Nutrition and immunity in the elderly

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Immune function declines with age, leading to increased infection and cancer rates in aged individuals. In fact, recent progress in the study of immune ageing has introduced the idea that rather than a general decline in the functions of the immune system with age, immune ageing is mainly characterized by a progressive appearance of immune dysregulation throughout life. Changes appear earlier in life for cell-mediated immunity than for humoral immunity. Thus, age-related modifications in cell-mediated immunity, i.e. changes in naive : memory T-cells, mature : immature T-cells, T-helper 1 : T-helper 2 cells are more important in the elderly than changes in humoral immunity, i.e. CD5 : CD5+ cells or length of antibody responses. Such evolution of the immune system has been linked to declining thymus function and to accumulative antigenic influence over the lifespan. In contrast, innate immunity (macrophage functions) is preserved or even increased during the ageing process. This finding shows that the 'primitive' immune system is less affected by the ageing process than the sophisticated specific immune system. The present review focuses on innate and cell-mediated immune changes with ageing. It provides evidence that primary changes (intrinsic modifications in the immune system) and secondary changes (resulting from environmental influences during the lifespan) exert different influences on the immune system. Primary changes, occurring in healthy individuals, seem less important nowadays than they were considered to be previously. For example, interleukin 2 secretion in some very healthy aged individuals is comparable with that in younger adults. Primary immune changes may not explain the increased incidence and severity of infections observed in the elderly population. Secondary immunological changes are far more frequent and are certainly responsible for most of the immune modifications observed in the elderly population. Environmental factors leading to secondary immune dysfunctions include not only antigenic influence, which is a reflection of diseases experienced over the lifespan, but also many other factors such as drug intake, physical activity and diet; factors for which important changes occur in the elderly population. Nutritional factors play a major role in the immune responses of aged individuals and the present review shows that nutritional influences on immune responses are of great consequence in aged individuals, even in the very healthy elderly.

Infection: Ageing: Immune system: Nutritional status

Age-related changes in the immune system have been the subject of investigation for several decades. There is obvious evidence, both from experimental and clinical data, that immune responses decline with the ageing process (Makinodan & Kay, 1980; Miller, 1992). More recently, it was reported that some immune functions, such as interleukin (IL) 6 production, do not decrease but rather increase with age (Kubo & Cinader, 1990; Erschler *et al.* 1993), leading to the new concept of age-related immune dysregulation (Kubo & Cinader, 1990; Ben-Yehuda *et al.* 1994; Lesourd & Meaume, 1994; Shearer, 1997).

Most investigations into immune ageing in human subjects rely on data from 'apparently healthy' aged individuals without checking for the possibility of under-lying diseases, which may be clinically not apparent. Some studies have attempted to check for this confounding factor. Most studies have investigated individuals selected on the criteria of the SENIEUR protocol (Ligthart *et al.* 1984) of the European Community's Concerted Action Program on Ageing (EURAGE). This protocol established selection criteria for immunogerontological studies taking into account:

(a) the living conditions of healthy volunteers;
(b) laboratory values for good health criteria;
(c) drug intake that may or may not be acceptable.

Some authors (Traill *et al.* 1985; Reibnegger *et al.* 1988), including our group (Lesourd *et al.* 1994; Mazari & Lesourd, 1998) found that the SENIEUR criteria were not precise enough to exclude the possibility of clinically hidden ongoing disease and/or of nutritional deficiency or other environmental factors influencing immune responses. New exclusion criteria (Table 1 shows the criteria we have used; Lesourd *et al.* 1994) included several biological variables supporting the diagnosis of undiagnosed ongoing diseases such as diabetes, atherosclerosis, minor sequelae, from recent (few years) previous diseases (even after apparently complete recovery, since they may have pushed the elderly individual towards a stage of frailty not always easy to recognize), and the presence of nutritional deficits, including low levels of vitamins and/or trace elements. Adding new criteria for checking the health of the volunteers allowed these researchers to compare groups of 'very healthy individuals', who fitted all additional criteria, with groups they termed 'apparently-healthy elderly', who fitted the SENIEUR criteria. It is obvious from these studies as well as from other studies (Mysliwska *et al.* 1998) that health and nutritional factors may influence immune functions of aged individuals, even in apparently-healthy individuals.

It appears that borderline difficult-to-characterize secondary immune deficiencies are quite common in aged individuals, and that part of what has been described as immune ageing (primary immune deficiencies) may be in fact related to secondary immune deficiencies, in particular to undiagnosed diseases and to low nutritional status. The importance of nutritional factors within the secondary immune deficiencies of aged individuals should be emphasized, since they are common in the elderly population; for example, one-third of the home-living self-sufficient population studied in the EURONUT/Seneca European Reference Study on Nutrition and Ageing had a low serum level

for at least one vitamin (Haller *et al.* 1996), which may be responsible for lower immune responses. Such a distinction between primary and secondary immune deficiency is important in order to understand how the immune functions age in an irreversible (primary immune deficiency) or reversible (secondary immune deficiency) manner. Furthermore, it may facilitate an understanding of the major influence of secondary immune deficiency on the immune system of aged individuals, and perhaps, as in the case of nutritional deficiencies, treatment of aged individuals in order to reverse secondary immune deficiency. Such nutritional treatment, using trace element–vitamin supplementation, has been shown to be effective in improving the immune responses of aged individuals, not only in institutionalized aged patients (Boukaïba *et al.* 1993; Galan *et al.* 1997), but even in the self-sufficient home-living apparently-healthy elderly (for review, see Lesourd *et al.* 1998). This form of supplementation is effective not only because it induces increased immune responses in self-sufficient home-living apparently-healthy elderly subjects, but it has also been claimed that it may be clinically effective in reducing the number of infections over 1 year (Chandra, 1992*a*).

The present review will first describe immune ageing *per se*, i.e. primary immune deficiency. Then we will focus on the influence of nutritional factors on immune ageing (secondary immune deficiency), showing that the immune deficiency observed in profoundly-undernourished elderly subjects may be present, to a lesser extent, in self-sufficient elderly subjects with micronutrient deficits. Finally, we will briefly focus on supplementation studies in healthy aged individuals in order to show that recommended dietary allowances (RDA) may be too low in the elderly, and that immune ageing, at least in human subjects, may be partly due to insufficient intakes in the general population. Adequate nutrition is an effective way, through energy restriction, to slow down the effect of age on the immune system in rodents (Venkatraman *et al.* 1994; Fernandes *et al.* 1991). Nutrition may also affect the human immune system of aged individuals from a different aspect, that of prevention and correction of nutritional deficit.

Table 1. Criteria for the selection of very healthy elderly*

1 SENIEUR criteria	In good health
	With no ongoing, developing or degenerative diseases
	Normal adult values for laboratory variables
	No drugs acting on the immune system
2 Additional criteria	No disease in the past 5 years
	No motor skill difficulties
	'Normal' physical activity: > 4 km walk/d
	No drug treatment for cardiac, neurological or psychotropical diseases (including depression)
	'Normal mental status' (Mini mental status of Folstein ≥ 28/30)
	Serum albumin ≥ 39 g/l
	No low values for trace elements (Zn and Se) or vitamins (B$_6$, folate, B$_{12}$, A and C)

* We used the criteria of the SENIEUR protocol (Ligthart *et al.* 1984), to which we added health criteria for the previous 5 years (Lesourd *et al.* 1994; Lesourd & Meaume, 1994) including drug intake and serum albumin (> 39 g/l). All subjects had normal values for serum or plasma trace element or vitamin levels tested; reference cut-off points used were those used in our laboratory together with EURONUT/Seneca values (Haller *et al.* 1996).

Primary immune deficiency in the elderly

In recent years numerous studies have investigated the age-related changes in immune responses. The most important changes will be presented, focusing on the most striking feature: modifications in cell-mediated immunity (T-cell functions). The data presented come from our group and concern the 'very healthy elderly' and the 'apparently-healthy elderly' we have studied in the past year. Thus, these results strictly represent the primary and secondary immune deficiency associated with the ageing process.

Decrease in new T lymphocyte generation

Lymphocytes are generated in bone marrow and mature as T-cells in the thymus. The ability of stem cells to undergo clonal proliferation declines with age (Tyan, 1981), as does thymocyte maturation in relation to thymus involution (Steinmann *et al.* 1985; Hirokawa *et al.* 1994). In fact,

thymus involution starts early in life and is greatly accelerated after the hormonal changes associated with puberty (Steinmann *et al*. 1985). Thymic tissue is progressively replaced by fat, and new T lymphocyte generation is almost absent in 60-year-old individuals. Thymus involution is probably of major significance during acute infections, since aged individuals do not replace the destroyed lymphocytes at the efficient levels observed for younger adults during lung infections (for review, see Lesourd, 1999).

Changes in peripheral blood lymphocytes

Lymphocyte numbers in peripheral blood decrease with age (Lesourd *et al*. 1994; Lesourd & Meaume, 1994; Huppert *et al*. 1998), but this change remains of minor importance (10–15 %) in nonagenarians when they are very healthy (Mazari & Lesourd, 1998), and may not be detectable at a younger age (Wick & Grubeck-Lowensteins 1997), even though contradictory results have been published recently (Huppert *et al*. 1998). In addition, T lymphocyte subset equilibrium changes with age. The modification occurring earlier concerned naive : memory cells which is often measured in human subjects as CD45RA : CD45RD. This type of modification, related to antigenic exposure, starts in infancy, increases greatly during childhood and early adulthood until age 30 years, and continues thereafter but at a far lower rate until death (Cossarizza *et al*. 1992). The transformation of naive CD4+ T lymphocytes to memory CD4+ T lymphocytes has also been shown to be accelerated in aged mice (Thoman, 1997). The rapid change in naive : memory T lymphocytes in early adulthood is linked to new antigen exposure, and the later change (slow ratio changes in the elderly) probably, at least in part, to the ageing phenomenon (Thoman, 1997).

Aged individuals express fewer mature T-cells (CD3+) and higher numbers of immature T-cells (CD2+CD3−; Table 2). This change, detectable only after age 50 years, slowly increases thereafter, even in the very old (> 90 years; Huppert *et al*. 1998). As synthesis of CD2 and CD3 molecules occurs during thymic maturation, the decrease in mature : immature cells is certainly dependent on thymic involution. Nevertheless, thymic function remains at a sufficient level to permit generation of new CD3+ lymphocytes until mid-adulthood. When thymus maturation is almost non-existent, then the mature : immature T-cell value in peripheral blood starts to decline. Very late in life, new CD3+ T lymphocytes may be generated in other organs, probably in the liver. Indeed, this process has been demonstrated in old mice (Abo, 1992; Nakayama *et al*. 1994), and the same process probably occurs in man (Lesourd *et al*. 1994; Mazari & Lesourd, 1998). Not only are immature T lymphocytes more numerous (in terms of percentage and absolute values; Lesourd *et al*. 1994; Mazari & Lesourd, 1998), but in addition immature T lymphocytes are also less mature: the proportion of the CD2+CD4−CD8− double negative population also increases in the very old (Mazari & Lesourd, 1998). While mature : immature T lymphocytes decreases with age in old individuals, natural killer (NK) cells increase (Ligthart *et al*. 1986; Alès-Martinez *et al*. 1988), and these factors are highly correlated (Mazari & Lesourd, 1998), even though it was claimed recently that levels of CD57+ cells remain constant (Huppert *et al*. 1998). Indeed, an increase in the NK population with age has been observed irrespective of the marker used to characterize NK cells, i.e. CD57+ cells (Ligthart *et al*. 1986; Lesourd & Meaume, 1994; Mazari & Lesourd, 1998), CD16+ cells (Goto & Nishioka, 1989; Krishnaraj, 1997; Utsuyama *et al*. 1997) or NK function (Alès-Martinez *et al*. 1988; Krishnaraj, 1997), although other reports have claimed that there is no change (Wick & Grubeck-Lowenstein, 1997; Huppert *et al*. 1998). The increases reported in NK were always associated with decreased CD3+ lymphocytes. In fact, the increase in immature CD2+CD3− cells represents part of the increased NK population (Alès-Martinez *et al*. 1988; Lesourd & Meaume, 1994).

Ageing is associated with a decrease in the CD8+ subset, but the CD4+ subset remains unchanged in very healthy elderly subjects (Lesourd *et al*. 1994; Lesourd & Meaume, 1994; Wick & Grubeck-Lowenstein, 1997). The decrease in

Table 2. Absolute counts of T-cell subsets‡ in peripheral blood of very healthy subjects§
(Mean values and standard deviations)

n …	Young adults 46		Young elderly (> 65–85 years of age) 30		Old elderly (> 90 years of age) 16	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	29·4	3·5	77·9	5·2	94·3	3·4
Lymphocytes (/mm³)	2210	470	1980	620	1830*	680
CD2+ (/mm³)	1980	310	1730**	410	1605**	470
CD3+ (/mm³)	1850	280	1510***	320	1360**†	380
CD2+ CD3 (/mm³)	130	130	220*	210	240*	250
CD57+ (/mm³)	210	135	390***	180	430***	205
CD4+ (/mm³)	1245	1190	1115*	260	1084*	290
CD8+ (/mm³)	670	145	460***	190	405***	220
CD45RA (/mm³)	1230	340	560***	180	380***††	200
CD45RO (/mm³)	760	235	1090***	420	1125***	470

CD45RA, memory T lymphocytes; CD45RO, naive T lymphocytes.
Mean values were significantly different from those for the young adults: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
Mean values were significantly different from those for the young elderly: †$P < 0.05$, ††$P < 0.01$.
‡ Quantified on freshly drawn blood, as previously described (Lesourd *et al*. 1994).
§ Healthy young adults (25–34 years of age) and elderly of different ages were selected according to the SENIEUR protocol (Ligthart *et al*. 1984) and additional criteria (Lesourd *et al*. 1994; Lesourd & Meaume, 1994).

the CD8+ subset is mainly associated with a decrease in CD8+ cells expressing high levels of CD8, partly compensated by an increase in CD8+ cells expressing low levels of CD8. The increase in the latter type of CD8+ cells (Lesourd *et al*. 1994; Mazari & Lesourd, 1998) suggests that they may be generated not in the thymus but elsewhere, perhaps in the liver as demonstrated in aged mice (Abo, 1992; Nakayama *et al*. 1994). We also found an increase in low-CD8 cells in aged mice (Barrat *et al*. 1997). Reports of a decreased CD4+ subset are consistently derived from studies of apparently-healthy elderly subjects, so may not be related to primary immune changes. In contrast, increased proportions of CD8+ cells have been reported in very healthy elderly subjects (Wick & Grubeck-Lowenstein, 1997), leading to confusing results even in this carefully-selected population. In any case, the observed changes are minor (< 20 %) and may not be sufficient to explain an immune deficiency state.

T-cell functions

Ageing is associated with impairment of T-cell functions, the most important being the decline in T-cell proliferation (Murasko *et al*. 1987; Nagel *et al*. 1988; Shearer, 1997) and the decrease in IL-2 synthesis (Rabinowich *et al*. 1985; Nagel *et al*. 1988; Shearer, 1997). Age-related decreased proliferation as well as decreased IL-2 secretion are, at least partly, related to the changes in T-cell subsets. Increases in memory T-cells may also be significant in the decreased lymphocyte proliferation observed, since memory cells are poor IL-2 secretors (Nagelkerken *et al*. 1991; Hobbs & Ernst, 1997). In addition, immature CD2+CD3− cells, which increase with ageing, have a lower capacity to replicate (Alès-Martinez *et al*. 1988; Lesourd & Meaume, 1994). Decreased lymphocyte reactivity is also at least partly associated with age-related increases in membrane viscosity (Huber *et al*. 1991), inducing signal transduction defects (Ca mobilization and/or protein phosphorylation) which increase with age (Ghosh & Miller, 1995). This process leads to lower entry of the cells into the cell cycle, and to lower proto-oncogene expression (*c-myc* gene; Gamble *et al*. 1990), and inhibits cell progression (Perillo *et al*. 1993).

Nevertheless, contradictory results have been reported: lymphocyte proliferation was reported to be higher in the nonagenarians than in younger (75–90 years) aged individuals and this difference was related to different genetic background in survivors (Proust *et al*. 1982; Yong-Xing *et al*. 1997). We have recently reported (Mazari & Lesourd, 1998) that proliferation of lymphocytes from very-carefully-selected healthy elderly subjects without any micronutrient deficiency is comparable with that from similarly healthy young adults. It is then possible that the reported age-related decline in T-cell proliferation may be mainly, if not totally, due to selection bias (studied population not completely healthy), different genetic background and/or difference in nutritional status (cf. p. 690).

Approximately 8 years ago, Kubo & Cinader (1990) reported that IL-2 does not decrease with age in some mouse strains, i.e. DBA1 and DBA2. A recent report has confirmed this finding in other strains (Engwerda *et al*. 1996). In human subjects it has been shown that serum IL-2 has the same relationship with health status in adult and young aged (60–70 years) individuals (Mysliwska *et al*. 1998), indicating that *in vivo* IL-2 secretion capacities are comparable. We recently reported (Mazari & Lesourd, 1998) that *in vitro* IL-2 release from phytohaemagglutinin-treated mononuclear cell cultures is comparable with lymphocytes from 25–34-year-old adults and those from 75–84-year-old very healthy elderly subjects having no micronutrient deficiency. In addition, we reported that in this very-carefully-selected healthy elderly population a minor micronutrient deficit such as low folate levels is associated with decreased IL-2 production and decreased lymphocyte proliferation (Mazari & Lesourd, 1998). Once again it appears that changes in immune function in aged individuals may be more related to environmental factors such as health and/or nutritional status than to ageing *per se*.

Since the early 1990s immune ageing has also been described as including a change in T-helper (TH) 1 : TH2 cells, due to decreased TH1 function (mainly a decrease in IL-2 or interferon-γ secretion) and increased TH2 function (IL-4 and IL-6 release; Kubo & Cinader, 1990). We have shown that the decreased IL-2 secretion and, therefore, the decrease in TH1 function is not as obvious in the very healthy elderly, and that many factors, all related to health or nutritional status, may explain the frequently observed decrease in IL-2 (Mazari & Lesourd, 1998). In addition, contradictory results have been reported for age-related interferon-γ variations which have been found to remain constant or to decrease (Chen *et al*. 1987; Sindermann *et al*. 1993) with age. In fact interferon-γ is also secreted by memory T-cells, a subset which increases with age (Sanders *et al*. 1998), and the apparently contradictory results may be related to different values for naive : memory T lymphocytes in the populations studied. The age-related decrease in the TH1 subset in the very healthy population needs to be confirmed in very carefully conducted studies, but is highly probable since the decreased CD8+ cytotoxic T-cell functions may be restored by exogenous IL-12, a TH1 cytokine (Mbawuike *et al*. 1997). In contrast, the more recently reported rise in IL-6 secretion by lymphocytes from aged individuals, a TH2 function, is probably an ageing phenomenon. IL-6 secretion is increased in aged individuals (Daynes *et al*. 1993; Erschler *et al*. 1993; James *et al*. 1997), even in the very-carefully-selected healthy elderly (Mazari & Lesourd, 1998). It has been reported recently that this phenomenon starts in middle age (between age 36 and 59 years; Mysliwska *et al*. 1998), and is more pronounced in the very old (nonagenarians) than in the young old (Table 3). Thus, the age-related change in TH1 and TH2 function is an age-related immune change which is probably due to cumulative antigenic influence throughout the lifespan, since cumulative antigenic influence has been reported to be associated with a lower TH1 : TH2 (Cakman *et al*. 1996). The decrease in TH1 : TH2 may be of great importance in age-related immune changes, since TH1 mainly induces maturation and activation of the cytotoxic T lymphocytes which decrease with ageing (Bruley-Rosset & Payelle, 1987; Mbawuike *et al*. 1997), while TH2 induces increased B lymphocyte immunoglobulin production which increases with ageing (Batory *et al*. 1984; Moulias *et al*. 1984).

Table 3. Lymphocyte functions from peripheral phytohaemagglutinin (PHA)-stimulated T lymphocytes† from very healthy individuals
(Mean values and standard deviations)

n …	Young adults† 24		Young elderly† 27		Old elderly† 17	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	28·6	3·9	79·4***	4·9	> 90***	
Albumin (g/l)	43·4	2·9	42·5	4·2	41·2	3·8
C-reactive protein (mg/l)	< 6		< 6		< 6	
Lymphocyte proliferation						
Purified PHA (1 μg/10^6 cells)						
[3H]Thymidine (cpm/10^3 cells)	110	28	98	29	77	32
Percentage of CD25+ cells at 72 h	66·2	7·8	59·8	7·9	42·3**	9·5
In vitro cytokine release after 22–24 h cultures						
IL-2 (ng/ml)	1·62	0·27	1·53	0·34	1·17	0·45
IL-6 (ng/ml)	1·32	0·13	1·75*	0·23	1·96**	0·34

IL, interleukin; cpm, counts/min.
Mean values were significantly different from those of young adults: *$P < 0.04$, **$P < 0.02$, ***$P < 0.001$.
† Procedures for PHA-stimulated cultures, and quantification of lymphocyte proliferation and of supernatant fraction cytokine levels were performed as previously described (Lesourd *et al*. 1994; Mazari & Lesourd, 1998).
‡ Young adults (25–34 years) and young elderly (75–84 years) were selected according to the SENIEUR protocol (Ligthart *et al*. 1984) plus additional criteria (Lesourd *et al*. 1994; Lesourd & Mearne, 1994). Old elderly were recruited using the same criteria except the age class did not fit the SENIEUR criteria.

Humoral immunity

Humoral immune responses are less severely affected by the ageing process than cell-mediated immunity (Lesourd, 1990*a*). Blood levels of immunoglobulins G and A are increased in the very old, indicating an effective TH2 response (Batory *et al*. 1984; Moulias *et al*. 1984). Primary antibody responses are decreased while booster responses are preserved (Moulias *et al*. 1985), indicating that changes in antibody production are mainly due to the acquisition of new TH cells (Miller, 1996). The decreased antibody response has also been shown to be associated with increased anti-idiotype antibodies (Arreaza *et al*. 1993), which lead to production of antibodies with lower antigenic affinity (Muller *et al*. 1986). The lower affinity of the antibody produced has also been related to changes in B-cell subsets: CD5+ lymphocytes which secrete autoantibodies increase with age, while CD5− lymphocytes which produce antibodies against foreign antigens decrease (Weksler, 1995). Thus, even though antibody production is mildly reduced in the aged individuals, the specificity and the affinity of the secreted antibody is reduced, leading to less-adapted antibody responses.

Monocyte–macrophage functions

Macrophage functions seem to be preserved or even enhanced with ageing. Antigen processing and presentation are comparable in young and old mice (Doria, 1988). IL-1 production is sustained in old mice (Goldberg *et al*. 1991) and human subjects (Nafziger *et al*. 1993), while IL-6 production is increased (Table 4). This finding represents another disequilibrium in the immune response of aged persons; preserved functions of accessory cells and decreased functions of T lymphocytes. This process may be of great consequence for the adaptive immune response to antigenic challenge. Indeed, as T-cell proliferation is decreased, T lymphocytes need to be stimulated more to react in a suitable manner to antigenic challenge (Lesourd & Mazari, 1997). The greater secretion of macrophage cytokines in response to antigenic challenge leads to greater or longer-lasting body metabolic changes in old individuals (Cederholm *et al*. 1997), since monokines play a central role in controlling body metabolism (Klasing, 1988; Lesourd, 1992). Monokines induce (directly or indirectly via hormonal secretions) muscle protein breakdown, which is particularly damaging in old individuals since ageing *per se* induces increased muscle protein catabolism (Fereday *et al*. 1997), and reduced protein synthesis (Welle *et al*. 1993; Yarasheski *et al*. 1993). Thus, any antigenic challenge or any disease will induce higher muscle protein breakdown in aged subjects than in adults, and the muscle destroyed is not fully rebuilt after recovery. Any disease leads to some muscle loss (protein body reserve). When disease follows disease, protein body reserves decrease, which will push the elderly into progressive and gradually increasing protein–energy malnutrition (PEM), and, therefore, to progressive and gradually reduced immune responses. As decreased immune responses increase the risk of infection, the disease-induced increase in muscle loss makes occurrence of new diseases more likely, which worsens muscle protein loss, worsening PEM and inducing lower immune responses. The macrophage–T lymphocyte imbalance is therefore part of the ageing process which precipitates aged individuals towards frailty.

In addition, it has been shown recently that the monocyte production of prostaglandin E₂, a suppressive factor for T lymphocytes, is enhanced with ageing and partly contributes to the age-related decrease in T-cell functions (Hayek *et al*. 1997). As lymphocytes of aged individuals are particularly sensitive to prostaglandin E₂ production (Goodwin, 1982), the increased metabolic production of prostaglandin E₂ from macrophages of aged individuals further promotes macrophage–T lymphocyte dysregulation. This process may

Table 4. Monocyte cytokine releases in lipopolysaccharide-stimulated cultures‡ from very healthy individuals
(Mean values and standard deviations)

	Young adults§ 36		Young elderly§ 27		Old elderly§ 16		PEM elderly§ 45	
n …	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	29·3	3·6	79·4***	4·9	>90***		85·3***	7·3
Albumin (g/l)	43·7	2·6	42·5	4·2	42·1	3·8	26·1***†††	4·4
C-reactive protein (mg/l)	<6		<6		<6		21·7††	6·3
Interleukin 1 (ng/ml)								
Spontaneous	ND		0·3	1·5	0·3	1·3	2·6	2·1
Stimulated	2·7	2·1	2·5	2·5	2·6	3·1	1·0	0·8
Interleukin 6 (ng/ml)								
Spontaneous	ND		ND		ND		0·24	0·11
Stimulated	1·22	0·31	1·45	0·27	1·56	0·39	0·78††	0·37

PEM, protein–energy malnutrition; ND, not detected.
Mean values were significantly different from those for young adults: ***$P<0.001$.
Mean values for PEM elderly were significantly different from those for the young elderly: ††$P<0.01$, †††$P<0.001$.
‡ Procedures for lipopolysaccharide-stimulated cultures and quantification of supernatant fraction cytokine levels were as previously described (Lesourd *et al.* 1994).
§ Healthy young adults (25–34 years) and young elderly (75–84 years) were selected according to the SENIEUR protocol (Ligthart *et al.* 1984) and additional criteria (Lesourd *et al.* 1994; Lesourd & Meaurne, 1994).

be the explanation for the observed moderate response when dendritic cells from elderly individuals are stimulated with influenza virus (Wick & Grubeck-Lowenstein, 1997).

Secondary alterations in immune responses in the elderly: influence of nutritional factors

Numerous studies have investigated immune responses in apparently-healthy elderly subjects and diseased elderly subjects. We would like to briefly review those data, particularly those concerning the influence of nutritional factors in healthy or apparently-healthy elderly subjects in whom immune responses are influenced by ageing and by environmental factors, especially diet.

Apparently-healthy elderly

We have focused our research on immune responses in different groups of aged subjects characterized by their health status. In addition, we checked all elderly subjects in apparently good health for nutritional status by simultaneous quantification not only of weight and BMI, but also several nutritional biological variables, including serum proteins (albumin and transthyretin) for protein status, serum Zn and Se for trace element status, and serum vitamins A, C, E, B_6 and B_{12} and folate for vitamin status. All subjects were classified for each nutritional variable as either normal according to our laboratory data for healthy adults, or as nutritionally deficient if any of the tested variables was under the cut-off point using the criteria of the EURONUT/Seneca study (Haller *et al.* 1996). According to our classification, we have been able to select very healthy elderly (SENIEUR protocol plus additional criteria; see Table 1) for whom all nutritional variables were in the normal range, and apparently-healthy elderly (SENIEUR protocol plus additional criteria; see Table 1) for whom only one nutritional variable was not within the normal range, i.e. albumin, Zn or folate levels. We were then able to measure the reciprocal influences of ageing *per se* (in the very

healthy elderly group) and the influence of nutritional factors on immune responses in apparently-healthy elderly subjects.

Decreased nutritional status (assessed as decreased serum albumin, although in the normal range (35–40 g/l)), is associated with important changes in T-cell subsets and in T-cell functions. In the groups of very healthy elderly lower levels of serum albumin are linked with lower levels of CD3+ mature T-lymphocytes, lower levels of CD8+, higher levels of CD2+CD4–CD8– double negatives and of CD2+CD3– immature T lymphocytes, and higher levels of CD57+ NK cells (Lesourd *et al.* 1994; Lesourd & Meaume, 1994). In addition, CD45RO memory cells are decreased, while CD45RA naive cells are increased (Mazari & Lesourd, 1998). The changes in T lymphocyte subsets are correlated with the decrease in albumin levels, indicating an influence of low nutritional status on those changes (Lesourd *et al.* 1994; Lesourd & Meaume, 1994). In addition, all elderly subjects with low albumin levels expressed decreased levels of CD4+ subset, a change we had not observed in the elderly with high serum albumin levels (>40 g/l; Mazari & Lesourd, 1998). Immune functions, as measured by lymphocyte proliferation and *in vitro* IL-2 release from phytohaemagglutinin-stimulated lymphocyte cultures, are also lowered when compared with values for very healthy elderly subjects with higher serum albumin levels. In addition, IL-6 release from phyto-haemagglutinin-stimulated cultures is also decreased and comparable with that of cultures performed on the same day from lymphocytes of healthy adult controls (Mazari & Lesourd, 1998). Thus, it appears that small changes in protein nutritional status, reflecting a small nutritional deficit, is associated in aged individuals with an increase in the age-related immune changes and with a general decrease in immune functions. In addition, we have shown that low folate levels (either serum or erythrocyte folates) are associated with decreased lymphocyte proliferation when measured by the kinetics of CD25 appearance in phyto-haemagglutinin-stimulated lymphocyte cultures, indicating

that decreased lymphocyte proliferation in elderly subjects may be due to minor short-term changes in nutritional status. The fact that decreased folate levels are not associated with decreased lymphocyte proliferation in healthy young adults (Mazari & Lesourd, 1998) indicates that immune responses of old subjects are more sensitive to nutritional influences than those of young adults. Thus, it is logical to ask what role nutrition plays in the reported data on immune ageing.

We also evaluated the influence of nutrition on monocyte functions in similar groups of elderly subjects (Lesourd *et al.* 1998; B Lesourd and L Mazari, unpublished results). Very healthy elderly subjects with lower BMI release lower levels of IL-1 and IL-6 from lipopolysaccharide-stimulated monocyte cultures than similar elderly subjects with higher BMI (Table 4). In addition, these subjects have increased serum C-reactive protein and α-1-glycoprotein acid levels, indicating a probable *in vivo* activation of monocytes, since acute-phase protein synthesis is boosted by monocyte cytokines even though we have been unable to detect any increase in serum cytokines. The decreased *in vitro* cytokine release may be due to limited capacity of monocyte secretions in aged individuals. In fact, we have reported that during acute pulmonary infections the rise in IL-1 from lipopolysaccharide-stimulated cultures is lower in aged individuals compared with young adult counterparts (Nafziger *et al.* 1993). Is the influence of *in vivo* stimulation of the immune system, even at low levels, playing a role on *in vitro* quantification of the immune system in the apparently-healthy elderly? Is such an influence part of the observed immune ageing in less carefully selected aged individuals? Further studies investigating the health status of aged individuals in a careful and extensive way will permit us to answer these questions.

Lipids probably play a role in the age-related changes in immune responses. During T lymphocyte proliferation induced by mitogens the membrane lipid composition changes, and this change is altered during lymphoblastoid transformation in the elderly (Stulnig *et al.* 1995). In addition, the change in membrane lipid composition observed in aged individuals is correlated with a decreased mitogenic response (Wick *et al.* 1991). This response was reported to be due to higher plasma membrane viscosity (Traill *et al.* 1985) in peripheral lymphocytes of healthy elderly subjects. Indeed, membrane viscosity is highly negatively correlated with mitogenic responses of T lymphocytes (Huber *et al.* 1991; Wick & Grubeck-Lowenstein, 1997). Changes in membrane viscosity are more pronounced in lymphocytes from unhealthy elderly subjects than healthy elderly subjects, the former also expressing lower proliferation ability (Wick & Grubeck-Lowenstein, 1997). Thus, it was postulated that part of the altered lymphocyte proliferation is due to increased membrane viscosity, even in the healthy elderly. In addition, membrane viscosity may be reduced by *in vivo* lipid-lowering drug treatment or by changes in dietary lipid, which reduce the free cholesterol to phospholipid and the *n*-3 : *n*-6 value respectively in the membrane; such treatments induce higher mitogenic activity (Wick *et al.* 1991). The influence of dietary lipid on the immune responses of healthy elderly subjects in comparison with young adults needs to be further investigated.

Several reports have addressed the question of the influence of micronutrient deficits in apparently-healthy self-sufficient home-living elderly subjects. Most of these studies have tried to determine the influence of nutritional supplementation on immune responses of aged individuals using supplementation with either one, a few, or many micronutrients. It was first shown that vitamin B_6 supplementation induces higher lymphocyte proliferation in aged subjects in relation to increased responses in vitamin B_6-deficient individuals (Talbott *et al.* 1987). Later, the influence of vitamin B_6 on immune responses of aged individuals was established by a depletion–repletion study in healthy elderly subjects (Meydani *et al.* 1991). The role of vitamin B_6 deficiency on immune responses of apparently-healthy elderly subjects (SENIEUR protocol) may be strongly suspected since vitamin B_6 deficiency is common in home-living self-sufficient elderly subjects. Indeed, up to 18 % of these subjects were shown to have insufficient intakes in the EURONUT/Seneca study (Amorin-Cruz *et al.* 1996; Lesourd *et al.* 1998) and up to 7 % to have low serum pyridoxal 5′-phosphate values (Haller *et al.* 1996). One-third of low-income elderly individuals have plasma pyridoxal 5′-phosphate levels below 30 nmol/l (Garry *et al.* 1982). In addition, Zn deficiency is extremely frequent in the elderly (Prasad *et al.* 1993). Both Zn (Keen & Gershwin, 1990; Cunningham-Rundles *et al.* 1991) and vitamin B_6 (Chandra & Sudhakaran, 1990; Rall & Meydani, 1993) deficiencies are associated with immunodeficiencies which closely resemble the age-related immune changes. Thus, it is more than likely that some of the carefully-selected elderly subjects (SENIEUR protocol) had a micronutrient deficit which may be of importance for their immune responses. The importance of such nutritional influences on the immune ageing reported remains to be determined. In addition, it has been shown in apparently-healthy elderly subjects that multi-micronutrient supplementation at one to three times RDA levels may induce greater immune responses (Bogden *et al.* 1990, 1994; Penn *et al.* 1991; Chandra, 1992*a*; Boukaïba *et al.* 1993), probably due to correction of minor micronutrient deficiencies (Bogden *et al.* 1990; Chandra, 1992*a*). The influence of minor micronutrient deficiencies on immune responses of aged individuals is obvious even in the healthiest individuals. Thus, studies investigating immune ageing must be performed in individuals who have been assessed for micronutrient status. Assessment must be performed simultaneously with immune measurements, since some micronutrient deficits (such as low folate levels) may occur within a few days.

Furthermore, very high levels of vitamin E supplementation (200–800 mg N-α-tocopheryl acerate/d) also lead to increased immune responses (Meydani *et al.* 1990, 1997) even though vitamin E deficiency is uncommon in the aged population (less than 1 % in the EURONUT/Seneca study (Amorin-Cruz *et al.* 1996; Lesourd *et al.* 1998) Vitamin E supplementation was associated with decreased free radical and decreased prostaglandin E_2 (Cannon *et al.* 1991; Meydani *et al.* 1995) production by monocytes; both factors which are increased in aged individuals (Lang *et al.* 1992; Harman, 1995; Hayek *et al.* 1997). Free radical production is enhanced during cell activation. Thus, any acute-phase

response induces increased free radical production. We (Mazari & Lesourd, 1998) have shown that very healthy elderly (SENIEUR protocol plus additional criteria) with lower BMI have increased C-reactive protein and α-1-glycoprotein acid levels, and as a consequence they would have increased free radical production which may contribute towards the lower immune responses observed in this group of elderly subjects. Thus, immune ageing *per se* must be studied in aged individuals without any detectable acute-phase responses, which has not yet been done.

Undernourished elderly

PEM exerts a strong influence on immune responses in aged individuals (Chandra, 1989, 1992; Lesourd *et al* 1990*a,b*). In this group, whatever the assay used for quantification of cell-mediated immunity, immune responses are always decreased compared with healthy or even apparently-healthy elderly subjects (for review, see Lesourd *et al*. 1998). This finding has been confirmed for the T-cell subsets which change with ageing: decreased CD3+ and CD8+ subsets, increased CD2+CD3−, double negative CD2+CD4−CD8− subset and CD57+ NK cells. In addition, CD4+ numbers are decreased in undernourished elderly subjects. T lymphocyte functions (lymphocyte proliferation, cytokine release from mitogen-stimulated cultures, cyto-toxic capacities and delayed type hypersensitivity etc.) are lowered. The decrease in immune functions is highly correlated with the intensity of the nutritional deficit (Chandra, 1989; Lesourd, 1990*b*; Lesourd *et al*. 1992), leading to profound immunodeficiency in elderly patients with severe PEM. It was shown that ambulatory elderly individuals with serum albumin levels lower than 30 g/l have lymphopaenia and peripheral blood CD4+ counts lower than 400/mm^3; a level associated with acute acquired immune deficiency syndrome in human immunodeficiency virus pathology (Lesourd, 1990*a*, 1995). PEM is also associated with greatly decreased vaccine antibody responses in the elderly population (Lesourd, 1990*b*, 1995; Chandra, 1994). PEM also has a major effect on innate immunity in the elderly (Lipschitz & Udupa, 1986; Rudd & Banerjee, 1989; Lesourd, 1999). It has been shown that PEM and ageing exert cumulative influences on immune responses in the elderly (Lipschitz & Udupa, 1986). In addition, immune changes observed in undernourished elderly subjects may be reversed by refeeding therapy (Chandra, 1992*a*; Lesourd, 1995, 1999; Lesourd & Mazari, 1997).

Not only does PEM exert a profound effect on the immune responses of aged individuals, but micronutrient deficiencies, mainly Zn and vitamin B$_6$ deficiencies (for review, see Lesourd *et al*. 1998), have the same effects. Nutritive therapy using vitamin B$_6$ (Talbott *et al*. 1987; Rall & Meydani, 1993) or Zn (Boukaïba *et al*. 1993), or a combination of several micronutrients (Penn *et al*. 1991; Fortes *et al*. 1993; Galan *et al*., 1997;) induces increased immune responses in aged patients, but may also have detrimental effects (high doses of Zn (Bogden *et al*. 1990) or RDA dose of vitamin A (Fortes *et al*. 1993)).

Conclusion

Restriction of food intake has been shown to increase lifespan and to slow down immune ageing in rodents. Conversely, there is strong evidence that undernutrition induces immunodeficiency irrespective of the nutrient affected. The present review provides evidence that human immune ageing is strongly influenced by nutritional factors, mainly low nutritional status in either macro- or micronutrients. Indeed, very-carefully-selected healthy elderly subjects have cell-mediated immune variables, i.e. peripheral blood T lymphocyte susbsets and/or T lymphocyte functions that resemble those of young adult controls. In addition, elderly subjects with protein or micro-nutrient deficiency exhibit cell-mediated immune responses that fit almost exactly those generally described as age-related changes in immune responses. Furthermore, micronutrient supplementation, at the RDA level but also at higher doses, enhance immune responses of aged individuals almost to levels comparable with those of healthy adults. There is strong evidence that nutritional status greatly influences immune responses of aged individuals, and that this is an important part of what is known as immune ageing. Carefully designed studies need to be conducted in order to separate the respective roles of primary immune deficiency (immune ageing *per se*) and nutritionally-induced secondary immune deficiency in aged subjects. In addition, the levels of micronutrients necessary to slow down the immune ageing process remain to be investigated.

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