Gino Doria
Daniela Frasca

# Genes, immunity, and senescence: looking for a link

Authors' addresses
*Gino Doria*[1], *Daniela Frasca*[2],
[1]Department of Biology, University of Rome "Tor Vergata", Rome, Italy.
[2]Laboratory of Immunology, AMB-PRO-TOSS, ENEA C. R. Casaccia, Rome, Italy.

Correspondence to:
*Gino Doria*
Chair of Immunology
Department of Biology
University of Rome "Tor Vergata"
Via della Ricerca Scientifica
00133 Rome
Italy
Fax: 39 6 30483644
e-mail: segrbio@utovrm.it

*Immunological Reviews 1997*
*Vol. 160: 159–170*

Immunological Reviews
ISSN 0105-2896

**Summary:** Aging is under the control of a small number of regulatory genes. Mice genetically selected for high immune responses, in most cases, exhibit longer life span and lower lymphoma incidence than do mice selected for low responses. The link between immunity and aging is further evidenced by the age-related alterations of the immune system, mostly of the T-cell population, in terms of replacement of virgin by memory cells, accumulation of cells with signal transduction defects, and changes in the profile of Th1 and Th2 type cytokines. Also, B cells exhibit intrinsic defects, and natural killer (NK) cell activity is profoundly depressed by aging. *In vitro* experiments indicate that IL-2, IFN-γ, and IL-4 production by mouse spleen cells changes with aging and may be upregulated by recombinant cytokines. These findings suggest possible cytokine interventions to prevent or treat age-related immune disorders, as they may affect the duration and the biological quality of life.

## Introduction

Aging is a process that changes healthy individuals into weak ones, with decreased performance of most physiological systems and increased susceptibility to diseases and death. All disabilities, including declines in cardiovascular and neurosensory functions, immune responsiveness, and resistance to neoplastic and degenerative diseases, display similar patterns in all mammalian species and affect the mean life span. Conversely, the rate of aging, as expressed by the maximum life span potential (life span of the longest-lived member of a very large population), varies across species in mammals by a 40-fold factor, from 3 years in rodents to about 120 years in humans. The mean life span, which is influenced by illness, has been enhanced greatly in humans since antiquity, owing to improvements in health and living conditions. However, on average, although people live longer today they still age at the same rate as in the past, as the age of the longest-lived survivor has remained the same irrespective not only of place but also, in a historical context, of time (1).

## Genes and aging

The aging rate is under genetic control, as supported by the following observations: a) All animal species have characteristic rates of aging; b) The progeny of animals from two inbred strains has a life span longer than that of either parental strain (heterosis); c) In humans, females live longer than males, as in most animal species. It is the opposite, however, in some mouse and rat strains; and d) Parents who die at ages beyond the average life expectancy have children who likewise are more likely to live longer.

It should be stressed, however, that non-genetic factors may also play a role in generating the impairments that lead to manifestations of aging. The cross-linking of collagen may be a kind of change that occurs over time and that is probably not directly influenced by a genetic program and yet might contribute to senescence changes. Thus, at the level of macromolecules, macromolecular stability may not require a genetic basis. The accumulation of lipofuscin and the denaturation of organelles and enzyme molecules having regular turnover rates may represent other examples of molecular changes occurring over time that do not necessarily require genetic information. Other non-genetic mechanisms are endocrine and neural factors which appear to regulate age changes in target cells. It should be pointed out, however, that the genetic control of these factors has not been ruled out (2).

The genetic control of the aging rate makes unlikely stochastic processes due to random events (3), and favors programed events encoded in part of the genome, as a master clock, and expressed as molecular mechanisms accounting for the synchrony of structural and functional changes in different cells and tissues of each member of a given species (4, 5). Identification of the genes and processes that set the aging rate differently in different species has been attempted using several approaches.

A limited gene theory of aging (6) has considered the role of the MHC that controls immunoregulatory cell functions and interactions. Furthermore, genes within the MHC together with other MHC-related genes regulating superoxide dismutase, mixed function oxidases, and the level of cyclic nucleotides have been postulated to constitute a multigene family exerting a multifactorial influence on the aging process (7). Besides affecting immune functions, this multigene family appears to regulate enzymes protecting from free radical-induced damage, which accumulates in aging (8), as well as cyclic nucleotides involved in cell proliferation and differentiation, which show marked age-related alterations (9). Experiments with congenic mice (6) have shown that, although genetically identical except for the short MHC region, the mice within each set display considerable variation in maximum life span. However, evidence was provided for a complex interaction between a particular allele and the overall genetic background, as a single allele promotes either longer or shorter life span depending upon the several backgrounds.

Using congenic mice, it has also been shown that the MHC influences the DNA repair capacity (10), a property which is positively correlated with maximum life span in several mammalian species (11). The role of DNA repair in cellular aging has recently raised great interest following the identification of cellular proteins that play a key role in DNA damage recognition and repair pathways (12). The damage is firstly recognized by nuclear proteins displaying helicase activity, and the DNA repair is then carried out under the control of other proteins. We have recently investigated the relationship between the helicase ku protein, involved in the earliest steps of double stranded DNA break recognition, and the mitotic responsiveness in aging. The helicase ku protein is a nuclear heterodimer composed of two subunits of 70 kDa (DNA binding) and 80 kDa. The heterodimer ku70/80 interacts with the serine-threonine protein kinase (DNA-PK) involved in the phosphorylation of nuclear targets which, in turn, facilitates the processes of DNA repair, recombination, transcription and replication (13). Our preliminary results from a survey on human populations of different ages (26–95 years) have shown that peripheral blood lymphocytes (PBL) from all elderly subjects (65–95 years) display low mitotic responses to phytohemagglutinin (PHA), but only a limited number exhibit reduced amounts of ku70/80, suggesting that age-related impairment in DNA repair is only marginally related to reduced expression of ku protein. As discussed elsewhere (D. Frasca, P. Barattini, C. Goso, S. Pucci, G. Rizzo, C. Bartoloni, M. Costanzo, A. Errani, L. Guidi, L. Antico, A. Tricerri, G. Doria, submitted), a higher frequency of DNA breaks is required for evident activation of repair processes or, otherwise, other steps of the cascade of events are more compromised than ku protein helicase activity in aging.

Studies on the role of the MHC in determining life span suggest that it could be the master genetic control region for a wide variety of functions involved in the aging process (6, 7, 9, 10). In these experiments, however, the role of other genetic factors, alone or in combination, in life span determination was not examined. In subsequent studies, different genetic regions on chromosomes 1, 2, 7, and 12 have, indeed, been found to interact between themselves and with environmental factors to influence longevity in recombinant inbred mice of 20 strains (14). The MHC, located on chromosome 6 in humans and 17 in mice, controls a variety of immune functions, and may play

**Table 1. Immunogenetic parameters of five selections**

| Selection | Agglutinin response to | Maximum interline difference in agglutinin titer ($log_2$) | Selection limit | Heritability | Number of loci | Multispecific effect |
|---|---|---|---|---|---|---|
| I | Sheep or pigeon RBC | 7.8 | F16 | 0.21 | 9–15 | large |
| II | Sheep RBC | 6.7 | F13 | 0.20 | 2–12 | intermediate |
| III | Salmonellae f | 6.3 | F16 | 0.18 | 7–18 | large |
| IV | Salmonellae s | 6.5 | F13 | 0.18 | 5–12 | large, null, reversed |
| V | BSA or RGG | 9.8 | F7 | 0.22 | 2–4 | small |

RBC = red blood cells
BSA = bovine serum albumin
RGG = rabbit gamma globulin

a major role in age-related immunologic alterations, as most of the diseases observed in senescence have an immunological pathogenesis associated with the decline of immune responsiveness and increased propensity to autoimmune reactivity (15).

The role of immune responsiveness in the aging process has been investigated in our laboratory on mice genetically selected for high (H) or low (L) antibody response (Biozzi mice). As described elsewhere (16), starting from distinct foundation populations (F0) of outbred mice, five selections were carried out by two-way assortative breeding for maximal or minimal agglutinin response to natural immunogens in each consecutive generation, so that assortative mating of the highest responder mice generated the H line while that of the lowest responder mice the L line. H or L antibody responsiveness resulted from the interaction of alleles, located at several independent loci, which accumulated progressively in H and L mice during the consecutive generations of selective breeding until homozygozity at all relevant loci was reached at the selection limit when the interline difference was maximal. The immunogenetic parameters in the five selections exhibited a remarkable similarity (*Table 1*).

Analysis of the antibody response in H and L lines, and interline hybrids of the five selections indicated that high responsiveness was incompletely dominant, to a variable extent, in Selections I, II, III, and IV, whereas it was incompletely recessive in Selection V. In all selections, the high or low effects of the selected alleles are not limited to the selection antigen but may also influence the immune response to unrelated immunogens non-cross-reactive with the selection antigen. This multispecific effect was large in Selections I and III, intermediate in Selections II and IV, and restricted in Selection V. Mapping analysis showed that some segregating loci in Selection I are linked to genes on chromosomes 2, 4, 8, 10, and 18, but also to genes certainly involved in major immune functions, such as genes on chromosome 6 coding for the TCR, Igk and CD8, genes on chromosome 12 coding for the IgH, and genes on chromosome 17 coding for the MHC, TNF-α, TNF-β, C2 and C4 (17).

Improvement of the effect of selection was obtained by assortative breeding from two foundation populations, F0H and F0L, each of which was produced by balanced frequency of the gene pools from the H or L lines of Selections I, II, III, IV, and V. After 16 generations of selection for primary or secondary responses to all antigens used in the original five selections, the difference between H and L lines in antibody responsiveness was remarkably amplified and the multispecific effect of the selection was generalized to several antigens. These results, obtained in Selection GP for general-primary and Selection GS for general-secondary responses, suggest that more genes with upward effects had accumulated in H mice or, rather, more genes with downward effects had accumulated in L mice during both selections.

Selective breeding was also carried out for mitotic responsiveness of lymph node cells to *in vitro* stimulation by PHA. The selection limit was reached after 10 generations, the realized heritability was 0.24, low responsiveness was incompletely dominant, and the character was under the control of 10–19 independent loci. These H and L responder mice also displayed a similar difference in responsiveness to concanavalin A (ConA), mixed lymphocyte reaction, and graft-versus-host reaction, but produced the same antibody titer when immunized with sheep red blood cells.

As reported elsewhere (18), whether selection breeding for a polygenic character, such as antibody responsiveness, also affects life span was investigated in Selections I, II, III, and GS. The life span was longer in H than in L mice of Selections I and II, but no difference was found between H and L mice of Selection III (*Table 2*).

The positive correlation between antibody responsiveness and life span was further analyzed in interline hybrids of Selection II and found statistically significant in most of these mouse populations. Moreover, the life span of the last surviving 20%,

**Table 2. Immune response, life span, and lymphoma incidence in high and low antibody responder mice**

| Selection | Line | No. of mice | Agglutinin titer ($log_2$) | Mean life span (days ± SD) | Lymphomas (%) |
|---|---|---|---|---|---|
| I | H | 23 | 12.7 | 723 ± 216 | 4 |
| | L | 47 | 4.9 | 562 ± 130 | 30 |
| II | H | 131 | 11.4 | 712 ± 148 | 14 |
| | L | 139 | 5.2 | 446 ± 110 | 35 |
| III | H | 189 | 12.7 | 611 ± 153 | 12 |
| | L | 130 | 6.2 | 622 ± 166 | 12 |
| GS | H | 195 | 12.8 | 615 ± 134 | 12 |
| | L | 187 | 3.6 | 381 ± 161 | 61 |

**Table 3. Immune response and life span in mice from Selection II**

| Mouse population | No. of mice | $Log_2$ agglutinin titer (mean ± SD) | Mean life span (days ± SD) | |
|---|---|---|---|---|
| | | | Total population | Last surviving 20% |
| H | 131 | 11.4 ± 0.8 | 712 ± 148 | 852 ± 64 |
| L | 119 | 5.2 ± 1.0 | 446 ± 110 | 544 ± 74 |
| F1 | 153 | 9.4 ± 0.7 | 649 ± 186 | 847 ± 62 |
| F2 | 191 | 9.8 ± 1.2 | 614 ± 183 | 781 ± 85 |
| BcH | 174 | 10.8 ± 0.9 | 630 ± 215 | 848 ± 71 |
| BcL | 102 | 7.5 ± 1.4 | 564 ± 158 | 708 ± 100 |

which was scarcely affected by early disease-induced mortality and mainly influenced by genes acting on the rate of physiologic aging, appeared as a polygenic character regulated by 3–7 independent loci. Of note, long life span was incompletely dominant in the total population, but life span was longer and completely dominant in the last surviving 20% (*Table* 3).

Thus, the results of this analysis suggest that antibody responsiveness and life span are polygenic traits regulated by a small number of the same or closely linked loci. In these studies, the incidence of spontaneous malignant lymphomas was found to be markedly higher in L than in H mice of Selections I and II, whereas no difference was found between L and H mice of Selection III.

The influence of immune responsiveness on life span and tumor incidence was also investigated in H and L mice of the GS selection at the F16 generation. It was found that the cumulative mortality rate was remarkably higher in L than in H mice, the difference being accounted for mostly by malignant lymphomas that were the major cause of death in L mice. The interline difference in life span and lymphoma incidence in mice of the GS selection was much larger than that observed in mice of Selections I and II (*Table* 2).

Mice genetically selected for high or low mitotic responsiveness to PHA exhibited low or high tumor incidence, respectively, but no difference in life span, suggesting that T-cell activity is restricted to immune surveillance of neoplastic transformation.

The results of these studies on genetic selection suggest that age-related immune dysfunctions have a significant impact on life span and disease. However, it is unclear how genetic selection, e.g. against antibody responsiveness, brings about an increased incidence of malignant lymphomas that are the predominant cause of death. It would be interesting to examine whether the lymphoma incidence could be decreased and the life span be prolonged by improving immune responsiveness in L mice. The results from such an experiment should indicate whether the negative effects of the selected genes are mediated by low immune responsiveness or are independent of immune dysfunctions. On the other hand, the hypothesis that age-related immune dysfunctions have a significant impact on life span and diseases is also supported by the study of centenarians, showing that healthy individuals who have reached the extreme limit of human life in good clinical condition are equipped with well preserved and efficient immune defence mechanisms (15).

### Aging of immune cell populations

Aging is characterized by decreased humoral and cell-mediated immunity to a large variety of exogenous antigens and by an increased propensity to autoimmune phenomena, suggesting an age-related dysregulation of the immune system (15, 19, 20). Alterations in cellular components of the immune system rather than in the extracellular milieu accounts for most of the variations of the immune response with aging (21). The decrease in immune reactivity with increasing age may reflect multiple events, affecting cell proliferation and differentiation, leading to reduction in cell number and function within immune cell populations (22).

Stem cells, the progenitors of lymphocytes and accessory cells, have been thoroughly investigated to detect changes in their proliferative and differentiative capacities, and have been found in most studies to be unimpaired by aging (20, 23–25). However, when competitive repopulation assays were used to detect subtle effects of aging on the ability of bone marrow stem cells to colonize the thymic stroma, marrow from older mice was found less able to initiate thymocyte maturation after *in vitro* transfer to a young thymic environment. Results from limiting dilution assays suggest an age-related loss of the prothymocyte frequency in the bone marrow (26). A recent survey on aging humans has also indicated reduced numbers of com-

**Table 4. T cells and T-cell subpopulations in the spleen of aging mice**

| Age | T cells | T-cell subpopulation, expressed as percentage of total T cells | | | | | |
|---|---|---|---|---|---|---|---|
| (months) | (%) | Total $CD4^+$ | Naive $CD4^+$ | Memory $CD4^+$ | Total $CD8^+$ | Naive $CD8^+$ | Memory $CD8^+$ |
| 3–4 | 30 | 75 | 59 | 14 | 40 | 92 | 25 |
| 12 | 30 | 51 | 48 | 23 | 37 | 87 | 48 |
| 24 | 25 | 50 | 16 | 38 | 27 | 57 | 82 |

T cells: $Thy.1^+$
Naive: $CD44^{low}$ $CD45^{high}$
Memory: $CD44^{high}$ $CD45^{low}$
Data adapted from (50, 53, 54)

mitted hematopoietic progenitor cells, as indicated by surface markers and functions (27).

Age influences both antibody and cell-mediated immune responses, T-cell responses being more severely affected than B-cell responses. T-cell proliferative responses to antigens and mitogens, and T-cell-mediated immune responses, such as cutaneous delayed-type hypersensitivity, mixed lymphocyte reactivity, and cell-mediated cytotoxicity, decrease with aging (28). Precursor frequency analysis of T cells, using limiting dilution techniques, indicates that the fraction of responding T-cell precursors declines with aging, but the progeny of each cell precursor maintains full proliferative capacity and immunocompetence (29).

Aging negatively affects the membrane structures involved in the early events of T-cell activation (30, 31). T cells from aged mice exhibit defects in calcium mobilization (32–35) and protein phosphorylation due to both tyrosine-specific (36, 37) and serine-threonine-specific (38) kinases which lead to changes in signal transduction pathways. The initial biochemical events following TCR stimulation, such as induction of the secondary messengers IP3 and DAG, have been described to be either severely compromised (39) or slightly decreased by aging, although the activity of phospholipase C, which generates IP3 and DAG, appears to be unaffected by aging (40). These contrasting results may reflect the use of different experimental procedures. Both the GTPase activity and the level of G proteins were found to change in lymphocytes from old individuals, the most significant alterations being found in the amounts of Gi and Gq subtypes (41). Studies on protein kinase C (PKC) isoforms ($\alpha, \beta, \gamma, \zeta$) revealed different profiles in lymphocytes from young and old individuals (41). Only PKCβ, but not the other isoforms, is able to translocate into the nucleus of activated lymphocytes from elderly subjects, whereas all the isoforms can translocate into the nucleus of activated lymphocytes from adult controls.

Signal transduction pathways mediated by the family of mitogen-activated protein kinases (MAPK) and the multifunctional MEK family, which is composed of MAPK/extracellular regulated kinases (ERK), have been found to be reduced in aging (42) in terms of both levels and duration of active MAPK. Since no age-related differences were found in the expression of $p42^{mapk}$/ERK2, $p44^{mapk}$/ERK1, or MEK, the impairment appears in the upstream inducers of MEK/MAPK activation. An increased expression of MAP phosphatase, which counteracts kinase activity, has also been reported to occur in aging (43).

Reduced activation of several transcriptional factors involved in the regulation of gene transcription has been found in aging. Among these factors, AP-1 and NF-AT are decreased in T cells from old individuals (42, 44). This age-related decline in the induction of DNA-binding activity of the transcription factors AP-1 and NF-AT has also been described in old rats and attributed to changes in the expression of the $p21^{ras}$ kinase-signaling pathway (45). Also, the nuclear NF-kB is decreased in aging and may be attributed to a higher activity of the IkB-α inhibitor in aging (46). Unlike c-fos, c-jun (47) and c-myc (44) are decreased in old individuals. OCT-1 seems to be unaffected by aging (46, 48).

The decreased T-cell immunity is also associated with shifts in T-cell subsets and cytokine secretion profiles. Changes in the splenic T-cell population have been shown to be a consequence of thymus involution (49) and to consist of a gradual decline in cell number with an increase of the $CD4^+/CD8^+$ cell ratio, due to a relative decrease in the $CD8^+$ cell number (50). Although the percentage of splenic $CD4^+$ T cells does not change greatly with age, the composition of this cell subpopulation is quite different in young and old mice. The peripheral pool of $CD4^+$ naive T cells ($CD44^{low}$ $CD45RB^{high}$ MEL-$14^{high}$ $3G11^{high}$), which has been shown to be predominant in young mice, decreases with age while that of memory T cells ($CD44^{high}$ $CD45RB^{low}$ MEL-$14^{low}$ $3G11^{low}$) increases (51, 52).

**Table 5. Peripheral blood lymphocytes in aging humans**

| Age (years) | Lymphocytes/mm³ | | | | | Percent of CD4⁺ | | Percent of CD8⁺ | |
|---|---|---|---|---|---|---|---|---|---|
| | CD3⁺ | CD4⁺ | CD8⁺ | B | NK | Naive | Memory | Naive | Memory |
| 20 | 1867 | 1150 | 753 | 323 | 268 | 56 | 42 | 73 | 26 |
| 40 | 1680 | 1040 | 673 | 267 | 285 | 35 | 63 | 60 | 39 |
| 60 | 1480 | 900 | 600 | 200 | 305 | 33 | 66 | 55 | 45 |
| 80 | 1330 | 700 | 533 | 133 | 323 | 23 | 76 | 53 | 47 |
| 100 | 1120 | 670 | 467 | 67 | 345 | 19 | 79 | 51 | 51 |

B cells: CD19⁺

NK cells: CD16⁺, CD56⁺, CD57⁺

Naive: CD45RA⁺

Memory: CD45RO⁺

Data adapted from (56, 57)

Similar changes in naive and memory T-cell subpopulations have also been found to occur in the peripheral pool of CD8⁺ cells of aging mice (53) (*Table 4*).

Studies on the role of the thymus in age-related changes of the naive and memory T-cell pools (54) have indicated that the young thymus has a greater propensity to provide naive T cells as compared to the old thymus, which, instead, favors the differentiation and maintenance of memory T cells rather than naive T cells. In humans, it is difficult to assess the contribution of thymic involution to changes in T-cell functions. However, there is evidence suggesting that even in very old individuals sufficient thymic function may be retained to allow naive T-cell differentiation (55). Analysis of PBL, as performed in aging humans (56, 57), has shown that the absolute numbers of CD4⁺ and CD8⁺ T cells, and of B cells, decrease with age, while the number of natural killer (NK) cells increases with age, from 20 to 100 years. As observed in the mouse, the percentage of naive T cells decreases while that of memory T cells increases with age in both CD4⁺ and CD8⁺ cell populations (*Table 5*).

The age-related alterations of the peripheral CD4⁺ T-helper-cell subsets in terms of cell-surface phenotype and cytokine production, as they emerge from the aging thymus, may produce suitable conditions for T-cell-mediated dysregulation of antibody responses. Hence, an unbalance within the CD4⁺ T-cell population may reduce B-cell proliferation and mutation rate during the antibody response, leading to the production of low affinity antibodies with increased cross-reactivity to self antigens (58–60). Although in some cases autoantibodies are of the IgM isotype, the response to many, if not all, autoantigens requires T-cell help (61). The appearance of autoreactive T cells in the periphery (62, 63) is often concomitant with the infiltration of organs by CD4⁺ T cells (64), although evidence of the organ-specific reactivity of these cells is lacking. It is likely that autoimmunity is induced and prevented by the interplay of autoreactive and regulatory CD4⁺ T-cell subsets (65).

The antibody response to exogenous antigens is decreased in aging, but it is uncertain to what extent this decline reflects changes in the performance of T cells needed to promote B-cell activation and differentiation (66) or intrinsic changes in B-cell functions (67). A negative effect of age on the signal transduction pathway involving $Ca^{++}$-dependent PKC has been demonstrated in murine B cells (68). Moreover, studies on B-cell hybridomas (69) and single B cells (70) have indicated that individual cells from aging mice are increasingly likely to produce antibodies with low affinity and cross-reactive specificity for foreign and self antigens. Limited data (71) suggest that aging may lead to major changes in the molecular processes by which antibody genes are assembled and then selected. Impairment of B-cell generation in bone marrow of old mice, as shown at the level of pro- and pre-B cells (72), may induce qualitative as well as quantitative alterations affecting tolerance and responsiveness of the mature B-cell population.

The ability of accessory cells to support T and B-cell activation seems unaffected by aging (19). However, the observed defects in the ability of follicular dendritic cells to process and present immune complexes may contribute to the decline of germinal center formation in old mice (58, 73). Furthermore, the defects in the transport of antigen into lymph node germinal centers by migrating dendritic cells may also contribute to decreased humoral and cell-mediated immunity (74).

Studies on NK-cell function in old mice have shown a profound loss of NK-cell function when spleen and lymph node cells were assayed, suggesting that a loss of NK-cell activity contributes to increasing sensitivity to neoplastic and viral diseases (75, 76). In humans, however, the number of NK cells in

peripheral blood has been shown to increase with aging (*Table* 5), while NK activity is decreased in the elderly (77). This loss of NK-cell activity has been attributed to the impairment in the phosphoinositide signaling pathway, in particular to reduced IP3 levels, whereas the intracellular content of perforin seems to be unaffected by aging (78).

### Cytokine production and expression of cytokine receptors in aging

#### Accessory cell-derived cytokines

Studies on monocyte/macrophage-derived cytokines have yielded controversial results, as the aging effects on these cell populations are extremely dependent upon variations in stimuli, culture conditions, and cytokine produced. IL-1 production by macrophages from old mice was found unchanged (19) or decreased (79) as compared to young controls. Moreover, it has recently been demonstrated that macrophages from old mice constitutively produce higher levels of both p35 and p40 IL-12 mRNA, as compared to young mice (80), which may account, at least in part, for altered production of T-cell-derived cytokines in aging. The spontaneous release of IL-8 by monocytes is reduced in aging but its lipopolysaccharide-induced release is higher in old as compared to young individuals (81). Moreover, the monocyte and lymphocyte-derived TNF-α seems to be increased in aging (82). TNFR p55 and p75 were found unaffected by aging in murine $CD8^+$ spleen cells (83).

#### T-cell-derived cytokines

T-cell proliferation declines with aging in both mice (19, 84–86) and humans (87, 88). Cell-cycle analysis of PHA-stimulated cells from old individuals indicates a decreased frequency of cells entering the S phase. The age-related impairment in the cell-cycle progression correlates with decreased expression not only of c-myc (G0/G1 progression marker) and c-myb (G1/S progression marker), but also of c-jun, IL-2 production, and IL-2R expression (47, 89, 90).

Alterations in T-cell proliferation with aging lead to dysregulation but not necessarily to reduction of cytokine production. Most of the results obtained in both humans and rodents show reduced IL-2 production and increased IL-4 production with aging (71, 87, 91–98). However, many controversial results concerning reduced or unchanged IL-4 release in aging have also been reported and primarily reflect differences in stimuli and methods of determination (91, 98, 99). Young naive T cells produce mainly IL-2 whereas young memory T cells mainly IL-4. In contrast, in old mice memory T cells produce twice as much IL-2 than naive T cells, while the overall level of IL-2 is significantly lower than that in young mice. Moreover, in old mice naive T cells produce twice as much IL-4 than memory T cells, while the overall level of IL-4 is at least the same as that in young mice. The number and/or affinity of IL-2R expressed on the surface of activated lymphocytes declines with aging in both mice and humans (100, 101). The release of the soluble form of the IL-2R (sIL-2R) in culture supernatants of activated human lymphocytes has been reported to decline (91, 102) or to increase (103) with aging. IL-4R mRNA has been described to be similarly expressed in murine $CD8^+$ splenocytes from young and old mice (83).

IFN-γ has been described to increase during aging (53, 70, 98, 104), although it was found decreased (91, 105) or unchanged (106) in some studies. The mRNA level for IFN-γR is unaffected by aging in murine $CD8^+$ splenocytes (83).

The release of hematopoietic cytokines such as IL-3 (107) and GM-CSF (67) seems severely affected by aging. However, IL-3 has also been reported to increase in aging (94). IL-5 increases with aging (94) while IL-6 has been reported to be unaffected (94) or increased (66) by aging.

Also the anti-inflammatory cytokine IL-10 increases in the serum of old, as compared to young, individuals (69), suggesting that the higher resistance to the septic shock in aging may be due, at least in part, to increased levels of this protective cytokine.

### Regulation of cytokine production

As recently reported (98), we have investigated the production of Th1-type (IL-2 and IFN-γ) and Th2-type (IL-4) cytokines by mitogen-activated spleen cells from young (3 months), adult (11–13 months), and old (19–22 months) mice and its regulation by recombinant cytokines (IL-1β, IL-2, IL-3, IL-4, IL-12, or IFN-γ). It was found that the production of IL-2, as protein in culture supernatant and mRNA extracted from cultured $CD4^+$ cells, is profoundly depressed by aging, whereas that of IFN-γ, as protein and mRNA, first declines and then increases with age. The production of IL-4, as protein, monotonically declines with aging whereas, as mRNA, first decreases and then increases above the level in young mice.

When spleen cells in culture were incubated with mitogens and with a recombinant cytokine at various concentrations, it was found that, by and large, cytokine production was enhanced when the level induced by mitogens alone was low (*Fig.* 1). This conclusion applied to IL-2 and IFN-γ production as protein and mRNA. The enhancing effect on IL-2 production was more pronounced upon addition of recombinant IL-12,

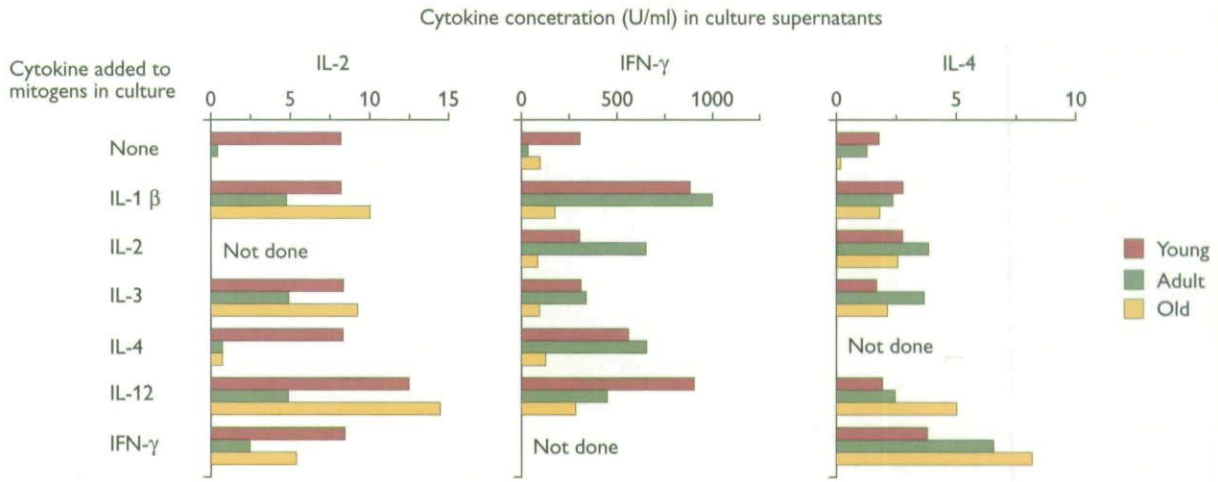


**Fig. 1. Maximum enhancement of cytokine production by addition of recombinant cytokines to culture of spleen cells from aging mice.** The spleen cells were incubated with mitogens and with a recombinant cytokine at various concentrations. Each recombinant cytokine was added at the contentrations of 0.001–60 U/ml. Mitogen addition was ConA (10 μg/ml) + PMA (50 ng/ml).

which is involved in Th1-cell amplification. Also, the addition of IL-4 increased IL-2 production, a finding that may result from the network of interactions among different cells and a variety of different cytokines. It may be envisaged that IL-4-induced amplification of the Th2-cell pool leads to increased release of IL-6, a pleiotropic cytokine with multiple effects on lymphocytes and accessory cells. IL-6 may, indeed, activate macrophages to release cytokines, such as IL-12 and IL-1, involved in Th1-cell maturation. Also, the level of IL-2-specific mRNA may be effectively upregulated by recombinant cytokines when spleen cells are derived from adult mice, but to a much lesser extent when spleen cells are derived from old mice. This finding may be related to changes in post-transcriptional regulation of IL-2-specific mRNA in aging. The stability of this mRNA may be increased in $CD4^+$ cells from old as compared to young or adult mice, leading to increased protein release in the culture supernatant. Preliminary results from our laboratory do indeed favor the hypothesis of an age-related increase in the half-life of IL-2-specific mRNA, as found when purified $CD4^+$ cells are activated by anti-CD3 and anti-CD28 mAbs (S. Pucci, G. Doria, S. Barile, C. Pioli, D. Frasca, submitted; C. Pioli, S. Pucci, S. Barile, D. Frasca, G. Doria, submitted).

Our results also show that the production of IFN-γ-specific mRNA and IFN-γ protein is lower, but can be increased by recombinant cytokines, in spleen cells from adult as compared to young or old mice. These findings are in line with others showing that the concentration of IFN-γ released in culture supernatants and the amount of mRNA are higher if activated spleen cells are from old as compared to young mice. Thus, unlike IL-2, the IFN-γ production seems to be independent of age-related differences in mRNA at the post-transcriptional level.

As to IL-4, we have demonstrated that the production of this Th2-type cytokine continuously decreases with increasing age, and could be enhanced by addition of recombinant cytokines only when spleen cells were derived from old mice. Conversely, the expression of IL-4-specific mRNA was decreased to a minimum at an intermediate age, but could be enhanced by *in vitro* treatment with recombinant cytokines only when spleen cells were derived from young mice. These results on the regulation of IL-4-specific mRNA may reflect possible changes in transcription rate as well as in post-transcription half-life and translation kinetics.

In conclusion, our results demonstrate the possibility for enhancing the synthesis and release of IL-2, IFN-γ and IL-4 when their production is deficient. Recombinant cytokines appear to be appropriate immunomodulators of clinical relevance. Since our data were obtained from a mitogen-activated heterogeneous lymphoid cell population, such as one made up

of whole spleen cells, they may be more readily related to in vivo conditions as compared to data obtained from the stimulation of purified cell populations. Activation of whole spleen cells with mitogens, indeed, induces responses of different cell subsets interacting in the cytokine network. It is our contention that the use of recombinant cytokines is a powerful approach for the effective regulation of immune functions in aging. Suitable protocols should be devised to increase immune responsiveness to exogenous antigens and to prevent expression of autoimmune reactivity.

## Concluding remarks

There is little doubt that maximum life span is genetically determined and that regulatory genes expressed in the immune system play a significant role in conditioning the duration and the biological quality of life. In fact, mice genetically selected for high immune responsiveness in most cases display longer life span and lower tumor incidence than do mice selected for low responsiveness (18).

During senescence, alterations of the immune system affect both antibody and cell-mediated responses. Within the T-cell population, aging leads to the replacement of virgin cells by memory cells, and to the accumulation of cells with signal transduction defects. Age-related changes in T-cell subsets and in the cytokine profile may produce suitable conditions for T-cell-mediated dysregulation of antibody responses characterized by the production of low affinity and self-reactive antibodies. Also, B cells exhibit intrinsic defects, and NK-cell activity displays a profound fall in old mice and humans.

The existence of a straight relationship between immunity and aging also stems from the application of methodologies known to decelerate the aging process, such as controlled caloric restriction without malnutrition, or mild long-term lowering of core body temperature. These studies have indicated that life span prolongation is associated with the delay of the immunodeficiency of normal aging or with the possible amelioration of the autoimmunity that develops with age (108). Hence, in line with the possible link between immunity and senescence, our results on the regulation of cytokine production in aging suggest practicable interventions with recombinant cytokines to prevent or treat immune disorders in aging. By this approach, maintenance of a normal balance of immune functions may prolong life span in healthy conditions.

## References


1. Miller RA. The biology of aging and longevity. In: Hazzard WR, ed. Principles of geriatric medicine and gerontology. 3rd ed. New York: McGraw-Hill, Inc; 1994. p. 3–18.
2. Hayflick L. Prospects for human life extension by genetic manipulation. In: Danon D, Shock NW, Marois M, eds. Aging: a challenge to science and society. Vol. 1 Biology. New York: Oxford Univ Press; 1981. p. 162–179.
3. Orgel LE. The maintenance of the accuracy of protein synthesis and its relevance to aging. Proc Natl Acad Sci USA 1963;**49**:517–520.
4. Jazwinski SM. Longevity, genes and aging. Science 1996;**273**:54–59.
5. Miller RA. The aging immune system: primer and prospectus. Science 1996;**273**:70–74.
6. Smith GS, Walford RL. Influence of the main histocompatibility complex on aging mice. Nature 1977;**270**:727–729.
7. Walford RL, Weindruch RH, Gottesman SRS, Tam CF. The immunopathology of aging. Annu Rev Gerontol Geriatr 1981;**2**:3–14.
8. Harman D. The aging process. Proc Natl Acad Sci USA 1981;**78**:7124–7128.
9. Tam CF, Walford RL. Cyclic nucleotide levels in resting and mitogen-stimulated cell suspensions from young and old mice. Mech Ageing Dev 1978;**7**:309–320.
10. Walford RL, Bergmann K. Influences of genes associated with the main histocompatibility complex on deoxyribonucleic acid excision repair capacity and bleomycin sensitivity in mouse lymphocytes. Tissue Antigens 1979;**14**:336–342.
11. Hart RW, Setlow RB. Correlation between deoxyribonucleic acid excision repair and lifespan in a number of mammalian species. Proc Natl Acad Sci USA 1974;**71**:2169–2173.
12. Jackson SP. The recognition of DNA damage. Curr Opin Genet Dev 1996;**6**:19–25.
13. Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement for DNA ends and association with ku antigen. Cell 1993;**72**:131–142.
14. Gelman R, Watson A, Bronson R, Yunis E. Murine chromosome regions correlated with longevity. Genetics 1988;**118**:693–704.
15. Franceschi C, Monti D, Sansoni P, Cossarizza A. The immunology of exceptional individuals. The lesson of centenarians. Immunol Today 1995;**16**:12–16.
16. Doria G. Biozzi mice. In: Roitt IM, Delves PJ, eds. Encyclopedia of immunology. London: Academic Press; 1992. p. 227–229.
17. Puel A, Mevel JC, Bouthillier Y, Feingold N, Fridman WH, Mouton D. Toward genetic dissection of high and low antibody responsiveness in Biozzi mice. Proc Natl Acad Sci USA 1996;**93**:14742–14746.
18. Doria G, Biozzi G, Mouton D, Covelli V. Genetic control of immune responsiveness, aging and tumor incidence. Mech Ageing Dev 1997;**96**:1–13.
19. Doria G, Adorini L, Frasca D. Immunoregulation of antibody responses in aging mice. In: Goidl EA, ed. Aging and the immune response. Cellular and humoral aspects. New York: Marcel Dekker, Inc; 1987. p. 143–176.
20. Doria G, Mancini C, Utsuyama M, Frasca D, Hirokawa K. Aging of the recipients but not of the bone marrow donors enhances autoimmunity in syngeneic radiation chimeras. Mech Ageing Dev 1997;**95**:131–142.
21. Makinodan T, Adler WH. Effects of aging on the differentiation and proliferation potentials of cells of the immune system. Fed Proc 1975;**34**:153–158.

22. Makinodan T, Albright JW, Good PI, Peter CP, Heidrick ML. Reduced humoral immune reactivity in long-lived old mice: an approach to elucidating its mechanisms. Immunology 1976;**31**:903–911.
23. Callard RE. Aging of the immune system. In: Kay MMB, Makinodan T, eds. Handbook of immunology in aging. Boca Raton, FL: CRC Press; 1981. p. 103–113.
24. Kay MMB, Makinodan T. The ageing immune system. In: Viidik A, ed. Lectures on gerontology. Vol. I: On biology of ageing. Part A. Orlando, FL: Academic Press; 1982. p. 143–171.
25. Harrison DE. Long-term erythropoietic repopulating ability of old, young and fetal stem cells. J Exp Med 1983;**157**:1496–1504.
26. Eren R, Zharhary D, Abel L, Globerson A. Age-related changes in the capacity of bone marrow cells to differentiate in thymic organ cultures. Cell Immunol 1988;**112**:449–455.
27. Keating A. The hematopoietic stem cell in elderly patients with leukemia. Leukemia 1996;**10**: S30–S32.
28. Thoman ML, Weigle WO. The cellular and subcellular bases of immunosenescence. Adv Immunol 1989;**46**:221–261.
29. Miller RA. Age-associated decline in precursor frequency for different T cell-mediated reactions, with preservation of helper and cytotoxic effect per precursor cell. J Immunol 1984;**132**:63–68.
30. Miller RA. Immunodeficiency of aging: restorative effects of phorbol ester combined with calcium ionophore. J Immunol 1986;**137**:805–808.
31. Thoman ML, Weigle WO. Partial restoration of ConA-induced proliferation, IL-2 receptor expression, and IL-2 synthesis in aged murine lymphocytes by phorbol myristate acetate and ionomycin. Cell Immunol 1988;**114**:1–11.
32. Miller RA, Jacobson B, Weil G, Simons ER. Diminished calcium influx in lectin-stimulated T cells from old mice. J Cell Physiol 1987;**132**:337–342.
33. Proust JJ, Filburn CR, Harrison SA, Bucholz MA, Nordin AA. Age-related defect in signal transduction during lectin activation of murine T lymphocytes. J Immunol 1987;**139**:1472–1478.
34. Philosophe B, Miller RA. Diminished calcium signal generation in subsets of T lymphocytes that predominate in old mice. J Gerontol A Biol Sci Med Sci 1990;**45**: B87–B89.
35. Philosophe B, Miller RA. Calcium signals in murine T lymphocytes: preservation of responses to PHA and to an anti-ly-6 antibody. Aging Immunol Infect Dis 1991;**2**:11–18.
36. Shi J, Miller RA. Differential tyrosine-specific protein phosphorylation in mouse T lymphocyte subsets. Effect of age. J Immunol 1993;**151**:730–739.
37. Garcia GG, Miller RA. Differential tyrosine phosphorylation of zeta chain dimers in mouse CD4 T lymphocytes: effect of age. Cell Immunol 1997;**175**:51–57.
38. Patel HR, Miller RA. Age-associated changes in mitogen-induced protein phosphorylation in murine T lymphocytes. Eur J Immunol 1992;**22**:253–260.
39. Miller RA. Aging and immune function. Int Rev Cytol 1991;**124**:187–216.
40. Utsuyama M, Varga Z, Fukami K, Homma Y, Takenawa T, Hirokawa K. Influence of age on the signal transduction of T cells in mice. Int Immunol 1993;**5**:1177–1182.
41. Fulop T. Signal transduction changes in granulocytes and lymphocytes with ageing. Immunol Lett 1994;**40**:259–268.
42. Whisler RL, Newhouse YG, Bagenstose SE. Age-related reductions in the activation of mitogen-activated protein kinases $p44^{mapk}$/ERK1 and $p42^{mapk}$/ERK2 in human T cells stimulated via ligation of the T cell receptor complex. Cell Immunol 1996;**168**:201–210.
43. Liu YS, Guyton KZ, Gorospe M. Age-related decline in mitogen-activated protein kinase activity in epidermal growth factor-stimulated rat hepatocytes. J Biol Chem 1996;**271**:3604–3607.
44. Whisler RL, Beiqing L, Wu L-C, Chen M. Reduced activation of transcriptional factor AP-1 among peripheral blood T cells from elderly humans after PHA stimulation: restorative effect of phorbol diesters. Cell Immunol 1993;**152**:96–109.
45. Palhavani MA, Harris MD, Richardson A. The age-related decline in the induction of DNA binding activity of the transcription factors AP-1 and NFAT is correlated to changes in the expression of $p21^{ras}$/MAP kinase signalling pathway. In: First International Conference on Immunology and Aging; 1996 Jun 16–19; Bethesda (MD). Bethesda (MD): NIH; 1996. p.S-13 (Abstract).
46. Ponnappan U. Regulation of transcription factor NFKB in immune senescence. In: 5th EUCAMBIS Congress on the Molecular Biology of Immunosenescence; 1997 May 14–18; Cordoba, Spain. 1997. p. 12 (Abstract).
47. Song LJ, et al. Expression of c-fos, c-jun and jun B in peripheral blood lymphocytes from young and elderly adults. Mech Ageing Dev 1992;**65**:149–156.
48. Trebilcock GU, Ponnappan U. Evidence for lowered induction of nuclear factor kappa B in activated human T lymphocytes during aging. Gerontology 1996;**42**:137–146.
49. Utsuyama M, Kasai M, Kurashima C, Hirokawa K. Age influence on the thymic capacity to promote differentiation of T cells: induction of different composition of T cell subsets by aging thymus. Mech Ageing Dev 1991;**58**:267–277.
50. Utsuyama M, Hirokawa K. Age-related changes of splenic T cells in mice. A flow cytometric analysis. Mech Ageing Dev 1987;**40**:89–102.
51. Ernst DN, et al. Differences in the expression profiles of CD45RB, Pgp-1 and 3G11 membrane antigens and in the patterns of lymphokine secretion by splenic CD4+ T cells from young and aged mice. J Immunol 1990;**145**:1295–1302.
52. Ernst DN, et al. Differences in the subset composition of CD4+ T cell populations from young and old mice. Aging Immunol Infect Dis 1990;**2**:105–109.
53. Ernst DN, Weigle WO, Noonan DJ, McQuitty DN, Hobbs MV. The age-associated increase in IFN-γ synthesis by mouse CD8+ T cells correlates with shifts in the frequencies of cell subsets defined by membrane CD44, CD45RB, 3G11 and Mel-14 expression. J Immunol 1993;**151**:575–587.
54. Kurashima C, Utsuyama M, Kasai M, Ishyima SA, Konno A, Hirokawa K. The role of thymus in the aging of Th cell subpopulations and age-associated alterations of cytokine production by these cells. Int Immunol 1995;**7**:97–104.
55. Steinmann G, Hartwig M. Immunology of centenarians. Immunol Today 1995;**16**:549–552.
56. Sansoni P, et al. Lymphocyte subsets and natural killer cell activity in healthy old people and centenarians. Blood 1993;**80**:2767–2773.
57. Cossarizza A, et al. CD45 isoforms expression on CD4+ and CD8+ T cells throughout life from newborns to centenarians: implications for T cell memory. Mech Ageing Dev 1996;**86**:173–195.
58. Miller C, Kelsoe G, Han S. Lack of B7-2 expression in the germinal centers of aged mice. Aging Immunol Infect Dis 1994;**5**:249–257.
59. Miller C, Kelsoe G. IgVH hypermutation is absent in the germinal centers of aged mice. J Immunol 1995;**155**:3377–3384.
60. Yang X, Streda J, Cerny J. Relative contribution of T and B cells to hypermutation and selection of the antibody repertoire in germinal centers of aged mice. J Exp Med 1996;**183**:959–970.
61. Weksler ME. The nature and significance of age-associated autoimmunity. In: Bona CA, Siminovitch K, Zanetti M, Theofilopoulos AN, eds. Molecular pathology of autoimmunity diseases. New York: Hardwood Acad Publ; 1993. p. 765–775.

62. Naor D, Bonavida B, Walford RL. Autoimmunity and aging: the age-related response of mice of a long-lived strain to trinitrophenylated syngeneic mouse red blood cells. J Immunol 1976;**117**:2204–2208.
63. Charreire J, Bach JF. Binding of autologous erythrocytes to immature T cells. Proc Natl Acad Sci USA 1975;**72**:3201–3205.
64. Hayashi Y, Utsuyama M, Kurashima C, Hirokawa K. Spontaneous development of organ-specific autoimmune lesions in aged C57BL/6 mice. Clin Exp Immunol 1989;**78**:120–126.
65. Fowell D, McNight AJ, Powrie F, Dyke R, Mason D. Subsets of CD4+ T cells and their roles in the induction and prevention of autoimmunity. Immunol Rev 1991;**123**:37–64.
66. Li SP, Verma S, Miller RA. Age-related defects in T cell expression of CD40 ligand and induction of in vitro B cell activation. Aging Immunol Infect Dis 1995;**6**:79–93.
67. Klinman NR. The basis for decreased B cell responsiveness with aging: the legacy and the challenge. Aging Immunol Infect Dis 1994;**5**:203–210.
68. Kawanishi H, Joseph K. Effects of phorbol myristate and ionomycin on in vitro growth of aged Peyer's patch T and B cells. Mech Ageing Dev 1992;**65**:289–300.
69. McElvoy SJM, Goidl EA. Studies on immunological maturation. II. The absence of high-affinity antibody producing cells early in the immune response is only apparent. Aging Immunol Infect Dis 1988;**1**:47–54.
70. Klinman NR. Similarities in B cell repertoire development between autoimmune and aging normal mice. J Immunol 1992;**148**:1353–1358.
71. Bangs LA, Sanz IE, Teale JM. Comparison of D, JH, and junctional diversity in the fetal, adult, and aged B cell repertoires. J Immunol 1991;**146**:1996–2004.
72. Zharhary D. Age-related changes in the capability of the bone marrow to generate B cells. J Immunol 1988;**141**:1863–1869.
73. Burton GF, Kosco MH, Szakal AK, Tew JC. Iccosomes and the secondary antibody response. Immunology 1991;**73**:271–276.
74. Holmes KL, Schnizlein CT, Perkins EH, Tew JC. The effect of age on antigen retention in lymphoid follicles and in collagenous tissue of mice. Mech Ageing Dev 1984;**25**:243–255.
75. Weindruch R, Devens BH, Raff HV, Walford RL. Influence of dietary restriction and aging on natural killer cell activity in mice. J Immunol 1983;**130**:993–996.
76. Albright JW, Albright JF. Age-associated impairment of murine natural killer activity. Proc Natl Acad Sci USA 1983;**80**:6371–6375.
77. Facchini A, Mariani E, Mariani AR, Papa S, Vitale M, Manzoli FA. Increased number of circulating Leu 11+ (CD16) large granular lymphocytes and decreased NK activity during human ageing. Clin Exp Immunol 1987;**68**:340–347.
78. Facchini A, et al. Signal transduction and perforin release after NK cell activation during ageing. In: 9th International Congress of Immunology; 1995 Jul 23–29; San Francisco. 1995. p.206 (Abstract).
79. Inamizu T, Chang M-P, Makinodan T. Influence of age on the production and regulation of IL-1 in mice. Immunology 1985;**55**:447–455.
80. Spencer NFL, Daynes RA. IL-12 directly stimulates expression of IL-10 by CD5+ B cells and IL-6 by both CD5+ and CD5- B cells: possible involvement in age-associated cytokine disregulation. Int Immunol 1997;**9**:745–754.
81. Clark JA, Peterson TC. Cytokine production and aging. Overproduction of IL-8 in elderly males in response to LPS. Mech Ageing Dev 1994;**77**:127–139.
82. Han D, Hosokawa T, Aoike A, Kawai K. Age-related enhancement of TNF production in mice. Mech Ageing Dev 1995;**84**:39–54.
83. Ernst DN, Sehv DW, Weigle WO. Aging, cytokine and cytokine receptor expression by mouse CD8+ cells. In: 9th International Congress of Immunology; 1995 Jul 23–29; San Francisco. 1995. p.196 (Abstract).
84. Krogsrud RL, Perkins EH. Age-related changes in T cell functions. J Immunol 1977;**118**:1607–1611.
85. Frasca D, Adorini L, Landolfo S, Doria G. Enhancing effect of IFN-γ on helper T cell activity and IL-2 production. J Immunol 1985;**134**:3907–3911.
86. Goso C, Frasca D, Doria G. Effect of synthetic thymic humoral factor (THF-γ2) on T cell activities in immunodeficient ageing mice. Clin Exp Immunol 1992;**87**:346–351.
87. Barcellini W, et al. Heterogeneity of immune responsiveness in healthy elderly subjects. Clin Immunol Immunopathol 1988;**47**:142–151.
88. Bartoloni C, et al. Immune parameters in a population of institutionalized elderly subjects: influence of depressive disorders and endocrinological correlations. Mech Ageing Dev 1991;**60**:1–12.
89. Gamble DA, Schwab R, Weksler ME, Szabo P. Decreased steady state c-myc mRNA in activated T cell cultures from old humans is caused by a smaller proportion of T cells that transcribe the c-myc gene. J Immunol 1990;**144**:3569–3573.
90. Pieri C, Recchioni R, Moroni F, Marcheselli F, Lipponi G. Phytohemagglutinin induced changes of membrane lipid packing, c-myc and c-myb encoded protein expression in human lymphocytes during aging. Mech Ageing Dev 1992;**64**:177–187.
91. Daynes RA, Araneo BA. Prevention and reversal of some age-associated changes in immunologic responses by supplemental dehydroepiandrosterone sulfate therapy. Aging Immunol Infect Dis 1992;**3**:135–154.
92. Chang M-P, Makinodan T, Peterson WJ, Strehler BL. Role of T cells and adherent cells in age-related decline in murine interleukin-2 production. J Immunol 1982;**129**:2426–2430.
93. Thoman ML, Weigle WO. Lymphokines and aging: interleukin-2 production and activity in aged animals. J Immunol 1981;**127**:2102–2106.
94. Hobbs MV, et al. Patterns of cytokine gene expression by CD4+ T cells from young and old mice. J Immunol 1993;**150**:3602–3614.
95. Nagelkerken L, Hertogh-Huijbregts A, Dobber R, Drager A. Age-related changes in lymphokine production related to a decreased number of $CD45RB^{hi}$ CD4+ T cells. Eur J Immunol 1991;**21**:273–281.
96. Kubo M, Cinader B. Polymorphism of age-related changes in interleukin (IL) production: differential changes of T helper subpopulations synthesizing IL-2, IL-3 and IL-4. Eur J Immunol 1990;**20**:1289–1296.
97. Kirman I, et al. Treatment of old mice with IL-2 corrects dysregulated IL-2 and IL-4 production. Int Immunol 1996;**8**:1009– 1015.
98. Frasca D, et al. Regulation of cytokine production in aging: use of recombinant cytokines to upregulate mitogen-stimulated spleen cells. Mech Ageing Dev 1997;**93**:157–169.
99. Candore G, Di Lorenzo G, Melluso M. γ-interferon, interleukin-4 and interleukin-6 in vitro production in old subjects. Autoimmunity 1993;**16**:275–280.
100. Frasca D, Adorini L, Mancini C, Doria G. Reconstitution of T cell functions in aging mice by thymosin α1. Immunopharmacology 1986;**11**:155–163.
101. Schwab R, Pfeffer LM, Szabo P, Gamble D, Schnurr CM, Weksler ME. Defective expression of high affinity IL-2 receptors on activated T cells from aged humans. Int Immunol 1990;**2**:239–246.
102. Frasca D, et al. Age-related modulation of cytokine production, IL-2R expression and function in a population of healthy subjects (22 to 97 years). Mech Ageing Dev 1994;**5**:3–12.

103. Orson FM, Saadeh CK, Lewis DE, Nelson DL. IL-2 receptor expression by T cells in aging humans.
Cell Immunol 1989;**124**:278–291.
104. Papciack SM, Collins GD, Adler WH. Increased expression of the IFN-γ gene in activated T cells from old mice is not due to age-related differences in mRNA regulation.
Aging Immunol Infect Dis 1995;**6**:107–123.
105. Born J, et al. Cytokine production and lymphocyte subpopulations in aged humans. An assessment during nocturnal sleep.
Mech Ageing Dev 1995;**84**:113–126.
106. Sindermann J, Kruse A, Frercks HJ, Schutz RM, Kirchner H. Investigations of the lymphokine system in elderly individuals.
Mech Ageing Dev 1993;**70**:149–159.
107. Chang M-P, Utsuyama M, Hirokawa K, Makinodan T. Decline in the production of IL-3 with age in mice.
Cell Immunol 1988;**115**:1–12.
108. Walford RL. Immunoregulatory systems and aging. In: Danon D, Shock NW, Marois M, eds. Aging: a challenge to science and society. Vol. 1 Biology. New York: Oxford Univ Press; 1981. p. 302–319.