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Progesterone-induced blocking factor (PIBF) modulates cytokine production by lymphocytes from women with recurrent miscarriage or preterm delivery

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Abstract

Spontaneous miscarriage and preterm delivery are common complications of pregnancy. Pro-inflammatory cytokines have been shown to be associated with recurrent spontaneous miscarriage (RSM) and preterm delivery (PTD) and these have led to exploration of ways to downregulate pro-inflammatory cytokines and/or to upregulate anti-inflammatory cytokines. Progesterone-induced blocking factor (PIBF) is a molecule with inhibitory effects on cell-mediated immune reactions. We have ascertained the effects of PIBF on secretion of selected type 1 and type 2 cytokines by peripheral blood mononuclear cells from healthy non-pregnant women, women undergoing normal pregnancy, women with unexplained RSM and women with PTD. Peripheral blood mononuclear cells from 30 women with a history of unexplained RSM, 18 women undergoing PTD, 11 women with normal pregnancy and 13 non-pregnant healthy women were stimulated with a mitogen in the absence and presence of PIBF after which the levels of cytokines released into culture supernatants were determined by ELISA. Production of the type 2 cytokines IL-4, IL-6 and IL-10 by lymphocytes from the RSM and PTD groups and of IL-4 and IL-10 by lymphocytes from healthy pregnant women was significantly increased upon exposure to PIBF, while the levels of type 1 cytokines were not affected. Ratios of type 1:type 2 cytokines were decreased, suggesting a shift towards Th2 bias. PIBF did not affect cytokine production by lymphocytes from non-pregnant women. Thus, PIBF acts on lymphocytes in pregnancy to induce a type 1 to type 2 cytokine shift by upregulating the production of type 2 cytokines.

Keywords: Recurrent spontaneous miscarriage; Th1; Th2; Cytokines; Progesterone-induced blocking factor; Preterm delivery

1. Introduction

Spontaneous miscarriage occurs at a frequency of 15–25% and is one of the most common complications of pregnancy. Only about 40–50% of the cases of recurrent spontaneous miscarriage (RSM) are explained by chromosomal abnormalities or other well-explained mechanisms, with a substantial proportion still classified as "unknown" or "unexplained" (Hill, 1991). Cell-mediated immune reactivity has been shown to have significant deleterious effects on the conceptus (Wegmann et al., 1993; Hill et al., 1995; Clark et al., 2005) and it is proposed that these effector cells actually exert their deleterious effects on the conceptus via

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cytokine action (Wegmann et al., 1993; Hill et al., 1995; Chaouat et al., 1996; Marzi et al., 1996; Raghupathy, 2001).

A dampening of type 1 or pro-inflammatory reactivity and augmentation of type 2 or anti-inflammatory immunity during pregnancy occurs during normal pregnancy (Guilbert, 1996; Wegmann et al., 1993; Raghupathy, 2001). Significantly reduced IL-2 and IFNγ mRNA expression has been demonstrated during normal human pregnancy with type 1:type 2 ratios revealing a shift to a pronounced type 2 status (Kruse et al., 2000). Significantly increased IL-4-producing T cells and significantly decreased IFNγ- and IL-2-producing T cells were demonstrated in normal pregnant women as compared to non-pregnant women suggestive of a type 2 shift in pregnancy (Kruse et al., 2000). Type 2 cytokine production predominates in the second and third trimesters of pregnancy (Jones et al., 1997) supporting the notion that successful pregnancy is correlated with, or perhaps even depends on, preferential stimulation of type 2 cytokine-producing T cells.

Cytokine patterns have been reported to be different in women with RSM as compared to normal pregnant women. Hill et al. (1995) have shown that PBMC of recurrent aborters stimulated with a human trophoblast antigen extract produce higher levels of the type 1 cytokines and embryotoxic activity as compared to normal pregnancy. These authors concluded that type 1 immunity to trophoblast antigens is associated with unexplained RSM and may play a role in reproductive failure while type 2 immunity may be the natural response to trophoblast in contributing to successful pregnancy. We have demonstrated that IL-4, IL-5, IL-6 and IL-10 are produced at higher levels at the end of the first trimester and at delivery in normal pregnancy than in RSM, while the levels of the type 1 cytokines IL-2, IFNγ and TNFα are uniformly higher in RSM than in normal pregnancy (Raghupathy et al., 2000). Ratios of type 1 to type 2 cytokines were higher in the RSM group as compared to the normal pregnancy group, indicating a greater type 1 bias in RSM and a greater type 2 bias in normal pregnancy. Jenkins et al. (2000) reported that continuing pregnancies were associated with increased levels of IL-10 and reduced levels of IFNγ, while pregnancies in the recurrent miscarriage group had lower levels of IL-10 and increased levels of IFNγ.

Cytokines have also been implicated in the pathogenesis of preterm delivery; Hillier et al. (1993) have reported that women with infection-related PTD had increased serum levels of pro-inflammatory cytokines. The presence of IFNγ in cervicovaginal fluid has been shown to be a risk factor for preterm labour in asymptomatic women (Chiileannain and Brennecke, 1998). Similarly, increased production of TNFα by placental cells (Steinborn et al., 1999) and by decidual tissues (Keelan et al., 1999) has been reported. We have demonstrated that significantly higher levels of the type 1 cytokines, IFNγ and IL-2, were produced by mitogen-stimulated peripheral lymphocytes from women with PTD than by lymphocytes from women with normal pregnancy who showed significantly greater production of the type 2 cytokines, IL-4, IL-5 and IL-10 (Makhseed et al., 2002). Dudley (1999) suggests that the production of pro-inflammatory cytokines could be a pathophysiologic basis for an "intrauterine inflammatory response syndrome".

Successful pregnancy, therefore, seems to be associated with a type 2 bias, while a shift towards type 1 cytokine dominance may be associated with conditions such as RSM and PTD. If maternal type 1 cytokine dominance is responsible for these complications it would be of scientific and clinical interest to develop modalities that shift the maternal cytokine balance from a pro-inflammatory profile towards a more anti-inflammatory one. One possible approach to this would be to use hormones and pregnancy-related molecules such as the progesterone-induced blocking factor (PIBF).

Pregnancy lymphocytes exposed to progesterone secrete PIBF, an immunoregulatory protein capable of blocking lymphocyte function *in vitro* (Szekeres-Bartho et al., 1995). The percentage of lymphocytes secreting PIBF is significantly reduced in women prone to recurrent miscarriage, as compared to women with normal pregnancy (Szekeres-Bartho et al., 2002). Women at pregnancy termination have serum PIBF levels lower than those of healthy pregnant women; similarly women who abort have low PIBF levels (Szekeres-Bartho et al., 1989). Sera obtained at the onset of spontaneous miscarriage contained significantly less PIBF than those of healthy pregnant women. Women with pathologic pregnancies do not show a rise in urinary PIBF levels that is observed in normal pregnancy (Polgar et al., 2004a) and there is a trend towards higher rates of miscarriage when PIBF is absent (Check et al., 2005). PIBF downregulates cell-mediated immune reactions such as cytotoxic T cell activity and the proliferation of allogeneically stimulated lymphocytes (Szekeres-Bartho et al., 1997). PIBF blocks NK activity (Szekeres-Bartho et al., 1997) and prevents fetal resorption induced by the transfer of NK cells (Szekeres-Bartho and Chaouat, 1990). Thus, PIBF appears to play important immunoregulatory roles in successful pregnancy (Szekeres-Bartho et al., 1989, 1995, 1997, 2002; Szekeres-Bartho and Chaouat, 1990; Polgar et al., 2004a; Check et al., 2005).