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QUARTERLY

Compensatory effect of TNFα on low natural killer activity in the elderly✪

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Regulatory effect of CD25, an activation antigen the α subunit of interleukin 2 receptor (IL2R) on the activity of natural killer (NK) cells was studied in fifty elderly (57–70 years old) and fifty young people (19–35 years old). Cytotoxic NK activity was assessed by ^{51}Cr release assay, the levels of interleukin 2 (IL2) and tumour necrosis factors α (TNFα) were measured using bioassays and expression of CD16 and CD25 proteins by flow cytometry. Low NK activity in the elderly was associated with decline of full health, lowered serum concentration of IL2 and increased production of TNFα during NK reaction. Inhibition of TNFα activity by anti-TNF monoclonal antibody suppressed exclusively NK activity of low NK responders. Moreover, stimulation *in vitro* of blood mononuclear cells, with TNFα induced in the elderly low NK responders a significantly higher increase of the CD25 expression on the surface of NK cells as compared with that in the elderly high responders. Since the CD25 molecule constitutes a subunit of the high affinity receptor, binding IL2 to immunocompetent cells, its increased expression on NK cells of low NK responders would enable them to bind even low amounts of the endogenous IL2 available in this group of the elderly. Thus, an overproduction of TNFα seems to be a mechanism compensating, in the non-fully healthy elderly, for the decreased IL2 production, promoting efficient cytotoxic reaction.

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Abbreviations: TNFα, tumor necrosis factor α; NK, natural killer cells; CD, cluster of differentiation; IL2, interleukin 2; IL2R, interleukin 2 receptor; LAK, lymphokine-activated killer cells; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; FCS, fetal calf serum; mAbs, monoclonal antibodies; PE, phycoerythrin; FSC, forward scatter; SSC, side scatter.

Ageing is associated with impaired specific immune response [1]. Deficient immune response can lead to the increased susceptibility of aged subjects to infections, cancers and autoimmune diseases [2]. The loss of the ability of T cells of the elderly people to generate an immune response equal to that of T cells of the young is unquestionably an age-related phenomenon. Lower concentration of secreted IL2 [3, 4] as well as lower levels of IL2 transcript [5], with simultaneously decreased induction of the transcriptional factors AP-1, NF-AT and NFκB [6, 7] – have been detected in the T cell cultures stimulated with mitogens and antigens. Moreover, T cells of the elderly people are characterised by a diminished expression of the alpha chain of IL2 receptor (p55 IL2R, CD25) [8–10].

In the healthy old people the decrease in T cell potential is compensated for by an elevated activity of a non-specific arm of the immune response. The most pronounced activation involves NK cells. Unfortunately, such a repolarisation of the immune response happens only in about 10% of the elderly people, while in the remaining population the immune response generally is weakened. Our previous papers showed that the level of NK activity correlates with health status of the individuals selected according to the Senieur Protocol criteria [11]. Elderly people who fulfil these criteria (optimally healthy) were characterised by high NK activity, while elderly people who did not meet all the criteria (not fully healthy) had low NK activity. We have also found that low NK activity and lack of a full health in elderly people are connected with low secretion of IL2 and high secretion of TNFα [12, 13].

The level of NK cytotoxic activity is regulated by several cytokines. IL2 is considered a main stimulatory factor for NK activity, however other cytokines play also an important (co)stimulatory role [14–16]. It has been shown that IL2 is necessary, but not sufficient, for optimal proliferation of NK cells [17]. Furthermore, although TNFα did not induce proliferation by itself, it could augment the IL2-induced proliferation of resting or ionomycin activated NK cells. TNFα and IL2 are known to have agonistic effects on the lymphokine-activated killer cells (LAK). They both are necessary if these cells are to achieve optimal proliferation and exhibit cytotoxic properties [16]. The two cytokines are also necessary for induction of expression of the activation antigen CD25 on LAK cells [17].

As TNFα potentiates many functions of NK cells – the intensity of production of this cytokine in the elderly people seems to be of importance. This production by the cells of old subjects is higher in comparison to that of the young, and may occur in the absence of any stimulant. Increased TNFα production has been found in the non-stimulated as well as stimulated cultures of peripheral blood mononuclear cells of aged individuals [18, 19].

In the light of these facts it is conceivable that a low NK activity in the elderly may be associated not only with an insufficiency of IL2 but also with an alteration in the production of TNFα.

It seemes of interest to check whether the age-associated overexpression of TNFα has a regulatory effect on the NK cytotoxic activity and whether this effect is realised through the CD25 molecule (activation antigen) on NK cells.

MATERIALS AND METHODS

Older volunteers (50 people aged 57–70) were recruited from The Geriatric Outpatient's Department in Gdańsk. The younger (50 people aged 19–35) ones were students and staff of our Department. All the volunteers were informed about the goal of the study and gave consent to it. Older volunteers had had during the last three years a full medical examination every 12 months by the physicians from the Geriatric Outpatient's Department. The laboratory tests were carried out in the Department of Biochemistry of the Medi-

cal University of Gdańsk. All volunteers were qualified into the study according to the Senieur Protocol criteria [11]. According to these criteria older volunteers were divided into the group of fully healthy people (called "healthy"), fulfilling all the criteria of the protocol (n = 8; all females, mean age 63.2 ± 6.5) and those not fulfilling all these criteria (called "almost-healthy") (n = 42; 30 females and 12 males, mean age 64.1 ± 4.2). The "almost-healthy" people were generally in a good physical and mental health. They led an independent and active life and regarded themselves as "healthy". They had minor symptoms: moderately elevated and controlled blood pressure, degenerative changes of the skeleton, without pain and without limitation of the movement. They did not suffer from acute and chronic inflammatory diseases, autoimmune or neoplastic diseases. They did not receive medicaments known to affect the immune system. Fifty young people who remained under medical care were divided in the same way as the older volunteers into two groups: "healthy" (n = 12; all females, mean age (30.6 ± 5.5) and those not meeting all the protocol criteria "almost -healthy" (n = 38; 20 females and 18 males, mean age 27.8 ± 8.8).

Isolation of peripheral blood mononuclear cells (PBMC). Human PBMC were obtained by centrifugation of heparinized venous blood samples on Lymphoprep (Nyegaard, Oslo, Norway) gradient. After three washings with phosphate buffered saline (PBS) mononuclear cells were suspended in a small volume of a culture medium containing RPMI 1640 (Gibco Life Technologies Inc., Gaithesburg, MD) supplemented with 5% fetal calf serum (FCS) (Flow Laboratories, Irvine, Scotland). The same RPMI 1640-FCS medium, supplemented as indicated, was used in further experiments.

Assessment of NK cytotoxic activity. K562 cells, the natural killer-sensitive erythromyeloid cell line, is routinely maintained in a continuous culture in our laboratory in the RPMI 1640-FCS medium. The K562 line is used as the target cells in biological tests measuring the cytotoxic NK activity. The cells were harvested during the log phase and used for the four-hour radioactive chromium release NK assay. A total of 2×10^6 K562 cells were incubated with 100 mCi of $Na_2{}^{51}CrO_4$ (Polatom, Świerk, Poland) at 37°C in a humidified atmosphere containing 5% CO_2 for 1 h. After incubation the cells were washed twice in RPMI 1640 supplemented with 10% FCS to remove the excess of $Na_2{}^{51}CrO_4$. As the next step, K562 cells were mixed with PBMC (effector cells) at a ratio of 1:25. To the selected samples of mixed cells, monoclonal antibodies (mAbs) against TNFα and anti-IL2 (Genzyme, Cambridge, MA) were added at the beginning of the reaction. The cell mixture was incubated for 4 h at 37°C in a humidified atmosphere containing 5% CO_2. After incubation the samples were centrifuged and the supernatants was collected. Radioactivity of the supernatants was counted in a LKB ultragamma counter. The following formula was used for calculations: percent of chromium release (% of cytotoxicity) = (experimental release – spontaneous release)/(maximal release – spontaneous release) × 100.

Incubation of PBMC stimulated with K562 cells. K562 cells, used in this test as stimulatory cells, were cultured in the RPMI 1640-FCS medium containing 100 μg streptomycin, 100 U/ml penicillin, 2 mM L-glutamate and 100 mM/ml of pyruvate (Gibco Life Technologies Inc., Gaithesburg, MD). PBMC (responders to the stimulation with K562 cells) were suspended in the same medium at a concentration of 1×10^6 cells/ml. Stimulatory and responder cells (K562 and PBMC) were mixed at a ratio of 1:1 (2×10^5 cells /100 μl), centrifuged and incubated for 4 h in a humidified atmosphere containing 5% CO_2 at 37°C. After incubation the supernatants were harvested and kept at 80°C until use.

Sera. Sera obtained from venous blood of the subjects tested were frozen and kept at 80°C until use.

Bioassay for TNFα. TNF-sensitive fibrosarcoma cell line WEHI 164, cell clone 13 (these cells undergo a dose-dependent apoptotic death under the influence of TNFα) (from Dr T. Espevik, University of Trondheim, Norway) was cultured for 24 h on 96-well plastic plates (Corning Science Products, Rochester, NY) at a concentration of 20 $\times$ 10^3 cells/well in the medium RPMI 1640-FCS supplemented with 2 mM L-glutamine, gentamycin and actinomycin-D (Sigma, St. Louis, MO) at a concentration of 1.0 mg/ml. A 10 μl portion of the supernatant (from above the mixture of K562 cells + PBMC) was added, in triplicate, to each well of the plate. Increasing concentrations of rTNFα (Genzyme, Cambridge, MA) were added, in duplicate, instead of the supernatants to the plate wells for obtaining a standard curve. Neutralising rabbit anti-TNFα antibody (Genzyme, Cambridge, MA) (1:10, 1:20, and 1:50) was added to the set of samples to check specificity of the test. To parallel control samples normal rabbit serum was added (1:50). After 24 h of incubation, 20 μl of MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma, St. Louis, MO) solution was added to each well of the plate. The plate was then incubated for another 4 h (37°C, 5% CO_2), 100 μl of isopropanol was added. Absorbance read at 570 nm on an automated plate reader (Multiscan MCC/340, Labsystems, Helsinki, Finland) fitted with titration standards of rTNFα. The anti-TNF antibody completely blocked the cytotoxic effect of the supernatants on WEHI cells. This assay had a detection limit of 1 pg/ml. The intra-assay coefficient of variation ranged from 5.5 to 13.8%, while the inter-assay coefficient of variation ranged from 15.5 to 22.3%.

Bioassay for interleukin 2 (IL2). IL2-dependent CTLL cells (from Institute of Immunology, Wrocław, Poland) (proliferating in a dose-dependent fashion under the influence of IL2) were cultured for 48 h on 96-well plastic plates (Corning Science Products, Rochester, NY) at a concentration of 20 $\times$ 10^3 cells/well in the RPMI 1640-FCS medium, 2 mM L-glutamate and gentamycin. A portion of 10 μl of the sera from the examined subject was added, in triplicate, to each well of the plate. Increasing concentrations of rIL2 were added, in duplicate, instead of sera to the wells for obtaining a standard curve. Neutralising rabbit anti-IL2 antibody (1:10, 1:20, and 1:50) was added to the set of samples to check specificity of the test. To parallel control samples normal rabbit serum was added (1:50). After 48 h of incubation, 20 μl of MTT solution was added to each well of the plate to penetrate into living cells. The plate was then incubated for another 4 h (37°C, 5% CO_2) and the 100 μl of isopropanol was added. Absorbance read at 570 nm on an automated plate reader from experimental wells fitted with titration standards of rIL2. The anti-IL2 antibody completely blocked the proliferative effect of the sera on the CTLL cells. The assay had a detection limit of 12.5 pg/ml. The intra-assay coefficient of variation ranged between 8.5 and 12.8%. The inter-assay coefficient of variation ranged from 16 to 25.%.

Stimulation of PBMC with TNFα. PBMC were suspended in the culture RPMI 1640-FCS medium at a concentration of 1 $\times$ 10^6 cells/ml. The cells were stimulated with human rTNFα (26 U/ml) (Genzyme, Cambridge, MA). Non-stimulated PBMC served as controls. Only sera negative for endotoxin were used in the experiments. Cells were incubated for 48 h in a humidified atmosphere containing 5% CO_2 at 37°C. Then cells were washed twice in cold PBS, kept on ice, and divided into samples of 30 $\times$ 10^4 cells for cytometry analysis.

Flow cytometry for surface staining: CD16 and CD25 molecules on PBMC. Surface staining of antigens was done on PBMC isolated from peripheral blood and for PBMC after stimulation *in vitro* with rTNFα. Monoclonal antibodies: anti-CD16 conjugated with phycoerythrin and anti-CD25 conjugated with

FITC (Immunotech S.A., Marseille, France) were used for surface staining of PBMC cells. Cells (30×10^4) were suspended after washing in 50 μl of PBS followed by 10 μl of anti-CD16-PE and 10 μl of anti-CD25-FITC monoclonal antibodies. For control of each set a separate sample was stained with 10 μl of IgG1-PE and 10 μl of IgG2-FITC. Samples were incubated in the dark, at 4°C for 30 min, then washed twice and suspended in 1 ml of cold PBS for immediate flow cytometry on an EPICS XL flow cytometer (Coulter, Hialeah, FL). Data from at least 10 thousand cells was collected from each sample and stored as listmodes; off-line analysis of these data was done using the WinMDI software (by Dr. Joseph Trotter, Scripps Institute, La Jolla, CA).

Statistical analysis. Statistical analysis was performed using the Excel 5.0 software (Microsoft), the statistical significance of differences was quantified by the unpaired T test (for comparing the groups) and paired T test (for assessing the effect of monoclonal antibodies on the NK cytotoxic activity).

RESULTS

Health status and NK activity in the young and elderly

An analysis of the NK activity and the health status of the elderly volunteers revealed that those characterised by full health (n = 8) had simultaneously higher NK (cytotoxicity 68 ± 22%) activity in relation to the "almost healthy" (n = 42) (cytotoxicity 32 ± 18%). Similar results were obtained with the young. The "healthy" (n = 12) young subjects had significantly ($P < 0.05$) higher NK activity (cytotoxicity 81 ± 16%) than those "almost healthy" (n = 38) (cytotoxicity 38 ± 22%). Basing on these results, in the further considerations the individuals with higher NK activity were called "high NK responders" and those with low NK activity "low NK responders".

Secretion of TNFα during the NK cytotoxic action in the groups differing in age and NK activity

Secretion of TNFα into the supernatants of the cultures containing mixtures of the stimulating cells (K562) with peripheral blood mononuclear cells as effectors, is shown in Table 1. The comparison of TNFα secretion during the NK action revealed that the levels of that cytokine released by the cells of the elderly with low NK activity were significantly higher than those of the elderly with high NK activity. There was no significant difference in the TNFα levels between the low and the high NK responders in the young group.

The statistical analysis confirmed a negative correlation between the level of TNFα released during the NK cytotoxic action and the intensity of NK activity in the elderly people ($r = -0.735$; $P < 0.05$). We did not find such a correlation in the young group.

Table 1. Level of TNFα (U/ml) in the cultures of PBMC stimulated with K562 cells.

The young and elderly subjects were divided into groups with low and high NK activity. The effector to-target cell ratio was 1 : 1, and the reaction was carried out for 4 h. Activity of TNFα was measured in the supernatants and expressed as U/ml.

NK activity	Young	Elderly
Low NK	3.01 ± 2.18 (n = 38)	8.7 ± 3.25 (n = 42)
High NK	6.18 ± 4.82 (n = 12)	3.72 ± 2.32 (n = 8)
Statistical significance	no difference	$P < 0.05$

Blood levels of interleukin 2 in the groups differing in age and NK activity

Both the young and the elderly people with low NK activity had significantly lower levels of IL2 in their sera in comparison with those of the age-matched subjects with high NK activity (Fig. 1). There was a positive correlation

(r = 0.766; $P < 0.05$) between the serum level of IL2 and the intensity of the NK reaction in the elderly.

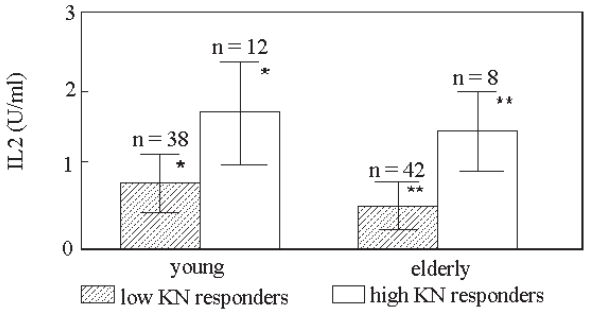

Figure 1. The level of IL2 in the sera of young and elderly people.

Bars represent arithmetical means of values obtained from the number of subjects (n) indicated, ± S.E.M. Asterisks show statistically significant differences at P = 0.043 (*) and P = 0.03 (**).

Effect of anti-TNFα and anti-IL2 antibodies on NK activity

To examine the role of TNFα and IL2 in the regulation of NK activity, anti-TNFα and anti-IL2 monoclonal antibodies were applied. An addition of anti-TNFα antibody to the NK reaction mixture significantly decreased the NK activity in the elderly people with low NK activity, while it had no effect on the intensity of the NK potential in the elderly with high NK activity. Monoclonal anti-IL2 antibody significantly decreased the NK activity both in the low and high NK responders (Fig. 2) (almost healthy and healthy subjects, respectively).

The results of this part of the study suggest that in the elderly people with low NK activity, a high production of TNFα during the NK reaction may be necessary for this reaction to reach the maximum possible intensity. The NK activity in those subjects remained, however, below that of the elderly with high NK activity.

Resting and activated NK cells in the young and elderly subjects

The percentages of resting ($CD16^+$ cells) and activated NK cells ($CD16^+CD25^+$ cells) were measured by flow cytometry in freshly obtained, non-stimulated PBMC of the young and elderly volunteers. There were no significant differences between groups of the young and elderly (young: $CD16^+$=13 ± 4.4% *versus* elderly: 19.3 ± 5% and young: $CD16^+CD25^+$= 0.7 ± 0.5% *versus* elderly: 1.7 ± 0.7). Similarly, the values for the high and low NK responders did not differ significantly within the age groups.

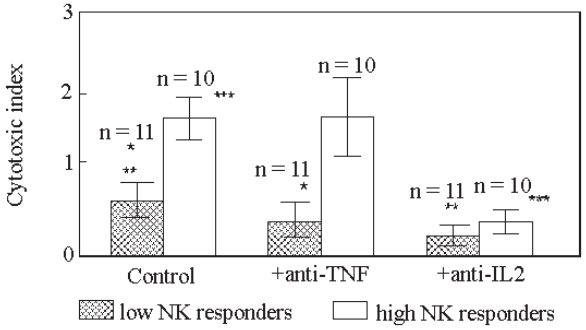

Figure 2. The effects of anti-TNFα and anti-IL2 monoclonal antibodies on NK cytotoxic activity in the elderly people.

The anti-TNFα and anti-IL2 mAb were added to the cellular mixtures containing PBMC + K562 cells at the start of the 4 h NK cytotoxic reaction. The spontaneous NK activity (mean ±S.D.) of the group with low NK response (n = 8) was assumed as the cytotoxic index = 1.0. All other values were calculated in relation to that value. The mouse anti-human TNFα mAb decreased NK activity at the concentration of 50 ng/ml while the mouse anti-human IL2 mAb had its maximal effect at the concentration of 100 ng/ml. The mean ±S.D. values of the cytotoxic indexes were as follows: low spontaneous NK = 0.69 ± 0.31; low spontaneous NK + anti-TNFα = 0.43 ± 0.17; low spontaneous NK + isotypic control mouse IgG1 (PharMingen, A Beckton Dickinson Comp., Warsaw) = 0.77 ± 0.28. Bars represent arithmetical means of values obtained from the number of subjects (n) indicated, ±S.E.M. Differences significant at P = 0.014 (*), P = 0.007 (**) and P = 0.0002 (***).

Activated NK cells in the elderly after stimulation with TNFα

To compare the effect of TNFα on NK numbers and phenotype in the elderly high and low NK responders, the cytometric tests were performed after 48 h incubation of PMBC with or without TNFα. In both groups examined after incubation of cells with TNFα there appeared cells with elevated values of the forward scatter (size) and slightly elevated side scatter (granularity) parameters (Fig. 3b) in relation to those for the non-stimulated population (Fig. 3a). Simultaneously, the double positive $CD16^+CD25^+$ cells (Fig. 3d) appeared which were not seen after incubation without TNFα (Fig. 3c).

It was therefore interesting to establish whether the double positive $CD16^+CD25^+$ cells remained within the major population of small lymphocytes with low values of both FSC and SSC, or whether they belonged to the bigger blastoid subpopulation. For this purpose, the graph displaying a plot of the FSC and SSC parameters was subdivided into two regions ("gates"): gate A – for the resting lymphocytes as in peripheral blood analysis and gate B – for lymphoblasts (Fig. 4a, 4b). Comparison of the presence of $CD16^+CD25^+$ population in gates A and B showed that these cells were localised almost exclusively in gate B, i.e., among the lymphoblasts (Fig. 4d) while they were absent in gate A (Fig 4c).

Effect of TNFα on CD16+CD25+ cells in elderly people differing in NK activity

After TNFα stimulation the percentage of $CD16^+CD25^+$ cells increased both in the elderly with low and high NK activity. In the cultures from the high NK responders the percentage of activated NK cells increased 3.7 times in relation to the values before stimula-

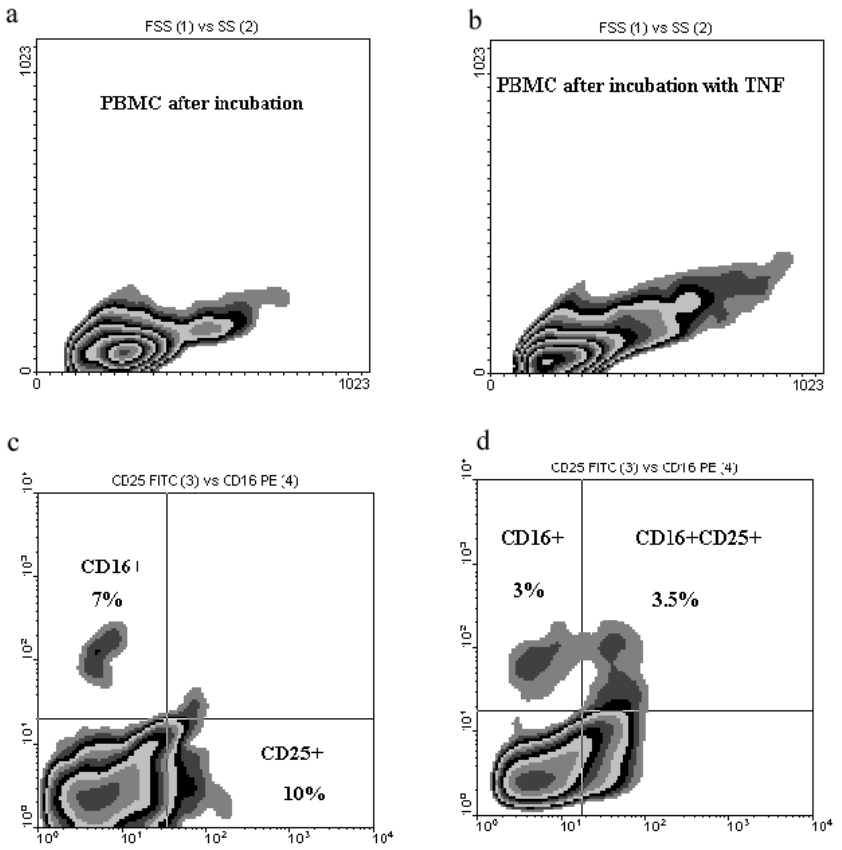

Figure 3. The effect of TNFα on resting and activated NK cells.

Panel **(a)** represents the forward and side scatter parameters of the non-stimulated, and panel **(b)** of the TNFα-stimulated PBMC. Activated NK ($CD16^+CD25^+$) are visible only after incubation of PBMC with TNFα **(d),** but not in a control, non-stimulated, sample **(c)**. The panels show typical result of the flow cytometric analysis in the form of a two-dimensional density plot.

tion. In the cultures from low NK responders an increase of activated NK cells was higher, reaching a value 20.85 times as high as the values before stimulation. The differences between the two groups were statistically significant ($P < 0.05$). The summary of these experiments is presented in Table 2.

These data indicate that the NK of the elderly low NK responders were more responsive to the stimulating effect of TNFα than the cells of the high NK responders.

DISCUSSION

In this study we have examined whether the age-associated overexpression of TNFα may have a regulatory effect on the NK cytotoxic activity and whether this effect on NK cells is realised through an activation antigen – the CD25 molecule. The results obtained showed that low NK activity in the elderly was associated with a lack of full health, a lower serum concentration of interleukin 2 and a higher production of TNFα during the NK reaction [12, 13]. Moreover, stimulation *in vitro* of blood mononuclear cells, with TNFα induced in the elderly low NK responders a significantly higher increase of the CD25 expression on the surface of NK cells as compared with the elderly high responders.

There is a consensus in the immunogerontological literature that production of TNFα is increased on ageing. An increased secretion of TNFα by cells of old individuals in the absence of any stimulant [19] as well as after a mitogenic stimulation of PBMC have been described [18]. This phenomenon has also been observed in the sera of elderly people [3, 20]. We have not come across any data analysing the changes of TNFα level during the NK cytotoxic reaction or its effect on that activity. It has been demonstrated, however, that TNFα may be secreted by NK cells after stim-

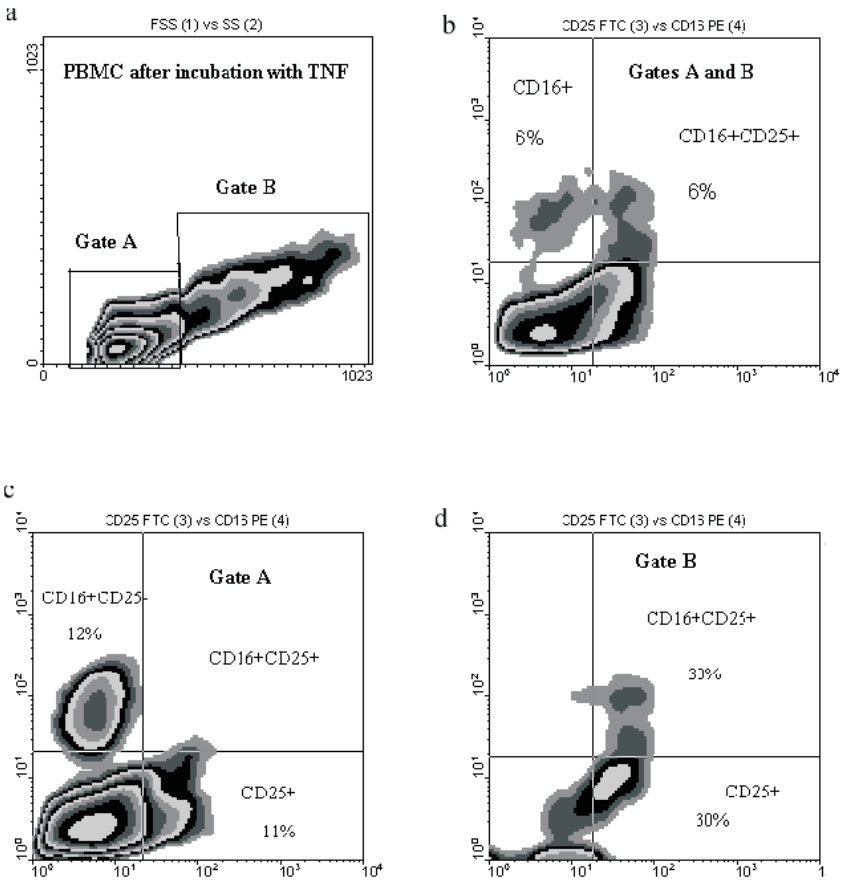

Figure 4. Characteristics of the TNFα activated NK cells.

Panel **(a)** shows "small cells" (Gate A) and "large cells" (Gate B). Panel **(b)** shows expression of CD25 *versus* CD16 in the total PBMC population. The activated NK ($CD16^+$-$CD25^+$) are visible only after incubation of PBMC with TNFα **(d)** but not in a control, non-stimulated, sample **(c)**. The panels are a typical result of the flow cytometric analysis in the form of a two-dimensional density plot.

ulation with carcinoma cells, transformed viral cells etc. [21, 22]. We had also earlier observed a negative correlation between the TNFα secretion and the intensity of NK activity in a group of elderly people, and lack of such a correlation in young individuals [12].

secreted to up-regulate the expression of CD25 molecule on the surface of $CD16^+$ NK cells. CD25 (p55, IL2R), the subunit of the IL2 receptor, can form, together with the beta and gamma chains, a high affinity receptor for IL2 on the surface of NK cells. In this way, this receptor might be able to bind low amounts of IL2, available in the elderly individuals.

Table 2. PBMC of the elderly low (n=42) and high (n=8) NK responders were stimulated for 48 h by TNFα.

The percentage of resting ($CD16^+$) and activated ($CD16^+CD25^+$) NK was determined in PBMC by flow cytometry.

Cells	High NK		Low NK	
	unstimulated	stimulated	unstimulated	stimulated
CD16+	19.3 ± 5	21.2 ± 4.2	13 ± 4.4	9.8 ± 2
CD16+CD25+	1.7 ± 0.5	6.3 ± 2.2	0.7 ± 0.5	14.6 ± 3
Increase of cell	3.7 ×		20.85 ×	

The results presented showed that the anti-TNFα monoclonal antibodies exerted an inhibitory effect on NK activity exclusively in the elderly low NK responders. This may indicate that an excessive secretion of TNFα during NK cytotoxic action in this group is necessary to maintain the highest possible level of NK activity.

The correlation between the level of NK activity and serum level of IL2 found in our study confirmed the role of IL2 in the stimulation of NK cells. The production of IL2 decreases during the ageing process. This phenomenon has been observed at different levels: at the level of secreted IL2, of IL2 mRNA and of IL2 receptors on the surface of T cells [4–10]. In our previous paper [13], we have found that not all elderly people are characterised by a low IL2 production. The level of IL2 production *in vivo* in the fully healthy elderly was similar as that in the healthy young people. Thus, the lower NK cytotoxic activity found in the majority of the elderly is primarily due to the IL2 insufficiency.

The coexistence of high levels of TNFα with an insufficiency of IL2 at a low NK cytotoxic response in the elderly leads us to hypothesize about the role of TNFα. If the endogenous IL2 production is low, high amounts of TNF are

In this paper we have shown that TNFα really contributes to up-regulation of the CD25 molecule on $CD16^+$ cells of the elderly and that this effect is more pronounced in the low NK responders.

Summarising, the results of our paper suggest that the age-related overexpression of TNFα, though associated with low NK activity, does not contribute to its suppression. This overproduction seems to be a mechanism compensating for the decreased IL2 production so as to achieve a possibly maximal activation for the cytotoxic effect of NK cells.

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