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Impaired NK-cell-mediated cytotoxic activity and cytokine production in patients with endometriosis: A possible role for PCBs and DDE

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Abstract

Endometriosis is a gynaecological disorder characterized by the presence and growth of endometrial tissue in ectopic sites. In this study we examined the immunological functions of patients with endometriosis and serum level of PCBs and *p,p′*-DDE to verify the impact of these environmental contaminants on the dysregulation of immune functions. We found that proliferative responses and immunoglobulin production were not dysregulated in patients with endometriosis while NK cell activity was significantly down-regulated in these patients. Moreover, a significant down-regulation of IL-1β and IL-12 production was found in patients with respect to controls. Serum levels of PCBs and *p,p′*-DDE were found to be significantly higher in women with endometriosis than in the control group, with respect to the sum of the congeners most prominent in human tissues. In particular, total PCBs concentration in patients with endometriosis and controls was respectively 330 and 160 ng/g fat with respect to the most abundant congeners, while *p,p′*-DDE concentration was of 770 and 310 ng/g fat. Moreover, we found that normal human PBMC pulsed with PCBs, *p,p′*-DDE and their combination showed a significant down-regulation of NK cell cytotoxic activity and IL-1β and IL-12 production. These findings suggest that changes in specific immune parameters correlate with elevated serum PCBs and DDE levels and endometriosis.

Keywords: Endometriosis; PBMC; Proliferative response; Immunoglobulin production; NK-cell-mediated cytotoxic activity; IL-1β; IL-12; PCBs; DDE

Introduction

Endometriosis is a gynaecological disorder characterized by the presence and growth of endometrial tissue in ectopic sites (Giudice and Kao, 2004).

The immune system is believed to be involved in the pathogenesis of endometriosis (D'Hooghe et al., 2003; Lebovic et al., 2001), and a lack of adequate immune surveillance in the peritoneum is thought to be a cause of the disorder. There is also evidence of compromised natural killer cells activity in peritoneal fluid and sera in women with endometriosis, which can facilitate the implant and growth of the ectopic tissue (Oosterlynck et al., 1991). High concentration of cytokines, growth factors, and angiogenic factors (Oosterlynck et al., 1991; Chegini, 2002; Gazvani and Templeton, 2002; Bedaiwy and Falcone, 2003) derived from the lesions themselves, secretory products of macrophages and other immune cells have been detected in the peritoneal fluid of women with endometriosis. Several pro-inflammatory cytokines such as IL-1, IL-8, TNF-α and INF-γ are secreted by endometriotic lesions (Harada et al., 2001; Rana et al., 1996; Van Le et al., 1992; Zarmakoupis and Rier, 1995). These cytokines modulate chemotactic factors, which in turn recruit macrophages and T lymphocytes to the peritoneum. These immune cells mediate the inflammatory reaction associated with endometriosis.

A postulated autoimmune aetiology of endometriosis derives from reports on increased polyclonal B cell activity,

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abnormalities in B and T cell functions, familial inheritance (Zondervan et al., 2002; Nothnicik, 2001), high B and T cells counts (Badawy et al., 1987) and reduced NK cells activity (Oosterlynck et al., 1991, Nothnicik, 2001). Moreover, high serum concentrations of IgG, IgA, and IgM autoantibodies (Nothnicik, 2001; Badawy et al., 1987; Gleicher et al., 1987) and antibodies to endometrium (Wild and Shivers, 1985; Grossinkinsky and Halme, 1993) have been described.

Some studies on women (Gerhard and Runnebaum, 1992; Louis et al., 2005) have shown an association between endometriosis and polychlorobiphenyls (PCBs), suggesting an involvement of these pollutants in the disease development. PCBs have been extensively used in a variety of industrial applications until early 1970s and, because of their improper and massive release into the environment, have become ubiquitous environmental contaminants. Their environmental and biological persistence, together with a high potential of bioaccumulation, causes PCBs to enter the food webs and biomagnify through the food chains. Human exposure mostly occurs via diet, with food of animal origin giving the most important contribution to the overall exposure. This large group of 209 different molecules or "congeners" includes congeners with dioxin-like structure and toxicity, and congeners with non-dioxin-like structure and toxicological activities. These latter are present in food, and hence in human tissues, in concentrations of up to three orders of magnitude higher than dioxin-like PCBs. In all human tissues, the four PCB congeners 118, 138, 153 and 180 dominate and account for at least 50% up to 80% of total PCB content (Wingfors et al., 2000; De Felip et al., 2004). A role of PCB exposure in the aetiology of endometriosis and/or in its progression has been hypothesised, on the basis of their effects on endocrine and immune system (Rier and Foster, 2002). Many PCBs and their metabolites have in fact endocrine-like activities which could affect endometriosis via the induction of inappropriate estrogen production in the endometrium (Connor et al., 1997; Kester et al., 2000). Moreover, PCBs could promote endometriosis via chronic stimulation of the expression and activity of the pro-inflammatory cytokines, such as IL-1, IL-6, IFN-γ and TNF-α involved in the cyclic regulation of endometrial remodelling, proliferation, and cell death (Rier and Foster, 2002).

Although numerous studies (Lebovic et al., 2001; Zarmakoupis and Rier, 1995; Oosterlynck et al., 1991; Harada et al., 2001) have shown alterations in cell-mediated and humoral immunity in patients with endometriosis, the importance of these changes remains obscure.

In this study we evaluate the immunological status of patients with endometriosis and correlate these functions with the blood level of the most abundant PCBs to verify the impact of these environmental contaminants on the dysregulation of immune function observed in endometriosis patients. We also explored a possible correlation between blood levels of *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE), the main metabolite of *p*,*p*′-dichlorodiphenyltrichloroethane (*p*,*p*′-DDT), present in human tissues at concentrations similar or higher to the most abundant PCBs, and the immunological functions of the patients with endometriosis, on the basis of the reported immunological dysregulation (Daniel et al., 2002) in women simultaneously exposed to this contaminant and PCBs.

Materials and methods

Patients

Eighteen women, aged 18 to 45 years, undergoing laparoscopy for a clinical suspicion of endometriosis or other benign gynaecologic conditions at the Department of Gynaecological Sciences, Perinatology and Child Health of the University of Roma "La Sapienza", were enrolled in the study. Selection criteria were: nulliparity, absence of endocrine, immunologic or other chronic diseases, absence of a clear professional exposure to environmental contaminants, no prior hormonal treatment except oral contraceptives. All women signed a written informed consensus to the study. A physician unaware of the indications of laparoscopy administered a questionnaire before surgery. The questionnaire documented the age, the education, the job, the medical, gynaecological and obstetrical history, the height and the weight, known exposure to organochlorines, the frequency and the amount of different kinds of food per week, and alcohol and smoking habits. Before laparoscopy, 40 ml of peripheral blood was collected from cubital vein: 30 ml was aspirated in vacutainer tubes containing ethylendiaminetetracetic acid, and stored at −20 °C until analysed for PCBs assessment, 10 ml was aspirated into a sterile heparinized tube and immediately employed in immunological studies. All patients underwent a 10-mm laparoscopy under general anaesthesia with surgical treatment of the condition and several peritoneal biopsies. Histological examination was performed on specimens and biopsies. Ten women (cases) had laparoscopically and histologically confirmed endometriosis whereas 8 subjects underwent laparoscopic treatment of other gynaecological conditions and their peritoneal random biopsies were negative for endometriosis (controls). Endometriosis was staged according to the revised classification of the American Fertility Society (rAFS); all endometriomas were completely excised by stripping, adhesions cut and peritoneal lesions coagulated with bipolar forceps.

Cell preparation

Human PBMC were isolated by Ficoll-Hypaque (Flow Laboratories, Irvine, UK) gradient separation of peripheral blood collected from cubital vein of endometriotic patients or control. Buffy coats from healthy volunteer blood donors women were obtained by the Transfusion Center of Università degli Studi "La Sapienza" Rome. K562 cell line was maintained in RPMI-1640 medium supplemented with 10% heat-inactivated foetal calf serum, L-glutamine and penicillin–streptomycin.

Proliferative response

PBMC from control or patients were resuspended at 1×10^6/ml in complete medium (RPMI-1640 supplemented with 10%

heat-inactivated human serum (blood type AB), L-glutamine and penicillin–streptomycin) and stimulated with different mitogens. The following doses were used: 10 μg/ml PHA, 1:50 dilution of PWM, 5 μg/ml ConA, 20 μg/ml. Normal human PBMC were stimulated with PHA and left untreated or treated with PCB. After 3 days, the cultures were pulsed for 18 h with 0.5 μCi/well of [^{3}H]-TdR (specific activity 5 Ci/mM, Radiochemical Inc., Amersham, UK). Cells were then harvested onto glass fibre filters and TdR incorporation was measured by liquid scintillation spectroscopy. The results are expressed as mean counts per minute (cpm)±SD of the mean of triplicate cultures.

Pokeweed mitogen (PWM)-induced immunoglobulin (Ig) production

PBMC were suspended at 1×10^6/ml in IMDM, supplemented with 10% heat-inactivated FCS, L-glutamine and antibiotics and stimulated with PWM at the final concentration of 1.25 μg/ml. The cultures were incubated at 37 °C in a humidified 5% CO_2 incubator. After 7 days, cells were assayed for IgM- and IgG-secreting cells (ISC) by ELISPOT assay (Sedgwick and Holt, 1988). To enumerate ISC, flat bottomed microtitre plates (96-well) (Dynatech M129A, Alexandria, VA, USA) were coated with 0.1 ml/well of optimal dilution of goat anti-human IgG and/or IgM (Southern Biotech) in carbonate–bicarbonate buffer, pH 9.7, overnight at 4 °C. Plates were washed twice with phosphate buffered saline (PBS) −0.05% Tween 20 (Sigma Chemical Co., St. Louis USA) and incubated for 1 h at 37 °C with PBS containing 3% gelatin (Sigma Chemical Co.) as blocking agent. The plates were rinsed, the cells were then added and incubated at 37 °C in 5%CO_2 incubator for 3 h. The wells were subsequently washed and then incubated overnight at 4 °C with 100 μl/well of optimal dilutions of alkaline phosphatase (AP)-conjugated goat anti-human IgG and/or IgM (Southern Biotech). After extensive washings with PBS–Tween, 100 μl of AP substrate 5-bromo-4-chloro-3-indolylphosphate (BCIP, Sigma Chemical Co.), 1 mg/ml, in 1 M 2-amino-2 methyl-1-propanol buffer (AMP, Sigma Chemical Co.) containing 5 mM $MgCl_2$, 0.01% Triton X-405 (Sigma Chemical Co.) and 0.01% NaN_3 was added to each well. The plates were incubated for 1 h at room temperature. The supernatants were then discarded and the wells rinsed with deionised water. The plates were allowed to dry and the spots were enumerated under a stereomicroscope with a 40-fold magnification. ISC were expressed per number of recovered cells at the end of the culture period.

Cytotoxic assay

K562 target cells (1×10^6) were labelled with ^{51}Cr by incubation with 100 μCi of $Na_2{}^{51}CrO_4$ (Amersham) for 1 h at 37 °C (Zarcone et al., 1989). Effector cells (E) were admixed with target cells (T) (1×10^4 cells/well) at several ratios ranging from 3.12:1 up to 25:1. The cytotoxic assay was performed on viable cells evaluated by the trypan blue dye exclusion test and the same number of cells was used in both control and patient samples.

All experiments were performed in triplicate. Cell mixtures were incubated for 4 h at 37 °C in bottom 96-well microtitre plates and centrifuged at 800×*g* for 5 min at the end of incubation period. One hundred microlitres of the supernatant was then collected from each well and counted in a gamma counter. Spontaneous release, maximum release and percentage of specific ^{51}Cr release were determined as described (Tilden et al., 1991).

Cytokine assay

Analysis of supernatants cytokine content was performed on PHA-stimulated PBMC from controls or patients, and on normal human PBMC untreated or treated with PCB. After 24 h of culture, supernatants were collected and IL-2, IL-1β, IL-6, IL-12, IFN-γ and TNF-α concentrations were measured by a sandwich enzyme-linked immunosorbent test (ELISA; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions, and results are expressed as picograms per millilitre or nanograms per millilitre as appropriate.

Analysis of PCBs and p,p′-DDE

Blood specimens from the 10 patients with endometriosis and 8 controls tested for NK-cell-mediated cytotoxic activity were analysed for the presence of PCBs 118, 138, 153, and 180 PCB congeners, and *p*,*p*′-DDE.

Ten millilitres of blood spiked with a ^{13}C mixture of labelled standards was added with a mixture of formic acid/2-propanol (4/1, v/v) and extracted with *n*-hexane. The extract was then subjected to treatment with concentrated sulphuric acid in order to destroy the lipidic load co-extracted with the analytical components. The organic phase from the acidic treatment was concentrated under a gentle nitrogen stream in order to undergo additional cleanup step by filtration on a multilayer column. Instrumental analysis was carried out by ion trap mass spectrometry (Polaris Q, Thermo Finnigan) coupled to high resolution gas chromatography.

Statistical analysis

Statistical analysis was calculated using a two-tailed Student's *t* test. A *p* value of less than 0.05 was considered as statistically significant.

Results

Comparison of immune parameters of endometriosis patients and controls

Proliferative response

PBMC isolated both from healthy controls and patients (End) were analysed for their capacity to proliferate in response to PHA, PWM and ConA stimulation. The results shown in Fig. 1A indicate that there was no significant difference in proliferative capacity between controls and patients with all the mitogenic stimuli utilised.

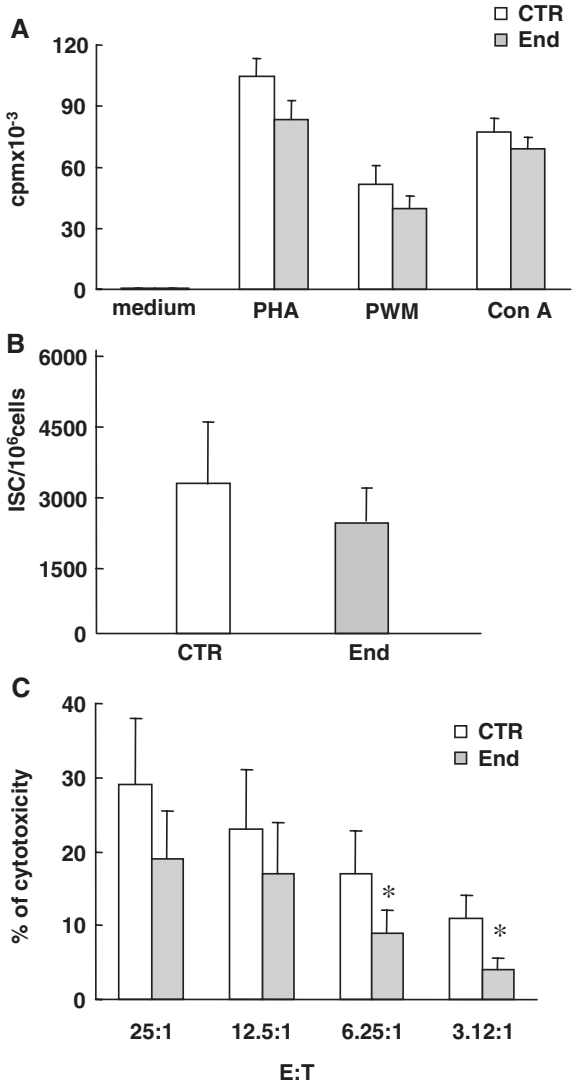

Fig. 1. (A) Freshly isolated PBMC from control (CTR) and endometriosis patients (End) were stimulated with PHA, PWM and ConA for 3 days. Data are expressed as mean cpm±SEM from 10 endometriosis patients and 8 controls. (B) Freshly isolated PBMC from control and endometriosis patients were stimulated with PWM and the number ISC. Data are expressed as mean±SEM from 5 endometriosis patients and 5 controls. (C) Freshly isolated PBMC from control and endometriosis patients. Results are expressed as percent of cytotoxicity (percent of specific ^{51}Cr release). Data are expressed as mean± SEM from 10 endometriosis patients and 8 controls. *$p<0.05$ vs. control. In all experiments the spontaneous release was less than 10% of maximum release.

PWM-induced polyclonal Ig production

We investigated T cell-dependent polyclonal induction of Ig production in PWM-stimulated cultures of PBMC from endometriosis patients and control donors. The results presented in Fig. 1B show no significant changes in the numbers of Ig-secreting cells.

NK-cell-mediated cytotoxic activity

Since a number of reports have measured NK cell activity both in peritoneal fluid and sera from women with endometriosis with varied results (Oosterlynck et al., 1991; Wilson et al., 1994; Ho et al., 1995; Kanzaki et al., 1992), we examined NK-cell-mediated cytotoxicity of PBMC from patients and control donors. The cytotoxic activity was measured against K562 target cells at different E/T ratio. As shown in Fig. 1C, a significant decrease of NK cell activity ($p<0.05$) was observed at 6.25:1 and 3.12:1 E/T ratio in patients with endometriosis with respect to controls.

Cytokine production

Since cytokines drive T, NK and B cell responses, and it has been reported an aberrant cytokine production by peritoneal leukocytes and endometrial cells in endometriosis (Harada et al., 2001; Rana et al., 1996; Van Le et al., 1992; Zarmakoupis and Rier, 1995) we analysed the pattern of the production of these mediators by PBMC from patients with endometriosis and controls. We found that IL-1β and IL-12 production was significantly down-regulated in patients with respect to controls. On the other hand, IL-2, IL-6, IFN-γ and TNF-α production was not dysregulated in patients with endometriosis (Fig. 2).

PCB and p,p′-DDE blood concentrations

Table 1 reports PCB and *p,p′*-DDE serum concentrations and the results of the statistical analysis. As shown, a significant increase in serum concentration level was observed in patients with endometriosis for *p,p′*-DDE and PCBs 118, 138, 153 and the sum of the four congeners analysed. This increase in serum concentration level was observed also for PCB 180, but not at the selected significance level ($p=0.071$).

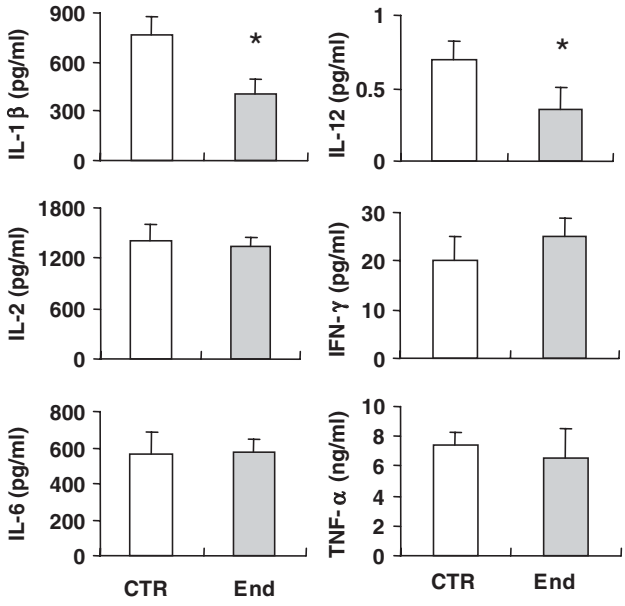

Fig. 2. Freshly isolated PBMC from control and endometriosis patients were stimulated with PHA and after 48 h cytokine supernatant release was determined. Results are expressed as pg/ml or ng/ml from and are means± SEM from 10 endometriosis patients and 8 controls (*$p<0.05$) vs. control.

Table 1
Serum *p,p′*-DDE and PCB concentrations (ng/g, lipid base) in Italian women with (cases) and without (controls) endometriosis

	Mean (SD)		*p* value[a]
	Cases (*N*=10)	Controls (*N*=8)	
p,p′-DDE	770 (550)	310 (140)	0.021
PCB 118	31 (9.2)	12 (4.4)	0.000034
PCB 138	83 (42)	42 (18)	0.012
PCB 153	170 (86)	72 (28)	0.0054
PCB 180	50 (21)	31 (20)	0.071[b]
∑PCBs[c]	330 (130)	160 (66)	0.0022

[a] Student's *t* test. *p* value of less than 0.05 was considered statistically significant.
[b] Not statistically significant.
[c] Sum of PCBs 118, 138, 153, and 180.

In vitro effect of PCB and DDE on normal human PBMC functions

It has been reported that PCB and DDE may exert several effects on the pathophysiology of endometriosis through a number of different pathways (Rier and Foster, 2002). Therefore, we tested in vitro the effect of PCB and DDE on normal human PBMC immune function to determine whether the elevated serum levels of PCB observed in patients with endometriosis correlated with ex vivo observed immune dysregulation. To this aim PBMC from healthy donors were pulsed for 24 h with the following PCB: PCB 153 (0.396 ng/ml), PCB 138 (0.206 ng/ml), PCB 180 (0.196 ng/ml), PCB 118 (0.098 ng/ml), DDE (1.2 pg/ml) and their combination (MIX), and proliferative response, NK-cell-mediated cytotoxic activity and cytokine production were assessed. The concentrations of PCB used ranged in patients' serum levels.

As shown in Fig. 3A neither PCB and DDE nor MIX exerted any effect on unstimulated or PHA-stimulated PBMC proliferative response.

In order to evaluate the effect of PCB and DDE or with their combination on NK-cell-mediated cytotoxicity, effector cells were pre-incubated overnight with these contaminants. As shown in Fig. 3B, a significant decrease of NK cell activity ($p<0.05$) was observed at 1.56:1 E/T ratio when PCB153 and *p,p′*-DDE were added while no change was detected after the exposure to PCB 138, PCB 180 or PCB 118. Moreover, MIX induced a significant down-regulation of NK-cell-mediated cytotoxicity at 12.5:1 E/T ratio.

Since inflammation may play an important role in the pathogenesis of endometriosis, we evaluated the impact of PCB and DDE on cytokine production. In vitro exposure of PHA-stimulated PBMC to PCB 153, 138, 180, 118, DDE and their combination (MIX) caused a down-regulation of IL-12 and IL-1β in culture supernatants while no significant decrease of IL-2, IL-6, IFN-γ and TNF-α was found in the same supernatants (Fig. 4).

Discussion

In this study we found that peripheral blood NK cell cytotoxic activity and IL-1β and IL-12 production were significantly down-regulated in patients with endometriosis

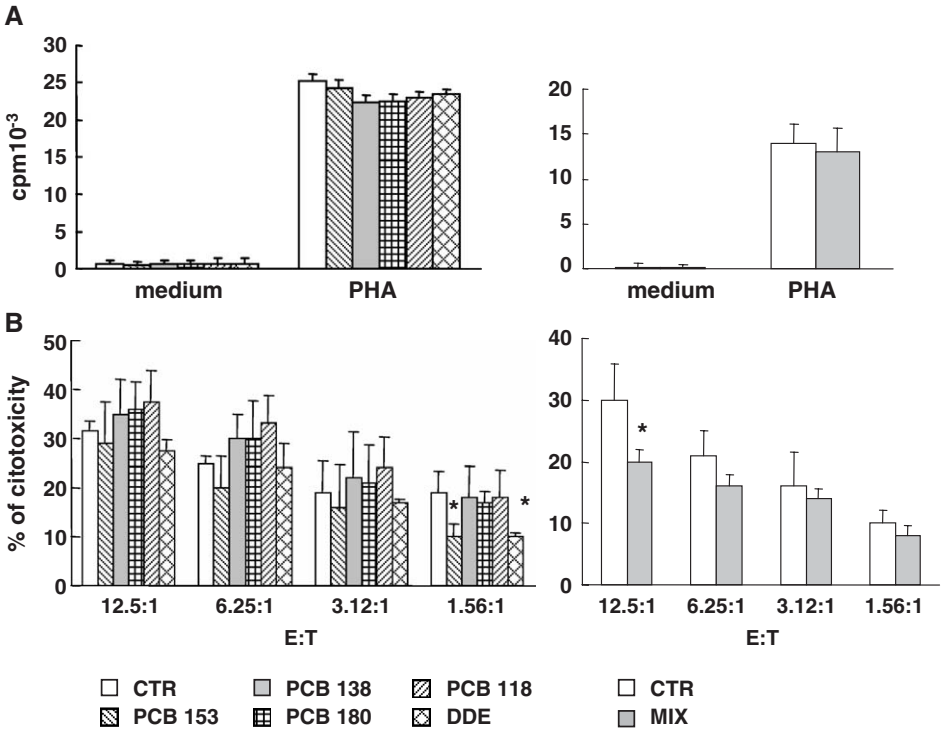

Fig. 3. (A) Effect of in vitro 118, 138, 153, and 180 PCB congeners, *p,p′*-DDE and MIX exposure on the PHA-induced proliferative response of normal human PBMC. Results are expressed as mean cpm±SEM from five independent experiments. (B) Effect of in vitro 118, 138, 153, and 180 PCB congeners, *p,p′*-DDE and MIX exposure on NK-cell-mediated cytotoxic activity. Data are expressed as mean±SEM from five independent experiments. *$p<0.05$ vs. control.

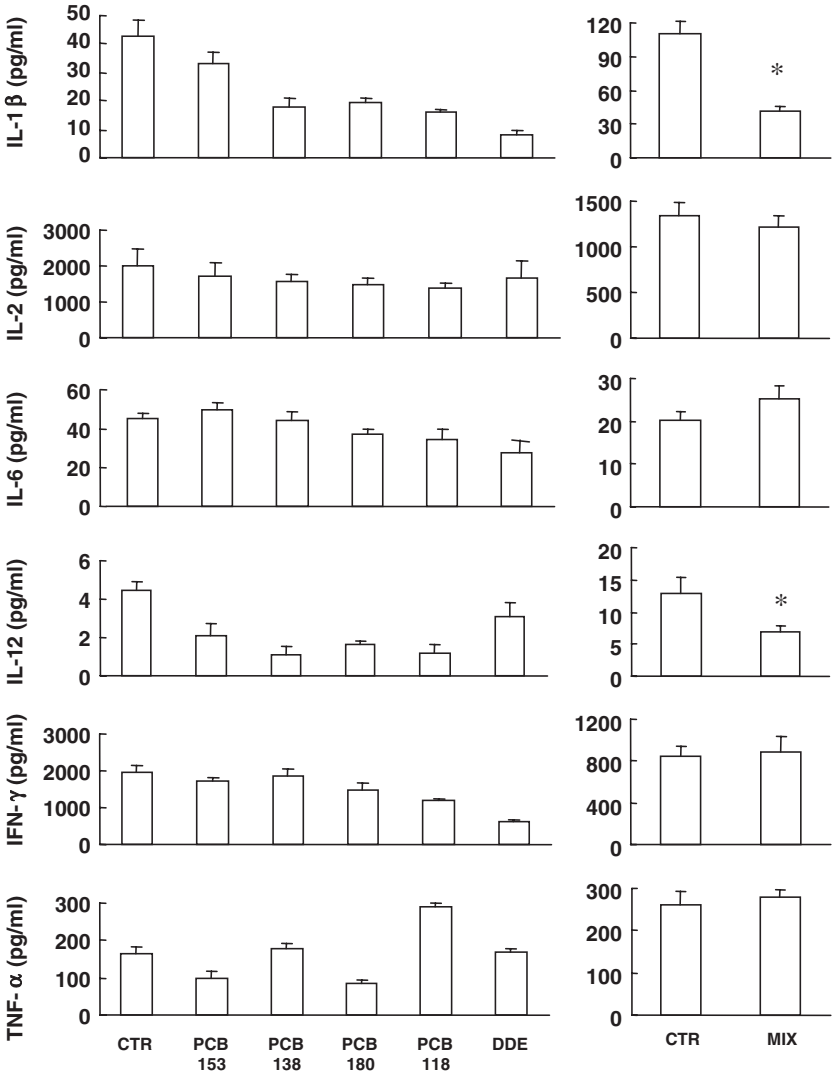

Fig. 4. Effect of in vitro 118, 138, 153, and 180 PCB congeners, *p*,*p*′-DDE and MIX exposure on cytokine release by normal human PBMC stimulated for 24 h with PHA. Results are expressed as pg/ml or ng/ml and are mean±SEM from four independent experiments. *$p<0.05$ vs. control.

with respect to controls. This matches nicely with higher serum levels of PCBs and *p*,*p*′-DDE in the same patients. Interestingly, we found that in vitro exposure of normal PBMC to PCBs, *p*,*p*′-DDE and their combination caused a significant decrease of NK cell activity and of IL-1β and IL-12 production.

Understanding the involvement of the immune system in the development of endometriosis may help to elucidate the pathogenesis and spontaneous evolution of this condition. At present, plausible hypotheses are both that pelvic inflammation and other immunological changes represent the consequence of endometriosis or that defective immunosurveillance may facilitate the survival of ectopic endometrial tissue and progression of disease. Oosterlynck and colleagues were the first to demonstrate decreased NK cell cytotoxic activity against autologous endometrial cells in women with endometriosis, which correlated with the stage of the disease (Oosterlynck et al., 1991). The same group later showed a significantly greater NK cell suppressive activity in the peritoneal fluid of infertile women with endometriosis than in fertile controls (Oosterlynck et al., 1993). Other studies confirmed these findings in serum (Kanzaki et al., 1992) and pelvic fluid (Ho et al., 1995). Prefumo et al. demonstrated that the reduced expression of CD54 on secretory endometrial cells could result in their reduced recognition and killing by NK cells (Prefumo et al., 2002). Deviating cytokine patterns have been reported in in vivo and in vitro studies in tissue (Bergqvist et al., 2001) and peritoneal fluid (Rana et al., 1996; Koga et al., 2005) of women with endometriosis. Endometriosis can be considered as an autoimmune disorder, associated with a dysfunction of natural immunity and can represent a human model of disease in which hormones, cytokines and the immune system influence each other (Matarese et al., 2003).

Besides the immunological/inflammatory aetiology of endometriosis other factors such as environmental pollutants seem to be involved in the pathogenesis of endometriosis (Rier and Foster, 2002). In particular, the possible role as risk factors of highly persistent, lipophilic and bioaccumulative organochlorinated environmental pollutants, such as dioxins and PCBs, has

been recently hypothesised, mainly on the basis of the results obtained in experimental studies (Rier et al., 1993, 2001). Results from the few clinical studies so far carried out to test the hypothesis of an association between endometriosis and these compounds are conflicting (Louis et al., 2005; Mayani et al., 1997; Heilier et al., 2004; Gerhard and Runnebaum, 1992; Eskenazi et al., 2002; Lebel et al., 1998), although discrepancies observed could be partly due to major differences in study design and methodology.

In our study we found a significant decrease of peripheral blood NK-cell-mediated cytotoxic activity and IL-1β and IL-12 production in patients with endometriosis with respect to controls, indicating the involvement of the immune response in the pathogenesis of disease. Our results nicely fit with previous reports indicating a compromised natural killer cell activity in peritoneal fluid and sera in women with endometriosis (Oosterlynck et al., 1991; Kanzaki et al., 1992). Moreover, in a previous study we observed that the number of NK cells was slightly higher in women with endometriosis or other gynaecological benign diseases compared to healthy controls (M.G. Porpora, personal communication). Therefore, the reduced cytotoxic activity exerted by NK cells is entirely ascribable to a real reduction in the killing efficiency of NK cells.

Moreover, here we demonstrated increased serum levels of PCB 118, 138, 153, and 180, the most abundant congeners in all human tissues, and of their sum. The observed increase was of statistical significance ($p<0.05$) for all of them, with the exception of PCB 180 ($p=0.07$). The causes of PCBs and DDE increase in women with endometriosis remain to be elucidated. Recently, we demonstrated that the difference in the observed serum levels could be determined by variability in toxicokinetics, possibly due to genetic polymorphism of the enzymes involved in PCBs and DDE metabolism and thus to the presence of isoforms with different metabolic capabilities (Porpora et al., in press).

Among the three PCBs for which a significant increase is observed, PCB 118 is the only congener with a dioxin-like structure and activity. Significantly increased serum levels of DDE were also observed in women with endometriosis. Altogether these data strongly suggest a correlation between the immune dysregulation observed in endometriosis patients and increased serum levels of PCBs and DDE in the same patients. Therefore, to better define this correlation we performed an in vitro analysis of the immunomodulating effect of these contaminants on normal human PBMC. We found that exposure to PCB 153, *p,p′*-DDE and their combination caused a significant decrease of NK cell activity ($p<0.05$) while no change was detected after the exposure to PCB 138, PCB 180 or PCB 118. Moreover, exposure of PHA-stimulated PBMC to PCB 153, 138, 180, 118, DDE and MIX caused a down-regulation of IL-12 and IL-1β in culture supernatants. This in vitro finding supports the hypothesis that these compounds may influence immune responses in women with endometriosis (Rier and Foster, 2002). Of relevance, we found that among the congeners tested those associated with down-regulation of IL-1β and IL-12 production are the most abundant in food and in human tissues. Moreover, most pronounced effects that we observed on both cytokine production and NK cell cytotoxic activity are those exerted by PCB mixture and DDE, therefore in conditions of real human exposure, such as that resulting from diet.

IL-12 and IL-1β produced by monocyte/macrophages have been demonstrated to have multiple effects on T cells and NK cells (D'Andrea et al., 1992; Chan et al., 1991; Cooper et al., 2001). These include both the activation of NK cell cytotoxic activity and the proliferation. We found a significant down-regulation of IL-12 and IL-1β when PBMC were pulsed with the MIX. This finding prompted us to hypothesise that PCBs and DDE act synergistically since a less evident down-regulating effect was observed when used as single agent. Hence, a role for PCBs and DDE in the impairment of NK cell function by indirect effect on the specific cytokine network cannot be ruled out.

Taken together, our results may contribute to clarify the role of persistent pollutants as PCBs and DDE in the dysregulation of the immune functions associated with the pathogenesis of endometriosis.

Of note, the in vitro effects of PCBs and DDE on normal PBMC immune function resemble the trend obtained ex vivo on endometriosis patients. Thus, our in vitro system could represent a useful model to test therapeutic manipulation (i.e. recombinant cytokine) of immune responses in vivo.

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