AN ENZYME-ASSESSED MICROPLATE-ASSAY FOR NEUTROPHIL ADHERENCE
I. IgA-Induced Adherence of Human PMNs

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Abstract—The binding of PMNs to extracellular matrix and cells is crucial to PMN host defense. Adherence mechanisms and the many families of molecules involved are major areas of study. We present here details of an enzyme-assessed microtiter plate assay for neutrophil adherence. This assay uses low numbers of cells (50,000/well) and permits analysis of several hundred wells in a short period of time, by using an ELISA reader. With this assay we observed 5- to 10-fold increases in the number of adherent human PMNs in response to nanogram amounts of LPS or as little as 5.0 μg/ml of aggregated IgA. Although fluoride blocked the LPS-induced adherence response, IgA-induced cell binding was largely unaffected.

INTRODUCTION

Adherence is crucial to the life-saving activities of neutrophils (PMNs) and the study of such attachment is important in helping us to understand adherence mechanisms and to characterize the molecules involved. A more convenient adherence assay would simplify these studies. Most existing assays are inconvenient because they require individual columns or slides for each sample and the time-consuming counting of individual cells or the use of isotope-labeled cells, which involves extra time and cell handling and adds the consequent problems of the expense, safety, and disposal of isotopes.

We previously reported that aggregated human secretory immunoglobulin

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A (sIgA), in the absence of complement activity, stimulated NBT-reduction by human PMNs (1). In order to answer our next question of whether IgA might induce PMN adherence, we applied an approach we developed earlier for quantitating PMN migration by relating cell number to cytoplasmic lactic dehydrogenase (LDH) activity (2). Here we describe an enzymatic microtiter plate method for rapidly measuring adherent cells. The assay uses only 50,000 PMNs/well and is rapid, convenient, and sensitive. Background PMN adherence is typically less than 5% of the starting population, in the presence of 10% serum. Our studies employing this assay reveal that aggregated IgA, bacterial lipopolysaccharide (LPS), and formyl-leucyl-phenylalanine (FMLP) induce 3- to 10-fold increases in the number of adherent PMNs through iodoacetamide-inhibitable cellular processes.

MATERIALS AND METHODS

Reagents. FMLP was obtained from CalBiochem Behring Corporation, San Diego, California. Stock solutions of FMLP were prepared at $2 \times 10^{-2}$ M concentrations in 0.1 N NaOH, brought to neutrality with HCl, diluted to $2 \times 10^{-5}$ M concentration in Hanks' balanced salt solution (HBSS), and stored frozen until further diluted and used (2). Ficol 400 was obtained from Pharmacia Inc., Piscataway, New Jersey. Sodium diatrizoate was obtained from Sigma Chemical Co., St. Louis, Missouri. Beef lung sodium heparin (10,000 units/ml) was obtained from Upjohn, Kalamazoo, Michigan. HBSS was diluted from Gibco 10x solution without NaHCO3 or phenol red, Chagrin Falls, Ohio. All HBSS used in this study contained 0.05 M (1,3-bis[tris(hydroxymethyl)-methylamino]-propane) (Bis-Tris-propane) (Sigma) and was brought to pH 7.3 and stored at 4°C. Phosphate-buffered saline (PBS) and colostral IgA were obtained from Sigma Diagnostics, St. Louis, Missouri. Salmonella typhimurium LPS (phenol extracted) catalog no. 3946-10, lot 665579, was obtained from Difco Labs., Detroit, Michigan. Myeloma IgA-1 (kappa) was generously provided by Dr. Richard Wistar at the Naval Medical Research Institute, Bethesda, Maryland and was heat-aggregated to slight opacity at 66°C (6 min).

PMN Preparation. The method used was a modification of the one-step method of Ferrante and Thong (3): Blood was drawn via venipuncture into a 60-ml syringe and expressed into 5 ml of HBSS containing 0.15 ml of heparin sodium (10,000 units/ml). The flask was swirled and the blood gently pipetted into six 16 x 125-mm plastic tubes preloaded with 5 ml of Ficoll-Hypaque solution and held at a 45° angle to minimize mixing. Ficoll-Hypaque consisted of 40 g sodium diatrizoate and 25.4 g Ficoll 400, brought to 267 ml with distilled water, passed through a 0.22 μm filter, and stored at 4°C. The tubes were spun at 312–372g for 45 min. The neutrophil-rich bands were removed, pooled, washed, and resuspended at $10^6$ PMNs/ml in HBSS and put in ice or held at room temperature for at least 30 min prior to use. Such preparations typically contained greater than 98% viable leukocytes, of which greater than 94% were PMNs. Remaining red blood cells (RBCs) (30–80%) were not removed since the RBCs were washed out of the wells prior to assay and because RBC LDH activity is less than 10% of the PMN LDH activity on a per cell basis. (Gabler, Bullock, Creamer, unpublished).

PMN Adherence Assay. The wells of Falcon Microtest III plates received 50,000 cells/well, HBSS, serum, and FMLP, LPS, IgA, or 10 mM iodoacetamide as indicated in the Results. Each well was brought to a final volume of 0.2 ml with HBSS. Plates were incubated with reagents and cells for 1 h at 37°C to induce cell adherence and then gently washed 2–5× with 0.2 ml/well PBS