Brief Definitive Report

PROSTAGLANDIN E PRODUCTION BY HUMAN BLOOD MONOCYTES AND MOUSE PERITONEAL MACROPHAGES*

By J. I. KURLAND and R. BOCKMAN

(From the Departments of Developmental Hematopoiesis and Calcium Metabolism, Sloan-Kettering Institute for Cancer Reseach, New York 10021)

The mononuclear phagocyte has been implicated in regulating the functions of lymphoid and hematopoietic cells and in most cases, these effects are mediated by soluble factors produced by circulating monocytes and tissue macrophages. In the same context, the E-series prostaglandins (PGE) have been shown to have significant physiological effects on the same functions of lymphoid and hematopoietic cells as does the macrophage. For example, both macrophages and PGE affect lymphocyte proliferation (1–6), facilitate thymocyte differentiation (7, 8), enhance erythropoiesis (9; Kurland et al., unpublished observations), limit myeloid stem cell proliferation (10–12) and are involved in various aspects of the inflammatory response. The possibility therefore exists that the modulation of immune and hematopoietic functions by macrophages may be mediated by PGE. It has been reported that guinea pig peritoneal exudate cells are a potent source of PGE (13), and a similar cell in murine peritoneal exudates which is believed to be a macrophage on the basis of adherence properties, converts radioactive arachidonic acid into PGE_2 (14). However, cells other than macrophages can adhere to a plastic surface, and in the absence of additional morphological, histochemical, or physical criteria, no definitive conclusions can be drawn concerning the actual cellular origin of PGE. Furthermore, no demonstration has heretofore been made of a similar PGE-producing cell in human peripheral blood. By employing cell separation techniques and histochemical identification, we have demonstrated that the monocyte and macrophage are the principal PGE-producing cells in human peripheral blood and murine peritoneal fluids, respectively, and that the synthesis of PGE can be modulated by endotoxic lipopolysaccharide.

Materials and Methods

Preparation of Murine and Human Cell Suspensions. Adherent mouse peritoneal cells were prepared as previously described (4, 11). Briefly, peritoneal exudate cells (PEC) were harvested from 2- to 3-mo-old female $B6D2F_1$ mice (The Jackson Laboratory, Bar Harbor, Maine). Varying numbers of PEC in McCoy's 5A modified medium containing 15% fetal calf serum (FCS) (Microbiological Associates, Bethesda, Md.) were allowed to adhere to 35-mm plastic culture

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dishes (Lux Scientific Corp., Newbury Park, Calif.) for 1.5 h at 37°C in a humidified atmosphere of 5% CO_2 in air, and the nonadherent PEC were then removed. The adherent cells, comprising ≅ 15% of the total peritoneal cell population, were >99% macrophages, as judged by staining for α-napthyl acetate esterase (15) and neutral red (16). The nonadherent cells were primarily comprised of small lymphocytes and peroxidase-positive polymorphonuclear leukocytes, and contained <0.1% cells positive for esterase. In addition, the adherent, but not the nonadherent PEC elaborated myeloid colony-stimulating factors (11, 12), lymphocyte-activating factor (4), and B-lymphocyte colony-stimulating factor (4, 17) macromolecules of characteristic macrophage origin.

Human peripheral blood from normal volunteers was separated into polymorphonuclear leukocytes (PMN) and mononuclear cells by buoyant density centrifugation in bovine serum albumin (12). The monocytes were separated from the light density lymphocytes by adherence to plastic dishes (12). The dense (>1.070 g/cm³) cell fraction contained >98% peroxidase-positive PMN and 0.2% α-naphthyl acetate esterase-positive cells. The nonadherent, light density (<1.070 g/cm³) cells comprised >99% small lymphocytes, 0.7% peroxidase-positive PMN, and 0.1% esterase-positive cells, and the adherent light density monocytes were >99% esterase-positive.

Murine Macrophage and Non-Macrophage Cell Lines. PGE was determined in media conditioned by the following murine cell lines: WEHI-3, a myelomonocytic leukemia (18, 19); J774, macrophage cell line (19); Abelson virus-induced RAW-264 macrophage tumor (20); SK-2, a spontaneous monocytic cell line (21); EL_4, a T-cell lymphoma (19); and RBL-3, a spontaneous B-cell lymphoma (22).

PGE Radioimmunoassay. Culture fluids were assayed for PGE by radioimmunoassay (23). Briefly, samples were extracted with petroleum ether and re-extracted with acidified ethyl acetate-iso-propanol. The silicic acid column effluent fractions containing PGE were sequentially eluted with benzene-ethyl acetate and varying concentrations of methanol, they were then dried, resolubilized in gelatin-tris buffer, and alkaline-hydrolized to quantitatively convert PGE_1 and PGE_2 to prostaglandin B (PGB) (24). Competitive binding between [³H]PGB, or the test supernate, and specific anti-PGB antibodies was measured using a double antibody radioimmunoassay (Clinical Assays Inc., Cambridge, Mass.).

Results and Discussion

There was a linear production of PGE by increasing numbers of adherent murine peritoneal cells (Fig. 1 A). Conversely, no PGE was detectable in media conditioned by nonadherent peritoneal cells, even at many times the concentration of adherent peritoneal macrophages. PGE production was linear with respect to the number of adherent human blood mononuclear cells which comprised >99% monocytes by morphology and histochemistry (Fig. 1 B). In contrast, no PGE was detectable in the supernates from either polymorphonuclear leukocyte or lymphocyte populations, even at four times the concentration of adherent monocytes. Indomethacin, a prostaglandin synthetase inhibitor (25), completely suppressed the levels of detectable PGE by both murine macrophages and human monocytes.

The effects of endotoxic lipopolysaccharide (LPS) on the production of PGE by murine peritoneal macrophages was examined (Fig. 2). There was progressive stimulation of PGE production with increasing concentrations of LPS from 0.1–20 μg/ml. The relationship was sigmoidal, and no further augmentation of PGE synthesis was observed at LPS concentrations greater than 20 μg/culture.

The capability to produce PGE by normal monocytes and macrophages was extended to populations of neoplastic mononuclear phagocytes (Fig. 3). The spontaneous CBA macrophage cell line, SK-2, and the murine myelomonocytic leukemia, WEHI-3, were constitutive producers of PGE, and like normal macrophages, their production of PGE was markedly augmented by endotoxin.

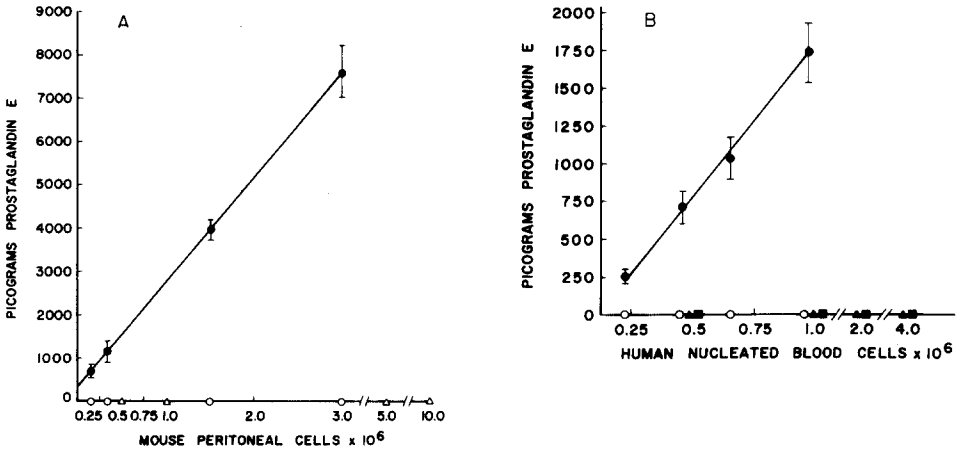

FIG. 1. Production of PGE by mouse peritoneal macrophages and human peripheral blood monocytes. (A) Various numbers of both adherent macrophages (●) and nonadherent peritoneal cells (△) were incubated in McCoy's medium containing 15% FCS for 48 h, and the supernatant media were removed for assay of PGE by radioimmunoassay. Indomethacin was added at a final concentration of 1.4×10^{-7} M at the initiation of replicate adherent macrophage cultures (○). The number of adherent cells are expressed as the actual number of esterase-positive PEC which adhered after the initial 1.5-h incubation determined by counting a designated area of the dish and multiplying this number by the ratio of the area counted to the total area. Triplicate cultures were used for each point and the results expressed as picograms of PGE ± SD. (B) Various numbers of human PMN (■) $\rho > 1.070$ g/cm³, lymphocytes (▲) $\rho < 1.070$ g/cm³, and monocytes (●) $\rho < 1.070$ g/cm³, were cultured in McCoy's 5A modified medium containing 15% FCS for 48 h, and the supernate was removed for determination of PGE. The monocytes refer to the actual number of adherent light density esterase-positive cells remaining adherent to the culture dish after 1.5 h and incubated in the absence (●) and presence (○) of 1.4×10^{-7} M indomethacin.

The RAW-264 and J774 macrophage tumors were not constitutive producers of PGE, but were induced to elaborate significant amounts of PGE after exposure to endotoxin. In contrast, however, neither the T-cell lymphoma, EL_4, nor the B-cell lymphoma, RBL-3, elaborated any PGE in the absence or presence of endotoxin.

Much confusion has heretofore existed concerning the cellular origin of PGE in murine hematopoietic tissues and human peripheral blood. It has been reported that the polymorphonuclear leukocyte in rabbit peritoneal exudates (26) and human peripheral blood (27), murine splenic and human blood lymphocytes (28), as well as human bone marrow cells (29), elaborated PGE in vitro. However, considering the omnipresence of the mononuclear phagocyte, as well as the high concentrations and lack of purity of the cell populations used, these previous findings did not exclude the blood monocytes and macrophages indigenous to these tissues as the cellular source of the prostaglandin. This is particularly true in light of the present findings that low numbers of monocytes and macrophages represent significant sources of PGE, whereas much higher numbers of purified populations of granulocytes and lymphocytes did not contribute any detectable PGE. The ability to elaborate PGE and/or be induced to produce PGE after exposure to endotoxin, was retained by neoplastic macrophages, but not T- or B-lymphoma cells. We therefore conclude that in

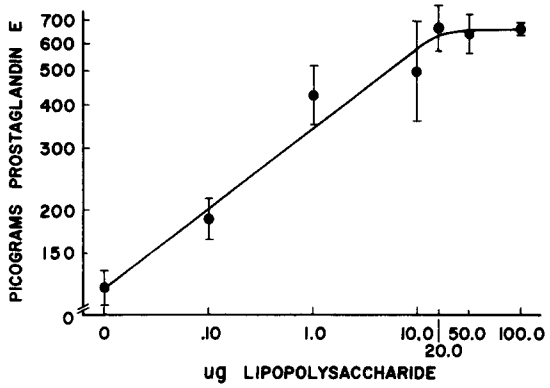

FIG. 2. Effect of endotoxic LPS on PGE production by adherent mouse peritoneal macrophages. 1×10^5 adherent neutral red and esterase-positive peritoneal macrophages were prepared in 35-mm culture dishes. Various concentrations of LPS from *Salmonella typhosa* (W0901; Difco Laboratories, Detroit, Mich.) were added to the macrophages, and the cells incubated in McCoy's 5A modified medium containing 15% FCS for 48 h. The supernatant media were harvested and assayed for PGE.

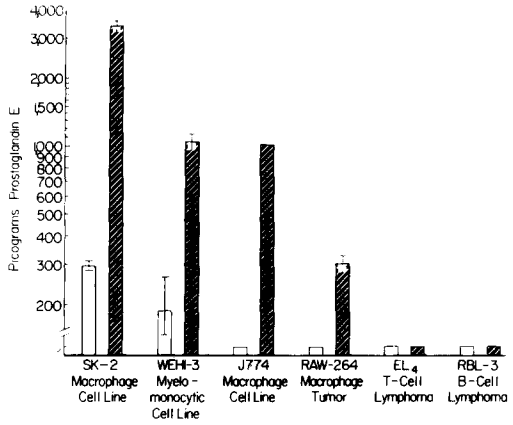

FIG. 3. Induction of PGE production by endotoxin in macrophage cell lines. 5×10^5 viable cells of the following monocytic macrophage and nonmacrophage cell lines, WEHI-3, J774, RAW-264, SK-2, EL_4, and RBL-3, were cultured for 48 h in the absence (open bars) and presence (closed bars) of 1 μg LPS, and the supernatant media assayed for PGE.

human peripheral blood and murine peritoneal fluids, there exists a cell population defined by accepted criteria as the blood monocyte and tissue macrophage which synthesize and release significant amounts of PGE, a function which extends also to neoplastic macrophages. Thus, the E-series prostaglandins may partially mediate some of the many regulatory functions of the mononuclear phagocyte in a variety of hematopoietic, immunological, and inflammatory responses.

Summary

Purified populations of both human peripheral blood monocytes and murine peritoneal macrophages synthesize and release Prostaglandin E in vitro. In contrast, prostaglandin E was detected in neither the supernate fluids from

cultures of highly enriched human lymphocytes and granulocytes, nor in nonadherent murine peritoneal cells. Macrophage prostaglandin E production was markedly enhanced by endotoxin, and completely suppressed by indomethacin. All neoplastic monocyte-macrophage cell lines examined elaborated prostaglandin E in vitro, either constitutively or after induction with endotoxin. In contrast, prostaglandin E production could not be detected from either a T- or B-cell lymphoma, whether or not they were treated with endotoxin. These findings thus indicate that the blood monocyte and tissue macrophage represent an important source of prostaglandin E, a function shared by both normal and neoplastic mononuclear phagocytes.

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