ACTIVATED RAT NEUTROPHILS
A Sequential Quantitative Assay for Aggregation and NBT Reduction

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Abstract—We present here a rapid, sensitive, and convenient approach for the analysis of activated Lewis rat PMNs based on detecting separately, or in tandem, PMN aggregation and PMN reduction of nitroblue tetrazolium (NBT). These responses are quantitated using an ELISA scanner which can rapidly measure optical densities of cell cultures in microtiter plates. Aggregation induced by as little as 0.005 μg/ml of phorbol myristate acetate (PMA), 0.01 μg/ml lipopolysaccharide (LPS), or a 1:160 dilution of lymphokine-containing rat serum can be detected employing this approach. NBT reduction was induced by as little as 0.01 μg/ml PMA. Blocking studies employing 2-deoxyglucose, iodoacetamide, and polymyxin B gave the expected results and confirmed that these assays detect cellular responses to soluble stimuli. Using this technology the effects of PMA and LPS on rat peritoneal exudate PMNs were evaluated. Rat PMNs appeared less sensitive to LPS than human PMNs and also reduced NBT more slowly following stimulation with PMA. Because of the slowness in NBT reduction following stimulation, NBT reduction can be evaluated, in tandem, after measuring aggregation. The simplicity of this system, coupled with the speed with which large numbers of microcultures can be read and the low number of cells required, make this approach for studying responses especially attractive.

INTRODUCTION

Neutrophils require several "skills" to carry out their essential role in protecting the host from the continual threat of bacterial disease. Upon "triggering,"

1This research was funded by grants from the Kroc Foundation and the Medical Research Foundation of Oregon.
2Kenneth O. Fennell received an N. L. Tartar Research Fellowship.
PMNs must aggregate to vascular walls and tissue, migrate along a chemotactic gradient, and accelerate oxygen-dependent bacterial activity. Several disease conditions exist where PMNs lack some functions while retaining other capabilities. It is not always clear if these differences reflect loss of some trigger mechanism or loss of a PMN subpopulation. Unfortunately, the term "triggering" has frequently been used to imply that PMNs have a single "off/on" switch which activates all of the above PMN activities. Some recent studies suggest, however, that this may not be true (1-3).

Assays of PMN activity are characteristically complex procedures that require significant investments in both time and cell numbers. Additionally, the use of human cells precludes certain studies that are possible with animal models. These realities tend to impose rather severe limitations on the number of variables that can be tested at one time employing cells from modest-sized blood samples. Methods which can conveniently test multiple stimulants over broad dose ranges with reasonable numbers of cells need to be developed for use with PMNs from common laboratory animals.

We recently reported an assay for activation of human PMNs employing an ELISA apparatus to measure OD loss which occurs due to cell aggregation. Only small amounts of blood are needed to analyze several hundred separate PMN suspensions (4).

We report here that peritoneally induced Lewis rat neutrophils undergo a similar stimulant-dependent loss of optical density (OD). This activity is blocked by inhibitors of glycolysis, 2-deoxyglucose, and iodoacetamide. Although the overall response of rat cells in this assay is similar to that of human cells, the relative activity of various stimulants differs. In addition, the variability between experiments is less probably due to strain heterogeneity and a more controlled environment. We have adapted a parallel assay developed by Pick et al. to measure the reduction of nitroblue tetrazolium (NBT) which reflects the "oxidative burst" following the activation of neutrophils (3). This activity is detected separately from aggregation or, for convenience, with the same cell population used to study aggregation.

MATERIALS AND METHODS

Reagents. Lipopolysaccharide B (LPS) Salmonella typhosa 0901 was obtained from Difco Laboratories, Detroit, Michigan N-Formyl-methionyl-leucyl-phenylalanine (F-MET) was obtained from CalBiochem Behring Corp., San Diego, California. Phorbol myristate acetate (PMA, Lot 71 FE16104), 2-deoxyglucose (2-DOG, Lot 12C-5200), nitroblue tetrazolium (NBT, Lot 36C-5034), and glycogen (Lot 70F-0195) were all obtained from Sigma, St. Louis, Missouri. Iodoacetamide (Lot 94710) was obtained from Pierce Chemical Co., Rockford, Illinois.

Preparation of Mono p-Azobenzenearsonic Acid-N-Chloroacetyl-L-tyrosine (ABA-T). In 20 ml distilled water 500 mg p-aminobenzenearsonic acid was dissolved by addition of 3 ml of 1 N