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Effects of Leptin, Interleukin-1α, Interleukin-6, and Transforming Growth Factor-β on Markers of Trophoblast Invasive Phenotype

Integrins and Metalloproteinases

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Phenotypic changes of integrin and metalloproteinase secretion of the invasive human cytotrophoblast are regulated by cytokines and growth factors, but how this occurs is not completely understood. We used 24-h cytotrophoblast cultures from first trimester pregnancies to investigate the effects of leptin and cytokines on the expression of the α2, α5, and α6 integrin subunits and on the activity of metalloproteinase-2 (gelatinase A) and metalloproteinase-9 (gelatinase B). The α2 subunit was marginally upregulated by leptin and interleukin-1α (IL-1α). All compounds tested upregulated, in some degree, the α5 expression. The α6 integrin subunit was massively upregulated, by leptin, interleukins, and transforming growth factor-β. None of the factors tested affected metalloproteinase-2 activity, but the activity of metalloproteinase-9 was upregulated by leptin and IL-1α. In conclusion, leptin and IL-1α actively induce some of the changes that cytotrophoblasts undergo to achieve a more invasive phenotype. A novel role for leptin is proposed during early pregnancy: leptin might be an autocrine/paracrine regulator of cytotrophoblast invasiveness during implantation and placentation.

Key Words: Cytotrophoblastic cells; leptin; integrins; metalloproteinases; cytokines.

Introduction

Implantation is a crucial event in viviparity. This process, which is dependent on the invasive properties of the trophoblast, is unique in humans and differs markedly from those found in other mammals. The invasive behavior of cytotrophoblastic cells (CTBs) is directly linked to their secretion of proteases, which digest the extracellular matrix (ECM) of the endometrium, and to their expression of integrins that allow anchorage and migration of CTBs *(1–3)*. These two processes characterizing the invasive phenotype of CTBs are under paracrine regulations by cytokines and growth factors *(4,5)*.

Binding of villous CTBs (vCTBs) to the villous basement membrane depends on the expression of a laminin receptor, the α6β4 integrin. This integrin is expressed in vCTBs in a polarized way toward the basement membrane containing laminin. Columns of invasive extravillous CTBs (evCTBs) modulate their integrin repertoire, expressing the α6β4 in a nonpolarized way in the proximal region, and becoming α6β4 negative and α5β1 positive (a fibronectin receptor) in the deeper layer of the placental bed *(6–8)*. Endovascular evCTBs express a α1β1 integrin, a collagen receptor. Thus, as CTBs migrate off the basement membrane into the decidua, they downregulate their α6 and β4 integrin subunits and upregulate their β1, α5, and α1 subunits *(6–11)*. The α1β1 integrin, which is present in purified CTBs *(8)*, is a major receptor for glycoproteins (laminin and collagen).

The use of CTBs obtained from first trimester pregnancies is a recognized model to understand trophoblast invasion during implantation of the human embryo *(1)*. CTBs cultured in vivo follow the same integrin expression pattern as in vivo *(12)*.

Integrins allow not only adhesion but are also transmembrane receptors that transduce inside the cells the changes of their immediate environment. In turn, the cell responds by secreting biologically active compounds, including proteases and matrix glycoproteins, which then modify the nature of the surrounding ECM *(13)*. During trophoblast invasion, HLA-G-positive evCTBs expressing the α6 integrin represent the invasive population of cells. The α6 integrin subunit–positive CTBs secrete more gelatinases, less fibronectin, but similar amounts of human chorionic gonadotropin (hCG) compared with the α5 integrin subunit–positive CTB phenotype *(14)*.

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Protease secretion is instrumental to CTB invasion. Matrix metalloproteinases (MMPs) are secreted by CTBs *(15,16)* and are capable of degrading the different collagens of the endometrial ECM. MMPs are a family of enzymes including gelatinases, collagenases, stromelysins, and membrane-type MMPs *(17)*. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are secreted by in vitro–cultured CTBs *(18)*.

Regulation of the invasive behavior of CTBs is not completely understood. Many molecules secreted locally, such as cytokines, growth factors, and ECM-glycoproteins, can mediate the CTB invasion *(1,5,17,19)*. The embryo could communicate with the maternal receptive endometrium through the interleukin-1 (IL-1) system *(5)* and lead to the secretion of MMP-9 by CTBs *(20–22)*. Human trophoblast synthesizes interleukin-6 (IL-6) *(23)*, which in turn stimulates protease activity in CTBs *(22)*. Trophoblastic release of hCG is stimulated by IL-1 through the activation of IL-6 and the IL-6 receptor system *(24)*. Transforming growth factor-β (TGF-β) stimulates the secretion of tissue inhibitors of metalloproteases (TIMPs) and limits CTB invasion *(25,26)*.

Leptin, a polypeptide product of the obesity (*ob*) gene *(27)*, has been found elevated in pregnancy *(28,29)*. Leptin is secreted by CTBs in cultures and upregulated by IL-1 *(30)* and IL-6 *(31)*. On the other hand, leptin increases the expression of fetal fibronectin and the activity of MMPs *(32)*. Furthermore, leptin and leptin receptor are expressed in the human endometrium, and leptin secretion has been found in conditioned media from blastocyst cultures and in media from blastocysts cocultured with endometrial epithelial cells *(33)*. In addition, leptin and IL-1 system actions might be related during the early phases of human embryo implantation. A potential autocrine/paracrine regulation of leptin secretion has been proposed in the early phase of human implantation *(34)*. We thus hypothesize that leptin might also be a regulator of the invasive phenotype of CTBs.

To obtain information on the mechanism involved in the differentiation of CTBs from noninvasive vCTBs to invasive evCTBs, we studied the in vitro effects of leptin, IL-1α, IL-6, and TGF-β on the integrin α2, α5, and α6 expression and activity of MMP-2 and MMP-9 by CTBs.

Results

Effect of Leptin and Cytokines on Integrin Expression by CTBs

In the absence of serum, the majority of CTBs cultured for 24 h were attached and round-shaped (Fig. 1). The addition of leptin or cytokines did not change the morphologic features of the cells but influenced in diverse degree the expression of α2, α5, and α6 integrins. Positive cells showed a strong to moderate staining for all integrins assayed. Aggregated cells were also observed with a strong to moderate staining in cytoplasm and focal contact of cells. A few multinucleated giant cells with strong staining in the cytoplasm were also observed. Contaminant spindle-shaped fibroblasts consisted of <3% of the total cells. The intensity staining for each integrin changed in the presence of some of these compounds. However, not all the cells were stained (Fig. 1). The data obtained on the staining for each integrin (i.e., the sum of strong, moderate, and weak stained cells) were statistically analyzed and are graphically represented in Figs. 2–4.

α2 Integrin Subunit Expression

The effect of IL-1α, IL-6, leptin, and TGF-β on α2 integrin expression is shown in Fig. 2A, 2B, 2C, and 2D, respectively. Thirty-six percent (±9.5 SEM) of CTBs cultured in control medium (Dulbecco's modified Eagle's medium [DMEM] alone) expressed the α2 integrin (Fig. 2A–D, first column). TGF-β and IL-6 had no effect on α2 expression (Fig. 2B,D). However, IL-1α (1–10 ng/mL; $p = 0.006$–0.016) and leptin (0.1 ng/mL; $p = 0.046$) induced an increase in α2 expression (Fig. 2A,C). Nevertheless, no dose-response effect could be observed ($r = 0.019$, $p > 0.05$).

α5 Integrin Subunit Expression

The effect of IL-1α, IL-6, leptin, and TGF-β on α5 integrin expression are shown in Fig. 3A, 3B, 3C, and 3D, respectively. Under basal conditions (control), 48.0 ± 2.9% (SEM) of CTBs expressed the α5 integrin subunit (Fig. 3A–D, first column). IL-1α significantly increased the expression of α5 at doses of 1.0 and 5.0 ng/mL ($p = 0.038$, $p = 0.022$, respectively; Fig. 3A). IL-6 significantly upregulated the α5 integrin subunit expression at concentrations between 0.1 and 5.0 ng/mL ($p = 0.004$ to $p = 0.021$, Fig. 3B). For both interleukins, the highest concentration tested (10 ng/mL) had no significant effect on α5 expression. Leptin induced a dose-dependent increase ($r = 0.510$, $p = 0.05$) of α5 expression and at a concentration of 10 ng/mL; the α5 expression was significantly ($p = 0.0006$) increased over that of the control (Fig. 3C). TGF-β had an effect similar to leptin: it dose dependently ($r = 0.815$, $p = 0.0002$) upregulated α5 integrin expression, and this expression was significantly increased over controls at doses of 5 and 10 ng/mL ($p = 0.045$, $p = 0.039$, respectively; Fig. 3D).

α6 Integrin Subunit Expression

Among the CTBs cultured in control medium, 29.5 ± 3.9% (SEM) were positive for the α6 integrin subunit (Fig. 4A–D, first column). All factors and all concentrations tested induced a significant ($p < 0.0001$) increase in α6 expression. After IL-1α (Fig. 4A) and IL-6 (Fig. 4B) supplementation, 100% of CTBs expressed the α6 integrin subunit. Leptin (Fig. 4C) and TGF-β (Fig. 4D) increased the α6 integrin expression similarly. About 80% of CTBs increased the α6 expression with the highest concentration (10 ng/mL) of these factors.

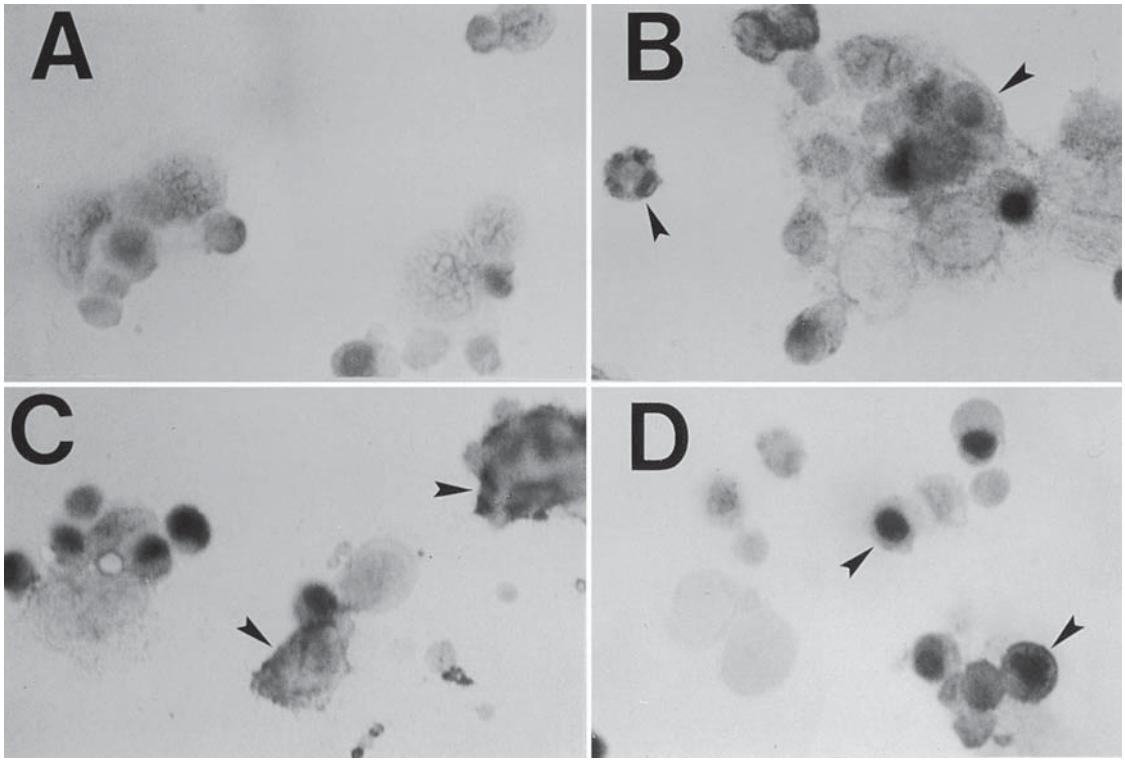

Fig. 1. Immunochemical staining for α2 (**B**), α5 (**C**), and α6 (**D**) integrin subunits (arrowhead) in CTBs cultured for 24 h in medium containing 10 ng/mL of leptin. In negative control (**A**), specific antiintegrin antibodies were substituted by irrelevant mouse IgGs. Magnification: ×400.

Metalloproteinase Activity

A typical zymogram obtained from the analysis of secreted MMP-2 and MMP-9 activity by CTB cultures with or without leptin (10 ng/mL) is shown in Fig. 5. The activity of MMP-2 (percentage of initial activity compared to control) in the supernatants from CTB cultures for 24 h was not significantly affected by the different concentrations (0.1–10 ng/mL) of leptin or cytokine supplements, as assessed by zymography (Fig. 6A–D).

By contrast, MMP-9 activity was significantly increased by IL-1 ($p = 0.003$–$p = 0.014$) and leptin ($p = 0.003$–$p = 0.037$) at 1–5 ng/mL (Fig. 7A and 7C, respectively). IL-6 (Fig. 7B) and TGF-β (Fig. 7D) exerted marginal effects on MMP-9 activity. However, these effects were not significant.

Discussion

Invasion of trophoblasts into the maternal decidua is crucial to implantation and placentation. In vivo, transient expression of an invasive phenotype is part of normal cytotrophoblast differentiation *(6)*. This process is characterized by biochemical changes in both maternal and embryonic tissues. Tissue remodeling is associated with changes in the constituents of the ECM and with changes in the expression of matrix glycoprotein receptors. In vitro observations indicate that in humans, decidua limits CTB invasion *(35,36)*. Therefore, the identification of endometrial or autocrine/paracrine trophoblast regulators that could lead the phenotypic changes of CTBs during the invasive phase of human implantation and placentation is the subject of intensive research *(17,37)*.

In this respect, the most relevant receptors are the integrins that bind laminin (α6β4) and fibronectin (α5β1) *(2,7, 19)*. A significant role for α2 integrin during CTB invasion has not been reported. This integrin is expressed in the fetal stroma and had a higher rate of detection in purified CTB *(8)*. The present investigation confirms and extends these data by showing α2 integrin expression in CTBs. However, α2 integrin expression was only affected by IL-1α and marginally increased by leptin.

Zymograms of MMPs used sodium dodecyl sulfate (SDS), which has important implications for the interpretation of results. This compound partially activates (without loss of the propeptide) the proform of MMPs so that (normally) inactive pro-MMPs will digest the substrate on the zymogram and appear as a digestion band. These SDS-induced artifacts need to be taken into account when interpreting zymography data *(37)*. However, we observed these changes but only recorded the data corresponding to the active form of MMP-2 (72 kDa) and MMP-9 (92 kDa).

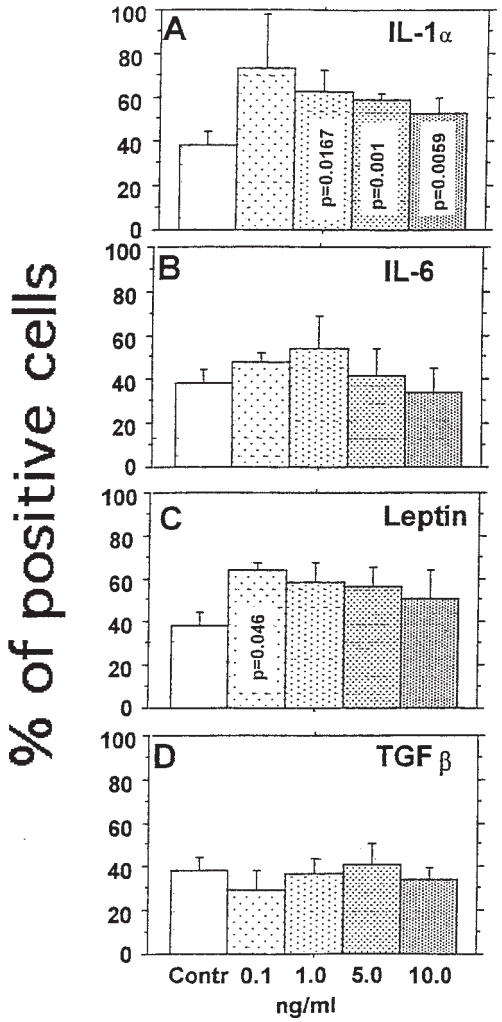

Fig. 2. Effects of IL-1α (**A**), IL-6 (**B**), leptin (**C**), and TGF-β (**D**) at doses of 0.1, 1.0, 5.0, and 10.0 ng/mL, respectively, on the expression of α2 integrin subunit on CTBs cultured for 24 h on glass. Replicate wells were assayed for each cytokine and leptin concentration, and the experiments were repeated at least three times with different CTB preparations. The expression of α2 integrin was assessed by immunocytochemistry with specific antibodies. α5 Integrin–positive cells were recorded in randomly chosen fields, and a total of 300 cells was counted in each field for each concentration (n = 6 per treatment).

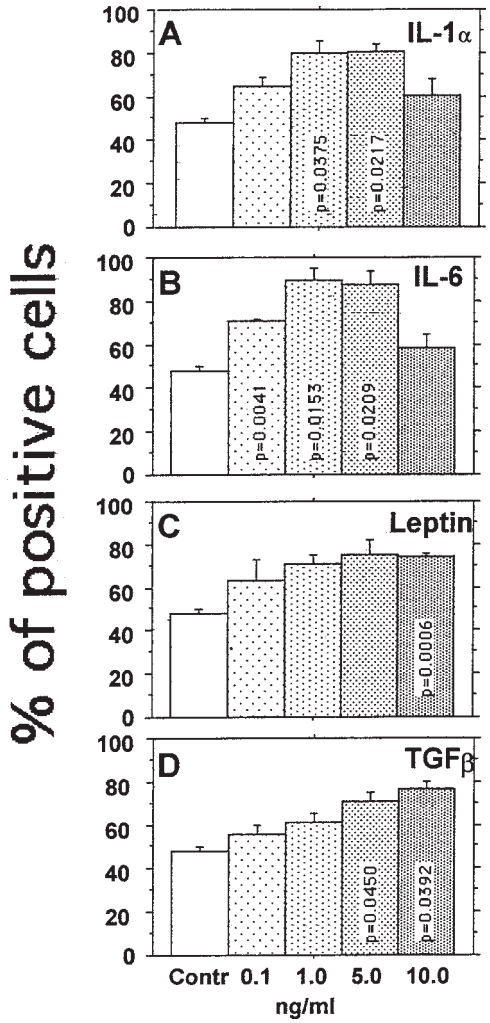

Fig. 3. Effects of IL-1α (**A**), IL-6 (**B**), leptin (**C**), and TGF-β (**D**) at doses of 0.1, 1.0, 5.0, and 10.0 ng/mL, respectively, on the expression of α5 integrin subunit on CTBs cultured for 24 h on glass. Replicate wells were assayed for each cytokine and leptin concentration, and the experiments were repeated at least three times with different CTB preparations. The expression of α5 integrin was assessed by immunocytochemistry with specific antibodies. α5 Integrin–positive cells were recorded in randomly chosen fields, and a total of 300 cells was counted in each field for each concentration (n = 6 per treatment).

Both CTBs and endometrium produce IL-1 *(38,39)*. This cytokine could be an important mediator of implantation *(40)*. The present investigation demonstrates that leptin and IL-1α increase α5 and α6 integrin expression and the gelatinolytic activity of MMP-9 but not of MMP-2 by CTBs. However, leptin could increase at higher concentrations (≥100 ng/mL) the immunoreactivity and activity of secreted MMP-2 *(32)*. The present results confirm that IL-1 stimulates the activation of MMP-9 *(20–22)*.

In addition to the adipose tissue, the human placenta is an important source of leptin *(41,42)*. Leptin is present in the cytoplasm of syncytiotrophoblast but not in the core villi *(42)*. This small peptide is expressed homogeneously in all cells of trophoblast columns, whereas its receptor showed a strong staining in the distal evCTBs *(32)*. CTBs cultured in vitro synthesize leptin, and its production is modulated by IL-1α, 17β-estradiol *(30)*, and IL-6 *(31)*, providing evidence for an autocrine/paracrine regulation of leptin production in the human placenta. Leptin receptor *(33,43, 44)* and leptin are expressed in the midsecretory human endometrium, and the blastocyst seems to regulate differentially leptin secretion in the presence of endometrial epithelial cells *(33)*. Therefore, a role for leptin at the early stages of human implantation might be anticipated.

Whether upregulations of integrin expression are instrumental to an increased MMP-9 activity remains to be dem-

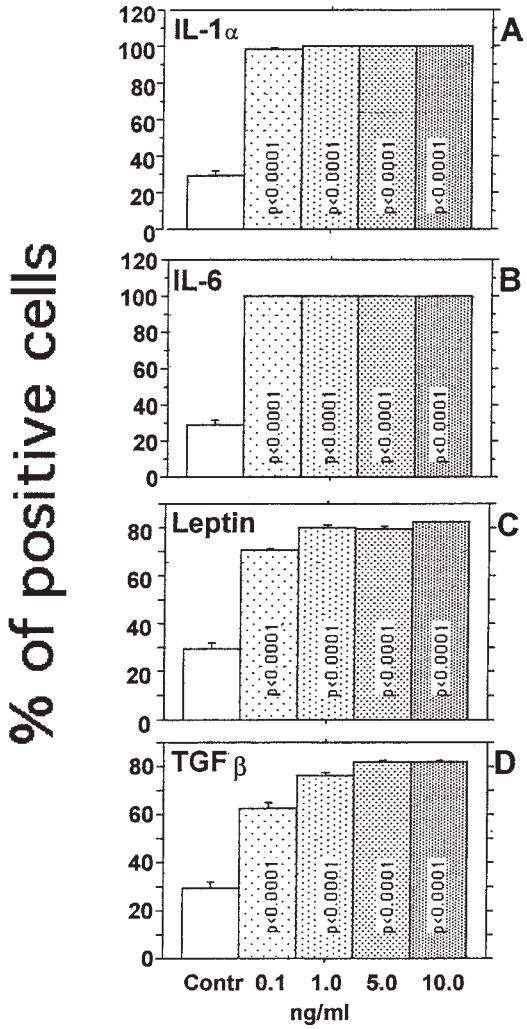

Fig. 4. Effects of IL-1α (**A**), IL-6 (**B**), leptin (**C**), and TGF-β (**D**) at doses of 0.1, 1.0, 5.0, and 10.0 ng/mL, respectively, on the expression of α6 integrin subunit on CTBs cultured for 24 h on glass. Replicate wells were assayed for each cytokine and leptin concentration, and the experiments were repeated at least three times with different CTB preparations. The expression of α6 integrin was assessed by immunocytochemistry with specific antibodies. α6 Integrin–positive cells were recorded in randomly chosen fields, and a total of 300 cells was counted in each field for each concentration (n = 6 per treatment).

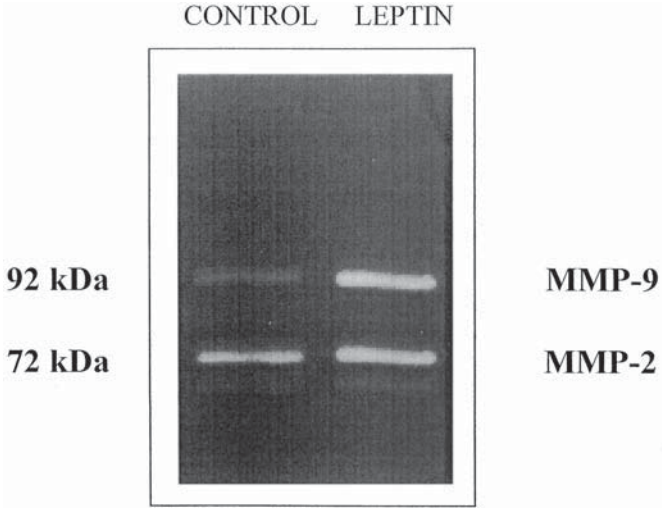

Fig. 5. Typical zymogram obtained for the quantitative measurement of the activity of secreted cytotrophoblastic MMP-2 (72 kDa) and MMP-9 (92 kDa) into medium with or without leptin (10 ng/mL). Incubation lasted 24 h.

onstrated in the particular case of CTBs, although indirect evidence attributes a causal relationship to these events *(14)*. Since leptin is secreted by CTBs in vitro *(30)*, its described effects on trophoblastic α5 and α6 expression and on MMP-9 activity define a novel role for this placental hormone as an autocrine (paracrine?) regulator of trophoblast invasion.

Although IL-6 exerts similar effects to IL-1α on α5 and α6 expression, it does not significantly affect MMP-2 and MMP-9 activity of CTBs grown on glass during 24 h. However, IL-6 stimulated both MMP-2 and MMP-9 activity in CTBs grown on plastic *(31)*. This discrepancy might be owing to the time (≥48 h) of CTB culture *(31)* and the nature of the matrices on which these cells were cultured *(18)*.

TGF-β is synthesized by endometrium and CTBs *(26,40)*. This growth factor is a putative modulator of IL-1 action and can also induce MMP-9 synthesis *(21)*. In addition, TGF-β decreased hCG and increased fetal fibronectin in CTBs *(22)*. In the present investigation, TGF-β exerted only a marginal effect on MMP-9 activity but upregulated α5 and α6 expression by CTBs. This is in concordance with previous findings that TGF-β upregulates the integrin expression (α5β1) and inhibits the migratory ability of the invasive trophoblast *(11)*. Interestingly, this growth factor has been shown to massively stimulate the secretion of TIMPs (TIMP-1) in CTBs *(25)*. Despite the fact that MMP-9 inhibition by TIMP-1 cannot be seen on zymograms (TIMP-1–MMP-9 complexes are dissociated), one might wonder if the increased TIMP-1 secretion is mediated by an increased expression of α5 and α6 integrin subunit.

In conclusion, the present results demonstrate that leptin, IL-1α, IL-6, and TGF-β induce the expression of α5 and α6 integrins by CTBs. However, only leptin and IL-1α upregulate the MMP-9, a rate-limiting enzyme for trophoblast invasion *(16,45)*. The present results do not demonstrate a causal relationship between integrin expression and upregulation of MMP-9. Leptin might be an autocrine/paracrine regulator of cytotrophoblast invasiveness during implantation and placentation.

Materials and Methods

Materials

DMEM, gentamycin, amphoptericin-B, L-glutamine, and trypsin were from Life Technologies, Basel, Switzerland. Penicillin was from Hoeschst-Pharma, Zurich, Swit-

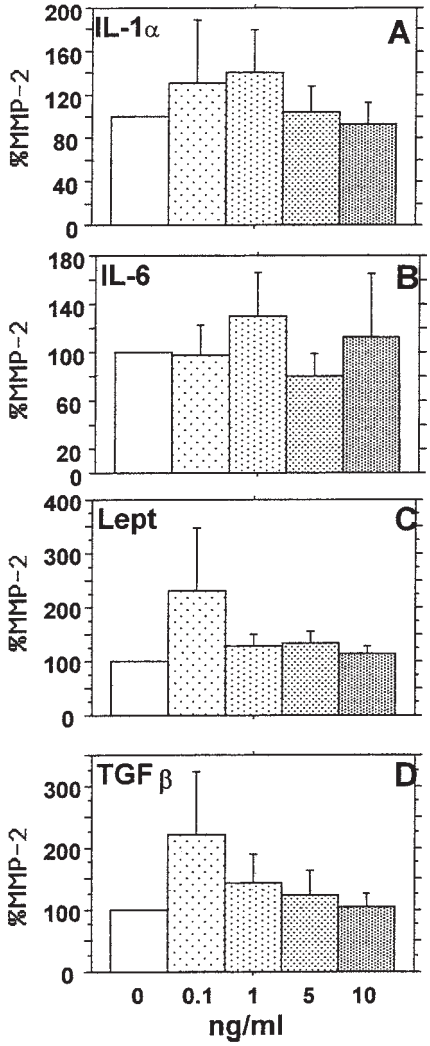

Fig. 6. Effects of IL-1α (**A**), IL-6 (**B**), leptin (**C**), and TGF-β (**D**) at doses of 0.1, 1.0, 5.0, and 10.0 ng/mL, respectively, on the activity of MMP-2 secreted by CTBs cultured for 24 h on glass. The activity of secreted MMP-2 was determined in the culture supernatants by zymography. Replicate wells were assayed for each cytokine and leptin concentration, and the experiments were repeated at least three times with different CTB preparations.

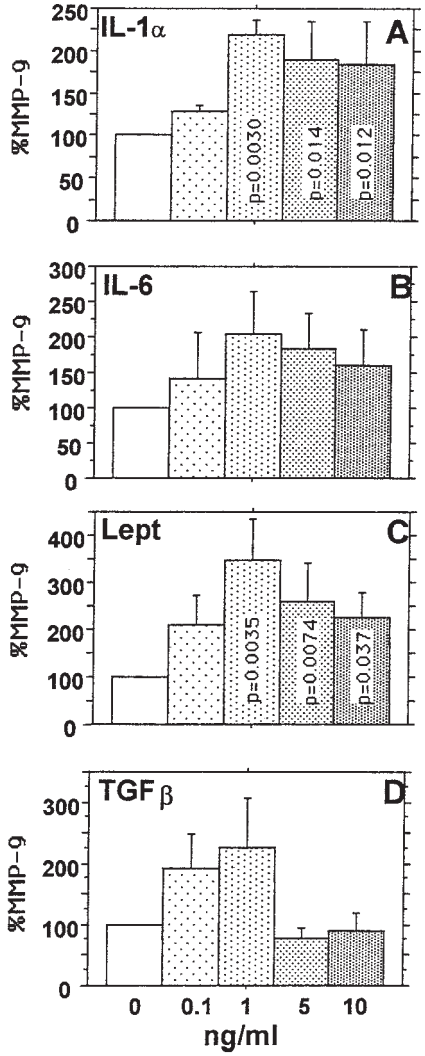

Fig. 7. Effects of IL-1α (**A**), IL-6 (**B**), leptin (**C**), and TGF-β (**D**) at doses of 0.1, 1.0, 5.0, and 10.0 ng/mL, respectively, on the activity of MMP-9 secreted by CTBs cultured for 24 h on glass. The activity of secreted MMP-9 was determined in the culture supernatant by zymography. Duplicate wells were run for each cytokine and leptin concentration, and the experiments were repeated at least three times with different CTB preparations.

zerland. Human IL-1α, IL-6, TGF-β, and recombinant human leptin were from R&D Systems, Bühlmann, Basel, Switzerland. The Dynabeads M-280 magnetic particles coated with sheep antimouse IgG were from Dynal, Milan, Geneva, Switzerland. Gelatin, Percoll, and high molecular weight standards were from Pharmacia Biotech, Dubendorf, Switzerland. All mouse antihuman integrin antibodies were from Serotec, Oxford, England.

The monoclonal antibody (MAb) CD49b (clone Gi9) recognizes the α2 integrin subunit of the VLA 2 complex (human endothelial cells and platelets). The MAb CD49e (clone SAM1) to the α5 integrin subunit recognizes the 120-kDa α-subunit of the VLA5 complex (platelets and myloid cells). The MAb CD 49f (clone 4F10) to the α6 integrin subunit recognizes glycoproteins Ic and IIa from both human and mouse platelets of the VLA6 complex (platelets, epithelial cells, monocytes, and T-lymphocytes). These antibodies have previously been tested for the evaluation of their specificity to recognize the aforementioned integrin molecules *(8,14)*.

Antimouse IgG-biotin conjugate, anti-human CD45, and fast-red substrate system were from Dako Diagnostics AG, Zug, Switzerland. Streptavidin–alkaline phosphatase conjugate (BioGenex Label) was from Stehelin, Basel, Switzerland. Other common reagents were from Sigma, Buchs, Switzerland.

Isolation and Immunopurification of CTBs

First trimester (6–12 wk) trophoblastic tissues ($n = 23$) were obtained from legal abortions, digested with trypsin, separated from blood cells and syncytia on a discontinuous Percoll gradient, and immunopurified using anti-CD45 (common leukocyte antigen) antibodies coated on magnetic particles as reported earlier *(18)*. Viability of CTBs was checked using the Trypan blue exclusion method and was always superior to 90%.

Culture Conditions

Between 2 and 3 × 10^5 CTBs/well were incubated in duplicate in eight-well glass culture plates designed for immunocytochemical studies (Nunc). Cells were cultured for 24 h (5% CO_2/37ºC) in DMEM with phenol red and containing 2 mmol/L of L-glutamine, 4.2 mmol/L of magnesium sulfate, 25 mmol/L of HEPES, 1% gentamycin, 1% amphopthericin B, 100 µg/mL of streptomycin, and 100 U/mL of penicillin *(18)*. Some culture media contained also one of the following compounds: IL-1α, IL-6, TGF-β, or leptin, at doses of 0.1, 1.0, 5.0, and 10.0 ng/mL, respectively. Media were collected after 24 h and the culture was stopped. Culture supernatants were kept at –20ºC or lyophilized and kept at 4ºC until assayed. Duplicate wells were run for each treatment with IL-1, IL-6, TGF-β, and leptin at the concentrations just described, and the experiments were repeated at least three times with different CTB preparations.

Immunocytochemical Studies of Integrin Expression

After 24 h of culture, the glass slides (bottom of culture plates) were washed with phosphate-buffered saline (PBS), immersed in acetone (–20ºC for 20 min), air-dried, and incubated at room temperature for 1 h with PBS containing 1% bovine serum albumin (PBS-BSA). Mouse monoclonal antiintegrin antibodies (α2, α5, and α6, diluted 1:50 in PBS-BSA) were added to the slides and incubated in a humid chamber at 4ºC overnight. The slides were then successively washed with PBS, incubated with biotin-labeled antimouse IgG (1:250 in PBS-BSA) for 30 min at room temperature, washed again, and reincubated with streptavidin–alkaline phosphatase conjugate (1:2 in PBS-BSA) for 30 min at room temperature. The immunocytochemical reaction was developed with the fast-red substrate system. Slides were counterstained with hematoxylin. The number of cells positive for each integrin subunit was recorded in a total number of 300 cells counted in a representative field selected in a random manner for each treatment and each replicate ($n = 6$ per treatment), and the result was expressed as a percentage.

Zymography

The activity of the secreted metalloproteinases, MMP-2 (72 kDa) and MMP-9 (92 kDa), was determined in the culture supernatants of each replicate ($n = 6$ per treatment) by zymography as reported previously *(18)*. Stained zymograms were scanned in an Apple Onescanner, and the surface of the digestion bands was measured by the NIH Image 1.60 program on a Power Macintosh 7100/66 computer. All zymograms were evaluated using the same preset standards, and the results were expressed as percentages of the respective controls (CTBs of the same preparation cultured in the absence of interleukins, leptin, or TGF-β).

Statistical Methods

Results are presented as means ± SEM. Comparisons among different treatments were made by analysis of variance using the Statview program (Abascus) on a Power Macintosh 7100/66 computer.

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References

1. Bischof, P. and Campana, A. (1997). *Early Pregnancy Biol. Med.* **3,** 81–95.
2. Aplin, J. D. (1993). *Placenta* **14,** 203–215.
3. Cross, J. C., Werb, Z., and Fisher, S. J. (1994). *Science* **266,** 1508–1518.
4. Tabibzadeh, S. and Babaknia, A. (1995). *Mol. Hum. Reprod. 1, Hum. Reprod.* **10,** 1579–1602.
5. Simón, C., Gimeno, M. J., Mercader, A., Frances, A., Garcia-Velazco, J., Remohi, J., Polan, M. L., and Pellicer, A. (1996). *Mol. Hum. Reprod.* **2,** 405–424.
6. Damsky, C. H., Fitzgerald, M. D., and Fisher, S. J. (1992). *J. Clin. Invest.* **89,** 210–222.
7. Burrows, T. D., King, A., and Loke, Y. W. (1993). *Hum. Reprod.* **8,** 475–484.
8. Bischof, P., Redard, M., Gindre, P., Vassilakos, P., and Campana, A. (1993). *Eur. J. Obstet. Gynecol. Reprod. Biol.* **51,** 217–226.
9. Korhonen, M., Ylanne, J., Laitineen, L., Cooper, H. M., Quaranta, V., and Virtanen, I. (1991). *Lab. Invest.* **65,** 347–356.
10. Damsky, C. H., Librach, C., Lim, K. H., Fitzgerald, M. L., Mac Master, M. T., Janatpour, M., Zhou, Y., Logan, S. K., and Fisher, S. J. (1994). *Development* **120,** 3657–3666.
11. Irving, J. A. and Lala, P. K. (1995). *Exp. Cell. Res.* **217,** 419–427.
12. Garbisa, S., Onisto, M., Mazzanti, L., Tranquilli, A. L., Pugnaloni, A., Cester, N., and Romanini, C. (1993). *Clin. Exp. Obstet. Gynecol.* **20,** 207–215.
13. Hynes, R. O. (1992). *Cell* **69,** 11–25.
14. Bischof, P., Haenggeli, L., and Campana, A. (1995). *Mol. Hum. Reprod.* **10,** 734–742.
15. Fisher, S. J., Cui, T., Zhang, L., Hartmann, L., Grahl, K., Guo-Yang, Z., Tarpey, J., and Damsky, C. H. (1989). *J. Cell. Biol.* **109,** 891–902.
16. Bischof, P., Martelli, M., Campana, A., Itoh, Y., Ogata, Y., and Nagase, H. (1995). *Early Pregnancy Biol. Med.* **1, 2**63–269.
17. Hulboy, D. L. and Rudolph, L. A. (1997). *Mol. Hum. Reprod.* **3,** 27–45.
18. Bischof, P., Friedli, E., Martelli, M., and Campana, A. (1991). *Am. J. Obstet. Gynecol.* **65,** 1791–1801.

19. Aplin, J. D., Haigh, T., Jones, C. J., Church, H. J., and Vicovac, L. (1999). *Biol. Reprod.* **60,** 828–838.
20. Librach, C. L., Feigenbaum, S. L., Bass, K. E., Cui, T. Y., Verastas, N., Sadovsky, Y., Quigley, J. P., French, D. L., and Fisher, S. J. (1994). *J. Biol. Chem.* **269,** 17,125–17,131.
21. Shimonovitz, S., Hurwitz, A., Barak, V., Dushnik, M., and Adashi, E. Y. (1996). *J. Clin. Endocrinol. Metab.* **81,** 3091–3096.
22. Meisser, A., Chardonnens, D., Campana, A., and Bischof, P. (1999). *Mol. Hum. Reprod.* **5,** 252–260.
23. Kameda, T., Matsuzaki, N., Sawai, K., Okada, T., and Saji, T. (1990). *Placenta* **11,** 205–213.
24. Masuhiro, K., Matsuzaki, N., Nishino, E., Taniguchi, T., Kameda, T., Li, Y., Saji, F., and Tanizawa, O. (1991). *J. Clin. Endocrinol. Metab.* **72,** 594–601.
25. Graham, C. H. and Lala, P. K. (1991). *J. Cell. Physiol.* **148,** 228–234.
26. Graham, C. H., Conelly, I., MacDougall, J. R., et al. (1994). *Exp. Cell. Res.* **214,** 93–99.
27. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). *Nature* **372,** 425–432.
28. Hardie, L., Trayhurn, P., Abramovich, D., and Fowler, P. (1997). *Clin. Endocrinol.* **47,** 101–106.
29. Schubring, C., Kiess, W., Englaro, P., Rascher, W., Dotsch, J., Hanitsch, S., Attanasio, A., and Blum, W. F. (1997). *J. Clin. Endocrinol. Metab.* **82,** 1480–1483.
30. Chardonnens, D., Cameo, P., Aubert, M. L., Pralong, F. P., Islami, D., Campana, A., Gaillard, R. C., and Bischof, P. (1999). *Mol. Hum. Reprod.* **5,** 1077– 1082.
31. Meisser, A., Cameo, P., Islami, D., Campana, A., and Bischof, P. (1999). *Mol. Hum. Reprod.* **5,** 1055–1058.
32. Castelluci, M., De Matteis, R., Meisser, A., Canullo, R., Monsurro, V., Islami, D., Sarzani, R., Marzioni, D., Cinti, S., and Bischof, P. (2000). *Mol. Hum. Reprod.* **6,** 951–958.
33. Gonzalez, R. R., Caballero-Campo, P., Jasper, M., Mercader, A., Devoto, L., Pellicer, A., and Simon, C. (2000). *J. Clin. Endocrinol. Metab.* **85,** 4883–4888.
34. Gonzalez, R. R., Simón, C., Caballero-Campo, P., Norman, R., Chardonnens, D., Devoto, L., and Bischof, P. (2000). *Hum. Reprod. Update* **6,** 290–300.
35. Bischof, P., Meisser, A., and Campana, A. (1998). *J. Reprod. Immunol.* **39,** 167–177.
36. Bischof, P., Meisser, A., Campana, A., and Tseng, L. (1998). *Placenta* **19,** 457–464.
37. Bischof, P. (1997). *Trophoblast Res.* **10,** 67–82.
38. Romero, R., Wu, Y. K., Brody, D. T., Oyarzun, E., Duff, G. W., and Durum, S. K. (1989). *Obstet. Gynecol.* **73,** 31–34.
39. Kauma, S. W., Matt, D., Strom, S., Eirman, D., and Turner, T. (1990). *Am. J. Obstet. Gynecol.* **163,** 1430–1437.
40. Simon, C., Frances, A., Piquette, G. N., el Danasouri, I., Zurawski, G., Dang, W., and Polan, M. L. (1994). *Endocrinology* **134,** 521–528.
41. Masuzaki, H., Ogawa, Y., Sagawa, N., Hosoda, K., Matsumoto, T., Hiroko, M., Nishimura, M., Yoshimasa, Y., Tanaka, I., Mori, T., and Nakao, K. (1997). *Nat. Med.* **3,** 1029–1033.
42. Senaris, R., Garcia-Caballero, T., Casabiell, X., Gallego, R., Castro, R., Considine, R. V., and Casanueva, F. F. (1997). *Endocrinology* **138,** 4501–4504.
43. Kitawaki, J., Koshiba, H., Ishihara, H., Kusuki, I., Tsukamoto, K., and Honjo, H. (2000). *J. Clin. Endocrinol. Metab.* **85,** 1946–1950.
44. Alfer, J., Muller-Schottle, F., Classen-Linke, I., von Rango, U., Happel, L., Beier-Hellwig, K., Rath, W., and Beier, H. M. (2000). *Mol. Hum. Reprod.* 6, 595–601.
45. Librach, C. L., Werb, Z., Fitzgerald, M. L., Chiu, K., Corwin, N. M., Esteves, R. A., Grobelny, D., Galardy, R., and Damsky, C. H. (1991). *J. Cell. Biol.* **113,** 437–449.