Immunosenescence: implications for cancer immunotherapy in elderly patients

REVIEW

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ABSTRACT

The aging process produces functional and developmental changes in the immune system. Those changes may occur at different levels or at different moments, from lymphopoiesis up to the final response of the immune system facing a certain disease. The response of the adaptive immune system is most strongly affected by the aging process, particularly at the level of the effector T-cells. These changes can have a negative impact on the immune response of elderly patients during cancer immunotherapy. The present paper is an updated review of the bibliography on the most important modifications produced in the immune system during aging, as well as on the relevance of these modifications for the design of new strategies for cancer immunotherapy.

Key words: Aging, immunosenescence, cancer, immunotherapy

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RESUMEN

Inmunosenescencia: implicaciones para la inmunoterapia de cáncer en los adultos mayores. Durante el proceso de envejecimiento ocurren cambios en el sistema inmune que afectan su funcionamiento y desarrollo. Estos cambios pueden ocurrir a distintos niveles o en diferentes momentos, desde la linfopoyesis hasta la respuesta final del sistema inmune frente a determinada enfermedad. La respuesta del sistema inmune adaptativo es la más afectada, particularmente la de las células T efectoras. Estos cambios pueden tener un impacto negativo en la respuesta inmune de los ancianos frente a la inmunoterapia de cáncer. Esta es una revisión actualizada de la bibliografía acerca de los cambios más importantes que ocurren en el sistema inmune durante el envejecimiento, y la relevancia que pudieran tener en el diseño de nuevas estrategias para la inmunoterapia de cáncer.

Palabras claves: envejecimiento, inmunosenescencia, cáncer, inmunoterapia

*I*ntroduction

The deterioration in the performance of the immune system due to aging has been named immunosenescence. One of the symptoms of this deterioration is an increased susceptibility to infectious diseases, cancer, and autoimmune disorders [1]. During aging there is a steady accumulation of functional defects in the immune system that gradually compromises its performance; for instance, the production of lymphocytes in the bone marrow and thymus decreases, ultimately reducing the amount of available naïve cells for replenishing the peripheral repertoire [2]. Additionally, there are oligoclonal expansions of effector T lymphocytes with specificities restricted to a narrow set of epitopes, related to infections acquired during the lifetime of the individual; the T-cell repertoire shrinks, and, in general, there is a decrease in the immune response. Although these age-related defects have been known for some time, most studies in this field have been limited to their description, and it is only recently that the focus has shifted towards the identification of the mechanisms underlying these processes. There has been a growing interest in recent years on the development of geriatric oncology, due to the evidence of several risk factors for cancer development at advanced ages, as well as the increase in life expectancy in many modern societies. A thorough analysis of all the information currently available on this topic would be useful for the design of new, specific strategies focused on increasing the efficacy of vaccinations in the elderly, restoring the immune function in immunocompromised individuals, and for the prevention and control of cancer.

This review describes the main findings of the last 10 years on the aging-related deterioration of the immune system, with the aim of providing information for the development of new schemes of specific immunotherapy for cancer treatment in elderly patients.

*E*ffects of aging on thymopoiesis

The thymus is the site for maturation and differentiation of T-cells, also known as "the immunological clock of aging". The involution of this organ, and the decrease in the output of T lymphocytes, are two major changes that take place in the immune system in the course of aging.

Thymus involution generally begins during puberty. However, it has been reported that the atrophy of the epithelial space begins during the second year of life in humans. Although there is functional thymic tissue at least until the age of 60, after 50 most of the parenchymal tissue has been replaced by fat. This involution results in a decrease in the production of T cells and their export to the secondary lymphoid organs additionally affecting the maintenance of the naïve cell repertoire in the peripheral T cell compartment [2].

The thymus receives a constant stream of incoming hematopoietic precursors derived from stem cells in

1. Solana R, Pawelec G. Molecular and cellular basis of immunoenescence. Mech. Ageing Dev 1998;102:115-29.

2. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. Nat Immunol 2004;5(2):133-9.

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the bone marrow. It has been observed that age brings about important changes, such as a decrease in replicative potential [3], which results in a diminished capacity for the renovation of stem cells. A number of authors consider that the changes with age in the compartment of intrathymic progenitor T cells are due, in part, to a decrease in the lymphopoiesis of T cells, since it has been shown that the bone marrow of older mice does not produce lymphocytes with high efficiency when transferred to a younger individual [4, 5]. This indicates that the intrinsic defects accumulating with age are deleterious for the potential development of these cells.

The thymus contains a heterogeneous population of stromal cells that support the development of T cells. It has been speculated that age leads to the selective loss of one or more of these populations, as well as to detrimental changes in their size and functionality. This may be attributed to a reduction in the synthesis of key factors for T cell lymphopoiesis, such as IL-7 [6, 7], which has been linked to thymus involution.

Several authors have noted that the organization and maintenance of the thymic microenvironment depends on signals produced in the mature T lymphocyte [8, 9]. Therefore, it is believed that the initial reduction in T cells due to age-related defects in the early progenitors of the T lineage might affect the viability or functionality of the stromal thymic cells, thus compromising their potential to contribute to thymic microenvironment. This would, in turn, have a negative impact on the progenitor cells that would lead to the reduction of the T cell population [10].

Phenotypic analyses of lymphoid cells and their microenvironment have proven that thymus involution is mainly caused by quantitative changes. Although the total volume of the thymus decreases with age, its main lymphocyte subpopulations are preserved during the lifetime of the individual, both in cortex and medulla.

It has also been proven that extrathymic factors, such as zinc, cathepsin L, melatonin, thyroid hormone and growth hormone, are important for the maintenance of thymic functionality [11-13]. The interaction between developing thymocytes and thymic stromal cells can be sensitive to changes in the endocrine system, since both express receptors to one or more hormones, including the growth hormone and insulin-like growth factor I [14, 15]. These hormones have an antiapoptotic effect on thymocytes and, additionally, stimulate the synthesis of cytokines by the thymic stromal cells.

Studies performed on rodents have shown that thymopoiesis is stimulated by these hormones [14, 16], and the administration of growth hormone in humans increases the size of the thymus. However, this procedure does not restore the amount of thymic T cells to the levels found in younger mice [14]. Other authors have reported that the administration of IL-7 to mice can also increase the number of thymic T cells, but again, not to the levels found in young individuals [17, 18]. These findings, considered together, evidence that thymic involution is a multi-factorial process, and illustrate why reversing the changes occurring in the population of peripheral T cells with simple interventions with hormones or similar treatments is hardly a trivial task.

T cells

The critical changes that characterize the immunosenescence process take place mainly in T cell populations. The three most important alterations observed are:

1. A decrease in the number of naïve cells, due to diminished levels of thymopoiesis.

2. An increase in the number of memory cells, leading to an increase in the production of cytokines.

3. The accumulation of dysfunctional activated effector cells with a limited repertoire [19].

The T cells produced in the thymus migrate to different sites in the secondary lymphoid organs. The number of these cells does not change much with aging but, there is a significant reduction in the responses mediated by CD4 and CD8 T cells [20, 21].

One of the main reasons explaining the reduction with age of cell-mediated immunity is the substantial reduction in the fraction of naïve T lymphocytes, with a simultaneous increase in memory T cells [22]. The net effect of the reduction of peripheral naïve T cells is the shrinkage of the available repertoire, and, consequently, a poorer response to the appearance of new antigens.

As mentioned above, the fraction of T cells with a memory phenotype increases with age. This change has been particularly well studied in CD4 T cells. The analysis of CD4 T cells obtained from aged animals which have been depleted of their peripheral populations and reconstituted with precursors from young individuals has shown that the new CD4 T cells in the old animals are mainly memory cells with a $CD44_{high}$, $CD45Rb_{low}$ phenotype, producing L-selectin and with a cytokine profile characterized by high levels of gamma IFN, IL-4 and IL-5. This suggests that the aged microenvironment of the host leads to an accelerated maturation of the naïve CD4 T cells towards a memory state [1].

It has also been reported that age produces not only alterations in the thymic microenvironment, but in biochemical and molecular mechanisms of the T cells as well: a decrease in transmembrane signaling upon the engagement of the T cell receptor, changes in tyrosine kinase activity due to a reduced activation of the raf-1 and MEK-ERK kinases, and a decrease in the CD28-mediated activation of the JNK kinase. Among other changes occurring downstream to TCR stimulation, there are reductions in transcriptional factors [22-24] that play an important role in the expression of genes involved in the induction of the immune response [25].

The proliferation of T cells requires the synthesis of IL-2 and the presence of its receptor (CD25) [26, 27]. However, aging reduces the expression levels of this receptor, and the reduction in the size of the naïve lymphocyte populations also reduces the production of IL-2 [28]. Additionally, the existence of a chronic inflammatory state at older ages, caused by an increase in the levels of IL-6 and TNF alpha, has been reported [29]. During this state the sensitivity of CD4+ and CD8+ cells to TNF, alpha-induced apoptosis increases. Chronic exposure to TNF alfa can lead to the loss of CD28 expression in naïve CD8 T cells and central memory cells. Whereas effector T cells are resistant to apoptosis under these conditions, naïve or central memory cells are not [30].

3. Geiger H, Van Zant G. The aging of lympho-hematopoietic stem cells. Nat Immunol 2002;3:329-33.

4. Tyan ML. Age-related decrease in mouse T cell progenitors. J Immunol 1977;118:846-51.

5. Yu S, Abel L, Globerson A. Thymocyte progenitors and T cell development in aging. Mech Ageing Dev 1997; 94:103-111.

6. Andrew D, Aspinall R. Age-associated thymic atrophy is linked to a decline in IL-7 production. Exp Gerontol 2001;37:455-63.

7. Ortman CL, Dittmar KA, Witte PL, Le PT. Molecular caracterization of the mouse involuted thymus: aberrations in expression of transcription regulators in thymocyte and epithelial compartments. Int Immunol 2002;14:813-22.

8. Klug DB, Carter C, Gimenez-Conti IB, Richie ER. Thymocyte-independent and thymocyte dependent phases of epithelial patterning in fetal thymus. J Immunol 2002;169:2842-5.

9. Shores EW, Van Ewijk W, Singer A. maturation of medullary thymic epithelium requires thymocytes expressing fully assembled CD3-TCR complexes. Int Immunol 1994;6:1393-402.

10. Min H, Montecino E, Dorshkind K. Effects of aging on early B and T-cell development. Immunol Rev 2005;205:7-17.

11. Mocchegiani E, Santarelli L, Muzzioli M, Fabriss N. Reversibility of the Thymic involution and of aged-related peripheral immune dysfuntions by Zinc supplementation in old mice. Int J Immunopharmacol 1995;17:703-18.

12. Kasai M, Shirasawa T, Kitamura M, Ishido K, Kominami E, Hirokawa K. Proenzyme from cathepsin L produce by thymic epithelial cells promotes proliferation of immature Thymocytes in the presence of IL-1, IL-7 and anti- CD3 antibody. Cell Immunol 1993;150:124-36.

13. Goya RG, Gagnerault MC, Sosa YE, Bevilacqua JA, Dardenne M. Effects of growth hormone and thyroxine on thymulin secretion in aging rats. Neuroendocrinology 1993;58:338-43.

14. Montecino-Rodríguez E, Clark R, Dorshkind K. Effects of insulin-like Growth factor administration and bone marrow transplantation in thymopoiesis in aged mice. Endocrinology 1998;139:4120-6.

15. Savino W, Dardenne M. Neuroendocrine control of thymus physiology. Endocrine Rev 2000;21:412-43.

16. Kelley KW *etal*. GH3 pituitary adenoma cell can reverse thymic aging in rats. Proc Natl Acad Sci USA 1986;85:5663-7.

17. Aspinall R, Andrew D. Thymic atrophy in the mouse is a sluble problem of the thymic environment. Vaccine 2000;18:1629-37.

18. Andrew D, Aspinall R. IL-7 and not stem cell factor reverses both the increase in apoptosis and the decline in thymopoiesis seen in aged mice. J Immunol 2001; 166:1524-30.

19.Motta M, Ferlito L, Malaguarnera L, Vinci E, Bosco S, Maugeri D, Malaguarnera M. Alterations of the lymphocytic set-up in elderly patients with cancer. Arch. Gerontol. Geriatr 2003;36:7-14.

Another important alteration taking place in T populations is the accumulation of terminally differentiated effector cells that are reactive against a particular virus, but have an extremely limited TCR repertoire [31]. Often there is an oligoclonal expansion, preferentially involving CD8 T cells [32, 33], which are reactive against Cytomegalovirus (CMV) and Epstein Barr (EBV) [34, 35]. In humans, this expanded population appears to be resistant to apoptosis, it has lost the expression of the CD28 co-stimulatory molecule, it has shortened telomeres, and it has suffered a reduction of its proliferative capacity [36].

Longitudinal studies in healthy, elderly individuals, have shown that aging produces an inversion of the CD4:CD8 ratio, associated to an increase in mortality [37]. It is known that doubling the number of CD8 T cells after repeated stimulation cycles leads to the appearance of activated effector cells with a CD28-, CD27-, CD45RA+, CD57+ phenotype [34, 38]. These dysfunctional CD8+ cells can reduce the repertoire of available T cells for responding to new infections or neoplasias.

Several authors have reported a specific decrease in the response to immunizations in elderly individuals, due to a reduction in naïve cells. As age increases, the number of naïve CD45RA+ CD8 T cells decreases, with an associated increase in CD45RO+ memory CD8 T cells [28] and a reduction in the expression of the CD27 and CD28 markers, which indicates a high degree of differentiation of CD8 T cells in these individuals [39-41].

It has been hypothesized that memory CD8 T cells might lose their capacity for replication due to telomere loss, which can restrict their functionality or persistence during aging [42]. The naïve and memory CD8 T cells from older individuals have significantly shorter telomeres than their homologous counterparts from younger individuals [43, 44], and this phenomenon, especially in the case of clones recognizing persistent antigens, can eventually limit their expansion. Since the latter can become problematic for older individuals who have had multiple encounters with the same antigens throughout their life, chronic viral infections can play a major role in the establishment of replicative senescence.

Replicative senescence and the role of antigenic load

The loss of CD28 in the T cells from aged individuals can have a substantial impact in their functionality, since this molecule is involved in several key processes; including the transcription of IL-2 genes IL-2 [45], apoptosis, cellular adhesion and the regulation of telomerase activity [46-49]. These cells are characterized by a reduced proliferative capacity and short telomeres, indicating that they may have reached a stage in which human cells can no longer proliferate and therefore, are irreversibly impaired in their capacity for growth and adoption of functional changes. The latter phenomenon is known as replicative senescence [50, 51].

Replicative senescence is typical of CD8 T cells. The expanded CD8+CD28- cells are usually oligoclonal, and most of them are positive for CD57. During cell culture experiments in which CD4 and CD8 T cells from the same donor have been subjected to identical stimulation protocols, it has been observed that there are no further increases of telomerase activity in the CD8 T cells after the fourth encounter with the antigen, and most of these cells do not express CD28. On the other hand, the CD4 cells from the same donor, having experienced the same number of replication events, show high levels of antigen-induced telomerase activity and the expression of CD28 in a high percentage of the population [49]. Senescent CD8 T cells remain viable and metabolically active for long periods of time, preserving their antigen-specific cytotoxic capacity [1, 50]. In spite of the loss of the CD28 co-stimulatory molecule –characteristic of T cell replicative senescence- these cells retain other T cell markers indicating their cellular lineage. Senescent T cells not only have shortened telomeres and undetectable telomerase activity, but also display a significantly reduced response to thermal shock and an increased resistance to apoptosis [50]. Being effector cells that have entered a terminal stage of differentiation, they produce gamma IFN, but their levels of IL-2 synthesis are markedly reduced, in contrast to CD8+CD28+ T cell clones in which the reverse is true [1].

It has been proven that there is a correlation between a high proportion of CD8+CD28- and CD45RA+CD27- T cells, and seropositivity to CMV [52, 53]. This seropositivity suggests that in healthy elderly individuals there is a bias of the CD8 T cell repertoire towards a large expansion of specific clones recognizing a restricted number of CMV epitopes which no longer express CD28 and CD27 [54]. Such a situation leads to the expansion and accumulation of dysfunctional virus-specific T cells, thereby reducing the repertoire of available functional T lymphocytes for responding to new antigens [55]. The chronic stimulation of the immune system (antigenic stress), caused by infections occurring earlier in life, is considered by many authors to be the most important cause of immunosenescence, which is characterized by a reduction in immunological space and a restriction of the T repertoire, and ends up being substituted by quiescent antigen-experienced cells [56].

There is evidence that a persistent exposure to infectious agents leads to a faster senescence of the immune system. This, as explained above, decreases the expression of important molecules for T cell activation and reduces their functional capacity, leading to a clonal expansion that fills the immunological space and resulting in telomere shortening, and the subsequent inability to proliferate. Most elderly persons have trouble coping with infections, due to senescence [57]. It is important to point out that in people reaching an age of 100 or more without significant health disorders, the functionality of the immune system is well preserved, with parameters resembling those of much younger persons [58]. This suggests that their longevity is due, at least in part, to a slower progress of immunosenescence, in spite of the remodeling that age imposes on the immune system.

The facts exposed above underscore the need for reducing the chronic stimulation of the immune system when treating infections that cause chronic antigenic loads. This includes the treatment of intestinal parasites and vaccination against the most common viral infections in man, such as EBV and CMV.

20. Effros RB, Cai Z, Linton PJ. CD8 T cells and aging. Crit Rev Immunol 2003;23:45-64.

21. Grubeck-Lobeinstein B, Wick G. The aging of the immune system. Adv Immunol 2002;80:243-84.

22. Lerner A, Yamada T, Miller RA. Pgp-I^{hi}T lymphocytes accumulate with age in mice and respond poorly to Concanavalin A. Eur J Immunol 1998;19:977-82.

23. Miller RA, García G, Kirk CJ, Witkowski JM. Early activation defects in T Lymphocytes from aged mice. Immunol Rev 1997; 160:79-90.

24. Hirokawa K. Age-related changes of signal transduction in T cells. Exp Gerontol1999;34:7-18.

25. Boren E, Gershwin ME. Inflamm-aging: autoimmunity, and the immune-risk phenotype. Autoimmunity Rev 2004; 3:401-6.

26. Chakravarti B, Abraham GN. Aging and T cell mediated immunity. Mech. Ageing Dev 1999;108:183-206.

27. Nagel JE, Chopra RK, Chrest FJ, McCoy MT, Schneider EL, Holbrook NJ, *et al.* Decreased proliferation, interleukin 2 synthesis, and interleukin 2 receptor expression are accompanied by decreased mRNA expression in phytohemagglutinin-stimulated cells from elderly donors. J Clin Invest 1998;81:1096-102.

28. Fagnoni FF, Vescovini R, Passeri G, Bologna G, Pedrazzoni M, Lavagetto G, Casti A, Franscechi C, Passeri M, Sansoni P. Sortage of circulating naive CD8(+) T cells provides new insights on immunodeficiency in aging. Blood 2000;95:2860-8.

29. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, *et al.* Inflammaging. An evolutionary perspective on immunosenescence. Ann NY Acad Sci 2000;908:244-54.

30. Wagner WM, Ouyang Q, Sekeri-Pataryas K, Sourlingas TG, Pawelec G. Basic biology and clinical impact of immunosenescence. Biogerontology 2004;5:63-6.

31. Hakim FT, Flomerfelt FA, Boyiadzis M, Gress RE. Aging, immunity and cancer. Curr Opin Immunol 2004;16:151-6.

32. Callahan JE, Kappler JW, Marrack P. Unexpected expansions of CD8-bearing cells in old mice. J Immunol 1993;151: 6657-69.

33. Shwab R, *et al.* Expanded CD4+ and CD8+ T cell clones in elderly humans. J Immunol 1997;158(9):4493-9.

34. Ouyang Q, Wagner WM, Wikby A, Walter S, Aubert, Dodi AI, Travers P, Pawelec G. Large number of dysfunctional CD8 + T lymphocytes bearing receptors for a single dominant CMV epitope in the very old. J Clin Immunol 2003;23:247-57.

35. Ouyang Q, Wagner WM, Walter S, Muller CA, Wikby A, Aubert G, Klatt T , Stevanovic S, Dodi T, Pawelec G. An age-related increase in the number of CD8 T cells carrying receptors for an immunodomnant Epstein Barr Virus (EBV) epitope is counteracted by a decreased frequency of their antigen-specific responsiveness. Mech. Ageing Dev 2003;124:477-85.

36. Effros RB. Long-term immunological memory against viruses. Mech Ageing Dev 2000;121(1-3):161-71.

B cells

The reduction of B cell lymphopoiesis, in a situation paralleling that of the thymus, may be produced both by intrinsic defects in B cell progenitors and by age-related changes that negatively affect the potential of the bone marrow microenvironment to support their growth and differentiation. The decrease in the generation of B cells of older mice reconstituted with bone marrow transplants from younger donors [59, 60], and an inadequate level of IL-7 secretion by the aging stromal cells [61], support the notion that aging brings about an accumulation of microenvironmental defects.

The steady input of B cells into the spleen, and their later selection within the marginal and follicular zones, guarantee a constant replenishment of splenic B cells, as well as the maintenance of repertoire diversity [62]. Aging produces alterations in peripheral B cells in two important ways: The number of B cells migrating from the bone marrow to the spleen is reduced, and there is an accumulation of cells of the B lineage in the transitional and mature compartments of the spleen [63, 64]. Many of the latter cells are experienced memory B lymphocytes, which have moved on to fill the peripheral niches left by the reduced production of follicular B cells [65].

These changes in the composition of the peripheral B cell population have a consequence for humoral immunity: a rise in the incidence of autoantibodies [66] and a concurrent reduction of antibody response to foreign antigens [1, 67]. In studies in mice, it has been observed that aging brings about a gradual decrease in the formation of germinal centers [68]. Several authors have reported that the duration of the humoral response in older persons is comparatively shorter than in young individuals, having less protective immunoglobulins with lower titers and decreased affinities [69-72]. Besides, it has been described that the proportion of IgM antibodies secreted by the B cells of elderly persons is higher, and that the set of V region genes being used is different to that employed at younger ages [73, 74]. Aging also decreases the expression of the B7 (CD86) co-stimulatory molecule [68], and propitiates the appearance of B cell receptor signaling defects [75].

The problems produced by aging on the functionality of the helper T cells can also contribute to the reduction of the capacity to mount a vigorous, high-affinity antibody response in the germinal reaction centers of elderly persons [68]. It has been found that the expression of CD40, an important molecule for the interaction of T and B cells, is reduced in the latter.

Recently, Eaton *et al.* reported a marked reduction on the expression levels of CD154 (also known as the CD40 ligand, or CD40L) in activated helper T cells from elderly mice [76]. Since the interaction between B and T cells via CD40 and CD145 is necessary for the formation of germinal centers and antibody class switching, this might be an explanation for the poor antibody responses observed in the vaccination of older persons. A study on elderly patients vaccinated against influenza virus showed that elder vaccinees fail to develop adequate antibody titers, thus remaining only marginally protected, or exposed to severe episodes of influenza infection in spite of the vaccine [77, 78].

*I*nnate immunity

The innate immune system also deteriorates with age in humans and animals. Although the studies published on this system are less advanced than those focusing on adaptive immunity, several age-related changes have been described. For instance, the increase in B-1 cells (producers of autoantibodies) with age might explain the increased frequency of self-reactive immunoglobulins in the elderly [65].

There is evidence demonstrating a reduction of the functional potential of monocytes and macrophages in older mice. The comparison of human monocytes from young and old donors showed that in the latter there was a reduction in the level of cytotoxicity against tumor cells after activation with LPS, which was associated to a decrease in IL-1 secretion and the production of oxygen reactive intermediaries, such as NO_2 y H_2O_2 [79]. Furthermore, there were alterations in the secretion of other chemokines and cytokines, such as IL-6 and TNF alfa, and a significant reduction of the expression of Toll-like receptors, both in spleen macrophages and in the activated peritoneal macrophages of old mice [80, 81]. These phenomena would have a negative impact on antigen presentation, and could trigger an inadequate activation of the innate or adaptive immune functions [82].

As in other cellular populations, the microenvironment in an older host can certainly influence the differentiation and the behavior of antigen-presenting cells. It has been observed that IL-10, a key cytokine that suppresses cell-mediated immunity and the maturation and function of dendritic cells, is up-regulated in healthy old individuals [83]. The process of aging also affects the migratory capacity of dendritic cells [84], and their numbers in epidermal decrease [85, 86].

Other studies related to the variations in innate immunity during aging indicate that the number of natural killer (NK) cells increases with age; however, their cytotoxicity is reduced [87]. In fact, it has been observed that NK cells from old persons secrete less IFN gamma and chemokines in response to IL-2 and IL-6 [88].

*T*he Immune Risk Phenotype as an integrating element

Longitudinal studies conducted on a Swedish population of very old adults (more than 80 years old) [89] analyzed the changes in several immunological parameters related to aging, monitoring their behavior in time with the goal of establishing predictive factors for longevity [90]. These analyses yielded a set of parameters whose value can be used to predict the mortality and morbidity of a relatively homogeneous population within a time period of two years [91]. This set of parameters, associated to a poor immune function [25], has been called the Immune Risk Phenotype (IRP) [92] (Table 1).

The seropositivity to CMV was later associated to the IRP [93, 94]. It has been described that in some older individuals there is a clonal expansion of CMV-specific CD8 cells. These clones are dysfunctional, more resistant to apoptosis, and occupy a relatively large portion of the immunological space, being therefore harmful for the immune system [95].

Currently, several authors have hypothesized that the advance towards an IRP or the permanence in this

37. Huppert FA, Pinto EM, Morgan K, Bryan C. Survival in population sample is predicted by proportion of lymphocytes subsets. Mech. Ageing Dev 2003;124: 449-51.

38. Wikby A, Johansson B, Olsson J, Lofgren S, Nilsson BO, Ferguson F. Expansions of peripheral blood CD8 T lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish NONA immune study. Exp Gerontol 2002;37:445-53.

39. Effros RB, Boucher N, Porter V, Zhu X, Spaulding C, Walford RL, Kronenberg M, Cohen D, Schachter F. Decline in CD28+ T cells in centenarians and in long-term T cell cultures: a possible cause for both in vivo and in vitro immunosenescence. Exp Gerontol 1994;29:601-9.

40. Effros RB, Pawelec G. Replicative senescence of T cells: does the Hayflick Limit lead to immune exhaustion? Immunol Today 1997;18:450-4.

41. Fagnoni FF, Vescovini R, Mazzola M, Bologna G, Nigro E, Lavagetto G, Franceschi C, Passeri M, Sansoni P. Expansion of cytotoxic CD8+CD28" T cells in healthy ageing people, including Centenarians. Immunology 1996;88:501-7.

42. Akbar AN, Soares MV, Plunkett FJ, Salmon M. Differential regulation of CD8+ T cell senescence in mice and men. Mech Ageing Dev 2000;121:69-76.

43. Cossarizza A, Ortolani C, Paganelli R, Barbieri D, Monti D, Sansoni P, Fagiolo U, Castellani G, Bersani F, Londei M, Franceschi C. CD45 isoforms expression on CD4+ and CD8+ T cells throughout life, from newborns to centenarians: implications for T cell memory. Mech Ageing Develop 1996;86:173-95.

44. Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M, Lansdorp PM. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J Exp Med 1999;190:157-67.

45. Jenkins MK, Taylor PS, Norton SD, Urdahl KB. CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. J Immunol 1991;147:2461-6.

46. Van Lier RAW, Brouwer M, De Jong R, Groot M, De Groot E, Aarden LA, Knapp W (editors). Functional properties of the human T-cell differentiation antigen CD28. Leukocyte typing, vol. IV. Oxford: Oxford University Press; 1989.

47. Azuma M, Phillips JH, Lanier LL. CD28- T lymphocytes. Antigenic and functional properties. J Immunol 1993;4:1147-59.

48. Sepulveda H, Cerwenka A, Morgan T, Dutton RW. CD28 IL-2-independent costimulatory pathways for CD8 T lymphocyte activation. J Immunol 1999; 163:1133-42.

49. Effros RB. Replicative senescence of CD8T cells: effect on human ageing. Exp Gerontol 2004;39:517-24.

50. Effros RB. Replicative senescence in the immune system: impact of Hayflick Limit on T cell function in the elderly. Am J Hum Gen 1998;62:1003-7.

51. Campisi J. From cells to organisms: can we learn about ageing from cells in culture? Exp Gerontol 2001;36:607-18.

Table 1. Immune Risk Phenotype (IRP)

IRP	Positive IRP	Negative IRP
CD4:CD8 ratio	< 1	> 1
Proliferative responses of T cells to mitogens	Low	Normal
Number of CD8+, CD28-, CD57+ T cells	Increased	Normal
Number of B cells	Decreased	Normal
Response to CMV	IgG seropositive	Seronegative

status with aging is greatly influenced by the nature of the individual response to CMV infection.

Studies with persons of more than 80 or 90 years old have confirmed the presence, in this population of a marked expansion of CD8 CD28- cells, with receptors for a CMV epitope that is immunodominant in humans [34]. A high proportion of these CMV-specific cells also expressed high levels of the lectin-like G1 receptor, which is a negative regulator for killer cells (KLRG-1) [96]. This molecule is present in terminally differentiated senescent cells which, in spite of retaining their cytotoxic functionality, have a reduced capability for the secretion of cytokines such as IFN gamma.

These studies in very elderly persons also showed that the IRP behaved independently from the health status of the individual during the time in which the measurements were taken (2 years) [97].

The clinical relevance, in a pragmatic sense, of these IRP parameters has been an issue for some years [98]. Recently, advocates of the IRP definition have defended its potential usefulness not only in the field of aging, but also in situations of chronic antigenic stress in young individuals, and specially in cancer patients [99], for the prediction of the duration of life and of the response to certain immunotherapies.

Immunosenescence and cancer

The incidence of cancer rises disproportionately at older ages, both in Cuba and in many developed countries. Cancer is one of the leading causes of death, especially in the age groups of 46 to 75. In spite of the existence of a large body of research on the topic, the relationship between aging and cancer has not yet been satisfactorily elucidated.

In earlier decades, the prevailing scientific opinion defended the concept that the conversion from a normal to a cancer cell was a relatively frequent event, with the resulting malignant cells being eliminated by the constant surveillance of the immune system of the host. According to this concept, the development of cancer cells into a clinically significant cancer would occur only if this immunosurveillance failed, due to aging or other reasons [100]. However, whether the rise in cancer incidence seen in the older population is mainly due to the deterioration of the immune system is still under discussion [100].

Although the idea of immunosurveillance was initially conceptualized by Paul Ehrlich in the first decade of the XX century [101], much of the research on the topic conducted ever since have, however, failed to prove the existence of such a system. Nonetheless, in recent years the concept that the immune system plays a critical role in the development of cancer has gained general acceptance [102]. The development of tumoral models in mice with molecularly defined immunodeficiencies has led to a progress in the understanding of the complex relationship between this system and the cancer process [103]. Studies on IFN gamma or perforins in knockout mice have shown that the absence of these molecules, which are paramount for the proper functionality of T cells, increases the susceptibility of the host both to spontaneous or chemically induced tumors [104, 105]. The NK and NKT cells are also actively involved in cancer immunosurveillance. One of the first experiments to prove this fact used a monoclonal antibody (anti NK 1.1) to deplete this population in the C57 BL/6 mouse strain, showing that the treated mice were more susceptible to methylcholanthrene-induced tumorigenesis than the undepleted controls [106]. The accumulated data gathered so far indicates that immunosurveillance is not restricted to animal models, but it is also present in human beings [102, 107].

The evidence suggests that at least some of the mechanisms used by cancer cells to escape the surveillance of the immune system might be more effective in older hosts. One of these mechanisms is related to the interaction between the Fas ligand (FasL) and its receptor (FasR) [108]. It has been reported that the increase in the expression of FasR in aged leukocytes may facilitate the immune escape of FasL-expressing tumors through the induction of apoptosis in tumor-infiltrating leukocytes.

Another mechanism allowing tumors to escape from the immune system is the secretion of immunosuppressive cytokines, such as IL-10 and TGF beta, by cancer cells. These cytokines can suppress the inflammatory response of T cells and cell-mediated immunity, which are indispensable for tumor control and the destruction of cancer cells. Usually, the production of these cytokines is higher in the leukocytes of older persons, and their higher concentration might thus reduce the immune response of the host against the tumor [109].

The usefulness of cancer immunotherapy may look controversial in the light of the relationship between immunosenescence and tumor escape from immunosurveillance. Unlike most vaccines against infectious agents, which are mostly prophylactic, the cancer vaccines currently under development can be used on a wide array of scenarios, ranging from cancer prevention and the treatment of pre-neoplastic lesions after early diagnosis, to their use in minimal residual disorders for preventing cancer recurrence after tumor removal [82].

However, so far the experiments in animal models have convincingly proven that the efficacy of antitumoral vaccination depends on the immunocompetence of the host [110, 111]. The reduction in immunocompetence derived from aging also imposes an immediate risk on the complex processes of tolerance to self. In the specific case of rheumatoid arthritis (RA), a predominantly adult autoimmune disease, a status of accelerated aging of the immune system has been described, in which the T cells have a senescent phenotype and the number of naïve T cells is reduced [112]. As a matter of fact, it is believed that the autoimmunity in RA is a side effect of immunodegeneration, associated to the inadequate remodeling of the T cell population that occurs with aging [113].

52. Looney RJ, Falsey A, Campbell D, Torres A, Kolassa J, Brower C, McCann R, Menegus M, McCormick K, Frampton M, Hall W, Abraham GN. Role of cytomegalovirus in the T cell changes seen in elderly individuals. Clin Immunol 1999;90:213-9.

53. Kuijpers TW, Vossen MT, Gent MR, Davin JC, Roos MT, Wertheim-van Dillen PM, Weel JF, Baars PA, Van Lier RA. Frequencies of circulating cytolytic, CD45RA+CD27"CD8+ T lymphocytes depend on infection with CMV. J Immunol 2003;170:4342-8.

54. Khan N, Shariff N, Cobbold M, Bruton R, Ainsworth JA, Sinclair AJ, Nayak L, Moss PA. Cytomegalovirus seropositivity drives the CD8 T cell repertoire toward greater clonality in healthy elderly individuals. J Immunol 2002;169:1984-92.

55. Ouyang Q, Wagner WM, Wikby A, Remarque E, Pawelec G. Compromised Interferon gamma production in the elderly to both acute and latent viral antigen stimulation: contribution to the immune risk phenotype? Eur Cytokine Netw 2002;13:392-4.

56. Franceschi C, Valensin S, Fagnoni F, Barbi C, Bonafe M. Biomarkers of immunosenescence within an evolutionary perspective: the challenge of heterogeneity and the role of antigenic load. Exp Gerontol 1999;34:911-21

57. Baarle Van D, Tsegaye A, Miedema F, Akbar A. Significance for virus-specific memory T cells responses: rapid ageing during chronic stimulation of he immune system. Immunol Let 2005;97:19-29.

58. Franceschi C, Monti D, Sansoni P, Cossarizza A. The immunology of exceptional individuals: the lesson of centenarians. Immunol Today 1995;16:12-6.

59. Labrie JE, Sah AP, Allman DM, Cancro MP, Gerstein RM. Bone marrow microenvironmental changes underline reduced RAG-mediated recombination and B cell generation in aged mice. J Exp Med 2004;200:411-23.

60. Li F, Jin F, Freitas A, Szabo PI, Weksler ME. Impaired regeneration of the peripheral B cell repertoire from bone marrow following lymphopenia. Eur. J Immunol 2001; 31:500-5.

61. Stephan RP, Reilly CR, Witte PL. Impaired ability of bone marrow stromal cell to support B-Lymphoiesis with age. Blood 1998;91:75-88.

62. Chung JB, Silverman M, Monroe JG. Transitional B cells: step by step towards immune competence. Trends Immunol 2003;24:342-8.

63. Kline GH, Hayden TA, Klinman NR. B cell maintenance in aged mice reflects both increased B cell longevity and decreased B cell generation. J Immunol 1999;162:3342-9.

64. Johnson KM, Owen K, Witte PL. Aging and developmental transcisions in the B cell lineage. Int Immunol 2002;14:1313-23.

65. Johnson SA, Rozzo SJ, Cambier JC. Aging-dependent exclusion of antigen-inexperienced cells from the peripheral B cell repertoire. J Immunol 2002;168: 5014-23.

66. Weksler ME, Szabo P. The effect of age on the B cell repertoire. J Clin Immunol 2000;20:240-9.

The remodeling of the immune system due to the aging process suggests that the vaccination models with proven efficacy in young adults may not be that efficient with an older target population.

It has been proven that in aged mice there is a reduction in the number of naïve T cells and their conversion to a memory phenotype [114-116], a deregulation of the Th1 and Th2 responses with a bias towards the Th2 phenotype, and a deterioration in antigen presentation to the T lymphocytes by the APCs, all of which illustrate the multiple deleterious effects of age in the immune system. These phenomena have a negative effect on the different cellular populations involved in the activation of the immune response against cancer [117, 118].

It has also been observed that the signals produced by the components of the innate immune system, which are required to lead the adaptive immune response, can be inadequate or misdirected in older individuals, having a negative influence on the clonal and specific adaptive response.

There is a consensus on the low efficiency of vaccines in the older adult. The latter is supported by the evidence from several vaccines against different infectious agents, such as the influenza virus [119, 120], the polysaccharide-based vaccine against pneumococci [121], tetanus [122], and combined Hepatitis A and B vaccines [123]. More than 50 cancer vaccines have been reported to enter the phase of clinical trials as of today. Most of these have been small, non randomized trials focused on the evaluation of safety, immunogenicity and long-term responses for each product. Table 2 shows some of the products of this field in more advanced development stages [124]. Although it can be stated that the technology for generating an immune response against tumor-associated antigens and to measure this response has, in general, been developed, the efforts invested in this direction have failed to achieve the cure, or at least an increase in the survival, of cancer patients.

Two of the possible causes for the failure of clinical trials in their most advanced stages are the use of experimental models that do not represent clinical reality, and the intrinsic complexity of the immune system. In fact, most cancer vaccine candidates have been selected using experiments in young, healthy mice, transplanted with exogenous tumoral cells. Unsurprisingly, most of these vaccines are very effective in these models [124].

There are no clinical data on the relationship between the efficacy of cancer vaccines and the aging process. Some direct evidences on the reduction of their efficacy in models of older animals have recently been published. A study on the effect of mammary tumor cells modified to produce IL-2 to induce an antitumoral response capable of rejecting the tumor showed that it was not possible to induce a specific immune memory against these cells in old mice [125].

The above results were recently confirmed in a study that evaluated the efficacy of a DNA vaccine against the HER2/neu oncoprotein in advanced ages. The study proved that the vaccination was much more effective in younger than in older animals, and that the reduction in the objective response in older mice was associated to the age-related deterioration of the immune function [126].

Table 2. Cancer vaccines: The Most advanced projects in clinical trials

Vaccine	Manufacturer	Anatomical location	Current status	Results
Melacine	Corixa	Melanoma	Market	No influence on survival; improvement on quality of life
Theratope	Biomira	Breast	Phase III	No influence on survival
Provenge	Dendreon	Prostate	Phase III	Not available yet
BEC-2	ImClone	Small-cell lung	Phase III	Not available yet
Canvaxin	CancerVax	Melanoma	Phase III	Not available yet
Oncophage	Antigenics	Kidney	Phase III	Not available yet

Many of the strategies adopted during the last decade in the field of cancer immunotherapy have been based on the use of interleukins and interferons, in addition to the so-called adoptive immunotherapy, based on the injection of autologous *in vitro*-expanded lymphocytes [127, 128]. All these immunotherapeutic tools have been shown to have severe limitations upon their introduction in clinical trials. A number of authors have recently suggested the possibility of using normally expressed self antigens as a target for cancer immunotherapy. Such is the case of the p185 protein, which is the product of the Her-2/neu oncogene and is also known as mucin 1; it is an epithelial glycoprotein that is overexpressed in 90% of adenocarcinomas. The same can also be said of the therapies based on the epidermal growth factor (EGF) and its receptor (EGF-R) [129-131]. These therapies have yielded mixed results in clinical trials, and whether immunotherapies using poorly immunogenic self antigens will be successful in the coming years remains unknown [124]. Recent, unpublished results from our group showed that the survival advantages conferred by vaccination with EGF to non-small cell lung cancer patients are confined only to individuals under 60 years old.

Although the data gathered so far is not enough, the available evidence suggests that the use of cancer vaccines in elderly patients may not be as effective as in young individuals, due to the effects of age on the activation of the specific immune responses. Therefore, there is a need for the development of techniques for the therapeutic manipulation of immunosenescence, with the goal of finding effective treatments against cancer in elderly patients. This goal will require a deeper understanding of the mechanisms underlying the process of immunosenescence.

Conclusions

The changes that take place with age on the immune system have been extensively described during the last decade. Much must still be discovered on the mechanisms underlying those changes, especially at the genetic and molecular levels. The data gathered so far indicate that adaptive immunity is the immune subsystem most highly affected by aging. The innate immune system seems to be only moderately affected, although it has been proven that age does affect some of its pathways. However, since it has become clear that an optimal immune response depends on a coordinated cooperation between both systems, a deterioration of one part will be obviously detrimental for the functionality of the other.

The age-related deregulation of the immune system may contribute to morbidity and mortality. The

67. Fisman DN, Agrawal D, Leder K: The effect of age on immunologic response to recombinant hepatitis B vaccine: A meta-analysis. Clin Infect Dis 2002;35:1368-75.

68. Zheng B, Han S, Takahashi Y, Kelsoe G. Immunosenescence and germinal center reaction. Immnol Rev 1997;160:63-77.

69. Makinodan T, Kay MM. Age influence on the immune system. Adv Immunol 1980;29:287-330.

70. Goidl EA, Innes JB, Weksler ME. Imunological studies of aging. II. Loss of IgG and high avidity plaque-forming cell and increased suppressor cell activity in aging mice. J Exp Med 1976;144:1037-48.

71. Zharhary D, Segev Y, Gershon H. The affinity and spectrum of cross reactivity of antibody production in senescent mice: The IgM response. Mech. Ageing Dev 1977;6:385-92.

72. Nicoletti C, Yang X, Cerny J. Repertoire diversity of antibody response to bacterial antigen in aged mice: III Phosphorylcholina antibody from young and aged mice differ in structure and protective activity against infection with *Streptococcus pneumoniae*. J Immunol 1993;150:543-9.

73. Riley SC, *et al*. Altered VH gene segment utilization in the response to phosphorylcholine by aged mice. J Immunol 1989;143:3798-805.

74. Nicoletti C, Cerny J. The repertoire diversity and magnitude of antibody responses to bacterial antigens in aged mice: I Aged associated changes in antibody responses differ according to the mouse strain. Cell Immunol 1991;33:72-83.

75. Whisler RL, Grants IS. Aged-Related Alterations in the activation and expression of phosphotyrosine kinases and protein kinase C (PKC) among human B cells. Mech Ageing Dev 1993;71:31-46.

76. Eaton SM, Burns EM, Kusser K, Randall TD, Haynes L. Age-related defects in CD4 T cell cognate helper function lead to reductions in humoral responses. J Exp Med 2004;200(12):1613-22.

77. Ershler WB, Moore AL, Socinski MA. Influenza and aging: age-related changes and the effects of thymosin on the antibody response to influenza vaccine. J Clin Immunol 1984;4:445.

78. Ershler WB, Moore AL, Socinski MA. Influenza and aging: age-related changes and the effects of thymosin on the antibody response to influenza vaccine. J Clin Immunol 1984;4:445.

79. McLachlan JA, Serkin CD, Morrey KM, Bakouche O. Antitumoral properties of aged human monocytes. J Immunol 1995;154:832.

80. Lloberas J, Celada A. Effect of aging on macrophage function. Exp Gerontol 2003;37(12):1325-31.

evidence supports the notion that a healthy immune system is necessary to protect the older population against infectious diseases and, possibly, autoimmune disorders, cancer, and other non-transmissible disorders of the adult.

Taking into account that the changes occurring in the immune system during aging are diverse and multiple, it is probable that a single therapeutic intervention will not be enough for achieving protection against the most frequent diseases of the elderly.

The next few years should show an emphasis on cancer immunotherapy, using animal models closer to clinical reality that take into account variables such as age and the effect of previous treatments. Once a validated model is obtained, it will be essential to combine several tools such as hormone or interleukin treatments with active immunotherapy, to restore some of the functions affected by the aging process. Another important field of research is the development of new adjuvants to improve the poor response to immunization in aged individuals.

There is still hope for the idea of using active vaccination as a therapeutic alternative against cancer, but its success will depend on the thorough understanding of the mechanisms of immunosenescence.

81. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. J Immunol 2002;169(9):4697-701.

82. Provinciali M, Smorlesi A. Immunoprevention and immunotherapy of cancer in ageing. Can Immunol Immunother 2005;54:93-106.

83. Rink I, Cakman I, Kirehner H. Altered cytokine production in the elderly. Mech Ageing Dev 1998;102:109.

84. Steger MM, Maczek C, Grubeck-Loebenstein B. Morphologically and functionally intact dendritic cells can be derived from the peripheral blood of aged individuals. Clin Exp Immunol 1996;105:544.

85. Uyemura K, Castle S, Makinodan T. The frail elderly: role of dendritic cells in the susceptibility of infection. Mech Ageing Dev 2002;123(8):955-62.

86. Sprecher, *et al*. Effect of aging on epidermal dendritic cell populations in C57BL/6J mice. J Invest Dermatol 1990;94(2):247-53.

87. Solana R, Mariani E. NK and NK/T cells in human senescence. Vaccine 2000;18(16): 1613-20.

88. Ginaldi M, De Martinis M, D´Ostilio A, Marini L, Loreto MF, Quaglino D. The immune system in the elderly: III. Innate Immunity. Imunol Res 1999;20:117-26.

89. Wikby, A, *et al*. Age-related changes in immune parameters in a very old population of Swedish people: a longitudinal study. Exp Gerontol 1994;29:531-41.

90. Aspinall R. Ageing and the immune system *in vivo*: commentery on the 16th session of British Society for Immunology annual Congress, Harrogate, December 2004. Immunity & Ageing 2005;2:5.

91. Ferguson FG, *et al*. Immune parameters in a longitudinal study of a very old population of Swedish people: a comparison between survivors and nonsurvivors. J. Gerontol. A Biol Sci Med Sci 1995;50:378-82.

92. Pawelec G, *et al*. The SENIEUR protocol after 16 years. Mech Ageing Dev 2001;122:132-4.

93. Olsson J, *et al*. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. Mech Ageing Dev 2000;121:187-201.

94. Pawelec G, Akbar A, Caruso C, Efrros R, Grubeck-Loebenstein, Wikby A. Is immnosenescence infectious? Trends Immunol 2004;25(8):406-10.

95. Pawelec G, Ouyang Q, Wagner W, Biol D, Wikby A. Pathways to a robust immune response in the elderly. Immunol. Allergy Clin North Am 2003;23:1-13.

96. Ouyang, Q, *et al*. Age-associated accumulation of CMV-specific CD8+ Tcells expressing the inhibitory killer cell lectin-like receptor G1 (KLRG1). Exp Gerontol 2003; 38:911-20.

97. Nilsson BO, *et al*. Morbidity does not influence the T cell immune risk phenotype in the elderly: findings in the Swedish NONA immune study using sample selection protocols. Mech Ageing Dev 2003;124:469-76.

98. Pawelec G, Ouyang Q, Colona-Romano G, Candore G, Lio D, Caruso C. Is human immunoenescence clinically relevant? Looking for immunological risk phenotype. Trends immunol 2002;23:330-2.

99. Pawelec G. Immunosenescence and human longevity. Biogerontology 2003;4: 167-70.

100. Cui Z, Willigham MC. The effect of aging on cellular immunity against cancer in SR/CR mice. Cancer Immunol Immunother 2004; 53:473-8.

101. Erlich P. Ueber den jetzigen stand der Karzinomfors-chung. Ned Tijdschr Geneeskd 1909;5:273.

102. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991.

103. Dunn GP, Old LJ, Schreiber RD. The Immunobiology of Cancer Immunosurveillance and Immunoediting. Immunity 2004;21:137-48.

104. Street SE, Cretney E, Smyth MJ. Perforin and inter-van feron gamma activities independently control tumor initiation, growth, and metastasis. Blood 2001;97:192-7.

105. Street SE, Trapani JA, MacGregor D, Smyth MJ. Suppression of lymphoma and epithelial malignancies effected by interferon gamma. J Exp Med 2002;196:129-34.

106. Smyth MJ, Crowe, NY, Godfrey DI. NK cells and NKT cells collaborate in host protection from methylcholanthrene induced fibrosarcoma. Int Immunol 2001;13:459-63.

107. Dunn GP, Old LJ, Schreiber RD. The Three Es of Cancer Immunoediting. Annu Rev Immunol 2004;22:329-60.

108. Walker PR, Saas P, Dietrich PY. Role of Fas Ligand(CD95L) in immune escape: the tumor cell strikes back. J Immunol 1997;158:4521.

109. Zhou D, Chrest FJ, Adler W, Munster A, Winchurch RA. Increased production of TGF-beta and IL-6 by aged spleen cells. Immunol Lett 1993;36:7.

110. Cavallo F, Signorelli P, Giovarelli M, *et al*. Antitumor efficacy of adenocarcinoma cells engineered to produce interleukin 12 (IL-12) or other cytokines compared with exogenous IL-12. J. Natl Cancer Inst 1997;89(14):1049-58.

111. Colombo MP, Forni G. Cytokine gene transfer in tumor inhibition and tumor therapy: Where are we now? Immunol Today 1994;15(2):48-51.

112. Goronzy JJ, Weyand CM. T cells senescence and contraction of T cell repertoire diversity-catalysts of autoimmunity and chronic inflammation. Arthritis Res Ther 2003;5:225-34.

113. Weyand CM, Fulbright JW, Goronzy JJ. Immunosenescence, autoimmunity and rheumatoid arthritis. Exp Gerontol 2003; 38(8):833-41.

114. Pawelec G, Solana R. Immunosenescence. Trend Immunol 1997;11:514.

115. Pawelec G, Hirokawa K, Fulop T. Altered T cells signalling in ageing. Mech Ageing Dev 2001;122:1613.

116. Kapasi ZF, Murali-Krishna K, Mc Rae ML, Ahmed R. Defective generation but normal maintenance of memory T cells in old mice. Eur J Immunol 2002;32:1567.

117. Donnini A, Argentati K, Mancini R, Smorlesi A, Bartozzi B, Bernardini G, Provinciali M. Phenotype antigen-presenting capacity and migration of antigen-presenting cells in young and old age. Exp Gerontol 2002;37:1097.

118. Pawelec G, Solana R, Remarque ED, Mariani E. Impact of aging on innate immunity. J Leukoc Biol 1998;64:703.

119. Provinciali M, Di Stefano G, Colombo M, Della Croce F, Gandolfi MC, Daghetta L, Anichini M, Della Bitta R, Fabris N. Adjuvant effect of low-dose interleukin-2 on antibody response to influenza virus vaccination in healthy elderly subjects. Mech Ageing Dev 1994;77:75.

120. Provinciali M, Montenovo A, Di Stefano G, Colombo M, Daghetta L, Cairati M, Veroni C, Cassini R, Della Torre F, Fabris N. Effect of zinc or zinc plus arginine supplementation on antibody titer and Lymphocyte subsets after influenza virus vaccination in elderly subjects. Age Ageing 1998;27:715.

121. Jackson LA, Neuzil KM, Yu O, Benson P, Barlow WE, Adams AL, Hanson CA, Mahoney LD, Shay DK, Thompson WW. Effectiveness of pneumococal polysaccharide vaccine in older adults. N Engl J Med 2003;348:1747.

122. Steger MM, Maczek C, Berger P, Grubeck-Lobenstein B. Vaccination against tetanus in the elderly: do recommended vaccination strategies give sufficient protection? Lancet 1996;348:762.

123. Wolters B, Junge U, Dziuba S, Roggendorf

M. Immunogenicity of combined hepatitis a and B vaccine in elderly persons. Vaccine 2003;21:3623.

124. Lage A, Pérez R, Fernández LE. Therapeutic cancer vaccines: At midway between Immunology and Pharmacology. Curr Cancer Drug Targets 2005;5:611-27.

125. Provinciali M, Argentati K, Tibaldi A. Efficacy of cancer gene therapy in ageing: adenoarcinoma cells engineered to release I-2 are rejected but do not induce tumor specific immune memory in old mice. Gene Ther 2000;7:624-32.

126. Provinciali M, Smorlesi A, Donnini A, Bartozzi B, Amici A. Low of DNA vaccination against HER-2/neu in ageing. Vaccine 2003; 21:843-8.

127. Rosemberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettighausen JE, Matori YL, Skibberg JM, Shiloni E, Vetto JV, Seipp CA, Simpson C, Reichert CM. Obsarvationson the systemic administration of autologus lymphokine-activated killr cells and recombinant IL-2, to patients with metastatic cancer. N Engl J Med 1985;313:1485.

128. Rosemberg SA, Lotze MT, Yang JC, Topalian SL, Chang AE, Schwartzentruber DJ, Aebersold P, Leitman S, Linehan WM, Seipp CA, White DE, Steinberg SM. Prospective randomized trial of high dose interleukin-2 alone or in conjunction with lymphokine activated killer cell for the treatment of patients with advanced cancer. J Natl Cancer Inst 1993;85:622.

129. Finn OJ. Cancer vaccines: between the idea and the reality. Nat Immunol 2003;3:60.

130. Ko BK, Murray JL, Disis ML, Efferson CL, Kuerer HM, Peoples GE, Ioannides CG. Clinical studies of vaccine targeting breast cancer. Clin Cancer Res 2003;9:3222.

131. Lollini P-L, Forni G. Cancer immunoprevention: tracking down persistent tumor antigens. Trends Immunol 2003;24:62.

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