

Interleukin 1 Promotes Tumor Cell Adhesion to Cultured Human Endothelial Cells

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Abstract

We report that IL 1 acts on the endothelium, inducing a long-lasting increase in its adhesivity to tumor cells. Selective pretreatment of cultured human umbilical vein endothelial cells (EC) with IL 1 caused a significant increase in adhesion of three human colorectal carcinoma (HT-29, HCC-P2988, and HCC-M1410) cell lines and one human melanoma (A-375) cell line. Tumor necrosis factor (TNF) was as effective as IL 1 in promoting tumor cell adhesion to EC, whereas IFN γ and IL 2 were inactive. The IL 1 and TNF induction of EC adhesivity was both concentration (threshold concentration 1 U/ml) and time dependent (peak 4–6 h), reversible within 24 h, and blocked by a protein synthesis inhibitor. The IL 1 and TNF action on EC may play a role in tumor cell lodgment.

Introduction

An important step in the metastatic process is the arrest of tumor cells in the venous or capillary bed of the target organ. It has been generally proposed that metastatic tumor cells adhere preferentially to the extracellular matrix underlying vascular endothelial cells (EC)¹ and specific receptors for subendothelial components have been defined (1–3). However, during the metastatic spread, the arrest and/or attachment of tumor cells to the vascular endothelium precedes their extravasation. It was hypothesized that specific recognition mechanisms might exist on the EC membrane and it was shown that EC from different organs are not alike but do express differences in terms of tumor cell recognition and adhesion (4, 5). Recent *in vitro* studies suggest that EC can be activated to express surface factors that mediate their adhesive interaction with circulating cells. Bevilacqua et al. (6) demonstrated that the inflammatory immunomediator IL 1 induces the synthesis of an EC surface activity that promotes polymorphonuclear cells and monocyte adhesion to them.

In this study, we report that IL 1 treatment of EC also increases the adhesion of different tumor cell lines. The IL 1 action on EC may play a role in the localization of tumor cells at the vascular level.

Methods

Cell cultures. EC were isolated from human umbilical vein and cultured as described (7).

The HT-29 human colon carcinoma (8), the A-375 human melanoma (9), and the murine UV-2237 fibrosarcoma (10) cell lines were cultured as described in the respective references. The HCC-P2988 and the HCC-M1410 lines, recently established from human rectal carcinomas (11), were maintained, in Ham's F12 medium supplemented with 10 ng/ml epidermal growth factor and 10% FCS. HT-29 (8), A-375 (9) and HCC-M1410 (11) produced lung tumor colonies in nude mice after intravenous injection. HT-29 was also metastatic to the lymphnodes after intravenous injection and HCC-M1410 was metastatic to the liver after intrasplenic injection. HCC-P2988 did not produce metastases after intravenous and intrasplenic injection (R. Giavazzi, unpublished results). UV-2237, when injected intravenously in syngeneic C3H-mice, produced lung tumor colonies (10). The non-tumorigenic human diploid embryo fibroblasts (MRC-5) were cultured in Eagle's basal medium supplemented with 15% FCS. All culture reagents were purchased from GIBCO-Europe, Paisley, Scotland. The tissue culture dishes were obtained from Cel-Cult, Flow Laboratories, Milan, Italy.

Lymphokine preparations and EC treatment. Human natural purified IL 1 was obtained from *Staphylococcus albus*-stimulated monocytes (Ultrapure IL 1; Genzyme Corp., Boston, MA). This preparation was used throughout the study unless otherwise specified. Human recombinant IL 1 α (specific activity 10³ U/ μ g) was kindly supplied by Dr. P. Lomedico (Hoffmann-La Roche Inc., Nutley, NJ). Human recombinant IL 1 β (specific activity 10⁴ U/ μ g) was obtained from Sclavo, Siena, Italy. Human recombinant tumor necrosis factor (TNF) (specific activity 10¹⁰ U/ μ g) was kindly supplied by Dr. Lin (Cetus Corp., Emeryville, CA) to Dr. P. Ghezzi (Istituto Mario Negri). IFN γ (specific activity 10¹⁰ U/ μ g) was kindly supplied by Dr. G. R. Adolf (Ernst-Boehringer, Vienna, Austria). IL 2 (specific activity 2 \times 10⁹ U/ μ g) was obtained from Biogen Glaxo, Geneva, Switzerland. A polyclonal antibody to IL 1 was a gift from Dr. C. A. Dinarello (Tufts University, Boston, MA) and a polyclonal antibody to TNF was a kind gift from Dr. Lin (Cetus Corp.). All IL 1 preparations were lacking any detectable endotoxin as determined by Limulus assay on samples containing 100 U/ml of IL 1.

For the adhesion assay, the EC were grown to confluence (1–1.5 \times 10⁵ in a 2-cm² culture well). EC wells were washed three times with PBS and incubated at 37°C with medium alone or medium containing IL 1 or other cytokines for the indicated times. The medium containing the cytokines was removed just before the adhesion assay and EC were washed twice with PBS. In certain experiments, EC were treated with emetine (Sigma Chemical Co., St. Louis, MO) (5 μ g/ml) for 1 h at 37°C, washed with PBS, and incubated with culture medium with or

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1. Abbreviations used in this paper: EC, endothelial cells; fn, fibronectin; lm, laminin; TNF, tumor necrosis factor.

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without IL 1. In preliminary experiments, this concentration of emetine blocked [35 S]methionine incorporation in EC by 95%.

Matrix proteins. Purified laminin (lm), and lm polyclonal antibody were obtained through the courtesy of Dr. L. Liotta, (Laboratory of Pathology, National Institutes of Health, Bethesda, MD). Human purified plasma fibronectin (fn) and polyclonal fn antibodies were prepared as described (7). The wells were coated with either fn or lm (10 μ g/ml) as described (7).

Adhesion assay. All tumor cell lines were labeled as described (12). Briefly, 10^7 living cells, suspended in MEM with 10% FCS, were incubated with 10 μ Ci of 111 Indium-oxine (Amersham International, Amersham, UK) for 15 min at room temperature. After this period, the cells were washed once with MEM containing 10% FCS and then resuspended in HBSS with 0.25% BSA at a concentration of 10^6 cells/ml (0.7–0.6 cpm/cell). EC, previously treated with cytokines or medium alone, were incubated for 30 min, unless otherwise specified, at 37°C with 300 μ l of the tumor cells suspension. At the end of incubation, the supernatant was carefully aspirated, the wells were washed twice with 1 ml of HBSS + 0.25% BSA to remove nonadherent tumor cells and incubated for 10 min at room temperature with 250 μ l of 1 M NaOH – 1% SDS, and the lysate was counted.

Electron microscopy. For scanning electron microscopy, EC were seeded on 13-mm-diam glass coverslips. Tumor cells were added as described above and allowed to adhere to EC for 30 min at 37°C; after this period, the cells were washed twice with 1 ml of PBS and fixed with 2% glutaraldehyde for 30 min at 22°C. The fixed cells were then washed twice with 1 ml PBS and maintained in PBS at 4°C for no longer than 24–48 h. For examination under an electron microscope, EC were dehydrated at the critical point in a Balzers Union CPD 010 (Balzers S. p. A., Milan, Italy). The specimens were coated with gold and scanned in a Philips SEM 505 electron microscope.

Results

Tumor cell adhesion to IL 1-treated EC. We first examined the kinetics of a tumor cell line (HT-29) adhesion to IL 1-treated EC (Fig. 1). HT-29 adhered more actively to IL 1-treated EC than to control cells. Adhesion was time dependent and reached a plateau within 30 min.

By scanning electron microscopy (Fig. 2), after 30 min of adhesion, the tumor cells were individually attached to the

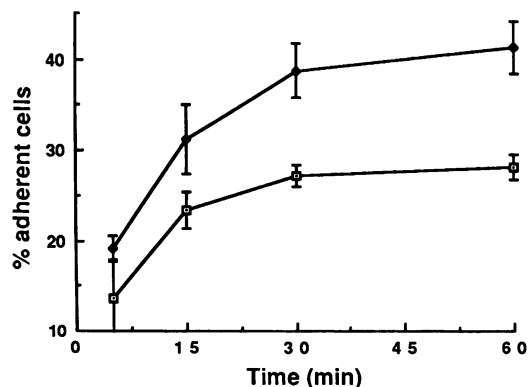


Figure 1. Time course of HT-29 cell attachment to EC preincubated with medium or IL 1. EC were treated with 10 U/ml IL 1 for 4 h at 37°C, HT-29 tumor cells were added, and cell adhesion to EC was measured at different times. The number of adherent tumor cells was expressed as percent of the total number of cells added. The values are means \pm SEM of four replicates. Comparable values were obtained in three additional experiments. \square , medium; \bullet , IL 1.

surface of both untreated and IL 1-treated EC. Tumor cell adhesion to IL 1-treated EC was visibly increased (Fig. 2, A and B). In three experiments performed on three different EC cultures, no EC damage, as evidenced by scanning electron microscopy, was apparent after IL 1 treatment and before or 30 min after tumor cell addition (data not shown).

Table I reports the adhesion of different cell lines to untreated and IL 1-treated EC. A significant increase in adhesion, varying between 91 and 180%, depending on the tumor line considered, was observed. The time course of adhesion of these cell lines was comparable. In all the experiments performed over > 1 yr, IL 1 was always effective in increasing tumor cell adhesion to EC, although there was some variability among experiments. For instance, the percent increase in HT-29 adhesion induced by IL 1 treatment of EC (10 U/ml for 4 h at 37°C) ranged from 35 to 98% in 20 separate experiments performed on separate EC cultures.

In contrast, MRC-5 and UV-2237 cell lines did not adhere significantly more effectively to IL 1-treated EC, compared with the untreated one (Table I).

The effect of IL 1 on EC adhesivity was apparently selective for EC. HT-29 adhesion to MRC-5 fibroblasts was unchanged by IL 1 up to 10 U/ml for 4 h treatment (not shown).

Characterization of the IL 1 and TNF effect on tumor cell adhesion to EC. TNF and IL 1, though biochemically and immunologically distinct, show overlapping biological activities (13). We thus evaluated the effect of the two cytokines on EC adhesivity.

For synthetic purposes, we report the data obtained with HT-29 as representative cells. Both cytokines showed a concentration- and time-dependent increase in EC adhesivity to HT-29 (Fig. 3). With both cytokines, EC adhesivity required 4–6 h to reach maximal values, then declined and returned to normal after 24 h. When 10 U/ml IL 1 was added to tumor cells at the beginning of the adhesion assay and maintained for its duration, it did not change tumor cell adhesion to untreated EC (not shown). We also verified whether IL 1 treatment induced EC to release active agents in the medium during the adhesion assay. After 4 h treatment with either 10 U/ml IL 1 at 37°C or medium alone, the EC were washed three times with buffer and incubated with HBSS for 30 min at 37°C (the maximal time of incubation of the adhesion assay). The conditioned medium was then collected and centrifugated, and HT-29 cells were resuspended in it. In this condition, the HT-29 cells' adhesion (30 min at 37°C) to untreated EC was unchanged.

The data reported in Fig. 3 were obtained with recombinant TNF and natural purified IL 1. When recombinant human α and β IL 1 preparations were used, they were equally active on a unit basis than the natural IL 1 in promoting HT-29 adhesion to EC. In contrast, human recombinant IL 2 (up to 100 U/ml for 4 h at 37°C) and human recombinant IFN γ (up to 1,000 U/ml for 4 h at 37°C) were ineffective. Polyclonal antibodies to human IL 1 and TNF (at a dilution of 1:100) added during EC incubation with the cytokines (10 U/ml for 4 h at 37°C) abolished the effect of the respective cytokine. Treatment of EC with the irreversible protein synthesis inhibitor emetine (see Methods) blocked the effect of IL 1 and TNF on EC adhesivity to tumor cells by 90 \pm 5%.

It has been suggested that during invasion, tumor cells adhere to subendothelial matrix proteins and preferentially to fn and lm (1, 3). To verify whether these matrix components

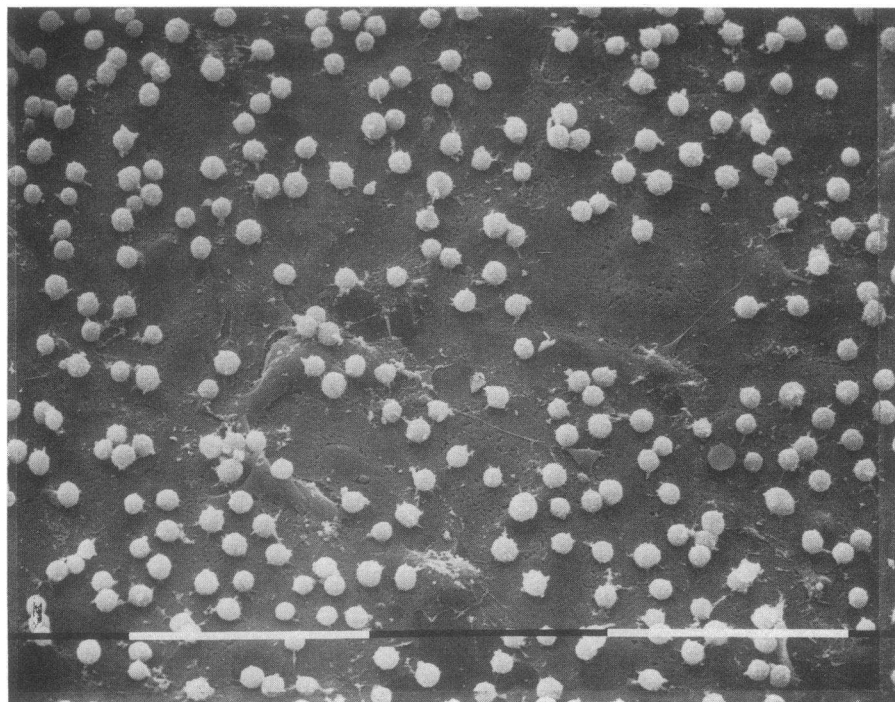
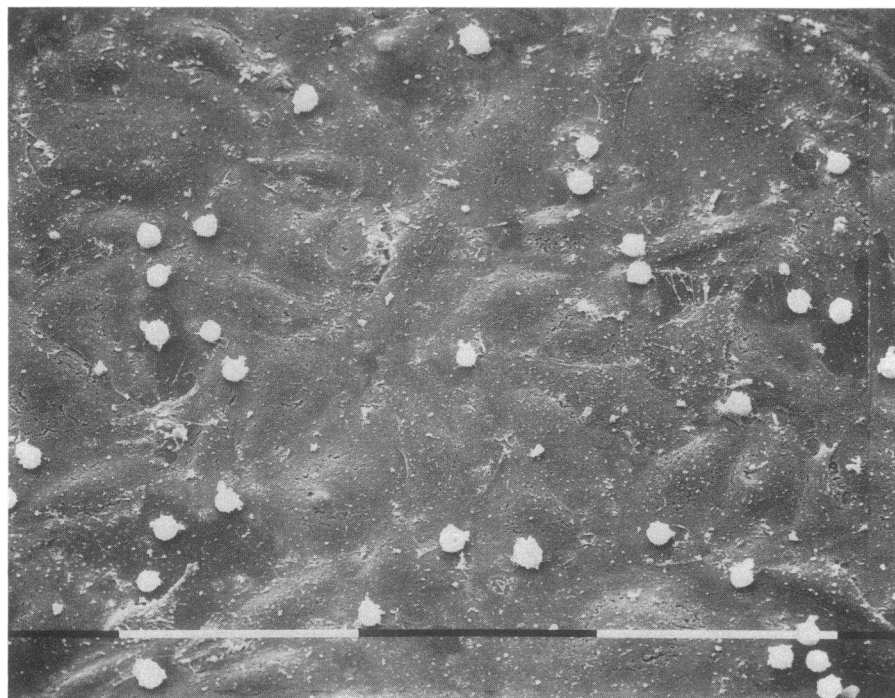


Figure 2. Scanning electron microscopy of HT-29 cells added to (*top*) untreated or (*bottom*) IL 1-treated EC. EC were treated with 10 U/ml IL 1 for 4 h at 37°C and HT-29 cells were added for 30 min at 37°C. $\times 312$.

could play any role in tumor cell adhesion to IL 1-treated EC, we studied the effect of specific fn and lm antibodies. Antibodies to either lm or fn were added to EC monolayers at dilutions of 1:200 and 1:20, respectively, for 1 h at 37°C and maintained for the 30-min duration of the tumor cells adhesion assay. Both antibodies did not change HT-29 adhesion to untreated and IL 1-treated EC, whereas they did inhibit tumor cell adhesion to purified lm and fn, by 45.8 and 64.2%, respectively.

Discussion

The factors associated with the lodgment of tumor cells in the microcirculation have been studied extensively. The direct tumor cell attachment to the endothelium has been described (14) and proposed as an active phenomenon mediated by specific recognition mechanisms (5). However, the relative importance of EC components versus tumor cell surface proper-

Table 1. Tumor Cell Adhesion to IL 1-treated EC

Cell line	Cells attached/ 10^3 EC		% increment
	-(IL 1)	+(IL 1)	
HT-29	253±22	485±45*	91
HCC-P2988	1,184±110	3,322±320*	180
HCC-M1410	401±40	883±81*	120
A-375	182±19	324±32*	78
MRC-5	488±45	567±50	16
UV-2237	419±42	367±32	-12

EC were treated with 10 U/ml IL 1 for 4 h at 37°C, before the adhesion assay. Cell attachment was determined after 30 min incubation. In each experiment, the number of EC was evaluated in separate wells run in parallel but without addition of tumor cells. The number of adherent tumor cells was evaluated by dividing the total counts per minute associated to the wells with the counts per minute of a single tumor cell. Percent increment was calculated as (tumor cells bound to stimulated EC - tumor cells bound to untreated EC)/(tumor cells bound to untreated EC × 100). The values are means±SEM of four replicates obtained in a typical experiment out of three performed.

* $P < 0.01$ vs. the values obtained in untreated EC, by t test.

ties are still unknown. In this paper, we show that two cytokines, IL 1 and TNF, can modify the EC properties and increase their reactivity to tumor cell adhesion. This effect is lasting, reversible, and protein synthesis dependent. It is not apparently related to chemotactic substances released by EC: when tumor cells were resuspended in IL 1-treated, EC-conditioned medium, there was no increase in their adhesion to untreated EC. A direct effect of IL 1 on tumor cells is also unlikely, since when IL 1 was added together with tumor cells at the beginning of the adhesion experiment, it did not have any effect on their adhesion.

It is also unlikely that the effect of IL 1 is mediated by nonspecific changes in the integrity of the EC monolayer (cell retraction and damage and exposure of subendothelial matrix). The scanning electron microscopy examination did not

show any significant sign of EC damage or matrix exposure after IL 1 treatment. In addition, the stimulation of adhesion to EC by IL 1 was not apparent using the MRC-5 or the UV-2237, both of which markedly adhere to subendothelial matrix proteins (15). Finally, antibodies to lm or fn did not inhibit tumor cell adhesion to untreated or IL 1-treated EC, whereas they did inhibit tumor cell adhesion to the corresponding purified proteins.

Other authors observed that after a few hours of adhesion to untreated cultured EC, tumor cells can induce their retraction and disruption of the intercellular junctions (1, 14). This effect was not apparent in this study, since shorter incubation times (5–30 min) were selected to study the initial attachment of tumor cells to the endothelium.

Among the cytokines tested, only TNF showed an effect comparable to IL 1, whereas IFN γ or IL 2 were inactive. This is in agreement with previous reports that show that IL 1 and TNF share many activities on EC, including changes in coagulant (16), fibrinolytic properties (17), and prostacyclin synthesis (18). IL 1 and TNF can also induce the synthesis and expression of EC membrane antigens that play a role in polymorphonuclear cell and monocyte adhesion (19). It would be tempting to speculate that the increased adhesion to EC induced by these cytokines is mediated by the same structures.

An interesting issue is whether IL 1 influences adhesion only of metastatic cells. All the lines but one used in this work are able to produce artificial metastases (8–11). Adhesion of the nonmetastatic line (HCC-P2988) was, however, equally influenced by IL 1 as were the other human lines. The comparison of the metastatic potential of the tumor cell lines used here is, however, very difficult for their difference in terms of origin, histology, and growth pattern. Further and more extensive studies comparing a large series of tumor cell lines in vitro and in vivo systems are needed to clarify this point.

In conclusion, in this study we present evidence that IL 1 and TNF can render EC reactive to tumor cell deposition. The biological relevance of this observation in vivo remains to be explained. IL 1 and TNF can be released in tissues and even reach appreciable levels in the circulation during inflammatory reactions or cell injury (13, 20, 21). Various cell types,

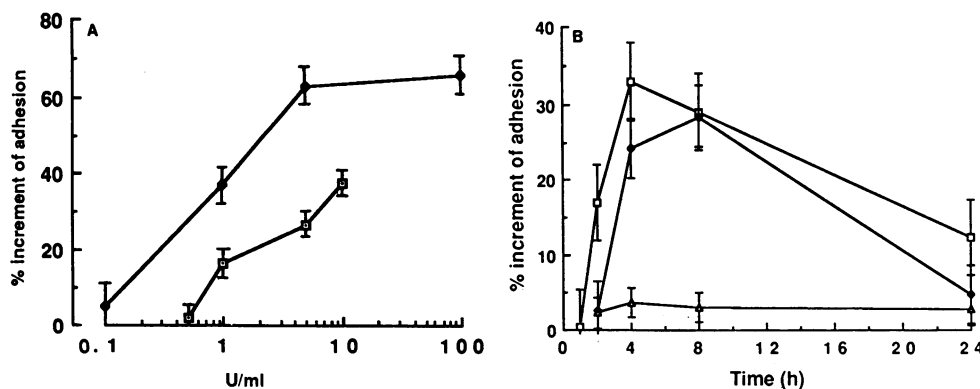


Figure 3. (A) Concentration and (B) time course dependence IL 1 and TNF stimulation of EC-tumor cell adhesion. (A) EC were incubated with increasing concentrations of IL 1 and TNF for 4 h at 37°C. Radiolabeled HT-29 cells were then added for 30 min at 37°C. □, IL 1; ●, TNF. (B) EC were incubated with 10 U/ml of IL 1 and TNF. After different times of incubation, EC were washed and radiolabeled HT-29 tumor cells were added for 30 min at 37°C. □, IL 1; ●, TNF; △, medium. Percent increment of tumor cell adhesion was calculated as described in the legend to Table I. The values are mean±SEM of at least three separate experiments.

including macrophages, EC, and tumor cells themselves, have the potential to produce and secrete these cytokines (20, 21). Macrophages, activated in damaged tissues, could represent powerful producers of these cytokines, and damage to tissues (e.g. by irradiation) has been shown to augment secondary localization of murine neoplasm (22).

Cancer cells of both hematopoietic and nonhematopoietic origin can produce IL 1 and TNF (20, 21). It has recently been reported that patients bearing different types of carcinoma and myeloma presented measurable levels of TNF in serum (23). A tempting speculation is that TNF released into the circulation from the primary tumor could facilitate tumor cell localization at the endothelial surface and eventually the metastatic process.

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References

1. Kramer, R. H., R. Gonzalez, and G. L. Nicolson. 1980. Metastatic tumor cells adhere preferentially to the extracellular matrix underlying vascular endothelial cells. *Int. J. Cancer*. 26:639-645.
2. Ruoslahti, E. Fibronectin and its receptors. *Annu. Rev. Biochem.* In press.
3. Liotta, L. A. 1984. Tumor invasion and metastases: role of the Basement Membrane. Warner-Lambert Parke-Davis Award Lecture. *Am. J. Pathol.* 117:339-348.
4. Auerbach, R., W. C. Lu, E. Pardon, F. Gumkowski, G. Kaminska, and M. Kaminski. 1987. Specificity of adhesion between murine tumor cells and capillary endothelium: an in vitro correlate of preferential metastasis in vitro. *Cancer Res.* 47:1492-1497.
5. Nicolson, G. L., and J. L. Winkelmake. 1975. Organ specificity of blood-born tumour metastasis determined by cell adhesion? *Nature (Lond.)*. 255:230-231.
6. Bevilacqua, M. P., J. S. Pober, M. E. Wheeler, R. S. Cotran, and M. A. Gimbrone, Jr. 1985. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J. Clin. Invest.* 76:2003-2011.
7. Dejana, E., S. Colella, L. R. Languino, G. Balconi, G. C. Corbascio, and P. C. Marchisio. 1987. Fibrinogen induces adhesion, spreading, and microfilament organization of human endothelial cells in vitro. *J. Cell Biol.* 104:1403-1411.
8. Price, J. E., L. M. Daniels, D. E. Campbell, and R. Giavazzi. 1988. Organ distribution of experimental metastases of a human colorectal carcinoma injected in nude mice. *Clin. & Exp. Metastasis*. In press.
9. Kozlowski, J. M., I. R. Hart, I. J. Fidler, and N. Hanna. 1984. A human melanoma line heterogeneous with respect to metastatic capacity in athymic nude mice. *J. Natl. Cancer Inst.* 72:913-917.
10. Kripke, M. L., E. Gruys, and I. J. Fidler. 1978. Metastatic heterogeneity of cells from an ultraviolet light-induced murine fibrosarcoma of recent origin. *Cancer Res.* 38:2962-2967.
11. Giavazzi, R., D. E. Campbell, J. M. Jessup, K. Cleary, and I. J. Fidler. 1986. Metastatic behavior of tumor cells isolated from primary and metastatic human colorectal carcinomas implanted into different sites in nude mice. *Cancer Res.* 46:1928-1933.
12. Frost, P., J. Smith, and H. Frost. 1978. The radiolabeling of lymphocytes and tumor cells with ¹¹¹Indium. *Proc. Soc. Exp. Biol. Med.* 157:61-65.
13. Dinarello, C. A., and J. W. Mier. 1987. Current concepts in lymphokines. *N. Engl. J. Med.* 317:940-945.
14. Kramer, R. H., and G. L. Nicolson. 1979. Interactions of tumor cells with vascular endothelial cell monolayers: a model for metastatic invasion. *Proc. Natl. Acad. Sci. USA*. 76:5704-5708.
15. Giavazzi, R., L. Liotta, and I. Hart. 1982. Laminin inhibits the adhesion of a murine tumor of macrophage origin. *Exp. Cell Res.* 140:315-322.
16. Bevilacqua, M. P., J. S. Pober, G. R. Majeau, W. Fiers, R. S. Cotran, and M. A. Gimbrone, Jr. 1986. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. *Proc. Natl. Acad. Sci. USA*. 83:4533-4537.
17. Schleef, R. R., M. P. Bevilacqua, M. Sawdey, M. A. Gimbrone, Jr., and D. J. Loskutoff. 1987. Interleukin 1 (IL-1) and tumor necrosis factor (TNF) activation of vascular endothelium: effects on plasminogen activator inhibitor (PAI-1) and tissue-type plasminogen activator (tPA). *Thromb. Haemostasis*. 58:17. (Abstr.)
18. Kawakami, M., S. Ishibashi, H. Ogawa, T. Murase, F. Takaku, and S. Shibata. 1986. Cachectin/TNF as well as interleukin-1 induces prostacyclin synthesis in cultured vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 141:482-487.
19. Pober, J. S., M. P. Bevilacqua, D. L. Mendrick, L. A. Lapierre, W. Fiers, and M. A. Gimbrone, Jr. 1986. Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. *J. Immunol.* 136:1680-1687.
20. Dinarello, C. A. 1984. Interleukin-1 and the pathogenesis of the acute-phase response. *N. Engl. J. Med.* 311:1413-1418.
21. Beutler, B., and A. Cerami. 1986. Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature (Lond.)*. 320:584-588.
22. Rappaport, D. S., and J. M. Brown. 1979. Influence of localized preinoculation irradiation of the foot of lymphatic metastasis from a primary tumor growing in the foot. *Radiat. Res.* 78:108-112.
23. Balkwill, F., R. Osborne, F. Burke, S. Naylor, D. Talbot, H. Durbin, J. Tavernier, and W. Fiers. 1987. Evidence for tumour necrosis factor/cachectin production in cancer. *Lancet*. ii:1229-1232.