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Short communication: Initial evidence supporting existence of potential rumen epidermal stem and progenitor cells

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ABSTRACT

The bovine rumen epidermis is a keratinized multilayered tissue that experiences persistent cell turnover. Because of this constant cell turnover, epidermal stem cells and their slightly more differentiated daughter cells, epidermal progenitor cells, must exist in the stratum basale of rumen epidermis. To date, these 2 epidermal cell populations and any unique cellular markers they may possess remain completely uncharacterized in the bovine rumen. An important first step in this new research area is the demonstration of the relative abundance and existence of markers for these cells in rumen tissue. A related second step is to document rumen epidermal proliferative responses to an extrinsic signal such as nutrient concentration within the rumen. The objectives of this experiment were to evaluate the extrinsic effect of diet on (1) gene expression of 6 potential rumen epidermal stem or progenitor cell markers and (2) rumen epidermal cell proliferation within the stratum basale. Twelve preweaned Holstein heifers were fed either a restricted diet (R) or an enhanced diet (EH). Animals on R received a milk replacer (MR) diet fed at 0.44 kg of powder dry matter (DM)/d (20.9% crude protein, 29.8% fat, DM basis) and EH received MR at 1.08 kg of powder dry matter/d (28.9% crude protein, 26.2% fat, DM basis). All calves had access to a 20% crude protein starter and were weaned during wk 7 of the experiment. Lifetime DM intake was 0.73 kg of DM/calf per day for R (5.88 Mcal of net energy/calf per day) and 1.26 kg of DM/calf per day for EH (10.68 Mcal of net energy/calf per day). Twenty-four hours before slaughter heifers received an intravenous dose of 5-bromo-2′-deoxyuridine to label proliferating cells. Heifers were slaughtered at 8 wk of age, and rumen samples from the ventral sac region were obtained and stored in RNA preservative and processed for routine histology. Quantitative real-time reverse transcriptase PCR was used to analyze relative abundance of genes. Candidate genes for markers of epidermal stem and progenitor cells were β1-integrin (*ITGB1*), tumor protein p63 (*TP63*), keratin-14 (*KRT14*), Notch-1 (*NOTCH1*), Leu-rich repeat-containing G protein-coupled receptor 5-expressing (*LGR5*), and musashi-1 (*MSI1*). All genes were detected in the rumen tissue; *ITGB1* was increased in EH compared with R. 5-Bromo-2′-deoxyuridine immunohistochemistry revealed that both R and EH rumen tissue had proliferating cells within the stratum basale of the rumen epidermis at the time of analysis. The EH diet resulted in an additive effect on cell proliferation. The percentage of cells in the stratum basale synthesizing DNA in preparation for mitosis nearly doubled (23.8 ± 2.4% for EH vs. 14.7 ± 2.0% for R) compared with calves fed R. This work represents the first attempt at characterizing rumen epidermal stem and progenitor cells. We demonstrated the relative abundance and existence of potential markers in rumen tissue and showed a rumen epidermal proliferative response to the extrinsic stimulus of nutrient concentration in the form of diet.

Key words: dairy calf, cell proliferation, mitosis, epidermis, epithelium

Short Communication

The epidermis of bovine rumen is a multilayered keratinized, stratified squamous epithelium. It is situated on top of the dermis, hence its name. The rumen epidermis is the first line of defense against microbial infiltration and also the first site for nutrient absorption; it therefore has barrier and absorptive functions. Rumen epithelium is composed of 4 strata, plus an outer layer of keratin and is collectively called the epidermis. Keratin is the most superficial layer, followed by the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. A basement membrane is situated below the stratum basale, followed by the dermis, which contains blood vessels (see Figure 2). The rumen epidermis is dynamic and must be maintained throughout life. Epidermal homeostasis requires cellular proliferation and differentiation events that originate in the stratum basale (Figure 2). From studies with other epidermal tissues, it is known that

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physiological cues induce cells in the stratum basale to detach from the underlying basement membrane, withdraw from the cell cycle, and begin the process of terminal differentiation and migration to the surface (Zouboulis et al., 2008). These committed cells traverse through the epidermal layers before being shed from the epidermal surface as cornified cells (Figure 2; Lavker and Matoltsy, 1970). In rumen epidermis, as with other types of epidermis, cellular death and loss through cornification occurs continuously. Thus, new differentiated cells must be generated throughout life to replenish lost cells. Because of this persistent epidermal cell turnover, it is hypothesized that epidermal stem cells and their slightly more differentiated daughter cells, epidermal progenitor cells (also known as transit-amplifying cells), must exist in the stratum basale of the rumen epidermis. Further, to function as true stem and progenitor cells, these candidate cells must be able to respond to extrinsic stimuli, such as nutrient concentration. Last, these cell populations must have unique cellular markers that distinguish them from other epidermal cells.

To date, these 2 epidermal cell populations and any unique cellular markers they may possess remain poorly understood in bovine rumen. Over the years, rumen epidermal cell proliferation has been quantified in numerous ways. Some methods include quantification of the proportion of stratum basale cells: with mitotic figures (Sakata and Tamate, 1978; Shen et al., 2004; Malhi et al., 2013), labeled with tritiated thymidine (Goodlad, 1981; Ohwada et al., 1984; Neogrády et al., 1989), Ki-67 labeled (Mentschel et al., 2001), or 5-bromo-2′-deoxyuridine (**BrdU**) and Ki-67 labeled cells (Baldwin et al., 2004), but an attempt has never been made to link these proliferation events to the presence of potential stem and progenitor cells. An important first step in this new research area is the demonstration of abundance and existence of these potential markers in rumen tissue. A related second step is to document rumen epidermal proliferative responses to the extrinsic signal of nutrient concentration within the rumen itself.

Regarding the existence of rumen epidermal stem and progenitor cell markers, β1-integrin (**ITGB1**), tumor protein p63 (**TP63**), keratin-14 (**KRT14**), and Notch-1 (**NOTCH1**) are documented epidermal stem and progenitor cell markers in other species and organs (Zouboulis et al., 2008; Ambler and Maatta, 2009; Eckhart et al., 2013). Also, Leu-rich repeat-containing G protein-coupled receptor 5-expressing (**LGR5**), and musashi-1 (**MSI1**), a RNA-binding protein, are commonly accepted intestinal stem cell markers in other animals (Barker, 2014; Clevers et al., 2014; Tan and Barker, 2014). Because the rumen has properties that are characteristic of both epidermis (barrier function) and intestine (nutrient absorptive function), we rationalized that presence of these markers may indicate not only the existence of bovine rumen epidermal stem or progenitor cells, but also that these markers are potentially specific for rumen epidermal stem or progenitor cells.

The primary objective of this experiment was to evaluate the extrinsic effect of diet on (1) gene expression of 6 potential rumen epidermal stem or progenitor cell markers and (2) rumen epidermal cell proliferation within the stratum basale.

The Virginia Tech (**VT**) Animal Care and Use Committee approved the live animal experimental portion of this work (protocol #14–045-DASC). Twelve Holstein heifers were used in an experiment that lasted approximately 2 mo; they were part of a larger experiment that dealt with effects of diet on mammary gland growth and full experimental details are reported (Geiger et al., 2016). Briefly, the 12 heifers used here were purchased from a single commercial dairy producer located ~145 km from VT; at VT, heifers were individually housed in outdoor hutches on crushed rock without bedding. Heifers had ad libitum access to water. The experimental unit was heifer. Heifers were approximately 1 wk old (5.3 ± 2.3 d; mean ± SD) at the time of arrival to VT, had serum total protein ≥5.5 g/dL, and weighed 40.4 ± 4.6 kg (mean ± SD). Heifers used here were in the original treatment groups of restricted (**R**) or enhanced (**EH**), which is in reference to the treatment diet (Geiger et al., 2016). In the original experiment, one heifer on EH died within 48 h of arrival to VT and was not replaced. The final animal numbers were R, n = 6, and EH, n = 5.

Heifers on R received fewer nutrients through milk replacer (**MR**) than heifers on EH. The MR fed to R heifers was 20.9% CP and 19.8% fat (DM basis), offered at 0.43 kg of MR powder/heifer per d (DM basis). The MR fed to EH heifers was 28.9% CP, 26.2% fat (DM basis), offered at 1.08 kg of MR powder/heifer per d (DM basis). Both MR were reconstituted to 13% solids and fed twice daily in equal portions at 0600 and 1700 h. During the eighth week of the trial, heifers were weaned from MR by removing one daily feeding. The weaning process lasted 7 d. Throughout the experiment, heifers had access to a 25.6% CP, 4.0% fat, and 19.8% NDF (DM basis) texturized calf starter. The amount of calf starter offered per calf was adjusted daily so that starter intake of R approximated that of EH (described in Geiger et al., 2016). Refusals of MR and starter, if any, were recorded daily and are reported elsewhere (Geiger et al., 2016). Likewise, heifer BW were obtained weekly and are summarized in Geiger et al. (2016).