蛻膜中 T 淋巴球 CD25 的表現和懷孕成功與否的關係
計畫編號：NSC-90-2314-B-002-290
執行期限：89 年 8 月 1 日至 90 年 7 月 31 日
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ABSTRACT

In the previous study, we demonstrate that the proportion of activated T cells ($CD69^+CD3^+$ and $HLA\text{-}DR^+CD3^+$) is higher in the endometrium and decidua after the luteal phase throughout early pregnancy compared with that of peripheral blood. However, there was no difference in the proportion of $CD25^+CD3^+$ lymphocytes between the endometrium and peripheral blood. In addition, the level of CD25 on $CD4^+$ and $CD8^+$ T lymphocytes is still unchanged, although levels of CD69 and HLA-DR are markedly increased. We also elucidate that the amount of activation molecules on local T lymphocytes is down-regulated after pregnancy compared with that during the luteal phase. In this study, we further verify that these decreases are restored for anembryonic pregnancies with both normal and abnormal karyotyping abortus. Furthermore dual activation marker analysis demonstrated that the expression of CD25 is dissociated from CD69 and HLA-DR on the same decidual lymphocytes. Because IL-2Rα plays a pivotal role in the development and propagation of functional T cells, its depressed expression may result in maternal tolerance to the fetal allograft.

INTRODUCTION

During a normal pregnancy, allogenic fetal tissues are exposed to the maternal immune system. Normally rejection reactions develop after allogenic recognition following the principle of transplantation immunology. Although the immune system is functional in the uterus and the embryo expresses paternal major histocompatibility complex (MHC) molecules, the conceptus nevertheless escapes the deleterious effect of maternal rejection. The exact mechanisms involved in maternal tolerance of foreign fetal antigens during early pregnancy have not been fully explored, but may be due to local tolerance or even perhaps suppression of maternal immune system.

T cells must change from a resting to an activated state during immune responses and lead to de novo synthesis of interleukin 2 (IL-2) and expression of IL-2 receptors (IL-2Rs) (Smith, 1988). There are three main IL-2 receptor subunits: IL-2Rα, IL-2Rβ, and IL-2Rγ (Waldmann, 1991). The IL-R2α is identified by the anti-CD25 monoclonal antibody. Interaction of IL-2 and its receptors triggers cellular proliferation culminating in the emergence of effector T cells that are required for the full expression of immune responses.

In this study, we further quantitatively measured and compared the molecular levels of activation markers on lymphocyte subpopulations in deciduae and peripheral blood of normal pregnancies and of anembryonic pregnancies with normal and abnormal karyotyping to elucidate the expression of T cell activation markers and to determine whether CD25 expression is dissociated from that of other activation markers in the same cells or different cells.

MATERIALS AND METHODS

Subjects

Thirsty pregnant women who had elective abortions of normal pregnancies due to multiparity, and 30 women who had anembryonic pregnancies of between 6 and 10 weeks of gestational age were enrolled in the study with informed consent and under the approval of the ethics committee of our hospital. An anembryonic pregnancy was identified by sonography, which failed to show a fetal pole when the gestational sac was greater than 25 mm.

Specimens

Decidual tissue and peripheral blood samples were taken from each pregnant

woman at the time of abortion. The fetal chorionic villi were karyotyped when anembryonic pregnancies were diagnosed. The decidual tissue was macroscopically separated from the chorionic villi, cut into small pieces, and passed through a 1.9-mm mesh to remove the residual blood without enzymatic treatment. These samples were then filtered through a 45.7-μm stainless steel mesh to remove tissue debris. The filtered solution was layered over a Ficoll-Paque PLUS gradient and centrifuged for 45 min. at 400 *g*. An enriched cell suspension was collected. Peripheral blood mononuclear cells (PBMCs) were also isolated by Ficoll-Paque PLUS sedimentation.

Immunophenotypic Analysis

The monoclonal antibody matchings (Becton Dickinson, San Jose, CA) were used as previous report. Furthermore, dual activation marker analysis of T lymphocytes were matched as anti-CD69/CD25/CD4, anti-CD69/CD25/CD8, anti-HLA-DR/CD25/CD4, and anti-HLA-DR/CD25/CD8. Three-color flow cytometric analyses were performed using a FACScan cytofluorimeter (Becton Dickinson) with computer interfacing to CellQuest software. Triggering was set on the forward scatter channel, and the threshold was adjusted to exclude debris. LeucoGATE was used to measure the proportion of lymphocytes in the sample being studied without any scatter gates. Then, the gate was set around the lymphocytes ($CD45^+CD14^-$) to exclude other cells from analysis. The Simultest control (mouse IgG1 FITC + IgG2a PE) was used for background control. For each experiment, 5000 endometrial or decidual lymphocytes and 10,000 peripheral blood lymphocytes were evaluated.

Mitogenic Activation of T Cells in Peripheral Blood

We used phytohemagglutinin (PHA) to activate peripheral blood mononuclear cells of 15 pregnant cases. Briefly, PHA (10 μg/mL) was added to 1 mL lymphocyte-enriched cell suspensions after collection, which then were incubated at 37 °C in a 5% CO_2-humidified incubator for 24 hours. The trend and correlation of surface markers after full activation was further analyzed by three-color flow cytometry as mentioned above.

Statistical Analysis

All results are expressed as the mean ± SD. Statistical significance was tested using paired and nonpaired Student *t*-test. A p value of less than 0.05 was considered significant.

RESULTS

Expression of Activation Markers in the Subpopulation of T Cells of Normal and Anembryonic Pregnancies

Among 30 abortuses of anembryonic pregnancies, there were 12 cases with aberrant chromosomes, most of which were autosomal trisomies. There was no difference in the mean fluorescence intensity (MFI) distribution of activation markers on the decidual T lymphocytes between anembryonic pregnancies with normal and abnormal karyotypes (Table I). We demonstrated that these MFIs of the local lymphocyte subpopulation are significantly reduced as pregnancies develop through the luteal phase (Figure 1). However, the MFIs of these lymphocyte subpopulation significantly increased again in anembryonic pregnancies, irrespective of their karyotypes.

In this study, there is no statistical difference existed in the MFI of CD25 between PBMCs and decidual lymphocytes during a normal pregnancy (Figure 1). Although the MFI of CD25 on local $CD4^+$ lymphocytes was significantly reduced after pregnancy compared with that in the luteal endometrium, it was restored for anembryonic pregnancies. Nevertheless, there was no significant change in the MFI of CD25 on $CD8^+$ lymphocytes between normal and abnormal pregnancies.

Co-expression of Activation Markers on Decidual $CD4^+$ or $CD8^+$ T Cells

To elucidate whether the dissociation of expression in activation markers occurs in different populations of T cells or on the same cell, we analyzed dual activation markers in the combination of CD25 with CD69, and CD25 with HLA-DR on decidual $CD4^+$ or $CD8^+$ T cells. Results demonstrate

that during pregnancy, most T cells expressed high MFI of CD69 and HLA-DR, but not for CD25, irrespective of $CD4^+$ or $CD8^+$ lymphocytes (Figure 2A). Furthermore, there was no difference when anembryonic pregnancies occurred. The expression of CD25 was dissociated from CD69 and HLA-DR on decidual T lymphocytes and this dissociation of activation markers occurred on the same cells rather than in different populations.

Immunophenotyping of PBMCs from Women with Normal Pregnancies after PHA Stimulation

To distinguish whether this dissociation of activation markers is due to systemic alterations of the immune system or to local effects, we used PHA to stimulate PBMCs from patients with normal pregnancies. After 24 hours of activation, the MFIs of CD25, CD69, and HLA-DR had simultaneously increased in both $CD4^+$ and $CD8^+$ groups (Figure 2B). These results indicate that after activation, T cells derived from PBMCs of a normal pregnancy are able to express simultaneously CD25, CD69 and HLA-DR activation antigens. However, in the decidua of normal pregnancies, CD25 expression is selectively down-regulated.

DISCUSSION

In this study, an analysis of dual activation markers during normal pregnancy has demonstrated that the activation profile of CD25 is dissociated from that of CD69 and HLA-DR on the same cells. This means that CD25 expression is greatly decreased or inhibited even though T cells are highly activated by the intense expression of CD69 and HLA-DR molecules. Therefore, a selective down-regulation in IL-2 receptor expression appears to be present in decidual lymphocytes of a normal pregnancy despite evidence of lymphocyte activation.

Although the fetus is a semi-allogenic transplantation during pregnancy, there is no rejection development at the fetomaternal interface. Delineating the immune reactions there and understanding the nature of the miraculous regulation of local immune system would contribute to the prevention of organ transplantation rejection. As we know, immunologic rejection has been shown to require intact $CD4^+$ helper T cells. Induction of IL-2 gene transcription and expression of IL-2R have also been shown to precede acute rejection. IL-2Rα are not expressed by resting T cells, but they appear after activation by interaction with alloantigens. The binding of IL-2 to these receptor subunits is important for clonal expansion and persistent viability of activated T cells. Therefore, the involvement of the IL-2/IL-2R system marks a critical step in the activation of alloreactive T cells.

In our previous study (Ho *et al.*, 1996), we demonstrated that CD69 and HLA-DR are highly expressed in the decidua, which indicates that the pregnancy is in a status of both early and persistent activation. However, CD25 expression is down-regulated, especially that of $CD4^+$ helper T lymphocytes. In this study, we further show that the MFIs of CD69 and HLA-DR on both $CD4^+$ and $CD8^+$ T cells also increase in the decidua. Nevertheless, the MFI of CD25 on these T cells in the decidua is still depressed. The reduced expression of CD25 on decidual lymphocytes may be a mechanism for suppressing the generation of potent lymphokine-activated killer (LAK) cells in the decidual environment, which would prevent the decidual lymphocytes from being too readily activated to kill the trophoblasts. In this way, we consider that the pregnancy may be under an immunosuppressive condition, and the fetus can survive in the uterus without rejection. However, this suppression is not complete, because decidual T cells still express high levels of CD69 and HLA-DR activation markers. This causes the decidual lymphocytes to secrete certain kinds of cytokines, in particular colony-stimulating factors, which may be important for trophoblast growth and fetoplacental development (Wegmann, 1987).

Investigation of the activation marker profile of decidual lymphocytes in anembryonic pregnancies can provide valuable information concerning the

functional change of decidual lymphocytes during a pathologic situation. Decidual LAK cells are unlikely to be present *in vivo* in a normal pregnancy, but it is not known if they can develop in, or are responsible for, failed pregnancies. We clearly demonstrate that the expression of CD25, especially on the $CD4^+$ lymphocytes, is markedly increased in anembryonic pregnancies. This altered phenotype may be indicative of an abnormal maternal immunological response in an anembryonic pregnancy. With further increased expression of CD69 and HLA-DR, these decidual lymphocytes may be activated to potentially kill the trophoblasts and then abortion occurs.

In conclusion, decreased expression of CD25 was found on activated $CD4^+$ and $CD8^+$ T lymphocytes derived from decidual tissue. Although high expression of CD69 and HLA-DR antigens, which may assist in the growth and development of the fetoplacental unit, decidual T lymphocytes are functionally inhibited and lose the ability of clonal proliferation and the capability of inducing cytotoxic activity due to down-regulated CD25 expression. Nevertheless, this characteristic of CD25 expression on decidual T lymphocytes can be a plausible mechanism to facilitate fetal survival within the potentially hostile maternal environment.

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Table I. Mean fluorescence intensity of surface activation markers CD25, CD69 and HLA-DR expressed on decidual $CD4^+$ and $CD8^+$ T lymphocytes in anembryonic pregnancies with normal and abnormal karyotypes

	Normal karyotype (n = 18)		Abnormal karyotype (n = 12)	
	PBMCs	Decidua	PBMCs	Decidua
MFI of activation markers on $CD4^+$ T cells				
CD25	12.9±3.2	23.5±13.9*	10.4±2.4	21.5±12.2*
CD69	6.2±1.5	52.6±30.9*	6.1±1.9	50.4±27.8*
HLA-DR	75.0±46.3	330.0±230.6*	69.0±33.4	357.1±253.2*
MFI of activation markers on $CD8^+$ T cells				
CD25	4.2±1.5	5.5±2.2	3.9±1.8	4.9±2.4
CD69	8.1±2.6	65.4±34.6*	8.1±4.0	54.1±33.1*
HLA-DR	129.8±99.8	350.1±228.5*	105.3±62.1	360.8±165.5*

* $P < 0.05$ compared to peripheral blood lymphocytes.

Figure 1. Mean fluorescence intensity (expressed as a logarithmic scale on the y-axis) of activation markers expressed in peripheral blood and on endometrial $CD4^+$ and $CD8^+$ T cells derived from the luteal phase and normal and anembryonic pregnancies.

*, $P < 0.05$ *vs.* peripheral blood; †, $P < 0.05$ *vs.* normal pregnancy.

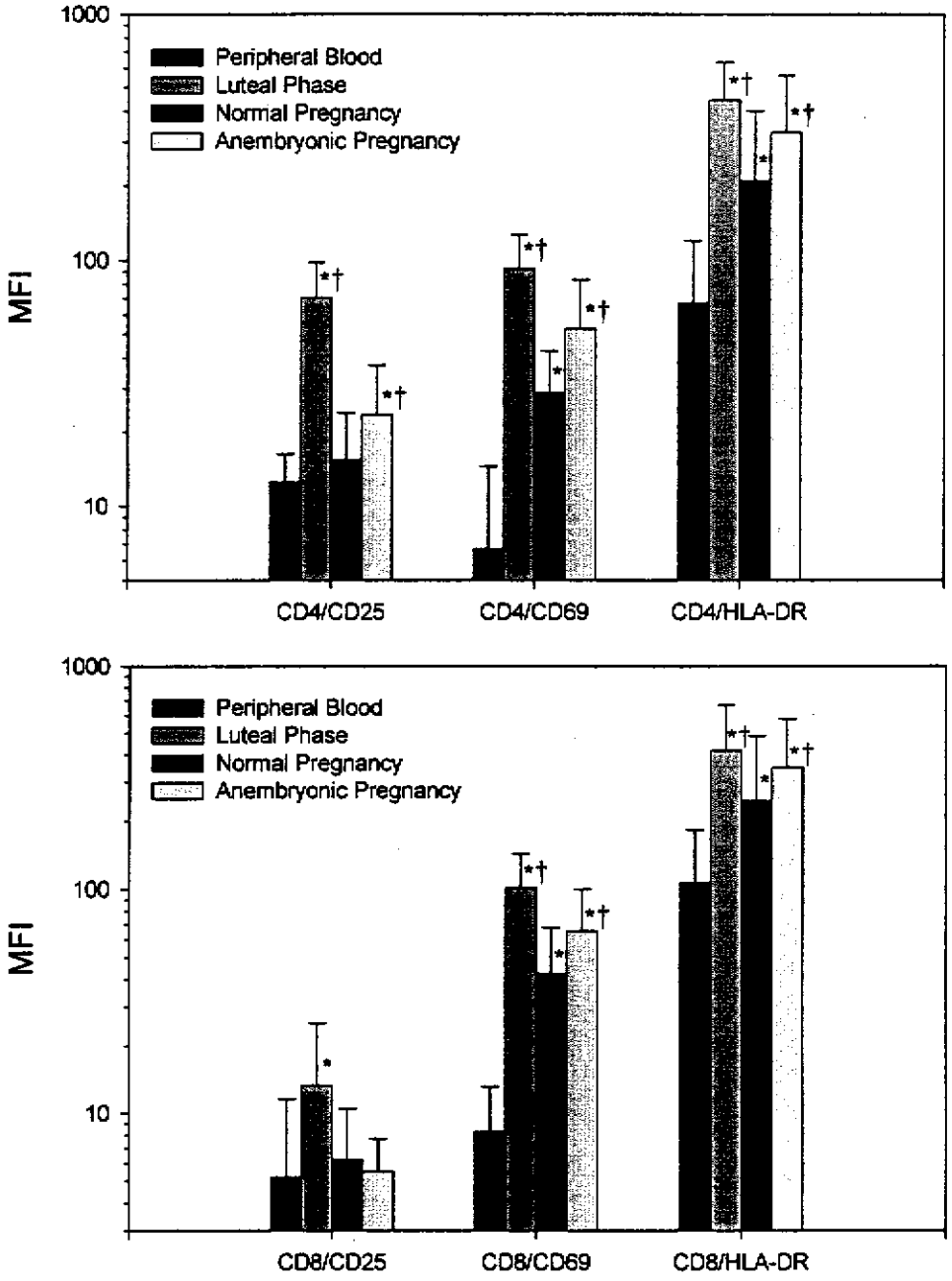

Figure 2. Representative dot-plots of activation marker expression on gated $CD4^+$ and $CD8^+$ T lymphocytes in the decidua (A, unstiumlated) and peripheral blood (B) during normal pregnancy after PHA stimulation.