A novel BoLA class II molecule with a tissue distribution different from BoLA-DR or BoLA-DQ molecules

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Original article

A novel BoLA class II molecule with a tissue distribution different from BoLA-DR or BoLA-DQ molecules

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Summary — This study was designed to investigate the lymphoid tissue distribution of a new BoLA class II molecule defined by a unique monoclonal antibody H42A. We immunostained various lymphoid organs of 4-month-old Holstein calves with this monoclonal antibody and compared its tissue distribution to the BoLA-DR and BoLA-DQ expressions. Our results demonstrate a unique tissue distribution of the H42A-defined molecules, restricted to epithelial cells of the thymic medulla but extending in the periphery to the different cells involved in antigen presentation (B-cells, macrophages and dendritic cells). The peculiar distribution of this new BoLA class II molecule suggests that it has a specific function.

BoLA-D / cattle / MHC / BoLA-DR / BoLA-DQ / tissue distribution

Résumé — Distribution tissulaire d'une molécule de classe II du BoLA distincte de BoLA-DR et -DQ. *Nous avons étudié la distribution tissulaire d'une nouvelle molécule de classe II du BoLA définie par un anticorps monoclonal unique, H42A, et l'avons comparée à la distribution des antigènes BoLA-DR et BoLA-DQ. H42A définit des antigènes exprimés uniquement sur les cellules épithéliales de la médullaire thymique, mais retrouvés en périphérie sur différentes cellules impliquées dans la présentation antigénique (lymphocytes B, macrophages, cellules dendritiques). Cette distribution particulière suggère une fonction spécifique liée à l'expression de la molécule H42A.*

BoLA-D / bovin / CMH / BoLA-DR / BoLA-DQ / distribution tissulaire

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INTRODUCTION

The major histocompatibility complex (MHC) encodes 2 types of extensively polymorphic cell surface glycoproteins, called class I and class II molecules, which present peptide antigens to T-lymphocytes. Class II molecules consist of one α monomorphic and one β polymorphic chain which associate non-covalently in the rough endoplasmic reticulum with a non-polymorphic invariant chain. They present antigenic peptides derived from endocytosed exogenous proteins to T-helper lymphocytes and are modulated by several positive and negative effector mechanisms (Benoist and Mathis, 1990). The regulatory role of class II molecules is reflected in their restricted tissue distribution. They are primarily expressed by B-cells, monocytes, dendritic cells and endothelia, except under the influence of the cytokine γ-interferon which induces class II expression in diverse cell types. Cell populations expressing class II antigens vary in different species and are likely to be associated with differences in the precise biological role played by these molecules. In dogs and horses, resting T-cells constitutively express class II antigens whereas the majority of murine T-cells do not appear to express class II genes although an endogenous synthesis of Ia molecules has been demonstrated on long-term T-cell lines and also on resting and stimulated thymic T-cells (Osborne and Rudikoff, 1983; Singh *et al*, 1984; Benoist and Mathis, 1990). In human primary lymphocyte cultures, a large proportion of activated T-cells become class II positive.

Bovine MHC class II antigens are found on all peripheral and tissue-derived B-lymphocytes (Letesson *et al*, 1983; Lewin *et al*, 1985; Lalor *et al*, 1986; Emery *et al*, 1987) as well as on activated bovine T-cells (Lalor *et al*, 1986). We recently provided evidence for the expression of 3 different bovine leukocyte antigen (BoLA) class II gene products recognized by different monoclonal antibodies (mAbs). These antigens were characterized as BoLA-DR (Ababou *et al*, 1993), BoLA-DQ and a third class II molecule with an unknown specificity precipitated by a unique mAb called H42A (Ababou *et al*, 1994). We defined this molecule as an αβ-heterodimer with an acidic 33 kDa α-chain and a 29 kDa β-chain consisting of several maturation intermediates with neutral and basic isoelectric points (pIs). The molecular weights and pI of the H42A defined molecules clearly differ from those of BoLA-DR and BoLA-DQ heterodimers. As no bovine DP equivalent was found (Bensaid *et al*, 1991), the H42A molecule likely represents the product of another BoLA class II gene product, as BoLA-DY (Groenen *et al*, 1989) or BoLA-DIB (Stone and Muggli-Cockett, 1990), whose protein expression was not established (Stone *et al*, 1993), or the product of a gene isolated from the bovine B-lymphoblastoid BL-3 cell line (Romano *et al*, 1989). This new gene codes for a molecule different from BoLA-DR, BoLA-DQ, BoLA-DIB and BoLA-DYA (HA Lewin, personal communication).

In order to further characterize the H42A-defined molecule, it was important to examine its tissue distribution. This was done by immunostaining bovine lymphoid organs. The particular distribution of the H42A molecules as compared to BoLA-DR and BoLA-DQ, suggested that their function was different from that of classical BoLA-DR and BoLA-DQ molecules.

MATERIALS AND METHODS

Antibodies

The 3 mAbs used in this study, TH14B, TH22A and H42A, were raised against conserved epitopes on MHC class II molecules (Davis *et al*, 1987). They have previously been described as recognizing respectively BoLA-DR (Ababou *et*

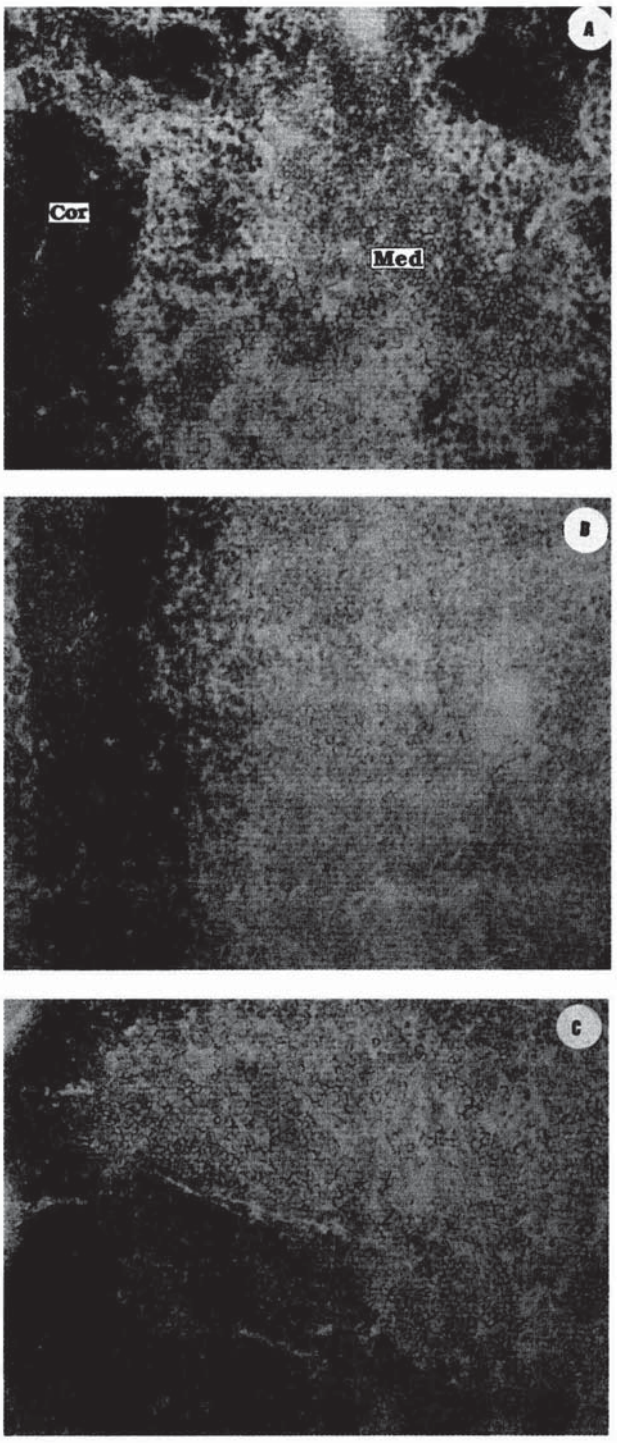

Fig 1. Immunoperoxidase staining of bovine thymus (x 100). The H42A (**A**) expression was not evidenced in the cortex (Cor) and was limited to aggregates of stellate cells with features of interdigitating cells in the medulla (Med), the site of marrow-derived class II positive antigen-presenting cells and epithelial cells. TH22A expression (**B**) was only limited to epithelial cells in the medulla. Conversely, the TH14B (**C**) expression was present throughout the medulla and focally in the cortica.

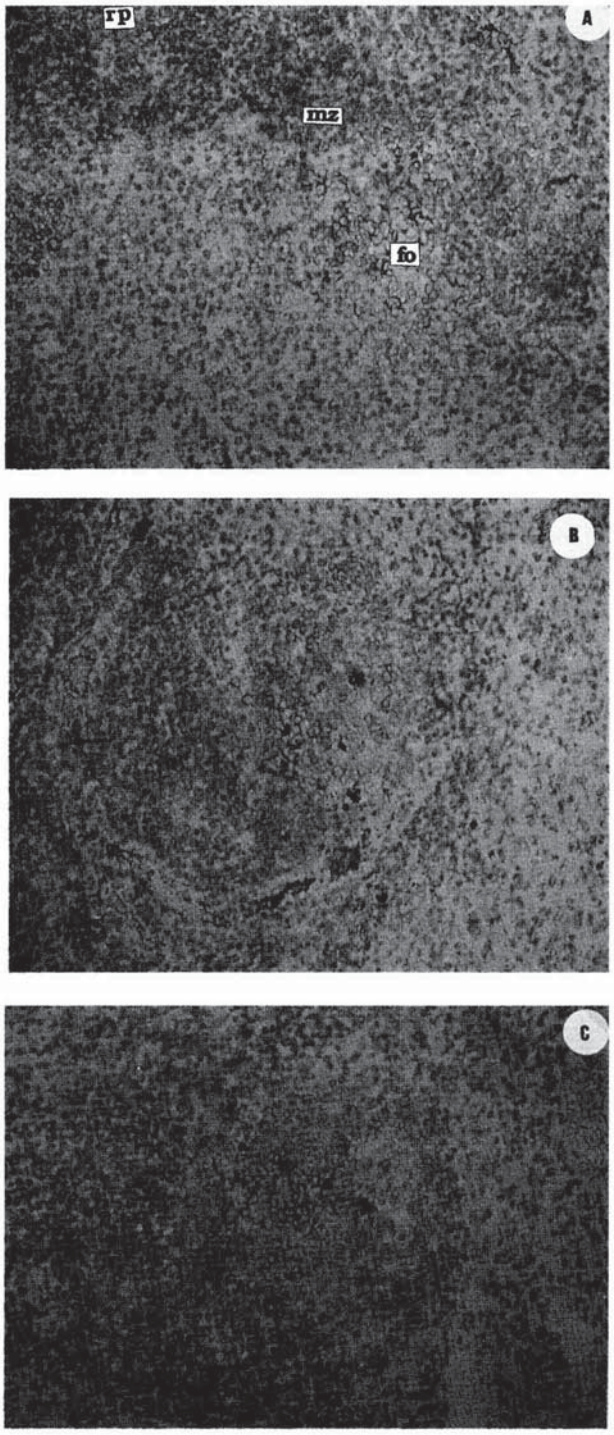

Fig 2. Immunoperoxidase staining of bovine spleen (x 100). A low expression was demonstrated by the 3 anti-BoLA mAbs in the red pulp (rp). In the white pulp, the H42A expression (**A**) was dense in the follicles (fo) and in the macrophages on the marginal zone (mz). The TH22A (**B**) and TH14B (**C**) expression was intense in areas of the white pulp enriched in B-cells and less in the other areas occupied by macrophages and interdigitating dendritic cells.

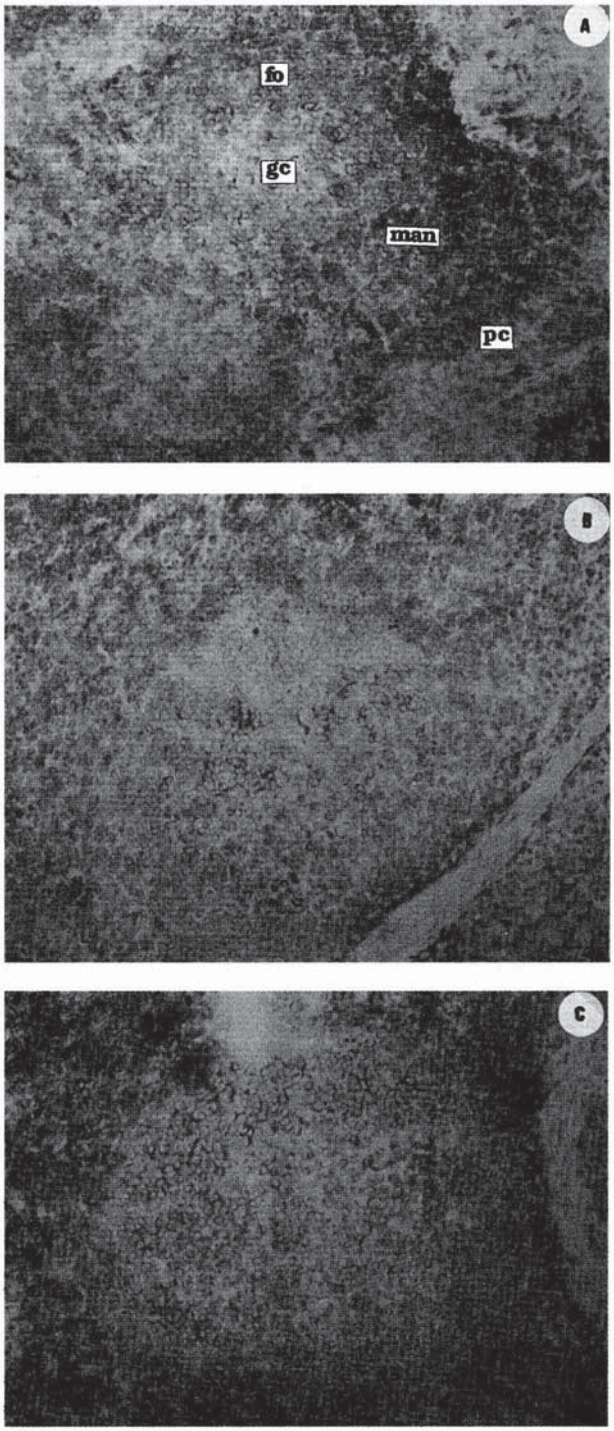

Fig 3. Immunoperoxidase staining of bovine mesenteric lymph nodes (x 100). The H42A (**A**) expression was prominent on the follicles (fo), on mature B-lymphocytes of the mantle zone (man), on germinal centres (gc), and on dendritic cells and macrophages of the paracortex (pc). The TH22A (**B**) expression was restricted to the germinal centres. TH14B (**C**) expression was similar to the H42A distribution.

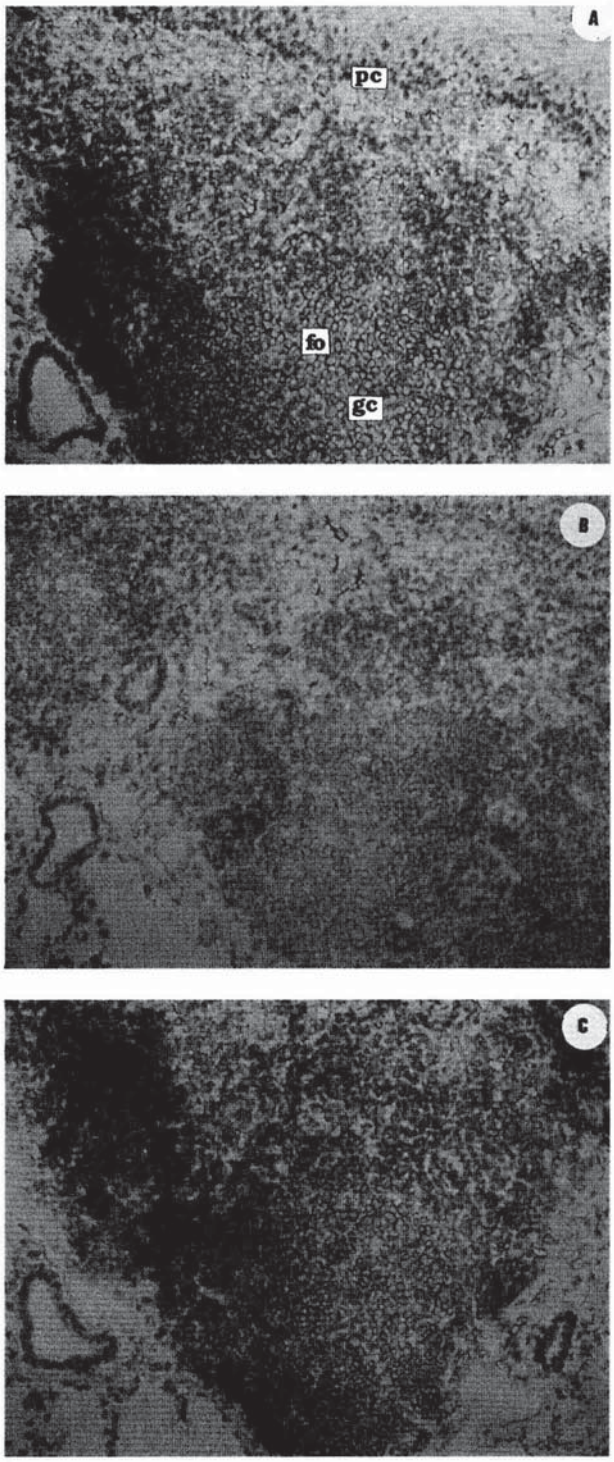

Fig 4. Immunoperoxidase staining of bovine tonsils (x 100). The H42A (**A**) antigen expression was intense in the follicle (fo) on dendritic cells of the germinal centres (gc) and in macrophages in parafollicular lymphoid cells (PC). The H22A (**B**) distribution was intense in the periphery of the cortex and faint in the follicles. Inversely, the TH14B (**C**) was very intense in the follicles and absent in the cortex.

al, 1993), BoLA-DQ and non-BoLA-DR or non-BoLA-DQ molecules (Ababou *et al*, 1994). All mAbs were titrated at 1 mg per ml.

Tissue samples

The thymus, mesenteric lymph nodes, spleen and tonsils of 4-month-old Holstein calves were studied. The tissues were rapidly snap-frozen in liquid nitrogen and stored at –80°C until use. Frozen tissue sections were cut to 4–6 μm thickness by cryostat (Microm, Heidelberg, Germany), air-dried overnight and subsequently fixed in acetone/methanol (V/V) for 15 min at room temperature. They were immunostained immediately or stored at –80°C.

Immunohistochemistry

The staining was performed using a peroxidase universal kit (Immunotech SA, France) based upon the ultrasensitive avidin/biotin affinity immunoperoxidase method. Briefly, fixed sections were washed in PBS at room temperature before incubation in PBS containing 0.3% H_2O_2 for inhibition of endogenous peroxidase activity followed by washing in PBS. The non-specific determinants were blocked by the protein-blocking agent (PBA) before specific staining. The sections were treated with the specific anti-BoLA class II mAbs diluted 1:200 in PBS at room temperature for 40 min in a humidified atmosphere. After washing in PBS, they were incubated for 40 min with 2 drops of a biotinylated sheep anti-mouse monoclonal antibody followed by washing in PBS. Incubation was then carried out for a further 20 min period with avidin–biotin–peroxidase complex. After washing twice in water, the peroxidase was developed by several drops of a complete chromogen solution for 20 min. After washing for 10 min in tap water, the sections were counterstained with hematoxylin prior to mounting and were observed under a conventional light microscope.

RESULTS AND DISCUSSION

We examined the tissue distribution of a new non-DR, non-DQ, BoLA class II molecule evidenced by a unique mAb, H42A. By using immunohistochemical staining techniques, we labelled frozen sections of various lymphoid organs with this antibody and compared the pattern and repartition of staining obtained with the BoLA-DR and BoLA-DQ expressions. No obvious variations were found between the 4-month-old Holstein calves studied. On the whole, frozen sections from 6 different animals were used.

In the thymus, the expression of the H42A defined antigens (fig 1A) was not observed in the cortex and was limited in the medulla to aggregates of stellate cells with features of interdigitating cells, the site of marrow-derived class II positive antigen-presenting cells, or epithelial cells that participate in the T-lymphocyte maturation. BoLA-DQ expression defined by the TH22A mAb (fig 1B) was also limited to the medulla and was very faint, involving cells devoid of filaments which likely represent epithelial cells. BoLA-DR expression, by contrast (fig 1C), was present throughout the medulla but present focally in the network of epithelial cells in the cortex. No labelling of thymic lymphocytes was obtained with any of the 3 different mAbs. The thymic distribution of molecules defined by H42A differed from BoLA-DR and BoLA-DQ expression and was comparable with the distribution of the murine H-2O molecule, recently described as a class II molecule with an unusual tissue distribution (Cho *et al*, 1991; Karlsson *et al*, 1991). Surprisingly, the BoLA-DR distribution was very close to murine H-2A expression (Karlsson *et al*, 1991).

In the spleen, a low class II-expression was found with the 3 anti-BoLA-class II mAbs in the red pulp. In the white pulp, the H42A staining (fig 2A) was dense in the B-dependent areas occupied by the follicles in the peripheral region, on the periarteriolar lymphocyte sheaths represented by interdigitating dendritic cells, and also in the sites occupied by macrophages on the

marginal zone. BoLA-DQ (fig 2B) and BoLA-DR (fig 2C) expression was prominent in areas of the white pulp enriched in B-cells and faint in the other areas of the white pulp occupied by macrophages and interdigitating dendritic cells.

In the lymph nodes, H42A antigen expression (fig 3A) was prominent on follicles and observed on mature B-lymphocytes of the mantle zone as well as on germinal centres, predominantly on dendritic cells and macrophages, and on scattered interdigitating cells of the paracortex. BoLA-DQ expression (fig 3B) was restricted to the germinal centres whereas BoLA-DR (fig 3C) had a very similar pattern of expression to H42A.

In the tonsils, H42A antigen expression (fig 4A) was very intense on the B-cells of the follicles and on the dendritic cells of the germinal centres. It was also prominent in the cortex on large cells with a clear nucleus, probably representing macrophages. Inversely, BoLA-DQ expression (fig 4B) was faint on B-cells of the follicles but more intense on scattered dendritic cells of the paracortex. BoLA-DR expression (fig 4C) was very intense on follicles and absent in the paracortex.

In conclusion, the H42A antigen distribution was very peculiar. It was previously determined that the positive selection of developing thymocytes may depend upon interaction between the $\alpha\beta$-receptors on these cells and major histocompatibility complex proteins bound to peptides found in the thymic cortical epithelium (Marrack *et al*, 1993). In the thymus, the absence of H42A antigens in the cortex suggested that these molecules might not be involved in the positive selection of T cells bearing $\alpha\beta$-receptors. The occurrence of H42A expression on a subset of medullary epithelial cells suggested that the H42A antigen might be involved in negative selection, as the negative selection process occurs in the deeper cortex, at the corticomedullary junction and in the medulla (Sprent *et al*, 1988). In the periphery, the H42A antigen expression, in contrast, was observed not only in the B-lymphocytes but also on macrophages and dendritic cells. A similar staining and distribution pattern (results not shown) were obtained with the rabbit anti-bovine S 100 antibody (Dako-Sébia, France). This antibody is known to recognize antigen presenting cells and to stain only the filaments of dendritic cells, macrophages and interdigitating cells. Altogether, these data suggested an essential role of the H42A-defined molecule in the antigen presentation to T-lymphocytes. The H42A molecule resembled the recently described murine class II H-2O molecule in its thymic distribution (Karlsson *et al*, 1991) but differed from it in the distribution pattern in the periphery. Indeed, the murine H-2O molecule was only expressed in the epithelial cells of the thymic medulla and seemed confined to B-cells in the lymphoid organs (Karlsson *et al*, 1991). As compared to H42A expression, BoLA-DR distribution was present throughout the thymic cortex and medulla, but was more restricted in its distribution in the periphery, involving predominantly B-cells. Similar results were recently reported for a different set of mAbs recognizing BoLA class II antigens (Taylor *et al*, 1993). BoLA-DQ, in contrast, was limited to thymic epithelial cells, but exhibited a peripheral distribution which was, on the whole, similar to BoLA-DR for the B-cell distribution, which extended to the dendritic cells in the tonsils. In view of the structural similarities between the H42A and BoLA-DR and BoLA-DQ molecules (Ababou *et al*, 1994), it would not have been surprising if the H42A molecules were recognized by T-cell receptors. Of particular interest would be investigations on the potential interactions of the H42A molecules not only with the $\alpha\beta$- but also with the $\gamma\delta$-T-cell receptors. Ruminants contain a substantial fraction of $\gamma\delta$-T-cells in their peripheral blood representing up to 50% of mononuclear cells in young animals

and decline to 10–25% in adults (Walcheck and Julita, 1993). γδ-cells exhibit a homing preference to epithelial-associated tissues with few cells accumulating in secondary lymphoid sites, such as peripheral lymph nodes (Walcheck and Julita, 1993). Such studies may help unravel the functioning of the ruminant immune system and the role imparted to the different lymphocyte populations for a host immune response.

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