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Reduced apoptosis in sheep ileal Peyer's patch is associated with low levels of follicle centre carbonic anhydrase reactivity

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Abstract

Apoptosis in lymphoid follicles of the ileal Peyer's patch (IPP) in 21 sheep of two different age groups was visualized by the TdT-mediated dUTP nick end-labelling (TUNEL) method, and quantified using computer-assisted image analysis. The IPP follicle carbonic anhydrase (CA) reactivity was evaluated in the same samples. No significant differences with respect to apoptosis and CA reactivity were found between sheep aged 5 and 11 months. Individual variation in apoptotic activity correlated with the follicle centre CA reactivity. The group of animals found to have predominantly atypical ileal lymphoid follicles (more than 80% of total number of follicles) with features resembling jejunal Peyer's patch follicles, had lower number of apoptotic cells and reduced CA reactivity compared to the rest of the animals. The differences in CA reactivity in the follicle centres probably represent a variation in the presence of CA rich ~50 nm membrane-bounded particles known to be a feature of the sheep IPP. The present results suggest that the particles are involved in the modulation of the lymphocyte proliferation of the IPP follicles.
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Keywords: Ileal Peyer's patch; Sheep; Apoptosis; Carbonic anhydrase

1. Introduction

The ileal Peyer's patch (IPP) of sheep is considered to be a central lymphoid organ, developing during the last 50 days of ontogeny and reaching its peak size at about two months of postnatal age (Lie et al., 2005b; Reynolds and Morris, 1983). The IPP has been shown to regress quite rapidly until 9 months of age. From this time point IPP involutes more slowly and largely disappear at the age of 2 years (Lie et al., 2005b). A role for the IPP in the diversification of the pre-immune antigen-receptor repertoire and expansion of early B-cell populations has been defined, mechanisms probably including gene conversion and somatic mutation (Jenne et al., 2003; Lucier et al., 1998;

Abbreviations: AILF, atypical ileal lymphoid follicle; CA, carbonic anhydrase; FAE, follicle-associated epithelium; IPP, ileal Peyer's patch; PBS, phosphate buffered saline; TBS, Tris buffered saline; BSA, bovine serum albumin; TUNEL, TdT-mediated dUTP nick end-labelling; VE, villous epithelium

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Reynaud et al., 1991). The high level of cell proliferation in the IPP is accompanied by extensive apoptosis (Reynolds, 1986; Motyka and Reynolds, 1991), probably serving to remove self-reactive and/or unsuitable B cells. The level of apoptosis in the IPP is far more prominent than what is seen in the other lymphoid tissues of sheep (Motyka and Reynolds, 1991). It has been estimated that the rate of B-cell emigration from the sheep IPP is only 5% of the rate of B-cell production (Pabst and Reynolds, 1986). The destroyed B cells are subsequently and efficiently removed from the follicles by macrophages (Bhogal et al., 2004).

Another characteristic feature of the sheep IPP is the carbonic anhydrase (CA) reactivity of the follicle-associated epithelium (FAE), domes and follicle centre (Landsverk et al., 1987). This enzyme reactivity has been shown to be present in the cell surface of the FAE, and in the membrane of ~50 nm particles frequently found in lacunal spaces between FAE cells and in association with lymphocytes in dome and follicle (Landsverk et al., 1987; Lie et al., 2005a). By their size, membrane structure and resistance to surfactants the particles resemble exosomes (Lie et al., 2005a; Collas et al., 2002).

In lymphocytes in follicle centres the CA rich particles are found inside cytoplasmic vesicles, in association with the outer mitochondrial membrane, the nuclear envelope, chromosomes during mitosis and freely in the cytoplasm (Landsverk et al., 1990). These particles have been shown to be closely associated to the transcytotic activity of the IPP FAE (Landsverk et al., 1990). Collas et al. (2002) have shown that the ~50 nm particles contain genomic 16 kb DNA from sheep. The link between the CA rich particles and FAE has recently been further strengthened in a study of experimentally B cell depleted sheep. In this study the FAE had numerous ~50 nm particles and typical CA reactivity, while the underlying rudimentary follicles showed very low CA reactivity (Lie et al., 2005a).

Factors produced by the FAE is likely to be important for the growth and function of the IPP, but the nature of such factors, and a possible involvement of the ~50 nm particles are still unresolved issues (Landsverk, 1987; Landsverk et al., 1987, 1990; Reynolds, 1997). An association between FAE and follicular CA reactivity was suggested in a study in which damage to the FAE during Eimeria-infection reduced the CA activity both in the FAE and in the dome and follicle (Aleksandersen et al., 2002). In these animals, a concomitant depletion of follicular lymphocytes and reduced follicular apoptotic activity was seen.

The present study was undertaken to study the levels of apoptosis of IPP B cells in two different age groups of young sheep, and to examine the possible relationship between the extent of cell death by apoptosis and CA reactivity in IPP follicles.

2. Materials and methods

2.1. Sheep and preparation of tissues

Twenty-one sheep were included in this study. The sheep used were a subgroup of animals sampled in a previous study, of which details are given in Lie et al. (2005b). Tissue samples were collected from the IPP at the attachment of the ileocaecal fold on the ileum. The animals were obtained from a local abattoir and were divided in two age groups, 5 months of age (sheep nos. 1–11) and 11 months of age (sheep nos. 12–21). One of the originally sampled 22 animals was excluded from further studies because no lymphoid follicles were identified in the sample obtained.

Tissue samples were collected at the time of evisceration and were immediately placed in phosphate buffered saline (PBS), pH 7.4 and kept on ice until further preparation. Frozen specimens were prepared by placing the intestinal mucosa onto thin slices of liver to improve the quality of sectioning and ensure proper orientation. Specimens were frozen in tetrachloroethane (R134a, Robert Schiessl GmbH, Oberhaching, Germany) chilled in liquid nitrogen. The frozen samples were wrapped in aluminium foil and stored at −70 °C until evaluation. Cryostat sections of the intestinal wall were collected on slides pre-coated with a solution containing 10% poly-L-lysine. Serial sections were obtained and kept for the runs of CA enzyme-histochemistry, the TUNEL (TdT-mediated dUTP nick end-labelling) procedure and BAQ44A-immunohistochemistry.

2.2. Carbonic anhydrase–enzyme reactivity

Ten micrometers thick sections were used. The sections were air dried for 1 h at room temperature and

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