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Age-dependent alterations of Fcγ receptor-mediated effector functions of human polymorphonuclear leucocytes

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SUMMARY

Changes in the effector functions in polymorphonuclear leucocytes (PMNL), harvested from blood of young and aged healthy subjects of both sexes, were studied. FCγ-receptor (FcγR)-mediated incorporation of IgG coated ^{51}Cr-HRBC significantly increased in the aged male group, while the phagocytosis of pre-opsonized fungi (*Saccharomyces cerevisiae* and *Candida albicans*) was independent of both the age and sex. However, the intracellular killing capacity of neutrophils obtained from aged male subjects significantly decreased toward ^{51}Cr-labelled *c. albicans*. The antibody-dependent cellular cytotoxicity (ADCC) was also impaired with ageing in both sexes. The age-dependent decrease in the effector functions of PMNL may be explained, among others, by the fact that during yeast cell incorporation the increased cAMP level does not return to the basic level in the old group. On the other hand, the cGMP level which increased in PMNL of aged subjects does not show any progressive increase as in the young subjects, but remains unchanged. The oxidative metabolism producing free radicals being necessary for the effective intracellular killing and ADCC diminished in PMNL of aged subjects of both sexes. The above findings indicate that the adaptation of cyclic nucleotide system and the oxidative burst to the cell activation becomes impaired with ageing.

Keywords polymorphonuclear leucocytes Fcγ receptors respiratory burst cyclic nucleotides ageing

INTRODUCTION

Polymorphonuclear leucocytes (PMNL) represent an important part of the host defence and their role against micro-organisms and tumour cells is well recognized. The increased incidence of infections and tumours in the elderly is also well documented (Gardner, 1980; Schneider, 1983).

It has been demonstrated (Fülöp *et al.*, 1984) that both the rosette formation and phagocytosis by the Fcγ receptor (FcγR) of human blood monocytes obtained from aged subjects increased while the ADCC activity of these monocytes decreased.

It is well known that the FcγR functions are regulated by the cyclic nucleotide system (Muschel *et al.*, 1977; Vogel *et al.*, 1981). The interdependent variations of the cAMP-cGMP ratio determine whether the target will be incorporated or not, i.e., which of the destructive mechanisms will be stimulated: the intracellular killing or antibody-dependent cellular cytotoxicity (ADCC) activity (Hafeman & Lucas, 1979; Gale & Zieghelboim, 1974).

Another important phenomenon playing a determinant role in the effectiveness of these killing activities is the oxidative burst providing the indispensable free radicals for these reactions (Rossi & Zatti, 1966; Babior, 1984).

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The present study was aimed to clarify the alterations of FcγR-mediated functions with ageing in PMNL and to reveal the functional state of the regulating mechanisms.

MATERIALS AND METHODS

Patients. As a part of a large screening program started between the inhabitants of a retirement home, 20 healthy old males (aged: 60–87 years) and 20 healthy old females (aged: 60–90 years) were selected on the basis of very careful physical and mental examinations confirmed by some routine biochemical, biological and radiological tests. They were all fully informed and consentient. Ten young males (aged: 20–25 years) and 10 young females (aged: 20–25 years), all medical students and 10 middle aged males (aged: 35–52 years) and 10 middle aged women (aged: 32–55 year) served as controls as volunteers.

Neutrophils. These were separated by Fycoll-hypaque density centrifugation followed by dextran sedimentaton of the PMNL rich pellet (Böyum, 1968). Residual erythrocytes were lysed with three cycles of hypotonic saline. Judged by morphological criteria, the neutrophil suspension was 95% pure, and 97% of the cells were viable by trypan blue exclusion.

Measurement of the FcγR-mediated phagocytosis. The experiments were carried out according to the method of Scribner & Fahrney (1976) with slight modifications. Type O, Rh positive human erythrocytes (10^9 cells) were incubated for 120 min with 100 μCi sodium chromate (Radioisotope Center, Swierk, Poland) at 37°C. After washing, the erythrocytes were treated with 0·1% of papain solution for 15 min and coated with the subagglutinating concentration of anti-D human IgG (National Institute of Hematology and Blood transfusion, Budapest, Hungary) for 30 min. After repeated washing the tracer erythrocytes were added (10^7 cells) to 10^6 neutrophils in 1·0 ml volume of RPMI 1640 (GIBCO medium) (final cell density). After 60 min incubation in ASSAB CO_2 incubator (5% CO_2, air 95%) at 37°C, the adhered erythrocytes were lysed by the addition, for 30 s, of ice cold distilled water, followed by addition of hypertonic NaCl solution. After repeated washings, PMNL were lysed with 0·2% of sodium dodecyl sulphate solution (0·1 ml) and the radioactivity of the 0·25 ml aliquots were determined in a NK 350 gamma scintillation counter.

Measurement of the ADCC by neutrophils. The ADCC activity of PMNL was measured in a system which was described above, however, the labelled and sensitized erythrocytes were added to the 10^6 PMNL in 10^6, $2{\cdot}5 \times 10^6$, $5{\cdot}0 \times 10^6$, $7{\cdot}5 \times 10^6$ and 10^7 quantities. Based on our preliminary experiments the measurement was carried out at 1:10 effector target cell ratio after 4 hours incubation in ASSAB incubator. After centrifugation, the radioactivity of the supernatants were counted and the number of lysed tracer erythrocytes per neutrophils was determined. In the control tubes the lysis of tracer erythrocytes without IgG were measured and the values were subtracted from the values of the individual test samples.

Measurement of yeast cell phagocytosis. Neutrophils were suspended in RPMI 1640 medium and 5×10^7 heat killed *Saccharomyces cerevisiae* or *Candida albicans* (strain obtained from the State Institute of Public Health, Budapest, maintained on 1% peptone and 2% glucose agar slopes) opsonized with fresh autolog serum were added to 5×10^6 neutrophils in 1·0 ml final volumes. After 60 min incubation at 37°C the cells were washed and from the pellet smears were prepared. After fixation and staining the number of incorporated particles per 200 neutrophils were determined.

Measurement of intracellular killing activity. The method of Yamamura, Boler & Valdimarsson (1976) using ^{51}Cr-*C.* albicans was carried out without any modification. For the determination RPMI 1640 medium containing 5% autologous serum was used. The amount of released chromium was calculated as % of the total releasable chromium obtained after treatment with deoxycholate and DNAse.

The determination of intracellular cAMP and cGMP levels. This was carried out before and during the yeast cell phagocytosis at 0, 15, 30, 60 and 120 min of incubation. In the control group the neutrophils were incubated without phagocytic stimuli. All incubations were performed in an ASSAB CO_2 incubator (CO_2 5%, air 95%, humidity 95%) at 37°C. For the cyclic nucleotide determination, the cells were prepared as it was described by Stabinsky *et al.* (1980). The

determinations were made according to the instructions enclosed in the radioimmunoassay kit (Amersham).

Chemiluminescence. This was measured at 37°C in siliconized counting vials with a reaction mixture consisting of 10^5 neutrophils and 10^{-7} M of Luminol (Fluka). The cells, the reagent and the vials were dark adapted before use and all experiments were performed under red light. The measurement was carried out in a Searle Isocap/300 liquide scintillation counter in the out-off coincidence mode; ct/min were recorded at 0·2 min intervals over a 40 min period and the area under the curve was integrated. The chemiluminescence was also measured by the same method under phagocytic stimulation using yeast cells.

RESULTS

In the first series of our investigations the FcγR mediated phagocytosis (Fig. 1) as well as the extracellular lysis of IgG coated human erythrocytes by neutrophils (Fig. 2) obtained from healthy young, middle aged and aged subjects of both sexes were evaluated.

As shown in Figs 1 & 2 the incorporation slightly increased with ageing in both sexes, however, this increase was significant ($P < 0·01$) only in the aged male group. On the other hand, the ADCC activity of neutrophils diminished significantly ($P < 0·01$) during ageing in both sexes. It should be noted that the FcγR-mediated polymorphonuclear functions of young subjects did not differ significantly from those which were found in the middle aged groups.

The phagocytosis of pre-opsonized *S. cerevisiae* and *C. albicans* by neutrophils was assessed only in the males, because the FcγR-mediated phagocytosis increased significantly only in this group. The results are shown in Table 1.

Despite of the unchanged incorporation, the intracellular killing capability of neutrophils obtained from the old persons decreased, however this decrease was significant ($P < 0·05$) only in the male group (Fig. 3). As we did not find any significant differences between the values of young and middle aged subjects, we omitted the middle aged subjects from our further investigations.

The significant decrease in both effector functions of neutrophils with ageing could partly be explained by the data presented in Fig. 4, indicating that during yeast cell phagocytosis, the

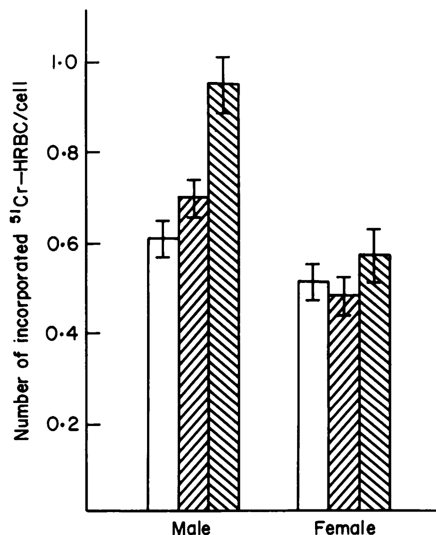

Fig. 1. Age and sex related alterations of the FcγR-mediated phagocytosis of human neutrophils. Each value represents the mean ± s.e. of 10 determinations. Each measurement was done in triplicate. □ = patients between 20–25 years; ■ = patients between 35–55 years; ▨ = Patients between 60–90 years. The difference between young and aged male groups was significant at a level of Student's *t*-test; $P < 0·01$.

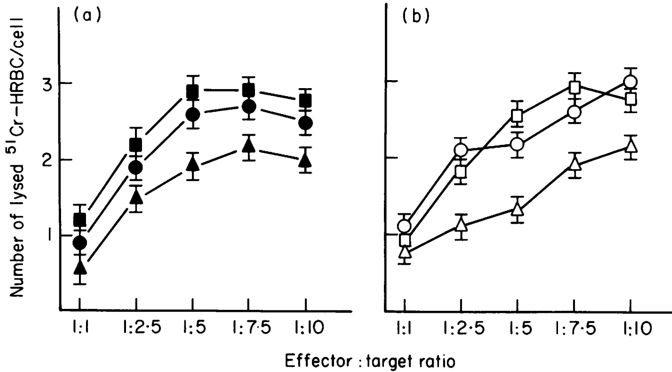

Fig. 2. (a) Age-dependent alterations of the ADCC activity of human neutrophils obtained from healthy males. ● = patients between 20–25 years; ■ = patients between 35–52 years; ▲ = patients between 60–87 years.
(b) Age-dependent alterations of the ADCC activity of human neutrophils obtained from healthy females. ○ = patients between 20–25 years; □ = patients between 35–55 years; △ = patients between 60–90 years.
Each point represents the mean ± s.e. of 10 determinations. Each measurement was done in triplicate. The differences between young and aged groups in both sexes were significant at 1 : 5–1 : 10 effector : target cell ratios at a level of Student's t-test; $P < 0{\cdot}01$.

Table 1. Phagocytosis of opsonized fungi by human PMNL

	Number of engulfed particle/neutrophil	
Micro-organisms	Young	Aged
S. cerevisiae	4·82 ± 0·16	4·56 ± 0·18
C. albicans	3·25 ± 0·11	3·42 ± 0·13

Data are presented as mean ± s.e. ($n = 10$).

produced chemiluminescence of neutrophils of aged male and female subjects was significantly ($P < 0{\cdot}01$) lower than that of the young groups. This finding shows a decreased generation of active oxygen species by neutrophils against the target cells with ageing.

In order to study the mechanisms regulating the effector functions of phagocytic cells, the alterations of cAMP (Fig. 5a) and cGMP (Fig. 5b) levels were measured during yeast cell phagocytosis in male subjects. The data obtained clearly show that the basic level of cAMP decreased with ageing. Nevertheless, the cAMP levels started to increase in the first 15 min in both young and aged subjects, while the return of the cAMP content to the basic level in the aged group delayed and remained at a high level during the 120 min of incubation (Fig. 5a). As against the basic cGMP content of neutrophils was elevated in the neutrophils obtained from aged males and it remained unchanged during the incorporation, whereas in the young group, the cGMP started to increase at the 30th min of incubation and this increase continued progressively up to the 120th min (Fig. 5b).

Therefore, the impairment of the extracellular cytotoxicity and the intracellular killing capacity of neutrophils with ageing may be the consequence of some dysregulation of the cyclic nucleotide system characterized by a delayed turn off in the cAMP level as well as by a loss of responsiveness of cGMP, and by an altered oxidative metabolism during cell activation by phagocytic stimuli.

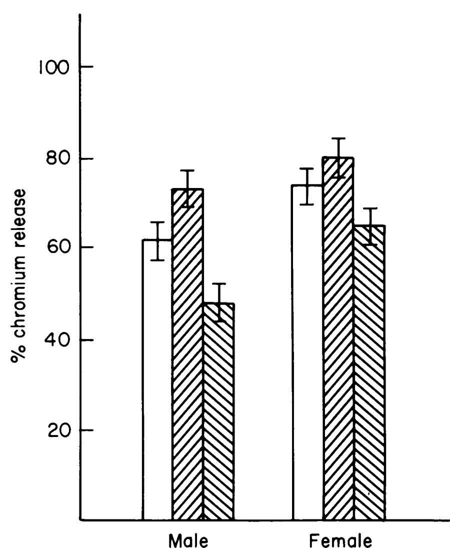

Fig. 3. The intracellular killing capacity of neutrophils obtained from young and aged subjects measured by ^{51}Chromium release from *C. albicans*. Each value represents the mean ± s.e. of 10 determinations. Each measurement was done in triplicate. □ = patients between 20–25 years; ▨ = patients between 35–55 years; ▧ = patients between 60–90 years. The difference between young and aged male groups was significant at a level of Student's *t*-test; $P < 0{\cdot}05$.

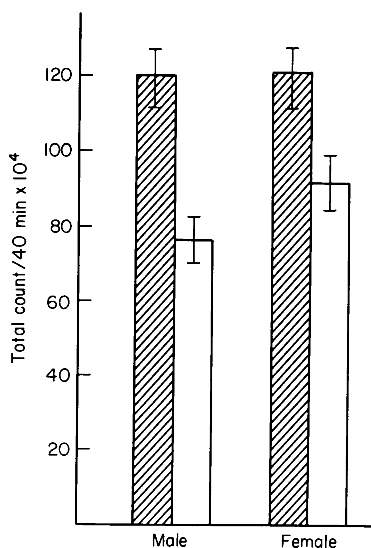

Fig. 4. Age and sex dependent changes of the phagocytosis stimulated chemiluminescence of human neutrophils. ▨ = patients between 20–25 years; □ = patients between 60–90 years. Each value represents the mean ± s.e. of 10 determinations. The differences between young and aged groups were significant at a level of Student's *t*-rest; $P < 0{\cdot}01$.

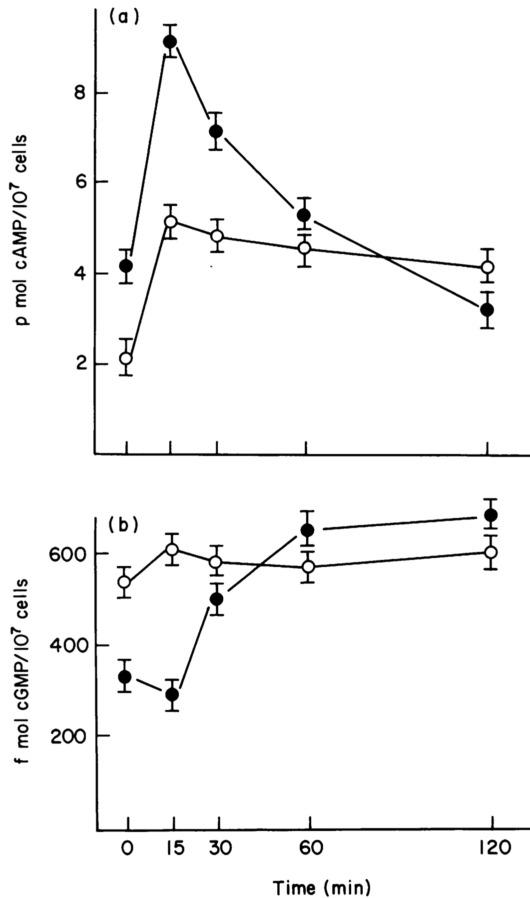

Fig. 5. Time-dependent alterations of the cAMP and cGMP levels during yeast cell incorporation by human neutrophils obtained from young and aged male subjects. (a) Alterations of cAMP levels with aging at the basic level and under phagocytic stimulation. (b) Alterations of cGMP levels with ageing at the basic level and under phagocytic stimulation. ● = patients between 20–25 years; ○ = patients between 60–87 years. Each point represents the mean ± s.e. of 10 determinations.

DISCUSSION

Several authors have described that the phagocytic activity as well as the intracellular killing capability of human PMNL against different micro-organisms remained unaltered with ageing (Palmblad & Haak, 1978; Nagel *et al.*, 1982), while others have demonstrated a slightly diminished intracellular killing function with similarly unaltered incorporation of the targets (Corberand *et al.*, 1981). In our experiments, the incorporation of opsonized yeast cells by PMNL of aged subjects was effective to the same extent as in the young subjects, in agreement with the above findings. Since in our system the incorporation required the co-operation of FcγR and C3bR, it seems that these receptor functions are not altered with ageing.

It should be pointed out that the cyclic nucleotide system regulates only the incorporation mediated by FcγR, while the C3bR mediated rosette formation and incorporation seems to be independent on both the Ca^{2+} transport and the cyclic nucleotides (Fóris, Füst & Medgyesi, 1983). Indeed, the early increase of cAMP following the stimulation of FcγR was observed in PMNL independently of age.

The unchanged phagocytosis with ageing does not involve that the effector functions, i.e., the intracellular killing and the ADCC activity of PMNL remained also unaltered. The decrease in

these effector functions of PMNL with ageing could partly explain the increased incidence of infections and tumours in the elderly. It should be pointed out that the differences found by us were more marked, with ageing, in males than in females, so why we made all our further investigations on male subjects. In the literature there are some data that the receptor functions (Burchinsky, 1984) and the immunological functions (Mascart-Lemone *et al.*, 1982) change differently in males than in females with ageing. In the following we try to elucidate the causes of such a decrease.

It is well known that the intracellular killing and ADCC activity of PMNL depend mainly, among other biochemical events (Gale & Zieghelboim, 1974; Katz *et al.*, 1980; Fleer *et al.*, 1978) on the activation of the oxidative metabolism resulting in the formation of oxygen free radicals (Clark & Klebanoff, 1975, 1977) the most important of which is the OH^-. Therefore, the metabolic burst seems to be essential for the maximal cytotoxic activity. The respiratory burst measured under stimulation by chemiluminescence significantly decreased in aged subjects. The identificaion of the exact biochemical mechanism underlying this alteration needs further studies. However, it seems to be evident that the decreased production of oxygen free radicals could play an important role in the diminished host defence of old subjects. It is controversial whether the early stimulation of the oxidative metabolism is under the control of cyclic nucleotide system (Smolen, Korchak & Wiessmann, 1980; Lehmayer & Johnston, 1978). On the other hand it is well known that the crucial point of both intracellular killing and ADCC activity is the fusion of lysosome membrane with either plasma or phagosome membrane (Ögmundsdottir & Weir, 1980). Both type of the above mentioned fusions are under the regulating effect of cyclic nucleotides and may be realized only at high intracellular level of cGMP and/or after a significant decrease of cAMP level (Wiessman, Dukor & Zurier, 1971; Lowrie *et al.*, 1980). Indeed in our present study a progressive increase of cGMP level was measured in the later phase of the incorporation in PMNL of young subjects when the initially rised cAMP level has already been normalized. On the contrary, in PMNl of aged subjects the increased cAMP level did not return to the basic level and the cGMP level did not increase.

Thus our findings concerning the cAMP/cGMP system in stimulated PMNL of aged subjects demonstrate unambiguously the impairment of adenylate cyclase 'turn off' and/or the exhaustion of phosphodiesterase, following the incorporation, although the more precise molecular basis of the assumed impairment requires further investigations. In this regard it is interesting to note that the age-dependent decrease of cAMP level found in resting PMNL by us and also by Tame & Walford (1980) in lymphocyes, could be probably explained only by a major increase of phosphodiesterase activity because the adenylate cyclase activity increased with ageing (Krall *et al.*, 1983).

Finally, based on our results we can conclude that both the intracellular killing and the ADCC activity being responsible for the host defence against pathogens and partly for the tumour destruction become impaired in neutrophils of aged subjects, while the FcγR activity with respect to the phagocytosis remains unchanged with ageing. Moreover, our findings suggest that the decrease of the effector functions of phagocytic cells in aged subjects are caused by the impaired adaptation of cyclic nucleotide system and oxidative metabolism to the activation of cells.

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