ELSEVIER

Arch. Gerontol. Geriatr. 36 (2003) 7–14

ARCHIVES OF GERONTOLOGY AND GERIATRICS

www.elsevier.com/locate/archger

Alterations of the lymphocytic set-up in elderly patients with cancer

M. Motta [a], L. Ferlito [a], L. Malaguarnera [b], E. Vinci [b], S. Bosco [a], D. Maugeri [a], M. Malaguarnera [a,*]

[a] *Department of Longevity Sciences, Urology and Neurology, University of Catania, Cannizzaro Hospital, Via Messina 829, I-95124 Catania, Italy*

[b] *Department of Biomedical Sciences, University of Catania, Via Androne 83, I-95100 Catania, Italy*

Received 17 January 2002; received in revised form 26 May 2002; accepted 29 May 2002

Abstract

It is assumed that the increased incidence of neoplastic pathologies with advancing age is correlated with the immunosenescence and with the altered immune-surveillance. The present study was aimed at evaluating the role of the immunocompetent system and immunosenescence in carcinogenesis. A pool of 99 subjects (38 females, 61 males) has been analyzed in three groups as follows. Group A: 51 elderly subjects with cancer (16 females and 35 males, average age 73.7 ± 7.5 years). Group B: 24 young subjects with cancer (12 females, 12 males, average age 49.5 ± 10.3 years). Group C: 24 elderly subjects without any clinical evidence of cancer (10 females, 14 males, average age 74.6 ± 6.3 years). Hemo-chromocytometric analysis and cytofluorimetric typifying have been performed in all subjects. A decrease of T ($CD3^+$)-lymphocytes has been observed in group A, if compared to group B ($P < 0.007$), and to group C ($P < 0.01$), The T ($CD4^+$)-lymphocytes were fewer in group A, than in group C ($P < 0.004$), and also the NK cells showed the same trend ($P < 0.002$). The numbers of leukocytes and monocytes increased in group A compared to group C ($P < 0.01$ and $P < 0.004$, respectively). Red cell numbers, hemoglobin and hematocrit values were lower in group A than in group B ($P < 0.03$, $P < 0.03$, $P < 0.01$, respectively), and also than in group C ($P < 0.007$, $P < 0.001$, $P < 0.01$, respectively), The results demonstrate that the alterations of the immunocompetent cells, particularly of the T-cell pool, may play an important role in the carcinogenesis of the elderly.

Keywords: Cancer in elderly; Immunity in elderly; Lymphocytes in cancer

* Corresponding author. Tel.: +39-095-726-2008; fax: +39-095-498-811/726-2011
E-mail address: malaguar@mbox.unict.it (M. Malaguarnera).

0167-4943/02/$ - see front matter
PII: S0167-4943(02)00053-5

1. Introduction

It is hypothesized that the deterioration of the immune system may contribute to the development of neoplastic and degenerative processes during aging. This hypothesis agrees with the facts showing that molecular and cellular components of the immune system tend to deteriorate with age up to level where coordinated immune functions decline. Such a decline may involve, although to various extents, both the unspecific and the specific immune functions, and in the filed of the latter, both the cellular and the humoral responses (Ginaldi et al., 1999a). Namely, the cellular equilibrium between the proliferation, survival and programmed cell death become more and more disturbed with advancing age. Because this equilibrium is so tightly regulated, even a slight shift of it may disturb the cellular homeostasis, with negative consequences for the immune functions, and causes an increased prevalence of pathological phenomena (Ginaldi et al., 2000). As a matter of fact, the tumor prevalence and the specific, malignant neoplastic mortality of a given age group continuously increase with age (Malaguarnera et al., 2000).

More than 50% of all tumors occur in patients older than 65 years (Carbone, 2000). In spite of a general age-dependent increase of the malignant neoplasias, the prevalence of various cancers is not uniform. Epidemiological studies in various parts of the world described that the most frequent tumors in the elderly are the bladder and prostate cancers in men, and the mammary cancer in women, whereas the subsequent prevalences are in both sexes the colorectal, the lung and the gastric cancer (Vercelli et al., 1999, 2000). In addition, an increase of prevalence of immunoproliferative diseases (lymphomas and myeloma multiplex) has also been observed in the elderly (Anisimov, 1985; Leone et al., 1999; Baraldi-Junkins et al., 2000).

Since the oncogenesis is an extremely complex phenomenon involving not only immunological factors, but also exogenous ones, like life style, stress, viruses and retroviruses, as well as various genetic factors, it is really very difficult to establish the exact roles played by the immuno-competent and the immunosenescent systems. One can assume that the increased prevalence of neoplastic pathologies with aging is correlated with the immunosenescence, i.e. with the complex alterations compromizing the immune-surveillance.

The present studies were aimed at evaluating the role of immuno-competent, and in particular, the immunosenescent systems in tumor development. Our attention was focused on three groups of patients (i) elderly patients with tumors, (ii) young patients with tumors and (iii) elderly patients without clinical evidence of tumors.

2. Subjects and methods

We studied 99 patients (38 females and 61 males) in three groups.

Group A consisted of 51 elderly subjects (16 females and 35 males) with tumors (average age was 73.7 ± 7.5 years). The occurrence of tumors in this group was the following: colon cc (5), endometrium cc (2), liver cc (9), laryngeal cc (2), mammary cc (3), pancreas cc (2), lung cc (7), prostate cc (4), rhino-pharyngeal cc (3), vulvar cc (2),

tonsillar cc (1), bladder cc (6), cholecyst cc (2), non-Hodgkin-lymphoma (1), chronic lympoid leukemia (1), and plasmacytoma (1).

The Group B consisted of 24 younger subjects (12 females, 12 males) (average age was 49.4 ± 10.3 years). The occurrence of tumors in this group was the following: colon cc (3), liver cc (2), mammary cc (2), mesotelioma (2), lung cc (2), kidney cc (3), testis cc (1), thyroid cc (1), bladder cc (1), apudoma (1), chondroma (1) endometrium cc (1), glioblastoma (1), ovarium cc (1), myeloma multiplex (1), melanoma (1).

The Group C consisted of 24 elderly subjects (10 females and 14 males) (average age was 74.6 ± 6.3 years) displaying no clinical evidence of neoplasias or other pathologies.

Hemocytometric analysis was performed in all patients, by using an instrument CELL-DYN 3000, (product of ABBOTT). The type distribution of the lymphocytes was established by using a Coulter EPICS XL cytofluorimeter. We have applied a quadruple antibody labeling (CD4–CD3–CD19–CD56 and CD45–CD3–CD4–CD8) with gating at CD45 (Cyto-Stat Antibodies). As an activation marker, the expression of HLA anti-genes by the T-cells was measured.

In particular conditions, like in cases of lymphoproliferative diseases, some further marker factors have also been evaluated.

We studied also a group of blood donors ($n = 24$) frequenting our hospital, as a control population. Their 'normal' values ($\pm$S.D.) were the following (all /μl):

T-cells ($CD3^+$ cells)	1460 ± 370
T-helper cells ($CD4^+$ cells)	950 ± 310
T-suppressor cells ($CD8^+$ cells)	530 ± 140
NK-cells ($CD3^-$, $CD56^+$ cells)	380 ± 120
B-cells ($CD19^+$ cells)	350 ± 110

Statistical analysis was carried out by means of the Student's t-test for unpaired data.

3. Results and discussion

It should be noted that because of the inhomogeneity of the study groups, it was not possible to create specific groups per diagnosis or sex. Therefore, the results obtained in the study groups are presented in Table 1, as a single pool of data per group. Although this method resulted in quite a large statistical scatter of data, the results allow us to reveal certain tendencies and draw some conclusions.

A greater part of the measured parameters displayed statistically significant differences, when comparing the groups A/B or A/C, whereas a smaller number of them did not differ significantly between the groups (Table 1).

Among the differences, we wish to emphasize those regarding the lymphocytes and their subtypes. Namely, in group A, the $CD3^+$, $CD4^+$, and NK-cells occurred less frequently than in the Group C, while in group B only the last two types of cells were less frequent.

Table 1
List of the measured values (mean ± S.D.)

Parameter	Unit	Group A	Group B	Group C	Significance	$P <$
Number of patients		51	24	24		
Age	years	73.7	49.4	74.6	A/B	0.01
		±7.5	±10.3	±6.3	A/C	NS
Blood counts						
Red cells	g/l	3.96	4.33	4.38	A/B	0.03
		±0.56	±0.82	±0.41	A/C	0.01
Hemoglobin	g/dl	11.6	12.7	13.2	A/B	0.03
		±1.90	±2.40	±1.21	A/C	0.01
Hematocrit	%	34.2	38.0	40.3	A/B	0.01
		±5.83	±6.83	±3.68	A/C	0.01
Mean cell volume (MCV)	Fl	87.1	88.4	90.4	NS	
		±7.60	±8.30	±4.23		
Platlets	K/μl	239.3	261.4	255.6	NS	
		±100.6	±110.9	±53.8		
White cells	K/μl	9.46	8.05	6.48	A/C	0.01
		±5.10	±3.90	±1.11		
Neutrophils	K/μl	6.74	5.32	4.02	A/C	0.02
		±4.80	±3.40	±1.09		
Lymphocytes	K/μl	1.83	1.92	1.81	NS	
		±1.86	±0.74	±0.41		
Monocytes	K/μl	0.73	0.64	0.30	A/C	0.01
		±0.43	±0.39	±0.24		
Eosinophils	K/μl	0.12	0.13	0.18	NS	
		±0.11	±0.08	±0.19		
Basophils	K/μl	0.03	0.07	0.09	A/B	0.01
		±0.05	±0.03	±0.18	A/C	0.01
Lymphocyte subsets						
T-cells ($CD3^+$)	/μl	1033.6	1433.4	1353.0	A/B	0.01
		±512.8	±620.3	±288.2	A/C	0.01
T-helper ($CD4^+$, Th)	/μl	603.6	537.6	865.8	A/C	0.01
		±334.7	±461.4	±238.1		
T-suppressor ($CD8^+$, Ts)	/μl	433.6	480.2	449.8	NS	
		±218.4	±355.5	±173.8		
$CD4^+/CD8^+$ ratio	2.021	2.510	2.300	NS		
		±1.200	±1.790	±1.290		
NK-cells ($CD3^-$, $CD56^+$)	/μl	157.3	141.1	288.5	A/C	0.01
		±85.6	±86.4	±224.5		
B-cells ($CD19^+$)	/μl	296.5	226.3	209.0	NS	
		±419.7	±171.4	±117.8		

Notes: $K = 10^3$; $M = 10^6$; fl = femtoliter, NK = natural killer cells.

Table 1 shows also some general blood composition changes, like increases in group A (white cells, monocytes) and decreases (basophils, red cells, hemoglobin, hematocrit, etc.) which were more accentuated, or even of different character, than in group B.

When interpreting these findings, one has to consider various facts. First, it is well established that aging itself causes certain alterations in the immune system, and these may influence the ability of maintaining the integrity of the organs, tissues, neurohormonal regulations, etc. to various extents. Second, it is also known that T-cells play a role in the neoplastic process, however, sometimes in a contradictory manner, i.e. they may inhibit or even stimulate the formation and growth of tumors (Malaguarnera et al., 2001a).

The decrease of $CD3^+$ cells in group A, i.e. in elderly persons with tumors, may be considered as an absolute modification of the lymphocytic functions. These cells derive from the $CD2^+$ cells, which after having con-tacted the antigens, become mature effector cells, being directly responsible for various functions of the T-cells. They are able to transmit the T-cell receptor (TCR)-activating signal intracellularly (Abbas, 1994; Robey and Allison, 1995).

The decrease of $CD3^+$ cells is known to be associated with that of NK-cells and of Th-cells, which has also been observed in our group A, resulting in an alteration of the complex functions controlling the anti-tumor immune response. As a matter of fact, the NK-cells and the cytotoxic T-lymphocytes (CTL) realize the anti-tumor immune response through a direct cytotoxic activity, as well as through an antibody-dependent, cell-mediated cytotoxicity (ADCC) (Rosenberg et al., 1987; Miller, 1991). These cells may be controlled by various cytokines like interferon-gamma (IFN-γ) and interleukin-2 (IL-2), which potentiate their activity, and also by prostaglandins (PG) which are of inhibitory effect on them (Heberman, 1985; Malaguarnera et al., 2001b). In addition, it is known that the IL-2-activated NK-cells represent a particular anti-tumor activity called lymphokine activated killer (LAK)-cells. These cells display a major adhesive capacity facilitating their migration in the tissues, and localization at the tumors and the metastases (Rosenberg et al., 1987). Aging increases the absolute number of NK-cells, and decreases their lytic activity, both at the endogenous and the lymphokin-inducible level (Miller, 1991; Paganelli et al., 1994; Rink and Seyfarth, 1997). Therefore, the anti-tumor immune protection becomes considerably weaker with age (Old, 1985; Rosenberg et al., 1987; Storkus and Dawson, 1991; Mariani et al., 1994).

From a functional point of view, therefore, the T-cells display a reduced response to receptor stimulations, a damaged capacity for proliferative response, and a decreased secretion of IL-2, due to a decrease of the $CD4^+$ T-cells (Ginaldi et al., 1999b).

The Th-cells are able to promote the antibody production in B-cells, the formation of cytotoxic T-cells (CTL), and also the production of certain cytokines regulating several phases of erythropoiesis and modulating the immune response (Malaguarnera et al., 2001b).

The Th1-cells producing IL-2, IFN and TNF, are considered as pro-inflammatory cells, involved in the cell-mediated immunity, while the Th2 cells which produce IL-3, IL-4, IL-6 and IL-10 are anti-inflammatory cells. These are able to supply the B-cells with the necessary cooperation for the antibody production, i.e. are involved in the humoral immune response (O'Mahony et al., 1998; Sangfelt et al., 2000). A decreased production of IL-2 has been described during aging, attributed to an

altered function of Th1-cells, and also an increase of IL-4 and IL-5 levels as a consequence of altered Th2 functions (Weksler and Schwab, 1992; Mosmann and Sad, 1996; Romagnani et al., 1997; Wick and Grubeck-Loebenstein, 1997). This explains why the reduced number of Th-cells causes increases and decreases in several cytokines controlling the neoplastic processes.

As against the above described situation, the Ts-cells are able to block the induction and/or function of Th-cells, i.e. they can protect the tumor cells against the immune defense, facilitating this way their growth (Robey and Allison, 1995). Likely the tumor cells defend themselves from the cytolytic cell attacks by activating the Ts-cells, attenuating this way the immune response of the organism. In addition, the reduction of the expression of class I HLA antigenes on their own surfaces, renders these cells non-attackable by the T- and B-cells (Elliott et al., 1989; Malaguarnera et al., 2001b).

Our studies did not reveal any significant difference in the Ts-cells in any of the groups. This finding might be of importance, if considering that the number of Th-cells decreased in the group A, compared to group C, resulting in that the ratio $CD4^+/CD8^+$ cells, although displayed some decreasing tendency in groups A/C, this decrease remained statistically insignificant (Table 1) at the given level of sampling. Nevertheless, this decreasing tendency would involve a decreased anti-tumor response (Segre et al., 1989; Thoman and Weigle, 1989). It seems that the main factor of the age-dependent decline of the anti-tumor immune response is due to a decreased number of the $CD4^+$-cells, and a series of functional alterations.

The changes in the general blood composition, like increased WBC and anemia in elderly with tumor seem to be more consequences than causal factors in the carcinogenesis, the extent of which depends also on the type and state of tumors, and also on the actually conducted chemotherapy, as well as on the response level to the treatment.

In conclusion, it seems to be sure that the altered immuno-surveillance is certainly involved in the anti-tumor defense in the elderly patients (group A), as compared to the younger ones (group B). Yet, there are still a number of controversies regarding the importance of the various types of alterations. A further and deeper exploration of these mechanisms might contribute to a more efficient intervention against the tumor growth.

References

Anisimov, V.N., 1985. Cancer and aging. Arch. Geschwulstforsch. 55, 421–428.

Abbas, A.K., 1994. Cellular and Molecular Immunology, 2nd ed.. Saunders, Philadelphia, PA.

Baraldi-Junkins, C.A., Beck, A.C., Rothstein, G., 2000. Hematopoiesis and cytokines. Relevance to cancer and aging. Hematol. Oncol. Clin. North Am. 14, 45–61.

Carbone, P.P., 2000. Advances in the systemic treatment of cancers in the elderly. Crit. Rev. Oncol. Hematol. 35, 201–218.

Elliott, B.E., Carlow, D.A., Rodrick, A.M., Wade, A., 1989. Prospective on the role of MCH antigens in normal and malignant cell development. Adv. Cancer Res. 53, 181–245.

Ginaldi, L., De Martinis, M., D'Ostilio, A., Marini, L., Loreto, M.F., Corsi, M.P., Quaglino, D., 1999. The immune system in the elderly: II. Specific humoral immunity. Immunol. Res. 20, 109–115.

Ginaldi, L., De Martinis, M., D'Ostilio, A., Marini, L., Loreto, M.F., Martonel-liu, V., Quaglino, D., 1999. The immune system in the elderly: III. Innate immunity. Immunol. Res. 20, 117–126.

Ginaldi, L., De Martinis, M., D'Ostilio, A., Marini, L., Loreto, M.F., Corsi, M.P., Quaglino, D., 2000. Cell proliferation and apoptosis in the immune system in the elderly. Immunol. Res. 21, 31–38.

Heberman, R.B., 1985. Multiple functions of natural killer cells, including immunoregulation as well as resistamce to tumor growth. Concepts Immunopathol. 1, 96–132.

Leone, G., Mele, L., Pusloni, A., Equitani, F., Pagano, L., 1999. The incidence of secondary leukemias. Haematologica 84, 937–945.

Malaguarnera, M., Di Mauro, S., Laurino, A., Motta, M., Di Fazio, L., Maugeri, D., 2000. The comorbidities of elderly oncologic patients. Arch. Gerontol. Geriatr. 30, 237–244.

Malaguarnera, L., Ferlito, L., Imbesi, R.M., Gulizia, G.S., Di Mauro, S., Maugeri, D., Malaguarnera, M., Messina, A., 2001. Immunosenescence: a review. Arch. Gerontol. Geriatr. 32, 1–14.

Malaguarnera, L., Ferlito, L., Di Mauro, S., Imbesi, R.M., Scalia, G., Malaguarnera, M., 2001. Immunosenescence and cancer: a review. Arch. Gerontol. Geriatr. 32, 77–93.

Mariani, E., Monaco, M.C., Cattini, L., Sinoppi, M., Facchini, A., 1994. Distribution of lytic activity of NK cell subset in the elderly. Mech. Ageing Dev. 76, 177–187.

Miller, R.A., 1991. Accumulation of hyporesponsive, calcium extruding memory T cells as key feature of age-dependent immune dysfunction. Clin. Immunol. Immunopathol. 68, 305–317.

Mosmann, T.R., Sad, S., 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol. Today 17, 138–146.

Old, L.J., 1985. Tumor necrosis factors (TNF). Science 230, 630–632.

O'Mahony, L., Holland, J., Jackson, J., Feighery, C., Hennessy, T.P., Mealy, K., 1998. Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. Clin. Exp. Immunol. 113, 213–219.

Paganelli, R., Sacla, E., Quinti, L., Ansotegu, I.J., 1994. Humoral immunity in aging. Aging 6, 143–150.

Rink, L., Seyfarth, M., 1997. Characteristics of immunologic test values in the elderly. Zeitschr. Gerontol. Geriatr. 30, 220–225.

Robey, E., Allison, J.P., 1995. T-cell activation: integration of signals from the antigen receptor and costimulatory molecules. Immunol. Today 16, 306–310.

Romagnani, S., Parrocchi, P., Delios, M.M., Romagnani, P., Annunziato, P., Piccini, M.P., Manetti, R., Sampognaro, S., Mavilia, C., Decarli, M., Maggi, E., Delprete, G.F., 1997. An update on human TH1 and Th2 cells. Int. Arch. Allergy Immunol. 113, 153–156.

Rosenberg, S.A., Lotze, M.T., Muul, L.M., Chang, A.E., Avis, F.P., Leitman, S., Linehan, W.M., Robertson, C.N., Lee, R.E., Rubin, J.T., 1987. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2, or high dose interleukin-2 alone. N. Engl. J. Med. 316, 889–897.

Sangfelt, O., Erickson, S., Grander, D., 2000. Mechanism of interferon-induced cell cycle arrest. Front. Biosci. 5, D479-D487.

Segre, D., Miller, R.A., Abraham, G.N., Weigle, W.O., Warner, H.R., 1989. Aging and immune system. J. Gerontol. 44, 164–168.

Storkus, W.J., Dawson, J.R., 1991. Target structures involved in natural killing (UK): characteristics, distribution, and candidate molecules. Crit. Rev. Immunol. 10, 393–416.

Thoman, M.L., Weigle, W.O., 1989. The cellular and subcellular bases of immunosenescence. Adv. Immunol. 46, 221–261.

Vercelli, M., Quaglia, A., Parodi, S., Crosignani, P., 1999. Cancer prevalence in the elderly. ITALPREVAL Working group. Tumori 85, 391–399.

Vercelli, M., Capocaccia, R., Quaglia, A., Casella, C., Puppo, A., Coebergh, J.W., 2000. Regulative survival in elderly European cancer patients: evidence for health care inequalities. Crit. Rev. Oncol. Hematol. 35, 161–179.

Weksler, M.E., Schwab, R., 1992. The immunogenetics of immune senescence. Exp. Clin. Immunogenet. 9, 182–187.

Wick, G., Grubeck-Loebenstein, B., 1997. Primary and secondary alterations of immune reactivity in the elderly: impact of dietary factors and disease. Immunol. Rev. 160, 171–184.