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MORPHOMETRIC INVESTIGATIONS OF PLASMOCYTES AND DETECTION OF IMMUNOGLOBULINS IN THE FEMALE RAT GENITAL TRACT DURING THE OESTROUS CYCLE

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The aim of this work was to determine the numerical density of plasma cells and detect the presence of immunoglobulins in all segments of genital organs during the oestrous cycle, from which one might assess the immunological reaction of virgin female rat genital organs. Wistar stock rats were killed at different stages of the oestrous cycle. Tissue clippings of organs were stained with methyl-green-pyronine according to Brachet for light microscopy and with FITC conjugated goat anti-rat-immunoglobulins for direct immunofluorescence according to Santa-Marie. Plasma cell populations were determined with the stereological method of numerical density according to Abercrombie.

The obtained values for numerical density of plasma cells in the oviduct, uterus and vagina showed highly significant variations during the oestrous cycle ($p < 0.001$).

Plasma cells were most numerous in the oviduct in oestrus, and in the uterus and vagina in postoestrus. Immunoglobulins were present in the oviduct, uterus and vagina. The localization and intensity of positive fluorescent reactions varied during the cycle. In the oviduct, fluorescence was maximal in oestrus in the epithelium, lamina propria and subserosa. In the uterus, it was maximal in postoestrus in the epithelium, glandular epithelium and lamina propria. In the vagina, it was most pronounced in oestrus in the epithelium and lamina propria.

The high significance of variations in the observed plasma cell population and different distribution of immunoglobulins indicate immunological variation in virgin female rat genital organs during the oestrous cycle.

Key words: plasmocyte, immunoglobulins, rat, oviduct, uterus, vagina

INTRODUCTION

The immunological response of female genital organs (FGO) is specific as opposed to other local mucosal immunological systems and varies during the oestrous cycle (Parr and Parr, 1990). Such variations are induced by the influence

of endocrinological parameters, particularly oestrogen and progesterone, which affect humoral as well as local immune response in the FGO region (Wira et al., 1980, 1983; Sullivan, 1983; Wira and Stern, 1986). The existence of a secretory immune system within the FGO is demonstrated through the presence of IgA producing plasmocytes (Kutehh, et al 1990). The presence of IgA and IgG is higher during the follicular and luteal phases and lower during ovulatiion (Verman and Ferrin, 1974). During ovulation the immunesuppression is associated with the variations in response to lymphokine stimulation (Watson and Zanecosky, 1990). Within the FGO, there is locally induced production of antibodies which is independent of the distribution of immunoglobulins within the circulation (Hogarth, 1982).Plasmocytes are infiltrated in the epithelium and stroma as a response to local antigen stimulation of lymphocytes (Hussein et al., 1983). The basic immunoglobulin of the local immune system is IgA synthesized in plasma cells from where it is transferred into the epithelial cells through receptor connections and bound to the secretory component. The membrane receptor, is then transported through the cytoplasm and released into the lumen via exocytosis (Bienstock et al., 1983). A hormone is considered to have a direct effect if it can activate a pure cell population in vitro. The direct effects of hormones on cell function have long been thought to be mediated via hormone - specific surface receptors. Oestrogen can elevate antibody (IgM) production in response to T dependent antigens, enhance T - dependent B-cell proliferation and the expression of surface immunoglobulins on plasma cells (Stanisz et al., 1994). It also has a pronounced effect on IgA transport in the genitourinary tract (Wira and Sullivan, 1982). Sex hormones have been shown to affect lymphocyte traffic to mucosal regions (Canning et al., 1983).

The local immune system of the FGO undergoes cyclic variations of immune-competent cells which are affected by the influence of hormones (Hanson et al., 1983).

MATERIAL AND METHODS

Sexually mature, virgin female rats, Wistar stock, 60 days old, were killed in specific phases of the oestrous cycle. Clippings of genital organs were fixed in Carnoy fixative cooled down to 4^0C. Paraffin moulds of organs were cut serially in 5 μm thick sections and stained with methyl-green-pyronine (MGP) according to Brachet, a histochemical method for staining nucleic acids. For stereological analysis of plasma cell number the numerical density of plasma cells (Nvpc) was determined in thick sections by the method of Abercrombie using the following formula

$$Nv = Na / (t + D) \ (mm^{-3})$$

According to this formula, one needs to count N particle profiles in test area At and calculate their ratio. The thickness of the histological slide t is taken into account. The average diameter of the plasmocyte profile D is obtained by the formula

$$D = 4 / Pi \times D \ (mm^{1})$$

For stereological analysis of plasmocytes, a Weibel multipurpose test system was used (M:42) and an ocular micrometer 5:100. The results obtained were statistically processed using the variance method. The percent isplay of these data is given with respect to phases of the cycle in the investigated organs. The histological slides for determination of immunofluorescence (IF) were obtained by slicing the paraffin moulds of tissue clippings from the Carnoy fixative, 5-6 μm thick and treatment by the Santa-Marie method for direct immunofluorescence. Slices were incubated with FITC labelled goat anti rat immunoglobulins (Nordic immunological laboratories). Dissolved conjugate in 1:10 titre was applied to the histological slides using a micropipette in 15 μl portions. The slides were washed in PBS and prepared for microscopy by mounting the cover glass using glycerine-PBS. The sections were photographed under the microscope Univar G, Reichert Austria using Ectachrome 160 film.

RESULTS

Plasmocytes stained by the MGP technique were histologically observed in all parts of the oviduct and especially in lamina propria and the serosal envelope. Nvpc values, obtained by the stereological processing of data measured during the whole cycle, reached 21% in prooestrus. The highest measured value occurred in oestrus, 30,5%, the most pronounced in the serosal envelope area. In postoestrus, NvPc was 29,9%, and decreased further in dioestrus to 18,6%. Plasmocytes were found in large quantities in lamina propria right under the basal membrane (Figure 1, A).

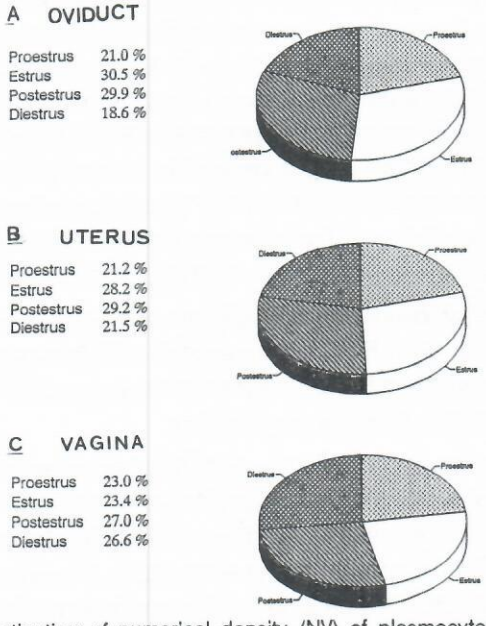

Figure 1. Percent of distinction of numerical density (NV) of plasmocytes in the phases of the oestrous cycle

The plasmocytes and Nvpc present the in uterus, during the rat oestrous cycle, demontrated relevant histological changes mostly in the endometrium. In prooestrus, because of greater proliferation, augmented pyroninophility of the cytoplasm of the cellular elements, was noted, as well as intensive staining of DNA.Plasmocytes were present in the lamina propria and between the tubular glands. In oestrus, plasmocytes were found in the interglandular stroma. An increased number of ribosomes in their cytoplasm was observed as a very pronounced pyroninophility. Endometrium thickness was generally increased in postoestrus and numerous plasmocytes were observed in lamina propria; pyroninophility was greatly reduced, and the mean diameter (D) of plasmocytes was 14,2 μm. Plasmocytes were located in groups around glandula uterina, and between the glandular cells. In dioestrus, the endometrium was in smooth regression and plasmocytes were observed mostly in the interglandular stroma in the direction of uterine gland propagation.

All the obtained stereological measurements are displayed as pie charts where the area of the whole circle represents the investigated phenomenon, and the areas of particular segments (sectors of the circle) parts of that whole, i. e. phenomenon. The plot shows that the Nvpc is the highest in postoestrus, 29,9%, then in oestrus,28.2%, falling to 21,5% in dioestrus, and 21.2% in prooestrus (Figure 1,B).

Dynamic changes of Nvpc and location of plasmocytes were observed during the cycle, in the vagina. Cytoplasm pyroninophility indicated a very active layer of epithelium basalis, and in prooestrus the plasmocytes were found especially in papillae of lamina propria, with 23% presence in this phase. In oestrus, a uniform distribution of plasmocytes was observed in lamina propria, and Nvpc was 23,4%. Plasmocytes in postoestrus were mature and occurred in extended intercellular epithelial spaces and in lamina propria, their cytoplasm being extremely pyroninophilic. In dioestrus, plasmocytes were located in lamina propria especially near the basal membrane. In the secretory phase of the cycle, Nvpc increased to 27.0% in prooestrus, and 26,0% in dioestrus (Figure 1,C).

The presence of different immunoglobulins in tissues of female genital organs was detected using FITC-conjugated G-A-M immunoglobulin which adheres to imunoglobulins of different classes of the appropriate animal species, in this case to rat blood serum immunoglobulins.

In the oviduct, the immunofluorescent reaction was positive and changed during the oestrous cycle. In prooestrus, it was very weak in stroma, and the epithelium was negative. In oestrus, a diffuse reaction in stroma was pronounced. It was negligible and non-specific in the epithelium. In postoestrus, the reaction was positive both in the stroma and in the epithelium while in dioestrus the reaction was negligible. (Figure 2)

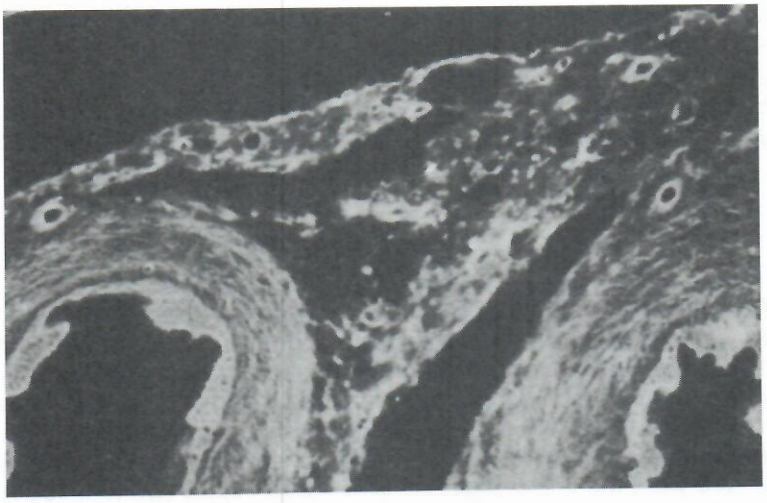

Figure 2. Postoestrous oviduct, direct fluorescence, 250 X. Positive reaction in postoestrus in stroma and epithelium

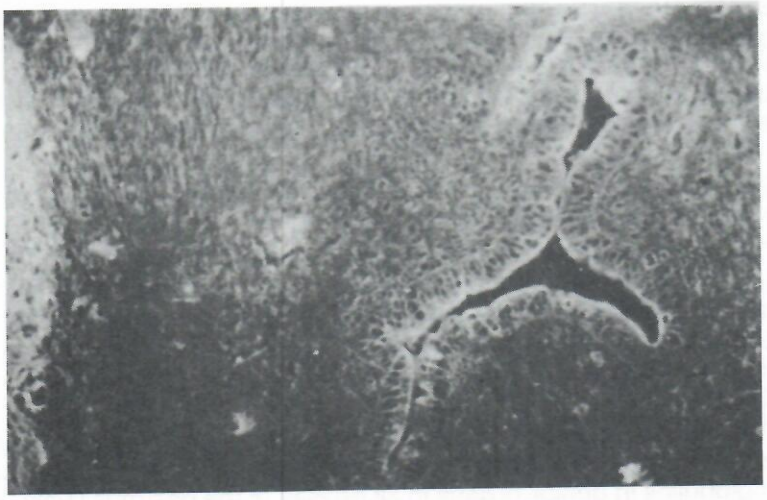

Figure 3. Postoestrous uterus, direct fluorescence, 250 X. Strong fluorescent reaction in lamina propria, glands and apical regions of the epithelium

The uterus gave a positive immunofluorescent reaction during the whole cycle. Small, oval, fluorescent areas were observed in proestrus, in lamina

propria. In oestrus, there were more immunofluorescent positive areas. In postoestrus, lamina propria, epithelium of the uterine glands and epithelium of the endometrium were fluorescent. Fluorescence of both epithelium and lamina propria was reduced in dioestrus (Figure 3).

The vagina gave a positive reaction in lamina propria and a negative reaction in the epithelium in all phases of the cycle. The weakest immunofluorescent reaction was in prooestrus, and it increased in the other phases of the cycle.

DISCUSSION

The values obtained for numerical density of plasma cells within the FGO of virgin rats showed significant variations during the oestrous cycle. Nvpc increased in the proliferative phase and reached its maximum during oestrus in the oviduct, and during postoestrus in the uterus and vagina. The highest Nvpc is in the oviduct (11.94×10^4 mm^{-3}). The infiltration by granulocytes, plasmocytes and lymphocytes occurred under the influence of steroids, and the most pronounced were the immunoglobulins of the IgA class (Cohen, 1984). Certain authors consider that the increase of plasmocytes in the secretory phase is influenced by progesterone (Murdoch et al., 1982), while the others claim that progestorene lowers the level of immunoglobulins in genital organs and their lowest number is in dioestrus (Sullivan and Wira, 1983; Wira and Stern, 1986). Progesterone reduces the immunological response of the uterus (Stephenson and Hansen, 1990). The immunological sensitivity of the uterus is highest in the luteal phase (Alexander, 1990). The other group considers that the lowest immunological sensitivity is in oestrus (Parr and Parr, 1991). Oestrogen stimulation was found to increase the presence of immunoglobulins in proestrus and oestrus (Wira and Sandoe, 1980; Wira and Sullivan, 1982; Sullivan and Wira, 1983).

Several authors relate the endocrine system and the immunereactivity of female genital organs (Tabibzadeh, 1990, 1991).

The results of our investigations obtained by measuring direct immunofluorescence, indicate increased amounts of immunoglobulins in the rat oviduct, uterus and vagina during oestrus. The pronounced presence continues in postoestrus,and decreases in dioestrus although in the vagina it is still well expressed, which complies with the results of other authors (Wira et al., 1986, 1987, 1989).

The direct immunofluorescence method which applies polyspecific antiserum to rat FITC conjugated immunoglobulins, indicated the presence of immunoglobulins in genital organs in all phases of the oestrous cycle. Intensity of the reaction in the oviduct, uterus and vagina was more pronounced during oestrus and postoestrus. It should be kept in mind that the intensity of the fluorescent reaction is not directly proportional to the quantity of immunoglobulins in the given tissue, but can be also a result of an artefact, i. e. non specific staining of the tissue.

Our results undoubtedly indicate that the quantity of immunecompetent cells and presence of immunoglobulins in certain parts of female rat genital organs is changed with respect to the phase of the oestrous cycle. This is directly under the influence of different hormones, which direct the morphological changes of the genital tract, too. We consider that within the framework of further considerations of genital tract dynamics which proceed under endocrine control it is relevant to follow immunological parameters as an inseparable system in the functioning of the female rat reproductive system.

REFERENCES

1. *Alexander, N. J.* 1990. Reproductive immunology: relevance to infertility practice. *Arch. Immun. Theor. Exp. (Warszaw). 38 (1-2),* 23-30.
2. *Bienenstock, J., Befus, A. D., McDermott, M., Mirski, S., Rosenthal, K., Tagliabue, A.* 1983. The mucosal immunological network. *Annals of the N. Y. Acad.of Sci. 409,* 164-171.
3. *Canning, M. B.* 1983. Hormonal regulation of immunoglobulins and plasma cells in the mouse uterus. *J. Endocrinol. 97,* 401-424.
4. *Cohen, J.* 1984. Immunological aspects of sperm selection and transport. *Butterworths, London.* 77-89.
5. *Hansson, L. A., Allstedt, B., Anderson, B., Carlsson, M. F.* 1983. Mucosal immunity. *Annals of the N. Y. Acadey of Sciences. 409,* 1-22.
6. *Hogarth, P. J.* 1982. Immunological aspects of mammalian reproduction. *Blackie & Son Ltd., Glasgow.*
7. *Hussein, A. M., Newby, T. J., Bourne, F. J.* 1983. Immunohistochemical studies of the local immune system in the reproductive tract of the sow. *Journ. Reprod. Immunol. 5,* 1-15.
8. *Kutehh, W. H., Blackwell, R. E., Gore, H., Kutehh, C. C., Carr, B. R., Mestecky, J.* 1990. Secretory immune system of the female reproductive tract. II. Local immune system in normal and infected fallopian tube. *Fertil. - Steril. 54(1),* 51-55.
9. *Murdoch, A., Buckley, C., Fox, H.* 1982. Hormonal control of the secretory immunal system of the human uterine cervix. *Journ. Reprod. Immunol.* 4, 23-30.
10. *Parr, E. L., Patt, M. B.* 1990. A comparison of antibody titers in mouse uterine fluid after immunization by several routes and the effect of the uterus on antibody titres in vaginal fluid. *Journ. Reprod. Fertil. 89(2),* 619-625.
11. *Parr, M. B., Parr, E. L.* 1991. Langerhans cells and T lymphocyte subsets in the murine vagina and cervix. *Biol. Reprod. 44(3),* 491-498.
12. *Stanisz, A. M., Galina Kataeva, Bienenstock, J.* 1994. Hormones and local immunity. Int. Arch. Allergy Immunol. 103, 217-222.
13. *Stephenson, D. C., Hansen, P. J.* 1990. Induction by progesterone of immunosuppressive activity in uterine secretions of ovariectomized ewes. *Endocrinology. 126(6),* 3168-3178.
14. *Sullivan, D. A., Wira, C. R.* 1983. Estradiol regulation of secretory component in the rat uterus. *Annals of the N. Y. Academy of Sciences. 409,* 882-884.
15. *Tabibzadeh, S.* 1990. Immunoreactivity of human endometrium: correlation with endometrial dating. *Fertil.-Steril. 54(4),* 624-631.
16. *Tabibzadeh, S.* 1991. Distinct subsets of stromal cells confined to unique microenvironments in human endometrium throughout the menstrual cycle. *Amer. Journ. Repr. Immum. 26(1),* 5-10.
17. *Vaerman, J. P., Ferin, J.* 1974. Local immunological response in the vagina, cervix and endometrium. *Diezfalusy. 281-305.*
18. *Watson, E. D., Zanecosky, H. G.* 1990. Immunosupressive properties of follicular fluid from preovulatory horse follicles. *Journ. Reprod. Fertil. 89(2),* 627-632.

19. *Wira, C. R., Sandoe, S. P.* 1980. Hormonal regulation of immunoglobulins: influence of estradiol on immunoglobulins A and G in the rat uterus. *Endocrinol, 106,* 1020-1026.

20. *Wira, C. R., Sullivan, D. A.* 1982. Effect of estradiol and progesterone on the secretory immune systemin the female genital tract. *Adr. Exp. Med. Biol. 138,* 99-111.

21. *Wira, C. R., Sullivan, D. A., Sandoe, C. P.* 1983. Estrogen mediated control of the secretory immune system in the uterus of the rat. *Annals of the N. Y. Acad. of Sciences. 409,* 534-551.

22. *Wira, C. R., Stern, J. E.* 1986. Estradiol regulation of the secretory immune system in the female reproductive tract: IgA in uterine and vaginal secretions of rats following portacaval anastomosis. *Journ. Steroid. Biochem. 24(1),* 33-37.

23. *Wira, C. R., Sandoe, C. P.* 1987. Specific IgG and IgA antibodies in the secretions of the female reproductive tract: effect of immunization and estradiol on expression of this response in vivo. *Journ. Immunol. 138(12),* 4159-4164.

24. *Wira, C. R., Sandoe, C. P.* 1989. Effects of uterine immunization and estradiol on specific IgA and IgG antibodies in uterine, vaginal and salivary secretions. *Immunol. 68,* 24-30.

MORFOMETRIJSKA ISTRAŽIVANJA PLAZMOCITA I OTKRIVANJE IMUNOGLOBULINA U ŽENSKOM GENITALNOM TRAKTU PACOVA TOKOM POLNOG CIKLUSA

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SADRŽAJ

Cilj ovog rada je da odredi numeričku gustoću plazmocita i prisustvo imunoglobulina u svim segmentima genitalnih organa tokom polnog ciklusa, na osnovu čega bi se imao uvid o mogućem stepenu imunološke reakcije polnih organa virgilnih ženki pacova. Pacovi Wistar soja su žrtvovani u različitim fazama polnog ciklusa. Tkivni isečci organa su bojeni Methyl-green pyronine metodom po Brachet-u za svetlosnu mikroskopiju, i sa kozjim antipacovskim imunoglobulinima obeležeenim FITC-om za direktnu imunofluorescenciju po Santa-Marie. Populacija plazmocita je određivana stereološkom metodom za numeričku gustoću, metodom po Abercrombie-u.

Dobijene vrednosti numeričke gustoće plazmocita u toku polnog ciklusa u jajovodu, materici i rodnici pokazuju visoko signifikantne promene ($p < 0.001$). Prisustvo plazmocita je najviše kod jajovoda u estrusu, a kod materice i rodnice u postestrusu. Imunoglobulini su prisutni u jajovodu, materici i rodnici, a razmeštaj i intenzitet fluorescentne reakcije se menja tokom ciklusa. U jajovodu, fluorescencija je maksimalna tokom estrusa u epitelu, lamini proprii i subserozi. U uterusu, maksimalna je tokom postestrusa u epitelu, žlezdanom epitelu i krznu. U rodnici, najviše je izražena tokom estrusa u epitelu i lamini proprii.

Visoka signifikantnost promena u posmatranoj populaciji plazmocita, kao i različita zastupljenost imunoglobulina ukazuje na promenljivost imunološkog statusa u polnim organima virgilne ženke u toku polnog ciklusa.