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ADRENOCORTICOTROPIN SECRETION FROM PERIPHERAL BLOOD MONONUCLEAR CELLS IN CYCLIC AND PREGNANT ANIMALS

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SUMMARY

A growing body of evidence suggests that a number of classical pituitary hormones are also produced and secreted from immune cells. The cross-talk between endocrine and the immune system is heightened during disease, stress and pregnancy. We studied the adrenocorticotropin (ACTH) secretion from peripheral blood mononuclear cells (PBMC) harvested from cyclic and pregnant animals. Our results indicate a significantly high secretion of ACTH from PBMCs in all stages of pregnancy compared to that in cyclic animals. This implies a new role for ACTH secreted from PBMCs in fetomaternal immune interactions.

Key words: ACTH; Lymphocytes; Pripheral blood mononuclear cells; estrous cycle; pregnancy.

INTRODUCTION

Hormonal control of immune functions and effects of cytokines on CNS and pituitary hormonal activity is known since decades (for review 1). In recent years, however, a growing body of evidence elucidates the production and secretion of classical pituitary hormones from immune cells in particular from peripheral mononuclear cells (hereafter, it will be referred to as lymphocytes). The later orchestrate adaptative immune responses via antigen ecognition and the secretion of cytokines and growth factors (2). The expression of hormones and their receptors on lymphocytes are believed to play an important role in the maintenance of homeostasis during normal physiological processes as well as pathological states (3). Adrenocorticotropin (ACTH) is one of the first hormones found to be *de novo* synthesized and secreted from lymphocytes (4, 5). Lymphocytic ACTH is apparently similar to its pituitary counterpart (6). Locally produced ACTH from lymphocytes has a powerful immune suppression activity independent of its capacity to stimulate glucocorticoid secretion (7). Adrenocorticotropin has been implicated in inactivating and immobilizing the ameoboid leukocytes (6, 7) and in inhibiting pro-inflammatory Th-1 cytokines (8, 9).

One of prerequisites for a successful pregnancy is the immunogenetical inertness of the mother. This is assumed to be achieved predominantly by the local production of regulatory signals with immunomodulatory properties (10, 11). In a series of experiments, we investigated the lymphocytic ACTH secretion in pigs and cows during estrous cycle and determined if the lymphocytic ACTH release is altered during pregnancy when the cross-talk between the immune and the endocrine system is heightened.

Lymphocytic ACTH secretion in cyclic and pregnant pigs and cows

The basal ACTH secretion is relatively high in cows and pigs during the cycle and does not alter in different stages of the cycle. Whereas no measurable ACTH production is observed in

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non-pregnant animals with ovarian cysts. The ACTH levels are low in cows with ovarian cysts regardless if the cysts produce more progesterone or more estrogens.

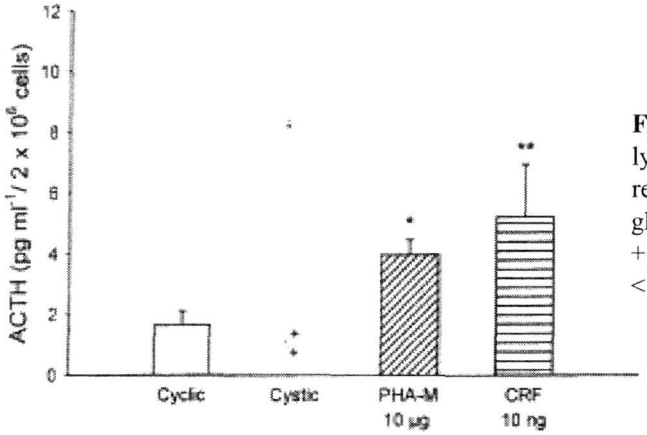

Figure 1: Mean ACTH secretion from lymphocytes in cyclic animals.Corticotropin-releasing hormone (CRH) and phytohemagglutinin (PH A-M) stimulate the ACTH release. + = below detection limit; * = le < 0.05; ** = P < 0.01 vs. cyclic animals.

The basal ACTH secretion in cyclic animals can be stimulated (Fig. 1) by adding CRH (corticotropin-releasing hormone) and/or phyto hemagglutinin from *Phaesolus vulgaricus* (PH A-M) to the incubation medium (12).

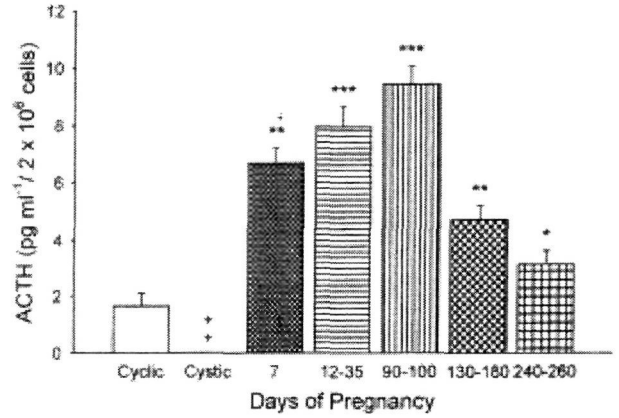

Figure 2 : Adrenocorticotropin secretion from lymphocytes is significantly higher in all stages of pregnancy than in cyclic animals. Lymphocytes harvested from animals with ovarian cysts secret very low levels (under the detection limit of the assay) of ACTH. + = below detection limit; * = le < 0.05; ** = le < 0.01; *** le < 0.001 vs. cyclic animals.

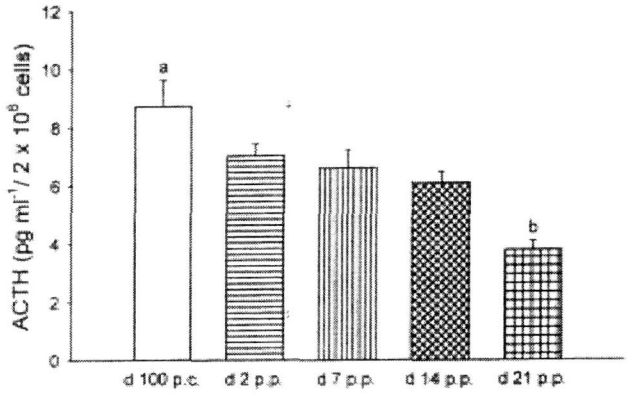

Figure 3 : Adrenocorticotropin release from lymphocytes on day (d) 100 of pregnancy (p.c.) and 2 to 21 days post partum (p.p.) in pigs, **a** and **b** significantly different = le < 0.01.

Interestingly, a marked increase in the secretion of ACTH is observed by lymphocytes obtained from pregnant cows and pigs (Fig. 2). In the pig, levels return to the baseline levels seen during cycle after the weaning of piglets (Fig. 3). In the cow, however, the basal levels are retrieved

within two weeks after parturition. Furthermore, the ACTH secretion is strictly correlated to nitric oxide (NO) release from lymphocytes (13).

The increased lymphocytic ACTH secretion beginning around day 7 of gestation (13) correlates well to the time at which bovine embryo expresses the paternal major histocompatibility complex molecules and is regarded as the time of immunological recognition of pregnancy (14). Another factor which is reported to be important for fetal survival is Interferon tau (IFN-τ), an antiluteolytic factor produced from trophoblast and is observed in maternal plasma from day 12 to day 35 of pregnancy. The peak levels can be measured around days 15-19 (15). ACTH production enhances around day 7 of pregnancy and maximum levels which reaches up to 600% that of non-pregnant animals are measured between days 90-100 after conception. This may indicate that the initiation of lymphocytic ACTH release is not dependent to IFN-tau secretion.

Progesterone plays apparently little or no role in the elevation of ACTH secretion from lymphocytes during pregnancy. Support for this assumption comes from the findings that lymphocyte harvested from animals during luteal phase or cows having ovarian cysts with low or high plasma progesterone levels produce very low amounts of ACTH.

Mechanisms controlling ACTH release from immune cells are still unknown, and contradictory data exist concerning CRH stimulation of ACTH release from human leukocytes (16). In our studies, a stimulatory effect of CRH on ACTH secretion from lymphocytes is noticed only in non-pregnant cyclic animals. Another observation in our present study is that PHA-M a plant lectin that stimulates lymphocytes by cross-linking B and T cell receptors, is also not effective in stimulating ACTH release from lymphocytes in pregnant animals. However, it enhances ACTH secretion from lymphocytes harvested in cyclic animals. On the other hand, we have shown that lymphocytic ACTH release is stimulated during a long-term stressful transportation or housing conditions (17). This indicate that the lymphocytes are refractory to secretagogues such as PHA-M and CRH during pregnancy or that the mechanisms regulating ACTH secretion from lymphocytes differ during pregnancy from those during the estrous cycle or both.

Multiple mechanisms have been proposed for tolerance of lymphocytes to fetal allograft (18, 19). It has been reported that downregulation of MHC class I and expression of Fas ligand on placenta are not the sole events for successful allogeneic pregnancy. Thus other factors must prevent a harmful maternal immune response (20-23). Ability of lymphocytes to secrete immunomodulators could be one of the possible mechanisms. Peripheral mononuclear cells have been shown to produce hCG as early as day 7-11 after embryo transfer. Production of hCG remains high during human pregnancy and is presumed to be vital for immunotolerance (24).

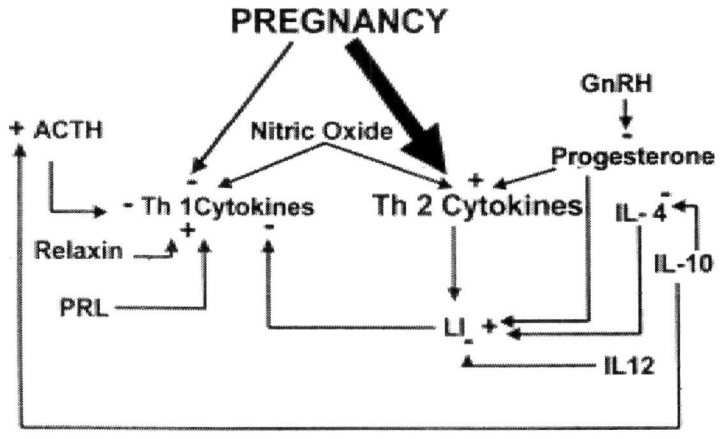

Figure 4 : Proposed role of ACTH, nitric oxide and cytokines in maintenance of pregnancy.

During pregnancy, there is a strong shift to T-helper 2 cytokines resulting in less production of Th-1 cytokines (25).This is proposed as an important mechanism of fetal survival, as increase in pro inflammatory Th-1 cytokines is associated with abortions (26-28). Both ACTH and NO are reported to cause a shift to Th-2 cytokines by increasing Th-2 cytokines and decreasing Th-1 cytokines (9, 29, 30) observed a strong positive correlation between production of ACTH and NO from lymphocytes during different reproductive states. The net result of cytokine-mediated self amplification and regulation is that once a T-cell immune response begins to develop along one pathway, namely Th-1 or Th-2, it tends to become progressively polarized in that direction (31).Th-2 shift can be induced by the presence of fertilized ovum (32) which progresses as the gestation proceeds (Fig. 4).

A shift to Th-2 cytokines has also been reported during HIV infection (33) and interestingly, it is observed that HIV infection also leads to increased production of ACTH from lymphoid cells (34, 35). Increased NO production from lymphocytes together with ACTH, as observed in the present study can act synergistically to skew the T-helper cytokines balance to Th-2.

The observed increase in the secretion of ACTH from peripheral lymphocytes is not reflected in the circulation, as plasma ACTH levels does not differ between non-pregnant and mid-pregnant cows. An increase in circulating ACTH levels could be obstructive by stimulating glucocorticoid secretion and causing a strong general immunosuppression, which can possibly predispose maternal immune system to infections. ACTH produced from lymphocytes could, however, have significant immunomodulatory effects by auto/paracrine mechanisms, as immune cells also express receptors for ACTH (8, 9). In addition, it has been shown that lymphocytes co-cultured with adrenocortical cells can stimulate adrenal androgen secretion via cell contact (36). The elucidation of significance of lymphocytic ACTH and NO secretion during pregnancy and clarification of trigger mechanisms need further investigation. Nevertheless, early appearance of increased ACTH and NO secretion from lymphocytes sustained till the very end of pregnancy sheds new light in the area of feto-maternal immune interactions and mechanisms governing the fetal survivability.

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