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Experimental Pneumococcal Meningitis: III. Chemotactic Activity in Cerebrospinal Fluid[1] (38989)

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(Introduced by R. G. Petersdorf)

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Evidence is accumulating that mortality in pneumococcal meningitis is related to inflammation in and around the meninges (1–3). Quantitative determinations of meningeal inflammation in a rabbit model of pneumococcal meningitis demonstrated an increase in leukocyte infiltration early in the infection with the peak at about the time the average animal dies (3). This study was undertaken to determine the stimulus for this influx of acute inflammatory cells. Neutrophil chemotactic activity was detected in cerebrospinal fluid (CSF) in the animal model of meningitis and the responsible factors were partially characterized.

Methods. The method of inducing pneumococcal meningitis in rabbits (intracisternal mucin plus iv pneumococci, Type III) and the clinical course of the infection have been described in detail (4). Groups of animals infected simultaneously were subjected to cisternal puncture either 24, 48, or 72 hr after infection. Supernatants of CSF from several infected rabbits were pooled, passed through a bacterial filter and tested undiluted for chemotactic activity for normal circulating rabbit granulocytes. Pooled CSF was concentrated five to tenfold by ultrafiltration (Amicon Model 12 with UM-2 filter, Amicon Corp., Lexington, MA) prior to fractionation by gel filtration on calibrated columns of Sephadex G-25 and G-75 (Pharmacia Fine Chemicals, Piscataway, NJ). Fractions of 2 ml eluted with 0.01 *M* phosphate buffered normal saline at 4° were assayed for chemotactic activity and protein content (absorbance at 280 mn).

Individual 72-hr CSF specimens were analyzed for total hemolytic complement (sensitized sheep erythrocyte hemolysis) and C4 (radial immunodiffusion against antibody to human C4, Meloy Laboratories, Springfield, VA). CSF and concentrated chemotactically active fractions from a Sephadex G-75 column were absorbed with antihuman C3 and C5 sera (Meloy Laboratories) or antitype III pneumococcal serum (Difco Labs., Detroit, MI) and then tested for chemotactic activity. Purified Type III pneumococcal polysaccharide (courtesy of Dr. George Kenney) at 10 and 50 μg/ml and supernatants of cultures of Type III pneumococci in medium 199 (BBL, Cockeysville, MD) were also tested for chemotactic activity.

Chemotaxis was measured by a previously described method (5) employing two-compartment chambers (Ahlco Machine Company, New Brunswick, CN, and Mark-it Corp., Chicago, IL) and rabbit blood granulocytes prepared by dextran sedimentation and labeled with ^{51}Chromium (6). Results were expressed as mean corrected CPM (5) of triplicate chemotaxis chambers $\pm$ SEM.

Results. Control CSF from noninfected rabbits had no granulocyte chemotactic activity (mean of 16 experiments 156 CPM $\pm$ 17 versus a mean for Gey's medium (6) controls of 234 CPM $\pm$ 51 in 8 experiments). In contrast, a progressive increase in chemotactic activity of CSF was observed following the development of meningitis (Fig. 1). The activity at 48 and 72 hr was consistently and significantly ($P < .01$) greater than control values. Rabbits given intracisternal mucin but no iv pneumococci developed a transient chemical meningitis

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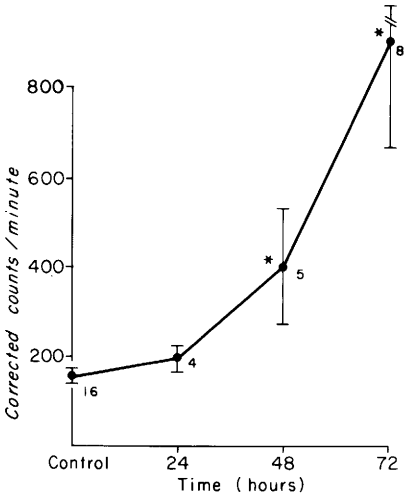

FIG. 1. Chemotactic activity in CSF of rabbits with pneumococcal meningitis. Each point represents the mean ± SEM of the number of experiments indicated. *Denotes values significantly different from controls ($P < .01$, t test).

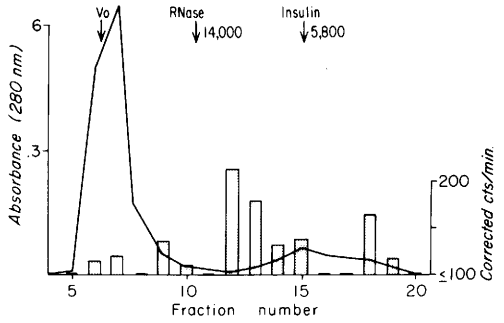

FIG. 2. Sephadex G-75 gel filtration of CSF from rabbits with pneumococcal meningitis. Absorbance at 280 nm (——) and chemotactic activity (stippled bars) of each fraction are plotted. Arrows indicate void volume (Vo) and elution position of marker proteins.

(4) and their CSF contained moderate chemotactic activity after 24 hr but none at 72 hr.

Chemotactic activity in infected CSF was not dialyzable and was stable at −80° for 2 weeks, 4° for 1 week and 56° for 30 min. Activity was abolished by heating at 100° for 15 min. Supernatants of CSF precipitated with 5% trichloracetic acid and both supernatants and redissolved precipitates of CSF saturated with ammonium sulfate were devoid of chemotactic activity.

Chromatography of infected CSF on Sephadex G-25 resulted in elution of most of the protein in the exclusion volume (mol wt greater than 10,000). Chemotactic activity was also concentrated in the exclusion volume, but a small amount of activity was present in fractions eluting after the marker protein insulin. On a Sephadex G-75 column (Fig. 2) most protein again eluted with the exclusion volume (mol wt > 50,000). However, the major peak of chemotactic activity occurred in fractions eluting between ribonuclease and insulin, corresponding to a mol wt of about 11,000; a smaller peak eluted after insulin, indicating a mol wt of approximately 3000.

Neither hemolytic complement activity nor C4 were detectable in infected CSF. Absorption of infected CSF or concentrated fractions comprising the 11,000 mol wt peak of chemotactic activity from a Sephadex G-75 column with antihuman C3 and C5 sera did not reduce chemotactic activity. Approximately 50% of the chemotactic activity was removed from an aliquot of infected CSF by absorption with antiserum to type III pneumococci. Significant chemotactic activity was detected in filtrates of overnight cultures of type III pneumococci and in solutions of purified Type III pneumococcal capsular polysaccharide.

Discussion. This study, showing that granulocyte chemotactic activity appears in CSF of rabbits with pneumococcal meningitis, adds another to the list of chemotactically-active biologic fluids from areas of inflammation (8–11). Note that the time course of appearance of chemotactic activity in CSF during infection closely parallels that of accumulation of the inflammatory mass of granulocytes within the meninges (3).

A cause-and-effect relationship between these two observations has not been established. Nevertheless, the detection of a potential mediator of central nervous system inflammation assumes importance in view of increasing evidence that meningeal inflammation is a determinant of mortality in pneumococcal meningitis. If this supposition proves correct in subsequent experiments, suppression of the inflammatory response

might be beneficial in the treatment of pneumococcal meningitis. Modification of the chemotactic stimulus is one possible therapeutic approach to this problem; characterization of CSF chemotactic factors and their origin would facilitate this approach.

Our preliminary work suggests that CSF chemotactic activity is attributable to relatively low molecular weight substances which are heat stable and either protein in nature or dependent upon protein for their activity. Chemotactic activity of infected CSF was party absorbed by specific pneumococcal antiserum, suggesting that a bacterial factor accounts for a portion of it. Pneumococci produce low molecular weight (<3600) chemotactic activity during growth (12).

Release of complement derived chemotactic factors in infected CSF might also be expected since pneumococci activate complement via the alternate pathway (13). Although complement activation products cannot be implicated on the basis of current evidence, CSF chemotactic activity detected in this model has characteristics in common with the potent chemotactic agent C5a. Both are stable at 56° and the mol wt of rabbit C5a (12,500) (14) is similar to that of the major CSF chemotactic peak separated by gel filtration. Clarification of the roles of microbial products and host factors in the inflammatory response in meningitis is critical and deserves further study.

Summary. Chemotactic activity was assayed in CSF of rabbits with pneumococcal meningitis to further characterize the inflammatory response in this infection. CSF chemotactic activity was detected in increasing levels for 72 hr after infection. Activity was stable at 56° and was inactivated by agents which denature proteins. Gel filtration demonstrated two chemotactically active fractions in infected CSF with mol wts of approximately 3000 and 11,000. Bacterial products appear to account for a portion of the observed CSF chemotactic activity, but the role of host factors remains to be clarified.

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