CHEMOTAXIS OF POLYMORPHONUCLEAR LEUKOCYTES IN RESPONSE TO SURFACE-BOUND COMPLEMENT-DERIVED CHEMOATTRACTANTS GENERATED IN SITU

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Abstract—Polymorphonuclear leukocytes (PMNLs) were allowed to migrate on slides with fixed yeast particles dotted about on the surface. Locomotion was quantified by counting the number of yeast particles in association with a PMNL. Yeast particles that differed in their ability to consume hemolytic complement differed also in their ability to generate chemoattractants. Addition of a complement source to yeast particles able to activate the complement system resulted in a chemotactic response, as well as when fluid-phase attractants were removed prior to the measurement of PMNL chemotaxis, indicating that the chemoattractants generated were adsorbed to the surface. Using an immunofluorescence technique, it was found that complement factor 5 coated a circular area around each yeast particle, provided that the particles were able to activate the complement system.

INTRODUCTION

Polymorphonuclear leukocytes (PMNLs) are of prime importance in the defence against invasive agents. To fulfill this role, the PMNL system is dependent on a number of steps, including migration towards the site of infection and phagocytosis of the invading agent (1). In order for locomotion of PMNLs to take place, cell adhesion to a supporting substratum is a prerequisite (2). It has been suggested that PMNLs (3) and other types of cells (4) may move directionally along a substratum by detecting surface differences, and PMNLs have been shown to be able to detect and respond to surface-bound concentration gradients of purified chemotactic substances (5, 6). In this report we present an experimental model, by which it is possible to investigate PMNL locomotion in response to surface-bound gradients of chemotactic complement components generated in situ.
MATERIALS AND METHODS

Polymorphonuclear Leukocytes (PMNLs). PMNLs were prepared from human peripheral EDTA-blood by dextran sedimentation and Hypaque-Ficoll gradient centrifugation (7). After separation, the remaining erythrocytes were removed by hypotonic lysis, and the PMNL washed and suspended in Krebs-Ringer phosphate buffer (KRG) supplemented with 10 mM glucose, and 1% human serum albumin (HSA, AB KABI, Stockholm, Sweden).

Preparation of Yeast Particles. Saccharomyces cerevisiae (boiled in a water bath for 30 min) was labeled with fluorescein isothiocyanate (FITC; BBL, Cockeysville, Maryland) in a 0.5 M carbonate buffer at pH 9.5. The mixture, containing 10⁸ yeast particles and 0.1 mg FITC/ml, was incubated at 37°C for 30 min and then washed four times in KRG. The FITC-conjugated yeast particles were serum opsonized by mixing equal volumes of yeast particles and normal human serum (NHS), followed by an incubation of the mixture for 45 min at 37°C. The yeast particles were washed four times in distilled water before use.

Consumption of Hemolytic Complement. To examine the ability of different yeast particles to activate the complement system, opsonized or unopsonized yeast particles were prepared as described above and mixed with an equal volume of NHS (diluted 1/9 in Tris buffer). These mixtures were incubated at 37°C for 45 min. After centrifugation, the remaining activity of hemolytic complement in the supernatants was determined as hemolysis of amboceptor-sensitized sheep erythrocytes, determined in a Beckman DU-2 spectrophotometer at 541 nm (8).

Preparation of Microscope Slides. A water suspension of FITC-labeled yeast was applied to multisport glass slides (0.1 ml/slot; Dynatech AG, Switzerland). The slides were air dried and briefly heat-fixed over a bunsen burner, and yielded 1 × 10⁴ yeast particles/cm². In order to build up surface-bound gradients of complement components, 0.1 ml NHS diluted in KRG was added to each spot. The slides were incubated at 37°C for different lengths of time and then washed in KRG.

PMNL Locomotion Measurement System. Locomotion of PMNL was quantitated on glass slides containing yeast particles as described by Coble et al. (9). Two different experimental procedures were used to study the effect of fluid-phase and solid-phase gradients of complement components on locomotion of PMNL on glass slides containing yeast particles.

For the fluid phase: To the yeast-coated slides were added 0.1 ml PMNL suspension (2 × 10⁵/ml). The PMNLs were allowed to adhere to the slides for 20 min at 37°C in a moist chamber, and then nonadhering cells were washed off with KRG. To each spot was then added 0.1 ml NHS diluted in KRG and, after an incubation at 37°C for 30 min, the cells were fixed in cold methanol. The number of PMNLs associated with a yeast particle was counted by the use of a Zeiss incident-light fluorescence microscope, provided with phase-contrast optics.

For the solid phase: To the slides prepared to obtain surface-bound gradients of complement components were added 0.1 ml PMNL suspension (2 × 10⁵/ml) to each spot, followed by an incubation for 30 min at 37°C. The cells were fixed as described above, and the number of yeast particles in association with PMNLs was counted. In some experiments, the PMNLs were added to the slides by cytocentrifugation of the cell suspension (0.5 ml; 2 × 10⁴/ml) by the use of a Cytospin I (Shandon Elliot; 500 rpm for 5 min) followed by incubation for 30 min at 37°C. The slides were fixed and counted as described above.

Detection of Surface-Bound C3 and C5. To detect deposition of complement components on the surfaces, yeast slides incubated with NHS, as described above, were examined with an immunofluorescence technique. Deposition of C3 was detected by using FITC-conjugated anti-human C3c (Behringwerke AG, Marburg; diluted 1/20 in PBS). Deposition of C5 was detected by using a sandwich technique with goat anti-human C5 antiserum (Capple Laboratories; diluted 1/20 in PBS) in the first step and FITC-conjugated anti-goat IgG (Capple laboratories; diluted 1/20 in PBS) in the second step. The slides were examined in a Zeiss incident light fluorescence