sites of BrdUrd incorporation (Fig. 1).

Other experiments tested the immunofluorescent staining in a more quantitative manner. Human lymphoblast cells (WiL2) were incubated briefly in medium containing BrdUrd, fixed in 70 percent ethanol, and stained in suspension. No cytoplasmic fluorescence was observed by fluorescence microscopy.

The cells were then analyzed by flow cytometry for light-scatter size and the amount of indirect immunofluorescence of the antibody to BrdUrd. Exposures as short as 6 minutes were sufficient to demonstrate the incorporation of BrdUrd (Fig. 2). The cells that were not labeled with BrdUrd exhibited about tenfold less fluorescence than the labeled cells (Fig. 2). With concentrated antibody preparations (20 mg/ml, compared to the 100 μg/ml routinely used), the fluorescence intensity was increased by only 0.8 percent, as judged by the fluorescence peak channel values of 3 and 4, respectively. In previous observations with heteroclonal rabbit antibodies to BrdUrd, careful titrations were necessary to arrive at a serum concentration that specifically stained the BrdUrd-labeled cells and did not stain the nuclei of unlabeled cells (*3*). Successively decreasing the protein concentration from 20 mg/ml to 10 μg/ml did not change the number of BrdUrd-labeled cells (the labeling index), but did cause decreases in the fluorescence intensity of individual cells. These observations demonstrate the importance of titrating to antibody excess in order to maximize the antibody binding to the BrdUrd in individual nuclei.

The results reported here indicate that monoclonal antibodies specific for BrdUrd can provide a sensitive method for detecting DNA replication in single cells in a manner analogous to the use of [^{3}H]thymidine. When the technique is used to measure cell proliferation kinetics, significant increases in statistical reliability and speed have been achieved (*9*). The fluorescence intensity per cell due to monoclonal antibody to BrdUrd was directly related to the amount of BrdUrd incorporated (*3*) and provides a rapid method for quantifying the rate of cellular DNA synthesis. The analytical applications of this monoclonal antibody detection method may be useful not only for measuring cellular DNA replication and DNA synthesis rate in vitro but for investigating DNA repair phenomena and DNA replication in situ.

HOWARD G. GRATZNER
Department of Surgery, University of Miami School of Medicine, Miami, Florida 33101

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Placental Mononuclear Phagocytes as a Source of Interleukin-1

Abstract. *Mouse and human placental tissue contains a large number of mononuclear phagocytes. These cells, isolated from placenta, were shown to produce the multifaceted immune factor interleukin-1. Activity in the supernatants of 48-hour mononuclear phagocyte cultures was associated with a 12,000- to 18,000-dalton protein, consistent with known interleukin-1 characteristics. Stimulation of phagocytosis with latex beads increased the production and release of interleukin-1 from these placental cells, which may be a useful source of this protein.*

Activated mononuclear phagocytes and macrophages are known to produce a number of immunologically important factors (*1*). Interleukin-1 (IL-1), a 12,000- to 18,000-dalton protein, is produced by macrophages and is responsible for a number of T and B lymphocyte actions (*2*). Recently, the effects of IL-1 were equated with the responses noted for other macrophage factors of similar molecular weight, such as leukocyte endogenous mediator (LEM). It was reported that LEM modulates the central nervous system, producing fever; the bone marrow, causing the release of neutrophils and stimulating granulopoiesis; and the liver, causing the production of acute-phase proteins (*3*). It was also shown that IL-1 is similar to endogenous pyrogen (EP), in that IL-1 binds to and elutes from an affinity column for EP (*4*). Further study of IL-1, LEM, and EP requires a source of cells that are activated or can be activated to produce sufficient quantities of mononuclear phagocyte culture supernatants.

Wood (*5*) showed that human placenta contains approximately 65 percent mononuclear phagocytes. He reported that most cells released from tissue rich in chorionic villi had receptors for the third component of complement (C3) and for Fc, and that they morphologically resembled mononuclear phagocytes and phagocytized opsonized erythrocytes. Wood (*6*) subsequently demonstrated that large amounts of immunoglobulin G (IgG) are bound within the placenta to Fc receptors on mononuclear phagocytes, suggesting a mechanism for the removal of incompatible antibodies.

Since, in pregnancy, many events mimic the effects of IL-1 or LEM (*7*), we questioned whether the mononuclear phagocytes in placenta could actively produce IL-1 or be stimulated to produce this factor. We initially examined placentas taken from C57B1/6 mice 2 days prior to term and subsequently human placentas collected after normal delivery. Whole mouse placentas and tissue rich in chorionic villi from human placentas were separately minced and enzymatically digested with trypsin and collagenase in calcium-free Hanks balanced salt solution for 30 minutes (*5*). The cell suspension was washed twice with medium (*8*) and cultured at 1.0×10^6 cells per milliliter on tissue-culture plates for 2 hours (*8*). The plates were then washed with medium to remove nonadherent cells and the percentage of adherent cells was estimated by counting the cells collected from the washing. Mononuclear phagocyte characteristics were determined by counting the number of adherent cells that phagocytized latex beads (*9*) and stained positively for nonspecific esterase (*10*). The adherent cell population (in human cultures a placenta can provide greater than 1×10^{11} adherent cells) was cultured for 48 hours, and the supernatants were collected, dialyzed (40 times), and filtered. The supernatant fluids were analyzed for IL-1 and interleukin-2 (IL-2) activity. The IL-1 activity was estimated by the thymocyte proliferation assay reported by Farrar *et al.* (*11*). Because this assay also detects IL-2, the samples were also examined for IL-2 activity by a conventional T cell growth assay (*12*) to ensure that the

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biological activity detected in the thymocyte proliferation assay was IL-1. There should have been no or little IL-2 activity in the culture supernatants since IL-2 is a T lymphocyte product. Gery *et al.* (*13*) reported that the production and release of IL-1 by peritoneal exudate cells could be enhanced by stimulating phagocytosis. We tested this concept by using latex bead ingestion to determine whether the release of IL-1 from placental mononuclear phagocytes could be increased.

The supernatant fluids from the mononuclear phagocytes activated by latex beads were chromatographed to determine whether the IL-1 activity was specifically in the 12,000- to 18,000-dalton fractions. One liter of culture supernatants from 48-hour cultures was concentrated (×500) with an Amicon ultrafiltration membrane system and applied to a calibrated gel filtration column (*14*). The protein was eluted with 0.1*M* phosphate-glycine buffer, *p*H 7.4, and collected in 2.0-ml fractions. The fractions were then assayed for IL-1, and the molecular weight of each active fraction was calculated from the calibration line.

Cultures of placental cells from C57B1/6J mice contained a mean of 67 percent plastic adherent cells after the 2-hour culture period. At 48 hours a mean of 86 percent of the adherent cells was capable of phagocytizing latex beads. The culture supernatant fluids from the mouse adherent placental cells contained a significant amount of IL-1 (Table 1), but did not contain discernible amounts of IL-2 activity.

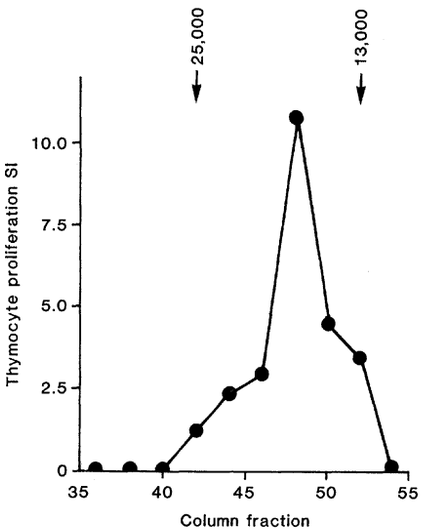

Fig. 1. The IL-1 activity in the supernatants of cultured mononuclear phagocytes derived from human placental tissue. Fractions of the supernatants were separated by gel filtration. The assays are reported at 1:16 dilutions on column fractions, this being the optimal dilution of seven dilutions tested.

Cultures of human placental cells contained 77 percent adherent cells after 2 hours. At 48 hours, a mean of 85 percent of the adherent cells phagocytized latex beads and a mean of 88 percent of the adherent cells was positive for the nonspecific esterase stain. Only IL-1 activity was measurable in the culture supernatants.

Table 1. Thymocyte proliferation and IL-2 assays of supernatant fluids from mouse and human placental mononuclear phagocytes. All assays are reported at 1:4 dilutions of placental cell supernatants; this was the optimal dilution of four dilutions tested. All means are from triplicate analyses. The stimulation index is calculated by dividing the radioactivity (counts per minute) in the experimental cultures by the radioactivity in the control cultures.

Assay and group	[^{3}H]Thymidine incorporation (count/min)*	Stimulation index
Mouse adherent placental cells		
Thymocyte proliferation		
Control	305 ± 33	
Supernatants (N = 18)	2895 ± 884	9.5
IL-2		
Control	1733 ± 195	
Supernatants	2046 ± 804	1.2
Human adherent placental cells		
Thymocyte proliferation		
Control	168 ± 42	
Supernatants (N = 8)	1949 ± 138	11.6
IL-2		
Control	1540 ± 282	
Supernatants (N = 8)	1935 ± 408	1.3
Thymocyte proliferation		
Control	168 ± 42	
Cells treated with latex beads (N = 3)	2638 ± 210	15.7
Cells not treated with latex beads (N = 3)	1462 ± 183	8.7

*Mean ± standard deviation.

Latex bead phagocytosis by the mononuclear phagocytes increased the production and release of IL-1. Chromatographic separation of the proteins in the culture supernatants indicated that IL-1 activity was present in the column fractions around 17,000 daltons (Fig. 1), which is consistent with the molecular weight of human IL-1 (*2*).

A number of physiological events in pregnancy may be related to IL-1 activity (*4*). Increases in body temperature in pregnancy may relate to the EP actions of IL-1 (*7*). Changes in albumin, insulin, glucagon, and a number of other serum hormone and protein changes in pregnancy may also be related to IL-1 (*7*). Understanding the mechanism for the production and release of IL-1 from the placenta to the maternal circulation may be of interest in studying complicated pregnancies where IL-1–related responses may be enhanced (*15*). Placental production of IL-1 may also influence the development of immune responses in the fetus.

Our results show that the tissue culture supernatants from mononuclear phagocytes of both mouse and human placentas contain factor IL-1. Compared to the cultures of mouse mononuclear phagocytes, cultures of phagocytes from human placentas appeared to contain a greater number of adherent, phagocytizing cells that produce significant amounts of IL-1. Gel filtration of the crude supernatants from human placental mononuclear phagocytes confirmed that the IL-1 activity was related to a 12,000- to 18,000-dalton protein. These data support the concept that placental mononuclear phagocytes produce IL-1 without stimulation, can be stimulated to produce and release greater quantities of IL-1, and can thus be used as a source of IL-1.

ARTHUR FLYNN
JAMES H. FINKE
MARY L. HILFIKER
Department of Molecular and Cellular Biology, Cleveland Clinic Foundation, Cleveland, Ohio 44106

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Circulating Somatostatin Acts on the Islets of Langerhans by Way of a Somatostatin-Poor Compartment

Abstract. *Somatostatin perfused in canine pancreases at 10 to 20 picograms per milliliter or 10 to 20 percent of the pancreatic vein somatostatin concentration inhibited insulin and glucagon secretion. This suggests that the high local concentration of endogenous somatostatin is not in contact with somatostatin receptors of the islets. The integrity of this separation may determine the sensitivity of islet cells to circulating somatostatin.*

It is generally believed that the interstitial space of the islets of Langerhans consists of a single compartment throughout which the islet hormones diffuse freely. If this were true, islet cell receptors for insulin, glucagon, and somatostatin would be continually exposed to maximum concentrations of those hormones, in which case only a relatively large change in their arterial concentration could have a biological effect on islet cells. Yet there is evidence that relatively small changes in the concentrations of exogenous insulin (*1*), glucagon (*2*), and somatostatin (*3*) can affect the secretion of islet cells. If a small change in arterial concentration of an islet hormone that is well below the concentration in the pancreatic vein could affect the secretion of other islet hormones, it would suggest that the hormone secreted into the venous circulation did not have access to the interstitial space containing the "arterial-side" receptors of the affected islet cells. We, therefore, systematically determined the relation between the venous efflux concentration and the minimal biologically effective concentration of two islet hormones: somatostatin, which is secreted by the D cells of the islets (*4*) and inhibits insulin (*5*) and glucagon secretion (*6*), and glucagon, which is secreted by the A cells of the islets and stimulates both insulin (*7*) and somatostatin (*8*) secretion. We used the isolated perfused canine pancreas preparation of Iversen and Miles (*9*).

In this system the baseline concentration of endogenous somatostatin in the venous effluent averaged ~ 100 pg/ml. Fujita (*10*) estimates that in the dog about 70 percent of the pancreatic venous effluent consists of blood that has not passed through islets; if this is so, the concentration of somatostatin in the islet interstitium near the D cells must be approximately 300 pg/ml or greater. We tested the effects of somatostatin in concentrations from 10 to 4000 pg/ml, that is, from 10 to 400 percent of the baseline somatostatin level in the venous effluent, upon insulin and glucagon secretion. Somatostatin significantly suppressed insulin secretion at a concentration of only 10 pg/ml, or 10 percent of the venous level (Fig. 1). Glucagon was significantly suppressed by a somatostatin concentration of 20 pg/ml, or 20 percent of the venous level (Fig. 1). Thus, exogenous somatostatin was biologically active on B cells at an arterial concentration that was only 10 percent of the level of endogenous somatostatin in the venous effluent—perhaps 3 percent or even less of the concentration of endogenous somatostatin in the interstitium surrounding the D cells. It seems unlikely that A and B cells could have responded to so small a rise in the arterial concentration of somatostatin had their somatostatin receptors been in contact with even the 100 pg/ml concentrations of endogenous somatostatin present in the venous effluent. Rather, it seems more plausible to suggest that the endogenous somatostatin that enters the venous circulation is secreted into a space that is separate from the space that contains the somatostatin

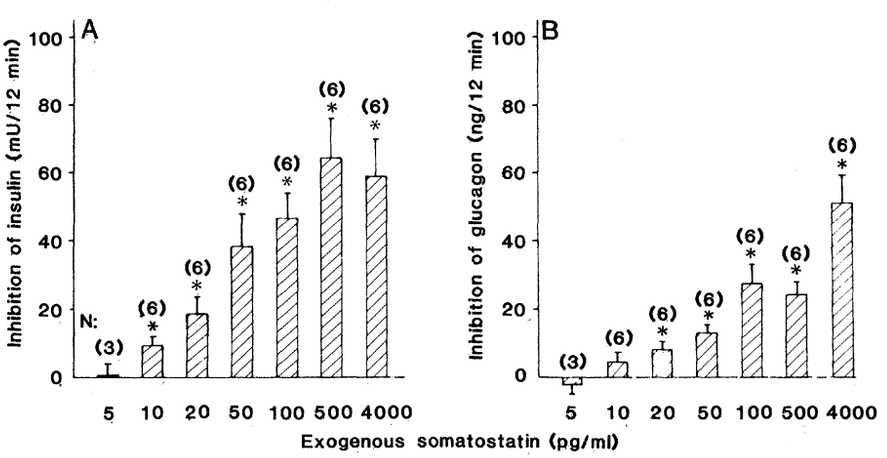

Fig. 1. Degree of inhibition of (A) insulin and (B) glucagon secretion by perfusion of various doses of somatostatin into isolated dog pancreas. The perfusate consisted of a Krebs-Ringer bicarbonate solution containing 4 percent dextran T-70 (Pharmacia Fine Chemicals), 0.2 percent bovine serum albumin, 5.5 m*M* glucose, and 10 m*M* arginine. The pancreatic effluent was collected at 1-minute intervals and frozen for subsequent radioimmunoassay for insulin (*15*), glucagon (*16*), and somatostatin (*17*) after a 40-minute equilibration period. Samples for determining basal concentrations of hormone secretion were collected for 15 minutes; somatostatin was perfused for the next 15 minutes. Inasmuch as the inhibitory effects of somatostatin on the hormone secretion required 3 minutes to reach a plateau, the degree of inhibition was calculated as a decrease in total insulin and glucagon secretion from the preceding basal secretion for the final 12 minutes of each 15-minute infusion period. The baseline concentration of endogenous somatostatin was ~ 100 pg/ml. Differences relative to the initial value (at 5 pg/ml) were regarded as statistically significant if Student's t (d.f. = 8) exceeded the $P = .05$ level (indicated by *).

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