Inhibition of lymphocyte proliferation by bovine trophoblast protein-1 (Type I trophoblast interferon) and bovine interferon-α1

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ABSTRACT


Bovine trophoblast protein-1 (bTP-1) is a Type I interferon secreted by the bovine trophoblast from about Day 15 of pregnancy. It is not known whether bTP-1 has functional properties in common with other interferons. The aim of the present study was to determine whether bTP-1 inhibits proliferation of lymphocytes induced by mitogens, mixed lymphocyte cultures (MLC) and interleukin-2 (IL-2) and, if so, whether this activity is similar to that of a related interferon, bovine interferon-α1 (bIFN-α1). Stimulation of lymphocyte proliferation caused by phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) was inhibited by bTP-1 and bIFN-α1 without any reduction in cell viability. Maximum or near-maximum inhibition (less than 50%) was achieved at concentrations of 0.5-5.0 nM of bTP-1 and bIFN-α1. Cells stimulated with PWM were less inhibited than cells stimulated with PHA and Con A. Both bTP-1 and bIFN-α1 inhibited MLC to a greater degree than lectin-stimulated cells (maximum inhibition was 78% or greater). Also, bTP-1 and bIFN-α1 slightly inhibited incorporation of [³H]thymidine ([³H]Tdr) induced by the combination of phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA), and calcium ionophore A23187. Finally, bTP-1 and bIFN-α1 had bimodal effects on incorporation of [³H]Tdr by IL-2-induced lymphocytes. Incorporation of [³H]Tdr was increased at 0.005 nM and 0.05 nM concentrations while higher concentrations caused a slight decrease in [³H]Tdr incorporation. Results confirm that bTP-1 inhibits lymphocyte proliferation in a manner similar to that caused by the leukocyte-derived interferon, bIFN-α1. Incomplete inhibition of mitogen-induced proliferation and differences in degree of inhibition between various stimulators suggest that bTP-1 and bIFN-α1 preferentially inhibit certain lymphocyte subpopulations. Local inhibition of lymphocyte proliferation caused by bTP-1 may help protect the allogeneic conceptus from immune responses to fetal antigens or regulate the release of cytokines from endometrial lymphocytes.

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INTRODUCTION

The Type I trophoblast interferons represent a group of interferon molecules produced by the trophoblast of ruminant conceptuses around the time that the corpus luteum is rescued from luteolysis and transformed into the corpus luteum of pregnancy (see Roberts et al., 1990; Bazer et al., 1991 for reviews). These interferons, which have been named ovine, bovine and caprine trophoblast protein-1 (oTP-1, bTP-1 and cTP-1 respectively), play a key role in preventing luteolysis during early pregnancy by blocking release of endometrial prostaglandin F2α (Vallet et al., 1988; Helmer et al., 1989a,b; Salamonsen et al., 1989; Miranda et al., 1990; Barros et al., 1991; Danet-Desnoyers et al., 1991). In fact, the ruminant trophoblast interferons were first described based on their antiluteolytic activity. It was subsequently discovered that oTP-1 and bTP-1 share significant sequence homology with Type I leukocyte interferons known as interferon-ω (or -α11) (Imakawa et al., 1987; 1989) and possess antiviral activity (Pontzer et al., 1988; Roberts et al., 1989; Klemann et al., 1990; Plante et al., 1990). It has been proposed (Hansen et al., 1991) that the Type I trophoblast interferons represent a class of Type I interferons distinct from interferon-ω because bTP-1 shows greater sequence similarity to its ovine equivalent (oTP-1) than it does to bovine interferon-ω (Imakawa et al., 1989; Charlier et al., 1991; Hansen et al., 1991), because the induction of bTP-1 gene expression by viruses is of much lower magnitude than for interferon-α1 and ω (Cross and Roberts, 1991), and because the promoter regions of the trophoblast interferons are organized differently than the promoter region of interferon-ω (Hansen et al., 1991).

While the major role of Type I trophoblast interferons during early pregnancy is to ensure continued function of the corpus luteum, interferon-like actions of these molecules on the endometrium may also be important. The interferons have a wide range of effects on target cells, including cell growth, oncogene expression and cellular differentiation (Clemens and McNurlan, 1985; Moritz and Kirchner, 1986; Salzberg et al., 1990). One potentially-important action of interferons is inhibition of lymphocyte proliferation (Mönnor et al., 1978; Shalaby et al., 1984; Biefeleldt Ohmann and Babiuk, 1986; Nagi and Babiuk, 1988; Niwano et al., 1989; Roberts et al., 1989; Weiss and Oostrom-Ram, 1990). The bovine and ovine endometrium are rich in a va-
riety of leukocytes (Van der Wielen and King, 1984; Gogolin-Ewens et al., 1989; Low et al., 1990). Endometrial leukocytes could play at least two roles: as cells participating in an anti-paternal immune response against the allogeneic conceptus, and as a source of cytokines that could act on the trophoblast to regulate its growth or function (Wegmann, 1988). It is known that oTP-1 can inhibit proliferation of ovine lymphocytes (Newton et al., 1989; Niwano et al., 1989; Roberts et al., 1989; Fillion et al., 1991), but the action of bTP-1 on lymphocytes has not been described. Additionally, the mechanism of action by which oTP-1 or bTP-1 inhibit proliferation has not been elucidated fully. The aim of the present study was to compare effects of recombinant bTP-1 and bovine interferon-α₁₁ (bIFN-α₁₁) on lymphocyte proliferation caused by mitogens and mixed lymphocyte reactions and to evaluate which stages of lymphocyte activation are susceptible to inhibition by bTP-1 and bIFN-α₁₁.

MATERIALS AND METHODS

Materials

Recombinant bTP-1 was prepared as described elsewhere (Klemann et al., 1990). Recombinant bovine interferon-α₁₁ (bIFN-α₁₁) and recombinant bovine interleukin-2 (IL-2) were gifts from Ciba-Geigy, Basle, Switzerland. Antiviral activity was $1 \times 10^8$ U mg⁻¹ for bTP-1 and more than $1 \times 10^8$ U mg⁻¹ for bIFN-α₁₁ as determined by the method of Pontzer et al. (1988). Phytohemagglutinin L (PHA), concanavalin A (Con A), pokeweed mitogen (PWM), Histopaque 1077, Dulbecco's Modified Eagle's Medium (DMEM), RPMI-1640 medium, calcium ionophore A23187 and phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA), were purchased from Sigma (St. Louis, MO). The $[^3]$H-methyl]thymidine (spec. act. 5.0 Ci mmol⁻¹) was from Amersham (Arlington Heights, IL). Iron-supplemented calf serum was from Hyclone (Logan, UT).

Preparation of lymphocytes

Blood mononuclear cells were obtained at slaughter or by jugular venipuncture from cows of various breeds. Suspensions of mononuclear cells (greater than 85% lymphocytes, the balance primarily monocytes) were prepared by density gradient centrifugation on Histopaque-1077 (Low and Hansen, 1988). The final concentration of cells was $1 \times 10^6$ ml⁻¹ in an RPMI-1640 medium modified to contain 10% (v/v) iron-supplemented calf serum, 1% (v/v) extra 200 mmol glutamine, 100 IU penicillin ml⁻¹, and 100 μg ml⁻¹ streptomycin.
Mitogen-induced proliferation

Effects of bTP-1 and bIFN-α1 on incorporation of $[^3\text{H}]$thymidine ([$^3\text{H}$]TdR) into mitogen-stimulated lymphocytes were determined as follows. Briefly, 100,000 cells in 100 μl were added to each test well of sterile 96-well plates. Various concentrations of bTP-1 and bIFN-α1 in 40 μl of modified RPMI-1640 were added to three to six replicate wells. Wells then received 0.4 μg PHA, 2.5 μg Con A or 2.5 μg PWM (dissolved in 10 μl modified RPMI-1640). Doses of mitogen used were those that gave maximum stimulation of proliferation in preliminary studies. After 48 h of culture (37°C, 5% CO₂), 0.1 μCi [$^3\text{H}$]TdR was added in 50 μl modified RPMI-1640. Cells were harvested 12–16 h later with a semi-automatic cell harvester (Brandel, Gaithersburg, MD) and repeatedly washed with 0.9% (w/v) NaCl and distilled H₂O. The radioactivity incorporated into newly synthesized DNA was determined by liquid scintillation spectrometry.

In the first study, lymphocytes were collected from each of three cows. For each cow, lymphocytes were cultured in triplicate with various combinations of mitogen and interferon in a $3 \times 2 \times 4$ factorial arrangement with three mitogens (PHA, Con A and PWM) and two interferons (bTP-1 and bIFN-α1) tested at four final concentrations (0, 0.5, 5.0 and 50.0 nM). This series of treatments was replicated three times with blood from three different cows being used for each replicate. In another study, the effect of 0, 0.005, 0.05, 0.5, 5.0 and 50.0 nM bTP-1 and bIFN-α1 on proliferation of PHA-stimulated cells was examined. For each replicate, treatments were applied to lymphocytes in triplicate; the experiment was replicated four times by using lymphocytes from a different cow for each replicate (i.e. treatments were tested using lymphocytes from four separate cows).

Mixed lymphocyte cultures

Two-way mixed lymphocyte cultures (MLC) were carried out by co-culturing $2.5 \times 10^5$ mononuclear cells from one cow with an equal amount of cells from another cow. Cells were added in a total volume of 100 μl and treatments added in 50 μl modified RPMI-1640 to give concentrations of bTP-1 or bIFN-α1 of 0, 0.005, 0.05, 0.5, 5.0 and 50.0 nM at the end of culture. After 5 days of culture, 0.4 μCi [$^3\text{H}$]TdR in 50 μl modified RPMI-1640 were added and cells harvested 12 h later. Triplicate determinations were made in each MLC and the experiment was replicated using three separate MLC.

Stimulation of lymphocytes by TPA and A23187

The general outlines of this procedure were described by Truneh et al. (1985). Procedures were similar to those for mitogen-induced proliferation.
except that 10 μl 400 nM TPA (in 10% (v/v) ethanol in modified RPMI-1640) and 10 μl of 12 μM A23187 (in 2% (v/v) DMSO in modified RPMI-1640) were added to the cells instead of mitogen, and treatments were added in 30 μl modified RPMI-1640. Thus, final concentrations were 80 nM TPA and 2.4 μM A23187. Effects of bTP-1 and bIFN-α1 (final concentration at end of culture was 0, 0.005, 0.05, 0.5, 5.0 and 50.0 nM) were determined in four separate lymphocyte assays, where each assay involved cells from a different cow.

**IL-2 induced proliferation**

Lymphocytes (2.5 × 10^6 mononuclear cells ml⁻¹) were cultured for 3 days in a 10-ml vol. of modified RPMI-1640 containing 2 μg ml⁻¹ PHA. Cultures were performed in 17×100 mm culture tubes at 37°C and 5% (v/v) CO₂. Cells were then washed four times with modified RPMI-1640. Viability was determined using the trypan blue exclusion test and concentration of lymphocytes was adjusted to 1×10⁶ live mononuclear cells ml⁻¹. Cells were then cultured as described for mitogen-induced proliferation except that IL-2 (final concentration 80 U ml⁻¹) was used instead of mitogen. Effects of bTP-1 and bIFN-α1 (0, 0.005, 0.05, 0.50, 5.0 and 50.0 nM concentration at end of culture) on basal and IL-2 induced proliferation of PHA-primed cells were determined using replicates from four individual cows.

**Controls**

Background incorporation of [³H]TdR for proliferation induced by mitogens and TPA/A23187 was determined from the incorporation by cells cultured without stimulators. For these assays, background incorporation was very low (less than 0.5% of maximum stimulation) and was ignored when calculating stimulation of other wells. For MLC, background incorporation was determined by culturing 5×10⁵ unmixed cells from individual cows under similar conditions. Values for background from the two lymphocyte donors cultured separately were averaged and subtracted from values for [³H]TdR incorporation by the MLC.

In all assays, wells were prepared without interferons to determine maximum stimulation. To allow proper statistical analysis, a series of wells to determine maximum stimulation were set up separately for bTP-1 and bIFN-α1. Values from these wells were considered as 0 nM bTP-1 and 0 nM bIFN-α1 for statistical analysis. For all assays, additional cultures were prepared to determine the effect of each treatment on cell viability. The [³H]TdR was omitted from these cultures and cell viability was determined at the end of culture using trypan blue exclusion. Finally, a negative control for lymphocyte inhibition (bovine serum albumin; 10 mg ml⁻¹) was included in assays.
to verify that inhibitory effects of bTP-1 and bIFN-α₁ on lymphocyte proliferation were not caused by the introduction of non-specific suppressive or cytotoxic elements.

Statistical analysis

Treatment effects on [³H]TdR incorporation into lymphocytes and on viability of cells were evaluated by least-squares analysis of variance with the General Linear Models program of the Statistical Analysis System (SAS, 1990). Triplicate determinations carried out within an assay were averaged before analysis. Treatments were arranged in multifactorial designs and included main effects of lymphocyte donor (three to four cows per experiment), type of interferon (bTP-1 vs. bIFN-α₁), concentration of interferon (0, 0.005, 0.05, 0.5, 5.0 and 50.0 nM) and, in one experiment only, mitogen type (PHA, Con A and PWM). All interactions were also included in the mathematical model. Data are presented as the least-squares means (i.e. averaged across all assays) ± pooled SEM.

RESULTS

Effects on mitogen-induced proliferation

Stimulation of lymphocyte proliferation caused by PHA, Con A and PWM was inhibited by bTP-1 and bIFN-α₁ (Table 1, concentration effect; P<0.03). There were no effects of either interferon on cell viability. The inhibition of lymphocyte proliferation caused by bTP-1 was similar to that caused by bIFN-α₁, i.e. there was no interaction between concentration and interferon type. The degree of inhibition was similar for all concentrations tested (0.5, 5 and 50 nM). The absolute inhibition caused by bTP-1 and bIFN-α₁ was least for PWM-stimulated lymphocytes (mitogen × concentration interaction, P=0.05). The maximum average inhibition caused by bTP-1 was 41% for PHA-stimulated cells, 36% for Con A-stimulated cells and 22% for PWM-stimulated cells; for bIFN-α₁, maximum average inhibition was 52%, 39% and 32% for PHA, Con A and PWM, respectively.

An additional experiment was performed with PHA-stimulated cells to further characterize the dose-dependency of inhibition (Fig. 1, top panel). In this experiment, bTP-1 and bIFN-α₁ were tested at concentrations as low as 0.005 nM. Lymphocyte proliferation was affected by concentration (P<0.001); all concentrations caused inhibition of lymphocyte proliferation and maximum inhibition occurred at 0.5–50 nM (maximum inhibition ranged from 24 to 33%). An interferon type × concentration interaction (P<0.05)
### TABLE 1

Inhibition of lectin-induced lymphocyte proliferation by bTP-1 and blFN-α1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PHA(^2)</th>
<th>Con A(^3)</th>
<th>PWM(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>([^{3}\text{H}]\text{TdR}) (dpm)</td>
<td>Viability (%)</td>
<td>([^{3}\text{H}]\text{TdR}) (dpm)</td>
</tr>
<tr>
<td>bTP-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (nM)</td>
<td>30667</td>
<td>68</td>
<td>50667</td>
</tr>
<tr>
<td>0.5 (nM)</td>
<td>21333</td>
<td>61</td>
<td>36333</td>
</tr>
<tr>
<td>5.0 (nM)</td>
<td>17667</td>
<td>64</td>
<td>32333</td>
</tr>
<tr>
<td>50.0 (nM)</td>
<td>18000</td>
<td>68</td>
<td>35667</td>
</tr>
<tr>
<td>bIFN-α1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (nM)</td>
<td>31667</td>
<td>61</td>
<td>45000</td>
</tr>
<tr>
<td>0.5 (nM)</td>
<td>18667</td>
<td>68</td>
<td>33000</td>
</tr>
<tr>
<td>5.0 (nM)</td>
<td>15333</td>
<td>61</td>
<td>28667</td>
</tr>
<tr>
<td>50.0 (nM)</td>
<td>17472</td>
<td>65</td>
<td>27667</td>
</tr>
</tbody>
</table>

\(^1\)Data represented the least-squares means of results from lymphocytes from three cows. The pooled SEM for \([^{3}\text{H}]\text{TdR}\) uptake = 1349 dpm. When data from all mitogens were analyzed as one data set, \([^{3}\text{H}]\text{TdR}\) incorporation was affected by concentration of interferon \((P<0.03)\) and the concentration × mitogen interaction \((P=0.05)\). There were no effects on viabilities (pooled SEM = 4.5%).

\(^2\)Incorporation of \([^{3}\text{H}]\text{TdR}\) by PHA-treated cells was affected by concentration of interferon \((P=0.05)\). Viabilities were not affected by any treatment.

\(^3\)Incorporation of \([^{3}\text{H}]\text{TdR}\) by Con A-treated cells was affected by concentration of interferon \((P<0.05)\). Viabilities were not affected by any treatment.

\(^4\)Incorporation of \([^{3}\text{H}]\text{TdR}\) by PWM-treated cells was affected by concentration of interferon \((P<0.01)\). Viabilities were not affected by any treatment.

occurred because bTP-1 caused greater inhibition at 0.5 and 5.0 nmol than bIFN-α1, while inhibition was similar at other concentrations. Cell viabilities at the end of culture were unaffected by bTP-1 or bIFN-α1.

### Mixed lymphocyte cultures

Both bTP-1 and bIFN-α1 inhibited MLC in a concentration-dependent manner \((P<0.01)\) without affecting viability at the end of culture (Fig. 1, middle panel). The interferon type × concentration interaction was not significant. Maximum inhibition occurred at 0.5–5.0 nmol. At these and higher concentrations, \([^{3}\text{H}]\text{TdR}\) incorporation was inhibited by greater than 75%.

### Induction of proliferation with TPA and A23187

The combination of TPA and A23187 was effective in inducing \([^{3}\text{H}]\text{TdR}\) incorporation in cells. The \([^{3}\text{H}]\text{TdR}\) incorporation was increased from 495 disintegrations per minute (dpm) for cells without TPA and A23187 to 87 431 dpm for lymphocytes treated with TPA and A23187. The incorporation of
Fig. 1. Concentration-dependent inhibition of lymphocyte proliferation by bTP-1 and bIFN-\(\alpha_1\) (IFN). Top panel: PHA-stimulated lymphocytes. Results are the least-squares means of results from four cows. Incorporation of \([^3]H\)TdR was affected by concentration of interferon \( (P<0.001)\) and the concentration \(\times\) interferon-type interaction \( (P<0.05)\). According to Duncan's analysis, all concentrations of bTP-1 and bIFN-\(\alpha_1\) were different from control values and 0.005 and 0.05 nM were different from 0.5, 5.0 and 50.0 nM. There was no effect of any treatment on cell viability at the end of culture (data not shown). The range for mean viability was 74–80% (SEM = 2.6%). Middle panel: mixed lymphocyte cultures. Results are the least-squares means of results from three MLC. The pooled SEM (2336 dpm) is shown as the error bars around each point. Incorporation of \([^3]H\)TdR was affected by interferon concentration \( (P<0.01)\) but not by the interferon type \(\times\) concentration interaction. According to Duncan's analysis, all concentrations of bTP-1 and bIFN-\(\alpha_1\) were different from control values and 0.005 and 0.05 nM were different from 0.5, 5.0 and 50.0 nM. There was no effect of any treatment on cell viability at the end of culture. The range for mean viability was 83–88% (SEM = 2.5%). Bottom panel: lymphocytes stimulated with TPA and A23187. Results are the least-squares means of results from four cows. Incorporation of \([^3]H\)Tdr was affected by interferon concentration \( (P<0.001)\) but not by the interferon type \(\times\) concentration interaction. According to Duncan's analysis, incorporation of \([^3]H\)Tdr by 0 nM > 0.005 nM > 0.05 nM > 0.5 nM > 5.0 nM and 50.0 nM. There was no effect of any treatment on cell viability at the end of culture. The range for mean viability was 59–71% (SEM = 2.9%).
Inhibition of lymphocytes by bTP-1 and IFN-α₁

[^3H]TdR was inhibited by bTP-1 and bIFN-α₁ in a concentration-dependent manner (P<0.001; Fig. 1, bottom panel). Inhibition was of similar magnitude for bTP-1 and bIFN-α₁ (no interferon type or interferon type × concentration interaction), and was apparent at the lowest concentration tested (0.005 nM). At the highest concentration (50 nM),[^3H]TdR incorporation was inhibited by 31% (bTP-1) and 34% (bIFN-α₁). Neither bTP-1 nor bIFN-α₁ caused cytotoxicity.

**IL-2-induced proliferation**

Basal incorporation of[^3H]TdR by PHA-primed lymphocytes cultured without IL-2 was unaffected by bTP-1 or bIFN-α₁ (Table 2). For cells stimulated with IL-2, however, bTP-1 and bIFN-α₁ had bimodal effects on incorporation of[^3H]TdR (Table 2). Both bTP-1 and bIFN-α₁ increased[^3H]TdR uptake at 0.005 and 0.05 nmol concentrations (P<0.05 by Duncan’s analysis), while higher concentrations caused a decrease in[^3H]TdR incorporation (overall effect of concentration, P<0.001). Responses of IL-2-induced cells was similar for the two interferons. The degree of inhibition of IL-2-induced proliferation was small. At the highest concentration tested (50 nM), inhibition of[^3H]TdR incorporation was 20% for bTP-1 and 24% for bIFN-α₁. Neither bTP-1 nor bIFN-α₁ altered cell death (Table 2).

**Table 2**

Inhibition of IL-2-induced proliferation of PHA-primed lymphocytes (least-squares means)

<table>
<thead>
<tr>
<th>Interferon</th>
<th>Concentration</th>
<th>[^3H]TdR incorporation (dpm)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>Stimulated^1</td>
</tr>
<tr>
<td>bTP-1</td>
<td>0 (nM)</td>
<td>3264</td>
<td>59412</td>
</tr>
<tr>
<td></td>
<td>0.005 (nM)</td>
<td>3136</td>
<td>64764</td>
</tr>
<tr>
<td></td>
<td>0.05 (nM)</td>
<td>3535</td>
<td>63790</td>
</tr>
<tr>
<td></td>
<td>0.50 (nM)</td>
<td>2919</td>
<td>56621</td>
</tr>
<tr>
<td></td>
<td>5.00 (nM)</td>
<td>3395</td>
<td>50952</td>
</tr>
<tr>
<td></td>
<td>50.00 (nM)</td>
<td>3935</td>
<td>48620</td>
</tr>
<tr>
<td>bIFN-α₁</td>
<td>0 (nM)</td>
<td>3343</td>
<td>59194</td>
</tr>
<tr>
<td></td>
<td>0.005 (nM)</td>
<td>4816</td>
<td>61389</td>
</tr>
<tr>
<td></td>
<td>0.05 (nM)</td>
<td>3171</td>
<td>60585</td>
</tr>
<tr>
<td></td>
<td>0.50 (nM)</td>
<td>3471</td>
<td>55506</td>
</tr>
<tr>
<td></td>
<td>5.00 (nM)</td>
<td>2938</td>
<td>50637</td>
</tr>
<tr>
<td></td>
<td>50.00 (nM)</td>
<td>2455</td>
<td>45152</td>
</tr>
</tbody>
</table>

SEM 568 1247 1107 4 3

^1[^3H]TdR incorporation affected by concentration (P<0.001). According to Duncan’s test, 0.005 and 0.05 nM > 0 > 0.5, 5.0 and 50.0 nM.

^2Viability affected by interferon type (P<0.03).
DISCUSSION

The present results confirm that bTP-1, like its ovine equivalent, oTP-1 (Newton et al., 1989; Niwano et al., 1989; Roberts et al., 1989; Fillion et al., 1991), inhibits lymphocyte proliferation caused by a variety of stimulators without also causing any associated cytotoxicity. Moreover, the inhibition of lymphocyte inhibition caused by bTP-1 was generally similar in characteristics and potency to that caused by the leukocyte-derived interferon, bIFN-α₁. Thus, bTP-1 and oTP-1, while they are most likely members of a distinct interferon family (Hansen et al., 1991), exert at least some biological functions similar to interferon-α. Such a result is to be expected because Type I trophoblast interferons have already been shown to induce antiviral activity (Pontzer et al., 1988; Roberts et al., 1989; Plante et al., 1990) and because Type I trophoblast interferons and interferon-α can compete for endometrial receptors (Stewart et al., 1987; Hansen et al., 1989) and exert similar actions on endometrial tissue (Salamonsen et al., 1989; Barros et al., 1991; Plante et al., 1992). Interferon-like activities displayed by the Type I trophoblast interferons may represent important aspects of embryonic regulation of endometrial function. Inhibition of lymphocyte proliferation caused by bTP-1 may help protect the allogeneic conceptus from immune responses to fetal antigens (Low et al., 1990) or regulate release of cytokines from endometrial lymphocytes. Such locally-derived cytokines have been postulated to play important roles in growth and function of the trophoblast (Wegmann, 1988).

Apparent saturation of lymphocyte inhibition occurred at concentrations of bTP-1 and bIFN-α₁ ranging from 0.5 to 5 nM. Most estimates of receptor affinities for Type I interferon-α molecules, including oTP-1 and bIFN-α₁, range from 0.01 to 0.5 nM (Godkin et al., 1984; Rubenstein and Orchansky, 1986; Hansen et al., 1989). In other studies with oTP-1 and bIFN-α₁, there was no evidence for saturation of anti-lymphocyte responses (Bielefeldt Ohmann and Babiuk, 1986; Nagi and Babiuk, 1988; Newton et al., 1989; Roberts et al., 1989). Except for MLC, the maximum inhibition of lymphocyte proliferation at saturation was usually no greater than approximately 50%. There is great variability in the magnitude of inhibition seen in other studies with oTP-1 (Newton et al., 1989; Niwano et al., 1989; Fillion et al., 1991) and bIFN-α₁ (Bielefeldt Ohmann and Babiuk, 1986; Nagi and Babiuk, 1988). This variability is due, in part, to the fact that inhibitory responses of interferon-α on lymphocyte proliferation depend upon the dose of mitogen used (Miörner et al., 1978; Nagi and Babiuk, 1988; Roberts et al., 1989). The degree of inhibition of mitogen-induced proliferation seen in the present study is within the range of inhibition seen in other studies with oTP-1 and bIFN-α₁. For example, Fillion et al. (1991) found that oTP-1 inhibited proliferation of PHA-induced cells by a maximum of 30–54% and Nagi and Babiuk
found that bIFN-α1 inhibited proliferation of peripheral blood lymphocytes treated with Con A by a maximum of approximately 25–30%.

While proliferation of lymphocytes induced by mitogen and TPA/A23187 was only partially inhibited by bTP-1 and bIFN-α1, these molecules caused almost complete inhibition of MLC. The predominant cells proliferating during MLC are CD4+ cells (Bach et al., 1989) and, therefore, it is likely that these cells are inhibited by bTP-1 and bIFN-α1 more than other cell types stimulated by mitogen or TPA/A23187. A similar conclusion was arrived at by Fillion et al. (1991), since oTP-1 inhibited human CD4+ T-lymphocytes but not CD8+ T-lymphocytes. Since CD4+ cells are associated with helper T-cells, and CD8+ with suppressor and cytotoxic cells (Mackay, 1988), such differential effects of oTP-1 on T-lymphocytes could conceivably promote inhibition of anti-maternal immune responses mediated by CD4+ cells without affecting proliferation of local CD8+-suppressor cells. Additional evidence for differential sensitivity of lymphocyte subpopulations is provided by the fact that PWM-stimulated cells were less inhibited by bTP-1 (Table 1), oTP-1 (Newton et al., 1989) and bIFN-α1 (Table 1) than cells stimulated with PHA, and in some cases, Con A. Both PHA and Con A stimulate T-cells through interaction with T-cell antigen receptor (Imboden and Weiss, 1988) while PWM stimulates T- and B-cells (Rouse and Babiuk, 1974).

The combination of TPA and A23187, which together mimic activation of the phosphatidylinositol second messenger system, has been shown to cause lymphocyte activation by inducing IL-2 receptor expression and IL-2 secretion (Truneh et al., 1985). There are two possible explanations for the mechanism by which bTP-1 and bIFN-α1 caused slight inhibition of TPA/A23187-induced proliferation. One possibility is that bTP-1 and bIFN-α1, which acts through stimulating metabolism of phosphatidylcholine (Pfeffer et al., 1990) or arachidonic acid (Hannigan and Williams, 1991), blocked the intracellular actions of TPA and A23187. There is precedence for such a mechanism in other systems, since oTP-1 (Salamonsen et al., 1989; Mirando et al., 1990) and bTP-1 (Danet-Desnoyers et al., 1991) block oxytocin-induced release of prostaglandins from endometrium and oxytocin increases prostaglandin release through activation of phosphatidylinositol breakdown (Flint et al., 1989; Mirando et al., 1990). A second possibility is that bTP-1 and bIFN-α1 do not block primary events induced by TPA/A23187, i.e. IL-2 secretion and development of IL-2 receptors, but instead act more distally in lymphocyte proliferation to inhibit cellular responses to IL-2 produced from TPA/A23187-activated cells. This second explanation is supported by several lines of evidence. First, Niwano et al. (1989) noted that human interferon-α did not reduce IL-2 secretion from human lymphocytes. Secondly, inhibition of mitogen-stimulated lymphocyte proliferation by oTP-1 (Newton et al., 1989) and human interferon-α (Niwano et al., 1989) could not be overridden with exogenous IL-2 (Newton et al., 1989; Niwano et al., 1989). Inhibition of Con
A-stimulated proliferation caused by bIFN-α₁ could be only slightly reversed with supplemental IL-2, and inhibition of PHA-stimulated cells was not reversed by IL-2 (Bielefeldt Ohmann and Babiuk, 1986). Thirdly, delaying addition of oTP-1 (Newton et al., 1989; Fillion et al., 1991), bIFN-α₁ (Nagi and Babiuk, 1988) and human interferon-α (Mjörner et al., 1978) until 24 h after addition of mitogen did not prevent lymphocyte inhibition, even though IL-2 secretion begins by 4 h after mitogenic stimulation and is maximal by 20 h (Weinberg et al., 1988). Finally, the present results demonstrate that higher concentrations of bTP-1 and bIFN-α₁ reduce somewhat the IL-2-induced proliferation of PHA-primed lymphocytes. Similar inhibitory responses have been found for oTP-1 (Newton et al., 1989).

Nonetheless, the inhibition of IL-2-induced proliferation caused by high concentrations of bTP-1 and bIFN-α₁ was slight and other mechanisms for suppression of lymphocyte proliferation are likely. The anti-proliferative actions of interferons are not limited to lymphocytes; growth of many cells are inhibited by interferon-α (Clemens and McNurlan, 1985; Fleishchmann and Fleishchmann, 1988; Ruszczak et al., 1990; Salzberg et al., 1990; Muro et al., 1991). Therefore, it is possible that the mechanism by which interferon-α inhibits lymphocytes may be a general mechanism rather than one specific for lymphocytes. In non-lymphoid cells, interferons inhibit cell proliferation through a delay in various phases of the cell cycle (Balkwill and Taylor-Papadimitriov, 1978; Creasy et al., 1980; Muro et al., 1991) and such a mechanism could be operative for lymphocytes also. It will be important to determine whether bTP-1 regulates proliferation of endometrial cells since such an effect would represent another mechanism by which the embryo would regulate the function of the underlying endometrium.

In summary, bTP-1 was found to inhibit proliferation of lymphocyte proliferation in ways characteristic of interferon-α. It should be pointed out that regulation of lymphocyte function by interferon-α in vivo is more complex than can be ascertained by in vitro tests of lymphocyte proliferation. For example, interferon-α causes a net movement of lymphocytes from peripheral blood to tissues (Griebel et al., 1989), perhaps by regulating binding of lymphocytes to endothelial cells (Issekutz, 1990). Future studies should be directed towards understanding effects of bTP-1 on endometrial lymphocytes in vivo as well as to evaluating other interferon-like actions of bTP-1 on endometrial tissue.

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