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Decline of T cell-related immune functions in cancer patients and an attempt to restore them through infusion of activated autologous T cells

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ABSTRACT

We developed a scoring system that can combine several immunological parameters and express the immune status of individuals as a simple numeral. T cell immune score was obtained by using 5 T cell-related parameters: number of T cells, ratio of $CD4^+$T cells to $CD8^+$T cells, number of naïve T cells, ratio of naïve T cells to memory T cells, and T cell proliferative index (TCPI). TCPI was calculated by using number of T cells and their proliferative activity. We assessed T cell immune score in 103 patients with colorectal cancer and 51 healthy age-matched controls.

The results were as follows: (1) T cell-immune score of patients in stages I–IV before surgery was significantly decreased as compared with controls. (2) The number of regulatory T cells in patients in stages I–IV gradually increased with disease progression. (3) T cell immune score was strongly suppressed after surgery, but were recovered to the initial level within a month. (4) Furthermore, restoration of immunological function was attempted in cancer patients by infusion of activated autologous T cells. The effectiveness was confirmed by an increase of TCPI in many cancer patients.

1. Introduction

A strong link exists between advanced age and an increased incidence of cancer (Ershler and Longo, 1997; Denduluri and Ershler, 2004). Many possible factors underlie this close link, among which the relationship between immunologic defects and the increased incidence of cancer associated with aging needs to be explored.

The concept of cancer immunosurveillance was first proposed by Burnet (1970). For many years since then, however, no convincing evidence has been reported in support of immunosurveillance system for spontaneous carcinogenesis in humans as well as animal models.

More recently, however, researchers have been able to use many types of genetically manipulated mice, and a large amount of data has suggested the involvement of both the innate and adaptive immune systems in cancer immunosurveillance. Ablations of NKT, γδT cells, NK cells, or αβ T cells, and IFNγ or IL12 all lead to increased susceptibility of the host to tumors (Dunn et al., 2002).

Nonetheless, it must be noted that the immune system can also promote tumor growth (Prehn, 1970). In fact, tumor growth and metastasis can be significantly more pronounced in young mice with high immune capacity than in old mice with depressed immune capacity (Hirayama et al., 1984, 1993). A similar age-related difference was also observed in the metastatic patterns of gastric cancer in human autopsy cases (Esaki et al., 1990).

Ageing of the immune system is well documented in animal models as well as humans. A large amount of data suggests that T cell-dependent immunity is the most susceptible to ageing (Makinodan and Kay, 1980; Hirokawa et al., 2006; Linton and Dorshkind, 2004; Deng et al., 2004). An extension in ageing population in many countries means that the population number of elderly people is continuously increasing. Autopsy examinations have revealed that the direct causes of death of these elderly people are a preponderance of infection as well as vascular disorders of the heart and brain, and cancer (Hirokawa et al., 2006).

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It is important to note that infection is the major cause of death in the elderly people. Moreover, more than 20% of cancer patients die of infection due to immunologic defects.

The severity of such immunologic defects is variable, with inter-individual differences. Thus, it is important to assess the severity of immunologic defects in patients suffering from various diseases, particularly for determining the treatment process.

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.

Here in this paper, we propose a scoring method for immunological parameters. By using this scoring method, several different parameters can be combined as a group and processed statistically. We assessed T cell-related immunological parameters of healthy people and patients with colorectal cancer, and confirmed a statistically significant decline in immune functions of cancer patients. In addition, we employed this method to assess the immunological improvement in cancer patients after infusion of activated autologous T cells.

2. Materials and methods

2.1. Assessment of immune parameters

2.1.1. Peripheral blood mononuclear cells

Two milliliters of blood was drawn into a tube with EDTA for hematological analysis and 8 ml of blood was drawn into a cell preparation tube (BD vacutainer, 362761) for the collection of mononuclear cells.

2.1.2. Flow cytometric analysis

Flow cytometric analyses were performed using a combination of monoclonal antibodies with two or three colors; CD3-RD1/CD20-FITC (T cells and B cells), CD4-FITC/CD8-RD1/CD45RA-ECD ($CD4^+$T cells, $CD8^+$T cells, and naive T cells), CD4-FITC/CD8-RD1/CD45RO-ECD (memory T cells), CD4-FITC/CD8-RD1/CD28-PC5 ($CD8^+$ $CD28^+$T cells), CD56-PE/CD16-FITC (NK cells), CD3-ECD/CD4-FITC/CD25-RD1 (regulatory T cells).

2.1.3. Proliferative response of T cells

The proliferative response of T cells to anti-CD3 mAb was assessed according to the MTS method (Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (Promega)).

2.1.4. T cells proliferation index (TCPI)

TCPI was calculated by using the following equation:

$$\text{TCPI} = \text{T cell proliferative activity} \times \left(\frac{\text{T cell number}}{1000}\right).$$

In this equation, T cell proliferative activity was obtained as optical density (OD) ranging between 0.95 and 2.0 by the MTS method mentioned above. TCPI and age showed a significant correlation: TCPI = $-0.0174 \times (\text{age}) + 2.5348$ (Fig. 1).

2.1.5. Scoring and grading of immunological parameters (Utsuyama et al., 2007; Hirokawa et al., 2007).

Each value of immunological parameters falls within a range specified in a database obtained from approximately 400 healthy people ranging in age from 20 to 100 years. Each parameter is scored into 3 grades based on its value. In particular, values in the range of cumulative frequency less than 10% of values observed for healthy subject are scored 1, which indicates a low immunity level; those between 10 and 40% are scored 2, which indicate a moderate immunity level; and those 40 or higher are scored 3, which indicates a sufficiently high immunity level. Since higher score of $CD4^+$T cells to $CD8^+$T cells ratios (CD4/CD8 ratios) are frequently observed in extremely aged people and patients suffering from diseases, values greater than 80% of the cumulative frequency are scored 2, which indicates a moderate immunity level.

In the present study, we selected five immunological parameters related to T cell functions and scored them. A total sum of five T cell-related parameters is referred to as the T cell immune score. These include the number of T cells per mm^3, ratio of $CD4^+$T cells to $CD8^+$T cells (CD4/CD8 ratio), number of naïve T cells per mm^3, ratio of naïve T cells to memory T cells (N/M ratio), and TCPI. Then, the T cell immune score was classified into five grades; grade V represents the sufficiently high level of immunity (score, 15); grade IV is the safety zone (score, 14–13); grade III is the observation zone (score, 12–10); grade II is the warning zone (score, 9–7) and grade I is the critical zone (score, 6–5).

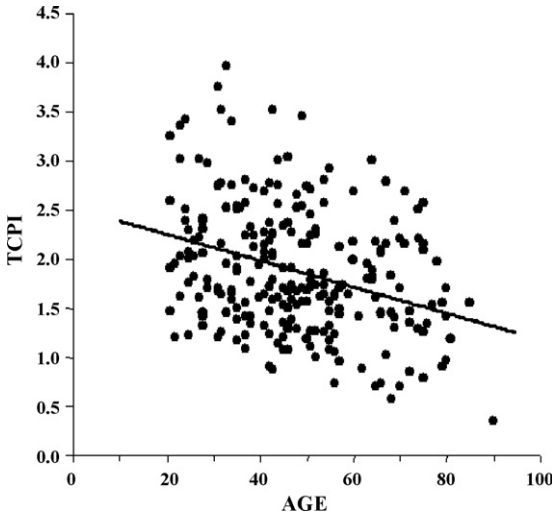

Fig. 1. Distribution of TCPI (T cell proliferation index) in healthy people (approximately 300 males and females) ranging in age between 20 and 90. $y = 0.011x + 2.469$; $R = 0.224$; $P < 0.0001$.

2.2. Cancer patients

We recruited 103 patients with colorectal cancer for the present study. Table 1 shows the progression stage, number of cases (sex ratio), average age ± S.E. and level of total protein in serum (g/dl). The control group included 51 cases of age-matched healthy people. These patients with colon cancer were examined to see the relationship between immunological status and progression stage of cancer. In addition, 14 patients with advanced cancer (Table 2) were examined to see the effect of the infusion of activated autologous T cells on the immune system. These 14 patients had been treated with either operation, chemotherapy, radiation, or combination of them and they hoped some beneficial result by the immunological intervention.

2.3. Preparation and infusion of activated autologous T cells

Activated autologous T cells were prepared by culturing mononuclear cells culture medium RPMI-1640 with immobilized anti-CD3 mAb in the presence of recombinant IL-2 (rIL-2) in a 175-cm^2 flask for 5 days, and then transferred to a 175 or 225-cm^2 flask without anti-CD3mAb in the presence of rIL-2 for 2 days. The cells were then cultured in culture medium AIM-V with rIL-2 for 7 days. Autologous serum was used for all the culturing processes at a concentration of 1–10%, depending on the proliferative conditions.

2.4. Ethical approval

This study was conducted in compliance with Declaration of Helsinki and applicable national laws and regulation, and was approved as no. 320 by the Ethics Committee of Tokyo Medical and Dental University. Written informed consent was obtained from all subjects.

2.5. Statistics

All statistical analyses were performed using StatView software. Statistical significance was defined as $p < 0.05$.

Table 1
Colorectal cancer cases and age-matched controls

Progression stage	Number (M/F ratio)	Age ± S.E.	TP (g/dl)
Stage 0	10 (M = 6, F = 4)	63.4 ± 4.0	7.33 ± 0.13
Stage I	18 (M = 14, F = 4)	62.4 ± 1.0	7.25 ± 0.17
Stage II	27 (M = 17, F = 10)	67.4 ± 2.1	7.14 ± 0.12
Stage III	30 (M = 20, F = 10)	65.1 ± 2.0	7.07 ± 0.11
Stage IV	18 (M = 11, F = 7)	63.4 ± 2.4	7.08 ± 0.14
Control	51 (M = 24, F = 26)	63.2 ± 2.0	NA

TP: total protein in serum; NA: not assessed. Standard level of TP in healthy control: 6.7–8.3 g/dl. Statistically no significant difference was observed in the level of TP among groups.