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## Increased Leukocyte Rigidity in the Elderly

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### ABSTRACT

We analysed leukocyte deformability and its relation to age and plasma lipids, albumin and gammaglobulin levels in a study group involving 21 (age: 65-88; female/male: 6/15) and a control group involving 20 healthy individuals (age: 18-62; female/male: 7/23). Leukocyte suspensions were pumped through polycarbonate filters with a nominal pore size of 5  $\mu$ m. In the study group, leukocyte rigidity was significantly higher than controls ( $0.22\pm 0.03$  vs.  $0.14\pm 0.05$ ,  $p<0.0001$ ). Also, we detected a moderate positive correlation between age and leukocyte rigidity. There was a weak negative correlation between leukocyte rigidity and albumin and triglyceride levels. There was no difference in blood chemistry parameters studied between the study and the control group. We conclude that leukocyte rigidity increases with aging. Since the quality of leukocyte membranes may have

significance with respect to various neutrophil and lymphocyte functions (extravasation, phagocytosis, T-cell receptor expression), we suggest that altered leukocyte deformability may participate in the pathogenesis of immunosenescence.

### Introduction

Infectious diseases are more frequent and more severe in the elderly compared to younger people(1). This is probably due to the gradual impairment of defense mechanisms with advancing age. On the other hand, there is considerable evidence that many aspects of the immune function may be related to membrane properties of neutrophils and lymphocytes, especially neutrophilic adhesion, extravasation, phagocytosis and T-cell

expression on the cell surface (2). Therefore it is possible that age related abnormalities of the cell membrane reflected by altered leukocyte deformability may contribute to immunosenescence. In the present study we investigated leukocyte deformability in individuals 65-years old or older (65-88) and compared the results with a control group involving individuals aged 18-62 years old.

## METHODS

The study group consisted of 21 individuals (age: 65-88; female/male: 6/15) and the control group consisted of 20 volunteers (age: 18-62; female/male: 7/23). None of the individuals in the study or control group had a history of chronic illness such as diabetes mellitus, chronic renal disease, and chronic liver disease; they had not smoked for the last 10 years.

Leukocytes were isolated from heparinated venous blood by Ficoll (Lymphoprep, Nycotron, Norway) gradient centrifugation at 700 g for 20 min. Red cells in the pellet were subjected to osmotic lysis (four 30s cycles of incubation in 0.2%NaCl followed by the addition of an equal volume of 1.6%NaCl). After centrifugation, leukocytes were re-suspended in Ringer solution.

Neutrophil deformability was analysed with the use of microfiltration technique in terms of cell rigidity (k) against pressure. Cell suspensions were pumped with a constant flow rate of 2 min/inch (6.05 ml/min) at room temperature (20-23°C) through polycarbonate filters (Nuclepore Corp. Pleasanton, Calif) with nominal pore sizes of 5 µm (Lot no. R7KM32882). The filtration pressure was measured on the upstream side of the filter with a pressure transducer (Model RM 6000 Nikon Kohden T400T, Tokyo Japan) connected to an amplifier and recorded on a polygraph (Nikon Kohden RM 6000). Then the data obtained was saved in a computer hard disk with the help of an analog-digital converter. Prior to the filtration of a cell suspension, buffer solution alone was filtered to obtain the filtration pressure for suspending medium (P<sub>0</sub>).

Cell rigidity was calculated according to the following formula showing the slope of the filtration pressure-time curve between 20 and 60 s after the start of pump (3):

$$K = \frac{\Delta P/t}{P_0} = \frac{(P_{60s} - P_{20s})/40s}{P_0}$$

Erythrocyte sedimentation rate was measured with the Westergreen method and the plasma cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol and gammaglobulin levels were analyzed

with a Roche modular system. Statistical analysis was performed with the SPSS program. Results were expressed as "mean ± standard deviation". The groups were compared with the student t test; for correlation analysis Pearson correlation coefficient was used.

## RESULTS

The mean age of the study control was significantly higher than the control group (73±7 and 43 ± 14, respectively, p<0.05). Leukocyte rigidity was found to be significantly increased in the study group compared to the control group (k= 0.22 ± 0.03 and k= 0.14 ± 0.05, respectively; p< 0.0001) (Table 1).

There was a significant and moderate positive correlation between leukocyte rigidity and age (p=0.001 and r=0.55) and a negative weak correlation between leukocyte rigidity and albumin (p=0.04, r= -0.37) in the study group. Combined data from the study and the control groups showed that there was a weak and negative correlation between leukocyte rigidity and triglyceride levels (p= 0.024 and r= -0.40).

There was not a statistically significant difference between the study group and the control group regarding the erythrocyte sedimentation rate, serum albumin, globulin, cholesterol, HDL-cholesterol levels and the leukocyte, lymphocyte and leukocyte counts (Table 1).

## DISCUSSION

In the present study we observed that leukocytes of the elderly were more rigid compared to the control group; that is, their ability to deform was reduced. We have also found that a positive correlation exists between age and leukocyte rigidity.

The general decline in the immune status of the elderly, termed immunosenescence, is associated with an increased incidence of bacterial and viral infections (1). The defects in defensive mechanisms against infectious agents may involve both cellular immunity and neutrophil function. Therefore interpretation of the results of the present study –i.e., decreased leukocyte deformability in the elderly- with respect to immunosenescence requires a brief description of the functional/structural properties of neutrophils and lymphocytes which may be potentially related to cell membrane properties.

Neutrophils constitute the first line of defense against various microorganisms and play a critical role in the early days of infection: when invaded by pathogenic microorganisms, the initial defensive mechanisms of the body include an increased number of neutrophils in the peripheral blood which then leave the circulation

(extravasation) and migrate to the focus of infection (chemotaxis) to phagocyte the invading agents (1).

A combination of defects in these critical steps may render the elderly susceptible to infections. It has been reported that the majority of elderly patients are able to mount a normal neutrophilia and there is no significant difference in neutrophil numbers between young and elderly patients during bacterial infections (4). Another target mechanism which is potentially subject to impairment during senescence is neutrophil margination, or chemotaxis. The migration process involves margination, rolling and endothelial adhesion of the leukocytes. In order to be able to pass through vessel walls (extravasation), the neutrophils must have been stimulated by chemotactic factors and adhere to the unstimulated endothelium (5, 6). The model of adhesive interaction between leukocytes and endothelium involves initial binding, stabilization of the adhesion, migration and diapedes, a complex process where many members of selectin, integrin and immune globulin (Ig) gene families participate (7). *In vitro* studies have shown that for binding and trans-endothelial migration, the neutrophils must have CD 18 molecules on their membranes to interact with the intracellular adhesion molecule-1 (ICAM-1) present on the endothelial cell surface (8,9). In various studies it was found that chemotaxis of neutrophils are not impaired by normal ageing and adherence of neutrophils to the endothelium is apparently normal (10, 11, 12). However, *in vivo* delivery of neutrophils into skin abrasions have been found to be decreased with reduced number of surface adhesion glycoprotein CD 11 (13). More recently, Wenisch et al reported that, in addition to a reduced phagocytic activity, a trend towards a decreased neutrophil chemotaxis was observed in the elderly (14). Therefore, although controversial at the present, defective neutrophil migration may be operative in the increased vulnerability of the elderly against infections.

Neutrophil bactericidal functions also deserve attention. It has been shown that in the early phase of chemotactic response, neutrophils are morphologically spherical; however, numerous projections begin to appear on the cell surface. Cell deformability increases in the next few minutes and then pseudopods rich in F-actin are formed after which the microorganism is phagocytosized (15, 16). These observations imply that the quality of the cell membrane may modify the efficiency of phagocytosis. The microorganisms “engulfed” by the neutrophil in a phagosome are destroyed either by lytic enzymes contained in the lysosomal granules or reactive oxygen species generated by NADPH oxidase (2). In an earlier *in vitro* study it has been shown that degranulation of lytic enzymes was reduced in the elderly after N-formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation

(17). Also, in studies where functional alterations of neutrophil functions were assessed in the elderly, it has been found that their neutrophils produce a lesser amount of free radicals upon stimulation with fMLP (18, 19). On the other hand, Angelis et al found no difference in respiratory burst activation as measured by luminol enhanced chemiluminescence between young and elderly people (4). Intriguingly, Wenisch et al showed that superoxide generation was suboptimal in response to *S. Aureus* whereas it was normal in response to *E. Coli*. Remarkably, they found that the number of phagocytized bacteria was decreased in an age dependent manner (14). These results suggest that even if the intracellular bactericidal activity is intact, or only partially defective, the mechanical engulfing of the microorganisms by the neutrophil may be impaired.

The aforementioned observations on neutrophil function, especially extravasation through the endothelium and alteration of cell shape during phagocytosis imply that membrane deformability may be critical for neutrophil activity. There is limited data concerning neutrophil deformability in older people. In an earlier study, Ciufetti et al. reported that the filterability of mononuclear cells decrease, whereas filterability of polymorphonuclear leukocytes do not change with age (20). In another study where the rheological properties of unfractionated leukocytes studied in different phases of acute bacterial infections, it was found that leukocyte rigidity was increased in the elderly during the onset and after 3 weeks of full clinical recovery (21).

Increased leukocyte rigidity may also be reflecting the quality of lymphocyte membranes, especially T cells. During the “adaptive” phase of the immune reaction, antigens are taken up in the periphery by antigen-presenting cells and presented as peptides to T cells which recognize them by their specific receptors (T cell receptors; TCR) and proliferate. It has been reported that TCR expression declines with age which may contribute to the pathogenesis of immunosenescence (22). Although not confirmed by other authors, O’Leary et al. found the density of CD3 on CD4 to be lower in the elderly (23). Also, the expression of CD28 is decreased in the elderly, leading to the hypothesis that age-associated impairment of CD28-mediated costimulation would have substantial impact on immune function (24, 25). Although difficult to prove, the increased leukocyte rigidity observed in the present study may be reflecting age related-abnormalities of the cell membrane, which may be associated with suboptimal TCR expression. In agreement with this view, Rivnay et al showed that membrane viscosity of the lymphocytes correlate with membrane cholesterol/phospholipid molar ratios which progressively increase with age (26). Interestingly, there was a concomitant decrease in response of

the lymphocytes to concavalin A stimulation. Their findings were reproduced by other studies leading to the hypothesis that alterations in lymphocyte plasma membrane structure with age can be implicated in abnormalities of membrane-bound receptor function associated with aging (27, 28). The reduced number of neutrophil surface adhesion glycoprotein CD 11 may also be consequence of similar membrane abnormalities (13).

Different from the studies quoted above, we assessed leukocyte deformability directly. Although the cell population analyzed involved unfractionated leukocytes, the increased rigidity may be attributed to neutrophils and/or lymphocytes which, although difficult to substantiate, may have some impact on immune function. It is also possible that abnormalities of leukocyte membrane associated with aging, is not cell-type specific; similar membrane lipid abnormalities are also observed in the erythrocytes of elderly with altered membrane mechanical properties. These alterations are frequently related to plasma lipid disturbances which may potentially affect both leukocytes and erythrocytes (29, 30).

In conclusion, in the present study we found that leukocyte deformability is reduced in a population of elderly where blood lipid parameters were not found to be significantly different than the controls, suggesting that the biological processes associated with aging per se can modify cell membrane mechanics independently. More detailed studies are needed to demonstrate whether leukocyte rigidity participates in immunosenescence or it is only a by-phenomenon of aging.

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**Table 1.** Leukocyte rigidity and other parameters in the study and the control group.

	Study group	Control group	P
Age (years) *	73±7	43±14	<0.05
Lymphocyte count (/mm <sup>3</sup> )	1772±544	2175±652	>0.05
PMN count (/mm <sup>3</sup> )	4791±2040	4134±1431	>0.05
ESR (mm/hour)	51±33	34±31	>0.05
Albumin (g/dl)	3.43±0.91	3.95±0.71	>0.05
Globulin (g/dl)	3.25±0.56	3.10±0.57	>0.05
Cholesterol (mg/dl)	191±48	193±40	>0.05
Triglycerides (mg/dl)	130±48	220±129	>0.05
HDL-cholesterol (mg/dl)	44±17	41±12	>0.05
Leukocyte rigidity*	0.22±0.03	0.14±0.05	<0.0001

\*: Significant; ESR: erythrocyte sedimentation rate