Mini Review

Novel insights into pathology of endometriosis from a disease model induced by autotransplantation of endometrium

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The pathogenesis of endometriosis, a gynecologic disorder associated with infertility, appears to involve immune responses. However, the details involved have not been clarified and treatment options are limited. Sugamata M. et al. found proliferation of stromal cells and infiltration of mast cells, consistent with human endometriosis, in peritoneal tissues adjacent to autotransplanted endometrium (endometriosis model). Subsequent studies using the endometriosis model have provided novel insights into the pathology of endometriosis. First, expression of cytokines and chemokines consistent with endometriosis in humans was found. This observation provided a focus on the contribution of immunoinflammatory response to development of endometriosis. Second, microarray analysis showed that an increase in expression of genes associated with cell adhesion and extracellular matrix precede formation of pathology of the endometriotic lesion and upregulation of expression of cytokines and chemokines. Moreover, it has been shown that the endometriosis model can be applicable to *in vivo* toxicity and preclinical testing. The pathology of endometriosis model was enhanced by exposure to diesel exhaust, a major air pollutant, and was inhibited by oral administration of drugs, including leukotriene receptor antagonist. Information provided by the endometriosis model will contribute to establishment of the methods for prevention and treatment of endometriosis. Further investigation using this invaluable tool could potentially help to protect many women against the effects of this currently incurable disease.

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Introduction

Endometriosis is a common gynecological disorder characterized by the growth of ectopic endometrium. The disease causes pelvic pain and dysmenorrhea and contributes, at least partially, to infertility. Despite the high prevalence, 6-10% of women of reproductive age, an efficient therapy that can prevent recurrence and does not interfere with fertility has not been established. Surgical intervention to remove endometriotic lesions combined with drug therapy to induce a hypoestrogenic state in patients is being used, but they still lead to a high recurrence rate, resulting in a chronic course of this disease and severe secondary side-effects[1]. It has been proposed that the retrograde seeding of endometrial cells during menstruation contributes, in part, to the initiation of development of endometriosis[2], but the details have not been clarified. To investigate the underlying biological mechanisms leading to the establishment of this disease, *in vitro* systems and experimental animal models have been developed. An endometriosis model induced by autotransplantation of endometrium in the rat has been developed as a simple, inexpensive and useful tool to study the natural history of endometriosis[3,4]. The autotransplantation technique was initially reported by Vernon and Wilson in 1985[5]. They observed growth and development of surgically transplanted endometrial tissue but did not observe any change in the peritoneal tissue attached to endometrium. Uchiide et al.[3] added minor modification to the surgical technique (Figure 1) and originally reported infiltration of allergic inflammatory-related cells into proliferative lesions in peritoneal stroma attached endometrium, not in the transplanted endometrium. Since then, the details of gene expression change in the proliferative lesion in peritoneal stroma of the autotransplantation model of endometriosis have been investigated.

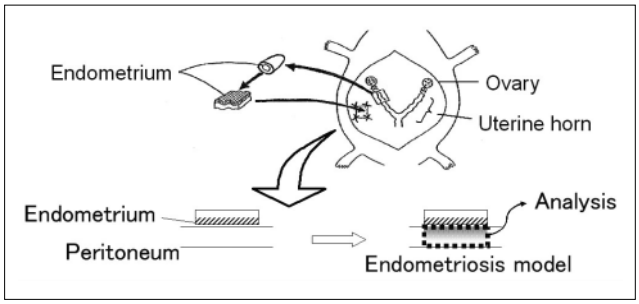

Fig.1 The procedure to induce the endometriosis model in mice and rats

Uterine transplants (5mm × 5mm) were attached to peritoneum via the surgical autotransplantation technique.

Induced allergic reactions in the endometriosis model

The endometriosis model was induced within 4 days post-transplantation, reached a maximum at 7 days, and declined within 14 days[3], whereas chronic lesions were formed by multiple stimuli with endometrium provided by retrograde menstruation in humans. In the disease model, the reactions induced by contact of endometrium and peritoneum can be observed. In the rat endometriosis model induced in the peritoneal tissue, infiltration of several types of cells, including plasma cells, lymphocytes and eosinophils as well as mast cells were observed[3], and this was observed also in endometriosis in humans[6]. Activated mast cells can promote fibroblast chemotaxis[7] and synthesis of collagen fibres[7-10], which can cause the stromal proliferation, that is observed in the lesion of type I allergy[11]. Along with induction of pathological features of endometriosis, cytokine expression is upregulated in the rat endometriosis model. It has been shown that expression of cytokines *Il6* and *Il10* and chemokines such as *Ccl2*, are increased, whereas *Il4* and *Ifng* are not increased[12]. The data showing the change of the expression of cytokines and chemokines is consistent with endometriosis in humans supported the usefulness of the disease model. However, increased expression of cytokines and chemokines is not likely to be a primary cause, because the upregulation remained at 14 days post-transplantation, when the lesion had already declined. To characterize the biochemical alterations that occur in early steps of development of the endometriosis model, the change of gene expression in peritoneal tissues at 24-96 hours post-transplantation has been analysed by microarray analysis[13].

Upregulated expression of genes associated with cell adhesion in the early steps of pathophysiology of the endometriosis model

Umezawa et al.[13] examined the change of gene expression that occurs, specifically in the peritoneal tissue adjacent to the endometrial transplant, excluding the factors occurring in this transplant. In this study, peritoneum only (excluding the transplant) was collected at 24-96 hours post-transplantation and subjected to microarray analysis. A sham-operated control was prepared through autotransplantation of white adipose tissue. Histopathological characteristics of endometriosis, i.e. stromal hyperplasia and infiltration of mast cells in peritoneal tissues, were induced within 96 hours post-transplantation. Microarray analysis showed the change of expression of hundreds of genes among the 13,728 genes analysed at each timepoint at 24-96 hours post-transplan-

tation. To interpret the large amount of data generated and to enable a functional analysis, microarray data were combined with Gene Ontology (GO) and Medical Subject Headings (MeSH) information. Of the upregulated genes, those involved in the inflammatory response, wound healing, leukocytes, cell adhesion and extracellular matrix were enriched at 24 and 48 hours post-transplantation. Those of ossification, cytokines, antibody-producing cells, dendritic cells, inflammation and infertility were enriched at 96 hours post-transplantation. A subsequent study using the quantitative reverse transcription-polymerase chain reaction (RT-PCR) showed an increase in the expression of various subsets of integrin, collagen and extracellular matrix (unpublished data). The results suggest that the factors occurring during early development of endometriosis are increase in adhesion molecules and inflammatory responses. It is of interest that an increase in adhesion molecules and extracellular matrix precedes formation of the pathology of the lesions. These factors can be activated in the earliest stage of development of endometriosis induced by autotransplantation of endometrium and may have a crucial role in the etiology.

A unique model of endometriosis

The advantages of the model described here include the fact that endometrial epithelium is transplanted autologously, not allogeneically, thus allowing investigation of allergic reaction (Figure 2). Several studies induced lesions in animals by allogeneic or heterologous transplantation of endometrial epithelium. However, the immune response in these cases differed from the lesions induced by autotransplantation. Moreover, proliferative lesions along with an increase in cytokine and chemokine expression were found in peritoneal tissues adjacent to the autotransplanted endometrium. This information is novel, as other researchers have focused only on the endometrial implants used. The findings from the endometriosis model are in agreement with those from endometriosis in humans. Further investigation is underway to develop a novel strategy for prevention and treatment, which involves regulation of expression of cytokine, adhesion molecules and hence the inflammatory reactions induced by autotransplantation of endometrium. It is hoped that this will reveal the reactions essential to induction of the endometriotic lesions.

Prospect for a novel strategy for prevention and treatment of endometriosis

The endometriosis model can be used for *in vivo* preclinical and toxicity testing. It is reported that *in utero* and postnatal exposure to diesel exhaust, a major air pollutant that can cause an allergic reaction[14,15], influence estrogen receptor expression and stimulate aryl-hydrocarbon receptor (AhR)[16], enhanced lesions of the endometriosis model[17]. Although it was known that allergic reaction[3], estrogen and AhR signal[18-20] contribute to the incidence of endometriosis, the original research using the endometriosis model showed the potential effects of diesel exhaust exposure on the pathology of endometriosis. Environmental pollutants, especially dioxins and dioxin-like toxicants such as polychlorinated biphenyl, are well known to be associated with the incidence of endometriosis[19,20]. However, there has been no experimental method for testing the effect of these toxicants on the pathology of endometriosis. Two markers to evaluate the effects are used here[17]; one is the extent to which abdominal muscles are affected by interstitial stromal cell hyperplasia, and the other is the level of Ccl2/monocyte chemoattractant protein (MCP)-1 in the serum. Clinical researchers have reported that the level of MCP-1 in serum is higher in women suffering from endometriosis[21-23], and the level is correlated with the severity of the disease[22]. The level of MCP-1 in serum is available as a biomarker of the severity in the rat endometriosis model, and this non-invasive marker will be helpful in evaluating toxic and/or therapeutic effects on endometriosis of rat models and humans.

As a tool for preclinical testing, a therapeutic effect of the leukotriene receptor (LT-R) antagonist[24], macrolide compound[25] and eicosapentaenoic acid[26] on endometriosis were found. Especially, the LT-R antagonist could dramatically inhibit infiltration and activation of mast cells in the lesion[24]. The authors think

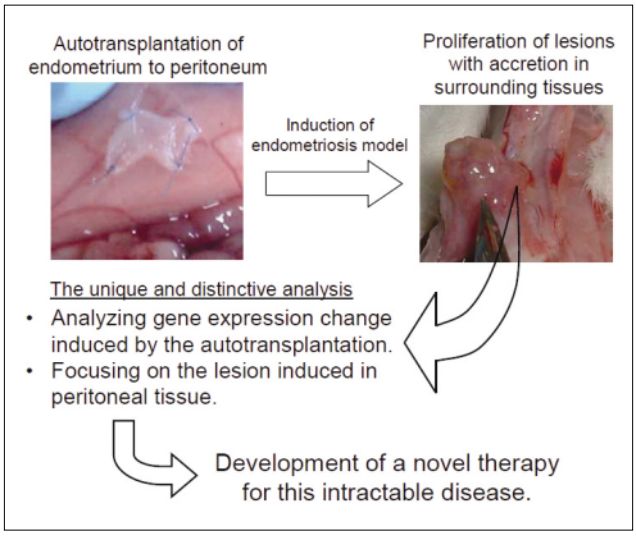

Fig.2 The novel strategy for investigating the pathogenesis of endometriosis

that the LT-R antagonist will be desirable curative medicine with minimal side-effects. Future studies using the endometriosis model and clinical research will contribute to the establishment of safe and effective therapy.

Conclusion

The model of endometriosis shown here has some advantages, including the fact that it is closer to human endometriosis because induced responses after autologous transplantation of endometrial tissue can be investigated. It is surprising that an upregulation of expression of cytokines and adhesion molecules is not induced by autotransplantation of control tissue (white adipose tissue) but is induced dramatically by autotransplantation of endometrium. As a tool for both toxicity testing and preclinical testing, information provided by the endometriosis model has the potential to contribute to the improvement of the environment and to human healthcare. Further investigation using this invaluable tool might help to protect many women against the effects of this currently incurable disease.

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Abbreviations

AhR, Aryl-hydrocarbon receptor; LT-R, leukotriene receptor; MCP-1, monocyte chemotactic protein-1.

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