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Monoclonal Antibodies against Human Blastocysts

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ABSTRACT

A panel of monoclonal antibodies against the implanting mouse blastocyst was tested for cross-reactivity to human blastocysts. The monoclonal antibodies were produced by intrasplenic immunization with living mouse blastocysts or egg-cylinders. Among the 13 anti-mouse blastocyst antibodies checked, 6 ones detected antigen epitopes also in human blastocysts. If the mouse-human cross-reacting antibodies detect similar antigens in blastocysts of the two species, the results imply that anti-mouse blastocyst antibodies with specific properties could be used for studying human blastocysts. This procedure opens for a way to obtain monoclonal antibodies for analysing human blastocysts.

INTRODUCTION

Earlier attempts to produce antibodies against mouse blastocysts required more than 1000 blastocysts as immunogen (9), but the introduction of splenic immunization (5) facilitated this work, since that method required only about 40 blastocysts (7). Antibodies against the human blastocyst, however, cannot easily be produced in a similar way due to, among other things, the need for blastocysts not only for immunization but also for the material-consuming screening of the mAbs obtained. Therefore, we tried another approach, namely raising monoclonal antibodies (mAbs) against mouse blastocysts and then searching for mouse-human cross-reacting mAbs.

Cross-reactivity between species implies that an antibody of one species detects the same antigen-epitope in another species. If the cross-reactivity appears at the same tissue location in the species, it could be that the antibody recognizes a developmentally conserved antigen, that is, a molecule which could have similar functions in the different species. It is already known that a mouse-human cross-reactivity occurs among the reproductive organs, among other tissues, in sperm cells (1) and oocytes (3).

The presence of a blastocyst cross-reactivity would offer a feasible way to obtain potentially interesting antibodies for analyses of the function of the human blastocyst. Therefore, we checked the immune responses of human blastocysts to a panel of mAbs against mouse blastocysts and found antibodies which cross-reacted with the human blastocyst. The present report describes the methods used to produce and select mouse-human cross-reacting anti-blastocyst mAbs.

MATERIALS AND METHODS

Monoclonal antibodies

The antibodies available for checking the mouse-human cross-reactivity had been produced after intrasplenic immunization with either mouse blastocysts or mouse egg-cylinders. In the form of blastocysts, the immunogen was zona-free and adhesive mouse blastocysts, reactivated from delayed implantation, since the aim was to obtain antibodies which detected the adhesive trophoblast of the implanting blastocyst. The blastocysts were deposited in the spleen tissue either in the living state or after drying on small pieces of a nitrocellulose (NC) membrane (blastocyst blots) (5). They were deposited in the spleen tissue in batches of about 10 blastocysts at each of the 3-5 immunizations which were performed at an interval of 4-6 weeks. The supernatants obtained were screened on blastocysts blots. Details of the procedures used have been published previously by Svalander and cowokers (7) and by Hjortberg and coworkers (2).

In the form of egg-cylinders, the immunogen used was obtained by growing blastocysts in-vitro to this stage. Since the blastocysts were grown on agarose-coated dishes and were unable to adhere to the agarose support, they floated freely in the medium and could easily be removed and transferred into the spleen. In this case, the aim was to obtain antibodies against the invasive trophoblast. The egg-cylinders were deposited in the spleen tissue either in their native state or dried on a small piece of NC membrane as an egg-cylinder blot. The immunization schedule used was 3-4 depositions with 6-8 egg-cylinders each time at intervals of 4-6 weeks between depositions. Screening was made on egg-cylinder blots. Details on the procedures have been published previously (6).

About 50 mAbs against mouse blastocysts were available for study. These mAbs had been characterized earlier in various ways and using this information, we selected a panel of 9 mAbs which were positive against blastocysts, activated from delayed implantation (adhesive, implanting blastocysts). The presence of cross-reactivity was ascertained by staining human blastocyst blots with the same immunohistochemical protocol. None of the antibodies used were positive for human serum. Each mAb was tested 2-3 times on human blastocyst blots.

Human Blastocysts

Ovarian stimulation was made with either human menopausal gonadotropin (hMG, Pergonal, Serono, Italy) or pretreatment with releasing hormone agonist (Buserelin) for 2 weeks prior to hMG stimulation. In certain patients purified FSH (Metrodin HP, Serono, Italy) was utilized for the controlled ovarian hyperstimulation. The ovarian response was monitored by serum estradiol determinations and transvaginal ultrasound to determine follicle number and size. The criterium for administration of human chorionic gonadotropin (hCG, Pregnyl, Organon, Holland) was the presence of at least one follicle with a diameter of >18 mm. Follicle aspiration was performed 36-37 h after the hCG-injection. At 4-6 h after aspiration, $5x10^4$ motile spermatozoa per oocyte were added and, 18-20 h later, the oocytes were checked for fertilization.

Morulae not suitable for transfer to patients (3 normally fertilized and cleaved oocytes were transferred 42-48 h after insemination) or for cryopreservation were cultured as "spare

embryos" in Earle's balanced salt solution (Medicult, Denmark), which was supplemented with 10% heat inactivated human serum three days after aspiration. Embryos growing in vitro were examined once a day and scored for the number of blastomeres. When the blastocysts reached the expanded stage, they were carefully pipetted free from their zonae, rinsed in phosphate buffered saline and mounted as blastocyst blots on NC membranes.

Immunohistochemistry

Immunostaining was initiated by blocking protein adhesion of the NC membranes by incubation in 3% bovine serum albumin in phosphate buffered saline overnight. The supernatants, diluted 1:1 in PBS (pH 7.6), were applied for 60 min. After rinsing, the blots were incubated for 15 min with biotin-conjugated goat anti-mouse polyvalent immunoglobulin antibody, diluted 1:500 with 1% normal goat serum (Sigma) in tris buffered saline (TBS), rinsed again and then incubated for 30 min in an 1:1000 dilution of strept-ABC complex containing alkaline phosphatase (Dakopatts, Denmark). The oocyte blots were stained with the substrate kit (Vector, USA), diluted 1:100 in TBS (pH 8.2) for 15-20 minutes, rinsed and mounted.

An irrelevant primary antibody was used as a negative control and an antibody against a retroviral protein (gp70) was applied as a positive control (4). The oocyte blots were scored subjectively in a preparation microscope.

RESULTS AND DISCUSSION

The antibodies against mouse blastocysts in the hybridome supernatants were detected by screening with adhesive, implanting mouse blastocysts, that is, blastocysts from a mouse in an experimental delay of implantation, which had been reactivated by an estrogen injection 18 h before recovery of the blastocysts. These blastocysts are zona-free, since they hatched during their period in delay. The blastocysts were used for immunohistochemical screening in the form of blastocyst blots, which means that newly flushed, living mouse blastocysts were placed on a small piece of nitrocellulose paper for drying and then staining. This procedure should preserve most of the antigens. The human blastocysts were also implanting and zona-free, since their zonae had been removed mechanically when they reached the expanded stage in culture before being prepared as blastocyst blots. Since human blastocysts were also screened as blastocyst blots, the condition of their antigens was probably similar to that of the mouse blastocyst antigens.

Thirteen mAbs to mouse blastocysts were tested for cross-reactivity with the blots of zona-free human expanded blastocysts. Antigen epitopes in the human blastocysts were detected by 6 anti-mouse blastocyst mAbs (Table 1). This finding demonstrates that mouse-human cross-reacting mAbs do occur. If the common antigen epitopes appear in similar types of molecule in the two species, the cross-reactivity could indicate both that the molecule is developmentally conserved and that it is related to the same function in the two cross-reacting tissues.

The cross-reacting anti-blastocyst antibodies have already been characterized for antigen location and functional effects in the mouse system. For instance, antigen location at the surface of the mouse trophectoderm was recognized by some mAbs (Table 1: N63, 9:23, 9:36) (Figs. 1 A and B). If these mAbs are also positive for the surface of the human blastocyst, they

would be appropriate for studies of the surface components of the human blastocyst at implantation. Further, antigens shedded

Table 1. Anti-mouse blastocyst mAbs cross-reacting with human expanded, zona-free blastocysts. The table shows their Ig-class and their stage specificity for nonadhesive, delayed and/or adhesive, implanting mouse blastocysts.

		Mouse Stage Specificity	
mAb	Ig-Class	Delay	Implant.
N63[ref 8]	IgM	+	+
3:3F1[ref 2]	IgG1	-	+
3:1[ref 6]	IgM	+	+
8:6[ref 6]	IgM	-	+
9:23[ref 6]	IgM	-	+
9:36[ref 6]	IgM	+	+

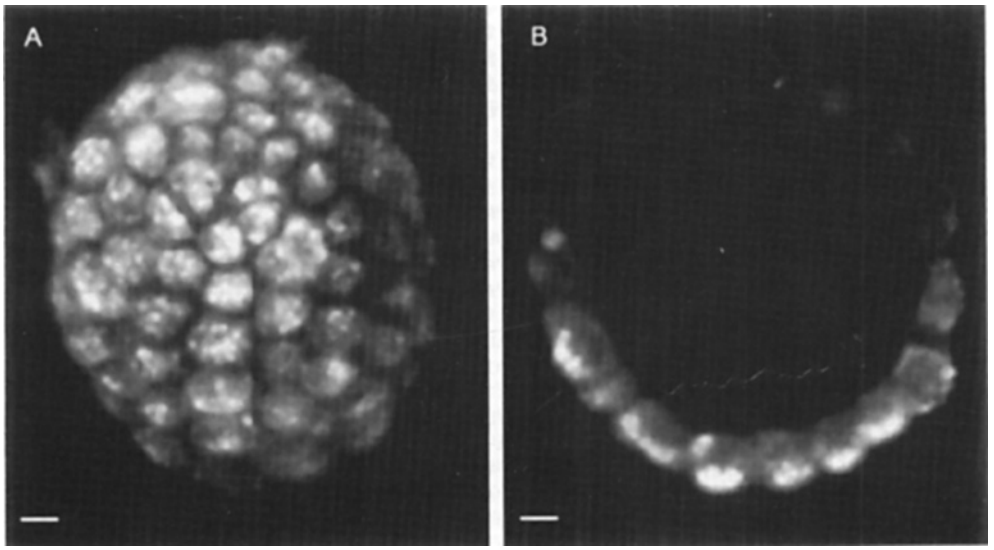

Fig. 1. Confocal laser scanning micrographs of a mouse blastocyst, labeled with a rhodamin marker which detects mouse monoclonal anti-blastocyst antibodies. A. The corresponding antigen epitope is located at the blastocyst surface as demonstrated by a projection of a series of optical sections. B. An optical section at the level of the inner cell mass shows that the antigen is present also intracellularily in the abembryonal pole of the blastocyst. The inserted line represents 5 μ.

by cultured mouse blastocysts into the medium were detected by some anti-mouse blastocyst antibodies (Table 1: N63, 3:1). We are now checking whether these mAbs are also positive for samples of medium from cultures of human blastocysts. If the cross-reacting mAbs detect epitopes with common function in the two species, then this opens for the possibility to use mouse blastocysts as a preparatory material for analyses of, for instance, adhesion molecules of the implanting human blastocyst.

In conclusion, our results imply that the described techniques are useful for obtaining monoclonal antibodies against human blastocysts and that now different types of antibodies can be made available for investigations of the human blastocyst.

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REFERENCES

1. Bronson, R.A., Cooper, G.W., Margalioth, E.J., Naz, R.K. & Hamilton, M.S.: The detection in human sera of antisperm antibodies reactive with FA-1, an evolutionarily conserved antigen, and with murine spermatozoa. Fertil Steril 52:457-462, 1989.
2. Hjortberg, M., Phil, M., Grönvik, K.-O. & Nilsson, B.O.: Production and determination of specificity of monoclonal IgG anti-mouse-blastocyst antibodies. J Reprod Immunol 23:83-92, 1993.
3. Jin, M., Johansson, L., Sundström, P. & Nilsson, B.O.: Characterization of mouse-hamster-human cross-reacting antioocyte monoclonal antibodies produced by intrasplenic immunization of mice with 12 zona-free mouse oocytes. Mol Reprod Develop 37:446-451, 1994.
4. Kättström, P.-O., Bjerneroth, G., Nilsson, B.O., Holmdahl, R. & Larsson, E.: A retroviral gp70-related protein is expressed at specific stages during mouse oocyte maturation and in preimplantation embryos. Cell Diff Dev 28:47-54, 1989.
5. Nilsson, B.O., Svalander, P.C., Andersson, J., Grönvik K.-O. & Larsson, A.: Methods for production and determination of monoclonal antibodies against surface components of adhesiv implanting mouse blastocysts. Upsala J Med Sci 91:317-322, 1986.
6. Olovsson, M. & Nilsson, B.O.: Methods to produce stage-specific monoclonal antibodies against mouse trophoblast cells by intrasplenic and in vitro immunizations. Hybridoma 12:305-315, 1993.
7. Svalander, P.C., Andersson, J. & Nilsson, B.O.: Intrasplenic immunization for production of monoclonal antibodies against mouse blastocysts. J Immunol Methods 105:221-227, 1987.
8. Svalander, P.C., Hjortberg, M., Grönvik, K.-O. & Nilsson, B.O.: Characterization of a trophectoderm surface epitope recognized by an anti-blastocyst monoclonal antibody. Cell Diff Dev 26:191-200, 1989.
9. Wiley, L.M. & Calarco, P.G.: The effects of anti-embryo sera and their localization on the cell surface during mouse preimplantation development. Dev Biol 47:407-415, 1975.