Phagocytosis of Carbon Particles by Theca Interna Fibroblasts in Hen Ovary

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The aim of this study was to determine whether the follicular cells phagocytose foreign agents in hens. White Leghorn laying hens were intravenously injected with carbon solution 22 hr before the expected time of ovulation. Then the ovarian stroma containing primary follicles (PF), white follicles (WF), the largest and third largest preovulatory follicles (F1 and F3, respectively), the largest postovulatory follicle (POF) and spleen were collected 20 hr after the injection. Tissues were processed for histological examinations. Carbon particles were observed in the cytoplasm of the theca interna fibroblasts of WF, F3, F1 and POF in the carbon injected birds. The population of cells containing carbon particles was significantly greater in F1 and F3 follicles than in the other follicles, and was greater in WF than in PF and POF. Carbon particles were also accumulated in the spleen. These results suggest that the fibroblasts in the theca interna of preovulatory follicles phagocytose foreign particle substances, and this activity is likely to be stronger during the rapid growth phase than the other phases.


Key words: follicle, theca interna, fibroblast, phagocytosis

Introduction

The hen ovary contains numerous primary follicles in the stroma, white and yellow preovulatory follicles and postovulatory follicles (Gilbert, 1979). Each yellow follicle shows a hierarchy in size and grows rapidly (Bahr and Johnson, 1991), and blood supply is well developed in these follicles (Gilbert, 1979). During their growth copious yolk substances are incorporated from the blood into the intrafollicular oocyte (Griffin et al., 1984; Johnson, 1986). Therefore, it is possible that the follicular wall is a tissue where various substances in the blood stream, including not only yolk precursors but also pathogenic agents, are accumulated. If accumulation of agents which are not responsible for the follicular growth occurs in the follicular wall, the follicular cells may need to remove them for the maintaining the follicular environment normal.
Phagocytic cells may play a role in such events. The aim of this study was to
determine whether phagocytic cells are located in the follicular wall.

**Materials and Methods**

White Leghorn hens regularly laying 4 or more eggs in a sequence were used. They
were kept in individual cages under a 14 hr light and 10 hr dark regimen, and provided
with feed and water *ad libitum*. They were intravenously injected with 0.5 ml carbon
solution (KURETAKE-SEISHODO Co., Japan) or 0.5 ml saline (control) 22 hr before the
expected time of ovulation (n=4 and 2 birds, respectively). The ovarian stroma
containing primary follicles (PF), white follicles approximately 5 mm in diameter (WF),
the largest and third largest preovulatory follicles (F1 and F3, respectively), the largest
postovulatory follicles (POF) were excised 20 hr after the treatment (2 h before the
expected time of ovulation). This time schedule was to maintain the injected birds as
long as possible within the time of the last ovulation to the next ovulation. The
spleen, which is known to contain the developed phagocytic cells, was also excised as
a positive control tissue. The follicular wall was prefixed with 4% paraformaldehyde
and 2% glutaraldehyde in phosphate buffer and postfixed with 1.3% osmium tetroxide
in phosphate buffer, followed by dehydration with acetone and embedding in Epoch
812 (OKEN Co., Japan). Sections (1μm thick) of them were stained with toluidine blue.
The spleen was fixed with 10% formalin, dehydrated and embedded in paraffin, and the
sections (6μm thick) were stained with hematoxylin and eosin. All sections were
examined under a light microscope.

The number of cells containing carbon particles in the inner theca tissue (20μm
thick from the inner boundary toward outside) was counted under an image analysis
computer system (MacAspect, Mitani Co., Fukui, Japan). This observation was
performed in 2,500 to 3,800μm² area, and the observed area covered the theca interna in
WF, F3, F1 and POF. The theca layer in PF had not been differentiated into the
 interna and externa layers. Then the cell number was calculated to be the cell number
in 1×10⁶μm². The average of two counts in one tissue was expressed as the cell
number in a tissue of a bird. The significance of the difference in the cell number
among the follicles was analyzed by one way ANOVA followed by Duncan's multiple
t test.

**Results**

The follicular wall consisted of the granulosa and theca layers, and the theca layer
was differentiated into the theca interna and externa in WF, F3, F1 and POF. Theca
interna contained fibroblasts, interstitial cells and capillary beds.

In the follicular wall of carbon injected birds, carbon particles were observed in the
theca interna in WF, F3, F1 and POF, but not in PF (Fig. 1a). These particles were
located in the cytoplasm of the theca interna fibroblasts (Fig. 1b). Occasionally,
macrophage–like cells, which contained carbon particles, were observed in the blood
vessels in the loose connective tissue coat (data not shown).

Absence of black particle was confirmed in the follicular wall of control birds
Fig. 1. Cross section of the wall of the third largest follicle obtained from a bird injected with carbon solution 20 hr before examination. (a) Arrow heads indicate the examples of carbon particles accumulated in the theca interna. Scale bar=30μm. (b) Magnified view of Fig. 1a. Note the presence of carbon particles in the cytoplasm of the theca interna fibroblasts (small arrow heads). Large arrow head shows a capillary. Scale bar=12μm. Toluidine blue staining.

Fig. 2. Cross section of the wall of the third largest follicle obtained from a bird injected with saline 20 hr before examination. No carbon particle is observed. Toluidine blue staining. Scale bar=30μm.
which were injected with saline (Fig. 2). Carbon particles were accumulated in the reticular cell–like or macrophage–like cells in the spleen in carbon injected birds (Fig. 3).

The population of theca interna cells containing carbon particles was significantly greater in F1 and F3 follicles than in PF, WF and POF, and was greater in WF than in PF and POF (P < 0.05) (Fig. 4).
Discussion

Carbon particles were localized in the cytoplasm of the theca interna fibroblasts in the birds which were injected with carbon solution 20 hr before examination. The number of cells was significantly greater in F3 and F1, which were the follicles of rapid growth phase, than in PF, WF and POF. Also, they were observed in the spleen cells and in the macrophage-like cells in the blood vessels that were known as the professional phagocytes. Therefore, we suggest that the theca interna fibroblasts phagocytose carbon particles as do the professional phagocytes. It is likely that their phagocytic function is stronger in the follicles of rapid growth phase than the follicles before the rapid growth phase or after ovulation.

The theca interna is a site where well developed capillary beds are formed (Gilbert, 1979). Phagocytic functions of the theca interna fibroblasts may play a role in maintaining the follicular environment normal, namely they may remove the pathogenic agents and substances in the blood that are not required for the follicular functions. Because carbon particles are inorganic particles, it remains to be determined whether the theca interna fibroblasts respond to soluble agents.

References