Analysis of the involvement of CCR5-Δ32 and CCR2-V64I variants in the development of endometriosis

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Several arguments support the proposal that the cytokine network plays a critical role in the aetiology of endometriosis. Among various chemokines, regulated-on-activation, normal-T-cell-expressed and -secreted (RANTES) and monocyte chemotactic protein 1 (MCP-1) concentrations have been shown to be increased in the peritoneal fluid of women with endometriosis. Some studies have demonstrated that, in the context of endometriosis, these chemokines are involved in apoptosis, angiogenesis and/or chemotaxis. Since the chemokines exert their effects by binding to their receptors, it would be plausible that factors affecting such interactions might play a role in the pathogenesis of endometriosis. Thus we postulated that the genes encoding CCR5 and CCR2, which are the receptors for RANTES and MCP-1 respectively, could be good candidate genes for the disease. We have used real-time PCR and FRET technologies to genotype and evaluate the variants CCR5-Δ32 and CCR2-V64I, as susceptibility factors in a cohort of Spanish women with endometriosis. No differences have been found in the frequencies of the two polymorphisms nor in the haplotype/genotype distribution between cases and controls. These data would suggest the lack of association between these polymorphisms and endometriosis in our population, although they do not permit us to discard completely a possible role of other variants within CCR5 and CCR2 genes in this pathology.

Key words: CCR5-Δ32/CCR2-V64I/endometriosis/genetics/polymorphisms

Introduction

Endometriosis is a common disorder characterized by the existence and proliferation of both endometrial stromal and glandular cells outside the uterine cavity, affecting between 5 and 15% of women of reproductive age (Goldman and Cramer, 1990). Although no single theory can fully account for the diverse clinical presentations of endometriosis, it is generally accepted that it results from retrograde menstruation with subsequent invasion/transplantation of exfoliated cells (Sampson, 1925). The aetiology and molecular mechanisms for the development of this disease are still unclear. However, a large body of laboratory and clinical studies suggests that hereditary genetic factors, in association with environment, are responsible for this gynaecological disorder (Bischoff and Simpson, 2000; Zondervan et al., 2001). An important general concept is that endometriosis is a local pelvic inflammatory process with an altered immunological function in the peritoneal environment (Harada et al., 2001). A wide array of cytokines has been shown to play a critical role in immunological surveillance, recognition and destruction of ectopic endometrial cells and possible facilitation of the implantation of ectopic endometrial tissues (Barcz et al., 2000). For instance, it is known that, among several chemokines, the endometrium produces RANTES (regulated-on-activation, normal-T-cell-expressed and -secreted), and MCP-1 (monocyte chemotactic protein 1) (Hornung et al., 2001; Jones et al., 1997), which, besides the regulation of leukocyte migration and function during menstruation, also display specific roles in apoptosis of endometrial, glandular and stromal cells (Garcia-Velasco et al., 1999). It has been observed that the concentration of RANTES in endometriosis varies depending on the severity of the disease. Some authors have postulated that, after T-cell activation in peritoneal fluid, secretion of RANTES may lead to the recruitment of peritoneal macrophages and more T-lymphocytes, further contributing to the progression of the endometriotic lesion (Khorram et al., 1993). Regarding MCP-1, its mRNA expression and protein production by endometrial glandular cells are found to be higher in women with endometriosis than in patients without the disease (Jolicoeur et al., 1998). Evidence suggests that, in the context of endometriosis, MCP-1 is involved in apoptosis, angiogenesis and chemotaxis (Kayisli et al., 2002).

Chemokines exert their effect by binding to their relevant receptors, whose expression levels may modulate their action. Just as the coordination of chemokine–chemokine receptor interactions plays an important role in the normal menstrual cycle and successful pregnancy, it would be plausible to speculate that the presence of factors affecting these interactions may result in cellular proliferation, migration and abnormal invasion. Among these factors, the genetic variants within the genes encoding chemokines and chemokine receptors would be excellent candidates. In the present report we have studied the involvement of two sequence variants in the genes encoding CCR5 and CCR2, which are the receptors for RANTES and MCP-1 chemokines, in endometriosis. More specifically, we have analysed and compared the distribution of the CCR5-Δ32 and CCR2-
V64I polymorphisms in a series of Spanish women with endometriosis and control subjects, in order to determine whether an association of these variants with the disease exists.

Materials and methods

Study participants
We have included in our study three different groups of women, following the recommendations for the design of epidemiological studies of endometriosis (Holt and Weiss, 2000; Antinòelo et al., 2003). Group 1 comprised 63 sporadic cases with surgically and histologically confirmed endometriosis in stage II-IV (American Fertility Society, 1995), operated in the Hospitales Universitarios Virgen del Rocío. Diagnosis of endometriosis was made by direct visualization of the pelvis during a laparoscopic surgical examination of excised tissue, and a later histological confirmation. Group 2 comprised 36 pre-menopausal women with a normal pelvis at hysterectomy, also operated on in the Hospitales Universitarios Virgen del Rocío. All women belonging to this group, were 45 years old or older, avoiding younger women who might develop the disease in later life. Group 3 comprised 110 women with an average age of 36 years old who came to the Hospital for other reasons, and did not present symptoms of endometriosis. All the subjects included in this study were Caucasian-Mediterranean, coming from the South of Spain. Informed consent was obtained from all participants in accordance with the regulations of the institutional review board for human subjects’ protection.

Analysis of the CCR5-Δ32 and CCR2-V64I variants

The genotyping of CCR5-Δ32 was made by real-time PCR and LightCycler™ technology, following the protocols previously described (Ruiz et al., 2001). With regard to CCR2-V64I, we used fluorescence resonance energy transfer (FRET) technology under the conditions previously reported (Royo et al., 2001).

Statistical analysis

Allelic frequencies of the analysed variants were determined and then compared between patients and controls. In addition, haplotypes comprising these two variants were constructed for every study participant, and the haplotype distribution was also compared between the groups. Two independent statistical analyses were performed, in which the group 1 (patients) was compared with groups 2 and 3 (controls) individually. Comparisons were performed using either \( \chi^2 \)-test analysis with the Yate’s correction, or Fisher’s two-tailed exact test when appropriate. Statistical significance was considered when \( P < 0.05 \).

Results

We have analysed 63 unrelated cases of women with endometriosis and compared them to 110 women without symptoms of the disease, and to 36 confirmed unaffected women, for the frequency of the CCR5-Δ32 and CCR2-V64I variants. The frequency data for each group are shown in Table I. Our results show that the differences in the frequencies of both polymorphic variants are not statistically significant when the group of patients was compared to the other groups (Table I).

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Discussion

The aetiology and pathogenesis of endometriosis remain poorly understood. It is likely that endometriosis is a common multifactorial disease, caused by an interaction between multiple gene loci and the environment. The identification of genetic loci conferring susceptibility to endometriosis may lead to a better understanding of disease aetiology and, in time, improved therapeutic strategies and diagnostic methods (Kennedy, 1997).

Several lines of evidence suggest that cytokines are in some way involved in the construction of the peritoneal environment that induces the development and progression of endometriosis (Harada et al., 2001). Since the cytokines exert their effects by binding their receptors, it would be plausible that factors affecting such interactions may play a role in the onset of the disease. In this sense, genes encoding cytokines and cytokine receptors represent good candidate genes for endometriosis. This is the first study in which the involvement of genetic variants within the genes encoding CCR5 and CCR2 chemokine receptors are evaluated in the pathogenesis of the disease. Our hypothesis was based on the facts that the natural ligands for the two receptors, RANTES and MCP-1, are found at high levels in the peritoneal fluid of women with endometriosis, and that these chemokines seem to be involved in endometrial angiogenesis, apoptosis, proliferation and differentiation (Kayisi et al., 2002). Thus, it would be plausible that variants in the genes encoding CCR5 and CCR2 receptors could modulate their transcription and expression in endometrium, and thereby affect the interactions with their ligands, contributing to the development of this complex trait. We have selected the particular polymorphisms CCR5-Δ32 and CCR2-V64I, because they have been previously found to be involved in some other
inflammatory and immunological diseases (see OMIM 601373 and 601267).

According to our data, neither CCR5-D32 nor CCR2-V64I seem to be involved in the susceptibility to endometriosis in our population. These data do not exclude, however, a role of CCR5/RANTES and CCR2/MCP-1 interactions in the pathogenesis of the disease. In fact, previous reports have largely shown the involvement of such chemokines in the apoptotic process associated with the progression of endometriosis, which could be due to still unknown genetic factors. Further studies must be performed in order to fully rule out the genes encoding the cytokine network as candidate genes in endometriosis.

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