rial generations. In the experimental samples, numbers of South-seeking cells increased with time until the ratio of South-seeking bacteria to North-seeking bacteria approached 1.0. No such changes occurred in the control samples. Thus, in natural habitats and in laboratory experiments neither cell polarity is selected in the absence of a vertical magnetic field. These findings complement previous observations that a predominant cell polarity is selected, depending on the sign of the vertical component of the ambient magnetic field (*4*). Because oxygen is toxic for these microaerophilic or anaerobic organisms (*1*), cells whose polarity causes them to be directed toward and kept in the sediments and away from the water surface will be favored.

Because of the horizontal orientation of the magnetic field at the geomagnetic equator, the motion of magnetotactic bacteria there will be directed horizontally. This could be advantageous to bacteria of either polarity in reducing detrimental upward migration, compared to random motion. Extended straight-line motion could also be advantageous as an escape response, for population dispersal, and as a means of outrunning chemical diffusion and finding more suitable environments (*5, 13, 14*). Their magnetism would also cause cells to localize in regions of high magnetic flux density surrounding materials with high magnetic susceptibility in the environment (*7, 15*); and bacteria that are within about 4 μm of each other will experience magnetic interactions stronger than thermal energy. These interactions may be advantageous but would not favor either polarity at any latitude.

Since the total intensity of the geomagnetic field in Brazil is less than one-half the intensity of the field in New England, Brazilian bacteria would need larger magnetic moments on the average to maintain the same ratio of magnetic-to-thermal energy and hence the same degree of alignment in water at ambient temperatures for the magnetotactic response (*7*). This hypothesis could be confirmed by detailed electron microscope studies of their magnetosomes or by observation of their swimming response in the magnetic field direction as a function of magnetic field strength (*16*).

Finally, the presence of magnetotactic bacteria at the geomagnetic equator implies their ability to survive periodic reversals or excursions of the geomagnetic field (*17*) even at latitudes with large inclination. Because of the nondipolar contribution to the geomagnetic field, the field intensity at any point on the earth's surface does not completely vanish during reversal, but does fluctuate in magnitude and rotate through zero inclination. During the reversal period of thousands of years, the distribution of magnetic dipole strengths of a bacterial population in a given locale could shift in response to decreasing and increasing field conditions, and the relative numbers of South- and North-seeking cells in the population could change in response to changes in the magnetic inclination.

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References and Notes

1. R. P. Blakemore, *Science* **190**, 377 (1975).
2. A. J. Kalmijn and R. P. Blakemore, *Animal Migration, Navigation and Homing*, K. Schmidt-Koenig and W. T. Keeton, Eds. (Springer-Verlag, New York, 1978), p. 344.
3. T. T. Moench and W. A. Konetzka, *Arch. Microbiol.* **119**, 203 (1978).
4. R. P. Blakemore, R. B. Frankel, A. J. Kalmijn, *Nature (London)* **286**, 384 (1980).
5. J. L. Kirschvink, *J. Exp. Biol.* **86**, 345 (1980).
6. R. B. Frankel, R. P. Blakemore, R. S. Wolfe, *Science* **203**, 1355 (1979).
7. R. B. Frankel and R. P. Blakemore, *J. Magn. Magn. Mater.* **15–18**, 1562 (1980).
8. World Data Center, National Oceanic and Atmospheric Administration, Boulder, Colo.
9. F. S. da Cruz and W. de Sousa, private communication.
10. D. L. Balkwill, D. Maratea, R. P. Blakemore, *J. Bacteriol.* **141**, 1399 (1980).
11. C. R. Denham, R. P. Blakemore, R. B. Frankel, *IEEE Trans. Magn.* **Magn-16**, 1006 (1980).
12. K. M. Towe and T. T. Moench, *Earth Planet. Sci. Lett.* **52**, 213 (1981).
13. R. P. Blakemore *et al.*, unpublished.
14. E. M. Purcell, *Am J. Phys.* **45**, 3 (1977).
15. R. P. Blakemore, D. Maratea, R. S. Wolfe, *J. Bacteriol.* **140**, 720 (1979).
16. B. D. Teague, M. K. Gilson, A. J. Kalmijn, *Biol. Bull. (Woods Hole, Mass.)* **157**, 399 (1979); A. J. Kalmijn, in preparation.
17. A. Cox, *Rev. Geophys. Sp. Sci.* **13**, 35 (1975).
18. We thank A. J. Kalmijn and V. Wescott for discussions; H. Maia, H. Lins de Barros, W. O'Brien, R. Caplan, and H. Stram for experimental assistance; and F. da Cruz and W. de Sousa for electron microscopy. Supported by the U.S. Office of Naval Research, the Organization of American States, and the Conselho Nacional de Desenvolvimento Cientifico e Technologico of Brazil, and NSF grant PCM-7922224 (to R.P.B.). The Francis Bitter National Magnet Laboratory is supported by NSF.

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Prostacyclin: A Potent Antimetastatic Agent

Abstract. *Metastasis is the principal cause of failures to cure human cancers. Prostacyclin is a powerful antimetastatic agent against B16 amelanotic melanoma cells. This effect, which may result from the platelet antiaggregatory action of prostacyclin, is potentiated by a phosphodiesterase inhibitor. Inhibitors of prostacyclin synthesis increase metastasis. Prostacyclin and agents that may increase endogenous prostacyclin production or prolong its activity are suggested as new antimetastatic agents.*

The ability of primary neoplasms to metastasize is a major obstacle in the search for a cure for human cancers. The development of a metastasis represents the terminal stage of an intricate series of events in which malignant cells, released from a primary tumor, disseminate to distant sites principally by way of the circulatory system (*1*). Most tumor cells dispersed by this route die (*1*). However, primary neoplasms have a mosaic of cellular potentials (*1*), and subpopulations of cells may have inherent biological properties that guarantee their survival. One of these properties may be the ability of the tumor cells to interact with and attach to host platelets (*2*), thus enhancing their potential to lodge in the microvasculature (arrest) and adhere to vascular endothelium. Alternatively, after arrest, tumor cells may initiate the formation of surrounding, protective platelet thrombi until extravasation is completed (*3*). Anticoagulant therapy with aspirin (*2, 4*), dipyridamole (*5*), heparin (*6*), and warfarin (*6, 7*) has been attempted in the hope of reducing metastasis. However, results to date are inconclusive. The studies we describe here were designed to determine if prostacyclin (PGI_2), the most potent antithrombogenic agent known (*8*), would reduce tumor cell metastasis.

Subcutaneous B16 amelanotic melanoma ($B16_a$) tumors were maintained in syngeneic C57BL/6J mice (Jackson Laboratory). Cell suspensions from primary tumors were obtained by a modification (*9*) of our described procedure (*10*).

Injection of $B16_a$ cells into their syngeneic host resulted in the consistent appearance of metastatic foci in the lung, liver, and spleen (Table 1). Metastatic

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foci were not observed in the kidney or brain. Intravenous injection of PGI_2 before tumor cell administration resulted in a 70 percent decrease in the number of metastatic foci in the lungs and totally prevented metastasis to the other organs examined (Table 1). The hydrolysis product of PGI_2, 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) was ineffective.

Since PGI_2 is a potent pulmonary vasodilator (*11*), such vasodilation may be partly responsible for the decrease in lung metastasis. However, prostaglandin E_2 (PGE_2), which is an approximately equipotent vasodilator but does not inhibit platelet aggregation (*12*), was ineffective in reducing metastasis (Table 1). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), was also without effect (Table 1). Prostaglandin D_2, which is also a potent inhibitor of platelet aggregation, also reduces metastasis of B16 melanoma cells (*13*). Our results (Table 1) with PGD_2 confirm that observation and indicate that PGI_2 is 3.5 times more effective than PGD_2 as an antimetastatic agent.

Moncada and Vane (*14*) have suggested that a therapeutic synergism would result from the use of PGI_2 with a phosphodiesterase inhibitor. Since the effect of PGI_2 is mediated, in part, by increasing concentrations of adenosine 3′,5′-monophosphate (cyclic AMP) in platelets, it follows that phosphodiesterase inhibitors, by slowing the breakdown of cyclic AMP, should potentiate the antithrombogenic action of PGI_2 (*14*). Therefore, we tested the effects of an intraperitoneal injection of the phosphodiesterase inhibitor theophylline (100 μg), given 30 minutes before the injection of PGI_2 and $B16_a$ cells. Theophylline alone was ineffective at this dose (Table 1). However, PGI_2 plus theophylline reduced metastasis 93 percent (Fig. 1), a fourfold reduction of metastasis compared to PGI_2 alone (Table 1).

The effects of PGI_2 on tumor cell arrest, retention, and distribution were evaluated with the use of [^{125}I]deoxyuridine-labeled $B16_a$ tumor cells. Labeled cells were injected with PGI_2 alone or with PGI_2 plus theophylline. The uptake of labeled cells in the lungs of control mice was rapid (5 minutes), but there was a gradual loss of these cells thereafter until approximately 10 percent of the injected dose remained at 20 hours. Tumor cell numbers in the liver remained constant for the first hour and gradually decreased thereafter (Table 2). Treatment with PGI_2 plus theophylline resulted in only a 6 percent decrease in the retention of lung tumor cells at 30 minutes, whereas the number of tumor cells in the liver was 31 percent greater than controls at the same time interval (Table 2).

These results indicate that PGI_2 may not significantly alter the initial entrapment of tumor cells in the lungs, but may alter the initial distribution pattern. At 20 hours after PGI_2 treatment the number of tumor cells in the lungs was 88 percent below the number in the corresponding controls. No detectable tumor cells remained in the livers of PGI_2-treated animals at 20 hours, whereas livers of the controls retained 23 percent of the injected dose at this time (Table 2).

This effect of PGI_2 on tumor cell distribution is not due to a direct effect on the tumor cells, inasmuch as treatment of tumor cells in vitro with PGI_2 (50 μg/ml for 1 hour) did not alter their ability to form lung tumor colonies (127 ± 20) when they were subsequently injected into mice; untreated cells produced similar numbers of lung tumor colonies (138 ± 17). Although some alteration of initial tumor cell distribution may occur, the cells are probably not retained in the organ of secondary arrest. This observation is supported by the absence of labeled tumor cells in the liver at 20 hours and the absence of liver tumor colonies in PGI_2-treated mice (Table 1). Studies with [^{125}I]deoxyuridine-labeled tumor cells indicate that PGI_2 does not alter initial tumor cell arrest but influences subsequent cell survival. Therefore, it is possible that the interaction of circulating tumor cells with platelets is of secondary importance. However, upon arrest and attachment to the endothelium, the initiation of thrombus formation, possibly by tumor cell damage to the endothelium (*15*), may assume paramount importance. If this process is prevented, the tumor cell may perish.

If PGI_2 prevents metastatic tumor colony formation, an inhibitor of endogenous PGI_2 synthesis should enhance metastasis. Hydroperoxy fatty acids in general are potent inhibitors of PGI_2 synthesis (*16*). Intravenous injection of 15-hydroperoxyarachidonic acid (15-

Table 1. Reduction of pulmonary and extrapulmonary metastasis of B16 amelanotic melanoma cells by prostacyclin. Aseptically removed subcutaneous tumors were diced and placed in sterile Eagle's minimum essential medium (MEM) buffered with sodium bicarbonate (15 m*M*) and Hepes (25 m*M*). The MEM used for tumor cell dispersion contained collagenase type III (Worthington, 1 mg/ml), deoxyribonuclease I (Sigma, 50 μg/ml), soybean trypsin inhibitor (Worthington, 100 μg/ml), and fatty acid–free human serum albumin (Sigma, 10 mg/ml). Cells were dispersed (once for 30 minutes and once for 60 minutes, 37°C) under air in a Dubnoff metabolic shaker (90 oscillations per minute). Supernatants were collected through cheesecloth and centrifuged (100*g*, 10 minutes), and the pellets were washed twice and resuspended in MEM. Final preparations consisted of monodispersed (> 99 percent) cells of high viability (> 95 percent) with low (4 to 7 percent) host stromal cell contamination. Cells were then suspended in MEM and injected intravenously into male C57BL/6J mice (20 to 25 g). The animals, housed under identical conditions of temperature, photoperiod, and feeding, were killed 3 weeks later. Lungs and livers were removed and fixed in Bouin's solution, and the number of metastatic foci in each organ was determined by using a Bausch & Lomb stereo zoom microscope. Kidney, spleen, and brain were also examined for gross metastatic foci. The PGI_2 was dissolved in 0.05*M* tris buffer, *p*H 9.37, immediately prior to use; 6-keto-$PGF_{1\alpha}$ was dissolved in MEM. Both compounds were injected intravenously (tail vein) in a volume of 25 μl. Controls received 25 μl of the appropriate buffer. $B16_a$ cells were injected at a dose of 3×10^5 per mouse. Theophylline was dissolved in MEM and injected intraperitoneally 30 minutes before the PGI_2. The 15-HPAA was synthesized from arachidonic acid with soybean lipoxygenase, and was purified by silicic acid column chromatography; 15-HPAA was stored at 0° C in hexane until immediately prior to use. For injection, 15-HPAA was taken to dryness under N_2, dissolved in absolute ethanol and injected intravenously in a volume of 25 μl. Control animals received 25 μl of absolute ethanol. The PGE_2, PGD_2, and $PGF_{2\alpha}$ were stored as stock solution in absolute ethanol (10 mg/ml) and diluted with MEM before use. They were injected intravenously (tail vein) in a volume of 25 μl; N.D., not determined.

Treatment	Lungs	Liver	Spleen
MEM control	144 ± 18*	10 ± 5	1 ± 0.5
Tris control	130 ± 9	8 ± 3	3 ± 1
Ethanol control	127 ± 14	9 ± 5	N.D.†
PGI_2 (100 μg)	43 ± 15	N.D.	N.D.
6-Keto-$PGF_{1\alpha}$ (100 μg)	126 ± 27	5 ± 3	N.D.
Theophylline (100 μg)	119 ± 8	7 ± 3	N.D.
Theophylline (100 μg) plus PGI_2 (100 μg)	10 ± 5	N.D.	N.D.
15-HPAA (100 μg)	380 ± 40	26 ± 9	4 ± 0.5
15-HPAA (100 μg) plus theophylline (100 μg) plus PGI_2 (100 μg)	39 ± 16	3 ± 2	N.D.
PGE_2 (100 μg)	139 ± 39	13 ± 6	N.D.
$PGF_{2\alpha}$ (100 μg)	121 ± 15	7 ± 3	2 ± 0.5
PGD_2 (100 μg)	102 ± 8	N.D.	N.D.

*Number of metastatic colonies on lung surface (bilateral), mean ± standard error; $N = 7$. †No detectable tumor colonies observed with Bausch & Lomb stereo zoom microscope.

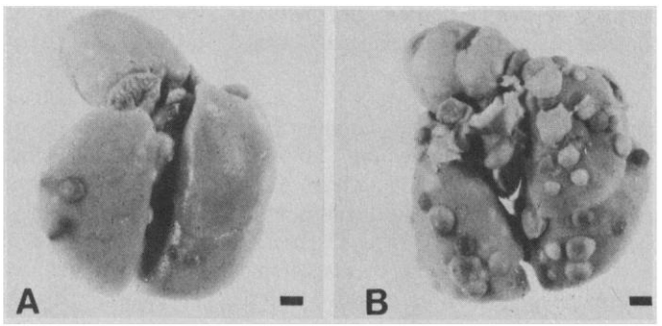

Fig. 1. Representative lungs from C57BL/6J mice receiving an intravenous injection of 3×10^5 B16 amelanotic melanoma cells. (A) Lung from a mouse injected with theophylline (100 μg, intraperitoneally) 30 minutes before it received PGI_2 (100 μg, intravenously). (B) Lung from a control mouse that received cells only. The injection of PGI_2 plus theophylline decreased lung tumor colony formation by 93 percent. Scale bar, 1 mm.

HPAA) 20 minutes before injection of $B16_a$ tumor cells resulted in a significant increase in the number of metastatic tumor foci in the lung, liver, and spleen (Table 1). Treatment with PGI_2 after administration of 15-HPAA reversed the effect of the latter on tumor colony formation (Table 1).

Prostacyclin, the most potent inhibitor of platelet aggregation (*8*), can also reverse secondary platelet aggregation (*14*) and inhibit mobilization of platelet fibrinogen-binding sites (*17*). On the basis of these results and ours, it appears reasonable to predict that the antimetastatic effect of PGI_2 would be further enhanced by an inhibitor of platelet thromboxane synthetase, since it is proposed that platelet-generated thromboxane A_2 (TXA_2) opposed the action of PGI_2 (*14*). In fact, such results have been obtained with $B16_a$ tumor cells (*18*) and have led to the hypothesis that metastatic tumor cells can alter the critical PGI_2-TXA_2 balance in favor of thrombosis; such results further suggest that TXA_2 synthetase inhibitors, PGI_2, or agents that augment PGI_2 synthesis or activity in vivo may function as antimetastatic agents (*19*).

Prostacyclin may function as a circulating hormone (*20*). Our studies demonstrate that animals treated with a known prostacyclin synthetase inhibitor have a decreased resistance to the establishment of successful metastatic foci. Normal prostacyclin production by healthy vascular endothelium may, therefore, function as a natural deterrent to successful tumor metastasis. It is known that tumor cells preferentially attach to damaged endothelium (*21*). The clinical association of microangiopathic hemolytic anemia and disseminated carcinoma is well established (*22*). It has also been demonstrated that decreased vascular endothelial prostacyclin production is associated with several experimental and clinical conditions associated with thrombotic or microangiopathic complications (*23*). Collectively, our results suggest that prostacyclin synthesis in vivo may play a vital role in preventing the spread of metastatic disease.

The half-life ($T_{1/2}$) of PGI_2 in aqueous buffer at physiological *p*H is very short (approximately 3 to 5 minutes). However, recent evidence demonstrates that the antiaggregatory effect of PGI_2 is considerably lengthened ($T_{1/2}$ = 60 minutes) in human plasma, presumably because it binds to human albumin (*24*). Wynalda and Fitzpatrick (*25*) have demonstrated that albumins of several mammalian species significantly prolong the $T_{1/2}$ of PGI_2. These results suggest that exogenous prostacyclin may be effective as an adjuvant chemotherapeutic agent in reducing the total number of metastatic tumor cells that survive hematogenous dissemination.

Table 2. Effects of prostacyclin on the retention and distribution of [^{125}I]deoxyuridine-labeled $B16_a$ melanoma cells. Tumor cell distribution studies were performed with $B16_a$ isolated as described in Table 1 and labeled with [5-^{125}I]deoxyuridine ([^{125}I]dU; New England Nuclear). Labeling was accomplished by incubating $B16_a$ cells (5×10^6 per milliliter) for 18 hours in a 75-cm^2 tissue culture flask at 37°C in the presence of [^{125}I]dU (0.3 μCi/ml; 1 Ci = 3.7×10^4 becquerels). The cells were washed, resuspended (1×10^6 cells per 100 μl) in MEM, and injected intravenously. The organs, removed at timed intervals, were extracted with 70 percent ethanol (three changes in 72 hours) and counted in a Searle 1185 gamma scintillation spectrometer. The data are expressed as counts per minute (mean ± standard error), $N = 7$.

Treatment	Lung	Liver
Killed after*		
5 minutes	7675 ± 109	340 ± 40
30 minutes	5027 ± 111	343 ± 7
60 minutes	3478 ± 74	343 ± 14
120 minutes	2470 ± 103	163 ± 13
20 hours	805 ± 42	77 ± 7
Theophylline plus PGI_2 plus cells†	4703 ± 185	450 ± 43
Theophylline plus PGI_2 plus cells‡	97 ± 22	0

*Time after tumor cell injection. †Theophylline (100 μg) was injected intraperitoneally 30 minutes before the PGI_2 (100 μg, intravenously); tumor cells were injected 10 minutes after the PGI_2; and the animals were killed 30 minutes after the cells were injected. ‡Theophylline (100 μg) was injected 30 minutes before the PGI_2; tumor cells were injected 10 minutes after the PGI_2; and the animals were killed 20 hours after the cells were injected.

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References and Notes

1. I. J. Fidler, *Cancer Res.* **38**, 2651 (1978); *J. Natl. Cancer Inst.* **45**, 773 (1970); *Methods Cancer Res.* **25**, 399 (1978); G. Poste and I. J. Fidler, *Nature (London)* **283**, 139 (1980).
2. G. J. Gasic, T. B. Gasic, N. Galanti, T. Johnson, S. Murphy, *Int. J. Cancer* **11**, 704 (1973); G. J. Gasic, P. A. G. Koch, B. Hsu, T. B. Gasic, S. Niewiarowski, *Z. Krebsforsch.* **86**, 263 (1976); E. Pearlstein, P. L. Salk, G. Yogeeswaren, S. Karpatkin, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4336 (1980).
3. S. Wood, *Pathobiol. Annu.* **1**, 281 (1971); P. Hilgard, *Br. J. Cancer* **28**, 429 (1973).
4. G. J. Gasic, T. B. Gasic, S. Murphy, *Lancet* **1972-II**, 932 (1972); S. Wood and P. Hilgard, *ibid.*, p. 1416.
5. H. Gastpar, *J. Med. (Clin. Exp. Theor.)* **8**, 103 (1977).
6. A. Lione and H. B. Bosman, *Cell Biol. Int. Rep.* **2**, 81 (1978).
7. H. C. Hoover and A. S. Ketcham, *Cancer (Brussels)* **35**, 5 (1975).
8. S. Moncada and J. R. Vane, in *Biochemical Aspects of Prostaglandins and Thromboxanes*, N. Kharasch and J. Fried, Eds. (Academic Press, New York, 1977), pp. 155–177.
9. B. F. Sloane, J. R. Dunn, K. V. Honn, *Science* **212**, 1151 (1981).
10. K. V. Honn, J. R. Dunn, L. R. Morgan, M. Bienkowski, L. J. Marnett, *Biochem. Biophys. Res. Commun.* **87**, 795 (1979).
11. A. L. Hyman and P. J. Kadowitz, *Circ. Res.* **45**, 404 (1979).
12. J. B. Smith, *Am. J. Pathol.* **99**, 743 (1980).
13. D. A. Stringfellow and F. A. Fitzpatrick, *Nature (London)* **282**, 76 (1979).
14. S. Moncada and J. R. Vane, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 66 (1979).
15. E. C. Chew, R. L. Josephson, A. C. Wallace, in *Fundamental Aspects of Metastasis*, L. Weiss, Ed. (North-Holland, Amsterdam, 1976), pp. 121–150.
16. J. A. Salmon, D. R. Smith, R. S. Flower, S. Moncada, J. R. Vane, *Biochim. Biophys. Acta* **523**, 250 (1978).
17. J. Hawiger, S. Parkinson, S. Timmons, *Nature (London)* **283**, 145 (1980).
18. K. V. Honn, in preparation.
19. ———, B. Cicone, A. Skoff, M. Romine, D. Gossett, K. Thompson, D. Hodge, *J. Cell Biol.* **87**, 64 (1980).
20. S. Moncada, R. Korbut, S. Bunting, J. R. Vane, *Nature (London)* **273**, 767 (1978).
21. R. H. Kramer and G. C. Nicolson, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5704 (1979).
22. M. C. Brain, J. G. Azzopardi, L. R. I. Baker, G. F. Pineo, P. D. Roberts, J. V. Dacie, *Br. J. Haematol.* **18**, 183 (1970).
23. G. deGaetano, G. Remuzzi, M. Mysliwiec, M. B. Donati, *Haemostasis* **8**, 300 (1979).
24. D. D. Pifer, C. M. Chesney, L. M. Cagen, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 323 (1980).
25. M. A. Wynalda and F. A. Fitzpatrick, *Prostaglandins* **20**, 853 (1980).
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