Short Communication: Lack of Stage-Specific Embryonic Antigen-1 Expression by Bovine Embryos and Primordial Germ Cells

S.-J. CHOI,[1] H. SHIM, and G. B. ANDERSON[2]
Department of Animal Science,
University of California, Davis 95616

ABSTRACT

The objective of this study was to determine whether stage-specific embryonic antigen-1, a cellular marker commonly used to identify murine undifferentiated embryonic cells, is also a useful marker for bovine pluripotent cells. Expression of stage-specific embryonic antigen-1 was examined by indirect immunohistochemistry on bovine preimplantation embryos and on primordial germ cells contained in the genital ridge. Expression of stage-specific embryonic antigen-1 was not observed in any of the cleavage-stage bovine embryos examined, including one-cell, two-cell, four-cell, eight-cell, morula, and blastocyst stages, nor in tissue sections of bovine genital ridges collected from embryos on d 34, 37, and 40 of gestation. As expected, expression of stage-specific embryonic antigen-l was detected on murine preimplantation embryos and on murine teratocarcinoma cells. Results of this study indicate that, unlike in the mouse, stage-specific embryonic antigen-1 is not a useful cellular marker for pluripotent bovine embryonic cells or bovine primordial germ cells.
(**Key words**: stage-specific embryonic antigen-1, embryos, primordial germ cells, bovine)

Abbreviation key: **DMEM** = Dulbecco's modified Eagle's medium, **EG** = embryonic germ, **ES** = embryonic stem, **PGC** = primordial germ cell, **SSEA-1** = stage-specific embryonic antigen-1.

INTRODUCTION

Cell-surface glycoconjugates are expressed and undergo substantial changes during embryogenesis (5, 14). Developmentally regulated carbohydrates are present in the form of glycolipids, glycopeptides, or proteoglycans that are recognized by antibodies or lectins, and these carbohydrates can be used as cell-surface markers to distinguish embryonic cells in the process of differentiation. Over the past several decades, a number of monoclonal antibodies have been produced that define antigens for specific stages of murine embryo development. Solter and Knowles (21) developed such a monoclonal antibody by the immunization of mice with irradiated F9 murine teratocarcinoma cells and named the antigen recognized by the monoclonal antibody stage-specific embryonic antigen-1 (**SSEA-1**). Hapten inhibition experiments revealed that the antigenic epitope has the Le^x structure (10), which is usually carried by the lactoserines such as poly-N-acetyllactosamine (embryoglycan) linked to high molecular mass glycoproteins (15). Competitive binding assays have demonstrated that SSEA-1 stabilizes compaction of morulae in mice, suggesting its involvement in intercellular recognition during embryogenesis (6).

Stage-specific embryonic antigen-1 has been detected at several stages of development in preimplantation mouse embryos, including on blastomeres of the eight-cell through blastocyst stages with the strongest expression at the morula stage (21). In blastocysts, expression of SSEA-1 was found in both inner cell masses and mural trophectodermal cells, but trophectodermal expression was only transient (21). Expression of SSEA-1 is restricted to the embryonic ectoderm and the visceral endoderm of mouse embryos at the egg cylinder stage (8). Stage-specific embryonic antigen-1 has also been found in various murine undifferentiated, pluripotent embryonic cells, including embryonal carcinoma cells, embryonic stem (**ES**) cells, and embryonic germ (**EG**) cells (8, 12, 13, 16, 20, 22), but not on their differentiated derivatives, suggesting that SSEA-1 is a specific cell-surface marker for undifferentiated cells. Moreover, SSEA-1 has also been reported to be expressed on primordial germ cells (**PGC**) in both mice (3, 8, 9) and pigs (23). Expression of SSEA-1 was found in both migratory and postmigratory PGC in mice at 8.5 to 14.5 d postcoitum and in pigs at 18 to 26 d of gestation.

Murine PGC have been shown to proliferate in long-term culture, ultimately producing pluripotent

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[1]Current address: Department of Animal Sciences, University of Illinois, Urbana 61801.
[2]Corresponding author.

EG cells that share common characteristics with ES cells, including morphology and the developmental capacity to produce chimeras after blastocyst injection (12, 16). Recently, we were successful at the isolation of porcine EG cells from cultured PGC (18). If available, bovine EG cells would be useful for site-directed manipulation of the genome in cattle. Several cellular markers can be used to facilitate identification of porcine PGC and their undifferentiated derivatives (17, 18), but no such markers have been described for bovine PGC. Detection of SSEA-1 has been useful to identify pluripotent murine cells, including PGC and ES, embryonal carcinoma, and EG cells; SSEA-1 expression on pluripotent bovine cells could prove to be useful as a marker to distinguish bovine PGC from surrounding somatic cells and to identify undifferentiated bovine embryonic cells in culture. The specific objective of this study was to determine whether SSEA-1, as in the mouse, could be detected on bovine preimplantation embryos and PGC.

MATERIALS AND METHODS

Preparation of Embryos, Tissues, and Cells

Bovine in vitro-produced embryos were obtained at the one-cell (n = 62), two-cell (n = 97), four-cell (n = 53), eight-cell (n = 29), morula (n = 62), and blastocyst stages (n = 78). Procedures for production of bovine embryos were as described previously for our laboratory (1). Zonae pellucidae were removed by a micromanipulator using a micropipette, and the embryos were cultured in Dulbecco's modified Eagle's medium (**DMEM**; Gibco, Grand Island, NY) at 39°C for 2 h. Bovine genital ridges were isolated from embryos on d 34 (n = 2), 37 (n = 2), and 40 (n = 2) of gestation as described by Cook et al. (2). Genital ridge-containing tissues were dissected from the body wall, and the genital ridge was isolated from the mesonephros in DMEM using fine forceps. The genital ridges were fixed in 10% buffered formalin for paraffin embedding, and histological sections were prepared at 6 μm. Embedded tissues were deparaffinized, cleared with xylene, and dehydrated through a graded series of ethanol solutions. Murine embryos (JU strain) (4) were collected at the two-cell (n = 30), four-cell (n = 30), eight-cell (n = 30), morula (n = 48), and blastocyst stages (n = 61) and were treated as described for bovine embryos, except culture was in Whitten's medium at 37°C.

Murine teratocarcinoma cells, F9 cells, were used as a positive control for the immunohistochemical assay for SSEA-1. The cells were cultured on 0.1% gelatin-coated 96-well tissue culture plates (Falcon, Franklin Lakes, NJ) in DMEM supplemented with 15% fetal bovine serum (Gibco), 1% L-glutamine (2 m*M*; Gibco), minimum essential medium nonessential amino acids (0.1 *M*; Gibco), 2-β-mercaptoethanol (0.1 m*M*; Sigma Chemical Co., St. Louis, MO), and 1% antibiotics (5000 U/ml of penicillin G; streptomycin, 5000 μg/ml of streptomycin; Gibco) at 37°C. The F9 cells were allowed to grow to confluence and were passed at 3-d intervals up to 10 passages. For the SSEA-1 assays, some F9 cells were fixed with 80% ethanol, and others were used without fixation.

Immunohistochemistry

An indirect immunofluorescence assay was carried out on embryos (21), tissue sections (7), and cells in culture as described previously (3) with modifications. All immunohistochemical tests were performed in 5% CO_2, 95% air at 37°C. Samples were preincubated with 5% heat-treated normal goat serum in PBS for 15 min at 37°C to block nonspecific binding. The fixed tissue sections and cells were rehydrated with PBS at room temperature. Samples were incubated with anti-SSEA-1 monoclonal antibody obtained from murine ascites fluid (1 mg/ml with 0.1% sodium azide; Kamiya Biomedical Co., Thousand Oaks, CA) diluted in PBS containing 2% BSA (1:50 dilution) for 1 h. After washing three times with PBS, all samples were treated with goat anti-mouse IgM (1 mg/ml of μ-chain specific antibody conjugated with fluorescein isothiocyanate in PBS with 1% BSA and 0.1% sodium azide; Sigma Chemical Co.) in PBS containing 2% BSA at (1:20 dilution) for 30 min. All samples were visualized by fluorescence microscopy after washing three times with PBS containing 2% BSA. As a negative control, representative samples of each type of specimen were incubated in secondary but not primary antibody after preincubation with 5% normal goat serum in PBS.

RESULTS AND DISCUSSION

Murine embryos at the two-cell and four-cell stages did not express SSEA-1, but expression of SSEA-1 was observed in all murine embryos from the eight-cell stage through the blastocyst stage. The strongest SSEA-1 expression was observed at the morula stage. Expression of SSEA-1 was detected in both inner cell masses and trophectoderm in blastocysts, and the intensity of expression appeared not to differ. Expression of SSEA-1 was consistently positive in F9 cells for both fixed and unfixed specimens. Expression of SSEA-1 was not observed in any bovine embryo at

any stage of development from one-cell stage through the blastocyst stage and was not observed in PGC and somatic cells in tissue sections of bovine genital ridges on d 34, 37, and 40. Figure 1 shows levels of fluorescence for representative murine (Figure 1, A and B) and bovine (Figure 1, C and D) embryos and illustrates the distinctive differences in SSEA-1 expression between the two species.

The consistent expression of SSEA-1 by murine preimplantation embryos from the eight-cell stage through the blastocyst stage, as previously described (21), demonstrates and confirms the validity of the immunohistochemical assay. The observation that no bovine embryos at any stages showed expression of the antigenic determinant recognized by the monoclonal antibody to SSEA-1 indicates that SSEA-1 is not a useful marker for undifferentiated bovine embryonic cells. In mice, expression of SSEA-1 has been reported to be localized in PGC in the genital ridge at 11 d postcoitum (8), and migratory PGC have also been shown to express SSEA-1 (3), but, in this study, bovine PGC in the genital ridge at 34, 37, and 40 d of pregnancy were not reactive to the monoclonal antibody to SSEA-1. Murine EG cells derived from long-term culture of PGC express SSEA-1 (12, 16), but results here suggest that SSEA-1 expression will not be useful to distinguish EG cells from embryonic cells that have undergone differentiation.

Failure to detect SSEA-1 expression by bovine embryos and PGC may result from SSEA-1 simply not being expressed in cattle. The possibility also exists that the monoclonal antibody to murine SSEA-1 does not recognize the antigenic determinant of an SSEA-1-like molecule on bovine cells, but we (17) and others (23) have observed that monoclonal antibody

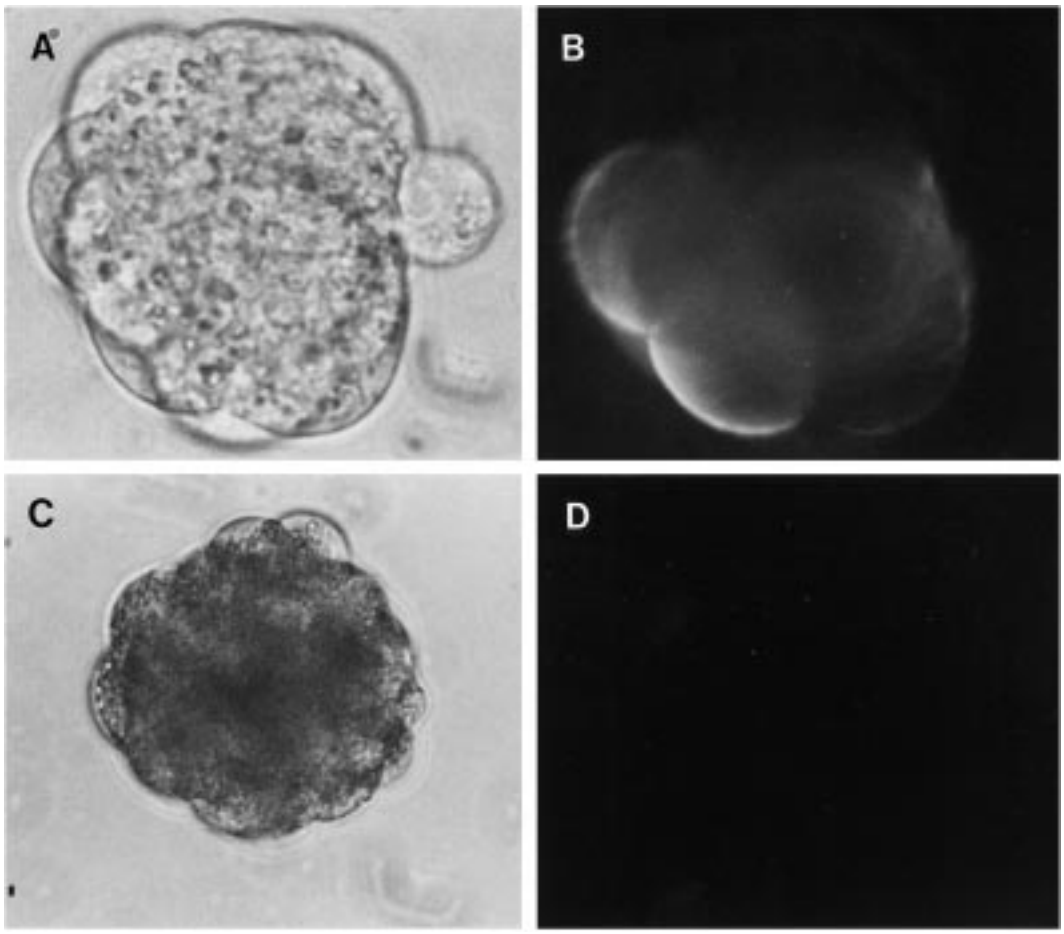

Figure 1. Expression of stage-specific embryonic antigen-1 (SSEA-1) by murine embryos but not by bovine embryos. Murine morula in bright-field (A) and dark-field (B) after indirect immunofluorescence assay for SSEA-1. Fluorescence in panel B indicates SSEA-1 expression (200×). Bovine morula in bright-field (C) and dark-field (D) did not show SSEA-1 expression when tested in the same assay (100×).

to SSEA-1 recognizes putative EG cells and PGC in pigs. Regardless of the explanation for why SSEA-1 was not detected, its expression should not be used to screen bovine cell preparations and cultures for pluripotent embryonic cells.

In summary, the consistent expression of SSEA-1 by murine but not bovine embryos and of SSEA-1 expression on both fixed and unfixed F9 cells but not on bovine PGC convinces us that SSEA-1 is not a useful marker for undifferentiated bovine embryonic cells and PGC. Stage-specific embryonic antigen-1, which is a useful marker for pluripotent embryonic cells and PGC in mice, appears not to be useful to identify putative pluripotent EG cells and PGC in cattle. Alkaline phosphatase activity has been used to detect PGC in cattle (11), but its histochemical assay is invasive, and its use to distinguish PGC from somatic cells is not fully reliable (S.-J. Choi and G. B. Anderson, 1997, unpublished). Failure to detect SSEA-1 in the current study leaves a specific cellular marker for undifferentiated bovine embryonic cells yet to be found.

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