Leukocyte Survival in Cerebrospinal Fluid

RUSSELL W. STEELE,* DANIEL J. MARMER, MARCUS D. O'BRIEN, SAMUEL T. TYSIN, AND CHRISTOPHER R. STEELE

Section of Immunology and Infectious Diseases, Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, Arkansas 72207

Received 28 October 1985/Accepted 23 January 1986

Delays in the laboratory examination of cerebrospinal fluid are commonly encountered in clinical medicine. The present studies were designed to evaluate changes in cerebrospinal fluid leukocyte counts relative to time elapsed before analysis. Neutrophil counts decreased most rapidly, being 68 ± 10% (standard error of the mean) and 50 ± 12% of initial values at 1 and 2 h, respectively. Lymphocyte and monocyte numbers were not significantly altered until 3 h.

Under ideal clinical circumstances, cerebrospinal fluid (CSF) obtained by lumbar puncture is rapidly transported to the pathology laboratory for analysis. Management decisions can then be made based on reported CSF parameters. There are, however, many circumstances which may delay analysis of spinal fluid, particularly if the patient is transferred to a referral center following a lumbar puncture which yielded cloudy CSF. It is also not unusual for specimens to remain in an emergency room for an hour or more while the patient is stabilized and treatment instituted, because these are priority concerns. Delays also occur in the clinical laboratory, where technicians may first complete other ongoing tests.

Because CSF is hypotonic (2) (specific gravity, 1.007), leukocytes lyse if left in this medium for extended periods. For most cases of bacterial meningitis, such a delay should not confuse interpretation of laboratory data. However, there are some situations, such as the differentiation of early bacterial meningitis from aseptic meningitis, in which cell counts may be more critical. We therefore analyzed survival of neutrophils, lymphocytes, and monocytes in CSF over periods comparable to the duration of delay in processing often encountered in clinical practice.

Lymphocytes and monocytes were separated from peripheral whole blood by using a Ficoll-Hypaque density gradient, and neutrophils were obtained by double-density gradient centrifugation as previously described (7). With this methodology, cell populations (mononuclear or neutrophil) were >90% pure. Acridine orange was used to differentiate cell types in the counting procedures.

CSF was pooled from samples sent to the clinical laboratory during the evaluation of possible meningitis in children. These samples had normal parameters, including fewer than 10 mononuclear cells per mm³. All fluids were centrifuged to remove cells and passed through Millipore membrane filters to eliminate other possible contaminants.

Sterile plastic tubes were used for all studies, because they are equivalent to containers normally used to collect CSF. In early experiments, cells were shown not to adhere to plastic, because the addition of trypsin did not increase the recovery of leukocytes. Purified leukocyte populations were adjusted to various concentrations from 0.4 × 10⁶ to 1.25 × 10⁶/ml (400 to 1,250/mm³) in CSF and placed at room temperature before reanalysis of cell counts. These experiments were repeated at least 11 times with different donors. Controls included similar cell populations maintained for the same time in buffered normal saline.

CSF neutrophils. To assure that the experimental conditions of this study accurately reflected the status of leukocytes in infected CSF, fresh spinal fluid was obtained from five patients undergoing therapy for culture-positive bacterial meningitis. All had a pleocytosis greater than 1,000/mm³ at the time of sampling. The CSF leukocytes were examined for viability and functional capacity as described below.

Viability and functional capacity of neutrophils. An assay using acridine orange as a vital stain was used to assess the viability of neutrophils and, at the same time, to directly visualize phagocytic and killing processes of peripheral blood or CSF cells. Live bacteria stain green with acridine orange, and this color changes to bright red when the bacteria are killed by phagocytes. Details of this assay were previously reported (9). Briefly, monolayers were first prepared by placing 5 drops of purified peripheral leukocytes or 5 drops of CSF obtained from meningitis patients onto flat glass cover slips and allowing attachment of these cells during 1 h of incubation at 37°C in 5% CO₂. The supernatant fluid was then removed by gentle washing with normal saline, and 2 drops of preopsonized Streptococcus pneumoniae type 14 containing approximately 10⁸ bacteria per ml in phenol-free Hanks balanced salt solution with 10% autologous human serum were placed on the cell monolayers. Cover slips were then incubated at 37°C for 90 min. After incubation, monolayers were washed twice with normal saline and stained for 1 min with 0.14% acridine orange in Gey balanced salt solution. Cover slips were mounted face down and examined by fluorescence microscopy. Approximately 100 neutrophils were counted, and the total number of bacteria phagocytized and the percent killed (red) and percent alive (green) were recorded. Viability of cells at all time points was >90%; therefore, enumerated cells were considered viable for data presentation.

No differences in leukocyte survival were seen according to the beginning cell count or to the leukocyte donor. Therefore, all data were combined for analysis. Changes in cell counts by time are shown in Table 1. To simplify interpretation, data are presented as percentage of the initial

* Corresponding author.
(zero time) leukocyte concentration. A more rapid decrease in the neutrophil population compared with mononuclear cells was evident, with only half of the neutrophils remaining by 2 h. Lymphocyte survival was similar to that in monocyte studies in that significant reductions ($P < 0.05$, compared with zero time) were not seen until 3 h in CSF.

Lymphocytes and monocytes maintained in buffered normal saline for 4 h at room temperature never demonstrated any change in concentration; in contrast, neutrophil concentrations decreased to 87 ± 2% (standard error of the mean) and 82 ± 5% at 3 and 4 h, respectively (both $P < 0.05$, compared with initial counts). At all time points, 0.5 to 4 h, neutrophils in CSF had significantly reduced concentrations compared with those in buffered saline ($P < 0.01$).

Viability of peripheral blood neutrophils 1 h after being added to CSF was 95.6 ± 2.4% compared with that of neutrophils recovered from patients with meningitis, which were 94.1 ± 5.3% viable ($P > 0.05$). The average numbers of bacteria phagocytized (4.7 ± 0.7 versus 1.1 ± 1.6) and percent killed (75.8 ± 11.5% versus 71.7 ± 7.2%) were not significantly different for the two neutrophil sources.

It is important in the practice of medicine to recognize those occasions in which a delay in the laboratory testing of specimens may alter results. Cell counts in CSF represent such a circumstance. Relevant clinical situations might best suggest times when the recalculation of counts following delay in CSF analysis might be considered. A minimal pleocytosis of 10 to 30 cells per mm$^3$ may be seen with tuberculous and fungal meningitis (3), central nervous system syphilis (5), and herpes encephalitis (8). This is not much different from the normal CSF counts in children of ≤10 mononuclear cells per mm$^3$ and adults of ≤5 cells per mm$^3$.

As a contrasting feature in the differentiation of Guillain-Barré syndrome from polymyelitis or asptic meningitis, a cell count less than 50/mm$^3$ is more compatible with the former, whereas a greater pleocytosis is more characteristic of the latter (1). Accurate cell counts are important in the management of partially treated meningitis, because adequacy of therapy is in part determined by this parameter in repeat lumbar punctures (4).

Analysis of CSF leukocytes from patients with meningitis for viability and functional capacity, i.e., phagocytosis and microbial killing, indicated that the present mixing experiments were comparable to the in vivo situation. These studies of recovered CSF neutrophils therefore support further interpretation of our in vitro data.

The decrease in neutrophil count at 1 h by 32% and at 2 h by 50% of the initial concentration suggests that clinical errors may occur if analysis of samples is delayed. It is not surprising that neutrophils lysed more rapidly than lymphocytes or monocytes, because under ideal circumstances neutrophils remain viable for only 7 h. Besides hypotonicity of CSF, low concentrations of proteins and lipids which normally function to stabilize leukocyte membranes would also contribute to cell destruction (6). Our buffered normal saline controls, which were isotonic but contained no plasma proteins and lipids, demonstrated that lack of membrane stabilization accounts for some neutrophil destruction, but only after 3 h.

These data emphasize the need for clinicians to assure the rapid analysis of CSF. If this cannot be achieved, corrections may be used for cell counts based on the duration of delay in examination.

**LITERATURE CITED**