Chemotaxis of Human Neutrophils and Monocytes Induced by *Cryptococcus neoformans*

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Chemotaxis of human neutrophils and monocytes was stimulated by sera activated with $\geq 1.25 \times 10^6$ *Cryptococcus neoformans*. Leukocytes from five renal transplant recipients had depressed chemotactic responses to *C. neoformans*-activated sera when compared with normal subjects ($P < 0.05$). Concentrations of cryptococcal capsular polysaccharide less than 1 mg/ml failed to generate chemotactic factors from sera.

Progressive infections caused by the encapsulated yeast *Cryptococcus neoformans* are usually associated with a scanty host inflammatory response, especially in brain (8, 12). *C. neoformans* activates the classical and alternative complement pathways in serum (5), generating factors chemotactic for rabbit neutrophils (10). Since human neutrophils and monocytes can kill *C. neoformans* (6), the degree of host chemotactic response may play a critical role in determining the outcome of cryptococcosis in humans. Therefore, generation of chemotactic factors by whole *C. neoformans* was studied, as well as effects of cryptococcal capsular polysaccharide on the process. To explore the potential biological significance of the process, results were compared by using leukocytes from normal volunteer subjects as well as from patients with conditions which are known to predispose to cryptococcosis.

A type A isolate to *C. neoformans* was maintained and used for preparation of capsular polysaccharide as in previous studies (5), as described by Bennett and Hasenclaver (1). Sera, neutrophils, and monocytes were prepared as in previous studies (4, 6). Subjects included normal volunteers, three patients receiving cytotoxic drugs and corticosteroid therapy for non-Hodgkin's lymphoma, and five clinically stable outpatient recipients of renal transplants. In this last group, all were receiving 100 to 150 mg of azathioprine without leukopenia. Four of five were receiving prednisone: one 30 mg/day, one 35 and 5 mg on alternate days, and two 10 mg/day (one with diabetes mellitus, one with successfully treated cryptococcal meningitis).

Chemotaxis assays were performed in modified Boyden chambers, each containing 2.3×10^6 neutrophils or monocytes per ml suspended in Gey medium (M. A. Bioproducts, Walkersville, Md.) with 2% bovine serum albumin above micropore filters. Sera were activated by incubation with killed *C. neoformans*, its polysaccharide, or nothing (control) for 30 min at 37°C followed by heat (56°C for 30 min), centrifugation, and dilution to 5% final serum concentration. Preliminary data verified that killed (100°C for 60 min) *C. neoformans* generated chemotactic factors as effectively as live organisms. Each time new batches of reagents were made, timed studies were performed to determine the optimum time period for neutrophil migration and to ensure that the leading front of neutrophils did not completely migrate through filters. Depending upon batches of reagents used, optimum migration was observed in individual experiments with incubations between 30 and 45 min. After incubation of neutrophils for 30 to 45 min at 37°C, filters (3-μm pore, nitrocellulose) were removed, fixed, and stained as described by Clark et al. (2), and the leading front of migrating cells was determined (2, 15). Monocyte chemotaxis was performed according to the procedure outlined by Lohr and Snyderman (11); after incubation for 90 min at 37°C, filters (5-μm pore, polycarbonate) were removed for counting of monocytes migrating to the bottoms of filters. Chemotactic indices were calculated from leukocyte migration in attractant divided by leukocyte migration in controls.

Chemotactic factors for neutrophils and monocytes from normal subjects were generated by activation of autologous serum with 1.25×10^6 *C. neoformans*. Monocytes prepared by the standard Hypaque-Ficoll separation procedure were used routinely, as there was no significant difference in results when 90% pure monocytes (prepared by elutriator) were used. Antibodies to human C5 but not to C3 (Meloy, Springfield, Va.) eliminated the chemotactic response of neutrophils and monocytes to *C. neoformans*-activated sera, and no chemotactic factors were

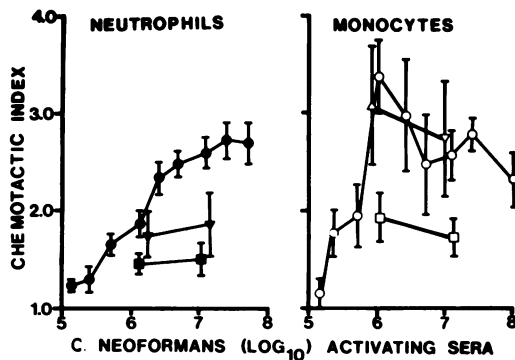NEUTROPHILS   MONOCYTES

C. NEOFORMANS (LOG₁₀) ACTIVATING SERA

FIG. 1. Chemotaxis induced by sera activated by various numbers of *C. neoformans*. Results are shown on the left for neutrophils from 24 normal subjects (●), 3 patients with non-Hodgkin's lymphoma (▲), and 5 renal transplant recipients (■). On the right, results for monocytes from these subjects are shown; normal (○), lymphoma patients (△), and renal transplant recipients (□). Each point denotes the mean, and vertical brackets denote the standard error of the mean for a set of observations.

generated from heated sera (56°C, 30 min). Compared with normal subjects, leukotaxis was depressed in five renal transplant recipients ($P < 0.005$ for neutrophils responding to autologous sera activated by 1.25×10^7 *C. neoformans*, $P < 0.02$ and $P < 0.05$ for monocytes responding to sera activated by 1.25×10^7 and 1.25×10^6 *C. neoformans*, respectively). Leukocyte migration did not differ significantly when normal serum was used to stimulate migration by cells from renal transplant patients, and sera from renal transplant patients did not affect migration by normal cells. These data, therefore, imply a cellular defect in the chemotactic response to cryptococcosis in these patients. Leukotaxis in three lymphoma patients did not differ significantly from normal values.

In five experiments, 2 mg of cryptococcal capsular polysaccharide per ml of sera induced a modest increment in neutrophil migration (chemotactic index 1.61 ± 0.21, $P < 0.05$); 1 mg of polysaccharide stimulated monocyte chemotaxis (1.50 ± 0.18, $P < 0.02$). Neither lower polysaccharide concentrations in serum nor polysaccharide or whole yeasts in the absence of serum were chemotactic. The presence of 1 mg of polysaccharide per ml of serum during activation by cryptococci combined with the addition of polysaccharide to incubations in the chemotactic chambers inhibited leukocyte migration (1.89 ± 0.12 versus 2.57 ± 0.24, $P < 0.05$). However, inhibition of chemotaxis was not significant if the polysaccharide was added in these concentrations either only to the activation step or to the incubation with leukocytes. Thus,

cryptococcal polysaccharide itself had minimal chemotactic activity, but when added to serum, it had the capacity to interfere with the normal, serum-dependent chemotactic response.

Hydrocortisone ($\geq 10^{-4}$ M) inhibited chemotaxis by monocytes only when present both during serum activation and in both sides of chambers (2.57 ± 0.24 reduced to 1.230 ± 0.10, $P < 0.01$).

C. neoformans yeast cells presumably generated C5a from unheated human serum, stimulating chemotaxis of human neutrophils and monocytes. Laxalt and Kozel showed comparable results with neutrophils and sera from experimental animals (10).

In contrast to intact *C. neoformans*, capsular polysaccharide generated chemotactic factors from sera only in high concentrations not generally found in cryptococcosis (3), although documented in rare patients (13). Higher concentrations of polysaccharide might cause even greater inhibitory effects, but the biological relevance of these effects would be questionable. Moreover, solutions of higher concentrations of polysaccharide have viscosities high enough to produce nonspecific effects on leukocyte function. Activation of complement by cryptococcal polysaccharide was described by Gadebusch (9) and then confirmed in this laboratory (5) by using polysaccharide prepared from a serotype A isolate. However, complement consumption in sera of cryptococcosis patients occurs only in association with fungemia (11), and Laxalt and Kozel noted no complement activation by ≤ 1 mg/ml concentrations of polysaccharide prepared from a serotype D isolate (10). Polysaccharide concentrations tested in our studies were higher, and currently available preparations of type A polysaccharide do activate complement, perhaps due to structural differences in the polysaccharides or to contamination with cell wall components (Thomas R. Kozel, personal communication). High concentrations of polysaccharide also inhibited the chemotactic response to cryptococcal cells in serum, similar to a phenomenon described by Drouhet and Segretain (7).

Immunosuppression, especially with azathioprine, may inhibit the chemotactic response to cryptococci, as all five renal transplant recipients were receiving azathioprine but not necessarily pharmacological doses of corticosteroids. Hydrocortisone added in vitro inhibited chemotaxis by normal monocytes only in a 10^{-4} M concentration. The biological implications of these observations are limited by the small number of patients studied and the use of healthy, normal subjects as controls; therefore, these data are preliminary. Testing of additional subjects, including patients with different stages of Hodgkin's and other lymphomas who are receiv-

ing different chemotherapeutic regimens, are needed. Abnormal chemotaxis has been associated with cutaneous anergy in cryptococcosis (14), and chemotactic responsiveness of host cells may prove to be a major factor determining the outcome of cryptococcosis.

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