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Four antigens expressed on most ovine cell types

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SUMMARY

Monoclonal antibodies have been produced that bind to four different antigens expressed on the surface of ovine lymphocytes as well as to a variety of other ovine cell types. These antigens have been characterized with respect to their tissue distribution and immunochemistry. The timing of appearance of these antigens within the ovine embryo is reported.

Many important cell surface antigens are not restricted in their distribution to cells of one particular lineage. This is well illustrated by several members of the immunoglobulin superfamily, including major histocompatibility complex (MHC) antigens, Thy-1 and MRC OX-2 antigens (Williams, 1985). Other molecules involved in cellular interactions or cellular activation that also have a broad distribution are LFA-3 (Gromkowski *et al.*, 1985) and the transferrin receptor (Jefferies *et al.*, 1985). The recent demonstration that the highly conserved protein ubiquitin is a component of a murine lymphocyte homing receptor, as well as being found in other cell surface proteins of unknown function (Siegelman *et al.*, 1986), suggests that other unidentified structures may also be found in a variety of cell surface antigens.

This report describes monoclonal antibodies that react with a variety of cell types, including lymphocytes. These antibodies were derived from two fusions following immunization of BALB/c mice with splenic cell suspensions from ovine fetuses of 50–65 days gestational age (Fusion 17) or thymocytes from ovine fetuses of 42–52 days gestational age (Fusion 46). Fusion, screening and characterization of the monoclonal antibodies were performed using the techniques described by Maddox, Mackay & Brandon (1985a, b). The monoclonal antibodies described in this report are all of the IgG1 subclass.

Three monoclonal antibodies were produced from Fusion 17 that reacted with subpopulations of lymphocytes as well as with a variety of other cell types. 17.3 and 17.12 were found to react with the same or closely related epitopes of a molecule that migrated on SDS–PAGE with a molecular mass of 55,000 (Fig. 1, Lanes e and f). A band of the same molecular mass is identified by these antibodies on Western immunoblots of non-reduced membrane proteins from lymph node, spleen and liver (data not shown). This antigen is weakly expressed on lymphocytes, being found primarily on B lymphocytes, as well as on connective tissue, a variety of epithelia (including intestinal and lung epithelium as well as kidney tubules), and the sinusoidal lining of the liver. Moreover, this determinant is also detected in ovine sera. The expression of the antigen defined by 17.3 and 17.12 on several ovine fibroblast cell lines demonstrates that it is synthesized by fibroblasts rather than being passively acquired from sera. 17.3 and 17.12 do not bind to macrophages or neuronal tissue. Both of these antibodies react with paraffin-embedded glutaraldehyde/paraformaldehyde-fixed tissue but not with paraffin-embedded alcohol-fixed tissue.

The third monoclonal antibody derived from this fusion, 17.15, is also weakly expressed on some lymphocytes. In contrast to 17.3 and 17.12, 17.15 stains a subpopulation of thymocytes as well as a subpopulation of peripheral T lymphocytes, but does not stain B cells. Immunoprecipitations from efferent lymph lymphocytes shows that 17.15 binds to an antigen with a relative molecular mass of 145,000 under reducing conditions (Fig. 1, Lanes k and l). This antigen has a faster mobility without reduction, suggesting the presence of intrachain disulphide bonds. 17.15 stains macrophages, connective tissue, ovine fibroblast cell lines, a variety of epithelia and the sinusoidal lining cells of the liver, but does not stain neuronal tissue. This antigen is not preserved during paraffin processing, nor in Western immunoblots.

The two monoclonal antibodies from Fusion 46 that react with both leukoid and non-leukoid tissues, 46.57 and 46.91, have been partially characterized. 46.57 is expressed on the majority of lymphocytes, but at a higher lever on B cells, ileal Peyer's patch lymphocytes and cortical thymocytes than on more mature T cells. Immunoprecipitations from lymphocytes reveal that the antigen detected has a relative molecular mass of 22,000 under both reducing and non-reducing conditions (Fig. 1, Lanes c and d). The antigen is detectable in Western

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Abbreviations: MHC, major histocompatibility complex; SDS–PAGE, polyacrylamide gel electrophoresis in sodium dodecyl sulphate.

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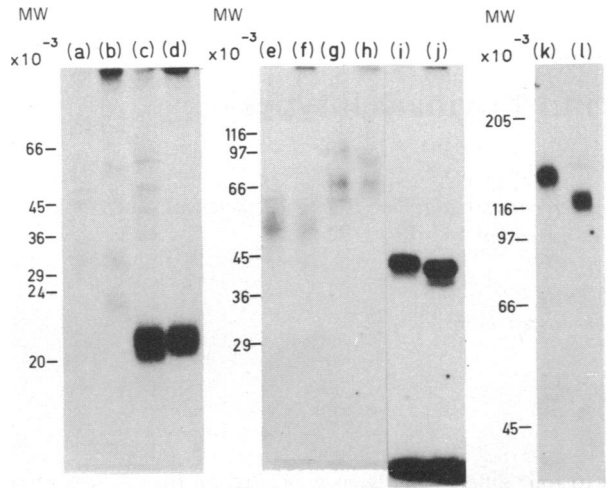

Figure 1. Immunoprecipitation of antigens from ^{125}I-labelled lymphocytes solubilized in Nonidet P-40. Antigens were immunoprecipitated with the following monoclonal antibodies: 28.1 (MHC II) (a) and (b); 46.57 (c) and (d); 17.3 (e) and (f); 46.91 (g) and (h); 41·19 (MHC I) (i) and (j); and 17.15 (k) and (l). Samples (a), (c), (e), (g), (i) and (k) were reduced with 2% dithiothreitol prior to SDS–PAGE; all samples were alkylated with iodoacetamide prior to polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS–PAGE). (a), (b), (c) and (d): 12% SDS–PAGE; (e), (f), (h), (i) and (j) 10% SDS–PAGE; (k) and (l) 6% SDS–PAGE. Purified antibodies of 17.3 and 17.15 were coupled to Sepharose 4B; supernatants of 46.57, 46.91, 28.1 and 41.19 were immunoprecipitated with sheep anti-mouse IgG coupled to Sepharose 4B.

immunoblots from non-reduced samples (with or without boiling), but is not detectable when the antigen has been reduced with dithiothreitol. As well as being expressed on lymphocytes, this antigen is also found on connective tissue, ovine fibroblast cell lines, a variety of epithelia and the sinusoidal cells lining the liver and brain.

46.91 defines an antigen that is weakly expressed on all lymphocytes, as well as being expressed on connective tissue, ovine fibroblast cell lines, a variety of epithelia, neurons, and sinusoidal cells lining the liver. Two chains with apparent molecular masses of 78,000 and 100,000 are immunoprecipitated by 46.91 from efferent lymph lymphocytes (Fig. 1, Lanes g and h). Western immunoblots of non-reduced material (with or without boiling) show that both bands are stained by 46.91. It is possible that the lower molecular mass band is either a precursor or a degradation product of the higher molecular mass product. Alternatively, these forms may be discrete proteins that both contain the epitope recognized by 46.91.

The appearance of these antigens was studied using immunohistological staining of cryostat sections of frozen embryos and fetal tissues. The earliest tissue studied, embryonic membranes (yolk sac or trophoblast) at Day 15 of gestation, was stained by 17.15, 46.57 and 46.91, but not by 17.3 or 17.12. At this stage of development this tissue does not express MHC antigens (J. F. Maddox, C. R. Mackay and M. R. Brandon, manuscript in preparation). The earliest embryos examined were at Day 19 of gestation; 17.3 stained scattered cells at this stage, while 17.15, 46.57 and 46.91 stained most embryonic tissues weakly and the liver more strongly. Class I MHC antigens were also found on a small number of cells within embryos of this age. Clusters of small cells with intensely basophilic nuclei were found in the ovine embryonic liver from Day 28 of gestation until birth. These cells were stained strongly by 17.3 and 46.57 and are probably primitive haemopoietic cells.

The function of these four antigens is unknown; however, the early appearance of these antigens on cells within the developing ovine embryo, coupled with their ubiquitous distribution, suggests that these antigens fulfil some important role on the cell surface.

Availability of monoclonal antibodies

The monoclonal antibodies described in this paper are available for research. Written enquiries should be directed to Ms K. MacRae, Dept. of Veterinary Preclinical Sciences.

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