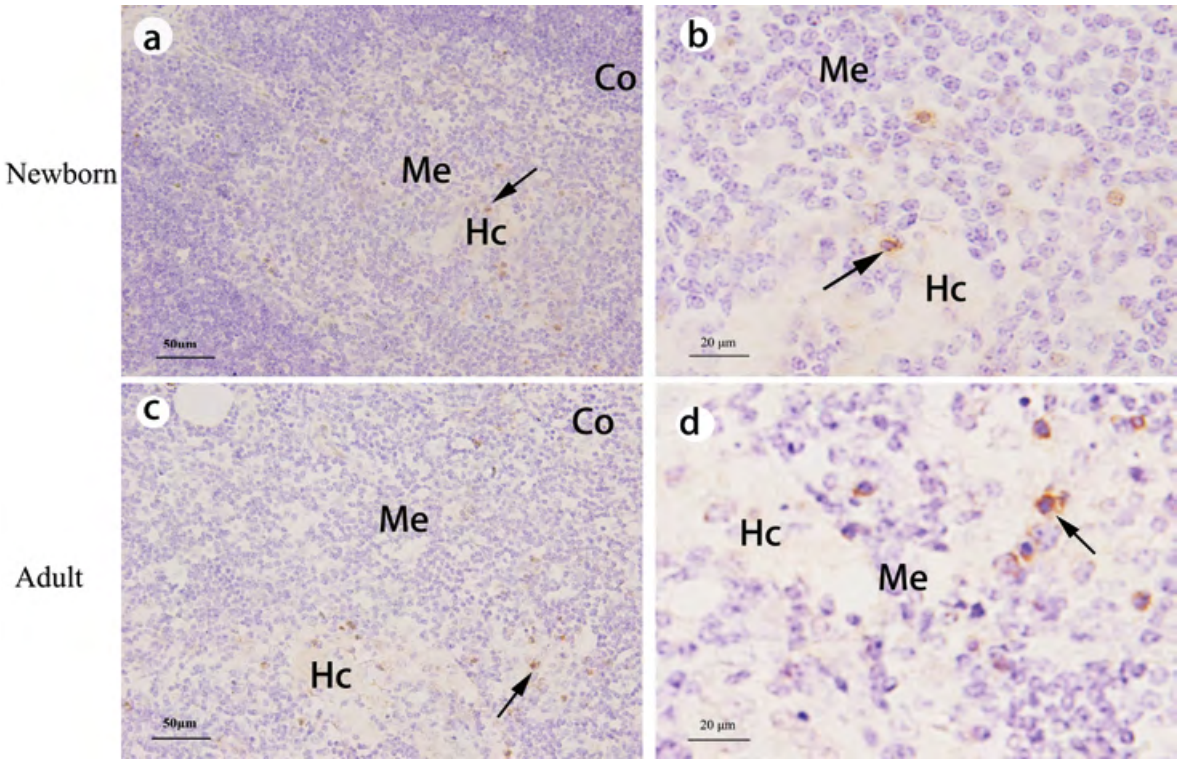

Figure 5. Immunohistochemical localization of CD79α-positive B lymphocytes in the thymus of newborn (**a**, **b**) and adult (**c**, **d**) yaks. Arrowheads indicate examples of positive cells (brown). Cortex (Co); Medulla (Me); Hassall corpuscles (Hc); Scale bar = 50 μm (400×) for panels a and c; 20 μm (1000×) for panel b and d.

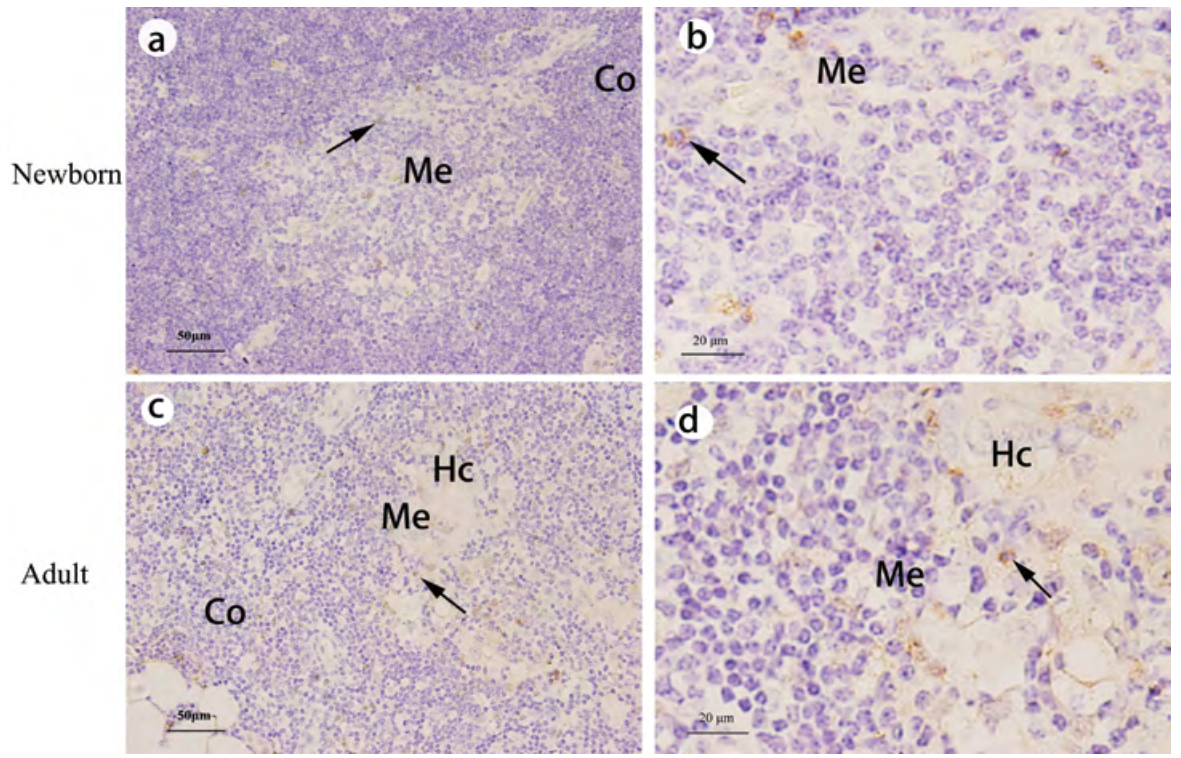

Figure 6. Immunohistochemical localization of IgA-positive plasmocytes in the thymus of newborn (**a**, **b**) and adult (**c**, **d**) yaks. Arrowheads indicate examples of positive cells (brown). Cortex (Co); medulla (Me); Hassall corpuscles (Hc); Scale bars: 50 μm (400×) for panels a and c; 20 μm (1000×) for panel b and d.

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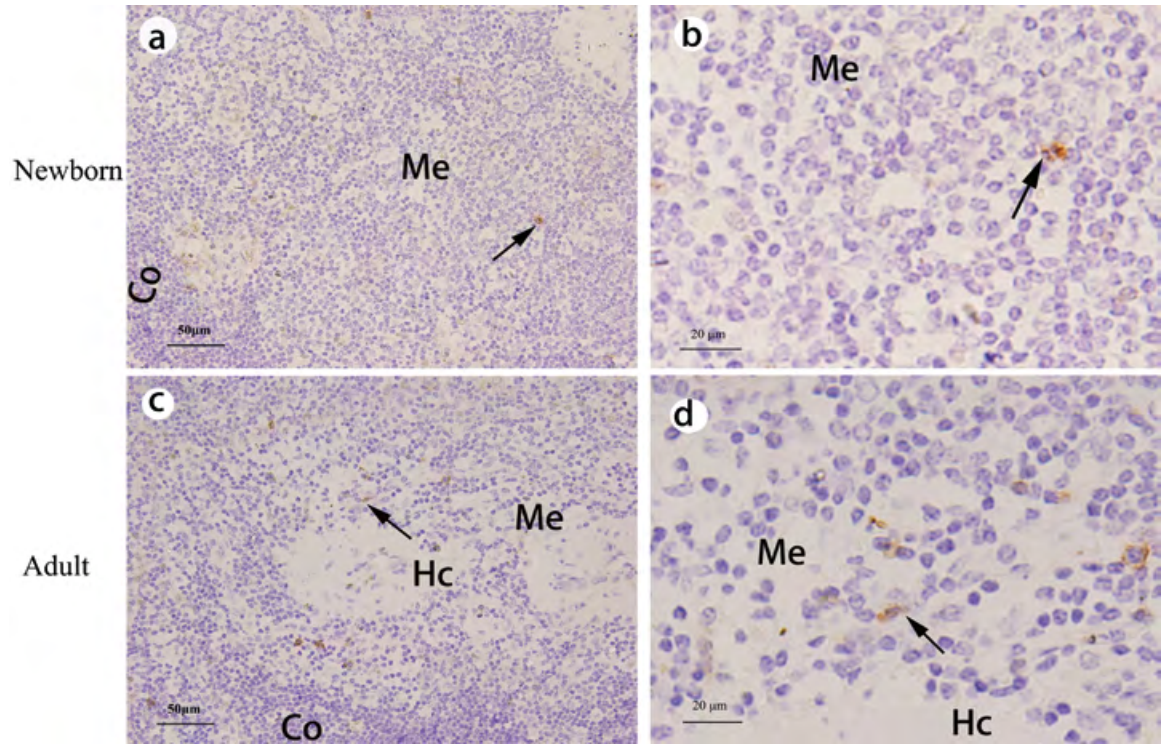

Figure 7. Immunohistochemical localization of IgG-positive plasmocytes in the thymus of newborn (**a**, **b**) and adult (**c**, **d**) yaks. Arrowheads indicate examples of positive cells (brown). Cortex (Co); medulla (Me); Hassall corpuscles (Hc); Scale bars: 50 μm (400×) for panels a and c; 20 μm (1000×) for panel b and d.

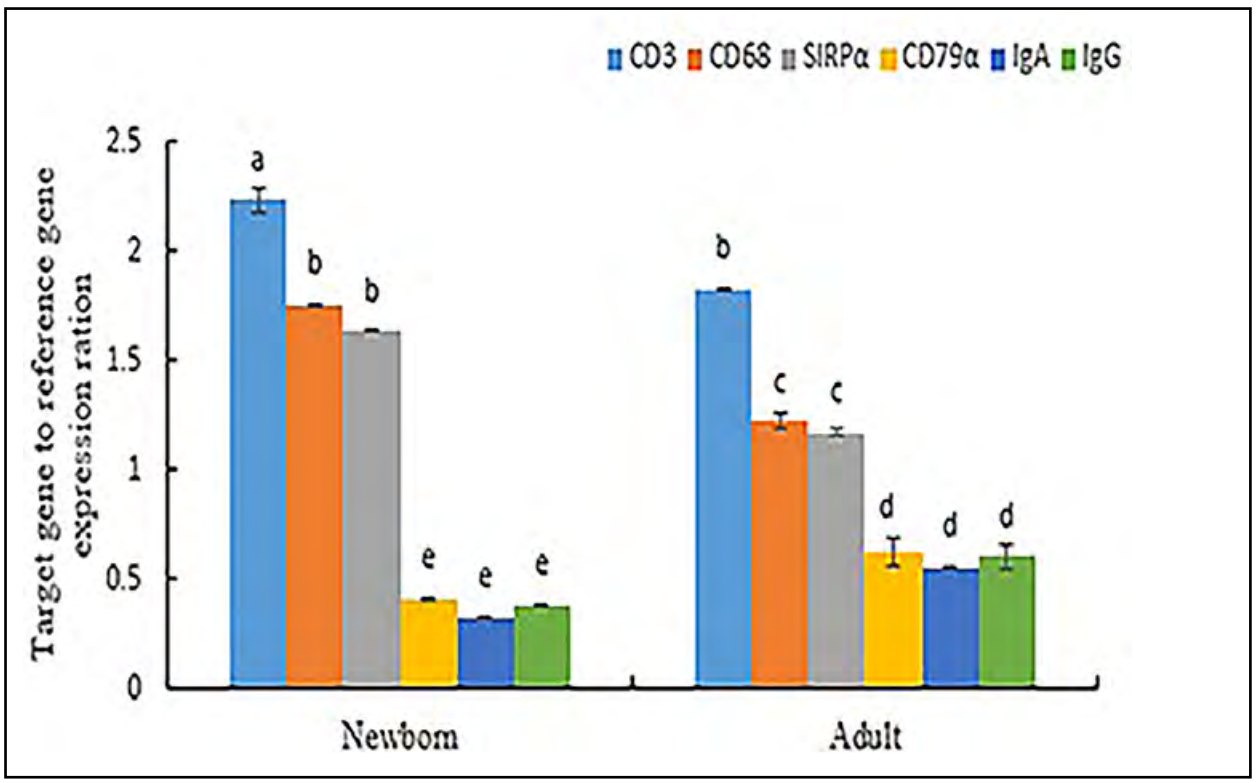

Figure 8. Relative abundance of *CD3ε, CD68,* SIRPα, *CD79α, IgA* and *IgG* mRNA in the thymus of newborn and adult yaks. Bars with different superscripts are significantly different ($P < 0.05$). Data are normalized and presented as ratio mean ± SE.

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tionally, the lowest expression levels were detected for *CD79α*, *IgA*, and *IgG*, and no significant difference was observed between them ($P > 0.05$). Interestingly, the mRNA expression levels of CD3ε, CD68, and *SIRPα* were lower in the thymus of adult yaks than in newborn yaks ($P < 0.05$). Further, *CD79α*, *IgA*, and *IgG* mRNA levels in the adult thymus were higher than those in newborn yaks ($P < 0.05$).

Discussion

The thymus is a primary lymphoid organ that provides a specialized microenvironment for T cell maturation. CD3 has been used as a marker of T lymphocytes [11]. In the present study, we observed that the expression of *CD3ε* mRNA was significantly higher than that of *CD68*, *SIRPα*, *CD79α*, *IgA* and *IgG* in newborn and adult yaks. Accordingly, we found that the frequency of CD3-positive T lymphocytes was higher than that of other immunocompetent cells in the yak thymus. This was consistent with data from bovines [3], humans [5], and rodents [6, 7], where the predominant cell population of the thymus was composed of T lymphocytes. Furthermore, we found that in each age group, the number of T lymphocytes in the cortex was higher than that in the medulla of the yak thymus. These results are similar to those reported for bovines [3], equines [4], humans [5] and mice [7]. Pearse *et al.* reported that the major activity of the thymus is to harbor T cells during their differentiation stages, and that the thymic cortex is the main area of T lymphocyte proliferation and division [1]. Additionally, we confirmed that $CD3^+$ T lymphocytes were more abundant in newborn yaks than in adults. One possible explanation is that the thymus undergoes progressive involution during adulthood. Thymic involution is macroscopically characterized by a decrease in organ weight and size, and the organ structure undergoes a loss of T lymphocytes [12]. Aspinall and Andrew observed that thymic masses diminish markedly and T cell numbers decline with age [13]. Based on these collective findings, we speculated that adipose and connective tissues accumulated in the adult yak thymus, leading to a decreased cell proliferation in the thymus.

Most macrophages can be detected by CD68 antibodies [14]. Similarly to the previous research [3–5, 7], the present study showed that macrophages are mainly located in the cortico-medullary junction and medulla of newborn and adult yak thymus. Surh and Sprent reported that macrophages participate in the removal of autoreactive thymocytes resulting from intrathymic selection events [15]. It was suggested that during the normal steady state, macrophages phagocytosed viable autoreactive thymic lymphocytes in the medulla of the yak thymus. Our results showed that in each age group, the frequency of CD68-positive macrophages was higher than that of SIRPα-, CD79α-, IgG-, and IgA-positive yak thymus cells. Moreover, a previous study showed that thymic macrophages could secrete some cytokines and present antigens to promote thymocytes' survival and proliferation [16]. Abundant thymic macrophages may regulate the maturation of developing thymocytes and phagocytose apoptotic thymocytes. Moreover, we found that thymic $CD68^+$ macrophages were more abundant in newborn yaks than in adult yaks. Similarly, Sminia *et al.* reported that the total number of macrophage precursors and their capacity to proliferate were reduced with age [16]. This might be correlated with the diminished number of thymic macrophages in adult yaks that we observed.

We used *SIRPα* as a dendritic cell marker [17] and showed that its mRNA expression level was significantly higher than that of *CD79α*, *IgG*, and *IgA* in both newborn and adult yak thymus. The frequency of $SIRP\alpha^+$positive dendritic cells was higher than that of CD79α-, IgG-, and IgA-positive dendritic cells. Thymic dendritic cells are unique antigen-presenting cells because of their role in generating central T-cell tolerance through deletion or functional inactivation (negative selection) of autoreactive thymocytes [18]. This indicated that the abundance of thymic dendritic cells could help medullary thymocytes differentiate and maintain tolerance in yaks. Moreover, we found that thymic $SIRP\alpha^+$ dendritic cells in yaks were mainly distributed in the cortico-medullary junction and medulla; however, they were not detected in the superficial cortex. This is in agreement with data of von Gaudecker *et al.* [19], who observed that dendritic cells were mainly distributed in the medulla. We conclude that the thymic medulla might be a major antigen-trapping site, where many antigen-presenting cells induce T cell maturation and differentiation. Additionally, we detected that $SIRP\alpha^+$ dendritic cell frequencies decreased with age in the yak thymus. Similarly, Agrawal *et al.* performed a morphometric analysis to evaluate the number of dendritic cells in the aged human thymus and showed an age-related reduction in the number of medullary dendritic cells [20]. As thymic cellularity undergoes a reduction as age increases, the number of dendritic cells also decreases. We assume that the reduction in the number of medullary dendritic cells might be related to a gradual loss in the induction of self-tolerance and T cell negativity in the adult yak thymus.

The existence of B lymphocytes as a constant part of murine and human thymic microenvironments has

recently been described [21, 22]. The B cell population is characterized by the expression of CD79α [24]. We found that *CD79α* mRNA levels were significantly lower than those of CD3ε, SIRPα, and CD68 in both newborn and adult yak thymus. The frequency of CD79α$^+$ B lymphocytes was lower than that of CD3ε-, SIRPα– and CD68-positive cells. Similarly, B cells are present at very low percentages in the human and mouse thymus. Thymic B lymphocytes constitute the resident intrathymic population, which locates to the thymus early during ontogenesis and is present in the postnatal and adult thymus [24]. Moreover, we found that thymic CD79α$^+$ B lymphocytes were mainly distributed in the cortico-medullary junction and medulla of newborn and adult yaks. Perera and Huang reported that thymic B cells are involved in autoantigen presentation to T cells and thymocyte negative selection to maintain central tolerance [24]. This indicates that the cortico-medullary junction and medulla of the yak thymus might serve as a site for thymocyte negative selection. We also found that the frequency of thymic CD79α$^+$ B lymphocytes increased with age. Similarly, Yamano *et al.* reported that an increasing number of B cells accumulate in the medulla, which is essential for the establishment of protective immunity against pathogens [23]. The thymus progressively shrinks with age, and damage occurs to the blood-thymus barrier. As a result, most thymocytes are exposed to antigens during aging. We speculated that in adults, the higher frequency of B cell subsets in the yak thymus might mediate humoral antibody responses and protect the thymus from infection.

IgA$^+$ and IgG$^+$ cells are important immunoglobulin secretory cells. Yamano *et al.* reported that despite being a specific site for T cell development, the human thymus also hosts plasmocytes [23]. Based on previous results [4, 5, 25], we investigated a few IgG and IgA expressing cells situated in the medulla and corticomedullary junction of newborn and adult yak thymus. Nunez *et al.* showed that plasmocytes spontaneously secrete Igs without additional stimulation [26]. However, these observations favor the possibility of a migratory origin of at least the bulk of thymic plasmocytes [27, 28]. The presence of plasmocytes, mainly in the cortico-medullary junctions, seems to support this view. Overall, it was speculated that the thymic IgA and IgG plasmocytes of yaks might have migrated into the thymus or originated from thymic parenchymal cells and might act as a sentry against blood-borne infectious agents.

Remarkably, we found that, in the same age group, there were no significant differences in the IgA$^+$ and IgG$^+$ plasmocyte frequencies or mRNA expression in the yak thymus. Conversely, Bianchi *et al.* reported that human thymus IgG$^+$ cells were the predominant subset and IgA$^+$ cells were present in a small population [29]. Wiener *et al.* stated that yaks living on highland plateaus have evolved and adapted to harsh environments and are less susceptible to disease, in contrast to the closely related low-altitude cattle (*Bos taurus*) [8–10]. Additionally, Huang *et al.* reported that yak lymphoid organs have adapted to high altitude conditions [30]. We speculated that the higher frequency of thymic IgA plasmocytes in yaks compared with other mammalian species might be related to plateau adaptation. It is unknown whether this observation is representative of the yak thymus, and further studies are required to confirm these findings. Interestingly, we detected that the IgA$^+$ and IgG$^+$ plasmocyte frequency and mRNA expression levels showed a significant increase with age. Nango *et al.* reported that plasmocytes are involved in autoantigen presentation to T cells in the thymus and are most efficient at complement fixation and antibody-dependent humoral immunity, which is relatively weak during the first year of life but progressively strengthens throughout adulthood [31]. It has been speculated that the greater concentration of thymic plasmocytes in the adult yak could result from an increase in antigen penetration into the thymus as this organ undergoes involution. Thymic plasmocytes might help to protect the thymus from infection from circulating antigens.

In conclusion, the present study provides a clear picture of the expression levels of immune cell markers in the thymus of highland plateau yaks. Our results suggest that the newborn yak thymus is well developed, with a majority population of T lymphocytes, macrophages, and dendritic cells, contributing to the maintenance of immune function. It is possible that newborn yaks have evolved and adapted to harsh environments and are less susceptible to disease. Moreover, a higher frequency of plasmocytes and B lymphocytes in the adult thymus was detected in adult yaks, suggesting that the adult yak thymus may better resist infections as this organ undergoes involution. There was no significant difference in the number of IgA and IgG plasma cells, which differs from observations in rodents and humans; this could be related to yaks' high tolerance or resistance to disease and the environment. Finally, this study provided morphological information regarding thymic immunoregulation in yaks.

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Conflict of interest

No conflict of interest was declared by the authors.

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