발생과 생식 제 3 권 제 1 호, 59~67 (1999)
Dev. Reprod. Vol. 3, No. 1, 59~67 (1999)

The Effects of Oviduct and Uterine Epithelial Cells on the Expression of Interleukin-1β Gene in Preimplantation Mouse Embryos

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생쥐 초기배아에서 Interleukin-1β 유전자의 발현에 미치는 수란관과 자궁내막세포의 영향

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ABSTRACT : To investigate the role of interleukin-1β (IL-1β) in the embryonic development, *in vivo* and *in vitro* expression patterns of IL-1β gene in the preimplantation mouse embryos were examined by RT-PCR, and the effects of explanted mouse oviduct and uterine epithelial cells on the expression of IL-1β gene in the preimplantation mouse embryos were examined by co-culture. IL-1β mRNA was detected in the embryos from 4-cell stage to blastocyst stage *in vivo* and from morula stage to hatching blastocyst stage *in vitro*. This transcript was not detected from the GV stage to late 2-cell stage *in vivo*, and not at the 4-cell and 8-cell stages *in vitro*. For the co-culture of late 2-cell embryos with the explanted mouse oviduct and uterine epithelial cells, oviducts and uterine epithelial cells were isolated at 48 hour after the hCG injection. The explanted oviduct and uterine epithelial cells in co-culture groups facilitated the IL-1β gene expression of the mouse embryos in comparison with the control. Taken together these results suggest that the presence of IL-1β plays an important role in preimplantation embryonic development. In addition, the up-regulation of IL-1β gene expression by the explanted oviduct and uterine epithelial cells demonstrates that embryonic expression of IL-1β gene may be regulated by the interaction with oviductal and uterine factor (s).

Key words : IL-1β , RT-PCR, Co-culture, Preimplantation mouse embryos.

요 약 : 초기배아의 발생과정동안 배아와 모체에서 발현되는 여러 cytokine은 착상을 위한 신호물질로 중요한 역할을 한다. 그 중 interleukin-1β (IL-1β)는 배아와 모체간의 상호 신호전달체로서 성공적인 착상을 위한 필수적인 요소로 작용한다고 알려져 있다. 따라서 본 연구에서는 초기배아의 발생과정에 있어서 IL-1β 유전자의 역할을 규명하기 위해 생쥐 초기배아에서의 IL-1β 유전자의 발현양상을 역전사중합효소연쇄반응을 통해 조사하였고, IL-1β 유전자의 발현에 미치는 수란관과 자궁내막세포의 영향을 밝혀보기 위해 공배양방법을 이용하였다. 그 결과 IL-1β *in vivo*에서는 4-세포기부터 포배기까지, *in vitro*에서는 상실배부터 부화중 포배기까지 발현하는 양상을 보였다. 또한 수란관과 자궁내막세포와의 공배양시 대조군과 비교하였을 때 실험군에서 IL-1β 유전자의 발현이 촉진되었다. 이러한 결과는 IL-1β 의 존재가 착상전 초기배아의 발생에 중요한 역할을 한다는 것을 의미한다. 또한 수란관과 자궁내막세포와의 공배양을 통해 IL-1β 유전자의 발현이 수란관과 자궁요소에 의해 조절됨을 확인하였다.

INTRODUCTION

The preimplantation mouse embryo produces many growth factors and cytokines during embryonic development to signal its presence to the maternal organism (Tazuke & Giudice, 1996). Appropriate signalling mechanisms between the preimplantation embryo and maternal endometrium are in part regulated by autocrine or paracrine cytokines

The present study was supported by a grant (HMP-98-M-1-0006) from the Good Health R & D project, Ministry of Health and Welfare of Korea, and in part by a grant (BSRI-97-4437) from the Basic Science Research Institute Program, Ministry of Education of Korea.

(Wegmann, 1991; Tabibzadeh et al., 1992). Although multiple studies have examined the existence and influence of various cytokines in embryos and endometrium (Chard, 1995; Simon et al., 1995), as yet, little is known about cytokine-production by preimplantation embryos.

The interleukin-1 (IL-1β) system is composed of two agonists, interleukin-1 (IL-1β) and interleukin-1 (IL-1β), one antagonist, the interleukin-1 receptor antagonist (IL-1β ra) and two membrane-bound receptors, interleukin-1 receptor type I (IL-1βR tI) and II (IL-1βR tII) (Dinarello, 1994). Both agonists are initially synthesized as precursor proteins of 31 kDa and the mature proteins have a molecular weight of 17 kDa. Although the amino acid sequences have a similarity of only 22%, they induce the same biological responses (Dower et al., 1986). The IL-1βR tI is found in low numbers on almost all cell surfaces, whereas IL-1βR tII is found primarily on white blood cells. Only the binding of either IL-1β or - to the IL-1β receptor type I results in signal transduction, with receptor type II and the soluble IL-1β receptor acting as competitors of the receptor type I (Clotta et al., 1993).

The IL-1β system comes into close relation with embryonic implantation. In humans, the IL-1βR tI has been detected in endometrium (Simon et al., 1993) and, more specifically, in endometrial epithelial cells with a maximal protein- and mRNA-expression during the luteal phase. All major components of the IL-1β system, namely IL-1β , IL-1βra and IL-1βR tI were detected immunohistochemically in single preimplantation embryo (De los Santos et al., 1996). Recently, IL-1β system mRNA was detected in single blastomeres of preimplantation embryos (Takacs & Kauma, 1996; Krüssel et al., 1998).

In mice, mRNA and protein of IL-1β and IL-1β have been detected and localized in endometrial epithelial cells (Takacs et al., 1988). The levels were increased from day 3 of pregnancy to make a peak at the time of implantation on day 4 (De et al., 1993). However, the temporal pattern of IL-1β gene expression and the regulation of its expression by female genital tract in embryos are not well documented.

The aim of this study was to investigate the role of IL-1β gene in the embryonic development. *In vivo* and *in vitro* expression patterns of IL-1β gene in preimplantation mouse embryos were examined by RT-PCR, and the effects of explanted mouse oviduct and uterine epithelial cells on the expression of IL-1β gene in preimplantation mouse embryos were examined by co-culture.

MATERIALS AND METHODS

1. Collection of the oocytes and the preimplantation embryos

ICR mice (Department of Biology, Hanyang University) were bred under the illumination condition of 14 hours light and 10 hours dark cycles. The female mice (10 to 12 week old) were superovulated by intraperitoneal injection of 5 IU of pregnant mare's serum gonadotrophin (PMSG, Sigma) followed by human chorionic gonadotrophin (hCG, Sigma) 48 hours later. Females were paired overnight with ICR males (10 to 12 weeks old). Mating was confirmed with the presence of a copulation plug and fertilization is considered to occur 12 hours after the hCG injection.

Oocytes at metaphase I (GV oocytes) were collected from unstimulated female mice by puncturing the ovary with a 26-gauge needle in HEPES-buffered medium 2 supplemented with 4 mg/ml bovine serum albumin (M2+BSA). Oocytes at metaphase II (ovulated oocytes) were collected from the ampullae of the oviducts by tearing with a fine forceps 18 hours after the hCG injection. Cumulus cells were removed by treatment with 0.1% hyaluronidase (Sigma) in M2 medium and washed several times.

Embryos of 1-cell, 2-cell, 4-cell, 8-cell, morula, and blastocyst stage were collected from either the oviduct or uterus by flushing with a fine Pasteur pipette 20, 48, 54, 65, 72, and 96 hours after the hCG injection, respectively.

2. Culture of the preimplantation embryos *in vitro*

Late 2-cell embryos were recovered from the oviducts by flushing with a fine Pasteur pipette 48 hour, after the hCG injection and the embryos were allowed to be dispersed into KSOM medium, which was a modified version of medium SOM (simplex optimized medium, Lawitts and Biggers, 1991) with higher K^+ concentration supplemented with 3 mg/ml BSA (Lawitts & Biggers, 1993).

The *in vitro* culture of late 2-cell embryos was performed in the medium under mineral oil (Sigma) at 37℃ in a humidified atmosphere of 5% CO_2 in air in plastic culture dishes (60×15 mm, Corning). Embryos of 4-cell, 8-cell, morula, early blastocyst, and hatching blastocyst stage were collected after 7, 21, 40, 50, and 72 hours culture, respectively.

3. Co-culture of late 2-cell embryos with explanted oviduct

In co-culture of late 2-cell embryos with the explanted mouse oviducts, the modified Whitten's medium supplemented with 3 mg/ml BSA was used (Hoppe et al., 1985). Late 2-cell embryos were collected from the oviduct by flushing 48 hours after the hCG injection.

Oviducts for co-culture were isolated 48 hours after the hCG injection and flushed with the medium. The isthmic and fimbrial regions of the oviducts were removed, and ampullae opened longitudinally with a fine forceps were washed several times with the medium. They were then carefully cleaned with sterilized filter paper to remove blood and other tissues, and placed on the bottom of plastic culture dishes. Each oviduct was added to a small co-culture drop (100 μl) of the medium, which was covered with mineral oil and equilibrated for at least 1 hour at 37℃ in a humidified atmosphere of 5% CO_2 in air. Late 2-cell embryos were added to each drop, and embryos of 4- and 8-cell stage for RT-PCR were collected at 7 and 21 hours culture, respectively.

4. Co-culture of late 2-cell embryos with uterine epithelial cells, and treatment of progesterone and/ or 17 β -estradiol

Uteri were removed from mature (10 to 12 weeks old) mouse and stripped of mesentery. Under a dissecting microscope (M3, Wild), each uterine horn was cut transversely into two equal segments, which were slit longitudinally to expose the endometrial surface. Epithelial cells were collected from the uterine lumen by scraping with a knife in modified Whitten's medium plus 10% heat-inactivated fetal bovine serum. The medium containing dissociated epithelial cells was transferred to a disposable sterile 15 ml cornical tube (Falcon). Five ml of medium was added to the tube, and the epithelial fragments in the supernatant were collected with a pipette. This procedure was repeated two or three times. The epithelial fragments were collected by centrifugation at 100× g for 5 min. The pellet was resuspended in medium. Resuspended epithelial cells (3.5×10^5/ml) were seeded on organ culture dish (60×15 mm, Falcon). The medium was quickly added to the dish, and cells were incubated at 37℃ in a humidified atmosphere of 5% CO_2 in air. The meidum was changed again on 24 hour (Tomooka et al., 1986).

Groups for co-culture of uterine epithelial cells were classified into five, a medium alone group, uterine epithelial cell co-culture group with or without progesterone (3.2×10^{-6}M) and 17 β-estradiol (3.7×10^{-5}M), and progesterone plus 17 β-estradiol (3.2×10^{-7}M and 3.7×10^{-5}M) treatment group (Lavranos & Seamark, 1989). Late 2-cell embryos were added to each co-culture group, and embryos of 4- and 8-cell stage for RT-PCR were collected after 8 and 22 hours culture, respectively.

5. Reverse transcription - polymerase chain reaction (RT-PCR)

All the solutions were prepared with distilled water treated with 0.1% diethylpyrocarbonate (DEPC, Sigma). After collection, embryos were washed three times with Ca^{2+} and Mg^{2+}-free PBS, counted, and transferred in a minimal volume to a chilled 1.5 ml eppendorf tube on ice. Trizol (Gibco) was immediately added to the tube. The sample was vortexed vigorously, and stored at −70℃.

Reverse transcription (RT) was conducted on 30 embryos equivalents. The reaction was carried out in 40 μl of 8 μl 25 mM $MgCl_2$, 4 μl 10× PCR buffer, 4 μl 10 mM dNTP mixtures, 2.5 pM oligo (dT) adaptor primer, and 20 units RNase-inhibitor (Takara). After the tubes (Gene Amp thin-walled tube) were incubated at 37℃, 5 units of AMV (avian myeloblastosis virus) reverse transcriptase XL (Takara) was added. The RT reaction was carried out in the 4800 PCR thermal cycler (Takara) by using a program with the following parameters: 42℃, 60 min; 99℃, 5 min; 4℃, ∞. After the reaction was complete, samples were either used directly for PCR or stored at −20℃.

First round of polymerase chain reaction (PCR) was conducted on 3 embryos equivalents. The reactions were carried out in 40 μl of 3.2 μl 25 mM $MgCl_2$, 4.0 μl 10× PCR buffer, 4.0 μl 2.5 mM dNTP mixtures, 2.5 units Taq polymerase (Takara), 12 pmol each of the appropriate outer 3' and 5' primers (Bioneer), 21.9 μl DEPC-treated distilled water, and 4 μl of the RT-product. After mixing all components in a 0.5 ml thin-walled tube, the reaction mixture was covered with 50 μl mineral oil. The basic PCR program used was incubation at 94℃ for 3 min, followed by a cycle program of 94℃ for 45sec, 54℃ for 45sec, 72℃ for 1 min. After completion of 35 cycles, the reaction was terminated at 72℃ for 10 min and cooled down to 4℃. First round PCR products were stored at −20℃ until the second round PCR (Krüssel et al., 1997).

For the second round PCR, 4 μl of first PCR-product were added to PCR mixture in thin walled tube and covered with 50 μl mineral oil. The reaction components and parameter of the second round PCR were identical with the first round PCR except for the number of cycles. The number of second round PCR was 18, and after completing the second round of PCR, the PCR products were run on 2.5% agarose gels (Takara) containing 0.5 μg/ml ethidium bromide and photographed under UV light.

The outer 5' and 3' primers for IL-1β were 5'-GGATGAGGACATGAGCACCTTC-3' and 3'-GGAAGACAGGCTTGTGCTCTGC-5'. The inner 5' and 3' primers were 5'-AGCCCATCCTCTGTGACTCATG-3' and 3'-GCTGATGTACCAGTTGGGGAAC-5', respectively (Huang et al., 1997). The IL-1β outer and inner primers gave rise to diagnostic fragments of 497 bp and 422 bp, respectively. The β-actin gene transcript was used as an internal control to confirm the efficiency of RT. The primer for β-actin was 5'-GTGGGCCGCTCTAGGCACCAA-3' and 3'-CTCTTTGATGTCACGCACGATTTC-5' (Rappolee et al., 1992).

6. Restriction enzyme digestion of IL-1β PCR product with BstxI

RNA corresponding to 30 embryos was subjected to reverse transcription. Three embryos equivalent were then subjected to 35 cycles of first round PCR using the outer primers for IL-1β and 0.3 embryo equivalent of RNA to 18 cycles of second round PCR using the inner primers. Thereafter, these products were treated with or without 5 units of BstxI (Taksra) at 37℃ overnight and then electrophoresed on 2.5% agarose gels. The agarose gel was photographed under UV light.

RESULTS

1. Identification of the PCR product by restriction enzyme digestion

The identity of the diagnostic fragment was confirmed by restriction enzyme analysis. From the cDNA sequence (Gray et al., 1986). the diagnostic fragment should carry an BstxI site, such that restriction enzyme digestion should give rise to two fragments, one 282 bp and the other 140 bp in length (Fig. 1).

2. Optimization of RT-PCR procedure with regard to number of cycles in the second round PCR

First round PCR products were amplified to optimize the number of cycles in the second round PCR. The cycle number of first round PCR was performed to 35. After completing the first round PCR, second round PCR was performed to 5, 10, 13, 16, 19, 22, 25, 30, and 35 cycles, respectively. The PCR products were in the log phase increment, and so the optimizing cycle of second round PCR was performed to 18 cycles (Fig. 2).

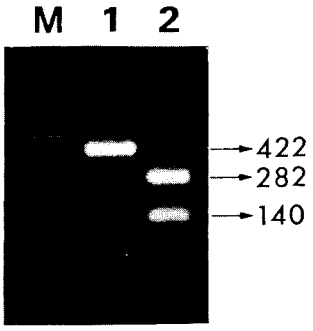

Fig. 1. Agarose gel electrophoresis of amplified IL-1β transcript subjected to BsxtI digestion. M, 100 bp ladder; Lane 1, amplified and undigested sample; the major band is 422 bp in length. Lane 2, amplified and digested sample; there are two bands, 282 and 140 bp.

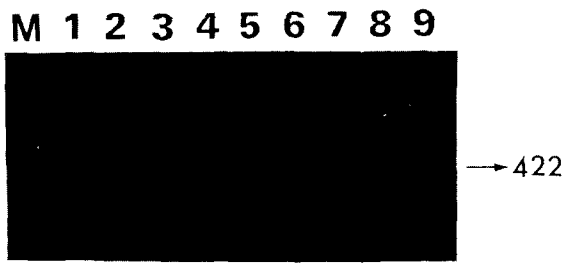

Fig. 2. Optimization of RT-PCR procedure with regard to number of cycles in the second round PCR. M, 100bp ladder; Lane 1, 5; Lane 2, 10; Lane 3, 13; Lane 4, 16; Lane 5, 19; Lane 6, 22; Lane 7, 25; Lane 8, 30; Lane 9, 35 cycles.

3. Expression patterns of IL-1β gene in the preimplantation mouse embryos *in vivo* and *in vitro*

Using the RT-PCR, IL-1β mRNA was detected in the embryos from the 4-cell stage to blastocyst stage *in vivo* (Fig. 3A) and from the morula stage to hatching blastocyst stage *in vitro* (Fig. 4A). This transcript was not detected from the GV oocyte stage to late 2-cell *in vivo*, and not at 4- and 8-cell stage *in vitro*. The amount of IL-1β mRNA *in vivo* was slightly increased in the morula and blastocyst stage in comparison with the 4- and 8-cell stage embryo, and so did *in vitro*. RT-PCR was also performed for β-actin gene as an internal control for this procedure (Fig. 3C, 4B), and performed for RNA from embryos that had not been reversely transcribed as a negative control for a possible genomic DNA contamination (Fig. 3B).

4. Effects of the explanted mouse oviduct on the development of late 2-cell embryos

Of 94 late 2-cell embryos co-cultured with the explanted oviduct, 87 (92.6%) developed normally to the blastocyst stage, with 33 (35.1%) hatching blastocysts and 19 (20.3%)

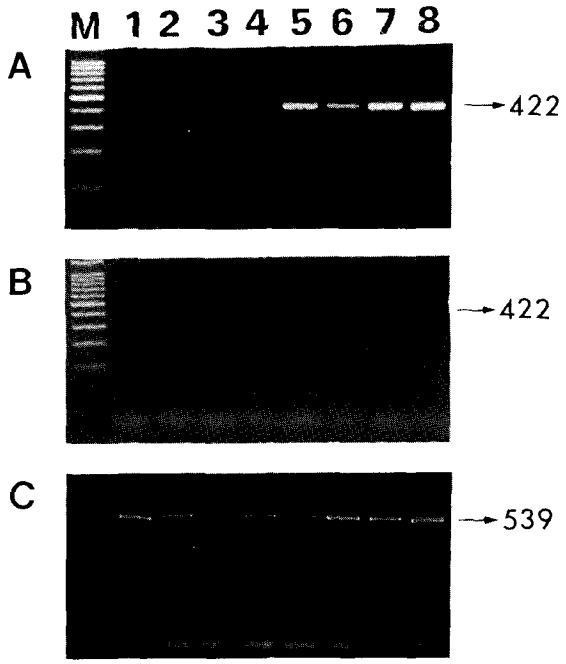

Fig. 3. Expression pattern of IL-1β mRNA in the oocytes and preimplantation mouse embryos *in vivo*. A; IL-1ββ. B; negative control. C; β-actin, positive control. M, 100bp ladder; Lane 1, GV oocyte; Lane 2, ovulated oocyte; Lane 3, 1-cell; Lane 4, 2-cell; Lane 5, 4-cell; Lane 6, 8-cell; Lane 7, morula; Lane 8, blastocyst.

hatched blastocysts (Table 1). After 72 hours' culture, the rate of hatching was significantly higher ($p<0.05$) in the medium alone (control) than in the co-culture group, and after 96 hours, the rate of hatched was significantly lower ($p<0.05$) for the co-culture group compared to the medium alone.

5. Effects of the explanted mouse oviduct on IL-1β gene expression in embryos

IL-1β mRNA was not detected in the embryos of me-

Table 1. Development of late 2-cell embryos co-cultured with explanted oviduct for 72 and 96 hours in mouse

Treatment	No. of embryos	% of developed embryos					
		72 hous			96 hours		
		M	ErB /ExB	HgB	ErB /ExB	HgB	HdB
Medium alone	100	0.0	50.0±0.1	33.0±0.1	33.0±0.1	32.0±0.0	28.0±0.1
Co-cultured with oviduct	94	0.0	76.6±0.0*	19.1±0.0*	37.2±0.1	35.1±0.1	20.3±0.1*

Degenerated embryos are not shown in this table.
Abbreviation : M, Morula ; ErB, Early blastocyst ; ExB, Expanded blastocyst; HgB, Hatching blastocyst ; HdB, Hatched blastocyst
Values are represented by mean±SD and obtained from three different experiments.
* : Significantly different from the control ($p<0.05$).

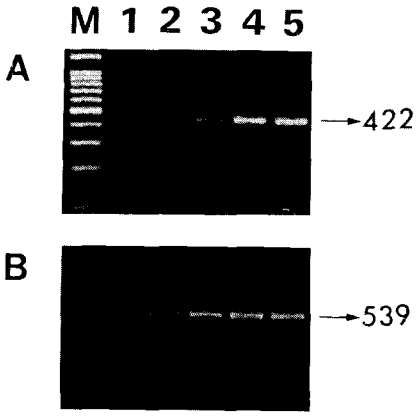

Fig. 4. Expression pattern of IL-1β mRNA in the preimplantation mouse embryos cultured *in vitro*. A; IL-1β. B; β-actin, positive control. M, 100 bp ladder; Lane 1, 4-cell; Lane 2, 8-cell; Lane 3, morula; Lane 4, early blastocyst; Lane 5, late blastocyst.

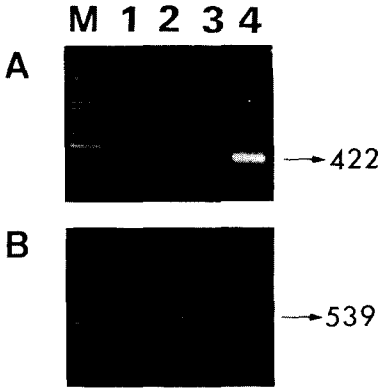

Fig. 5. Expression pattern of IL-1β mRNA in the preimplantation mouse embryos co-cultured with the explanted oviduct. A; IL-1β. B; β-actin, positive control. M, 100bp ladder; Lanes 1 and 3, 4-cell; Lanes 2 and 4, 8-cell; Lanes 1 and 2, embryo cultured with medium alone; Lanes 3 and 4, embryo co-cultured with the explanted oviduct.

dium alone. However, it was only detected at the 8-cell stage embryo, not 4-cell stage in oviduct co-culture group (Fig. 5A). Embryos at each stage expressed β-actin gene, which confirmed the integrity of the RNA and the RT-PCR process (Fig. 5B).

6. Effects of progesterone and/ or 17 β-estradiol on the development of late 2-cell embroys co-cultured with uterine epithelial cells

After 72 hours culture, the rate of blastocyst formation was higher in the co-culture group than in the medium alone except for the co-culture group without steroid hormone treatment. But, there was no significancy in the rate of blastocyst formation.

The rate of hatching was higher in all the co-culture group than in the medium alone. However, it was significantly higher ($p<0.05$) in the co-culture group treated with progesterone plus 17β-estradiol (40.3%) than in the medium alone (18.5%), and there was not significant difference between the other co-culture groups (Table 2).

7. Effects of the uterine epithelial cells on IL-1β gene expression in embryos

IL-1β mRNA was not detected in the embryos of medium alone. However, it was detected at the 4-cell and 8-cell stage embryos of all co-culture group irrespective of

Table 2. Effects of progesterone and/or 17β-estradiol on the development of late 2-cell mouse embryos co-cultured with uterine epithelial cells for 72 hour

Treatment	No. of embryos	% of developed embryos		
		M	ErB /ExB	HgB
Medium alone	65	10.8±0.1	61.5±0.0	18.5±0.0
NH	62	11.3±0.1	51.6±0.1	27.4±0.1
P_4	62	8.1±0.0	54.8±0.0	30.6±0.0
E_2	61	8.2±0.1	50.8±0.1	34.4±0.1
P_4+E_2	62	4.8±0.2	50.0±0.1	40.3±0.1*

Degenerated embryos are not shown in this table.
NH, P_4, E_2, and P_4+E_2 are co-cultured group with uterine epithelial cells.
Abbreviation: NH, Not treated hormone; P_4, Progesterone treatment; E_2, 17β-estradiol treatment; P_4+E_2, Progesterone plus 17β-estradiol treatment; M, Morula ; ErB, Early blastocyst ; ExB, Expanded blastocyst; HgB, Hatching blastocyst
Values are represented by mean±SD and obtained from three different experiments.
* : Significantly different from the control ($p<0.05$).

progesterone and/or 17 β-estradiol treatment (Fig. 6A).

DISCUSSION

In this study, to investigate the role of IL-1β in embryonic development, *in vivo* and *in vitro* expression pattern of IL-1β mRNA was examined by RT-PCR, and the effects of explanted mouse oviduct and uterine epithelial cells on the expression of IL-1β gene were examined by co-culture.

IL-1β has been detected at the protein level in the human preimplantation embryos by immunohistochemistry (De los Santos et al., 1996). Recently, the presence of IL-1β mRNA has been detected in a pools of and single preimplantation mouse embryo by RT-PCR (Takacs & Kauma, 1996; Kr ssel et al., 1997).

In the present results, IL-1β mRNA was detected from the 4-cell stage to blastocyst stage *in vivo* (Fig. 3A) and from the morula stage to hatching blastocyst stage *in vitro* (Fig. 4A). Embryonic development prior to the middle of the 2-cell stage depends mainly on protein and RNA synthesized during oogenesis (Hou & Gorski, 1993). Thus, the embryonic genome in mouse is inactive until after the first cleavage. These results demonstrate that IL-1β mRNA is transcribed after activation of embryonic genome because the IL-1β is detected from the 4-cell stage onwards *in vivo*. Furthermore, the difference of expression pattern *in vivo* and *in vitro* suggests that the embryonic expression of IL-1β gene is regulated by oviductal factor(s) because the 4- and 8-cell embryos develop in the oviduct.

This possibility was ascertained by co-culture with the explanted oviduct. In the experiment on the late 2-cell embryos co-cultured with the explanted oviducts, the expression of IL-1β gene was facilitated in the co-culture group. It was only detected at the 8-cell stage embryo, not 4-cell stage (Fig. 5A). This result shows tha the IL-1β may be regulated by the interaction with oviductal factor(s). Many studies have been reported that the embryonic development was significantly enhanced by co-culture with oviductal epithelial cells (Ouhibi & Hamidi, 1990) or explanted oviduct (Minami & Bavister, 1988; Menezo, 1989). However, there is no reports about the IL-1β gene expression pattern in the preimplantation mouse embryos co-cultured with oviduct.

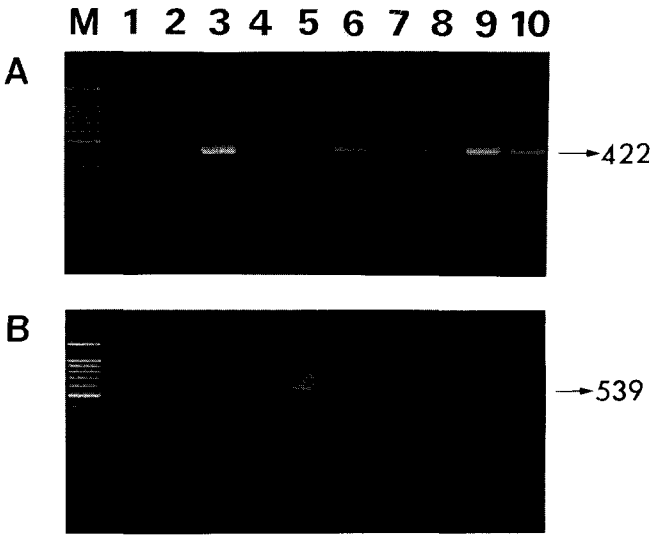

Fig. 6. Expression pattern of IL-1β mRNA in the preimplantation mouse embryos co-cultured with uterine epithelial cells. A; IL-1β. B; β-actin, positive control. M, 100bp ladder; Lanes 1, 3, 5, 7 and 9, 4-cell; Lanes 2, 4, 6, 8 and 10, 8-cell; Lanes 1 and 2, embryo cultured with medium alone; Lanes 3 and 4, embryo co-cultured uterine epithelial cells; Lanes 5 and 6, co-cultured embryo from progesterone treatment medium; Lanes 7 and 8, co-cultured embryo from 17β-estradiol treatment medium; Lanes 9 and 10, co-cultured embryo from progesterone plus 17β-estradiol treatment medium.

Previously, studies on the co-culture of embryos with uterine cells have been reported (Sakkas & Trounson, 1990) and uterine cells may be helpful to enhance the preimplantation embryonic development (Freeman et al., 1993). Although it was unclear that the expression of IL-1β gene was influenced by steroid hormone *in vivo*, steroid hormone was known to play an important role in the maintenance of the uterine cells and the preimplantation embryonic development (Lavranos & Seamark, 1989).

In the experiment on the late 2-cell embryos co-cultured with the mouse uterine epithelial cells, the expression of IL-1β mRNA was identical to that of in vivo. IL-1β mRNA was detected at the 4- and 8-cell stage embryo of all the co-culture group irrespective of progesterone and/or 17 β-estradiol treatment (Fig. 6A).

Undoubtedly, the morphological and functional differentiation of uterine epithelial cells are under the control of these steroids and the status of uterine epithelial cells at the

time of tissue collection has possibly an important factor for supporting the embryonic development. This fact implicates that the IL-1β gene expression may be also regulated by uterine factor(s), and it is more sensitive and effective than oviductal factor. However, it may be not different from the oviductal factor.

After 72 hours culture, the rate of hatching was higher in all the co-culture group with uterine epithelial cells than in the medium alone. However, it was significantly higher for the co-culture group treated with progesterone plus 17 -estradiol compared to the medium alone (Table 2). These results were similar to the reports of other co-culture studies.

In summary, these findings demonstrate that the presence of the IL-1β plays an important role in the preimplantation embryonic development, and co-culture with the explanted oviducts or uterine epitehlial cells facilitates to the IL-1β gene expression in the preimplantation mouse embryos. However, the expression pattern of IL-1β gene alone cannot explain the exact role of IL-1β system in embryonic development and implantation process. Therefore, to elucidate the exact role of IL-1β system, all major components of the IL-1β system, namely IL-1β , IL-1βra and IL-1βR tI must be examined by their expression pattern and co-culturing effects.

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