Immunology 1990 **69** 277–281

The effect of human placental protein 14 (PP14) on the production of interleukin-1 from mitogenically stimulated mononuclear cell cultures

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Accepted for publication 25 October 1989

SUMMARY

Crude human decidual tissue extracts containing placental protein 14 (PP14) were shown to inhibit the production of interleukin-1 beta (IL-1) from mitogenically stimulated mononuclear cell cultures. The inhibition was dose-dependent over the range of PP14 concentrations investigated (0–8·0 mg/l) and was effective on both phytohaemagglutinin-(PHA) and lipopolysaccharide- (LPS)-induced IL-1 secretion. Using these culture systems, a PP14 concentration of 1·0 mg/l induced a 34% suppression of IL-1 secretion following LPS stimulation and 22% following PHA stimulation. For PHA stimulation the suppression of IL-1 secretion was effective throughout the culture period investigated (0–89 hr). Individual crude decidual extracts inhibited the incorporation of [^{3}H]thymidine into PHA-stimulated lymphocytes, such inhibition being partially reversed by the addition of exogenous recombinant IL-1 to the cultures.

These results suggest that the previously reported immunosuppressive activity of PP14 may be mediated by the suppression of IL-1 secretion.

INTRODUCTION

The developing fetus in human pregnancy may be regarded as a foreign allograft in that semi-allogeneic fetal tissue comes into direct contact with the maternal immune system. The non-rejection of the fetus in normal pregnancy has been attributed to a number of mechanisms, one of which may be the presence of immunomodulatory molecules within the feto-placental unit. One such protein with potential immunosuppressive properties is placental protein 14 (PP14), a 42,000 MW glycoprotein that was originally isolated from term placental tissue (Bohn, Kraus & Winckler, 1982).

PP14 is secreted by the decidualizing endometrium and appears in the maternal circulation at peak levels during the first trimester of pregnancy (Julkunen *et al.*, 1985). Previous studies have shown PP14 to exhibit potential immunosuppressive activity on both allogeneically stimulated (Bolton *et al.*, 1986, 1987) and mitogenically stimulated (Pockley *et al.*, 1988) peripheral blood lymphocytes.

Mitogenic and allogeneic activation of lymphocytes leads to the production of a range of soluble mediators of the immune response. Central to the response is the production of interleukin-1 (IL-1) and interleukin-2 (IL-2) and the expression of IL-2 receptors on activated lymphocytes. Interleukin-1 has two forms, IL-1 alpha and IL-1 beta, with less than 30% amino acid homology but similar biological activities. These molecules induce a broad spectrum of biological changes in various tissues, acting as a central mediator of host defence mechanisms during inflammation, tissue injury and infection. Whilst many nucleated cell types produce IL-1, the predominant source of the cytokine is cells of the monocyte/macrophage lineage. One of the many biological activities of IL-1 is its ability to activate lymphocytes. This may be mediated by increasing lymphocyte responsiveness to IL-2 via an induction of 70,000 MW IL-2 receptor chains which combine with the already present 55,000 MW (Tac) protein to form high-affinity receptor sites (Oppenheim *et al.*, 1986).

Recent work has shown that PP14 inhibits the production of IL-2 by phytohaemagglutinin-stimulated perpheral blood lymphocytes (Pockley & Bolton, 1989). It is only the interaction of IL-2 with the high affinity form of the IL-2 receptor which elicits the biological activity of this cytokine (Robb, Munck & Smith, 1981; Robb, Greene & Rusk, 1984). As yet it is not known whether PP14 directly inhibits IL-2 production, whether it affects the high affinity IL-2 receptor, or whether it is acting at an earlier stage in the immune response. PP14 does not appear to inhibit the interaction of IL-2 with the IL-2 receptor once the receptor has been expressed (Pockley & Bolton, 1988).

In this study we have investigated the effects of PP14 on the production of IL-1 from mitogenically stimulated peripheral blood mononuclear cell cultures in order to identify the level of the immune response that is modulated by PP14.

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MATERIALS AND METHODS

PP14 preparations

Extracts of decidual tissue separated from the uterine aspirates of elective terminations of normal pregnancies at 10–14 weeks gestation, and identified by subsequent histological examination, were prepared in phosphate-buffered saline containing 0·1 mmol/l phenylmethylsulphonyl fluoride (PBS) (10 ml/g wet weight of tissue). Following extensive dialysis against PBS (100 vol, three changes) at 4° for 24 hr, extracts were stored at −20° before use. Aliquots (1·0 ml) of extracts were treated with an equal volume of CNBr-activated Sepharose-4B (Pharmacia Biotechnology, Milton Keynes, Bucks, U.K.) coupled to a monoclonal anti-PP14 antibody of previously reported specificity (Bolton *et al.*, 1987; Pockley & Bolton, 1989) and immunoadsorbed, by mixing for 3 hr at room temperature followed by centrifugation (300 *g*, 5 min) and aspiration, to specifically remove PP14. Controls were prepared by similarly treating decidual extracts with CNBr-activated Sepharose-4B coupled to glycine. IL-1 was undetectable or present at the limit of detection (20–30 pg/ml) of the ELISA system used to measure this cytokine in the crude decidual extracts.

PP14 was purified from decidual tissue by a modification of the method of Westwood *et al.* (1988). Briefly, decidual tissue obtained as above was homogenized with 50 mmol/l Tris-HCl buffer, pH 7·2, containing 0·1 mmol/l phenylmethylsulphonyl fluoride (Tris buffer). After removal of the cell debris by centrifugation (5000 *g*, 30 min at 4°), the extract was applied to a TSK DEAE 5PW ultrapac column (7·5 × 75 mm; Pharmacia) and eluted with a linear 0–1·0 mol/l sodium chloride gradient in Tris buffer. PP14-containing fractions were identified by the radioimmunoassay described previously (Bolton *et al.*, 1983). After desalting on a column of Sephadex G-25 (0·9 × 12 cm; Pharmacia) eluted with Tris buffer, the PP14-containing fractions were chromatographed on a 5-ml column of Reactive Blue 2–Sepharose CL-6B (Sigma, Poole, Dorset, U.K.) in Tris buffer, to remove contaminating albumin. The PP14-containing fractions were rechromatographed on TSK DEAE 5PW as above and the PP14 peak stored at −20°.

Mitogenic responses

Human peripheral blood mononuclear cells were isolated from the heparinized whole blood of normal volunteers by density gradient centrifugation, as described by Boyum (1968), using Lymphopaque lymphocyte separation medium (Nycomed UK Ltd, Sheldon, Birmingham, U.K.). The isolated lymphocytes were washed three times in Dulbecco's modification of Eagle's medium (DMEM; Northumbria Biologicals, Cramlington, Northumberland, U.K.) and finally resuspended at a concentration of 1×10^6 viable cells per ml (as assessed by trypan blue dye exclusion) in SF1 serum-free hybridoma growth medium (Northumbria Biologicals) containing 3·7 g/l $NaHCO_3$, 200 IU/ml penicillin and 200 μg/ml streptomycin.

For the mitogenic stimulation assay, 100 μl of the washed cell suspension (1×10^5 cells/well) were incubated in the presence of 50 μl of the PP14 preparation, 50 μl of PHA (2·5 μg/ml final concentration; Sigma Chemical Co.) and 50 μl of growth medium in 96-well microtitre culture plates (Linbro, Flow Laboratories, Irvine, Ayrshire, U.K.). For investigating the reversal of the suppressive activity by the addition of recombinant IL-1, 50 μl of recombinant IL-1 (rIL-1; Cistron Biotechnology, Pinebrook, NJ) were added to the respective cultures to give a final concentration of 5 U/ml. Units were as defined by the manufacturer on the basis of thymocyte proliferation assays.

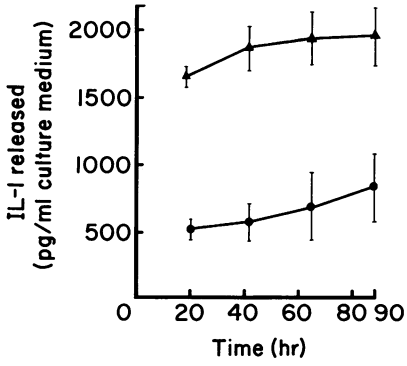

Figure 1. The kinetics of IL-1 production from PHA-stimulated peripheral blood lymphocyte cultures incubated either with crude decidual extracts in which the PP14 had been removed by immunoadsorbtion (▲) (PP14 concentration less than 1·0 mg/l) or with non-immunoadsorbed extracts (●) (PP14 concentration 8·0 mg/l).

The plates were incubated at 100% humidity, 37° and 5% CO_2 for 72 hr. Six hours prior to the termination of the cultures, the cells were pulsed with 1 μCi of [^{3}H]thymidine (90 Ci/mmol; Amersham International, Amersham, Bucks, U.K.) and on termination were harvested onto glass fibre filters using a semi-automated cell harvester (Skatron AS, Lier, Norway). The degree of lympho-proliferation was assessed by liquid scintillation counting (LKB Rackbeta, Pharmacia). All cultures were performed in triplicate. The data presented in this study were pooled from a number of experiments using different cell donors.

Preparation of cytokine-containing supernatants

Peripheral blood mononuclear cells were isolated as described earlier. 10^6 cells were cultured in 24-well culture plates (Linbro, Flow Laboratories) in the presence of 200 μl of PP14 preparation and 200 μl of PHA (5 μg/ml final concentration) or lipopolysaccharide (LPS; 20 μg/ml final concentration; Sigma Chemical Co.) for the times indicated in the results section. Control cultures contained PBS in place of the PP14 preparation and DMEM in place of the mitogen solution. At the termination of the culture period, the culture supernatants were harvested and stored at −20° until assay.

Cell culture supernatants were assayed for IL-1 beta and tumour necrosis factor alpha (TNF), according to the manufacturer's recommendations using the respective ELISA kits (IL-1, Cistron Biotechnology; TNF, T-Cell Sciences, Cambridge, MA).

RESULTS

The production of IL-1 and TNF from control cultures (cultures stimulated in the presence of immunoadsorbed PP14 preparations, either decidual extract or purified material), was taken as 100% and the production from cultures stimulated in the presence of PP14 preparations treated with control adsorbent compared to these values.

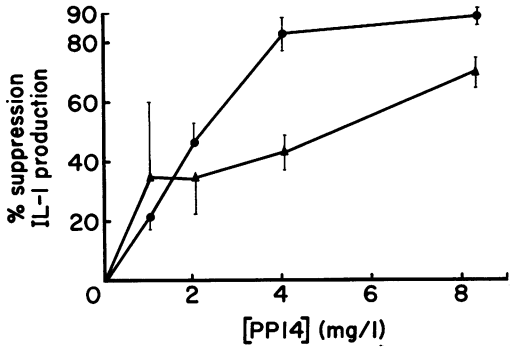

Figure 2. The effect of PP14 on the production of IL-1 from PHA-(●) and LPS-(▲) stimulated peripheral blood lymphocyte cultures after 72 hr of culture. Results presented are the means of four experiments.

Interleukin-1 was detectable at a mean concentration of about 1·7 ng/ml in the supernatants of PHA-stimulated mononuclear cell cultures after 18 hr of incubation, the earliest time-point investigated. The levels of IL-1 in the supernatant tended to increase (from 1·7 to 2·0 ng/ml) during the culture period of 90 hr (Fig. 1), although the increase was not statistically significant. Incubation of the mononuclear cell cultures with a crude decidual extract containing 8·0 mg/l of PP14 inhibited the production of IL-1 compared to the immunoadsorbed control (PP14 concentration $<1{\cdot}0$ mg/l; Fig. 1) by about 60–70%. Similarly, purified PP14 preparations suppressed IL-1 accumulation in culture supernatants (data not shown). PP14 inhibited the accumulation of IL-1 in the culture supernatants throughout the culture period (Fig. 1). Measurement of TNF in the same culture supernatants after 72 hr culture showed no such inhibition of accumulation of this cytokine by PP14 ($1{\cdot}04\pm0{\cdot}21$ ng/ml stimulated in the absence of PP14; $1{\cdot}47\pm0{\cdot}36$ ng/ml stimulated in the presence of 8·0 mg/l of PP14; means ± SD, $n=3$).

The suppressive activity of PP14 on IL-1 release was dose-related over the range of PP14 concentrations investigated (0–8·0 mg/l; Fig. 2). A PP14 concentration of 8·0 mg/l induced a marked suppression of IL-1 release ($87{\cdot}5\pm0{\cdot}7\%$) and this was not due to a cytotoxic effect of PP14, as cell viability (assessed by trypan blue exclusion) in the presence of the decidual extract was comparable to that of control cultures containing no decidual extract. The inhibitory activity of PP14 was not specific for PHA-stimulation of mononuclear cells as the levels of IL-1 in the cell culture supernatants following stimulation by LPS were also suppressed by PP14 in a dose-dependent manner (Fig. 2). Using these culture systems, a PP14 concentration of 1·0 mg/l induced a 34% suppression of IL-1 secretion following LPS stimulation and 22% suppression following PHA stimulation (Fig. 2).

Radiolabelled PP14 did not bind significantly to PHA coupled to CNBr-activated Sepharose-4B. In addition, the suppressive activity of the decidual tissue extracts on [^{3}H]TdR uptake into PHA-stimulated mononuclear cells was not dependent on PHA concentration (data not shown). These observations would appear to eliminate the possibility that PP14 exerts its effect of inhibiting lympho-proliferation by complexing with PHA and thereby making the lectin unavailable for inducing cell transformation.

Table 1. The effect of the addition of exogenous recombinant IL-1 (5 U/ml final concentration) on the inhibition of [^{3}H]thymidine uptake into PHA-stimulated lymphocytes by five individual decidual tissue extracts (PP14 concentrations 2·0–8·0 mg/l)

		Inhibition of [^{3}H]TdR uptake (%)	
Decidual extract	[PP14] (mg/l)	Without IL-1	With IL-1
DE A	5·0	$30{\cdot}0\pm2{\cdot}0$	$25{\cdot}0\pm4{\cdot}2$
DE B	4·8	$62{\cdot}0\pm8{\cdot}3$	$12{\cdot}0\pm2{\cdot}3$
DE C	4·0	$46{\cdot}0\pm7{\cdot}2$	$25{\cdot}0\pm2{\cdot}9$
DE D	8·0	$48{\cdot}0\pm6{\cdot}4$	$33{\cdot}0\pm2{\cdot}8$
DE E	2·0	$41{\cdot}0\pm9{\cdot}0$	$30{\cdot}0\pm3{\cdot}0$
Mean		$45{\cdot}4\pm11{\cdot}6$	$25{\cdot}0\pm8{\cdot}0$*

Results expressed are means of three experiments. Incorporation of [^{3}H]TdR into stimulated control cultures 140, 618 ± 3863 c.p.m.

* $P<0{\cdot}001$ (paired Student's t-test of individual data points).

To investigate whether inhibition of IL-1 secretion may be a factor in the observed suppression of [^{3}H]-TdR uptake into PHA-stimulated lymphocytes by PP14 (Pockley *et al.*, 1988), peripheral blood mononuclear cells were stimulated by PHA in the presence of five individual crude decidual extracts (PP14 concentrations 2·0–8·0 mg/l) and in the presence and absence of 5 U/ml of recombinant IL-1 beta. The incorporation of [^{3}H]TdR into control cultures (incubated with immunoadsorbed decidual extract, PP14 concentration $<1{\cdot}0$ mg/l) was taken as 100% and the incorporation into test cultures (containing unadsorbed decidual extract) compared to these values. After 72 hr of culture the decidual extracts inhibited the incorporation of [^{3}H]TdR into the stimulated cells ($45{\cdot}4\pm11{\cdot}6\%$; Table 1). Addition of 5 U/ml of recombinant IL-1 at the initiation of the culture period significantly reduced the suppressive activity of the crude decidual extracts to 25·0% ($P<0{\cdot}001$, paired Student's t-test of the individual data points).

From Table 1 it can be seen that DE B, which contained a similar concentration of PP14 to DE A, exhibited over twice the inhibitory activity. Furthermore, IL-1 reversed the suppressive activity of DE B twice as effectively as the suppressive activity of DE A. The reasons for these observations are currently unexplained. It would be too simplistic to suggest that PP14 is the sole immunomodulatory protein present during pregnancy. Although PP14 may be the major such factor normally present in crude decidual tissue extracts, in this case other molecules present may have contributed to the data obtained.

DISCUSSION

There is increasing evidence that the human uterus is a site of production of immunomodulatory factors. Nakayama *et al.* (1985) have demonstrated inhibition of allogeneically stimulated lymphocytes in the presence of first trimester pregnancy endometrium, and Wang *et al.* (1987) have demonstrated the

release of soluble factors capable of suppressing both the mitogenic and allogeneic stimulation of lymphocytes from secretory phase endometrium. The results of Bolton *et al.* (1987) and Pockley *et al.* (1988) indicate that the decidual protein PP14 suppresses lympho-proliferation, and Matsui, Yoshimura & Oka (1989) have identified a factor with a molecular weight between 43,000 and 67,000 released from dispersed decidual cell suspensions with a similar activity, an observation that is in accordance with PP14 being the factor responsible.

In an investigation of the mode of action of PP14 in immunosuppression, Pockley & Bolton (1989) found that PP14 appeared to inhibit the secretion of IL-2 from PHA-stimulated lymphocyte cultures, with the addition of exogenous rIL-2 partially reversing this inhibitory activity. Matsui *et al.* (1989) similarly provided evidence for the inhibition of IL-2 production in relation to their soluble decidual factor.

Interleukin-1 is central to the immune response, controlling lympho-proliferative responses to various stimuli by modulating the affinity of IL-2 receptors on the surface of stimulated lymphocytes (Oppenheim *et al.*, 1986). Furthermore, peripheral blood mononuclear cells can respond to IL-2 stimulation by the production of both IL-1 alpha and beta from monocytes (Numeroff, Aronson & Mier, 1988). It could, therefore, be possible that the suppression of IL-1 secretion by PP14 observed in this study is secondary to the previously reported suppression of IL-2 (Pockley & Bolton, 1989). However, the concentrations of IL-2 required to generate IL-1 (around 10 U/ml) are in excess of those generated from lymphocytes following PHA stimulation (1·1 U/ml; Pockley & Bolton, 1989). In addition, a suppression of IL-1 secretion mediated by a suppression of IL-2 secretion would not explain the inhibitory effect of PP14 on LPS-induced IL-1 production. We therefore conclude that the key action of PP14 in suppressing lympho-proliferation is by inhibiting the prodution and/or release of IL-1.

A number of naturally occurring IL-1 inhibitors have been described (reviewed by Larrick, 1989); however, most of these molecules appear to inhibit the action of IL-1 at the level of IL-2 production rather than its secretion. Similarly, using the allogeneic mixed lymphocyte reaction, Domingo, Moreno & Palomino (1988) have shown that pregnancy serum interferes with IL-1 activity by rendering IL-2 producer cells unable to synthesize IL-2 and subsequently to proliferate. The mode of action of PP14 would appear to differ from these inhibitory factors.

The secretion of IL-1 is regulated by several second messenger pathways. A major regulator of IL-1 synthesis and release is prostaglandin E_2 (PGE_2; Kunkel, Chensue & Phan, 1986a) and PGE_2 has been implicated in the immunosuppressive activity of human decidual cells (Parhar, Yagel & Lala, 1989). Exogenous PGE_2 suppresses the release of both macrophage-derived IL-1 and TNF (Kunkel *et al.*, 1986b). Although it is possible that the immunosuppressive activity of PP14 may be mediated by PGE_2 associated with the PP14 molecule, this is unlikely since the decidual extracts used in this study were extensively dialysed prior to incorporation into the culture systems and a similar activity was found with purified PP14. Furthermore, concentrations of PP14 which suppressed IL-1 production from PHA-stimulated mononuclear cell cultures had no suppressive effect on TNF secretion.

The mechanism by which PP14 inhibits IL-1 secretion is currently under investigation. As PP14 appears early in pregnancy, it may be associated with the process of implantation and in maintaining the early conceptus which could be particularly susceptible to rejection by the maternal immune system.

ACKNOWLEDGMENTS

This work was funded by Sterivet Laboratories Ltd, Ontario, Canada. The authors would like to thank C. Dalton for her expert technical assistance.

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