Immunohistochemical Study of HLA-DR Antigen in Endometrial Tissue of Patients with Endometriosis

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Summary: In order to evaluate the expression of HLA-DR antigen in glandular cells in eutopic and ectopic endometrium in patients with endometriosis, 19 infertile patients with endometriosis were analyzed immunohistochemically by labelled streptavidin biotin (LSAB) method. Nineteen infertile patients without endometriosis were studied as controls. The results showed that the expression of HLA-DR antigen in the glandular cells in both eutopic and ectopic endometrium was increased significantly as compared with that in the controls ($P<0.01$). It is likely that aberrant expression of HLA-DR antigen in endometriotic tissue is involved in abormal immunogenesis of endometriosis.
Key words: HLA-DR antigen; immunohistochemistry; endometriosis

Several autoimmune phenomena have been recognized in endometriosis including an increased number of peritoneal macrophages and their activation[1]. Furthermore, autoantibodies, especially those specific for phospholipids or endometrium are frequently positive in endometriosis[2, 3]. These findings indicate that endometriosis is an autoimmune disease. However, the reason why macrophages are activated and autoantibodies are highly positive in this disease remain unclear. The present study was to investigate the expression of HLA-DR antigen in glandular cells in eutopic and ectopic endometrium in endometriosis.

1 MATERIALS AND MEHHODS

1.1 Subjects

Fifty endometrium samples were obtained from 38 women with regular menstruation who underwent diagnostic laparoscopy for infertility. All the patients had no history of habitubal abortion and autoimmune diseases. According to the diagnosis, they were divided into two groups; endometriosis group ($n=19$): All patients with endometriosis were minimal or mild endometriosis as staged according to the revised American Fertility Society classification[4]. Nineteen eutopic endometrium samples (proliferative phase 11, secretory phase 8) and 12 ectopic endometriun samples (proliferative phase 6, secretory phase 6) were obtained. Control group ($n=19$): 15 were chronic pelvic inflammatory and the remaining 4 unexplained infertility. Nineteen endometrium samples were obtained by curettage.

1.2 Treatment of the Samples

All samples were embedded in optimal cutting temperature compound (OCT), and snap-frozen in liquid nitrogen immediately. Serial cryostat 4 μm sections of endometrium were cut for use.

1.3 Immunological Staining

The sections were stained using labelled streptavidin biotin (LSAB) method. In brief, the sections were fixed in acetone and then washed with PBS. Monoclonal mouse anti-human HLA-DR antibody (ZYED Biotechnology, USA) was added and incubated overnight at 4 ℃. After washing with PBS, the second antibody (goat anti-rabbit IgG, Vector corporation, USA) was added and incubated for 1 h at 37 ℃. Then the HRP-Streptprotein complex was layered on the slides. After washing with PBS, DAB kit was used to develop the color reaction and examined under a light microscope.

1.4 Evaluation of Staining

The postive staining was brown. Evaluation of the staining was performed only on the glandular cells in eutopic and ectopic endometrium. Surface epithelium, stromal cells, lymphocytes, and endothelial cells in vessels in the eutopic and ectopic endometrium were excluded from the present study. Each section was divided into five degrees according to the percent of HLA-DR expression on the glandular in eutopic and ectopic endometrium: −/+/++/+++ as shown in table 1[5]. The relative intensity of HLA-DR positive staining was scored on 0/0.5/1/2/3, where 0 equals negative, 0.5±, 1+, 2++, 3+++.

Table 1 Criterion of intensity of HLA-DR antigen expression on glandular cells (%)

Expression	−	±	+	++	+++
HLA-DR	<2	2—5	5—19	20—49	>50

1.5 Statistical Analysis

The results was analyzed by Kruskal-Wallis test, and P value less than 0.05 were considered to be statistically significant.

2 RESULTS

The relative intensity score in each group in proliferative or secretory phase was shown in table 2. In each phase, the expression of HLA-DR in glandular cells in eutopic and ectopic endometrium were higher significantly than in control, in which χ^2-value were 13.30 and 9.90, respectively ($P<0.01$). However, no significant differences in each group were observed between the two phases.

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Table 2 Expression of HLA-DR antigen in each group in the proliferative or secretory phase

Degrees	Eutopic		Ectopic		Control	
	Proliferative	secretory	Proliferative	secretory	Proliferative	secretory
−	0	0	0	0	2	2
±	0	0	0	0	2	2
+	2	2	1	1	5	3
++	2	2	2	1	1	2
+++	7	4	3	4	0	0
Relative intensity	2.45	2.25	2.33	2.50	0.80	0.89

3 DISCUSSION

3.1 Effect of HLA-DR Expresson in the Autoimmune Diseases

Expression of the HLA-DR antigen is usually limited in mature B cells, macrophages, and activated T cells. In the process of an immune reaction, a key factor appears to be the expression of HLA-DR antigen on the cell surface. HLA-DR positive cells are initially recongnized by macrophages, which activate T cells. Activated T cells secrete cytokines such as INF-γ and stimulate B cells to produce antibodies. In fact, there is no expression of HLA-DR in normal nonimmunocompetent cells, whereas increased expression of HLA-DR is observed in the target organs of several autoimmune diseases, such as Hashimmune disease[6], and juvenile diabetes mellitus[7]. Organ-specific antibodies are also found in peripheral blood in patients with this diseases. It is suggested that aberrant expression of HLA-DR antigen is involved in the pathogenesis of autoimmune diseases.

3.2 Expression of HLA-DR Antigen in Normal Endometrium

Throughout the cycle, HLA-DR expression is positive in endometrium in human uterus, especially in glandular epithelium, surface epithelium, lymphocytes, and endothelial cells of the vessels. Tabibzadeh[8] has reported that the expression of HLA-DR on glandular cells in endometrium was variable throughout the cycle, HLA-DR antigen was stained weakly in early proliferative phase, and strongly in late proliferative phase. The expression of HLA-DR was decreased in secretory phase and absent in gestational endometrium. But we found no difference in expression between the two phases in the present study. The inconsistency and conflicting results might be related to the selection of patients and control group studied or the difference of method used.

3.3 Aberrant Expression of HLA-DR in Endometriosis

The pathogenesis of endometriosis remains unclear. It has been reported that macrophages were increased and activated in endometriosis[1]. The organ non-specific antibodies (such as antiphospholipids antibodies) and organ specific antibodies (such as antiendometrial antibodies) are frequently positive in peritoneal and peripheral blood in patients with endometriosis[2, 3]. Furthermore, it has been demonstrated marked deposition of Igs or complement components in the ectopic endometrium as well as in the eutopic endometrium in endometriosis[9, 10]. These findings indicate that endometriosis is an autoimmune disease. However, the reason why macrophages are activated and autoantibodies are highly positive in the disease remain unclear. The present study clearly revealed an increased expression of the HLA-DR antigen in the glandular cells of ectopic and eutopic endometrium in endometriosis, consistent with results reported by Ota[11]. From the above, it can be postulated that aberrant expression of HLA-DR antigen in glandular cells in ectopic and eutopic endometrium may be recognized by macrophages that would in turn activate helper T cells and stimulate B cells to produce antibodies and involved in the pathogenesis of endometriosis.

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