ANIMAL EXPERIMENTATION

Effect of Different Concentrations of Recombinant Leukemia Inhibitory Factor on Different Development Stage of Mouse Embryo In Vitro

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Purpose: *To assess the influence of different concentrations of recombinant human leukemia inhibitory factor (LIF) on the in vitro development of mouse embryos.*
Methods: *The 2- to 4-cell embryos of CB6F1 mice were cultured in the human tubal fluid (HTF) media containing different concentrations of LIF. Mouse embryos were divided into seven groups: (1) HTF; (2) 1500 IU/ml LIF; (3) 1000 IU/ml LIF; (4) 750 IU/ml LIF; (5) 500 IU/ml LIF; (6) 250 IU/ml LIF; (7) 125 IU/ml LIF. The embryonic numbers of different stages including 5–8 cell, 9–16 cell, morula, blastocyst, and hatching blastocyst were recorded.*
Results: *The percentage of early embryo stage (2-cell embryos to 6- to 16-cell stages) in all groups were nonsignificantly different. There were higher formation rates of preimplantation embryos (morula to hatching blastocyst) in groups 2, 3, 4, and 5 than in groups 1, 6 and 7.*
Conclusions: *LIF has positive effects on preimplantation embryo development and has nonsignificant influence on the early embryo development. The lowest concentration of LIF which could provide the optimal embryo development is 500 IU/ml.*

KEY WORDS: Leukemia inhibitory factor; recombinant LIF; coculture.

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INTRODUCTION

Leukemia inhibitory factor (LIF) plays an important role in the embryo development (1). LIF has been shown to enhance in vitro blastocyst development in mice (2, 3), in vitro blastocyst hatching in sheep, and increased pregnancy rates for in vitro cultured embryos transferred back into recipient ewes (4). Michell *et al.* (3) applied the recombinant LIF (r-LIF) in mice, which also enhanced the growth of mice embryos. Currently, most authors agree on the benefit of LIF for later stages of human embryogenesis and during implantation (1, 5). Stewart (6) demonstrated that LIF primarily controls proliferation in the preimplantation blastocyst. We previously had demonstrated that the 1000 IU/ml LIF enhances preimplantative embryo development, including blastocyst, expanded blastocyst, and hatching blastocyst states (1).

However, there have been few reports on the influence of different concentration of LIF on the in vitro development of embryos, with most investigators adopting 1000 IU/ml LIF as the standard concentration for culture media supplement (1,7,8). No investigator has ever demonstrated the impact of different concentrations of LIF on the embryo development. This study was designed to clarify these issues.

MATERIALS AND METHODS

Female CB6F1 mice (Charles River, Wilmington, MA) that were between 6 and 8 weeks old were admin-

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istered intraperitoneally with 10 IU pregnant mare serum gonadotropin (PMSG) for superovulation; this was followed 46–48 hr later by the intraperitoneal administration of 10 IU human chorionic gonadotropin (hCG). The mice were then mated to mature BDF1 male mice and checked by vaginal plug 20 hr later. Mice were killed 44–48 hr after hCG injection. After disinfecting with 70% alcohol and opening the abdomen wall, the Y-shaped uterus, ovaries (below the kidney, light yellowish), and oviducts (whitish color) were identified. The oviducts were excised as follows: clamping cornus, dissecting the peritoneum and fat between ovary and tube, and then cutting the whole oviduct from the proximal end.

After washing and flushing of the oviduct from the ampulla or proximal end of oviduct, the 2- to 4-cell embryos could be selected and collected under 100× microscopy. All mouse embryos were cultured in the 30-μl microdroplets of human tubal fluid (HTF) containing different concentrations of LIF. According to the different LIF concentrations, mouse embryos were divided randomly into seven groups: group 1, HTF; group 2, HTF + 1500 IU/ml LIF; group 3, HTF + 1000 IU/ml LIF; group 4, HTF + 750 IU/ml LIF; group 5, HTF + 500 IU/ml LIF; group 6, HTF + 250 IU/ml LIF; and group 7, HTF + 125 IU/ml LIF. In group 1, the embryos were collected for the control database.

The embryos were cultured in an incubator at 37°C with 5% carbon dioxide and 95% humidity for 120 hr. The mediums in all groups were replaced every 48 hr. The embryo development was evaluated daily by morphological observation under a light microscope. The number and percentage of embryos reaching the 5- to 8-cell, 9- to 16-cell, morula, blastocyst, and hatching blastocyst stages were recorded at 24, 48, 72, 96, and 120 hr after embryo collection. The χ^2 test and logistic regression were used to compare the embryonic development in different groups. A P value of $< .05$ was considered statistically significant.

RESULTS

There was nonstimulatory and noninhibitory effect of LIF when added to the culture medium at early cleavage stages (2- to 4-cell to 6- to 16-cell stage) of mouse embryos. The percentage of 2-cell embryos reaching the stage of 2- to 4-cell, 5- to 8-cell, and 6- to 16-cell stages in each groups were nonsignificantly different (Table I). After the initial embryo development stage, there were higher formation rates of morula, blastocyst, and hatching blastocyst in groups 2 (77.1%, 65.6%, 33.6%), 3 (76.6%, 67.6%, 35.9%), 4 (74.1%, 63.3%, 33.8%), and 5 (75.4%, 68.1%, 32.5%) than those in groups 1 (52.7%, 33.0%, 14.3%), 6 (66.4%, 49.0%, 14.7%), and 7 (54.5%, 35.2%, 16.7%).

The formation percentage of morula, blastocyst, and hatching blastocyst in groups 2, 3, 4, and 5 were nonsignificantly different. The formation rates of morula, blastocyst, and hatching blastocyst between group 6 (66.4%, 49.0%, 14.7%) and group 7 (54.5%, 35.2%, 16.7%) were statistically similar to those of the group 1 (52.7%, 33.0%, 14.3%) (Table I).

DISCUSSION

In an attempt to enhance the in vitro development of embryos, many investigators have utilized coculture of the early embryos with a variety of cell types including tubal epithelial cells (9), uterine fibroblasts (10), and Vero cells (11). These cells secrete growth factors or other embryotropic products that foster embryonic growth (12). One of these potential growth factors is LIF. Many authors have demonstrated that the benefit of co-culture may be due to LIF production by feeder cells (2). This glycoprotein that is secreted by the endometrium during the implantation window plays a part of signaling between the endometrium and the blastocyst (14). In this series, we used recombinant LIF instead of LIF released from the coculture cells for mouse embryo culture. The advantage of recombinant LIF for coculture over LIF released from coculture cells is its convenience and commercial availability, as well as the absence of virus infection risk.

Numerous literature about the effects of LIF application on the embryo development has been reported. Fry *et al.* (4) reported that addition of human LIF to culture medium increased sheep blastocyst formation and hatching by fourfold. Dunglison *et al.* (15) further demonstrated that LIF significantly enhances the blastocyst formation of human embryos (18.4 to 43.6%). However, most investigators have adopted the LIF concentration of 1000 IU/ml for supplementing the culture media (1,7,8). No investigator has ever investigated the effects of different concentrations of LIF on the in vitro embryo development. In this series, we first compared the effects of different concentrations of the LIF and observed the lowest concentration of LIF being 500 IU/ml for significantly enhancing preimplantative embryo development. The addition of 250 and 125 IU/ml LIF resulted in better but nonstatisti-

Table I. Embryo Development Between Seven Groups

	2- to 4-cell embryo[a]	5- to 8-cell embryo[a]	9- to 16-cell embryo[a]	Morula[b]	Blastocyst[b]	Hatching blastocyst[b]
Group 1 (control, HTF)[c]	112	98 (87.5%)	82 (73.2%)	59 (52.7%)	37 (33.0%)	16 (14.3%)
Group 2 (1500 IU/ml LIF + HTF)[d]	131	116 (88.5%)	105 (80.2%)	101 (77.1%)	86 (65.6%)	44 (33.6%)
Group 3 (1000 IU/ml LIF + HTF)[d]	145	130 (89.7%)	119 (82.1%)	111 (76.6%)	98 (67.6%)	52 (35.9%)
Group 4 (750 IU/ml LIF + HTF)[d]	139	122 (87.8%)	110 (79.1%)	103 (74.1%)	88 (63.3%)	47 (33.8%)
Group 5 (500 IU/ml LIF + HTF)[d]	191	174 (91.1%)	153 (80.1%)	144 (75.4%)	130 (68.1%)	62 (32.5%)
Group 6 (250 IU/ml LIF + HTF)[c]	143	129 (90.2%)	109 (76.2%)	95 (66.4%)	70 (49.0%)	21 (14.7%)
Group 7 (125 IU/ml LIF + HTF)[c]	156	140 (89.7%)	116 (74.4%)	85 (54.5%)	55 (35.2%)	26 (16.7%)

[a] Nondifference in the early embryo development (2- to 4-cell to 9- to 16-cell stage) between seven groups.
[b] The higher development of preimplantation embryos (morula to hatching blastocyst) in groups 2, 3, 4, and 5 than those in groups 1, 6, and 7.
[c] Nonsignificant difference in the preimplantation embryo development between groups ± 1, 6, and 7.
[d] Nonsignificant difference in the preimplantation embryo development between groups 2, 3, 4, and 5.

cally different development of preimplantative embryo than that of the absence of LIF.

There is controversy as to the effect of LIF on the different development stages of embryo development. Michell *et al.* (3) demonstrated that r-LIF enhances blastocyst formation and decreases embryo fragmentation even in the two-cell stage. In contrast, Jurisicova *et al.* (16) demonstrated that r-LIF in standard medium does not enhance the development of early stage human embryos. In this series, our data are consistent with our previous report (1) that r-LIF benefits the development of preimplantation embryos. We did not find the obvious benefit of LIF in the early stage of embryo development (2- to 16-cell stage). The main reason for this may be the different requirements during different embryo development stage, since as the embryo grows, the requirement for growth factor and nutrition are increased.

In conclusion, this series provides information about the effects of different concentrations of LIF on the embryo development in different stages. There was nonstimulatory effect of LIF when it was added to the culture medium at early cleavage stages of mouse embryos. In the preimplantative stage, LIF assisted with some degree of stimulatory effect on the embryos (morula, blastocyst, and hatching). Furthermore, 500 IU/ml is the lowest concentration of LIF that provided the optimal enhancement as the higher concentrations. However, a further application and larger series of LIF on the human embryo development is indicated. This may lead to the design of a defined medium preparation that will replace coculture cells as a safer and more practical means of stimulating the in vitro development of human embryos.

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