Effects of blocking Le^Y oligosaccharide on cell surface to MMPs secreted by blastocysts and epithelial cells in mouse *in vitro*

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Abstract In order to understand the role of Le^Y oligosaccharide antigen (Le^Y) during implantation, the relationship of Le^Y on the cell surface with matrix metalloproteinase (MMPs) secreted by blastocysts and monolayer epithelial cells during implantation in the mouse *in vitro* was studied by monoclonal antibody (mAb) AH_6, directed to Le^Y[Fuc α1-2 Gal β1-4 (Fuc α1-3) GlcNAc-], and gelatin zymography. The results showed that MMPs secretion was reduced after Le^Y on the cell surface of either epithelial cells or trophoblasts was blocked. It indicated that MMPs expression which played an important function during the process of implantation were regulated by Le^Y. Therefore, it was considered that Le^Y could regulate embryos invasion by some mechanism.

Keywords: mouse, Le^Y oligosaccharide antigen, matrix metalloproteinases, implantation, monoclonal antibody.

THE organs of the adult reproductive system can undergo a series of tissue and function remodeling during menstruation, ovulation, implantation, and uterine, breast involution. Much of this remodeling is attributed to the action of MMPs.

Investigators have detected that mother and fetus can produce MMPs and their tissue inhibitors (TIMPs) in certain numbers and sorts. The expression of MMPs and TIMPs is in a coordinated and balanced manner. It ensures that the trophoblast invasion is precisely regulated to be confined spatially to the uterus and temporally to early pregnancy. It is known that[1] uterine tissue can secret MMP-1, -2, -3, -7, -9 and -11, and embryos produce MMP-1, -2, -3, -9, and -11 during implantation *in vitro*.

We know that many glycoconjugates participate in implantation, and our previous studies[2—4] have revealed that Le^Y on the cell surface played a key role during recognition and attachment between embryos and uterine epithelial cells. Recently, we observed that the expression of epidermal growth factor receptor (EGF-R) changed after Le^Y on the blastocyst was blocked by mAb AH_6 (Wang *et al.*, unpublished). EGF shows a maximal concentration before normal embryos implantation and regulated MMPs expression[5]. Based on the above results, we were engaged in a primary exploration on the relationship between strong expression of MMPs and Le^Y on the cell surface during implantation.

Uterine epithelial cells and blastocysts in the period of implantation were used as sample in this study, and gelatin zymography was employed to define MMPs expression. Meanwhile, the samples were pretreated with mAb AH_6 and the relationship of Le^Y with MMPs secretion was examined.

1 Materials and methods

(ⅰ) Animals. Matured female mice of Kunming white strain were purchased from the Experimental Animal Center of the Institute of Animal Science. Females were induced to superovulation by regular method and mated with the same strain male. The presence of copulatory plug the next day morning was considered as the 1st day of pregnancy. Females were killed on the 4th day of pregnancy and blastocysts and uterine epithelial cells were cultured *in vitro*.

(ⅱ) Reagents. Monoclonal antibodies AH_6 and αLe^b were kindly supplied by Dr. Hakomori in the Biomembrane Institute (Seattle, USA) and Dr. Guo NH in NIH of USA, respectively. The carbohydrate structures recognized by these mAbs are listed in table 1. Otherwise specially noted, all other reagents were purchased from Sigma (St. Louis, USA).

Table 1 Carbohydrate structure recognized by monoclonal antibody used in this study

Antibody	Antigen	Carbohydrate structure
AH_6	Le^Y	$\begin{matrix} & & & & & & & & & & \text{Fuc}\alpha1 \\ & & & & & & & & & & \uparrow \\ \text{Fuc}\alpha1\rightarrow & 2\text{Gal}\beta1\rightarrow & 4\text{GlcNAc}\beta1\rightarrow & 3\text{Gal}\beta1\rightarrow & \text{R} \\ & 3 & & & \end{matrix}$
αLe^b	Le^b	$\begin{matrix} & & & & & & & & & & \text{Fuc}\alpha1 \\ & & & & & & & & & & \uparrow \\ \text{Fuc}\alpha1\rightarrow & 2\text{Gal}\beta1\rightarrow & 3\text{GlcNAc}\beta1\rightarrow & 4\text{Gal}\beta1\rightarrow & \text{R} \\ & 4 & & & \end{matrix}$

(ⅲ) Blastocysts collection and monolayer epithelial cells preparation.

(1) Blastocysts collection and culture *in vitro*. As described by Zeng *et al*.[6], medium with or without mAb $AH_6/\alpha Le^b$ was used to culture blastocysts for 3 h *in vitro*. Then blastocysts were washed with prewarmed medium (1 mL/time) without mAbs for 3 times to remove excess mAb. After that, blastocysts were continually cultured in the medium without mAb (1 blastocyst /μL). Cultured medium was collected at different time (6, 12 and 24 h) and stored at −20℃.

(2) Uterine epithelial cells preparation and culture *in vitro*. Epithelial cells harvested as described by Zeng *et al*.[6] and Wang *et al*.[7] were placed in a 24-well sterile plastic plate and incubated at 37℃ under 5% CO_2 in air in a humidified chamber. After monolayer of epithelial cells (cultured about 18 h later) was formed, the culture medium was changed to simple medium, without fetal calf serum (FCS, Tianjin) and with or without $AH_6/\alpha Le^b$, for another 6 h culture. Then the cells were washed like blastocysts. After that, cells were cultured in medium without FCS and mAb. The aliquots of Culture medium were collected at different time (3, 6, 12, 24 and 48 h) and stored at −20℃.

(ⅳ) Determination of MMPs secretion by gelatin zymograph. This step was carried out as described by Liu *et al*., and the results are shown in photographs.

2 Results

2.1 MMPs secreted by blastocysts and the effect of mAb AH_6 on its secretion

The results showed that there are two MMPs secreted by blastocysts; that is MMP-2 (72-ku gelatinase A and its activity form 67-ku) and MMP-9 (92-ku gelatinase B). It caused MMP-9

production to decrease dramatically from the 6th hour to the 24th hour when blastocysts were pretreated with mAb AH_6 (fig. 1), but mAb αLe^b had no effect on MMPs secretion (data not shown).

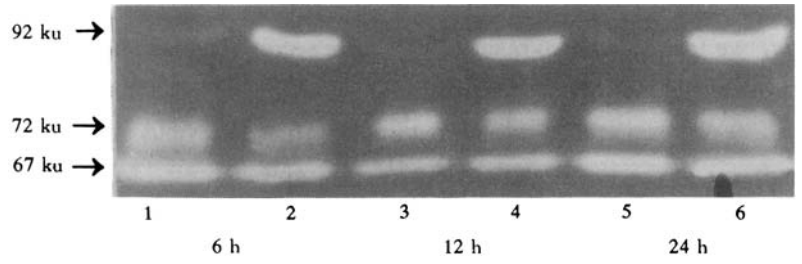

Fig. 1. Effect of monoclonal antibody AH_6 on MMPs secreted by blastocysts. MMPs secretion was determined by gelatin zymograph. 1, 3, 5, mAb AH_6 pretreated groups; 2, 4, 6, control groups.

2.2 MMPs secreted by monolayer epithelial cells and the effect of mAb AH_6 on its secretion

Gelatin zymograph showed that there are two MMPs produced by monolayer epithelial cells; that is MMP-2 (72-ku gelatinase A) and MMP-9 (92-ku gelatinase B) (fig. 2). It caused MMPs secretion to change when Le^Y on the cell surface was blocked by mAb AH_6. The most obvious effect was on MMP-2 secretion. The activity of MMP-2 was decreased after 3 h, especially during the 12th hour to the 24th hour its secretion was reduced significantly and MMP-9 secretion was also inhibited, but mAb αLe^b had no effect on MMPs secretion (data not shown).

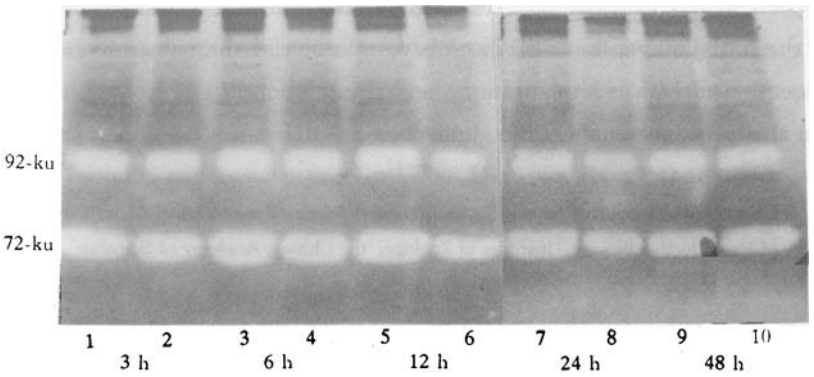

Fig. 2. Effect of monoclonal antibody AH_6 on MMPs produced by monolayer epithelial cells. MMPs secretion was determined by gelatin zymograph. 1, 3, 5, 7, 9, Control groups; 2, 4, 6, 8, 10 mAb AH_6 pretreated groups.

3 Discussion

Implantation is a process initiated by fertilization of ovum which ultimately leads to the embedding of blastocyst in the underlying stroma and placenta formation[8]. Successful implantation of the blastocyst is dependent on the synchrony between the developmental program of the embryo itself and the complex series of molecular and cellular events that are induced in the pregnant uterus by estrogen and progesterone. During this period of time, MMPs expression increased gradually and approached to a maximal value when developing embryos gained the same invasion behavior as

tumor cells. However, unlike tumor cell invasion, embryo invasion is precisely regulated to be confined spatially to the uterus and temporally to early pregnancy. After implantation, MMPs concentrations decreased because of embryo's losing its invasion ability. All these results suggested that implantation depended on the MMPs existing to a certain extent[1].

In our study, we found that two main MMPs were secreted by blastocysts and monolayer epithelial cells *in vitro*; that is MMP-2 (gelatinase A) and MMP-9 (gelatinase B). It indicated that MMP-2 and MMP-9 played a crucial role during implantation. Previous studies have revealed that MMP-9 is a key factor in successful implantation[1]. In pre-eclampsia, MMP-9 expression is dramatically reduced in cytotrophoblasts isolated from pre-eclamptic placentas. During implantation, MMP-2 is one of some important factors[1]. In addition, MMPs participate in the process of dramatic changes in tissue morphology and extensive matrix remodeling during embryo development[1,9].

Our previous studies have revealed[2—4] that it induced implantation failure after the recognition and attachment between mother and fetus were blocked by mAb AH_6. Our present data indicated that it took an effect not only on the recognition and attachment between mother and fetus but also on the embryo invasion process by some signal transductive pathway after Le^Y on the cell surface was blocked by mAb AH_6. At the same time, we also selected another mAb αLe^b(directed to Le^b oligosaccharide antigen, whose oligosaccharide structure is similar to Le^Y and also contains two fucose residues) to pretreat blastocysts or monolayer epithelial cells. The results showed that mAb αLe^b had no effect on MMPs secretion. It indicated that the role of Le^Y in implantation has carbohydrate specificity. In conclusion, Le^Y plays a role not only in the period of recognition and attachment at the initial stage, but also in embryo invasion at the subsequent stage. The mechanism and the location of Le^Y in implantation, and the relationship of Le^Y with some other factors participating in this process remain to be studied.

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