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RED BLOOD CELLS FROM ALZHEIMER PATIENTS AND FROM NORMAL SUBJECTS DISCERNED BY CELL ELECTROPHORESIS IN AN AQUEOUS POLYMER SOLUTION

Harry Walter[1], Kim E. Widen[1] and Stephen L. Read[2]

[1]Laboratory of Chemical Biology, Veterans Affairs Medical Center, Long Beach, CA 90822

[2]John Douglas French Center for Alzheimer's Disease, Los Alamitos, CA 90720

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Blood from patients diagnosed as having Alzheimer disease and from subjects without memory impairment or dementia was collected in citrate. The erythrocytes were washed and electrophoresed in phosphate-buffered saline as well as in a number of polymer solutions in phosphate-buffered saline. The electrophoretic mobilities of red cells from Alzheimer patients and from normals were indistinguishable when measured in phosphate-buffered saline. In the dextran-rich bottom phase obtained from a dextran-poly(ethylene glycol) aqueous phase system, but not in a dextran solution alone, the electrophoretic mobilities differ ($P < 0.001$). In a poly(ethylene glycol) solution the mobilities also differ although, on a percentage basis, less so than in the bottom phase. It would appear that a differential adsorption of appropriately selected polymer(s) on the Alzheimer and normal red blood cells renders surface differences electrophoretically detectable.

Numerous reports in the literature relating extra-neuronal abnormalities in Alzheimer disease (AD) [for a review see (1)] including altered properties in red blood cell (RBC) membranes (2-5) prompted us to test, by partitioning and by electrophoresis, whether such alterations are detectable at the cell surface.

Partitioning of cells in dextran-poly(ethylene glycol) (PEG) aqueous two-phase systems is a sensitive method for the detection of differences in surface properties (6). Examination of the partitioning behavior of RBC from AD patients and from subjects without memory impairment or dementia ("normals") revealed, however, that AD RBC partition ratios fall within the range associated with RBC obtained from normals (7). Electrophoresis in phosphate-buffered saline (PBS) also revealed no difference in electrophoretic mobilities (EPM) between RBC from AD and normals.

An unrelated study in our laboratory in which possible correlations were sought between the partitioning behavior of RBC from different species in a dextran-PEG

Abbreviations: AD, Alzheimer disease; EPM, electrophoretic mobility; PBS, phosphate-buffered saline; PEG, poly(ethylene glycol); RBC, red blood cells.

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aqueous phase system and these cells' relative EPM (6) in PEG-rich top or dextran-rich bottom phase prompted us to also examine the EPM of RBC from AD and normals in these polymer solutions. It was in this manner that we found the striking difference between the EPM of AD and normal RBC in dextran-rich bottom phase reported here.

EXPERIMENTAL METHODS

The aqueous two-phase system, made as previously described (6), contained 5% (w/w) dextran T500 (Pharmacia-LKB, Piscataway, NJ), 3.5% (w/w) PEG 8000 (PEG, Union Carbide, Long Beach, CA), 0.15 M NaCl + 0.01 M Na-phosphate buffer, pH 6.8. The system was mixed and permitted to settle in a separatory funnel overnight at 21-24°C. Top and bottom phases were then separated with the material at the interface being discarded. The PEG-rich top and the dextran-rich bottom phases were centrifuged at 12000 x g for 15 min to ensure that phase separation was complete. Top phase was removed leaving all remaining bottom and some top phase behind in the centrifuge tube. Bottom phase was pipetted from the middle of the tube being careful to keep any residual top phase from entering the pipette.

All patients met criteria for Dementia (8) and for Probable or Possible AD (9). Consent was obtained from patients and respective responsible parties per institutional guidelines and approved procedures. Patients were selected after thorough evaluation including physical and mental status examination, blood studies, electrocardiography, electroencephalography (EEG), and brain imaging with x-ray CT scan or magnetic resonance imaging of the brain (10,11). The majority also had functional imaging with quantitative EEG and/or Single Photon Emission Computed Tomography (SPECT). Subjects had been followed longitudinally for a minimum of six months (maximum 9 years) in a specialized dementia program to establish progression of dementia and to clarify the role of any identified concomitant medical disorders. Blood in these experiments was not obtained during acute medical illness. Confirmation of clinical diagnoses of Probable AD post mortem from the clinical program (not including any members of the sample reported here) has been 100% to date (unpublished data).

Blood from AD patients and from normals (comprising some spouses of Alzheimer patients, some other elderly individuals and some younger donors who were employees of the VAMC) - spanning the age range from 22 to 83 years - was collected in citrate vacutainer tubes. [Note: blood collected in heparin, either because the molecule is highly charged or acts in a different part of the clotting cascade, does not yield the differences in EPM between AD patients and normals reported here.]

RBC were washed three times with PBS (0.15 M NaCl + 0.01 M Na-phosphate buffer, pH 6.8) and a suitable cell aliquot was, finally, suspended in PBS. Aliquots of the latter cell suspensions were diluted 1:1 (by weight) with top or bottom phase (of the above-indicated phase system), 8% (w/w) dextran in PBS or 5% (w/w) PEG in PBS for EPM measurements (see below).

Cell microelectrophoresis was carried out in a cylindrical chamber (Rank Brothers Ltd., Cambridge, UK) at 25 ± 0.2°C with transillumination (12). Measurements were made using an applied voltage of 50.0 resulting in a field strength ranging from 2.28 to 2.58 V/cm depending on the suspending medium used. In each sample the rates of migration of ten RBC were obtained at the stationary level for the calculation of EPM in μm/sec/V/cm (12). The rates of migration were observed in alternate directions. The viscosities of the various suspending media were estimated by means of an Ostwald viscometer immersed in a tank thermostated at 25 ± 0.2°C. The EPM of the RBC in the different media were corrected to the viscosity of water.

The EPM obtained in the different suspending media are presented, in each case, as the mean ± SD with the number of individuals in parentheses. P values were obtained by one way analysis of variance (ANOVA). EPM in bottom phase of RBC from individual AD patients and normals are depicted in a histogram.

RESULTS

Table I presents the viscosity-corrected EPM in five different suspending media of RBC from AD patients and from normals. In a standard suspending medium (i.e., PBS) there is no difference in the mobilities of these two cell populations. In the 1:1 diluted dextran-rich bottom phase as well as in a 2.5% (w/w) PEG 8000 solution, statistically significant differences in mobilities are observed.

Despite the clear differences in mobilities between RBC from AD patients and normals in (diluted) dextran-rich bottom phase as well as in a PEG solution no such differences can be discerned in a dextran solution in PBS or in (diluted) PEG-rich top phase from the above-indicated phase system (Table I). See **Discussion** below.

The data obtained in bottom phase are presented in greater detail in Fig. 1. It is apparent that the mobilities of RBC from AD patients and from normals fall essentially under two distinct distribution curves. In other experiments RBC from

TABLE I

Viscosity-corrected electrophoretic mobilities of red blood cells (RBC) from Alzheimer patients and from normal individuals in five different suspending media[1]

Suspending medium	Normal RBC	Alzheimer RBC	
PBS[2]	-1.08 ± 0.01 (23)	-1.08 ± 0.01 (23)	N.S.
top phase[3]	-1.82 ± 0.03 (12)	-1.81 ± 0.03 (12)	N.S.
bottom phase[3]	-4.56 ± 0.07 (23)	-4.87 ± 0.09 (23)	$P < 0.001$
dextran[4]	-4.32 ± 0.13 (13)	-4.30 ± 0.12 (13)	N.S.
PEG[5]	-1.41 ± 0.02 (14)	-1.46 ± 0.02 (14)	$P < 0.001$

[1] Data present the mean electrophoretic mobilities, EPM, (μm/sec/V/cm) ± SD with the number of individuals given in parentheses.
[2] PBS, phosphate-buffered saline was composed of 0.15 M NaCl + 0.01 M Na-phosphate buffer, pH 6.8.
[3] Top and bottom phases were from a system containing 5% (w/w) dextran T500, 3.5% (w/w) poly(ethylene glycol) (PEG) 8000, 0.15 M NaCl and 0.01 M Na-phosphate buffer, pH 6.8. Top phase is PEG-rich and bottom phase, dextran-rich. Top and bottom phases were diluted 1:1 with the indicated RBC suspension in PBS followed by measuring cell EPM.
[4] 4% (w/w) dextran T500 solution in PBS.
[5] 2.5% (w/w) PEG 8000 solution in PBS.

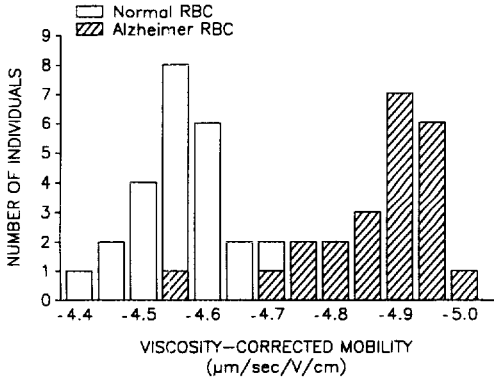

Fig. 1. Histogram of viscosity-corrected electrophoretic mobilities of RBC from patients with Alzheimer disease and from normal subjects in dextran-rich bottom phase (diluted 1:1 with a PBS suspension of the respective RBC) from an aqueous two-phase system composed of 5% (w/w) dextran T500, 3.5% (w/w) PEG 8000, 0.15 M NaCl + 0.01 M Na-phosphate buffer, pH 6.8. In the data presented RBC from each Alzheimer patient were run side by side with RBC from a different normal subject.

normals younger than or overlapping with AD patients in age (i.e., 22 to 62 vs. 68 to 83 years of age) were found to have indistinguishable EPM.

DISCUSSION

A large number of reports have described abnormalities in AD associated with blood and tissues, findings which raise the possibility that the disease is systemic (1). AD is diagnosed as outlined under **Experimental Methods**. Diagnosis can only be confirmed by brain tissue examination usually post mortem.

Fig. 1 shows that RBC from patients diagnosed as having AD have a significantly higher EPM in the dextran-rich bottom phase than do red cells from normal individuals. A few of the normal RBC samples were obtained from spouses of Alzheimer patients, some others from individuals in the same age range as the patients, and the majority came from younger individuals. No difference in electrophoretic behavior among RBC from normals of different ages was observed.

Note that there is one patient diagnosed as having AD (out of an overall total of 36 AD patients examined thus far) whose RBC EPM falls into the middle of the normal group. This patient has a mild AD, a state in which diagnosis is most difficult. It is not known whether the normal RBC EPM reflects the mild state of the disease, a misdiagnosis or an anomaly.

The difference in EPM between RBC from AD patients and from normal individuals is evident not only in the dextran-rich bottom phase from the indicated phase system but also in a PEG 8000 solution in PBS. Thus it seemed strange that no difference in mobility between these cell populations was found in either a dextran solution in PBS or the PEG-rich top phase (Table I). This phenomenon appears to be due to the generally observed dextran "contaminant" which becomes

readily visible to the naked eye at the interface between dextran-rich and PEG-rich phases in such two-polymer aqueous phase systems. The interface is discarded when the two phases are separated and the resulting bottom phase is therefore depleted in the material. Collection of bottom phase together with the interface (also followed by a 1:1 dilution with PBS) results in a suspending medium in which mobility differences between AD and normal RBC cannot be found thereby suggesting an inhibitory nature for the contaminant. The contaminant, as do many particulate materials (6), may well partition between the interface and the PEG-rich top phase rendering the latter also unable to discern differences between the two cell populations.

RBC tested from two patients with Parkinson's disease had the same EPM in the dextran-rich bottom phase as normal individuals. The altered EPM of RBC from AD is, hence, not a phenomenon shared by RBC in all neurologic diseases.

Human young and old RBC (from normals), prepared by a centrifugation method (13), have identical EPM values in buffered saline but display a statistically significant difference in EPM in a dextran solution (14). It appears that certain surface differences between cell populations that either are too small to be detected or are otherwise not reflected by electrophoresis in PBS are amplified or revealed by electrophoresis in polymer solutions as a consequence of a differential adsorption of polymer on the cells' surfaces. (The surface differences detected between young and old RBC and between AD and normal RBC also differ in that the difference can be discerned in dextran solutions in the former but not in the latter case).

CONCLUSIONS

We report a simple and rapid way in which RBC from AD patients and from normals can be discerned: cell electrophoresis in certain aqueous polymer solutions [e.g., in the bottom, dextran-rich, phase from a dextran-PEG aqueous phase system, (6)]. In such suspending media AD RBC have a significantly higher EPM than do RBC from normals.

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