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Erythrocyte Aging Characteristics in Elderly Individuals With Beginning Dementia


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SENSILE dementia of the Alzheimer type (SDAT) is a syndrome with a heterologous etiology (20,22). However, the final neuropathological lesions, plaques, and tangles, are identical for all patients. Because plaques, tangles, and cell loss (or shrinkage) are also observed—be it to a much smaller extent—in healthy individuals of advanced age, the pathophysiology of SDAT has been characterized as an accelerated and/or disturbed neuronal aging process (20).

However, too little is known about the normal neuronal aging process to falsify this hypothesis. In contrast, the final stages of the erythrocyte life are well defined (14). Structural changes in band 3, the erythrocyte anion transporter [AE1 of the anion exchanger gene family; (4)], do not only lead to characteristic functional changes, but also to neoantigen activity and binding of autologous IgG. Cell-bound IgG, either or not in combination with complement activation (16), leads to recognition and removal of old erythrocytes by the immune system (14).

Earlier, we measured some parameters of this paradigm (IgG content, anion transport characteristics, AE1 breakdown) in erythrocytes from patients in advanced stages of senile dementia of the Alzheimer type (SDAT), multifactorial dementia (MID), and from age-matched control donors (3). Our results led us to the conclusion that the erythrocyte aging process is disturbed in patients with SDAT. We reached an identical conclusion with regard to the erythrocyte aging process in donors with Down’s syndrome (4).

Recent findings support the hypothesis that Alzheimer-specific changes can be found at the level of the erythrocyte membrane (23). The changes in erythrocytes from individuals with Down’s syndrome who showed no signs of dementia (4) indicated that aberrant erythrocyte aging characteristics might already be detectable before the neuropsychological symptoms of dementia. Such a finding might be important for early diagnosis. Therefore, we measured erythrocyte aging characteristics (anion exchange, AE1 breakdown, IgG content) in elderly individuals with beginning dementia, and compared them with age-matched, old controls and with much younger control donors. Here we present the results of these investigations.

METHOD

Subjects

The subjects were 27 patients with senile dementia of the Alzheimer type (SDAT), 17 patients with multifactorial dementia (MID), 9 patients with mixed dementia (SDAT/MID), and 2 control groups of subjects without dementia: one age-matched (AMC; N = 14) and one young control group (YC; N = 8). The YC were coworkers of the authors (28 ± 5 years). All other subjects were residents of local homes for the elderly, who took part in a longitudinal study on psychiatric symptoms in dementia. The present study comprises patients in the more beginning stages of dementia than the patients from a psychogeriatric institution examined previously (3). Informed consent was obtained from all patients and control donors in the study.

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presented in Table 1.

The demographic characteristics are by the Consortium to Establish a Registry for Alzheimer’s Disease (12). Furthermore, they had undergone standardized examinations using the Clinical Assessment Battery as developed by the Consortium to Establish a Registry for Alzheimer’s Disease (17), including medical history, current medication, heteroanamnesis, neuropsychological investigation, physical and neurological examinations, a scheme for laboratory investigations, and a psychiatric diagnosis using the Geriatric Mental State Examination (6). Using this information, the demented subjects received a psychiatric diagnosis using the Geriatric Mental State Examination (6). According to their Hachinski score (10), they were divided into three subgroups: SDAT (score 0-4), SDAT/MID (score 5 and 6), and MID (score ≥7). The age-matched control group consisted of subjects who did not fulfill the DSM-III-R criteria of dementia. The following exclusion criteria for demented and control subjects were used (17): communication problems interfering with test completion (severe hearing problems, severe aphasia), cognitive impairment caused by alcohol abuse, Parkinson’s disease requiring medication, major depression, specific neurological disease with impairment caused by alcohol abuse, Parkinson’s disease requiring medication, major depression, specific neurological disease with dementia (with the exception of cerebrovascular disorders and Alzheimer’s disease), or an incomplete Hachinski score.

All subjects were scored on two cognitive screening instruments, the Mini-Mental State Examination (7) and the Short Blessed Test (12). Furthermore, they had undergone standardized examinations using the Clinical Assessment Battery as developed by the Consortium to Establish a Registry for Alzheimer’s Disease (17), including medical history, current medication, heteroanamnesis, neuropsychological investigation, physical and neurological examinations, a scheme for laboratory investigations, and a psychiatric diagnosis using the Geriatric Mental State Examination (6). Using this information, the demented subjects received a diagnosis of dementia compatible with the DSM-III-R criteria (1). According to their Hachinski score (10), they were divided into three subgroups: SDAT (score 0-4), SDAT/MID (score 5 and 6), and MID (score ≥7). The age-matched control group consisted of subjects who did not fulfill the DSM-III-R criteria of dementia. The following exclusion criteria for demented and control subjects were used (17): communication problems interfering with test completion (severe hearing problems, severe aphasia), cognitive impairment caused by alcohol abuse, Parkinson’s disease requiring medication, major depression, specific neurological disease with dementia (with the exception of cerebrovascular disorders and Alzheimer’s disease), or an incomplete Hachinski score.

**Clinical Measurements**

All subjects were scored on two cognitive screening instruments, the Mini-Mental State Examination (7) and the Short Blessed Test (12). Furthermore, they had undergone standardized examinations using the Clinical Assessment Battery as developed by the Consortium to Establish a Registry for Alzheimer’s Disease (17), including medical history, current medication, heteroanamnesis, neuropsychological investigation, physical and neurological examinations, a scheme for laboratory investigations, and a psychiatric diagnosis using the Geriatric Mental State Examination (6). Using this information, the demented subjects received a diagnosis of dementia compatible with the DSM-III-R criteria (1). According to their Hachinski score (10), they were divided into three subgroups: SDAT (score 0-4), SDAT/MID (score 5 and 6), and MID (score ≥7). The age-matched control group consisted of subjects who did not fulfill the DSM-III-R criteria of dementia. The following exclusion criteria for demented and control subjects were used (17): communication problems interfering with test completion (severe hearing problems, severe aphasia), cognitive impairment caused by alcohol abuse, Parkinson’s disease requiring medication, major depression, specific neurological disease with dementia (with the exception of cerebrovascular disorders and Alzheimer’s disease), or an incomplete Hachinski score.

**Blood Collection**

Five to ten milliliters of blood, collected with EDTA as an anticoagulant, served for all assays. The assays were performed on the major erythrocyte fraction, representing “middle-aged” cells (2). Separation of plasma, platelets, leukocytes, and young, middle-aged and old red blood cells was performed on the day of collection by differential centrifugation and density separation (3).

**Erythrocyte-Bound IgG**

The amount of membrane-bound immunoglobulin G (IgG) was measured on intact erythrocytes using an enzyme-linked antiglobulin test (3).

**Anion Transport Characteristics**

Erythrocyte anion transport characteristics were determined by measuring initial rate of efflux of 35S-labeled sulfate (10 mCi/mmol; Amersham, Buckinghamshire, UK) under exchange conditions as a function of the sulfate concentration (2,3,13). $K_m$, the sulfate concentration at which the rate equals half the maximal velocity $V_{max}$, was determined from the initial, linear part of the velocity vs. concentration graph (2,3).

**Immunochemical Analysis**

Erythrocyte membranes were prepared by hypotonic lysis in the presence of EDTA, EGTA, and disopropylfluorophosphate as protease inhibitors (3). Band 3 polypeptides were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblotting using standard techniques described before (3). The reactivity of the antisera against human erythrocyte band 3 (MK1B3 against the whole protein and PMB3 against the membrane domain) have been described before (3,4). Quantitative analysis of the extent of band 3 fragmentation was performed with a densitometer (GS-670) and analysis program (Molecular Analyst) from Bio-Rad Laboratories (Hercules, CA).

The clinical and neuropsychological data of the patients were only revealed and combined with the data from the biochemical studies after the latter had been completed.

**RESULTS**

**Erythrocyte-Bound Immunoglobulin G**

The amount of erythrocyte-bound IgG did not differ significantly between the demented and healthy, age-matched donor groups (Fig. 1), and was in the same range as reported before for healthy, old-aged people [3.6 ± 0.9 fgm/1000 cells; (3,4)]. This value is higher than that found for a relatively young (28 ± 5 years) control group (1.8 ± 0.8 fgm/1000 cells), confirming earlier results (4). In contrast with earlier findings in patients in an advanced stage of dementia of the Alzheimer type (3), we did not find a significant increase in erythrocyte-bound IgG for the SDAT group. Exclusion of the extremely high IgG values (Fig. 1) does not significantly affect these conclusions.

**Anion Transport Characteristics**

The mean values of $V_{max}$ and $K_m$, of sulfate exchange did not differ significantly between the various donor groups, and were not different from the previously reported values for erythrocytes of young or aged control groups [Table 2; (3,4)]. It is noteworthy, however, that 9 of the 11 donors with $V_{max}$ values that were significantly lower than the mean (control) value (12.1 ± 2.9 $\mu$mol/1010 cells/min), belong to the patient groups (6 SDAT; 2 SDAT/MID; 1 MID). The numbers of individuals with a $K_m$
FIG. 1. Erythrocyte immunoglobulin G content of patients with senile dementia of the Alzheimer type (AD), multiinfarct dementia (MID), mixed dementia (MID/AD), and age-matched controls (AMC). Erythrocyte-bound IgG was measured as described before (3). Mean values ± SD in fgm/1000 red blood cells (rbc) are: AMC, 3.2 ± 3.9 (n = 12); AD, 2.4 ± 1.8 (n = 28); MID, 2.7 ± 2.3 (n = 15); MID/AD, 1.9 ± 1.6 (n = 9).

significantly lower than the normal value (0.9 ± 0.2 mM sulfate) and with a normal Vmax, earlier found to be characteristic for advanced SDAT patients (3), were not significantly different between the groups (SDAT, 6 out of 28; MID, 5 out of 15; SDAT/MID, 2 out of 9; AMC, 5 out of 22). In the whole donor group, there was a highly significant, negative correlation between Km and Vmax (r = −0.46, p = 0.0001; n = 65), as found for physiologically aged erythrocytes (2).

Erythrocyte Band 3 Fragmentation

The extent of band 3 fragmentation can be deduced from the presence of polypeptides that are reactive with antibodies against erythrocyte band 3, and that have an apparent molecular weight smaller than 95 kDa, the molecular weight of native band 3 (24, 8, 13, 14). Band 3 fragmentation increases with cellular aging (2,14). An immunoblot comparison of young and healthy, old donors indicated that, in general, band 3 fragmentation is also increased in the total erythrocyte population of healthy, old donors (Fig. 2), although there is considerable variation in the old age group (Bosman et al., in preparation). When we compared the erythrocyte band 3 patterns of demented donors with those of age-matched control donors using an antiserum specific for the membrane domain of band 3, our first results indicated an increased band 3 fragmentation in erythrocytes from demented donors, apparently superimposed on the aging-related increase (Fig. 3). However, quantitative analysis of a higher number of donors (n = 32), in which the relative densities of all immunoreactive bands with a molecular weight smaller than 95 kDa were compared, did not reveal a significant difference between age-matched controls and the demented groups. We also found no visible differences in the extent of band 3 fragmentation between the patient and age-matched donor groups with respect to the presence or absence of specific bands (data not shown). However, increased fragmentation of band 3 seems to be correlated with an increase in erythrocyte IgG content. For example, the mean IgG content of the erythrocytes analyzed in lanes 1–4 in Fig. 3 is 2.1 ± 0.8 vs. 1.0 ± 0.1 fgm/1000 cells of those of lanes 5–8. In a larger sample, erythrocytes with apparently increased band 3

TABLE 2
ANION TRANSPORT CHARACTERISTICS OF ERYTHROCYTES FROM SDAT PATIENTS COMPARED WITH VARIOUS CONTROL DONORS†

<table>
<thead>
<tr>
<th>Donor Group</th>
<th>Vmax (gm/s)</th>
<th>Ks (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMC</td>
<td>12.1 ± 2.9</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>SDAT</td>
<td>11.6 ± 3.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>MID</td>
<td>11.8 ± 2.2</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>SDAT/MID</td>
<td>11.2 ± 2.5</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

† Vmax and Ks of sulfate transport of erythrocytes were measured under exchange conditions as described before (3), and are given as mean ± SD. AMC, age-matched controls; SDAT, senile dementia of the Alzheimer type; MID, multiinfarct dementia; SDAT/MID, mixed dementia.

FIG. 2. Band 3 fragmentation patterns in erythrocyte membranes from young and old, healthy individuals. Immunoblot analysis of erythrocyte band 3 was performed as described in the Method section. YC: 18-8, 50-4, 54-3, 55-1; AMC: 10-7, 60-3, 63-4, 64-2. For abbreviations, see Fig. 1.

FIG. 3. Immunoblotting analysis of band 3 fragmentation in erythrocyte membranes of patients with dementia and age-matched control donors. Erythrocyte membrane proteins were analyzed for the presence of polypeptides that are immunologically related to band 3 as described in the Method section. Numbers under the lanes are numbers of donors with the following diagnosis: SDAT: 45-1, 45-2, 45-4; MID: 45-3; AMC: 45-5, 60-1, 60-2, 60-3.
fragmentation had a significantly higher IgG content (2.5 ± 0.5 fgm/1000 cells; n = 19) vs. when compared with erythrocytes showing less fragmentation (1.6 ± 0.6 fgm/1000 cells; n = 17).

**Blood Chemistry**

Measurement of erythrocyte, thrombocyte, and leukocyte numbers, and related parameters (hemoglobin, mean cellular volume, etc.) did not indicate any disturbances in cellular homeostasis in the patient groups as compared with the control groups. The serum concentrations of sodium, potassium, chloride, bicarbonate, and calcium, as well as those of glucose, creatinine, and urea, were within the normal range. There was a highly significant (r = 0.52, p < 0.001, n = 47) positive correlation between V_max of sulfate exchange and hemoglobin content. We found no significant difference between the patient and control groups in the extent and type of medicines used. There was no significant correlation between any of the erythrocyte aging parameters and use of medicine, with one notable exception. The value of the V_max of sulfate exchange was significantly correlated with the use of acetylsalicylic acid (r = 0.46, p < 0.001, n = 57).

**DISCUSSION**

The erythrocyte aging process seems to be accelerated in aged individuals. The increased percentage of reticulocytes, the increased percentage of erythrocytes containing IgG, and the increase in erythrocyte IgG content in old rats are all considered to be signs of increased removal and compensatory erythrocyte formation in aged individuals (15). Our present findings, which show an increase in erythrocyte IgG content in healthy old people and an increase in breakdown of erythrocyte band 3, confirm this conclusion for aging humans. The increased fragmentation of band 3 in the elderly supports the hypothesis that breakdown of band 3 accompanies the generation of senescent erythrocyte-specific antigens, and, thus, in the binding of IgG (2,14). The Km and V_max values of sulfate exchange, however, did not differ significantly between old and young donors. As sulfate transport is 10,000 times slower than that of the physiological-chloride/bicarbonate exchange (18), these parameters may not be sensitive enough to detect small changes. Together, these data indicate that in old individuals the rate of the erythrocyte aging process is increased. An increased susceptibility to (calcium-activated) proteases, possibly due to a decrease in antioxidant activity, has been suggested for the erythrocyte aging process (13), as well as for cellular aging in general (11,19). A more detailed description of the structural differences in band 3 and its fragmentation products in old vs. young erythrocytes, and in erythrocytes from young vs. those of old donors, will be instrumental in understanding the underlying processes.

Despite the large increase in knowledge about the composition of plaques and tangles in Alzheimer's disease-affected brain areas, the processes that underlie neuronal degeneration and death are far from understood (20,22). The neuropathological and the neuropsychological characteristics of AD indicate a disturbance and/or acceleration of the normal neuronal aging process (5). In addition, numerous data indicate that AD/SDAT may have specific systemic manifestations (24). From these observations, we postulated that acceleration or disturbance of the cellular aging process in patients with AD/SDAT might be detectable at the level of the erythrocyte membrane. Our first results did indeed show an increase in erythrocyte IgG content, a change in band 3 fragmentation patterns, and a decrease in the Km of sulfate exchange in AD/SDAT, but not in multifactorial dementia patients, when compared with a group of age-matched control donors (3). These findings were supported by reports of protein-related differences at the surface of erythrocytes between control and SDAT patients (23), and of irregularly shaped erythrocytes in some SDAT patients (9). Thus, the membrane characteristics of patients in advanced stages of AD/SDAT led us to the conclusion that the erythrocyte aging process might be disturbed in these patients (3). Measurements of the same parameters in individuals with Down's syndrome of various ages and in various stages of dementia resulted in the hypothesis that changes in the erythrocyte aging process might be detectable before the onset of dementia (4). However, in the present study we found no disease-specific differences in any of the erythrocyte aging characteristics in aged patients with beginning dementia, compared with age-matched controls. This difference between the findings in two donor groups, who were in the same age range but in different stages of dementia, could be due to various causes: 1) Although the methods used by us to establish the diagnosis of dementia and SDAT are as accurate as presently possible (see the section Clinical Measurements in the Method section), it can not be excluded that statistically significant differences may not be apparent because of this problem. 2) It is generally acknowledged that SDAT is a heterogeneous disease (20,22). The patients in the advanced stages of dementia described before (3) were of the same age as the patients in the beginning stages described here. Thus, these two groups may represent another illustration of the heterogeneity in age of onset and/or rate of progression of SDAT that may be reflected by the erythrocyte aging process. 3) More likely, a general disturbance of the cellular aging system in AD/SDAT underlies neuronal degeneration and cell death and may affect neuronal and erythrocyte aging equally. The constant renewal of the erythrocyte population could prevent detection of such a disturbance until at an advanced stage, i.e., after a long period of disease progress. 4) An advanced stage of dementia might run parallel with a deficiency in the maintenance of general, organismal homeostasis. A small disturbance of the already increased rate of erythrocyte aging in the elderly (15) might very well result in changes in erythrocyte homeostasis, and, thus, in the values of the erythrocyte aging-related parameters.

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