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Animal Experimentation

Anti-Macrophage Inhibitory Factor Antibody Inhibits PMSG-hCG-Induced Follicular Growth and Ovulation in Mice

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***Purpose*:** To investigate the effect of an anti-MIF antibody on PMSG-hCG-induced murine follicular growth and ovulation and to determine whether MIF plays an essential role in this process.

***Methods*:** Mice were primed with an intraperitoneal injection of pregnant mare serum gonadotropin (PMSG) and were treated with an anti-rat MIF antibody and human chorionic gonadotropin (hCG) to induce ovulation. After that, the ovulated ova were counted. The ovaries were studied using standard histological procedures.

***Results*:** Ovaries treated with the anti-MIF antibody showed reduced numbers of growing follicles surrounded by granulosa cells and theca cells with a little proliferation compared with the control. The average numbers of ova collected from mice treated with the anti-MIF antibody were reduced compared with those collected from control mice.

***Conclusions*:** Anti-MIF antibody inhibits the follicular growth and ovulation in mice, and MIF may play an important role in the inflammatory reactions during follicle growth and ovulation.

KEY WORDS: Anti-macrophage inhibitory factor antibody; cytokine network; follicle growth; macrophage migration inhibitory factor; ovulation.

INTRODUCTION

Follicle growth and ovulation are coordinated with the proliferation of granulosa cells and theca cells with inflammatory changes (1). The histological changes in follicular growth and ovulation suggest that cytokines, as one of the major mediators in the reproductive process, are involved in the regulation of ovarian function. Previous reports also suggested that thecal macrophages might be involved in the regulation of follicular growth and ovulation, as well as being important for the normal progression of the estrous cycle (2). In growing mouse follicles, progesterone secretion from granulosa cells is enhanced by coculture with macrophages (3). This observation supported the idea that macrophage might play an important role in the local control of follicular growth as one of the key cells in the immune responses in the process of ovulation.

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is produced by T-cells and macrophages, and inhibits the migration of macrophages from inflammatory sites (4). It has been considered that MIF is expressed in activated T-lymphocytes, anterior pituitary cells, macrophages, and granulosa cells (5). For many years, MIF has been considered to be associated with acute inflammatory responses and increases in secretion of

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cytokines (tumor necrosis factor-α, interferon-γ, interleukin-1β) (4).

However, the functions of MIF in follicular growth and ovulation remain unclear, although MIF, with its ubiquitous effects, is one of the key cytokines in the inflammatory process.

In this study, we examined whether an anti-MIF antibody inhibited PMSG-hCG-induced murine follicular growth and ovulation, and investigated the possibility that MIF plays an essential role in the process of follicular growth and ovulation.

MATERIALS AND METHODS

Preparation of Anti-MIF Antibody

A polyclonal anti-rat MIF antibody was generated by immunizing New Zealand white rabbits with rat recombinant MIF as described (6). Because only a single amino acid residue is different between rat and mouse MIF, the anti-rat MIF immunologically cross-react with mouse MIF (6).

Animal Experiments

Immature 20-day-old female ICR (SLC, Hamamatsu, Japan) mice were injected intraperitoneally with 50 μg ($n = 13$), or 100 μg ($n = 11$) of the anti-rat-MIF-antibody, or 100 μg ($n = 13$) of nonimmune rabbit IgG from the 20th to the 23rd day after birth consecutively. In 21-day-old, these mice were primed with an intraperitoneal injection of 5 IU of pregnant mare serum gonadotropin (PMSG) (Sigma, St Louis, MO, USA) to produce multiple preovulatory follicles. Forty-eight hours later, the mice, treated with the anti-rat MIF antibody were admistrated 5 IU of human chorionic gonadotropin (hCG) (Mochida Pharmaceutical Co. Ltd., Tokyo, Japan) to induce ovulation. Approximately 12 h after administration of hCG, mice were killed by cervical dislocation. The cumulus mass was easily visualized within the ampulla of each follopian tube. The ampullae of the uterine tube were excised, and the ovulated ova were released and counted under a dissecting microscope. Further flushing of the oviduct and uterus revealed the absence of any additional oocytes. The numbers of oocytes in the cumulus mass recovered from each side were recorded. The ovaries were immediately fixed in 10% buffered formalin at room temperature for 24 h and then transferred to 70% ethanol at 4°C. For histological analysis, fixed tissues were paraffin embedded, sectioned at 4 μm and stained with hematoxylin and eosin according to the standard histological procedures. This research was approved by the Research Committee of the Hamamatsu University School of Medicine, Japan.

Statistical Analysis

Data of ovulation were analyzed using Student's t test. All statistical analysis was carried out using Statview 4.0 Software (Abacus Concepts, Berkeley, CA). Differences were considered to be statistically significant if $p < 0.05$.

RESULTS

Histological examination showed that ovaries from mice treated with the anti-MIF antibody and nonimmune IgG retained the mixture of ruptured and unruptured follicles as demonstrated in Fig. 1. However, in ovaries treated with the anti-MIF antibody, there were reduced numbers of growing follicles with immature ova surrounded by granulosa cells and theca cells compared with the control. In addition, the granulosa and theca cells in ovaries from the anti-MIF antibody group showed little proliferation. As shown in Fig. 2, the average numbers of ova collected from mice treated with the anti-MIF antibody after the induction of ovulation were significantly reduced, by approximately 55%, in mice injected with 100 μg and 67% in those injected with 50 μg compared to those collected from mice treated with nonimmune IgG.

DISCUSSION

In the present study, we have shown that the anti-MIF antibody inhibited the follicular growth and ovulation induced by PMSG-hCG in a mouse model. Recent studies on humans have revealed that MIF levels are increased in serum and follicle fluid in response to hCG or LH stimulation (7). The functional role of MIF in the ovary may be to stimulate the induction of growth factors during follicular growth and ovulation via activation of macrophages. Our data showed that the number of ova collected at PMSG-hCG induction of ovulation were suppressed in mice treated with the anti-MIF antibody compared with controls. Furthermore, histological examination showed retarded proliferation of granulosa and theca cells in ovaries from mice treated with the anti-MIF antibody. Theses histological changes suggested that the

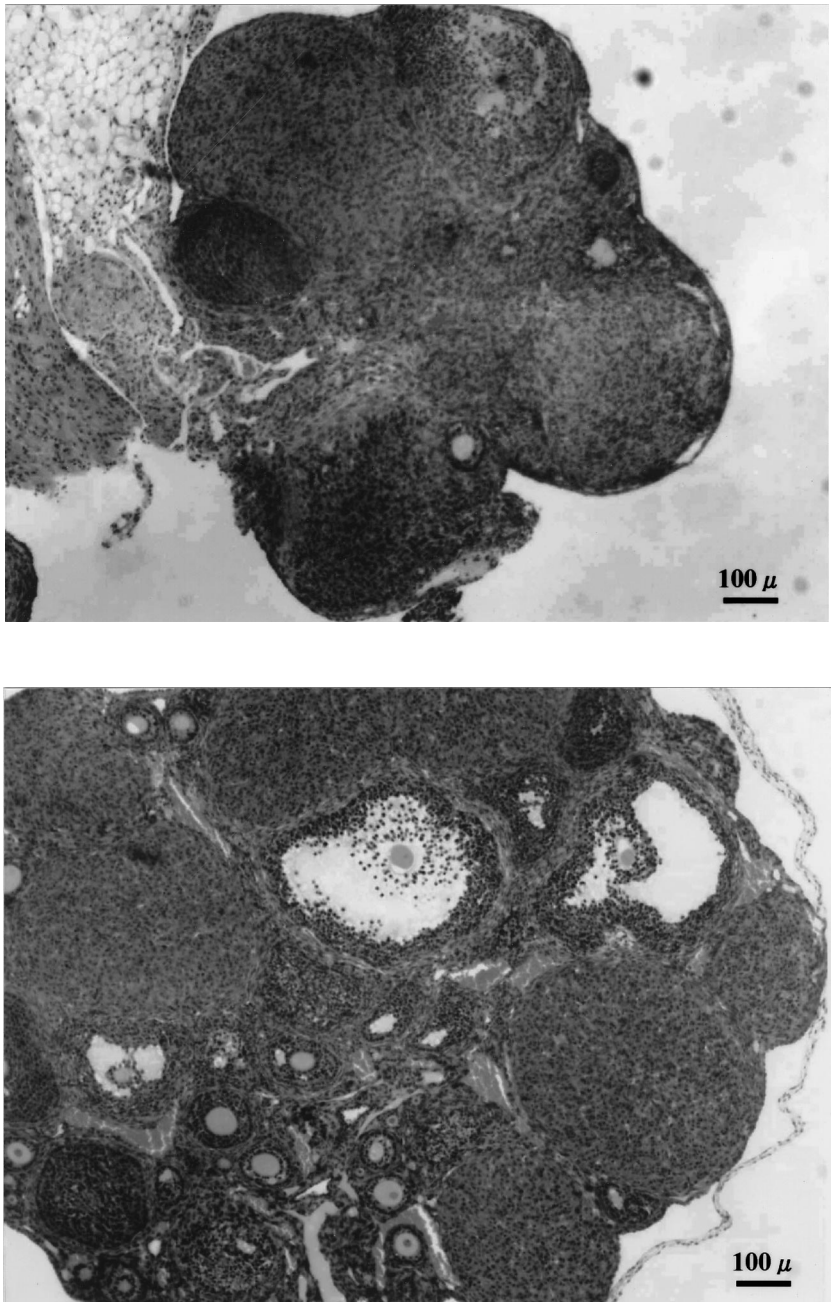

Fig. 1. Histological changes in ovarian tissues in the anti-MIF antibody-treated groups and the control group after induction of ovulation. Immature 20-day-old female ICR mice were injected intraperitoneally with 50 μg ($n = 13$), or 100 μg ($n = 11$) of the anti-rat-MIF-antibody, or 100 μg ($n = 13$) of nonimmune rabbit IgG from the 20th to the 23rd day after birth consecutively and then, mice were injected with 5 IU of hCG after 48-h administration 5 IU of PMSG. The oocytes and the ovarian tissues were examined as described in Materials and Methods. (A) Ovary from a mouse treated with anti-MIF antibody; (B) Ovary from a mouse treated with nonimmune IgG.

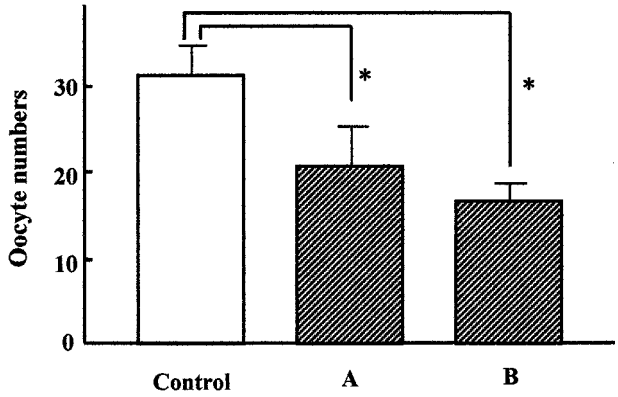

Fig. 2. The numbers of oocytes after induction of ovulation from mice treated with the anti-MIF antibody and from mice treated with nonimmune IgG. (A) The numbers of oocytes collected from mice treated with 50 μg of anti-rat MIF antibody ($n = 13$); (B) The numbers of oocytes collected from mice treated with 100 μg of anti-rat MIF antibody ($n = 11$). Control: The numbers of oocytes collected from mice treated with 100 μg of nonimmune IgG ($n = 13$). Error bars indicate standard deviation. $^*p < 0.001$ vs. (control).

anti-MIF antibody inhibited follicular growth by regulation of proliferation of granulosa and theca cells. These results support the possibility that MIF is involved in the process of follicle growth and ovulation.

The mechanisms of follicle growth and ovulation are similar to cell growth, angiogenesis, and wound repair for the next cycle. It has been shown that MIF stimulates cell proliferation and MIF mRNA is upregulated in parallel with angiogenesis and wound repair (8,9). MIF has been detected in human vascular endotherial cells (10). These observations suggest that MIF may contribute to follicle growth and ovulation.

It was previously reported that VEGF was commonly expressed during follicle growth and ovulation (11,12). In addition, it has also been shown that anti-MIF antibodies suppress the expression of VEGF in the tumor vasculature (13). There are reports that gonadotropins, oocyte growth factors, and cytokines act on follicular growth and ovulation (14–16). Once the follicle enters the growth cycle, some growth factors such as IL-1, IL-6, IGF, GM-CSF, and TGF-α, etc., play important roles (17). These factors are closely related to macrophages, which are connected to MIF in the ovary. In our experiments, the local regulation of cytokines by theca macrophages remained undetermined. However, we speculate that the inactivation of MIF by the anti-MIF antibody may affect the functions of cytokines and growth factors derived from macrophages. In assisted reproductive technology, we must hyperstimulate ovaries to retrieve ova. Therefore, as a side effect of this treatment, ovarian hyperstimulation syndrome (OHSS) is one of the most dangerous iatrogenic problems. In the future, we may be able to use anti-MIF antibodies for OHSS treatment as clinical medication.

In conclusion, our present study suggests for the first time that anti-MIF antibodies inhibit follicular growth and ovulation and that the cytokine network is involved with respect to MIF and macrophages. Although further experiments are required to determine the precice roles of MIF and cytokines, our findings will contribute to the investigation of the reproductive mechanisms in follicular growth and ovulation.

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