Uptake of immunoglobulin and albumin by granulated metrial gland cells in vitro

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Summary. Single cell suspensions of metrial gland tissue from rats at Day 14 of pregnancy were prepared for maintenance in vitro. During the first 2 days of culture IgG was detected in glycoprotein granule-containing granulated metrial gland (GMG) cells. Albumin was also detected in GMG cells at the same stages. The IgG and albumin were not detected during the next 4 days in culture. When metrial gland cells, maintained in vitro for 5 days, were incubated with rat serum for a further 24 h, IgG and albumin were detected in GMG cells. When similar cultures were incubated for 24 h with purified rat IgG or purified rat albumin, GMG cells were positive for IgG and albumin respectively. Albumin was not detected in GMG cells in wax sections of metrial gland tissue, although IgG has previously been demonstrated. The uptake of serum proteins by GMG cells in vitro has been clearly shown but the difference in IgG and albumin content of these cells in paraffin-wax sections indicates that the means by which IgG accumulates intracellularly may be different in vitro and in vivo.

Introduction

In the pregnant rat a metrial gland develops at each implantation site by about Day 11 of gestation. The number of cells in the gland reaches a maximum at around Day 14 and thereafter declines. Most of the cells present are granulated metrial gland (GMG) cells or fibroblast-like stromal cells. The GMG cells are bone marrow-derived (Peel et al., 1983; Mitchell & Peel, 1984) and contain cytoplasmic immunoglobulin (Ig) G, both in pregnancy (Bulmer & Peel, 1977a, b; Mitchell et al., 1980) and in the deciduomata of pseudopregnancy (Mitchell et al., 1981). In the studies by Mitchell et al. (1980, 1981) IgG and the characteristic glycoprotein granules were demonstrated in the same GMG cells but no investigations were carried out to determine whether other serum proteins were present.

The function of the metrial gland has long been disputed but it has been suggested that the IgG in GMG cells might play a role in the immunology of pregnancy (Peel & Bulmer, 1977). It is of importance, therefore, to know whether the IgG is present as a result of endocytosis or of in-situ synthesis. Experiments have been carried out to determine whether GMG cells are able to endocytose IgG and albumin.

Materials and Methods

Animals. Virgin random-bred Wistar rats were mated with males of their own strain and Day 1 of gestation was defined as the day on which spermatozoa were detected in vaginal smears. On Day 14 rats were bled by cardiac puncture under ether anaesthesia and killed by cervical dislocation.

Single cell preparations. Metrial glands were dissected from at least 6 implantation sites of 6 pregnant rats at Day 14 of gestation. Tissue was prepared under aseptic conditions using a collagenase dispersion method (Bray et al.,...
Viability of cells was assessed using a nigrosin dye-exclusion test (Hudson & Hay, 1976): only preparations of >90% viability were used for cultures.

**Culture method.** Metrial gland cells (3 × 10^6) from single cell preparations were cultured in plastic dishes (Sterilin, Middlesex, U.K.) each containing a glass coverslip (25 mm²) in 3 ml of Minimum Essential Medium with Earle’s salts (Gibco, Paisley, U.K.) supplemented with 10% heat-inactivated fetal calf serum (Flow, Rickmansworth, U.K.), 1% 200 mg/ml-glutamine, 2% penicillin-streptomycin solution (Flow; 5000 i.u./ml and 5000 ng/ml respectively) and progesterone (Sigma, Dorset, U.K.; 200 ng/ml). Cultures were maintained in a carbon dioxide incubator (5% CO₂, 95% air) at 37°C in a humid atmosphere. Media were changed daily. Cultures of metrial gland cells from 2 rats were maintained for 1, 2, 3, 4 or 5 days before fixation, whilst cultures from other rats (N = 4) were maintained for 5 days and then cultured for a further 24 h with autologous rat serum (1:1 dilution in culture medium), purified rat IgG (Nordic, Maidenhead, U.K.; 4 mg/ml culture medium), purified rat albumin (Nordic; 5 mg/ml culture medium) or in culture medium alone. Some cultures were then tested for viability using the nigrosin dye-exclusion test. At the end of the culture period coverslips were removed, washed thoroughly in Hank’s balanced salt solution (HBSS) (Flow), broken into pieces and fixed in 10% neutral buffered formalin, 5% glacial acetic acid in ethanol, or saturated alcoholic mercuric chloride (SAMC). Coverslip fragments fixed in 10% neutral buffered formalin were stained with 1% toluidine blue, or reacted for glycoprotein by the periodic acid–Schiff (PAS) technique after diastase digestion. Coverslip fragments fixed in 5% glacial acetic acid in ethanol or SAMC were reacted by an immunofluorescence method for IgG or albumin.

**IgG demonstration.** An indirect immunofluorescence method was used for IgG localization on coverslip fragments fixed in 5% glacial acetic acid in ethanol. The primary antibody was rabbit anti-rat IgG (Nordic; 1/80 or 1/160) and the secondary layer was sheep anti-rabbit Ig conjugated to fluorescein isothiocyanate (Nordic; 1/20 or 1/40). Preparations were mounted in PBS/glycerol AF1 (Citifluor, London, U.K.) to reduce fading.

Some coverslip fragments fixed in SAMC were reacted with the TAS method (after 1% diastase digestion) to demonstrate glycoprotein before the immunofluorescence procedure. Controls showed that this procedure did not affect immunoreactivity.

Spleen tissue from some pregnant rats was quenched in liquid nitrogen-cooled 2 methyl butane and sections (7 µm thick) were cut on a cryostat, air dried and fixed in 5% glacial acetic acid in ethanol to serve as a positive control tissue for IgG demonstration.

**Albumin demonstration.** A similar immunofluorescence method to the one described above for IgG was used for albumin localization, except that the primary antibody was rabbit anti-rat albumin (Nordic; 1/80 or 1/160). Conceptuses from some pregnant rats were fixed in SAMC, processed to paraffin wax and sections (5 µm thick) were cut. Some sections were reacted by the PAS method after 1% diastase digestion for glycoprotein. Some sections were reacted using a peroxidase–antiperoxidase technique. The primary antibody was rabbit anti-rat albumin (Nordic; 1/160). The bridging anti-globulin was sheep anti-rabbit Ig (Nordic; 1/80) and rabbit peroxidase-antiperoxidase (Nordic; 1/200) was used as the third layer. Antibody binding sites were revealed by incubating sections for 10 min at room temperature in 10 mg diaminobenzidine tetrahydrochloride in 10 ml 0.2 M-Tris–hydrochloric acid buffer, pH 7.6 with 0.1 ml 1% hydrogen peroxide. Endogenous peroxidase activity was inhibited with a 1.7% solution of 30% (100 volume) hydrogen peroxide in methanol before application of the immunoperoxidase technique.

The yolk sac and other placental sites in sections of conceptuses acted as positive controls for albumin localization.

**Results**

In cultures stained with toluidine blue it was clear that the majority of cells present were large, flattened cells (Fig. 1) but GMG cells were not readily identified. In cultures reacted by the PAS method (after diastase digestion) small numbers of small, round cells were present and were identified as GMG cells by their possession of glycoprotein granules (Fig. 2). The GMG cells were often located above the plane of the large, flattened cells, upon which they appeared to be resting. There was no change in appearance of either cell type throughout the culture period.

In 1- and 2-day cultures it was possible to demonstrate IgG- (Fig. 3) or albumin-containing cells. Such cells were not present in cultures maintained for 3, 4, 5 or 6 days. In 1- or 2-day cultures fixed in SAMC it was possible to demonstrate IgG and glycoprotein granules, or albumin and glycoprotein granules in the same cells, and to identify them as GMG cells. Virtually all the IgG- or albumin-positive cells in these cultures were identified as GMG cells.

In cultures maintained for 5 days and incubated for a further 24 h with rat serum cells were detected in which both IgG and glycoprotein (Fig. 4a) or albumin and glycoprotein (Fig. 4b) were present. The form and distribution of these cells identified them as GMG cells. In such preparations...
Fig. 1. A 2-day culture of metrial gland cells stained by toluidine blue. Large flattened cells are predominant. ×190.

Fig. 2. A 1-day culture of metrial gland cells reacted for glycoprotein. Some granulated metrial gland cells are arrowed. ×380.

Fig. 3. A 1-day culture of metrial gland cells reacted for IgG. Some positive cells are arrowed. ×480.
Fig. 4. A 6-day culture of metrial gland cells after 24 h in (a) rat serum reacted for glycoprotein (red) and IgG (yellow), (b) rat serum, reacted for glycoprotein (red) and albumin (yellow), (c) purified rat IgG, reacted for glycoprotein (red) and IgG (yellow) and (d) purified rat albumin, reacted for glycoprotein (red) and albumin (yellow). Granulated metrial gland cells are arrowed. × 480.
only occasional cells were detected which contained glycoprotein, but no IgG or albumin. Very few cells were detected which contained IgG or albumin but no glycoprotein.

In cultures maintained for 5 days and incubated for a further 24 h with purified rat IgG or purified rat albumin glycoprotein-positive cells could be detected which contained IgG (Fig. 4c) or albumin (Fig. 4d). The form and distribution of these cells identified them as GMG cells. In such cultures it was possible to detect cells with IgG but no glycoprotein; only very occasionally were albumin-positive, glycoprotein-negative cells seen. No non-viable cells were identified in the control cultures tested for nigrosin dye exclusion on the 6th day of culture.

Fig. 5. Albumin distribution (a) reaction product is widely distributed and some of the negative granulated metrial gland cells are arrowed, and (b) in cells of the visceral endodermal yolk sac. × 380.

In paraffin-wax sections of conceptuses, reaction product for albumin was detected only extracellularly (Fig. 5a) and comparison of paraffin-wax sections reacted with the PAS method after 1% diastase digestion showed by their distribution that GMG cells were negative for albumin. Albumin was also detected lining the maternal blood spaces in the labyrinthine placenta and in the visceral endodermal layer of the yolk sac (Fig. 5b). In cryostat sections of spleen, IgG-containing plasma cells were detected in immunofluorescence preparations. In cultures or sections reacted by immunofluorescence or immunoperoxidase techniques no reaction was detected when the primary antibody was omitted.

Discussion

IgG has been demonstrated in GMG cells in culture (Fig. 3) but after 2 days it was lost from the cells. The explanation for these observations remains to be established but it provided an opportunity to determine whether endocytosis of IgG could occur. It was clear from the form, distribution and glycoprotein content of the cells in vitro that were able to take up IgG from rat serum (Fig. 4a) or from purified rat IgG (Fig. 4c) that these cells were GMG cells. Mitchell (1986) showed that the endocytosis of IgG by GMG cells in vitro occurred irrespective of whether autologous or heterologous serum was used. GMG cells were also shown to take up albumin (Figs 4b & 4d) and this indicates that the uptake may be attributable to a non-specific mechanism.
The uptake of purified rat IgG or purified rat albumin excludes the possibility that a serum factor(s) stimulates synthesis or endocytosis of these serum proteins by GMG cells. Passive entry of serum proteins into the cell cytoplasm, regarded as an indicator of dead or dying cells (Isaacson & Wright, 1979) is an unlikely explanation for the endocytosis because in cultures subjected to the nigrosin dye-exclusion test non-viable cells were not detected. Receptor-mediated endocytosis of IgG by GMG cells is unlikely since Bray et al. (1978) found that this cell type does not express Fcγ receptors. Bray et al. (1978) noted that the erythrocyte antibody rosetting technique used did not demonstrate Fcγ receptors on B lymphocytes (Shevach et al., 1973) and so it is possible that GMG cells bearing Fcγ receptors would not be detected by this method.

The finding that cells of the metrial gland, other than GMG cells, were able to take up purified rat IgG, but not purified rat albumin to any extent, may indicate that the IgG uptake by these cells is specific and receptor-mediated. At least some of these cells might be the Fcγ receptor-bearing fibroblast-like stromal cells described by Bray et al. (1978), especially in the light of observations of Craggs (1981) that such cells can endocytose human IgG in vitro.

The uptake of serum proteins by GMG cells in vitro in the present study contrasts with the in-vivo studies of Sharma & Peel (1979) in which fluorescein isothiocyanate-labelled serum was injected intravenously into pregnant rats: GMG cells did not take up the fluorescein-conjugated proteins but fibroblast-like stromal cells showed evidence of uptake. Furthermore, the uptake of albumin by GMG cells in vitro does not accord with the distribution of albumin in paraffin-wax sections of metrial gland tissue (Fig. 5a) in which only extracellular reaction was seen. This contrasts with the results of the study by Mitchell et al. (1980) in which IgG was demonstrated in GMG cells in paraffin-wax sections.

Although it is clear that GMG cells can endocytose IgG and albumin in vitro it may not be the mechanism by which IgG accumulates in vivo. Since IgG, but not albumin, can be detected in GMG cells in paraffin-wax sections of tissue fixed immediately, it remains possible that GMG cells can synthesize IgG.

References


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