EFFECT OF PROSTAGLANDINS ON THE PRODUCTION OF INTERLEUKIN-2

R. S. Rappaport and G. R. Dodge
Wyeth Laboratories, Inc.
Philadelphia, Pennsylvania

INTRODUCTION

A substantial body of evidence implicates prostaglandins as potent, local regulators of the immune response (1). It is well documented, for example, that prostaglandins, especially of the E-series, inhibit lectin or antigen-induced lymphocyte proliferation in vitro (2-5). Recently, it has become evident that T-cell proliferation is dependent on the production and utilization of a soluble protein known as T-cell growth factor (TCGF) or Interleukin-2 (IL-2) (6-8). Current evidence suggests that IL-2 is produced by one subset of T-lymphocytes (producer cells) and that it acts upon another subset of T-lymphocytes (responder cells via specific IL-2 receptors (9,10). The precise sequence of events which regulate the production of IL-2 and the development of specific receptors are not completely understood.

In the present report, we describe the effect of several prostaglandins on IL-2 production by mitogen-stimulated normal human lymphocytes. We also describe the effect of a prostaglandin synthetase inhibitor in the same system.

MATERIALS AND METHODS

Preparation of Lymphocytes. Peripheral venous blood was drawn from normal fasting men and women into Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing heparin. The blood was processed within two hours of collection. To reduce volume and achieve partial separation of cells, whole blood was centrifuged
at 1200 x g for 4 minutes. After removing the plasma, the white cell layer was removed and diluted with an equal volume of Hank's Balanced Salt Solution (HBSS). Mononuclear cells were isolated by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. The resultant lymphocyte-rich bands were washed 3 times in HBSS and suspended in HEPES-buffered RPMI-1640 medium (M.A. Bioproducts, Walkersville, MD) containing streptomycin (100 μg/ml), penicillin (100 units/ml), Fungizone (2.5 μg/ml) and 10^{-5}M mercaptoethanol (Complete Medium). Cell viability was routinely assessed by the trypan blue exclusion method and determined to be >90%.

Production of IL-2. Lymphocytes, at a concentration of 10^6 cells/ml, were cultivated in complete medium supplemented with 2% human AB serum (Grand Island Biological Co. Grand Island, NY) for 48 hr at 37°C in an atmosphere of 5% CO_2, 95% humidity. In some experiments, the lymphocytes were first passed through glass wool (Pyrex wool; Corning Glass Works, Corning, NY) to remove glass-adherent cells. Prostaglandins (Sigma Chemical Co., St. Louis, MO) or indomethacin (Merck Sharp and Dohme, West Point, PA) were added to various cultures containing 1 μg/ml phytohemagglutinin (PHA) (Wellcome Reagents, Ltd., Beckenham, England) at the beginning of the cultivation period. After incubation, culture supernatants were sterilized by passage through 0.45 μm Acrodisc filters (Gelman Sciences, Inc., Ann Arbor, MI) and stored frozen until assayed.

Measurement of IL-2. IL-2 levels were determined by measuring the ability of IL-2-containing fluids to stimulate proliferation of sensitized normal human lymphocytes. The assay cells (0.3 x 10^6 per ml) were cultured in Complete Medium with 10% heat-inactivated fetal calf serum (Δ-FCS) (Grand Island Biological Co., Grand Island, NY) and 1 μg/ml PHA for 7 days at 37° in 5% CO_2. Prior to assay, the cells were washed three times with HBSS, counted, and suspended (10^6 cells per ml) in Complete Medium containing 40% Δ-FCS. Aliquots of 10^3 cells (100 μl) were distributed in the wells of Linbro microtiter plates (Flow Laboratories, Inc., Hamden, CO) and test samples (100 μl per well) were added in quadruplicate. The plates were incubated for 48 h at 37° in 5% CO_2. The cultures were incubated with 1 μCi/well of [3H] thymidine (New England Nuclear, Boston, MA) and 16-18 hr later, they were harvested on glass fiber filters using a Titertek Cell Harvester (Flow Laboratories, Inc.). The filters were counted in a liquid scintillation counter. Data were evaluated using the following equation:

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\frac{\text{c.p.m. control sample} - \text{c.p.m. test sample}}{\text{c.p.m. control sample}} \times 100
\]

where the control sample represents the IL-2-containing culture.