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REVIEW

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Immunoprevention and immunotherapy of cancer in ageing

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Abstract Over the last few years there has been a growing interest in geriatric oncology, mainly because of the evidence that advanced age is the greatest risk factor for the development of cancer and that, since the elderly population is rapidly expanding, so too will the number of cancer patients. This forecast necessitates the development of new and more specific strategies for the prevention and cure of cancer in the elderly and as a result an ever-increasing need for oncologists, geriatricians and researchers to work closely together. The increased incidence of cancer in elderly people has been related to the age-associated changes occurring in the immune system, the so-called immunosenescence. This phenomenon is best characterised by a remodelling of the immune system, which appears early on and progresses throughout a person's life and mainly involves a decrease in cellular functions. This review aims to provide a rationale for the development of specific immunotherapeutic and immunopreventive regimens for the elderly. We also include a discussion on the influence that immunosenescence has on the growth of tumours and the effectiveness of immunogene therapy and cancer vaccination following a brief analysis of the age-related alterations of the cell populations involved in antitumour immunity.

Keywords Adaptive immunity · Cancer · Cancer vaccines · HER-2/neu · Immunosenescence · Innate immunity

Immunological changes of ageing

Experimental and clinical data have demonstrated that ageing is associated with immune system dysregulation, which generally correlates with a decrease of immune functions, even though the activity of some immune effectors has been shown to increase in the elderly. Age-associated immune alterations have been related to the increase of infections, tumours and autoimmune diseases in the elderly [1–4]. For many years, on the evidence that postpuberal thymus involution may be relevant for specific immunity, studies on immunity during ageing have concentrated on the adaptive response and its hallmarks. For a long time the phylogenetically ancient defense mechanism, also known as the innate immune system, has been considered as a separate entity from the adaptive immune response and has been regarded as a factor of minor importance in the hierarchy of immune functions. For the past few years, however, interest in innate immunity has grown enormously, based on new knowledge of its integration with specific immune effectors and its key role in stimulating the subsequent, clonal response of adaptive immunity [5]. Major histocompatibility complex (MHC) unrestricted cytotoxic cells, besides their direct killing of target cells, seem then to play a pivotal role in providing the signals that are required to direct an adaptive immune response. For this reason, age-related alterations of the cellular components of innate immunity might be involved in the impairment of the adaptive immunity in the elderly. In what follows, we do not intend to provide an extensive review of the characteristics of immunosenescence, but briefly examine the major age-related alterations of adaptive and innate components of the antitumour immune response.

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T cells

Experimental data have revealed the evidence of immune responses to tumours and have shown that T cells are a critical mediator of tumour immunity. Naïve T cells, i.e. lymphocytes which have never encountered their specific antigen, are essential for the induction of primary immune responses against new tumour

antigens, and for the efficient generation of T-helper cell type 1 (Th1)-cell immunity which promotes cytotoxic T lymphocyte (CTL)-mediated responses. Destruction of tumour cells by specific CTLs has been demonstrated numerous times both in vitro and in vivo for a variety of tumours.

It is generally assumed that age-associated immune impairment is mainly due to a profound reshaping of adaptive immunity which is related to thymic involution [2, 3, 6–8]. Many investigators have examined age-related changes in T-cell subsets, in the hope of obtaining clues to the cellular basis of age-associated changes in immune functions. Most of this literature suggests that, in mice and in humans, ageing leads to an increase in the proportion of memory T cells, and a reciprocal decrease in T cells with the naïve phenotype. The numerical change in lymphocyte representation is accompanied by alterations of both T-cell–mediated and T-cell–dependent functions. Thus, proliferation to mitogenic lectins and alloantigens, the generation of cytolitic effector cells, delayed-type hypersensitivity, primary and secondary antibody responses are diminished in the old [4]. Furthermore, diminished and/or altered cytokine patterns have been described in old age with consequent dysregulation of Th1 and Th2 responses, with a shift to the Th2 phenotype [9]. Under these circumstances, the leukocytes of the elderly have been found to produce fewer Th1 cytokines, such as interleukin 2 (IL-2) and IFN-γ and, conversely, higher amounts of Th2 cytokines, such as IL-1, IL-6, IL-8 and IL-10, than those of young donors [10].

B cells

B cells contribute to the antitumour humoral responses by differentiating into antibody-secreting plasma cells. Both IgM and IgG antibodies have been shown to destroy tumour cells through either activation of complement or interaction with lytic cells which possess surface receptors for the Fc portion of the antibody (antibody-dependent cell-mediated cytotoxicity, ADCC). Though the number of circulating B cells has not been reported as changing with age, recent studies have demonstrated a decline in B lymphopoiesis in aged mice which reflects the loss of very early B-lineage precursors [11]. Furthermore, B cells from aged donors have shown a decreased expansion in response to antigens [12], whereas, the ability of aged B cells to differentiate into high-affinity, isotype-switched, antibody-secreting cells seems to be well preserved [12]. The functional ability of B cells to mount specific antibody responses to primary and secondary antigens decreases in the elderly [4]. The antibody response is reported as being lower in older adults than it is in younger adults following in vivo immunization with a flu vaccine [13]. Similarly, antitetanus toxoid antibody production was found to be significantly lower in adults over 65 years of age than in younger subjects [14]. Most of the age-associated changes of an antibody response have been related to the decline in T-lymphocyte function arising during ageing. However, the diminished ability of purified B cells to respond to isolated T-helper cells or to T-cell–derived helper factors, provides evidence of a decline in intrinsic B-cell function [4].

Antigen-presenting cells

Antigen-presenting cells (APCs), and primarily dendritic cells (DCs), which have been labelled as the most potent APCs, are essential in activating specific T cells and in turn initiating an adaptive immune response. Immature APCs are found in peripheral tissues where they capture antigens for processing and deliver them to lymphoid organs. Mature APCs are conversely specialised in presenting processed antigens to T lymphocytes [15]. The migration of APCs from the site of antigen deposition to lymphoid organs is considered to be a critical initial step during the induction of an immune response.

Though some studies show ageing does not affect the number of APCs or their function, several others have demonstrated the occurrence of age-related alterations of their antigen-presenting capacity [16–19]. Ageing may affect the antigen-presenting capacity of APCs by influencing their antigen-processing capacity, the presence of costimulatory signals on their surface, the levels of cytokines in their microenvironment, or their migratory capacity.

The central event in the activation of a cellular immune response to tumour cells is the presentation of antigenic peptides by APCs to CTLs through MHC class I molecules. The efficient generation of MHC class I–binding peptides depends on the intracellular immunoproteasome-mediated proteolysis machinery. A variety of experimental data have provided evidence for an age-related impairment of proteasome structure and function. The loss of proteasome activity was found to depend on at least three different mechanisms: decreased proteasome expression, alterations and/or replacement of proteasome subunits, and formation of inhibitory cross-linked proteins [20]. To date, few data exist on age-related changes of immunoproteasomes. This circumstance is likely due to the experimental difficulties involved in carrying out studies in this field. However, available data suggest that there is a general decrease in immunoproteasome content [21]. Hence, it seems that one of the first alterations in APCs in ageing may affect the crucial step of antigen presentation—i.e., the degradation of endogenous proteins, and then the generation of peptides for presentation by MHC class I molecules.

A second defect occurring in APCs that has been described during ageing is the expression of costimulatory molecules and the regulation of their activity. It is common knowledge that a signal received through the antigen T-cell receptor (TCR) is not sufficient on its own for the activation of naïve T lymphocytes since a second

costimulatory signal is required to induce effective immune responses. This costimulatory activity, present in APCs, seems to be required to signal the presence of a non-self antigen to antigen-specific receptors on T lymphocytes. Though the total number and the expression of MHC I and II, CD80, and CD86 both on immature and mature APCs do not seem to differ significantly in young and old mice [18, 22, 23], DCs in germinal centres of aged mice were found to lack expression of important costimulatory ligands such as CD86 [17], which would promote the induction of anergy in the antigen-specific T cells with which they interacted. Among the factors that regulate the expression of costimulatory activity on APCs are sets of receptors of the nonclonal innate recognition system called pattern-recognition receptors (PRRs). Of these, toll-like receptors (TLRs) are receptors that recognize conserved molecular patterns, which are shared by large groups of microbial components and are perfectly capable of distinguishing between self and non-self pathogen-associated structures and in turn of signalling the presence of a pathogen to the APCs [24]. A decreased TLR expression and function was recently demonstrated on APCs from aged mice [25]. In this study, both splenic and activated peritoneal macrophages from aged mice expressed significantly lower levels of all known murine TLRs (TLR 1–9), and produced lower levels of cytokines when stimulated with known ligands for TLRs when compared with young mice [25]. The reduced representation of TLRs on APCs from aged mice may have important implications in ageing. Macrophages and DCs specifically bind to apoptotic cell-associated molecular patterns through TLRs and mediate the efficient phagocytosis of apoptotic bodies [26]. Moreover, TLRs expressed on APCs are receptors for heat shock proteins (HSPs) and mediate HSP signalling [27]. HSPs are highly conserved proteins from prokaryote to eukaryote; various types of stress induce expressions of HSPs which function as chaperones improving antigen processing and presentation through binding to short peptides. The chaperoning of antigenic peptides into APCs via complexing with tumour cell–derived HSPs, has been described as a very effective way of immunising against a range of different tumours. This evidence suggests that a decreased TLR expression and function during ageing may have an impact on the antigen-presenting function resulting in an impaired immune activation of both innate and adaptive responses.

Antigen-presenting cell maturation and function are influenced by the surrounding cytokine milieu. As has been reported for other cell populations, the microenvironment existing in the "old" host may certainly influence the differentiation and performance of APCs. It has been observed that IL-10, a key cytokine which suppresses cell-mediated immunity and DC maturation and function is elevated in healthy old people [10]. The inhibitory effects of IL-10 on the accessory functions of APCs, such as the conversion of immature DCs into tolerating APCs, or the suppression of IL-12 production by activated DCs, or the down-regulation of CD40 expression on DCs, may be strengthened by the higher levels of this cytokine found in old age. For example, recombinant IL-10 was found to markedly inhibit IL-12 production in cells from elderly subjects [28], while the production of cytokines relevant for the differentiation and functional activity of APCs, like IL-4 and IL-12, declines in frail elderly people along with APC function [29, 30]. These findings suggest that age-related changes in cytokine levels may not only directly influence immune responses but may also alter the balance and maturation of APC subsets.

The migratory capacity of DCs has also been found to be affected by the ageing process [23]. A lower expression of the mRNA for the migratory CCR7 chemokine receptor was found in APCs from old mice, and a lower lymphocyte cytotoxicity and a reduced number of $CD8^+$ T cells producing IFN-γ were induced by APCs from aged mice in comparison to APCs from young animals [18]. The fact that CCR7 was greatly increased in mature APCs up to the levels found in young animals and that in vivo migration of APCs to regional lymph nodes was higher in old than in young mice, suggests that an increased migratory capacity of old APCs may be required to balance their reduced antigen presentation to cytotoxic lymphocytes [18]. The latter assumption is further emphasised by the fact that the lower CTL cytotoxicity induced by APCs from old mice has been attributed to an age-related defect of antigen presentation rather than to an intrinsically lower frequency of CTLs. Evidence that the precursors of CTL (pCTL) frequency always improved when the source of APCs was changed from old to young animals [31], and that the transfer of young T lymphocytes to old mice was unable to correct the deficit in T lymphocyte responsiveness observed in aged animals [32] is consistent with this suggestion. These results lead us to suspect that various factors are implied in the immunosenescence and that an impairment in APC function, in addition to an alteration of cytotoxic lymphocyte function [2, 3], may also be involved.

Macrophages and polymorphonuclear leukocytes

Macrophages are important cellular constituents of innate immunity which, besides their phagocytic and tumouricidal functions, have the capacity to influence the priming environment. They kill bacteria, viruses, parasites and tumour cells either directly or through the release of mediators, such as IL-1, IL-6, TNF-α, IFN-γ, which, in turn, activate other immune cells. Early studies conducted in ageing mice or in human subjects showed normal macrophage function [33, 34]. More recent studies, however, suggest that macrophage number and function may indeed alter with ageing. A significant expansion of $CD14^{dim}/CD16^{bright}$ circulating monocytes, which are considered to show phenotypic evidence for activation, has been reported to occur in elderly

people [35]. The constitutive or induced production of IL-1, IL-1 receptor antagonist, and IL-6, was found to increase in monocytes from elderly subjects [35, 36]. However, in a recent study conducted in young and old subjects screened using the Senieur protocol, no age-related difference was noted in the total amounts of IL-1β and in IL-6 serum levels after normalising for circulating monocytes [37]. Monocytes from old donors, when compared with monocytes from young subjects, displayed decreased cytotoxicity against tumour cells after LPS activation, the impairment being associated with a decrease in IL-1 secretion and production of reactive oxygen intermediates such as NO_2 and H_2O_2 [38].

Alterations in macrophage number and function have also been described in old rats and mice. An age-associated impairment in TNF-α production was found in rat macrophages [39]. Similarly, macrophages from aged mice, in vitro activated with IFN-γ and LPS, exhibited reduced antitumour activity and impaired capacity to produce TNF, IL-1 and nitric oxide, critical monokines and effector molecules able to directly inhibit tumour growth [40, 41]. A lower expression of the production of MHC class II gene was found on the cell surface of macrophages from old mice after incubation with IFN-γ [42]. Finally, recent findings have been reported on the expression of TLRs on macrophages in ageing mice [25]. Both splenic and activated peritoneal macrophages from old mice have been found to express significantly lower TLR levels. These cells also secreted lower levels of IL-6 and TNF-α when stimulated with known ligands for TLR compared to those in young mice.

Long regarded as mere phagocytes, in current publications polymorphonuclear leukocytes (PMNs) have been described as important effectors in the induction of immune responses; these studies view PMNs as active participants in the antitumour immune surveillance. PMNs have been found among the cell populations more represented in the tumoral infiltrate after in vivo immunisation both in young and in old age [32, 43, 44]. The peritumoural and intratumoural release of cytokines attracts PMNs, as demonstrated by the fact that mice challenged with IL-2–engineered tumour cells were able to reject the tumour because of direct killing by activated PMNs and macrophages both in young and old age [32, 43]. Several studies show that the neutrophil number in blood and neutrophil precursors in bone marrow, as well as the response to GM-CSF and IL-3, are not lowered in the healthy elderly—albeit, the proliferative response of neutrophil precursor cells to G-CSF was found reduced [45–47].

In vitro studies of leukocyte chemotaxis have revealed that migratory responses of neutrophils from healthy old subjects are either unaltered [48, 49] or only slightly reduced [50, 51]. The data from the literature have reported differences arising from the effects of age on PMN phagocytosis, with several studies showing a dramatic decrease in the PMN phagocytic activity from aged individuals [52, 53]. Several groups have examined neutrophil microbicidal activity and, though data are often conflicting [54, 55], the bulk of evidence supports a decline in cytotoxicity toward bacteria and yeast with age [50, 55, 56].

Natural killer cells

Natural killer (NK) cells are naturally occurring, large, granular, lymphocyte-like killer cells that are able to kill various tumour cells and that play a pivotal role in the resistance to tumours. Whereas an age-related impairment of both endogenous and cytokine-induced NK-cell activities has been commonly reported in mice, the changes occurring in human NK-cell activity with advancing age remain to be fully elucidated. Data from the literature demonstrate differences in line with the enrolment criteria used to select the elderly subjects for the study and the use of either total lymphocyte populations or purified NK cells.

Overall, both the total and the relative number of circulating NK cells were found to be significantly increased in healthy elderly people in comparison with young adult ages. The increased percentage of NK cells in the elderly is mainly related to the higher number of the $CD56^{dim}$ population, which represents the mature NK-cell subset, with a decreased $CD56^{bright}$ to $CD56^{dim}$ ratio. [57, 58]. The age-related increase of NK-cell number has been considered as a compensatory mechanism for the decreased cytolytic activity *per cell* found in elderly subjects, as is highlighted in purified NK cells through sorting or after cloning of NK-cell precursors by limiting dilution [59].

There are few and contradictory findings on the responsiveness of NK cells from elderly humans to the boosting action of IFN or IL-2. Decreased [60] or unchanged [61] stimulation of NK activity by IL-2 and a reduced or unchanged IFN-boosting effect have been found in peripheral blood lymphocytes from old humans [62]. The simultaneous analysis of the boosting effect of IL-2 or IFN-α in a ''healthy'' elderly population yielded no change in the increase of young and elderly NK cytotoxicity against the NK-sensitive K562 cell line [58, 63]. In one of these papers, the use of IL-12, similarly to that of IL-2 and IFN, was found to enhance NK cytotoxicity to the same degree in both young and elderly subjects over a wide range of doses and incubation times [58]. A gradual decline in both IFN-inducible and IL-2–inducible NK activities appeared with increasing age in humans selected through the Senieur protocol [64]. The defect was more evident with IFN than for IL-2 responsiveness of NK cells, suggesting that, as observed in mice, the NK-cell response to IFN is more affected by age than the IL-2 one. By contrast, other data showed that the response of NK cells to IL-2 was impaired when proliferation, expression of CD69, and Ca^{2+} mobilisation were considered [57]. Consistent with these findings NK cells from elderly people gave a decreased proliferative response to IL-2 and a parallel impaired expression of the CD69-activation antigen [65].

Besides their endogenous activity and short-term cytokine-mediated activation, the role of NK cells as precursors of the so-called lymphokine-activated killer (LAK) cells has also been extensively studied after long-term activation with IL-2. LAK-cell activity is mediated by broadly reactive cytotoxic cells which are not major histocompatibility complex restricted and are capable of lysing fresh and cultured tumours, including both NK-sensitive and NK-resistant targets. In human subjects, most of LAK-cell activity is associated primarily with cells of the $CD56^+$ and $CD16^+$ phenotype, the precursor of which is similar to NK cells. The effects of ageing on the in vitro induction of LAK-cell activity has not been widely studied. Studies conducted in healthy subjects not selected as per specific immunogerontology protocol guidelines revealed that LAK-cell activity induced by short-term activation with IL-2 [66, 67] or with IL-2, IL-12, or IFN-α, for longer incubation times [68], declined with age. When the kinetics of the development of LAK cells was evaluated in young and old healthy subjects screened following the Senieur protocol, no age-related difference was found in terms of proliferative capacity, cytotoxicity against Daudi tumour cells, and expression of p55 or p75 IL-2 receptors [69]. In contrast, the proportion of $CD56^+$ and $CD16^+$ cells reached higher levels in old than in young donors, this fact demonstrates that an increased number of cytotoxic cells are required in old subjects to obtain the same levels of LAK-cell activity present in young age, further suggesting that a lower cytolytic activity *per cell* was present in old age [69]. The continuous culture or the short pulse of peripheral blood mononuclear cells with IL-2 was able to develop significant levels of LAK cell cytotoxicity even in elderly cancer patients [64].

Their direct MHC-unrestricted cytotoxic effects apart, NK cells have been shown to represent one of the first lines of defence during the early stages of immune activation because of their inducible secretory function. NK cells synthesise many cytokines and chemokines that can positively or negatively modulate their activity and that of cells of the adaptive immune response. A lower production of IFN-γ, IL-8 and chemokines was observed in either resting or activated NK cells taken from healthy elderly subjects in comparison with those from young subjects [70, 71].

Regardless of mechanism, the defect of NK activity in aged mice does not represent an irreversible process, since it may be recovered by hormonal and nutritional treatment [6]. Among hormonal factors relevant for NK function, it has been observed that thymic peptides or thyroid hormones, but not the pineal hormone melatonin, were able to restore the crippled NK cytotoxicity of spleen cells from old mice [72–75]. Among the nutritional factors, either zinc or a lipid mixture, which increases membrane fluidity, called ''active lipids'', were able to recover the impaired NK function in aged animals [76, 77]. Whether endocrine and nutritional factors have an additive effect or act through the same intracellular mechanism remains to be seen, though the first possibility seems more likely since the action of TSH and thyroid hormones is specifically directed toward lymphokine-boosted NK activity, while active lipids are able to prevent age-associated impairment of basal NK cytotoxicity [74, 78]. Apart from IFN and IL-2, the effect of cytokines on the development of cytotoxic cells during ageing has scarcely been investigated. In a study conducted using IL-12, the cytokine was able to boost both endogenous and IL-2–induced NK-cell activity in young and old mice. The levels of cytotoxicity were lower in old than in young animals although the relative increase of IL-12 plus IL-2 versus IL-2 alone was greater in old mice [79]. These data confirmed and extended previous findings obtained in humans and show that IL-12 is able to enhance NK cytotoxicity to the same degree in both young and elderly subjects, whereas the induction of IL-2–activated cytotoxic cells decreased in elderly compared to young individuals [58].

Compared to studies on NK activity, fewer have been conducted on the effects of ageing on the generation of LAK cells by IL-2 in mice. In several papers, no age-related difference in the cytolytic response of murine spleen cells to culture with IL-2 was found [77, 80, 81], though the inclusion of IFN-γ during the culture with rIL-2 augmented the activity induced in young mice but, by contrast, inhibited the generation of LAK activity from bone marrow cells of old mice [82]. Two other papers, on the other hand, described a decline in IL-2–induced LAK activity in aged mice [66, 83].

T lymphocytes bearing the $\gamma\delta$ TCR

T lymphocytes bearing the $\gamma\delta$ TCR represent a minor population of human peripheral lymphocytes (1–10%), most of them expressing the $CD3^+$ $CD4^-CD8^-$ phenotype [84]. The ability of $\gamma\delta$ T cells to respond to nonprocessed and nonpeptidic phosphoantigens in a MHC-unrestricted manner is an important feature distinguishing them from $\alpha\beta$ T cells [85]. In human peripheral blood, two main populations of $\gamma\delta$ T cells have been identified based on the composition of the TCR. The predominant subset expresses the Vδ2 chain associated with Vγ9 and represents 70% of the circulating $\gamma\delta$ T cells in adults, while a minor subset (approximately 30%) expresses a Vδ1 chain linked to a chain different from Vγ9. At birth the Vδ1 population predominates, while in adults there is a shift towards Vδ2 T lymphocytes, probably due to a selective response to environmental stimuli such as commonly encountered bacteria [86]. Although little is known about the physiological significance of $\gamma\delta$ T cells, their marked reactivity toward mycobacterial and parasitic antigens as well as tumour cells suggests that $\gamma\delta$ T cells play a role in antiinfectious and antitumoural immune surveillance [84]. Moreover, it has been shown that in healthy donors, $\gamma\delta$ T cell stimulated with nonpeptidic phosphoantigens such as isopentenylpyrophosphate (IPP), produced high levels of cytokines, especially IFN-γ and TNF-α.

Because of their cytokine production, it has been suggested that $\gamma\delta$ T cells are involved in coordinating the interplay between innate and adaptive immunity and in particular in guiding the establishment of acquired immunity, thus contributing to the definition of $\alpha\beta$ T-cell responses toward the Th1 or Th2 phenotype.

The data from the literature on numerical or functional changes of $\gamma\delta$ T cells during ageing are scarce and fragmentary. It has been reported that the complexity of the $\gamma\delta$ T-cell repertoire decreases with age as a consequence of the expansion of a few T-cell clones [87]. The analysis of $\gamma\delta$ T-cell number and function in elderly people and in centenarians has demonstrated an age-dependent alteration of $\gamma\delta$ T lymphocytes, with a lower frequency of circulating $\gamma\delta$ T cells, an altered pattern of cytokine production, and an impaired in vitro expansion of these cells [88]. The decrease in the $\gamma\delta$ T-cell number was due to an age-dependent reduction of Vδ2 T cells, whereas the total number of Vδ1 T cells was unaffected by age. As a result, the Vδ2/Vδ1 ratio was inverted in old subjects and centenarians. A higher percentage of $\gamma\delta$ T cells producing TNF-α was found in old donors and centenarians whereas no age-related difference was observed in IFN-γ production. After in vitro expansion, a twofold lower expansion index of $\gamma\delta$ T cells, and particularly of the Vδ2 but not of the Vδ1 subset, was found in old people and centenarians in comparison with young subjects, demonstrating the existence of a proliferative defect in $\gamma\delta$ T lymphocytes from aged subjects. In contrast, the cytotoxicity of sorted $\gamma\delta$ T cells was preserved in old people and centenarians [88]. Interestingly, these cells were found more activated in the elderly than in young subjects, as determined by the increased expression of the early activation marker CD69 on $\gamma\delta$ T lymphocytes from old subjects, suggesting that the high level of basal activation of $\gamma\delta$ T cells was due to the "inflamed" environment of the elderly host [89].

Tumour-induced immunosuppression in aged individuals

Age-related alterations of the immune effectors, which, as described above, characterise the phenomenon of immunosenescence, influence the ability of an aged subject to react against exogenous antigens and, in particular, reduce the capacity of antitumoural immune defences in the elderly. Besides these, other factors may contribute to triggering spontaneous tumour growth without being rejected by the immune system: passive mechanisms, such as the lack of either distinctive antigenic peptides or the adhesion and costimulatory molecules needed to elicit a primary T-cell response; and active mechanisms through which tumours can avoid or evade immune attack. Some evidence suggests that at least some of the mechanisms used by cancer cells to escape immune clearance might be more effective in ageing. One of these is related to the Fas ligand / Fas receptor (FasL/FasR) interaction. FasL is a key molecule in normal immune development, homeostasis, modulation, and function and acts by inducing apoptosis of sensitised cells through interaction with its own FasR receptor, expressed on their surface. To date, the expression of functional FasL has been reported in several distinct lineages of tumours [90]. Various studies have demonstrated significant increases in the FasR expression with age, either as percentages of T cells or as mean fluorescence intensity [91]. An increased FasR expression on aged leukocytes might facilitate the immune escape of tumours expressing FasL in elderly patients by promoting the apoptosis of tumour infiltrating leukocytes.

Another mechanism which enables tumours to evade immune rejection is the release by tumour cells of immunosuppressive cytokines. Many tumours produce TGF-β, or IL-10 or other cytokines which tend to suppress inflammatory T-cell responses and cell-mediated immunity, which are needed to control tumour growth and to destroy tumour cells. In old subjects, these suppressive cytokines released by tumour cells may synergize with immunosuppressive cytokines (TGF-β, IL-10 and others) which are already overproduced by leukocytes up to elevated concentrations able to impair antitumour immune responses. Furthermore, IL-6, another cytokine overproduced in the elderly, has been reported to increase the expression of the TGF-β receptor, thus facilitating this mechanism of tumour immune escape [92].

Prostaglandins are other factors that have been involved in cancer-induced immune suppression. Tumour cells produce prostaglandins which can inhibit various immune functions. The above examples lend weight to the idea that immune suppression induced by tumour cell–derived prostaglandins may have particular implications in ageing, since lymphocytes from elderly subjects are now known to be sensitive to inhibition by prostaglandins in comparison with lymphocytes from younger individuals [93].

Several mechanisms of active immune escape have been proposed to explain the incapacity of the immune response in rejecting tumours. It seems that these mechanisms of immune escape play an important role in the aged host even though the exact relevance of tumour-induced immunosuppression in the early phases of tumour development in the elderly remains to be proven.

Immunoprevention of cancer in ageing

As a means of disease prevention and control, vaccines have proved to be highly effective and a financially viable solution. Because of the success of antimicrobial vaccination programmes in completely or almost completely eradicating human infections, the development of an antitumour vaccine is now being considered. Unlike most vaccines for infectious agents, which are solely and purely prophylactic, cancer vaccines may have a wide field of application: including prevention of cancer where the risk of cancer has been predicted through the

evidence of genetic defects or the presence of relevant risk conditions, treatment of preneoplastic lesions diagnosed early on through sophisticated modern techniques, and application to minimal residual disease for the prevention of any recurrence of cancer after the removal of a tumour. In any case, cancer vaccinations are today the most intriguing possibility in activating an immune response capable of effectively hampering the progression of the preclinical stages of a tumour.

In recent years, experimental data have shown the effectiveness of anticancer vaccination models which can potentially elicit a potent immune response and induce immune memory against tumour antigens [44, 94, 95].

Most data on the preventive potential of vaccines have been drawn from studies performed in mice transplanted with parental tumours or in transgenic mice. The use of transgenic mouse models spontaneously developing cancers is certainly preferable since murine models of cancer involving the challenge of mice with a bolus of tumour cells provide information that, while informative, may not be entirely relevant to cancer development in humans, where the tumour is initiated by the clonal expansion from a single in vivo cell.

An experimental model which is widely used in studies on cancer immunoprevention is represented by murine tumours overexpressing the rat HER-2/*neu* protooncogene or its mutated transforming form. The HER-2/*neu* oncogene encodes a 185-kDa receptor-like thyrosine kinase that was found to be overexpressed in several types of human adenocarcinomas, especially in breast tumours, and which was correlated with short time to relapse and poor survival of breast cancer patients [96, 97]. In healthy individuals the HER-2/*neu* receptor, which is involved in organogenesis and epithelial growth, is highly expressed during foetal development, while it is present at low levels in adult tissues. HER-2/*neu* is a self-antigen with poor immunogenicity due to immunological tolerance, but weak humoral [98, 99] and cytotoxic [100, 101] immune responses directed against HER-2/*neu* antigen have been detected in patients with HER-2/*neu*–expressing mammary and ovarian tumours. These observations demonstrate that tolerance to this oncoprotein is not absolute and could be circumvented by using potent active vaccines enhancing to therapeutic levels the ineffective naturally occurring anti-HER-2/n *eu* immunity. Though the efficacy of active vaccination might be limited by the nature of HER-2/*neu*, targeting a self-tumour antigen offers the remarkable advantage of avoiding the problem of the emergence of tumour-specific antigen-loss variants which are usually obstructive when targeting tumour-specific non-self antigens. The genetic instability of tumour cells which can elude immune surveillance by activating mechanisms of phenotypical change is one of the reasons why immunotherapy is ineffective [102], but when the presence of a tumour antigen on tumour cells is the prerequisite for their tumorigenicity, as is the case with HER-2/*neu*–expressing tumours, cells that have lost the antigen are unable to grow and cause tumours.

So far the experiments performed in murine models of HER-2/*neu*–expressing mammary carcinomas have clearly demonstrated that the efficacy of antitumour vaccination depends on the immunocompetence of the host [44, 103]. Indeed, the rejection of tumours was related to the immune effectiveness of mice, and no protection against the tumour challenge was obtained in physically or chemically immunosuppressed hosts [103], whereas an increased antitumoural response was observed to enhance immunological effectiveness through adjuvants [104].

The remodelling of the immune system taking place during ageing suggests that vaccination models which proved efficacious in young adult age may be not wholly efficient in old age. In particular, at least three main characteristics of immunosenescence may determine an age-related disadvantage for the potential application of cancer vaccinations in the elderly (Fig. 1). First, the possibility of inducing an effector-cell population in response to a vaccine depends on the recognition of the vaccine antigen by naïve T cells. It has been clearly shown that old mice give weaker primary responses than young mice because of an age-dependent reduction of the pool of naïve T cells and of the fact that the conversion to memory phenotype is compromised with age [3, 105, 106]. Second, in contrast to vaccines against infectious agents, in which the generation of neutralising humoral immunity is the most important feature, the major focus in cancer immunoprevention has been on the generation of Th1-cell immunity which promotes CTL responses. The ageing process appears to be accompanied by a dysregulation of Th1 and Th2 responses, with a shift to the Th2 phenotype [9]. This dynamic change toward a type 2–dominant state may imply that a particular vaccine strategy may not be equally efficacious in young adults and in the elderly. Third, the defect of antigen presentation by APCs to T lymphocytes, which has been reported in aged mice, strongly suggests the existence of an age-associated multistep defect in which the different cell populations involved in the activation of anticancer immunity are all affected [18, 107].

The new light shed on innate immunity and on its integration with specific immune effectors over the past few years emphasizes a further disadvantage affecting preventive approaches in the elderly. The signals that are produced by the components of the innate system required to direct the adaptive immune response may be insufficient or erroneous in aged individuals and might thus adversely influence the specific clonal adaptive response. It is thus that the age-related alterations of $\gamma\delta$ T cells, NK cells, and the expression of TLRs on APCs, may be relevant for their implications in the activation of inefficient specific T-cell–mediated responses.

The poor effectiveness of vaccinations in the elderly has been reported in several infectious diseases. Influenza virus vaccines have generally proven limited in

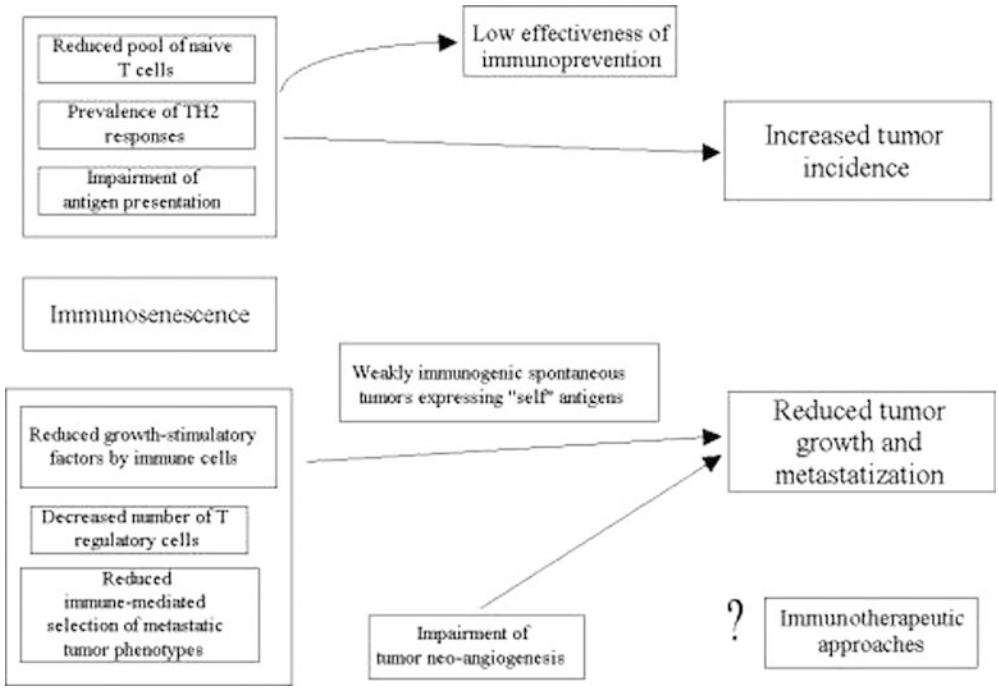

Fig. 1 Immune mechanisms involved in the increased tumor incidence and reduced tumor growth and metastatization present in ageing

preventing morbidity and mortality among the elderly. This has been accounted for by the lower immunological protection that influenza vaccines may confer on older adults compared with younger persons [108, 109]. Also, the effectiveness of the pneumococcal polysaccharide vaccine in reducing the risk of pneumonia was found to be deficient in the elderly [110]. Many older people do not have immunity to tetanus, against which vaccines have been available for decades [111]. Finally, the measure of antihepatitis virus and anti-HBs in elderly people after a combined hepatitis A/B vaccination has underlined the decreased response to vaccination with increasing age [112].

But, while these results explain the decreased responsiveness of elderly patients to antimicrobial vaccines, few data exist on the effectiveness of cancer vaccines with ageing.

Several papers have described the occurrence of age-related changes in the immune system which contribute to increased susceptibility to cancer, indirectly suggesting their potential influence on the success of cancer vaccines. Age-associated, changing patterns of T-cell subsets which predict resistance to spontaneous lymphoma, mammary carcinoma, and fibrosarcoma have been reported in mice [113]. Numerical and functional alterations of $\gamma\delta$ T lymphocytes, similar to those found in patients with primary melanoma [114], have been reported in healthy elderly subjects [88]. Some direct evidence of the decreased efficiency of cancer vaccines with ageing have been recently described in mouse models. A study was conducted on the efficacy of IL-2–engineered mammary tumour cells to induce an immune response capable of rejecting the tumour and of inducing specific immune memory in young and old mice. In this study, it was found that mammary adenocarcinoma TS/A cells engineered to release IL-2 were rejected in both young and old mice, whereas, unlike what occurred in young mice, it was not possible to induce a specific immune memory against TS/A cells in old animals [32]. Whereas the rejection of IL-2–transduced cells was attributed to the good infiltration of neutrophils and macrophages, the defect in memory acquisition was correlated with a reduced representation of both $CD4^+$ and $CD8^+$ lymphocytes in the tumoural infiltrate in old mice.

The age-related decreased effectiveness in inducing memory against tumour cells was recently confirmed in another paper which adopted a different experimental approach. The antitumoural vaccination with DNA plasmids codifying HER-2/*neu* in old mice demonstrated that effectiveness in inducing protective immunity against a lethal challenge with syngeneic tumour cells overexpressing HER-2/*neu* was lower in old mice than it was in young animals [115]. The reduced number of objective responses observed in old mice was associated with an age-related impairment of several immune responses. Although further evidence in other experimental models will be needed, present knowledge suggests that the application of anticancer vaccination in ageing patients may not be as effective as it is in young age because of the existence of age-related defects in the activation of specific immune responses making it necessary to develop specific approaches for the immunoprevention of cancer in advanced age.

Immunotherapy of cancer in ageing

Various approaches have been tried in the past with the aim of activating an immune response able to provoke

the regression of established tumours. The general consensus of opinion that has been drawn from the great bulk of experimental designs performed is that rejection of an established cancer is a difficult, if not impossible, task for the immune system. Certainly, among the causes involved in this failure is the typology of patients used for immunotherapeutic trials, which plays a pivotal role. To properly assess the effectiveness of an immunotherapeutic approach, trials should target patients with nonmetastatic disease and not, as has generally been the case, patients with advanced cancer in whom chemotherapy or radiotherapy has proven ineffective.

Most immunotherapeutic approaches adopted in the last decade have been based on the use of interleukins and interferons and on the so-called adoptive immunotherapy (AI) of cancer, based upon injection of in vitro–manipulated autologous lymphocytes, such as LAK cells or tumour-infiltrating lymphocytes (TILs) [116, 117]. Although these immunotherapeutic tools seemed very effective in the first experimental models tested, they had great limitations once introduced in clinical trials. The cytokine-based immunotherapy of cancer has never been widely used in elderly patients, mainly because of the suspected low responsiveness of ''old'' lymphocytes to stimulation with lymphokines and of the risk of intolerance to high-dose cytokine regimens. Though no definite data exist on the clinical response and the toxicity of these immunotherapeutic tools in the elderly population, there is some evidence which supports the reduced in vivo responsiveness of cytotoxic cells from aged mice to cytokines [118], even though the development of IL-2–induced cytolytic activity was not compromised in elderly subjects who were selected through immmunogerontological protocols [69]. As regards the increased risk of relevant adverse effects in the elderly, it has been reported that the intravenous administration of IL-2 in patients over 65 years of age with metastatic renal carcinoma resulted in higher cardiac toxicity in comparison with younger patients [119]. For this reason, efforts have been concentrated on the development of regimens able to reduce treatment-related systemic toxicity, while preserving therapeutic effects. One of these therapeutic approaches was based on the induction of antitumour cytotoxic cells through short ''pulses'' with cytokines. The pulsing procedure using either IL-2 or IL-12 proved efficacious in developing cytotoxic cells from old donors showing a phenotype and a lytic activity not significantly different from those obtained culturing lymphocytes in the continuous presence of the cytokines [64, 79].

The few papers on the use of immunostimulants as a cure for cancer in aged animals have shown lower efficacy of these therapeutic tools in comparison with young animals. In experimental studies performed in mice it has been shown that the treatment of Lewis lung peritoneal carcinomatosis with biological response modifiers significantly prolonged the survival of young mice but not of aged mice more than 24 months old [41]. The defect was associated with a reduced antitumour activity of peritoneal macrophages from old mice. In another tumour model, indomethacin and/or rIL-2 in vivo treatments were found ineffective in tumour-bearing aged mice in either stimulating NK-cell number and function or in increasing life span [118]. In this study, the authors concluded that some resistance to an immunotherapic approach which has already proved very effective in tumour-bearing young adult mice [120] was present in old animals.

Recent reports suggest it is possible to use normally expressed ''self-antigens'' as targets for human tumour immunotherapy [121–123]. This approach is based on immunological principles which focus on circumventing tolerance, a primary mechanism of tumour immune escape. The premise for such a possibility is that the autoimmune consequences of this therapeutic approach are tolerable and not life limiting, in other words, they may affect functions that are not necessary for survival or that can be readily replaced. In murine models, the therapy of very early mammary carcinomas has been accomplished by immunising animals against the self-protein p185, which is the product of the HER-2/*neu* oncogene. Up until now these results have been obtained in murine models transplanted with HER-2/*neu*–overexpressing tumour cells and in current studies in transgenic mouse models [123, 124]. Immunisation of cancer patients with HER-2/*neu* peptides generated $CD8^+$ and $CD4^+$ T cells responsive to HER-2/*neu* and, as appears from the preliminary results, resulted in some effective clinical response [122, 125]. In these studies, despite the induction of anti-HER-2/*neu*–specific immune responses, there was no evidence of autoimmunity directed against tissues expressing basal levels of the protein [125]. Studies on the immunotherapy of spontaneous carcinomas targeting the self-antigen mucin 1 (MUC1) are also in progress. MUC1 is an epithelial mucin glycoprotein which is overexpressed in 90% of all adenocarcinomas, including those of the breast, lung, pancreas, stomach, colon and ovary. Both in experimental mouse models transgenic for human MUC1 and in clinical studies in cancer patients, immunisation against human MUC1 increased the number of mucin-specific CTL precursors and induced some objective responses [122, 126–128]. In another recent study performed in patients with metastatic melanoma, the adoptive transfer of highly selected tumour-reactive T cells directed against overexpressed self-derived differentiation antigens after a nonmyeloablative conditioning regimen, resulted in the persistent clonal repopulation of T cells and in the regression of the patients' metastatic melanoma [129].

Whether the immunotherapy against weak immunogenic spontaneous tumours expressing self-antigens will be pursued successfully in the coming years is not known. However, an important question is whether the remodelling associated with the immunosenescence may prove to be an advantage or disadvantage for the

potential application of these kind of immunotherapeutic approaches in the elderly. Indeed, if, on the one hand, the remodelling of immune functions determines an impairment of the processes involved in immune-mediated anticancer defences and limits the use of immunotherapy in ageing; on the other hand, as is explained below, the generalised phenomenon of senescence may bring out some mechanisms that favour the growth of tumours and consequently might lend support to immunological anticancer strategies.

Though there is a higher frequency of malignant tumours in the aged, many naturally occurring tumours in humans and laboratory animals are less aggressive with advancing age and permit longer host survival [31, 130]. For example, breast, lung, and colon cancers often have a more benign course in the elderly with slower growth and decreased metastasis. B16 melanoma cells inoculated in old mice have a slower growth and metastatisation which increase the survival time of the animals [31]. Aged mice inoculated with erythroleukemia cells survived the growth of the tumour longer than young adult or infant mice inoculated with an equivalent number of tumour cells [120]. Several factors may explain the slower growth rate and the reduced aggressiveness of cancer in the old (Fig. 1). Weak or nonimmunogenic tumours, like spontaneous tumours in humans, may trigger a low immune response which is unable to reject the tumour but, on the contrary, may cause tumour growth enhancement due to the production of nonspecific growth-stimulatory factors by immune cells. In old individuals, the induction of a weaker immune response and the consequent reduced production of growth factors may result in less fertile "soil" for tumour cells [31]. An incomplete immune response may contribute to the selection of metastatic phenotypes inside the tumour cell population by the selection of specific cancer-cell clones with metastatic potential [131]. Also in this case, the weaker immune response induced in old age might reduce the risk of metastatic cancer-cell clone selection. The growth of solid tumours depends on the establishment of an adequate vascular system. The fact that certain poor immunogenic tumours do not grow well in old hosts but grow aggressively in young hosts has been related to age-associated deficits in tumour vascularisation and, in particular, to a lack of angiogenic factors or the presence of host inhibitors [132, 133]. Another piece of evidence that may influence the success of immunotherapy against self-antigens in the elderly is the suspected reduced representation of cells with potential suppressive activity. Growing evidence has demonstrated that a population of CD4 T cells which constitutively express the IL-2 receptor α chain (CD25), may function as regulatory cells capable of down-regulating immune responses to self-antigens. In vivo depletion of $CD4^+CD25^+$ regulatory cells resulted in suppression of tumour growth in various tumour models [134, 135]. A recent paper has reported that the number of T-regulatory cells progressively decreases with increasing age of mice [136]. Even though this observation requires further investigation, it correlates well with the age-dependent increase of autoantibodies and autoimmune diseases. The decrease of $CD4^+CD25^+$ regulatory cells in the elderly may favour the induction of reactive immunity against self-antigens rather than the activation of tolerogenic mechanisms. It seems, then, that various factors may be involved in reduced tumour growth and metastatisation in the elderly, particularly in the case of spontaneous tumours which express "self" antigens and are weakly immunogenic (Fig. 1). This age-related advantage might be further exploited in the development of immunotherapeutic approaches for the elderly. A reduced T-regulatory cell number, for example, might favour the application of immunotherapeutic procedures capable of enhancing the CTL response specific for cancer-associated "self" antigens.

Conclusions and perspectives for future directions

Age-related changes in the immune system are well documented and concern primarily the adaptive immune responses, including alterations in T-cell phenotype and functions and a reduced ability of B cells to mount specific antibody responses. The innate immune system seems only moderately affected by age, even though some alterations at the level of most of the components of the innate pathway have been demonstrated. Recently, it has become increasingly clear that the adaptive and innate immune systems cooperate at several levels in ensuring the optimal immune response and that any alteration of the innate system will have an impact upon the function of the adaptive immune system and vice versa. In this view, the age-related alterations of the components of the innate immune system may certainly be implicated in the activation of less efficient T-cell–mediated immune responses. More studies are required to provide further insight into innate-adaptive immune interactions and to define the impact that each branch of the immune system has on the functional activity of the other immune component, in order to design tailored protocols to optimise the antitumour immune response in the elderly.

Over the last few years the use of immunological measures to prevent cancer in experimental mouse models has demonstrated the possibility of preimmunising mice through new vaccines against even a poor or apparently nonimmunogenic tumour. Preventive antitumour vaccination is currently considered in humans for the prevention of the reappearance of the cancer after a primary tumour resection. On the basis of the data obtained in experimental mouse models, the strategies of immunoprevention which were effective in young adult age do not seem to be applicable in old individuals. Attempts at finding adjuvants to improve the low effectiveness of immunisation in the elderly are needed, and studies targeting this are currently being performed. It is noteworthy that while investigating new approaches

for treatment in old age the "old" environment may be either unfavourable or advantageous for some types of immunotherapy. On the one hand, some sides of immunosenescence may negatively influence the success of immunotherapeutic approaches: (a) the impairment of various cell populations of both innate and adaptive immune systems certainly represent a disadvantage for the application of immunotherapy in old age; (b) the potential nonspecific activation of immune functions in elderly subjects could determine the risk of losing the age-related immune privilege, which, it has been suggested, may be involved in the reduced growth and metastatisation of cancer in the elderly, without improving the deranged specific immune responses; and (c) at least some mechanisms of tumour immune escape might be favoured in old age, thus increasing the age-related immune suppression. On the other hand, the suspected lower representation of T-regulatory cells in old age may result in a lower risk of activation of tolerogenic mechanisms and may favour the possible generation of high levels of tumour-specific immunity through the infusion of competent T cells, i.e. adoptive T-cell therapy.

Efforts aimed at designing specific protocols for the prevention and cure of cancer in the elderly should take into account either the advantages or the disadvantages offered by the senescent immune system; the application of the immune strategies so defined for the elderly and the optimal management of the old cancer patient will require an upgraded interface bringing together researchers, oncologists and geriatricians.

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