Assessment of Age-related Decline of Immunological Function and Possible Methods for Immunological Restoration in Elderly

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Abstract: The immune system plays an important role in protection against infection and in the maintenance of the internal environment of the body. However, such important immune functions are known to decline with age in many mammals, including humans. It is a matter of clinical importance that the incidence of various age-associated diseases such as infections, cancer and vascular disorders increases with a decrease in immunological vigor. The extent of immunologic decline is variable and exhibits wide inter-individual variations. Thus, it is important to assess the extent of immunologic decline in both patients suffering from various diseases and in healthy people in order to maintain healthy conditions. To this end, we have developed a scoring system that analyzes immune parameters according to a database of known age-associated immune changes obtained from a healthy population. Using this scoring system, we can combine several different immunological parameters and express the immune status of individuals as a simple numeral.

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After determining immunological vigor for individuals, it is necessary to replenish immune defects and restore them to normalcy for individuals with depressed immunological scores. This chapter provides methods of immunological restoration in animal models and introduces some similar attempts in humans. The effect of any immunological restoration varies with the individual and must therefore verified. Currently, the proposed immune scoring system proposed is useful to determine whether the methods employed are effective for the restoration of immune functions.

1 Introduction

A decade ago, centenarians used to appear mainly in fairy stories. Nowadays centenarians are not uncommon and there are more than 30,000 centenarians in Japan. In 1900 when Soseki Natsume, a Japanese writer, was in London, the mean life span of the male population in England was 44 years old and that in Japan was 45 years old. In many countries, the mean life span has increased steadily during the first half of the 20th century, however, this has not been the case in Japan. The increase in the mean lifespan was outstanding in the second half of the 20th century (after the Second World War) in Japan. This pronounced improvement is attributed to rapid progress of medicine including antibiotics and sufficient food supply including the supply of proteins.

WHO reports that a "healthy" life span is generally 6–10 years shorter than the mean lifespan. This means that many elderly people suffer from some diseases for several years before death.

It is well known that the incidence of cancer, cardiovascular disease, neurovascular disease and infection increases with age. Autopsy examinations have revealed that the largest cause of death in the elderly is infections such as bronchopneumonia and urinary tract infection (Table 1a and 1b). The occurrence of severe acute respiratory syndrome (SARS) in south Asia and China in the winter of 2003 clearly indicated that the fatality rate was high, approximately 50%, in people over 65 years of age (Table 1c) (Hirokawa et al. 2006). With regard to infection, Pawelec et al. (2004) suggested that chronic antigenic stimulation could lead to an increased prev-

Table 1a Causes of death in a hospitalized geriatric population: an autopsy study of 3000 patients

Bronchopneumonia	42.9%
Malignant neoplasms	28.1%
Pulmonary thrombo-embolism	21.2%
Acute myocardial infarction	19.6%
Urinary tract infection	12.3%
Acute cerebrovascular disease	6.5%
Internal hemorrhage	5.5%
Congestive cardiac failure	3.3%

The data, based on 3000 consecutive autopsies (1758 females/1242 males: mean age 80.3 years) performed from 1972 to 1992 in Geneva Geriatric Institutions (Mac Gee W).

Table 1b   Major causes of death in autopsy cases of elderly persons at Tokyo Metropolitan
            Geriatric Hospital

	People over 60 years	People over 70 years
Infections	39.2%	27.6%
Vascular diseases in brain and heart	29.7%	43.1%
Malignancies	18.7%	22.4%
Others	12.4%	6.9%

The data are based on 923 autopsy cases (570 females/353 males) of people over 60 years of age.

alence of senescent dysfunctional T-cells, and therefore contribute to more general
alterations in the immune system.

Furthermore, elderly people face an indefinite number of problems such as pain
in the shoulder, arm or leg, dizziness, staggering and headaches. In addition, chronic
inflammatory symptoms such as bronchitis, laryngitis or adenoiditis are very com-
mon in the elderly.

An age-related increase in various diseases is causally related with the age-related
decline of immune functions (Hirokawa et al. 2006). Indefinite problems are attribut-
able to variable causes including various diseases and psychological stress, but one
of the major causes is inadequate adaptation to variable stresses from the external
environment. Nervous, endocrine and immune systems work together to maintain
the internal environment, when the body is exposed to the stresses from physical,
psychological and biological surroundings. In the elderly, however, the function of
all these three systems declines with age, resulting in improper adaptation to stress.

Therefore, restoration of immunological function would be quite helpful for the
elderly not only to prevent infection, but also maintain their internal environment at
the time of exposure to stress. In other words, immunological restoration is expected
to be effective for the improvement of the QOL in the elderly.

Immunological restoration requires 2 steps. The first is to assess immunological
parameters or functions to determine the extent of the age-related decline of immune
functions of each individual. To this end, we require a measuring method to express
the level of immunological vigor as a simple numeral that anybody can understand.
The second is to select adequate methods to restore immunological functions and to
check the effectiveness of the selected immunological restoration by the measure-

Table 1c   Fatality rate of SARS in Hong Kong

Age	Fatality rate (%)
24 years and below	0%
25–44 years	6%
45–64 years	15%
65 years and above	52%
Total	14–15%

WHO report. Consensus document on the epidemiology of severe acute respiratory syndrome
(SARS). 17 October 2003, http://www.who.int/csr/sars/guidelines/en/

Fig. 1 Proper assessment of immunological functions is essential to verify the effectiveness of immunological restoration
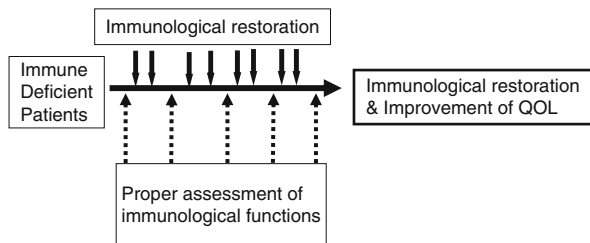
ment method mentioned above, because the effectiveness of immunological restoration differs based on the individual and the method of restoration (Fig. 1). Thus, this chapter deals with the following; (1) the measurement method to assess the immunological level of individuals, (2) causes of immunological suppression, (3) possible methods of immunological restoration in elderly people.

2 Assessment of Immunological Level as a Whole

The immune system comprises various functions and consists of many types of cells that perform various functions, and it is difficult to select immunological parameters that are suitable for the assessment of immune functions in healthy people and patients suffering from various diseases.

From a functional viewpoint, there exist parameters such as cell mediated immunity, humoral immunity, cytokine production, proliferative activity of T-cell and B-cells, antigen presentation of dendritic cells and so on. Cells comprising the immune system are T-cells and their subpopulations, B-cells and their subpopulations, NK-cells, NKT-cells, macrophages and dendritic cells. None of these may be excluded for the assessment of the immunological level as a whole.

Another important aspect is to determine which immune cells or parameters play a key role in the age-related decline of immune function. We have been studying the immunological aspect of aging for many years and have confirmed that immune functions are susceptible to aging, diseases and stress. For the past 30 years, many studies including ours have shown that the age-related decline mainly occurs in T-cell-dependent immune functions and is relatively small in functions of other cells such as B-cells, macrophages and NK-cells (Makinodan and Kay 1980; Hirokawa 1992; Linton and Dorshkind 2004; Hirokawa et al. 2006). Therefore, when considering the age-related change of immunological functions, the immunological assessment can be focused on parameters that are related to T-cell-dependent functions. We have reported that the age-related change in the T-cell-dependent immune system is observed in a decrease in the T-cells number, a change in the T-cell subpopulations and a qualitative change in T-cells such as a decline in proliferative capacity and a change in cytokine production (Fig. 2).

Therefore, the number of whole T-cells and their subpopulations, and the proliferative activity of T-cells are useful parameters to assess the extent

Fig. 2 Age-related decline in T-cell-related immune parameters

(a) A significant decline is observed between the 2nd and 3rd decades. The number stays at almost the same level through the 6th decade and declines after the 7th decade

(b) The age-related decrease in the number of naïve T-cells (open columns) with a concomitant increase in the number of memory T-cells (grey columns)

(c) Proliferative response of lymphocytes to varicella-zoster virus (VZV) peaks in the 1st decade and gradual decline thereafter. (The graph was originally made by Prof. S. Kishimoto)
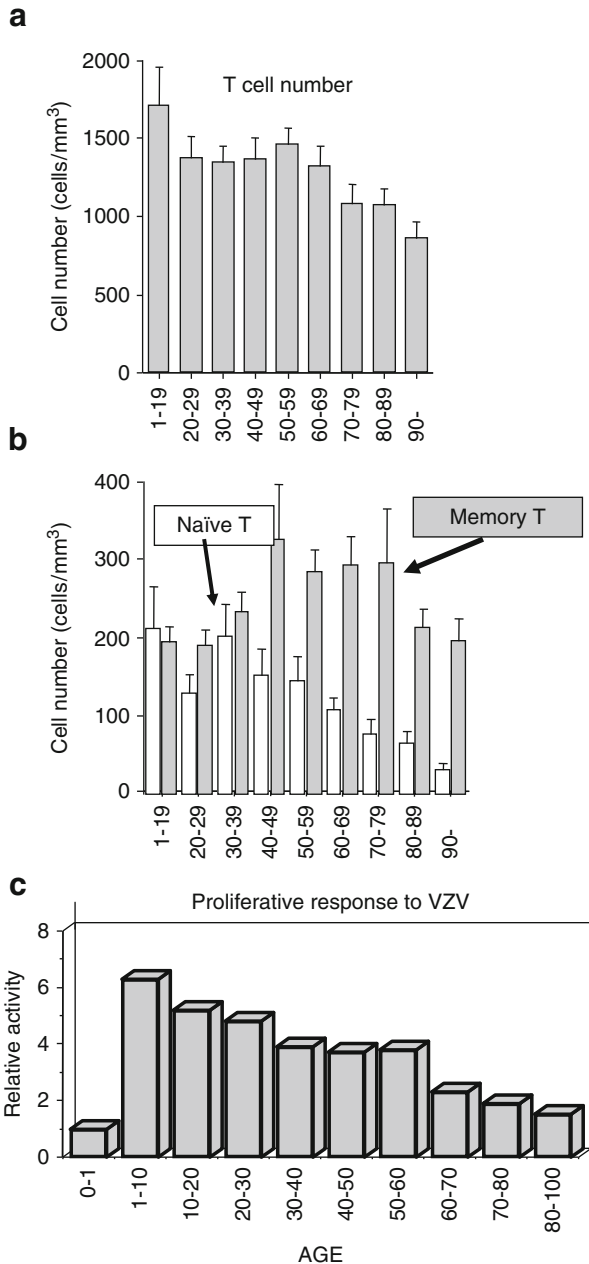
of the age-related decline of immune functions. Hence, we performed flow cytometric analysis for 7 parameters reflecting T-cells and their subpopulations; number of T-cell ($CD3^+$ cells), number of $CD4^+$ cells, number of $CD8^+$ cells, the ratio of $CD4^+$ cells to $CD8^+$ cells (CD4/CD8 ratio), number of naïve T-cells

(CD4$^+$CD45RA$^+$ cells), number of memory T-cells (CD4$^+$CD45RO$^+$ cells), and the ratio of naïve T-cells to memory T-cells (N/M ratio). The proliferative activity of T-cells was measured by nonspecific stimulation of T-cells by anti-CD3 monoclonal antibody.

We defined a new indicator, T-cell proliferation index (TCPI) using the number and proliferative activity of T-cells, as described later.

In addition to T-cells, the number of B-cells and NK-cells was included for this assessment, since these cells are counterparts of immune functions.

Cytokine production is another important aspect of immunological function. Among many cytokines, information on Th1/Th2 balance is helpful for understanding health and disease condition. Therefore, we employed 3 interleukins; IL-2 and IFNg as the Th1 group, and IL-4 as the Th2 group.

Here, we have ten parameters to assess the extent of age-related decline in immunological functions (hereafter referred to as the scoring of immunological vigor: SIV) of individuals as shown in Fig. 3. Since it is difficult to imagine the immunological level of each individual by merely looking at the numbers of the 10 parameters, we have tried to represent the immunological level of each individual
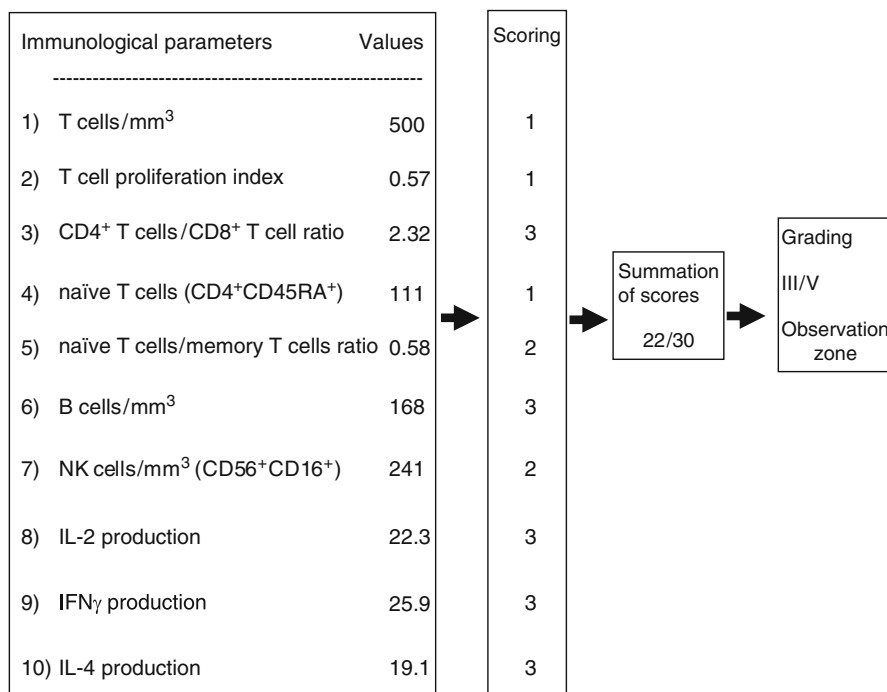
Immunological parameters	Values	Scoring
1) T cells/mm^3	500	1
2) T cell proliferation index	0.57	1
3) CD4$^+$ T cells/CD8$^+$ T cell ratio	2.32	3
4) naïve T cells (CD4$^+$CD45RA$^+$)	111	1
5) naïve T cells/memory T cells ratio	0.58	2
6) B cells/mm^3	168	3
7) NK cells/mm^3 (CD56$^+$CD16$^+$)	241	2
8) IL-2 production	22.3	3
9) IFNγ production	25.9	3
10) IL-4 production	19.1	3

Summation of scores 22/30

Grading III/V Observation zone

Fig. 3 Process of scoring and grading immunological parameters. Values of immunological parameters are given a score from 1 to 3. Sum total of 10 scores is named scoring of immunological vigor (SIV-10). SIV-10 is then classified into 5 grades as shown in Table 2. It is generally difficult to determine immunological status of individuals by a mere list of figures of 10 parameters. By the scoring system, 10 parameters can be grouped and expressed numerically as immunological score or grade

in a plain style. The value of each immunological parameter falls within a range specified in a database and each parameter was scored into 3 grades based on its value. In particular, values in the range of a cumulative frequency less than 10% of values observed for healthy subject were scored 1, which indicates a low immunity level; those between 10% and 40% were scored 2, which indicates a moderate immunity level; and those with 40% or higher were scored 3, which indicates a sufficiently high immunity level. Since higher scores of CD4/CD8 ratios are frequently observed in very old people and patients suffering from diseases, values greater than 80% of the cumulative frequency were scored 2, which indicates a moderate immunity level.

We employed 3 options to assess SIV; the first using 10 parameters, named SIV-10; the second using 7 parameters excluding cytokine production, named SIV-7 and the third using 5 parameters composed of T-cell-related functions, named SIV-5 or T-cell immune score.

Values of SIV-10, SIV-7 and SIV-5 were then classified to 5 grades (V~I) according to the total score. V: sufficiently high, IV: safety zone, III: observation zone, II: warning zone, I: critical zone (Table 2). The observation zone indicates that the level of SIV is average, but needs attention to move up into the safety zone. The warning zone indicates that the level of SIV is not sufficient to maintain health and considerable effort is required to increase the level of SIV. The critical zone indicates that the susceptibility to infection is so high that the individual needs to be admitted to an aseptic isolator.

3 T-cell Proliferation Index and Assessment of Immunological Age

We defined a new parameter, T-cell proliferation index (TCPI) which was calculated by using the number and proliferation activity of T-cells. The number of T-cells is essential for the maintenance of immune function. In addition, T-cell proliferation is the most essential function of T-cell immunity, including the process of antigen recognition and sequential division of T-cells. There are 4 types of number and proliferative capacity of T-cells. (1) both are sufficient; (2) number is sufficient,

Table 2 Scoring of immunological vigor (SIV) and grading

Scoring			
SIV-10	SIV-7	SIV-5	
10 parameters	7-parameters (without cytokines)	5parameters (T-cell related)	Grading
30 ~ 29	21	15	Grade V sufficiently high
28 ~ 26	20 ~ 18	14 ~ 13	Grade IV safety zone
25 ~ 22	17 ~ 13	12 ~ 10	Grade III observation zone
21 ~ 17	12 ~ 10	9~ 7	Grade II warning zone
16 ~ 10	9 ~ 7	6 ~ 5	Grade I critical zone

Fig. 4 Distribution of TCPI against age. Approximate values of 400 people are displayed
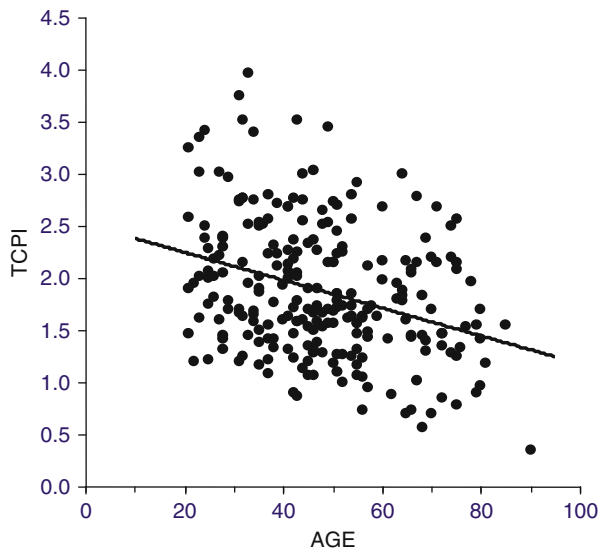
but proliferative capacity is insufficient; (3) number is insufficient and proliferative capacity is sufficient; (4) both are insufficient. Therefore, we arrived at the conclusion that we needed a new parameter which reflected both the number and proliferative capacity of T-cells. The new parameter was TCPI and was calculated by the following equation.

TCPI = T-cell proliferation activity x (T-cell number per μL/1000)

Figure. 4 indicates the distribution of TCPI according to age. Although, there is a wide individual variation, the age-related decline of TCPI is obvious and the following equation was obtained.

TCPI = − 0.0174 x (Age) + 2.5348

In other words, we can determine age of individual by the value of TCPI using the following equation. Since the age is calculated from the number of T-cells and their proliferative activity, the age obtained by this equation is hereafter referred to as the immunological age (IA).

IA = (2.5348 – TCPI)/0.0174

4 Scoring of Immunological Vigor (SIV) Shows Wide Range of Individual Variation

Immunological vigor of 400 healthy people and 300 cancer patients were assessed using the method mentioned above (Fig. 5a). Age-related related decline as observed in SIV-7 was apparent in the healthy population. However, there was a wide individual
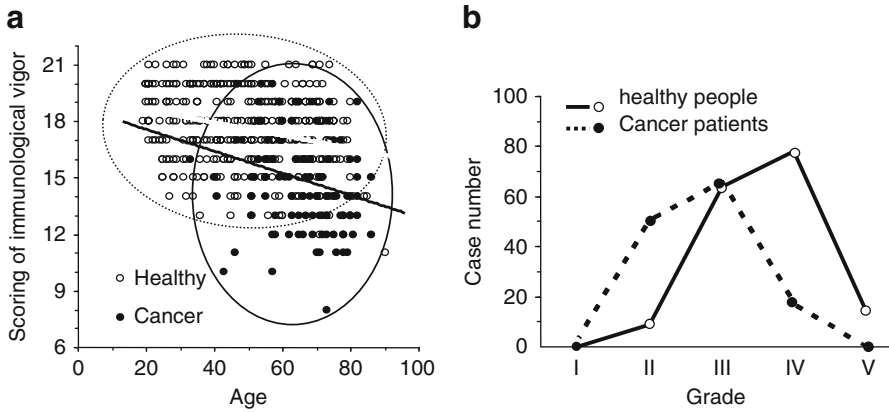
Fig. 5 Comparison of SIV-10 (a) and grade (b) between healthy people (400 cases) and cancer patients (300 cases)

variation. The level of SIV-7 ranged between 21 and 14 in the 3rd decade and between 21 and 12 in the 7th decade, indicating that individual variation is greater than the difference between young and old people. But when observing SIV-7 in cancer patients, we found that the distribution of values was apparently different from that in healthy people and the age-related decline in cancer patients was much steeper than in healthy people. However, it is again apparent that the SIV-7 level of cancer patients showed a wide range of individual variation, although the values were lower as compared with those for healthy people (Hirokawa et al. 2007a, 2007b).

Based on the SIV-7 grade, the difference in SIV-7 between healthy people and cancer patients was much obvious (Fig. 5b). Most healthy people belonged to Grade IV and III, while cancer patients belonged to Grade III and II, indicating that the immunological deficient state is more serious in cancer patients. Thus, considerable attention should be given to the immunological state of cancer patients during treatment (Hirokawa et al. 2008).

Figure. 6 shows radar graphs of 3 example cases showing the 10 immunological parameters, IA, SIG-10, immunological grade and immunological zone. This figure shows how the immune status of individuals can be understood.

5 Variable Causes Suppress Immunological Functions

As presumed from the wide individual variation of immunological vigor, immunological functions are changeable by variable causes: i.e., aging, stress, diseases, life style, food, genetic background etc.

Aging is unavoidable cause of immunological decline. However, the rate of immunological decline by aging might be accelerated or decelerated by surrounding environmental factors which can be controlled by appropriate intervention. For
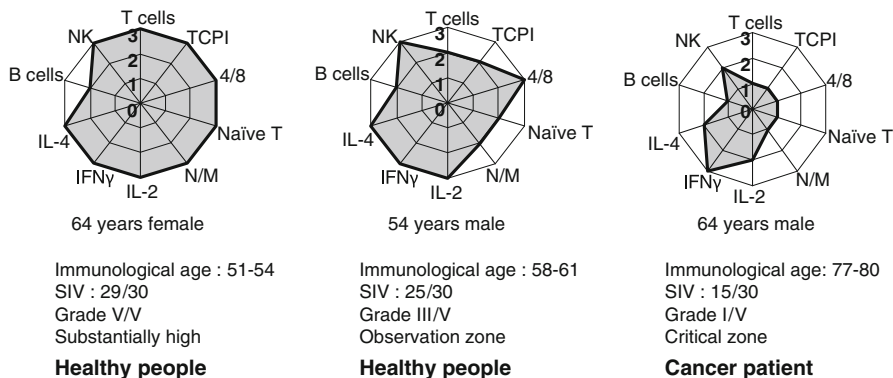
Fig. 6 Radar graph shows 3 examples assessed by immunological age, SIV and grade

instance, caloric restriction extended the life span of animal models and enhanced the immunological function of old animals (Heilbronn and Ravussin 2003; Utsuyama et al. 1996).

Stress is the major cause of immunological decline. Any forces that disturb homeostasis can be stressors. In other words, stress is life and life is stress (Chrousos GP et al. eds; Stress 1995).

Various diseases including minor ones are also factors suppressing immunological functions. Incidence of cancer is known to increase with the advancement of age and this is partly caused by a decrease in immune surveillance (Dunn et al. 2002). In fact, a patient with colonic cancer has a low level of SIV (Hirokawa et al. 2008). But it is obvious that SIV generally recovers in a certain interval after the removal of cancer lesion. Thus, it is likely that the presence of cancer suppress the immunological function of the individual. It is also well known that diabetes mellitus suppresses immunological function. Various therapies for many kinds of diseases can down-regulate immunological functions. One example is surgical operation. Chemotherapy for cancer and steroid therapy for autoimmune diseases suppress immune functions.

6 Possible Methods of Immunological Restoration

Now we can assess the immunological level by SIV, as mentioned above. As observed in Fig. 5, there is a wide individual variation in the SIV levels. We presume that individuals with high SIV can live longer and those with low SIV are susceptible to diseases. Further longitudinal studies are necessary to say something for the prognosis of people with high or low SIV. Roberts-Thomson et al. reported that individuals with reduced immune functions had significantly greater mortality than those whose immune functions were within the normal range. Centenarians in Okinawa have significantly high immune functions as compared with controls. Considering these data, it may be inferred that it is desirable to enhance SIV level of individuals with a lower SIV level.

Here we would like to present our preliminary experience of immunological restoration in humans and its possible methods as determined from experiments using animal models (Hirokawa et al. 2002).

6.1   Coping with Stress

In daily life, a variety of stresses down-regulate immune functions and in fact, the stresses are the major cause of immunological suppression in our life. Stresses originate from communication problems at home, office and school, from diseases or injury, from treatment of diseases or from natural disasters. There are 2 solutions for stress: (a) avoid stress. (b) alleviate the effect of stress.

(a) Avoid stress.

Needless to say, it is best to avoid stress if possible. Unfortunately, however, most stresses are unavoidable. But it is worthwhile to reconsider the cause of stress and analyze whether or not it is avoidable.

(b) Alleviate stress.

It is interesting to note that the same level of stress has very serious effects in some people, but not in others. This means that the magnitude of stress is dependent on the response of individuals. For instances, an academic examination would not be as serious for students who have studied beforehand as it would be for those who have not studied.

Singing, running and chattering with friends are mood-altering activities. Sleeping, if possible, is another good activity. A good way to alleviate stress is to devote time to some hobby. In this respect, we studied the effect of music in humans and we found that playing the drums in a group could enhance NK activity (Wachi et al. 2006).

6.2   Improvement of Life Style

Habits indicative of an improper life style are poor sleep, overwork, smoking, excessive drinking, irregular meals, deviated food habits and insufficient physical exercise. We interviewed more than 50 of young people who showed a lower level of SIV and over 50% of them had improper work habits.

Nutrition is an important factor for immunological restoration. For the last 50 years, the mean life span of Japanese people has extended from 50 to over 80 years. This rapid increase in life span is partly due to the improvement of food intake, especially, protein. Today, people are worried about metabolic syndromes and are making an effort to not eat too much. But too much of anything is harmful.

More than 70 years ago, MaCay (1935) reported the elongation of life span in rats by caloric restriction. Many studies have since shown that caloric restriction is effective on several counts; i.e., elongation of lifespan, enhancement of immunological function and alleviation of autoimmune diseases.

One argument about food restriction or caloric restriction is that it only appears to be effective in animals that are reared in an artificial environment such as a laboratory animal colony (Utsuyama et al.1996). Most laboratory animals are fed highly nutritional chow without being given enough space or tools for physical exercise. In other words, immunological improvement by food restriction might be seen only in overfed animals that do not perform physical exercise. In order to test this conjecture, we employed an automatic feeding device that was electrically interlocked with a running wheel: the device provided constant running exercise to the animal and fed a determined amount of diet to each rat (lchikawa et a1. 2000).

Rats were individually reared in this automatic feeding device for 18 months from 2 to 20 months of age. At the age of 20 months, the rats were sacrificed and examined for various indices including immunological functions. The body weight was almost the same between rats fed ad libitum and those given 80%-restricted diet. Their body weight was significantly greater than that of rats given 60%-restricted diet. The body weight of those given 80%-restricted diet together with physical exercise was almost the same as that of rats given 60%-restricted diet without physical exercise. High proliferative response of T-cells was observed in some of rats given 80%-restricted diet together with physical exercise (Fig. 7).

Meanwhile, studies are ongoing to observe the effect of caloric restriction on various physiological parameters in monkeys (Roth et al. 2002). A report provided evidence that CR can delay immune senescence in nonhuman primates, potentially contributing to an extended lifespan by reducing susceptibility to infectious disease (Messaoudi et al. 2006).

In humans, the effects of CR on life extension are actually present; it is estimated that CR extends life by 3–13 years. This extension is much smaller than those achieved by medical and public health intervention, which have been known to extend life by about 30 years in developed countries of the 20th century (Everitt and Le Couteur, 2007).
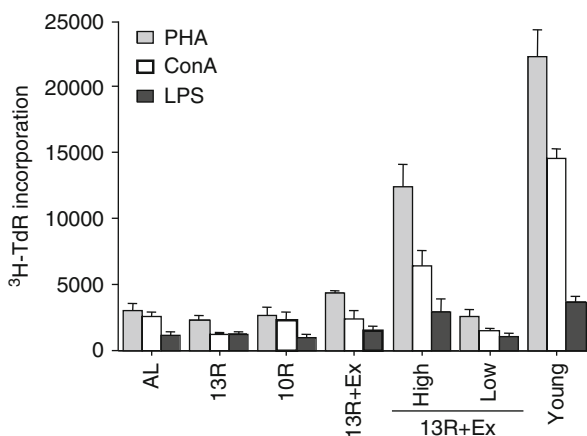

Fig. 7 Effect of long-term exercise and caloric restriction on lymphocyte proliferation in rats

6.3   Anti-oxidant Chemicals

It is now commonly believed that oxidative stress, such as that caused by reactive oxygen species and free radicals, is the major cause of aging phenomena and various diseases (Harman 1956; Ames et al. 1993; Wakikawa et al. 1999). The decline of immune function is one of major aging phenomena and constitutes the background of various diseases occurring in the elderly people (Hirokawa 1998). Many investigators assume that oxidative stresses play an important role in the progression of immunological decline during the course of aging (Martin et al. 1996; Meydani et al. 1998). Thus, it has been expected that enzymes or substances that compete with oxidative stress are candidates for antiaging medicines. Anti-oxidant enzymes are present in our body, e.g., SOD and catalase. It is possible to enhance the production of these antioxidant enzymes by appropriate physical exercise. In addition, there are many kinds of supplements that are commercially available as antioxidant chemicals, although effectiveness of most of them has not been proved scientifically.

Flavonoids or polyphenols are representative antioxidants contained in many kinds of vegetables, fruits and other foods. Extracted or enriched flavonoids and polyphenols are commercially available as supplements. The effectiveness of these supplements requires further study.

Vitamin E (VE) works as an antioxidants. Research groups have found conflicting effects of VE. In our experiment using aging mice, we confirmed that VE enhances the immune functions of young, but not old mice (Wakikawa et al. 1999) (Fig. 8). Other reports indicated that VE supplementation was not effective in humoral immune response modulation in young, middle-aged and elderly women (Park et al. 2003). On the other hand, Meydani's group reported the positive effect of VE in both human and animal experiments (Meydani et al. 1998; Marko et al. 2007). These reports suggest that the effectiveness of supplements is not uniform, but differs with the individual and the genetic background.

The effectiveness of any antioxidants and related supplements usually varies with the individual. Therefore, immunological assessment as performed in an objective
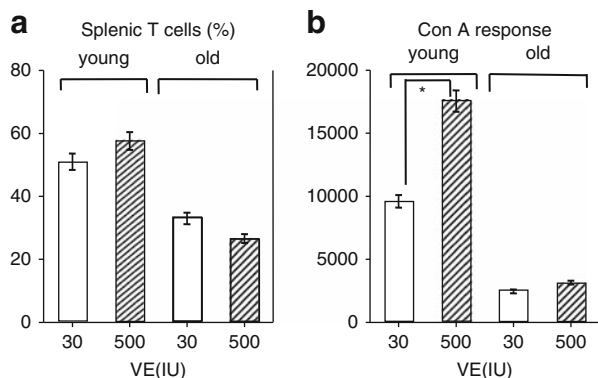

Fig. 8 Effect of vitamin E on splenic T-cells (a) and Con A response (b) of young and old mice

manner, such as determining SIV, is always necessary to verify the effectiveness of chemicals and supplements on the immune system.

6.4 Vaccine

Vaccines are very useful to protect children who are susceptible to various infectious diseases due to immaturity of the immune system. The same effect could be expected for the elderly people with impaired immune functions. However, vaccination in very old people may not work due to depressed immune functions. Thus, vaccination should be started latest before the age of 60, so that the individual still have sufficient capacity to respond to pathogenic organisms. Alternatively, attempts should be made to enhance or stimulate the depressed immune capacity of elderly people at the time of vaccination.

Susceptibility of old mice to infection is clearly observed in experimental infection with influenza virus. Old mice died of infection at a 10-fold lesser dose of influenza virus. 01d mice that survived after exposure to low doses showed strong immunity to the same virus and became resistant to the second infection with a high dose of influenza virus (Hirokawa and Utsuyama 2002). These data suggest that vaccination is useful to protect the elderly people from various kinds of infection.

In humans, vaccination against pneumococcus and influenza virus is already clinically conducted for elderly people. As stated in the earlier section, there is individual variation even in elderly people. Those having higher SIV can respond to vaccination and show sufficient immunity against bacteria and virus. Problem is in finding a way to enhance immunological functions of the elderly people with lower SIV so that they can effectively respond to vaccination.

Another point to be considered is the route of vaccination. Since the nose and mouth are the major entries for respiratory infection, their mucosal immunity is important. Using a mouse model, we found that the route of vaccination is important. Vaccination through the intranasal route provides high level of IgA production in nasal mucosa, but that through the intravenous and intraperitoneal routes does not. (Table 3). (Asanuma et al. 2001).

Table 3 Difference in the levels of IgA antibody specific to influenza virus (ng/mouse) between vaccination route and age

	3 months old	18 months old
1) i.n. → i.n.	172 ± 42	54 ± 50
2) i.v. → i.p.	2 ± 2	2 ± 2

1) Mice were administered intranasally (i.n.) with A/PR/8/34 vaccine (10mg) and boosted 3 weeks later i.n. with the same vaccine. Antibody was assessed 1 week after the boosting

2) Mice were administered intravenously (i.v.) with A/PR/8/34 vaccine (10mg) and boosted 3 weeks later intraperitoneally (i.p.) with the same vaccine. Antibody was assessed 1 week after the boosting

6.5   *Japanese Herbal Medicines*

A group of Japanese herbal medicines, called 'Kampo-Hozai' have been used to improve the physical condition of patients suffering from various diseases (Utsuyama et al. 2001). Among more than 100 kinds of Kampo-Hozais, Hochuekki-to(TJ-41) is a drug used to recover immune function and has been reported to be useful in healing infections (Yamaoka et al. 2000; Mori et a1. 1999), oncostatics-induced leukopenia (Kaneko et al. 1999), and allergies (Suzuki et a1. 1999). Juzen-taiho-to (TJ-48) is useful not only for the recovery of immune function (Abe et a1. 1998), but also for the enhancement of antitumor effects (Saiki et al. 1999; Onishi et al. 1998; Utsuyama et al. 2001).

We tested the effectiveness of Japanese herbal medicines in young and aged mice. The data indicated that Hochu-ekki-to (TJ-41) was effective in the restoration of impaired immune functions of aged mice, in terms of the number of T-cells and NK-cells, and anti-SRBC antibody response. However, it was not effective in enhancing the immune functions of young mice (Fig. 9).

Juzen-taiho-to was also effective in increasing the number of T-cells and NK-cells in aged mice, although a significant increase was not observed in young mice. Functionally, however, NK activity increased both in young and old mice. A significant decrease was also observed in the number of metastatic pulmonary colonies of B16 melanoma cells both in young and old mice treated with Juzen-taiho-to for 16 weeks (Fig. 10).

These results suggested that some Japanese herbal medicines are useful in the restoration of impaired immune functions of old mice and could be recommended for the elderly people with immunological problems.

We performed preliminary clinical trial of TJ-41 to observe its effect on immunological function in humans. The results showed that the effect varied with the individual. Approximately half of the people who were given TJ41 showed a positive effect, but another half did not show any effect. Fig. 11 shows an example case of the positive effect of TJ41. Therefore, in these cases also, the assessment of immunological parameters is necessary to verify the effectiveness of herbal medicines for immunological restoration.
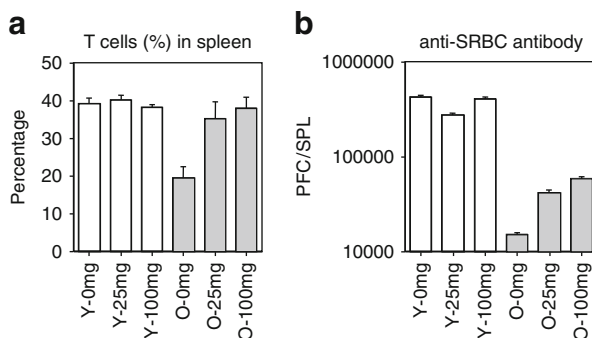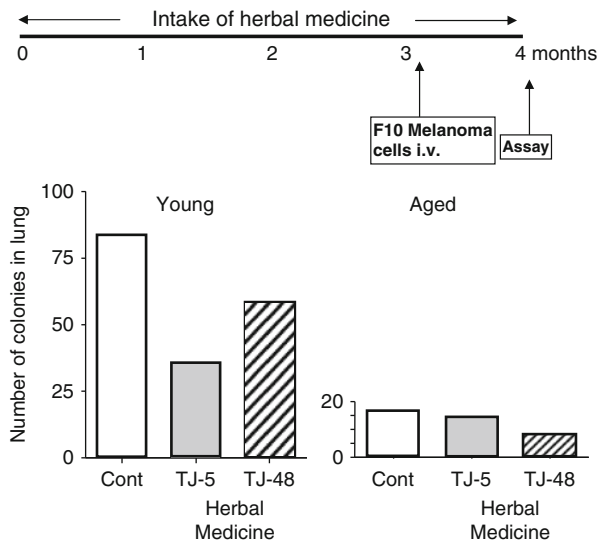
Fig. 9   Effect of Japanese herbal medicine (TJ-41) on splenic T-cells (a) and anti-SRBC response in young and old mice

Fig. 10 Effect of Japanese
herbal medicine (TJ-48) on
tumor metastases to lungs
in young and old mice. Top
figure shows experimental
protocol. TJ-5 is another
Japanese herbal medicine,
Anchusan

6.6   Hormones

After gonadectomy, hypertrophy of the thymus was observed in aging C57BL/6 mice, ranging in age from 4 to 20 months. The mice had been gonadectomized 1 month before the sacrifice, and the magnitude of thymic regeneration was more pronounced in males than in females (Fig. 12). However, enhancement of anti-SRBC antibody response was observed only in females, but not in males regardless of age. Gonadectomy brought about not only thymic hypertrophy but also an increase in T-cells and B-cells in the spleen. An increase in T-cell subpopulations was proportional in female mice, but disproportional in male. The disproportional increase of T-cell subpopulations could account for the failure to enhance the anti-SRBC antibody response in male mice (Utsuyama and Hirokawa 1989).

Gonadectomy also resulted in the thymic hypertrophy in male and female young Wistar rats, but not in those that had been previously hypophysectomized (Utsuyama and Hirokawa 1989).
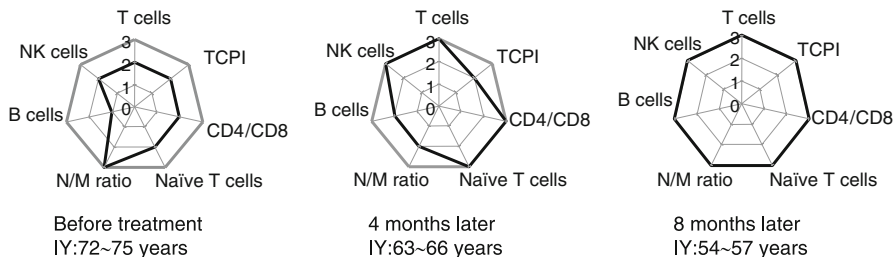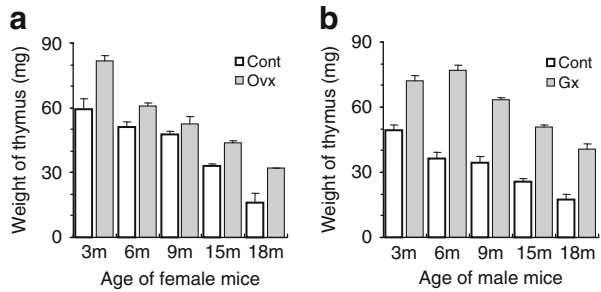

Fig. 11 Effect of TJ-41 on immunological parameters and immunological year (IY) in 66 years old male. Full recovery was observed 8 months later

Fig. 12 Effect of ovariectomy (a) and orchidectomy (b) on the weight of the thymus at various ages. All mice were sacrificed 1 month after the gonadectomy and the wet weight of the thymus was measured

Elderly males undergoing orchidectomy for prostatic carcinoma demonstrated an increase in circulating T-cell numbers, particularly naive (TREC+) T-cells. Chemical castration by administration of LHRH antagonist was also effective in activation of thymic regeneration in mice and humans (Sutherland et al. 2005).

In females, hormone replacement therapy (HRT) by estrogen has been prescribed to postmenopausal women for prevention of a variety of medical conditions including osteoporosis, cardiovascular diseases, stroke and Alzheimer's disease; yet HRT is often associated with altered immune parameters (Fahlman et al. 2000; Stopinska-Gluszak et al. 2006). HRT is now going to be reconsidered, since estrogen is closely related with carcinogenesis of mammary cancer.

Growth hormone plays a key role in the development and aging of the thymus and T-cell-dependent immune system (Hirokawa et al. 1998, 2001). Activation of the immune functions occurred in both males and females by administration of GHRH (Khorram et al. 1997; Koo et al. 2001). Since elderly people frequently have occult carcinoma in thyroid, prostate and other organs, we need to consider the possibility that an increased level of growth hormone may stimulate proliferation of tumor cells, giving rise to the clinical manifestation of the occult carcinoma (Perry JK 2006).

6.7   Immunological Enhancement by a Low Dose of Anti-cancer Drug

Cyclophosphamide (CY) is an antitumor drugs commonly used for the chemotherapy of human cancer. It is also known to be a potent immunosuppressive drug in human and experimental animals. It is interesting how CY influences the impaired immunological function in aged mice (Ishiyama et al. 1999).

Aged mice treated with a low dose of CY showed significantly enhanced immune capacity in terms of T-cell proliferation and T-cell-dependent antibody response (Fig. 13). In these mice, the total cell numbers of T-cells increased in both in the thymus and spleen, as compared with those in nontreated mice. Treatment with a low dose of CY induced apoptosis of thymocytes in the atrophic thymus of aged mice and this was followed by an increase in proliferation of thymocytes and an increase in thymocytes and splenic T-cells. Treatment with a high dose of CY also
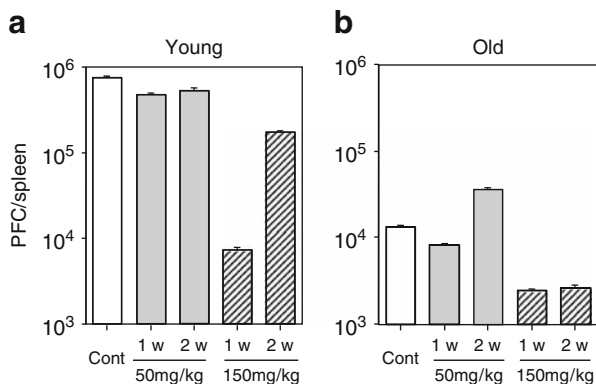
induced apoptosis in the thymus, but suppressed the proliferative capacity, thereby, not leading to an enhancement of immune capacity.

CY in young mice, however, suppressed immune capacity regardless of the dose. Thymocytes and splenic T-cells of young mice were more susceptible to CY than those of aged mice and decreased in number after treatment with even a low dose of CY.

For human application, Berd et al. (1984) reported that a low dose of CY enhanced cell-mediated and humoral immunity in patients with advanced cancer. We examined SIV in patients with colorectal cancer before and after chemotherapy and found that 8 out of 13 patients showed a trend of increased SIV 8 weeks after the start of chemotherapy (Fig. 14).

6.8 Grafting of Cells and Tissues

In animal experiments, the level of immune functions of aged mice can be restored to a level approaching that of young adult mice by grafting both newborn thymus and bone-marrow from young donors (Hirokawa et al. 1976, 1982; Hirokawa and Utsuyama 1989). The results suggest that intrinsic cellular change of the immune system is more responsible for the immune deficiencies in the aged than the environmental or structural tissue changes including connective tissues and humoral factors. In thymus grafting, however, thymic stromal tissues rather than thymocytes are important for the restoration of T-cell-dependent immune system. For human application, the transplantation of bone-marrow cells is becoming easier, but it is almost impossible to find donors of young thymuses. Further, donors need to have the same MHC type as the recipients. Thymus transplantation may be performed, if an autologous thymus can be partially removed at a young age and stored in liquid nitrogen.

Meanwhile, researchers have been accumulating data on molecules of thymic epithelial cells that are essential for T-cell differentiation (Zuniga-Pfucker 2004; Utsuyama et al. 2003). Accordingly, in the near future it may be possible to reconstruct an artificial thymus using easily available cells of individuals.

Fig. 14 Patients with colorectal cancer, (13 cases), treated by chemotherapy. Eleven out of 13 cases showed a trend of increase in SIV-7, 8 weeks after the onset of chemotherapy
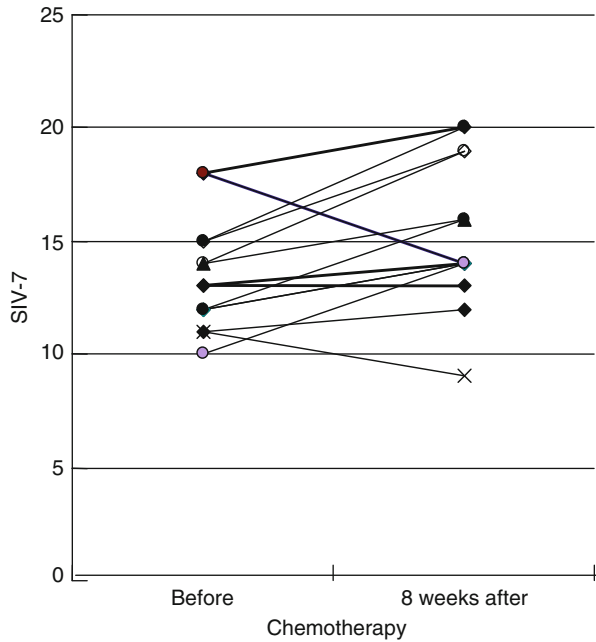
6.9   Infusion of Activated Autologous T-cells

Cell transfer method using young and old mice showed that 10% of the age-related decline can be attributed to cellular environment and 90% to changes intrinsic to the old cells (Price and Makinodan 1972). Many studies including ours (Hirokawa et al. 2006) have revealed that the intrinsic cellular changes are mainly observed in T-cells; i.e., decrease in number, changes in composition of subpopulations and qualitative changes such as proliferative activity and cytokine production.

In fact, T-cells from old individuals do not proliferate efficiently in vitro, but the proliferation can be promoted in the presence of anti-CD3 and IL-2. Thus, activated T-cells in vitro are expected to restore the declined immune functions of aged mice and humans. This treatment was already employed for cancer treatment as an immunotherapy, e.g., lymphokine activated killer cells (LAK) (Rosenberg 2001).

6.9.1   Animal Models

We tested the effect of infusing activated T-cells using young and old mice (Hirokawa et al. 2007). In this study we employed a congenic combination of B10.Thy1.1 mice (young and old) as donors and C57BL/6 Thy1.2 mice (young and old) as recipients, to determine how many activated T-cells survived in the recipients. The mice were sacrificed 11 days and 25 days after the infusion of activated T-cells and used for immunological assessment. For the infusion of activated T-cells, splenic lymphocytes were expanded 10- to 15-folds in the presence

of immobilized anti-CD3 monoclonal antibody and IL-2. Lymphocytes activated in this way were composed of mostly T-cells in which approximately 70 to 80% were CD8⁺ T-cells and 7 to 14% were CD4⁺ T-cells. The activated T-cells prepared from old mice donors contained many more CD8 T-cells. Although CD4+ T-cells were smaller in number than CD8+ T-cells, most of them expressed a phenotype of naïve T-cells. After infusion of activated T-cells, the absolute number of T-cells significantly increased in the spleen of the recipient mice, especially of old mice. In the peripheral blood and spleen, donor-type Thy-1.1 T-cells were significantly more numerous in old recipients than in young ones. In addition, the number of donor-type T-cells that survived was significantly high in the spleen than in the peripheral blood in both young and old recipients.

The magnitude of antibody formation against SRBC did not change significantly in young recipients. About half of the old recipients, however, showed a significant enhancement of antibody formation. It is of importance to note that such an enhanced antibody formation was observed in old recipients infused with activated T-cells either from young or old donors.

6.9.2 Trials in Human Cancer Patients

T-cells from peripheral blood of healthy people can be easily expanded more than 1000-fold in vitro in the presence of immobilized anti-CD3 monoclonal antibody (MoAb) and IL-2. The infusion of activated autologous T-cells has been widely used for cancer patients as a form of immunotherapy (Rosenberg 2001), but without significant impact on cancer treatment in many cases. However, it can be expected that activated T-cells expanded in vitro in a nonspecific manner may improve the immune deficient status of elderly people and cancer patients. T-cells expanded in a nonspecific manner may contain harmful T-cells exhibiting autoimmune activity. In this respect, a recent paper reported that the infusion of activated autologous T-cells did not enhance or promote autoimmune activity (Yamaguchi et al. 2004).

In the next step, we examined cancer patients in the advanced stage; patients with tongue cancer (1 case), esophageal cancer (2 cases), lung cancer (4 cases), gastric cancer, pancreatic cancer (3 cases), colon cancer, appendical cancer and ovarian cancer. All the patients were in the advanced stages of cancer, with multiple metastases, and they underwent an infusion of autologous activated T-cells (so-called LAK cells).

The activated autologous T-cells prepared in the study comprised T-cells (99%), and the CD4/CD8 ratio was approximately 2–3. The proportion of NK-cells was less than 1% in the activated T-cells.

The number of cell per infusion was approximately 5×10^9, and the infusion was repeated 5–6 times for 10 weeks. Various parameters were examined before and after the infusion of activated autologous T-cells.

Figure15 shows the immunological parameters before and after the infusion of activated autologous T-cells in an advanced cancer patient. Most of the immunological parameters that were examined improved after the infusion, except for the ratio of T-cell subpopulations.
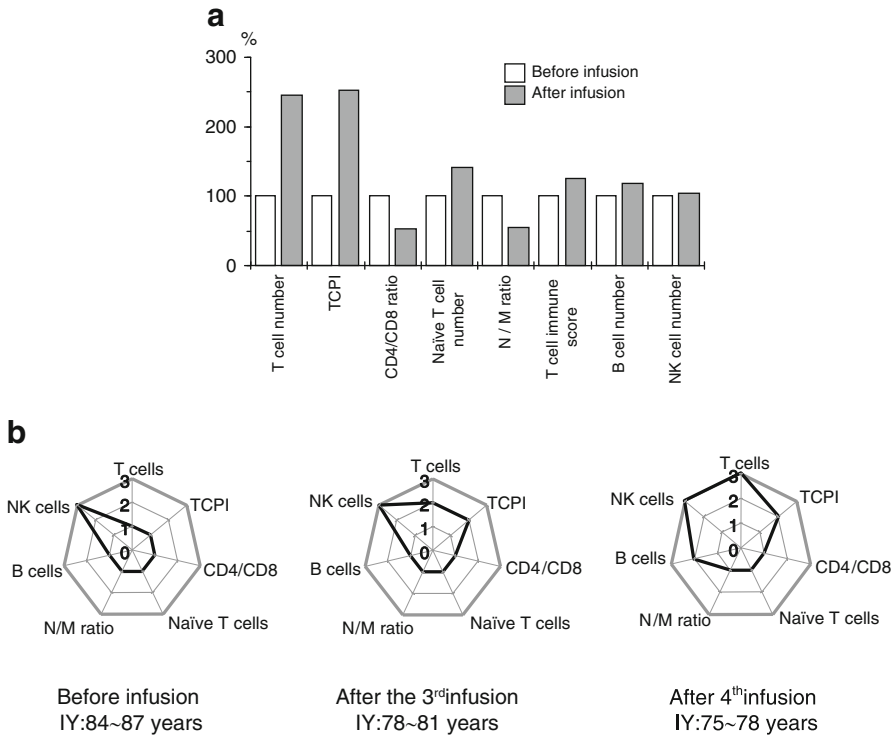
Fig. 15 Two cases of cancer patients, treated with infusion of activated autologous T-cells (a) Improvement of immunological parameters was observed in 60-years-old male with lung cancer. Improvement was observed in most parameters except for the ratios of T-cell subpopulations such as the CD4/CD8 ratio and N/M ratio (b) Infusion of activated autologous T-cells gradually improved radar graph patterns and immunological year (IY) in 78-years-old female with lung cancer

We examined the effect of the infusion of activated autologous T-cells in 14 cases and found that the most pronounced improvement was observed in the T-cell proliferation index (TCPI). The values varied across cases and were unsuitable for statistical analysis. After the infusion of activated autologous T-cells, an improvement in TCPI was observed in 11 out of 14 cases. The average value of TCPI before the infusion was 1.02, which is apparently lower than that observed in colonic cancer stages I to IV (1.21). After the infusion, the average TCPI was increased to 1.49, although it was definitely lower than that of healthy controls (1.70). It was not obvious whether the infusion of activated autologous T-cells was effective in reducing the tumor size, but most of the patients revealed that in general, they experienced an improvement in their health status after the infusion.

Improvement of immunological function can not be expected in cancer patients whose T-cell expansion in vitro is less efficient. Thus, we have to consider the source of T-cells. A good technique is to obtain T-cells not from patients suffering from cancer, but from individuals in healthy condition. For this purpose we need to obtain peripheral blood lymphocytes from healthy peoples and these lymphocytes should be kept in the frozen state. The process can be named as T-cell-bank and the system to establish T-cells bank is now under way.

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