Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 656–660, January 1989
Medical Sciences

The interleukin 2 gene is expressed in the syncytiotrophoblast of the human placenta

(lymphokines/growth factors/fetal allograft/extraembryonic)

KEITH D. BOEHM*, MICHAEL F. KELLEY*, JOSEPH ILAN†‡§, AND JUDITH ILAN*†¶

Departments of *Reproductive Biology, and of †Developmental Genetics and Anatomy, ‡Department of Medicine–Oncology Division, Case Western Reserve University School of Medicine, and §The Developmental Biology Center, Case Western Reserve University, Cleveland, OH 44106

Communicated by Lester O. Krampitz, October 3, 1988 (received for review July 1, 1988)

ABSTRACT **The lymphokine interleukin 2 is an important immune system regulatory glycopolypeptide. It is produced by antigen- or mitogen-stimulated T lymphocytes and is required for the proliferation or clonal expansion of activated T lymphocytes. In this report, it is demonstrated by RNA transfer blot hybridization that the poly(A)$^+$ RNA population of the human placenta contains a 0.85-kilobase RNA transcript that specifically hybridizes to a human interleukin 2 cDNA probe. By using hybridization histochemistry *in situ*, it is further shown that interleukin 2 RNA transcripts are localized, primarily, to the syncytial (syncytiotrophoblast) layer of the human placenta. Possible roles for syncytiotrophoblast-produced interleukin 2 are suggested and discussed.**

Interleukin 2 (IL-2), or T (thymus-dependent)-cell growth factor, is a 15,500-dalton glycoprotein that is produced by T lymphocytes after antigen or mitogen stimulation and is required for proliferation of activated T cells, which possess specific receptors for this growth factor (recently reviewed, refs. 1–9). Furthermore, IL-2 potentiates the effector function(s) of activated T cells, natural killer cells, and other cytotoxic effector cells (10–17) and has been implicated in T-cell differentiation (18). IL-2 also affects other cells possessing its receptor, such as B lymphocytes [including potentiation of immunoglobulin secretion (19–21)] and macrophages, and it stimulates or increases the production of other cytokines, such as interferon, B-cell growth factors, and colony-stimulating factors (17, 21). Thus, IL-2 plays a major role as a central regulator or mediator of immune response and, through indirect mechanisms, affects hematopoietic cell proliferation and maturation.

The placenta is a unique, multifunctional organ upon which the developing embryo and fetus are dependent for survival and growth. Immunologically, the feto-placental unit has been viewed as an allograft (reviewed, refs. 22–26) and more than one hypothesis has been forwarded to explain the survival of this special graft (22–26). It was recently reported (27) that molecules sharing antigenic determinants with IL-2 were present in the human placental syncytiotrophoblast. In this report, evidence is provided that the human placental syncytiotrophoblast expresses the gene encoding the central immune regulatory molecule, IL-2, thereby indicating a possible active role for the placenta in the control of local immune function, at the interface between the host mother and the developing fetal allograft.

METHODS

Human Placental RNA Preparation. Preterm placentas were obtained from therapeutic pregnancy terminations at the MacDonald Hospital for Women, University Hospitals of Cleveland. Near-term and term placentas were obtained from cesarean sections at the same institution, within 20 min of delivery. Permission to use placental tissue was granted by the Case Western Reserve University School of Medicine and University Hospitals of Cleveland Institutional Review Board for Human Investigation. Some placentas obtained were frozen in liquid nitrogen and stored at −70°C and others were processed immediately upon receipt. Placentas from first trimester were pooled due to the relative paucity of tissue obtained from each pregnancy.

Extraction and isolation of total RNA were accomplished by using guanidinium isothiocyanate (Fluka; purum) as described (28), and poly(A)$^+$ RNA was selected from the total RNA by passage (twice) through oligo(dT)-cellulose (type 3, Collaborative Research) columns (29).

RNA Transfer Blot Hybridization Analysis. Placental poly(A)$^+$ RNA from differing stages of gestation was separated by electrophoresis through 1% agarose/formaldehyde denaturing minihorizontal submarine slab gels, transferred to nitrocellulose, and baked under vacuum for 2 hr at 80°C as described (30). Total RNA from placenta or an RNA ladder (Bethesda Research Laboratories) was separated simultaneously on the same minigels in an adjacent lane and stained with ethidium bromide for RNA size determination. Nitrocellulose-bound poly(A)$^+$ RNA was hybridized with an [α-^{32}P]dCTP nick-translated 700-base-pair (bp) human IL-2 coding region cDNA fragment (Oncor, Gaithersburg, MD) (1–5 × 10^7 cpm/μg of DNA, 1 × 10^6 cpm/ml) in a solution containing 35% (vol/vol) formamide, 0.9 M NaCl/90 mM trisodium citrate, 0.1% sodium pyrophosphate, 0.1 M sodium phosphate (pH 7.0), 1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 0.1% sodium dodecyl sulfate, 10% dextran sulfate, 10 mM vanadyl ribonucleoside complex, and 10 μg of sheared and denatured *Escherichia coli* DNA per ml at 42°C for 18–20 hr. Posthybridization washes were accomplished in a solution composed of 0.015 M NaCl/1.5 mM trisodium citrate/0.1% sodium dodecyl sulfate at 50°C. Nitrocellulose was then exposed to Kodak XAR-5 radiographic film in the presence of two DuPont Cronex Lightning Plus intensifying screens for varying periods of time (120 hr for the transfer blot shown) to obtain autoradiograms. On some transfer blots, poly(A)$^+$ RNA (kindly provided by D. Kaplan, Department of Pathology, Case Western Reserve University) from mitogen-stimulated human Jurkat (31, 32) leukemia T cells (an IL-2-producing T-cell line) was used as an IL-2 RNA transcript positive control (data not shown). Results presented are representative of four separate experiments.

***In Situ* Hybridization Analysis.** Portions of second-trimester and term placentas (obtained as previously described) were fixed in either 10% buffered formalin, 2%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL, interleukin; T, thymus-dependent; CSF, colony-stimulating factor.

¶To whom reprint requests should be addressed.

glutaraldehyde, or 4% paraformaldehyde immediately upon receipt. Fixed samples were then embedded in paraffin wax and sectioned on a standard microtome at 5 μm. The placental histologic sections were then placed on microscope slides pretreated with poly(L-lysine). Following deparaffinization in xylene, the slide sections were exposed to proteinase K (1 μg/ml) in a solution of 20 mM Tris·HCl, pH 7.4/2 mM $CaCl_2$ for 30 min at 37°C. As a control for specificity of hybridization to tissue RNA, some sections in each experiment were treated at this point in the procedure with ribonuclease A (1 mg/ml) in a 0.5 M NaCl/10 mM Tris·HCl, pH 8.0, solution for 1 hr at 40°C. Sections were then exposed to 0.1 M triethanolamine (pH 8.0) for 2 min at room temperature, followed by treatment with 0.25% (vol/vol) acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min at the same temperature. Hybridization was performed in a solution composed of 70% (vol/vol) formamide, 10 mM Tris·HCl (pH 7.5), 1 mM EDTA, 300 mM NaCl, 2% dextran sulfate, 250 μg of sheared and denatured *E. coli* DNA per ml, and 250 μg of yeast t-RNA per ml at 37°C for 16–20 hr in a humidified container. The dCTP[^{35}S]/[^{3}H]dATP, dGTP, TTP nick-translated IL-2 cDNA fragment (described above) [specific activity, 1–5 × 10^7 cpm/μg of DNA] was added in the hybridization solution to each slide histologic section (3–5 ng of radiolabeled cDNA fragment per section). Posthybridization treatments included two 30-min and one 60-min wash in 0.3 M NaCl/30 mM trisodium citrate at 37°C. The placental histologic sections were then immersed in either Ilford K.5D or K.2 nuclear track emulsion in the dark. After drying, the tissue sections were left at −20°C for 2–3.5 wk (as described for photomicrographs shown). Sections were then developed (Kodak D-19), fixed, and counterstained (hematoxylin/eosin Y) for viewing and photomicrography. In some experiments similarly nick-translated pBR322 plasmid (lacking insert) was used on a few sections as a control for background, nonspecific binding and on other sections, hybridization was performed without any radiolabeled cDNA present, to control for artifactual grain formation (data not shown). Results presented are representative of four separate experiments.

RESULTS AND DISCUSSION

A 0.85-kb (kilobase) IL-2 RNA transcript was detected by RNA transfer blot hybridization analysis in the poly(A)$^+$ RNA populations of human placentas from all three major stages of gestation (Fig. 1). This transcript is in the size range reported by others to be present in the RNA populations of activated human T lymphocytes (33) and leukemia T-cell lines (32). Although it appears (Fig. 1) that the relative abundance of the IL-2 RNA transcript is greatest during the first trimester of pregnancy (Fig. 1, lane 1) and is lowest during the second trimester (Fig. 1, lane 2), with a slight increase by term (Fig. 1, lane 3), other placental samples examined (data not shown) varied considerably from this pattern of expression. Therefore, no conclusions can be made, at the present time, as to IL-2 RNA transcript relative abundance in the human placenta at each major stage of gestation. A large sampling would have to be performed to account for individual placental sample variation.

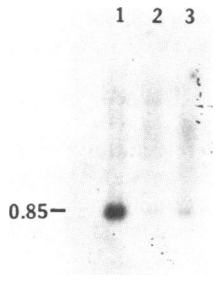

FIG. 1. Autoradiogram of hybridization of IL-2 cDNA probe to transfer blot of human placental poly(A)$^+$ RNA from differing stages of gestation. The poly(A)$^+$ RNA was electrophoresed on an agarose gel, transferred to nitrocellulose paper, and autoradiographed. Probe specific activity was 1.2 × 10^7 cpm/μg of DNA. The gestational stages of the placental poly(A)$^+$ RNA are first trimester/11–12 wk pooled (lane 1), second trimester/29 wk (lane 2), and term (lane 3). Lanes 1 and 3 each contain 2.5 μg of poly(A)$^+$ RNA, and lane 2 contains 3.0 μg of poly(A)$^+$ RNA. The size of the hybridized transcript is indicated in kb.

As the human placenta is composed of, and contains, a multiplicity of tissues and cells, it was of interest to determine the site of expression of the IL-2 gene, especially since activated T lymphocytes may be present in this extraembryonic organ. It has been shown, however, that human placental tissue, itself, has a low level of permeability to lymphocytes, and murine placental tissue is virtually impenetrable to these cells (ref. 22 and references therein).

By utilizing the method of hybridization histochemistry or *in situ* hybridization analysis, IL-2 RNA transcripts were localized primarily to the syncytiotrophoblast layer of the human placenta (Fig. 2, arrowheads), indicating that the IL-2 gene is expressed in the human placental syncytiotrophoblast. This finding can be correlated with, and is substantiated by, the report of Soubiran *et al.* (27) that a polypeptide (or possibly polypeptides) sharing several epitopes with IL-2 is present in the human placental syncytiotrophoblast.

As a control for specificity of hybridization to tissue RNA, treatment of placental histologic sections with ribonuclease A was done prior to performance of *in situ* hybridization. This experiment resulted in a very substantial reduction of the hybridization signal detected (Fig. 3) in the placental histologic sections (as compared with that of sections not pretreated with ribonuclease A—i.e., Fig. 2). This reduction in hybridization reactivity indicates that the radiolabeled IL-2 cDNA is not binding nonspecifically but instead is interacting, specifically, with the RNA present in the fixed tissue sections.

The *in situ* hybridization results reported here (Figs. 2 and 3) provide evidence that the IL-2 gene is expressed in the human placental syncytiotrophoblast. As the syncytiotrophoblast is an amitotic, multinucleate cytoplasmic mass with a continuous membrane (a syncytium) that forms a layer, it would be most unlikely that viable, activated T lymphocytes could enter the syncytiotrophoblast and, therefore, be responsible for the IL-2 RNA hybridization signal (Fig. 2) detected autoradiographically. Furthermore, the distinctive morphology of lymphocytes would make them readily apparent in placental histologic sections. No lymphocytes or other blood cells could be seen in the syncytiotrophoblastic layer of placental sections.

IL-2 has generally been believed to be an antigen- or mitogen-induced T-lymphocyte- or T-cell line-specific product (34, 35). Besides the detection of human placental (syncytiotrophoblastic) IL-2 RNA transcripts reported here and the detection of immunoreactive IL-2 in human syncytiotrophoblast and amnion (27), only a couple of other indications of extra-T-cell IL-2 production have been reported (36, 37). Dreno *et al.* (36) demonstrated immunoreactive IL-2 in granular layer keratinocytes of the human skin, and Walker *et al.* (37) have demonstrated IL-2 RNA transcripts in, and IL-2 release from, *Staphylococcus aureus* stimulated murine B-lymphoma cell lines.

T-cell IL-2 production is a transient event, triggered by antigen or mitogen stimulation (38–40). This induction has further been shown to be dependent on increased rates of IL-2 gene transcription (33, 41, 42). In this report, it is shown that IL-2 RNA transcripts are present in the placental RNA population of each major stage of pregnancy (Fig. 1), leading

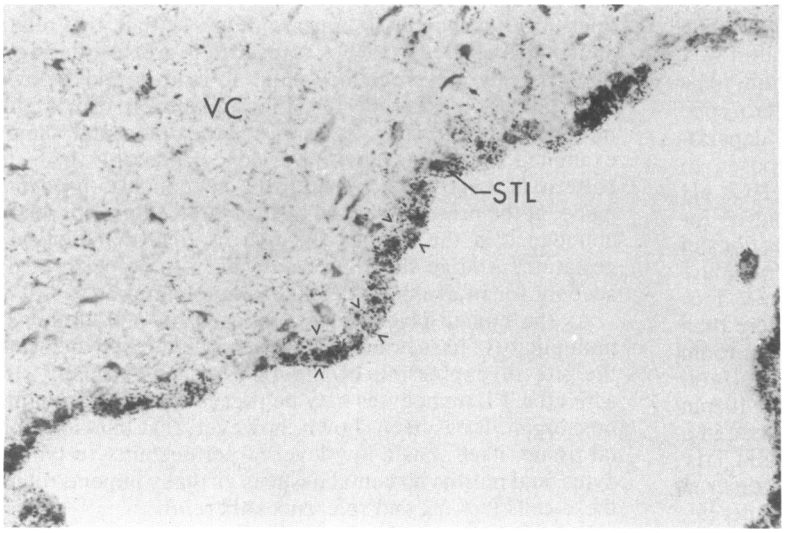

FIG. 2. A second trimester human placental histological section hybridized with IL-2 cDNA probe. Specific activity of the probe was 1.1×10^6 cpm/μg of DNA. Exposure time for development was 3.0 wk. Grain densities (arrowheads) in the syncytiotrophoblastic layer (STL) represent IL-2 RNA transcripts. Some grains are also present in the villus core (VC), and minor nonspecific binding of the IL-2 cDNA probe can be seen in the space not containing tissue. (×45.)

to the suggestion that the IL-2 gene in the placenta is expressed throughout the entire period of gestation. This apparent constitutive expression of the IL-2 gene in the placenta raises some interesting questions. (*i*) Is the seemingly continuous presence of placental IL-2 RNA transcripts due to IL-2 gene transcription (analogous to the induction of IL-2 in the activated T cell) or to stabilization of IL-2 RNA transcripts, or both? (*ii*) Is the apparent constitutive placental IL-2 gene expression truly constitutive (i.e., without inducing stimulus and, perhaps, a tissue-specific mode of expression) or is the syncytiotrophoblast actually in a constant state of "activation?" (*iii*) If the syncytiotrophoblast is in a constantly "activated" state, are the possible stimuli for and mechanisms of activation identical or similar to those of the T cell? (*iv*) Is the apparent constitutive syncytiotrophoblast IL-2 gene expression observed a consequence of alterations in IL-2 genomic regulatory elements and/or the nuclear proteins that interact with them?

In regard to this last question, regulatory regions upstream of the IL-2 gene have been identified (42–46). Possible positive regulatory upstream enhancer elements have been shown to be required for transcription (42–44), and T-cell-specific DNase-hypersensitive upstream sites in close proximity to or within these enhancer-like sequences have been demonstrated (45). Recently, Nabel *et al.* (35), in an effort to determine the mechanism(s) responsible for T-cell-specific IL-2 gene transcription, examined the binding of proteins from nuclear extracts of resting and activated Jurkat cells (and IL-2 nonproducing, non-T cells) to radiolabeled DNA probes derived from the upstream IL-2 genomic regulatory region in electrophoretic mobility shift assays. They identified a site [near one of the previously identified T-cell-specific DNase-hypersensitive sites (45)] that negatively regulates IL-2 gene expression in resting T cells. The nuclear protein complex that binds to the area of DNA containing this site is altered only in activated T cells. The authors suggested that negative regulation at this site, via interaction with its binding protein(s), is likely to be important in T-cell-specific IL-2 gene expression. It would, therefore, be of interest to clone and sequence the human placental IL-2 gene and its flanking regions for identification of possible alterations (from that of T cells) in regulatory sequences or sites. As the placental IL-2 gene is apparently continuously expressed, there may be alterations in these elements. Alternatively, these regions of the IL-2 gene may be no different than those described from T cells, and other regulatory regions may be present in the placental gene or its flanking regions. It is also possible that, if the placental gene and its regulatory sequences are identical to those of the T cell, the IL-2 gene negative regulation site binding protein complex, identified by Nabel *et al.* (35), that is modified in activated T cells, would only be present in its modified form in placental nuclei. By using placental or, preferably, syncytiotrophoblast nuclear protein extracts in the system of Nabel *et al.* (35), one could address this latter speculation.

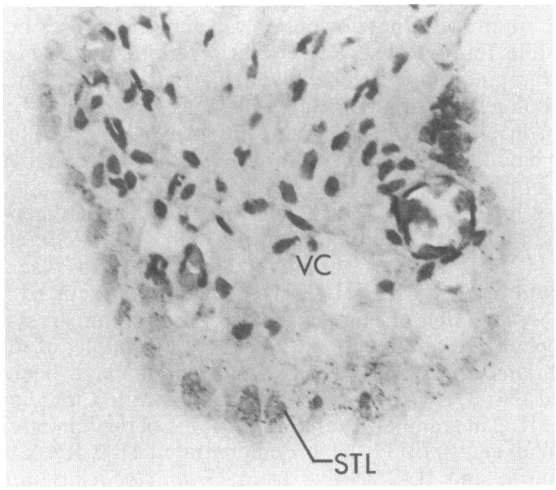

FIG. 3. A second trimester human placental histological section hybridized with IL-2 cDNA probe. Specific activity and exposure time as in Fig. 2. Grains, representing IL-2 RNA transcripts, are greatly diminished (as compared with those seen in Fig. 2) in number in the syncytiotrophoblastic layer (STL) due to ribonuclease pretreatment, demonstrating specificity of hybridization to tissue RNA. Very few grains are present in the villus core (VC). (×90.)

Whether or not the IL-2 gene in the placenta is expressed constitutively, with or without syncytiotrophoblast activation, the most interesting and intriguing question remaining is: What would be the function(s) of placental-derived IL-2?

As part of its unique multifunctionality, the placenta must be viewed as also possessing an immunological component. The special allograft status of the feto-placental unit is now a quite well-established, albeit controversial, concept (see recent reviews, refs. 22–26). IL-2, an important regulator of immune function produced by the syncytiotrophoblast, would most certainly play a major role in any scheme of immune interaction(s) postulated to exist, between the host mother and the feto-placental graft. It is obvious that any local maternal or relatively mature fetal lymphocyte or lymphoid cell possessing IL-2 receptors [i.e., activated T lymphocytes (1–18), some B lymphocytes (19–21, 47), and resident macrophages (21)] would potentially be affected (1–

21) by syncytiotrophoblast-derived IL-2, and by more indirect means other hematopoietic cells (21) would possibly also be affected. Also, fetal T- and/or B-lymphocyte maturation might be affected. IL-2 produced by the placenta would potentially be in competition for receptors with T-cell-derived IL-2, complicating its interaction(s) with blood-borne cellular elements. Whether the syncytiotrophoblast IL-2 participates in activation (10–17, 19–21) and/or suppression (48) of local immune mechanisms is an important question to be answered for gaining a greater understanding of potential mechanisms mediating the rejection or maintenance of the feto-placental allograft in the host mother.

As the placenta is in intimate contact with the uterus, factors produced by it may also affect certain uterine cells that participate in local immune mechanisms. Therefore, placental IL-2 may participate in the modulation or regulation of the proliferation and/or differentiation of specific IL-2 receptor-bearing uterine cells. The placental-derived IL-2 may also regulate/modulate the production of immune regulatory molecules by specific uterine cells. A variety of suppressor cells that inhibit lymphocyte activation have been reported to be resident in the uterine decidua (49, 50). IL-2 has been purported to induce the generation of suppressor T cells (48). Therefore, syncytiotrophoblast-derived IL-2 may likewise affect the decidua-associated suppressor cell population(s). This would be of importance to fetal allograft nonrejection. Of interest here is the report that first trimester human decidua contains a T-lymphocyte population lacking IL-2 receptors (51), which means that these uterine-associated cells would be unable to respond to placental-derived IL-2. Further, decidual suppressor cells have been shown to produce immunosuppressive factor(s), which may be important mediator(s) of their inhibitory effect on lymphocyte activation (52, 53). Syncytiotrophoblast-produced IL-2 may affect the production and release of these immunosuppressants. Recently, it was also reported that uterine luminal and glandular secretory epithelial cells express the colony-stimulating factor 1 (CSF-1) gene and produce CSF-1 (54), which, in turn, most likely interacts with its receptor(s) (the product of the c-*fms* protooncogene) located on placental membranes, to possibly affect the development and function of this extraembryonic organ. As IL-2 may potentiate the production of CSFs (21), placental-derived IL-2 might enhance the production of CSF-1 by uterine secretory epithelial cells, completing a paracrine circle from uterus to placenta and back again.

Another possible role for syncytiotrophoblast-derived IL-2 might be modulation or regulation of the production of immunoregulatory molecules by the placenta itself. Placentas or trophoblasts have been reported to produce a variety of immunoregulators. Soluble inhibitory factor (also a product of T lymphocytes) is released by the placenta (55). It has the ability to suppress lymphocyte proliferation and antibody synthesis. Other immune regulatory or hematopoietic factors reported to be produced by the placenta are granulocyte-macrophage CSF (GM-CSF) (56), hematopoietic survival and stem cell growth factor (57), colony-stimulating activity (58), transforming growth factor B (TGF-B) (59, 60), and IL-1 (61–63). This latter factor, IL-1, has been reported to be produced by purified cytotrophoblasts (63), placental cells/tissue pieces (62), and resident placental macrophages (61). IL-1 is a stimulus for T-lymphocyte IL-2 production (1–9, 64), and, therefore, placental macrophage or cytotrophoblast-derived IL-1 may stimulate the expression of IL-2 by the syncytiotrophoblast. The other factors listed may also feed back upon the syncytiotrophoblast, or they may affect other cellular/tissue elements found in the placenta. For instance, GM-CSF and IL-3 have been shown to stimulate the proliferation and phagocytic capabilities of murine placental macrophage-like cells (65). Lastly, a factor(s) present in umbilical cord serum stimulates IL-2 production (66) and, thus, may be an important "fetal stimulus" for syncytiotrophoblast IL-2 production.

An additional role for IL-2 derived from the syncytiotrophoblast might be as a placental growth and/or maturation factor. Placental cytotrophoblasts, if they possess IL-2 receptors, might respond to syncytiotrophoblast-produced IL-2 in a paracrine manner, by proliferating and/or differentiating, ultimately, possibly, forming additional syncytiotrophoblast. Possible autocrine effects of this IL-2 would most likely concern perhaps subtle aspects of syncytiotrophoblast differentiation and/or maturation, as it apparently has no proliferative potential. At present, there is no evidence for the presence of IL-2 receptors on cyto- or syncytiotrophoblasts (27).

A final possible mode of action for placental IL-2 may be as a mobilizer of local immune function in response to the presence of infectious agents, such as bacteria or viruses. Nelson *et al.* (67) provided evidence that ≈80% of "normal" human placentas examined contained reverse transcriptase activity, which was associated with retrovirus-like particles observed in the tissue of some of the placentas. The syncytiotrophoblast-derived IL-2 may, therefore, participate in the suppression of invading viruses. One major potential mechanism whereby placental IL-2 might assist in responding to viral infection would be via stimulation of production of interferon (17, 21) or some similar protein produced during mammalian pregnancy, such as ovine trophoblast protein 1 (68, 69). This protein appears to be an interferon-α produced by the conceptus and is important in sheep maternal recognition of pregnancy. Recently, it was also shown to possess antiviral activity (69). Thus, placental syncytiotrophoblast-produced IL-2 would most likely be important in protecting the feto-placental unit when infection occurs *in utero*.

In conclusion, evidence is presented in this report that expression of the IL-2 gene is not T-cell-specific, as its transcription occurs in the human placenta, and has been localized to the syncytiotrophoblast.

Appreciation is heartily expressed for the professional histotechnological expertise provided by Ms. Claudia Garner and for the manuscript word processing of Ms. Joyce Olson. This work was supported, in part, by an American Heart Association, Northeast Ohio Affiliate, fellowship grant to K.D.B., Grant HD-18271 from the National Institutes of Health to Judith Ilan, Grant 284-b-87 from the Diabetes Association of Greater Cleveland, and NIH Grant CA-43703.

1. Smith, K. A. (1988) *Adv. Immunol.* **42,** 165–179.
2. Smith, K. A. (1988) *Science* **240,** 1169–1176.
3. Smith, K. A. (1986) in *Progress in Immunology VI*, eds. Cinader, B. & Miller, R. G. (Academic, New York), pp. 440–448.
4. Waldmann, T. A. (1986) *Science* **232,** 727–732.
5. Waldmann, T. A., Kozak, R. W., Tsudo, M., Oh-ishi, T., Bongiovanni, K. F. & Goldman, C. K. (1986) in *Progress in Immunology VI*, eds. Cinader, B. & Miller, R. G. (Academic, New York), pp. 553–562.
6. Dinarello, C. A. & Mier, J. W. (1986) *Annu. Rev. Med.* **37,** 173–178.
7. Greene, W. C. & Leonard, W. J. (1986) *Annu. Rev. Immunol.* **4,** 69–95.
8. Mertelsmann, R. & Welte, K. (1986) *Immunobiology (Stuttgart)* **172,** 400–419.
9. Smith, K. A. (1984) *Annu. Rev. Immunol.* **2,** 319–333.
10. Henney, C. S., Kuribayashi, K., Kern, D. E. & Gillis, S. (1981) *Nature (London)* **291,** 335–338.
11. Domzig, W., Stadler, B. E. & Herberman, R. B. (1983) *J. Immunol.* **130,** 1970–1973.
12. Hefeneider, S. H., Conlon, P. J., Henney, C. S. & Gillis, S. (1983) *J. Immunol.* **130,** 222–227.
13. Flomenberg, N., Welte, K., Mertelsmann, R., O'Reilly, R. & Dupont, B. (1983) *J. Immunol.* **130,** 2635–2643.

14. Merluzzi, V. J., Savage, D. M., Mertelsmann, R. & Welte, K. (1984) *Cell. Immunol.* **84,** 74–84.
15. Grimm, E. A., Ramsey, K. M., Mazumder, A., Wilson, D. J., Djeu, J. Y. & Rosenberg, S. A. (1983) *J. Exp. Med.* **157,** 844–897.
16. Henney, C. S., Kern, D. E. & Gillis, S. (1982) *Transplant. Proc.* **14,** 565–569.
17. Farrar, W. L., Johnson, H. M. & Farrar, J. J. (1981) *J. Immunol.* **126,** 1120–1125.
18. Kern, D. E., Gillis, S., Okada, M. & Henney, C. S. (1981) *J. Immunol.* **127,** 1323–1328.
19. Swain, S. L., Dennert, G., Warner, J. F. & Dutton, R. W. (1981) *Immunology* **78,** 2517–2521.
20. Leibson, H. J., Marrack, P. & Kappler, J. W. (1981) *J. Exp. Med.* **154,** 1681–1693.
21. Farrar, J. J., Benjamin, W. R., Hilfiker, M. L., Howard, M., Farrar, W. L. & Fuller-Farrar, J. (1982) *Immunol. Rev.* **63,** 129–166.
22. Gill, T. J., III (1988) in *The Physiology of Reproduction*, eds. Knobil, E. & Neill, J. D. (Raven, New York), pp. 2023–2042.
23. Jacoby, D. R., Olding, L. B. & Oldstone, M. B. A. (1984) *Adv. Immunol.* **35,** 157–208.
24. Chaouat, G. (1984) *Ann. Immunol. (Paris)* **135D,** 301–351.
25. Möller, G., ed. (1983) *Immunol. Rev.* **75.**
26. Gill, T. J., III & Repetti, C. F. (1979) *Am. J. Pathol.* **95,** 463–570.
27. Soubiran, P., Zapitelli, J.-P. & Schaffar, L. (1987) *J. Reprod. Immunol.* **12,** 225–234.
28. Mills, N. C., D'Ercole, A. J., Underwood, L. E. & Ilan, J. (1986) *Mol. Biol. Rep.* **11,** 231–236.
29. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
30. Shen, S.-J., Wang, C.-Y., Nelson, K. K., Jansen, M. & Ilan, J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9179–9182.
31. Kaplan, J., Tilton, J. & Peterson, W. D., Jr. (1976) *Am. J. Hematol.* **1,** 219–223.
32. Weiss, A., Shields, R., Newton, M., Manger, B. & Imboden, J. (1987) *J. Immunol.* **138,** 2169–2176.
33. Efrat, S., Pilo, S. & Kaempfer, R. (1982) *Nature (London)* **297,** 236–239.
34. Smith, K. A. (1980) *Immunol. Rev.* **51,** 337–357.
35. Nabel, G. J., Gorka, C. & Baltimore, D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2934–2938.
36. Dreno, B., Jacques, Y., Barriere, H. & Soulillou, J. P. (1986) *J. Invest. Dermatol.* **86,** 359–362.
37. Walker, E., Leemhuis, T. & Roeder, W. (1988) *J. Immunol.* **140,** 859–865.
38. Efrat, S. & Kaempfer, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2601–2605.
39. Arya, S. K. & Gallo, R. C. (1984) *Biochemistry* **23,** 6685–6690.
40. Granelli-Piperno, A., Andrus, L. & Steinman, R. M. (1986) *J. Exp. Med.* **163,** 922–937.
41. Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R. & Hamuro, J. (1983) *Nature (London)* **302,** 305–310.
42. Fujita, T., Shibuya, H., Ohashi, T., Yamanishi, K. & Taniguchi, T. (1986) *Cell* **46,** 401–407.
43. Yamada, G., Fujita, T., Hatakeyama, M. & Taniguchi, T. (1986) in *Progress in Immunology VI*, eds. Cinader, B. & Miller, R. G. (Academic, New York), pp. 469–475.
44. Durand, D. B., Bush, M. R., Morgan, J. G., Weiss, A. & Crabtree, G. R. (1987) *J. Exp. Med.* **165,** 395–407.
45. Siebenlist, U., Durand, D. B., Bressler, P., Holbrook, N. J., Norris, C. A., Kamoun, M., Kant, J. A. & Crabtree, G. R. (1986) *Mol. Cell. Biol.* **6,** 3042–3049.
46. Williams, T. M., Eisenberg, L., Burlein, J. E., Norris, C. A., Pancer, S., Yao, D., Burger, S., Kamoun, M. & Kant, J. A. (1988) *J. Immunol.* **141,** 662–666.
47. Nakagawa, T., Nakagawa, N., Goldstein, H., Volkman, D. J. & Fauci, A. S. (1986) *J. Immunol.* **137,** 3175–3182.
48. Ting, C.-C., Yang, S. S. & Hargrove, M. E. (1984) *J. Immunol.* **133,** 261–266.
49. Clark, D. A., Slapsys, R. M., Croy, B. A. & Rossant, J. (1983) *J. Immunol.* **131,** 540–542.
50. Daya, S., Clark, D. A., Devlin, C. & Jarrell, J. (1985) *Fertil. Steril.* **44,** 778–785.
51. Bulmer, J. N. & Johnson, P. M. (1986) *Immunology* **58,** 685–687.
52. Bolton, A. E., Clough, K. J., Stoker, R. J., Pockley, A. G., Mowles, E. A., Westwood, O. M. R. & Chapman, M. G. (1987) *Lancet* **i,** 593–595.
53. Daya, S., Rosenthal, K. L. & Clark, D. A. (1987) *Am. J. Obstet. Gynecol.* **156,** 344–350.
54. Pollard, J. W., Bartocci, A., Arceci, R., Orlofsky, A., Ladner, M. B. & Stanley, E. R. (1987) *Nature (London)* **330,** 484–486.
55. Wolf, R. L. (1988) *Pediatr. Res.* **23,** 212–218.
56. Cukrova, V. & Hrkal, Z. (1987) *J. Chromatogr.* **413,** 242–246.
57. Hiraoka, A., Ohkubo, T. & Fukuda, M. (1987) *Cancer Res.* **47,** 5025–5030.
58. Ruscetti, F. W., Chou, J. Y. & Gallo, R. C. (1982) *Blood* **59,** 86–90.
59. Frolik, C. A., Dart, L. L., Meyers, C. A., Smith, D. M. & Sporn, M. B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3676–3680.
60. Derynck, R., Jarrett, J. A., Chen, E. Y., Eaton, D. H., Bell, J. R., Assoian, R. K., Roberts, A. B., Sporn, M. B. & Goeddel, D. V. (1985) *Nature (London)* **316,** 701–705.
61. Flynn, A., Finke, J. H. & Hilfiker, M. L. (1982) *Science* **218,** 475–477.
62. Flynn, A., Finke, J. H. & Loftus, M. A. (1985) *Immunopharmacology* **9,** 19–26.
63. Main, E. K., Strizki, J. & Schochet, P. (1987) in *Trophoblast Research*, eds. Miller, R. K. & Thiede, H. A. (Plenum, New York), Vol. 2, pp. 149–160.
64. Hamblin, A. S. (1988) *Immunology* Suppl. 1, 39–41.
65. Athanassakis, I., Bleackley, C., Paetkau, V., Guilbert, L., Barr, P. J. & Wegmann, T. G. (1987) *J. Immunol.* **138,** 37–44.
66. Bessler, H., Sirota, L., Dulitzky, F. & Djaldetti, M. (1986) *J. Reprod. Immunol.* **9,** 103–110.
67. Nelson, J., Leong, J. & Levy, J. A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6263–6267.
68. Imakawa, K., Anthony, R. V., Kazemi, M., Marotti, K. R., Polites, H. G. & Roberts, R. M. (1987) *Nature (London)* **330,** 377–379.
69. Pontzer, C. H., Torres, B. A., Vallet, J. L., Bazer, F. W. & Johnson, H. M. (1988) *Biochem. Biophys. Res. Commun.* **152,** 801–807.