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Mononuclear Cell Subpopulations in Human Follicular Fluid From Stimulated Cycles

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ABSTRACT: **The study of lymphocyte subsets from human follicular fluid (FF) provides an opportunity to evaluate immunological features of the ovary. We investigated the mononuclear cell subsets in FF and peripheral blood obtained at the time of laparoscopy from ten in vitro fertilization (IVF) patients. Midcycle nonpregnant peripheral blood was used as the control. A marked increase in the proportion of monocytes ($CD14^+$) was observed in FF. Although FF was enriched with $CD8^+$ lymphocytes, a decrease in the proportion of $CD4^+$ lymphocytes was observed. "Memory" T cells in FF, identified by the $CD4^+$ $CD45R^-$ phenotype, predominated over "naive" T cells ($CD4^+$ $CD45R^+$) at a ratio of 2:1, which differs from the ratio yielded by control blood samples (1:1). The percentage of activated T cells ($CD3^+$ $HLA\text{-}DR^+$ cell) increased significantly in FF. When lymphocyte subsets were studied in the peripheral blood of IVF patients, changes similar to but less significant than those in FF were found. These data support the concept that lymphocytes play an important role in ovarian physiology. (Am J Reprod Immunol. 1990; 22:127–129.)**

Key words: Flow cytometry, Lymphocyte subsets, Monocytes

INTRODUCTION

Gonadal steroids[1] and hormones such as human chorionic gonadotrophin[2] (hCG) used in ovarian stimulation are known to have immunological properties. This may be reflected as changes in mononuclear cell subpopulations because hormonal receptors are selectively distributed among lymphocyte subsets, such as $CD4^+$ (helper/inducer) T-lymphocytes and $CD8^+$ (suppressor/cytotoxic) T-lymphocytes.[3]

Previous studies,[4,5] although with discrepant results, have shown that mononuclear cells are present in follicular fluid (FF) from in vitro fertilization (IVF) patients. Hill et al.[4] reported a preponderance of $CD8^+$ cells in FF; however, Droesch et al.[5] showed an overall increase in $CD4^+$ cells over $CD8^+$ lymphocytes in FF, as compared with peripheral blood.

Recently, $CD4^+$ T-lymphocytes were separated into at least two different maturational stages: $CD4^+$ cells expressing the CD45R marker have been reported to include "naive" cells, which do not proliferate in response to antigen in vitro, and $CD4^+$ $CD45R^-$ cells, which exhibit a "memory" proliferative response to soluble recall antigen and provide help for antibody production.[6] Also, the expression of histocompatibility leukocyte antigen class II (HLA-DR) appears to be linked to T-cell activation.[7]

In an attempt to broaden the understanding of ovarian immunoregulation, we studied the lymphocyte subsets, including $CD4^+$ subpopulations and $HLA\text{-}DR^+$ T cells, in peripheral blood and FF obtained from women undergoing laparoscopic oocyte harvesting for IVF.

MATERIALS AND METHODS

FF and peripheral blood samples were obtained at the time of laparoscopy from ten normal menstruating women with tubal infertility. The patients were treated with clomiphene citrate, human menopausal gonadotropin, and hCG for ovarian stimulation before collecting the oocytes for IVF and embryo transfer. Induction of multiple ovulation for IVF, monitoring technique, and ovum pickup were as described previously.[8] Oocytes were graded as mature, intermediate, or immature according to oocyte maturity and the degree of expansion of the corona-cumulus complex.[9] Midcycle nonpregnant peripheral blood was used as the control. Only FF samples free of visible blood contamination were used. FF from follicles that contained a mature oocyte were pooled, and peripheral blood and FF were processed simultaneously.

Monoclonal Antibodies (MAb)

The MAbs used in this study (Becton Dickinson, Mountain View, California) are shown in Table I.

Immunofluorescent Phenotypic Analysis

Mononuclear cells were isolated by Ficoll-Hypaque centrifugation. Double staining was performed by pairing a fluorescein isothiocyanate (FITC)-conjugated MAb against one surface antigen, with another MAb, directly conjugated to phycoerythrin (PE), directed against the second antigen. Mononuclear cells (8×10^5) were suspended in 1 ml of RPMI 1640 (GIBCO, Grand Island, NY). Then 50 µl of cell suspension were incubated for 30 min on ice with 5 µl of a FITC-conjugated anti-CD45, anti-CD3, or anti-CD4 MAb, and 5 µl of PE-conjugated anti-CD14, anti-CD19, anti-CD8, anti-CD45R, or anti-HLA DR MAb. After incubation, cells were washed twice with saline buffer and fixed in 1% p-formaldehyde/0.15 M phosphate buffer saline solution (pH 7.2) and stored until analysis. Cells were analyzed by flow cytometry (FACscan IV, Becton Dickinson Monoclonal Center, Mountain View, California).

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TABLE I. Cell Specificity and Characteristics of MAbs

MAb	Specificity
Leu4	CD3 (pan T cell)
Leu3a	CD4 (helper/inducer T cell)
Leu2a	CD8 (suppressor/cytotoxic T cell)
LeuM3	CD14 (monocytes)
Leu12	CD19 (B cell)
Antileucocyte	CD45 (pan leucocyte)
Leu18	CD45R ($CD4^+$ $CD45R^+$ = "naive" T cell; $CD4^+$ $CD45R^-$ = "memory" T cell)
Anti-HLA-DR	HLA-DR ($CD3^+$ $HLA\text{-}DR^+$ = activated T cell)

One-way analysis of variance was used to test for statistical significance.

RESULTS

Mononuclear cells were present in FF at a mean concentration of 2.08×10^5 cells/ml. The percentage of monocytes ($CD\ 14^+$ cells) was significantly increased in FF (37.7 ± 7.9%), as compared with peripheral blood from IVF patients (5.4 ± 1.0%; $P<0.001$). Table II clearly demonstrates that FF from IVF patients is enriched with $CD8^+$ T cells, the percentage of $CD8^+$ cells being twofold higher in FF than in peripheral blood from the control group. The percentages of $CD8^+$ T cells also increased in blood of the same group of patients ($P<0.01$).

$CD4^+$ cell comprised 41.5% of lymphocytes in control blood, whereas only 31.1% ($P<0.01$) and 18.4% ($P<0.001$) of the cells were $CD4^+$ in blood and FF from IVF patients, respectively. In contrast, there was no difference in the concentration of T cells ($CD3^+$ cells), because the decrease in $CD4^+$ T cells was compensated by for the increase in the proportion of $CD8^+$ T cells.

"Memory" T cells identified by the $CD4^+$ $CD45R^-$ phenotype in FF and blood from IVF patients predominated over "naive" T cells ($CD4^+$ $CD45R^+$) at a ratio of 2:1, which differed from the ratio obtained in control blood (1:1).

We found no alterations in the proportions of $CD19^+$ cells (B-lymphocytes) in this study. Nevertheless, the percentage of activated T cells ($CD3^+$ $HLA\text{-}DR^+$ cell) was significantly increased in peripheral blood ($P<0.01$) and FF ($P<0.001$) from IVF patients.

DISCUSSION

The results of this study confirm previous findings that mononuclear cells are present in human FF obtained from stimulated cycles[4,5] and show an increase in the proportion of monocytes ($CD14^+$ cells) in FF. Evaluations of the percentage of T-lymphocyte subsets in FF have yielded conflicting findings, including a predominance of $CD8^+$ T cells[4] and an increase in the proportion of $CD4^+$ T cells.[5] This discrepancy in T-lymphocyte subsets may be due to the use of fluorescence microscopy, as individual variations in the threshold level for positivity of fluorescent cells may partially account for the conflicting results. To our knowledge, this is the first study of mononuclear cells in FF carried out by automated flow cytometry analysis. The finding of a significant increase in the percentage of $CD8^+$ T lymphocytes and a decrease in the proportion of $CD4^+$ T cells are in agreement with Hill et al.[4] and supports previous evidence of immunoregulation in the follicle. This factor may be influential in the development of the ovarian follicle[10] and in the maintenance of self-tolerance.[4]

The number of activated T-lymphocytes increased in FF, as indicated by the elevated proportion of $CD3^+$ $HLA\text{-}DR^+$ cells. The "naive" T cells ($CD4^+$ $CD45R^+$) mature into functional "memory" helper cells ($CD4^+$ $CD45R^-$) by activation mediated by T cell-derived lymphokines.[6] We observed an increase in the memory/naive T cell ratio in FF. These findings suggest that $CD4^+$ cells in FF are activated, due possibly to the participation of $CD4^+$ lymphocytes in an immune response against antigenic ovarian components. When T-cell subpopulations are experimentally altered, autoimmune diseases are induced in different organs, including the ovary.[11] Our results suggest that this response is controlled in the ovarian follicle by a selective reduction of $CD4^+$ lymphocytes and an increase in suppressor T cells. The raised numbers of monocytes in FF may be an additional mechanism for this immunosuppressive state, as release of monocytic prostaglandins inhibit lymphocyte responses.[12]

The mechanism leading to these cellular changes are still unknown. Lymphocytes incubated in vitro with FF can inhibit the response of autologous lymphocytes to mitogens, which suggests that FF can induce suppressor cells in vitro.[13] Different inducers of suppressor cells, e.g., histamine[14] and prostaglandin E,[15] are present in FF. In addition, hCG appears to reduce the proportion of $CD4^+$ cell[16] and induce suppressor T cells,[2] both mechanisms by which hCG may contribute

TABLE II. Percentage of Lymphocyte in FF and Peripheral Blood of IVF Patients*

	Peripheral blood		
Cells	Control (n = 10)	IVF women (n = 10)	FF (n = 10)
$CD19^+$ (B-Lymphocytes)	10.1 ± 5.6	7.7 ± 1.7	11.0 ± 5.3
$CD3^+$ (T-lymphocytes)	65.3 ± 10.2	64.0 ± 5.2	58.7 ± 13.7
$CD3^+$ $HLA\text{-}DR^+$ (activated T cell)	2.1 ± 2.0	12.5 ± 2.7*	25.2 ± 5.6**
$CD4^+$ (Helper/inducer T cell)	41.5 ± 8.1	31.1 ± 5.6*	18.4 ± 7.0**
$CD8^+$ (suppressor/cytotoxic T cell)	23.4 ± 7.0	34.4 ± 3.8*	46.7 ± 8.4**
$CD4^+$ $CD45^+$ ("naive" T cell)	20.5 ± 10.1	13.2 ± 4.9	3.8 ± 2.1**
$CD4^+$ CD45R ("memory" T cell)	18.2 ± 11.2	21.8 ± 7.5	7.9 ± 2.6**

Values represent % mean ± SD.
*$P < 0.01$ vs. control group.
[b]$P < 0.001$ vs. control group.

to the immunosuppression responsible for the survival of the fetal transplant.

Ovarian granulosa cells express HLA-DR antigens,[17] and this expression can be increased by in vitro exposure to τ-interpheron.[18] The factor(s) responsible for the induction of HLA class II antigen expression on ovarian granulosa cells in vivo may be the same as in the expression on lymphocytes from FF. The notable presence of HLA class II antigens in the ovarian follicle could be related to follicular development, as suggested Bukovsky et al.[10] Moreover, $CD8^+$ T-lymphocytes are able to express HLA-DR antigens in the context of an immunosuppressive response in order to maintain self-tolerance.[19]

The changes in lymphocyte subsets in the peripheral blood of IVF patients reflect more significant changes at the ovarian level, inasmuch as the former may result from different concentrations and/or rates of production of immunoregulatory factor(s) by the ovarian follicle.

CONCLUSIONS

The data presented here suggest an immunoregulation in the ovarian follicle. This factor may be influential in the development of the ovarian follicle and in the maintenance of self-tolerance.

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