compared to immunization with p245–270. As a consequence, immunization with bCII induces strong CIA, whereas immunization with p245–270 leads to the regulation of the disease. These data further suggest that the production of the clinical manifestations of autoimmunity depend upon the balance between the pathogenic effector and regulatory populations. Each population undergoes its own development and then interaction will be decisive in determining the clinical outcome, as proposed earlier (36).

Autoimmune rheumatoid arthritis in humans and CIA have several similarities, e.g. susceptibility depends on the genetic background in both diseases. However, common T cell repertoires have not been identified in rheumatoid arthritis patients (37,38) except for a few reports showing a predominant TCR usage of $V_{\alpha}11$, $V_{\alpha}14$, $V_{\alpha}28$ and $V_{\beta}7$, $V_{\beta}9$, $V_{\beta}17$ in the early phase of rheumatoid arthritis or a common usage of the TCR $V_{\beta}14$ in juvenile rheumatoid arthritis (39,40). Some reports have demonstrated successful vaccination with peptides derived from the TCR in rheumatoid arthritis patients (41,42). However, it is difficult to find an effective vaccine region for each patient, because one has to identify the dominant TCR repertoire for each patient. Our vaccination method, which is based upon vaccination with the dominant autoantigenic determinant, is superior in that we do not need to identify the dominant TCR repertoire for each patient. We assume that it is possible to find a functional TCR peptide region corresponding to the immunoregulatory B5 region commonly shared by different strains of mice.

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Abbreviations

ALP	alkaline phosphatase
APC	antigen presenting cell
bCII	bovine type II collagen
CFA	complete Freund's adjuvant
CIA	collagen-induced arthritis
CII	type II collagen
EAE	experimental autoimmune encephalomyelitis
HEL	hen egg lysozyme
IFA	incomplete Freund's adjuvant
NOD	non-obese diabetic

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Senescence-associated decline of lymphocyte migration in gut-associated lymphoid tissues of rat small intestine

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Abstract

Scenescence-induced changes in gut-associated lymphoid tissues may contribute largely to the impaired immune responses during aging. Age-related changes in lymphocyte recirculations were investigated in Peyer's patches of rat small intestine. Cell dynamics of labeled T lymphocytes were observed under an intravital fluorescence microscope and compared between young and aged rats. Lymphocyte transport through intestinal lymph was decreased in aged rats. The lymphocyte rolling and adherence in postcapillary venules (PCV) of Peyer's patches was also significantly impaired in aged rats, with decreased expression of L-selectin on lymphocyte surfaces. Immunohistochemical analysis revealed a significant decrease in the CD8-positive cell population in Peyer's patches of the aged group, although mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expression in postcapillary venules was unaltered. Lymphocyte adoptive-transfer studies indicated that although both the donor and recipient factors influence the adherence of T cells, the former may play a predominant role in the age-related change. This study clearly demonstrated in situ that T cell migration into Peyer's patches is significantly decreased in the aged intestine, which may reflect the impaired immune responses in the aging process.
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Keywords: Peyer's patches; Adhesion molecules; L-Selectin; MAdCAM-1; Lymphocyte subset; Adoptive-transfer

1. Introduction

It is well established that aging is associated with systemic immunodeficiency. Advancing age is associated with B and T lymphocyte dysfunctions and the T cells in the elderly are often characterized by altered phenotypes, reduced responses to mitogens and impaired cytokine production (Ernst et al., 1993; Flurkey et al., 1992; Okumura et al., 1993). It is likely that these age-related T cell deficits contribute to diminished B cell and antibody responses (Zharhary, 1986). The gastrointestinal tract in the elderly is particularly susceptible to infectious and inflammatory diseases, suggesting that mucosal immune defenses are compromised (Schmucker et al., 1996, 2001). For example, the IgA titer in the intestinal lumen declines 15–20% between maturity and senescence in mice (Lim et al., 1981). Furthermore, Schmucker et al. (1988) demonstrated significant declines in the intestinal IgA antibody response to cholera holotoxin in rats. They also suggested that the decline in the number of specific antibody-containing cells in the intestinal mucosa of old immunized rats in comparison to that in young animals suggested that ageing compromises the migration of putative IgA plasma cells to the intestinal mucosa (Schmucker et al., 1988; Van der Heijden et al., 1988). Recently, Koga et al. (2000) demonstrated that age-associated alterations in antigen-induced cytokine responses occur earlier in the mucosal immune system than in the systemic immune compartment. Despite reports of

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age-associated changes in the mucosal immune system, the mechanisms underlying mucosal immunosenescence remain poorly understood.

During immune surveillance, naive lymphocytes preferentially enter secondary lymphoid organs where they sample sequestered antigens before returning to the circulation. Naive lymphocytes usually migrate very efficiently from the blood into secondary lymphoid tissues, such as lymph nodes and Peyer's patches, by extravasating through the endothelium of specialized postcapillary or high endothelial venules (HEV) (Butcher and Picker, 1996; Girard and Springer, 1995). Lymphocytes are thought to enter secondary lymphoid organs through a cascade of steps (Springer, 1994). In mice, the $\alpha4/\beta7$ heterodimer is the principal homing receptor that mediates the tissue-specific binding of lymphocytes to venules in the gut-associated lymphoid system by interacting with the endothelial mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (Berlin et al., 1993; Briskin et al., 1993; Fujimori et al., 2002). Furthermore, studies have shown that there are several adhesion molecules with overlapping functions (Bargatze et al., 1995). Recently Schmucker et al. (2000) reported the effect of ageing on the expression of lymphocyte homing molecules in the rat intestine. However, we are unaware of any data concerning ageing affects on lymphocyte–endothelial interactions in Peyer's patches.

Recently, we developed a method for monitoring the dynamic in vivo process of lymphocyte migration into rat small intestinal Peyer's patches using intravital microscopy and fluorochrome carboxyfluorescein diacetate succinimidyl ester (CFSE) (Miura et al., 1995). In the present report, this procedure was employed to investigate how ageing modifies the initial interaction of T lymphocytes with postcapillary venules (PCV) endothelium and to investigate the possible contributions of changes in the expressions of $\alpha4$-integrin, L-selectin, and endothelial MAdCAM-1 expression to the age-related changes of T lymphocyte–endothelial cell adhesive interactions.

2. Methods

2.1. Collection and labeling of T lymphocytes

Male Fischer 344 rats from the National Institute on Aging colony (Harlan–Sprague–Dawley, Inc., Indianapolis, IN) were used in this study. The animals were segregated into young adult (12 weeks) and older (>77 weeks) age groups and maintained on standard laboratory chow (Oriental Yeast Mfg., Ltd., Tokyo) and water ad lib. All animals were handled according to the guidelines of the Keio University, School of Medicine, Animal Research Committee. The rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg), and the main mesenteric lymphatic duct was cannulated as described by Bollman et al. (1948). After cannulation, the animals were maintained in a Bollman's cage and isotonic saline was infused via the jugular vein (2.4 ml/h) to replace the fluid and electrolytes lost due to lymphatic drainage. Lymph samples were collected in ice-cold vials containing heparin (6 units/ml), 5% fetal bovine serum, and RPMI 1640 medium (pH 7.4; GIBCO, Grand Island, NY). The lymphocytes were washed three times with RPMI 1640, containing 1% penicillin, streptomycin, and 0.1% bovine serum albumin before separation and labeling.

A T cell-rich fraction of lymphocytes from mesenteric lymph was obtained using a nylon-wool column. Lymphocytes (1×10^8) in 20 ml of RPMI medium with 1% fetal bovine serum were incubated at 37 °C for 1 h and subsequently passed over a nylon-wool column (Kanto Kagaku, Japan) and the pass-through fraction was designated the T cell-rich fraction. Lymphocyte viability was unaffected as assessed by trypan blue exclusion.

In some experiments, T lymphocytes were separated into CD4-positive and CD8-positive subpopulations using magnetic cell sorting (MACS). T lymphocytes (1×10^7 cells) were suspended in 90 μl of phosphate buffered saline containing 0.5% bovine serum albumin and 5 mM ethylenediaminetetraacetic acid (EDTA), and incubated with 10 μl of anti-rat CD4-labeled or anti-rat CD8-labeled MACS microbeads (Miltenyi Biotech, Bergish Gladbach, Germany) for 15 min at 6 °C. The treated T cells were passed through a separation column (type LS, Miltenyi Biotech), which was placed in the magnetic field of a MACS separator (MidiMACS, Miltenyi Biotech). The magnetically labeled CD4- or CD8-positive cells were retained in the column, whereas the unlabeled CD4- or CD8-negative cells passed through. The CD4- or CD8-negative cell populations were used for migration studies. The purity of each lymphocyte subpopulation was confirmed by flowcytometry, and the cells were resuspended in RPMI containing 5% fetal calf serum on ice until used. Immediately prior to transfer, suspended cells (1×10^8 in 20 ml RPMI) were incubated in carboxyfluorescein diacetate succinimidyl ester (CFDASE, Molecular Probes, Eugene, OR) for 30 min at 37 °C.

2.2. Intravital observation of lymphocyte migration in rat Peyer's patches

The abdomen was opened via a midline incision and 12 cm segment of the ileum near the ileocecal valve was placed gently on a plastic plate for observation. The intestine was kept warm and moist by continuous superfusion with physiologic saline at 37 °C. Two small proximal and distal incisions were made and warm Krebs–Ringer solution (pH 7.4) was instilled via a tube in the proximal end in order to maintain luminal pressure at 15 cm H_2O. Areas of the microcirculation in Peyer's patches were observed from the serosal side using an intravital microscope (Diaphot, TMD-2S (Nikon, Tokyo)) equipped with a TV-video tape recording system. The fate of the fluorescence-labeled lymphocytes was observed on a TV-monitor via a fluorescence microscope equipped with a silicon intensified target image

tube camera (SIT) with contrast enhancement (C-2400-08, Hamamatsu Photonics Co., Shizuoka, Japan) following excitation at 470–490 nm and emission at 520 nm (Miura et al., 1995).

Lymphocytes (3×10^7 cells in 1 ml RPMI) were injected into the jugular vein of recipient rats over 3 min. The migration of CFSE-labeled T lymphocytes through the Peyer's patch microvasculature was recorded on S-VHS video tapes during the first 20 min and at 10 min intervals for the subsequent 60 min. Lymphocyte behavior in the PCV's can be classified into two different types according to the pattern of their interaction with the vascular walls as assessed by frame analysis. Lymphocytes that adhered to the wall but exhibited movement along the vessel wall were classified as "rolling" lymphocytes. The percentage of rolling lymphocytes per total number of entered cells was calculated. Lymphocytes that adhered to the vessel wall without movement following transient rolling for more than 30 s were classified as "sticking" lymphocytes. The number of sticking lymphocytes was determined in 1 mm^2 fields containing PCVs 25–50 μm in diameter.

2.3. Immunohistochemical study

The small intestine was removed, fixed in periodate–lysine–paraformaldehyde (PLP) and used for immunohistochemical study using labeled streptavidin biotin method. Segments of ileal mucosa containing Peyer's patches were vertically embedded in OCT (Sakura Fineteck Inc., Tokyo, Japan) and 7 μm cryostat sections were used for immunohistochemistry. The primary antibodies were directed against MAdCAM-1 (OST-2) (Hokari et al., 2001), rat macrophage (ED-1), CD4 and CD8. They were visualized by streptavidin-FITC and examined by fluorescence microscopy. Vessels positive for MAdCAM-1 in the lamina propria and submucosa were calculated using an image analyzer and expressed as the positively stained area per unit area. The infiltrated cells were expressed as the number of CD4-, CD8- or ED-1-positive cells per mm muscularis mucosa.

2.4. Fluorescence-activated cell sorter analysis

T lymphocytes (1×10^6) were washed in Hank's balanced salt solution containing 0.2% bovine serum albumin and 0.1% NaN_3. This medium was used throughout the staining procedure and all incubations with antibodies were performed at 4 °C for 30 min. Antibodies against rat L-selectin (HRL3, IgG1) (Seikagaku Co., Tokyo), α4-integrin (MRα4-1) (PharMingen, San Diego, CA) and CD18 (WT.3) (PharMingen) were used. Lymphocytes (2×10^5) were incubated in antibodies directed against α4-integrin or CD18, washed in 400 μl Hank's balanced salt solution, centrifuged at $1500 \times g$ for 30 s (3×), incubated with 1 ml FITC-labeled anti-mice IgG, washed twice, and resuspended for analysis. L-selectin was visualized by using a primary antibody (HRL3) and a secondary antibody, FITC-conjugated anti-hamster IgG (Cappel, West Chester, PA). Flow cytometry was performed using FACSort (Becton Dickinson, Mountain View, CA), and dead cells identified their failure to exclude propidium iodide dye, were excluded from the data pool.

2.5. Statistics

All results were expressed as the mean ± S.E.M. The differences among groups were evaluated by one-way ANOVA and Fisher's post hoc test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Age-related changes in lymphoid cell subsets and endothelial adhesion molecules in Peyer's patches

The total number of Peyer's patches in the small intestine was 15.6 ± 2.4 in young adult rats and 11.8 ± 2.5 in aged animals, but the decline was not statistically significant. However, there was a significant increase in the total area of Peyer's patches in the aged group (11.2 ± 2.1 mm^2) in comparison to that in young adults (5.35 ± 2.9 mm^2).

Fig. 1 shows mesenteric lymph flow in cannulated rats and lymphocyte flux (number of collected lymphocytes per hour) in the mesenteric lymphatics. Although there was no difference in lymph flow between the young adult and the aged groups, a significantly lower lymphocyte flux was observed in the mesenteric lymph in the aged rats in comparison to the young adults.

We compared the distribution of lymphocyte subsets and ED-1-positive cells in the Peyer's patches by immunohistochemistry. Fig. 2 shows a representative picture of CD4- and CD8-positive lymphocytes in Peyer's patches of young adult and aged animals. There was a marked decrease in the

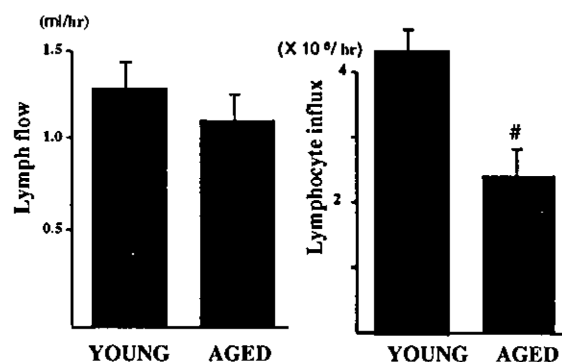

Fig. 1. Mesenteric lymph flow and lymphocyte flux in mesenteric lymphatics of young adult and aged rats. (#) $P < 0.05$ vs. young adults; data expressed as the mean ± S.E.M. for six animals.

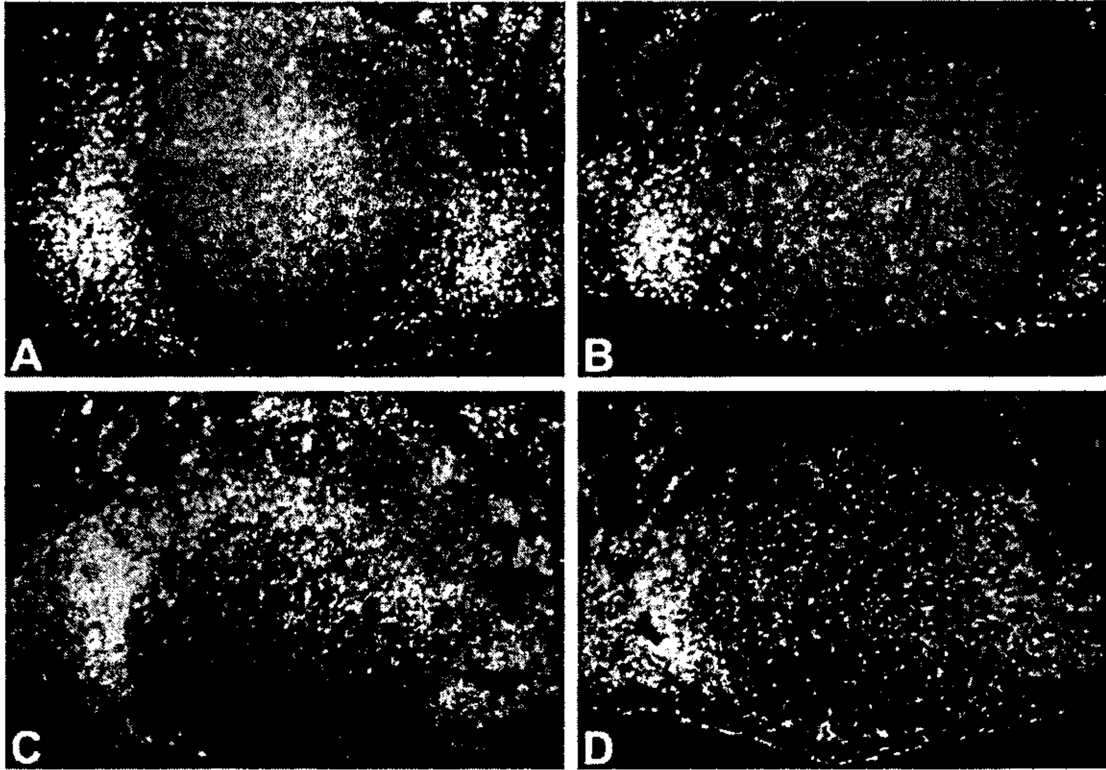

Fig. 2. Immunohistochemical staining of CD8- and CD4-positive cells in the Peyer's patches of young adult and aged rats. (A) CD8-positive cells in young adults (100×). (B) CD8-positive cells in aged rats, showing a marked decline, especially in the interfollicular zones of the Peyer's patches in the aged group. (C) CD4-positive cells in young adults (100×). (D) CD4-positive cells in aged rats (100×). There was also a decline in the number of CD4 cells in the Peyer's patches of aged rats, although these declines were not statistically significant vs. values in the CD8 cells.

number of CD8 cells, especially in the interfollicular zones of Peyer's patches in the aged rats. There were also fewer CD4 cells in the Peyer's patches of the aged rats, but the decline was not statistically significant in comparison to the decline in the CD8 cells. There was also a slight decline in the number of B cells in the aged rats, but there was no difference between the number of ED-1 cells in the young adult group in comparison to that in the aged group (data not shown).

We examined MAdCAM-1 expression in the venular endothelium of Peyer's patches using immunohistochemistry. Fig. 3A shows the immunohistochemical staining of MAdCAM-1 in young adult and aged rat small intestines. Fig. 3B includes the quantitative data. There was significant constitutive expression of MAdCAM-1, primarily in the postcapillary venules of Peyer's patches in both young adult and aged rats. Furthermore, there was no quantitative difference in MAdCAM-1 expression between these two groups.

3.2. Age-related changes in T lymphocyte migration into Peyer's patches

Some fluorescence-labeled and transferred lymphocytes exhibited a characteristic rolling behavior on the surface of PCV endothelium in Peyer's patches. However, more than 70% of the labeled cells passed through the Peyer's patches without rolling or adhesion. The total number of lymphocytes entering the PCVs within 10 min of transfer into the jugular vein did not differ significantly between young and aged animals, 1.3 ml/h versus 1.05 ml/h, respectively. However, at 10 min after transfer, the percentage of rolling cells from young donors in young recipients (28.1 ± 4.8) was significantly greater than that of rolling cells from aged donors in aged recipients (12.1 ± 4.3, $P < 0.05$), suggesting diminished lymphocyte–endothelial interaction in the old animals.

Some lymphocytes adhered to PCV endothelium (Fig. 4A). On the one hand T cells from young donors adhered selectively to the PCVs of Peyer's patches in young recipients. On the other hand, in old recipients fewer sticking lymphocytes from old donors were observed at 20 min postinfusion. The number of sticking lymphocytes gradually increased during the 40 min post transfer observation period. Fig. 4B compares the time-course of changes in the number of cells sticking to PCVs of Peyer's patches. The data for lymphocytes inside the microvascular walls and the data for lymphocytes located along the microvascular walls are included. The number of young donor adherent cells transferred into young rats increased, especially during the

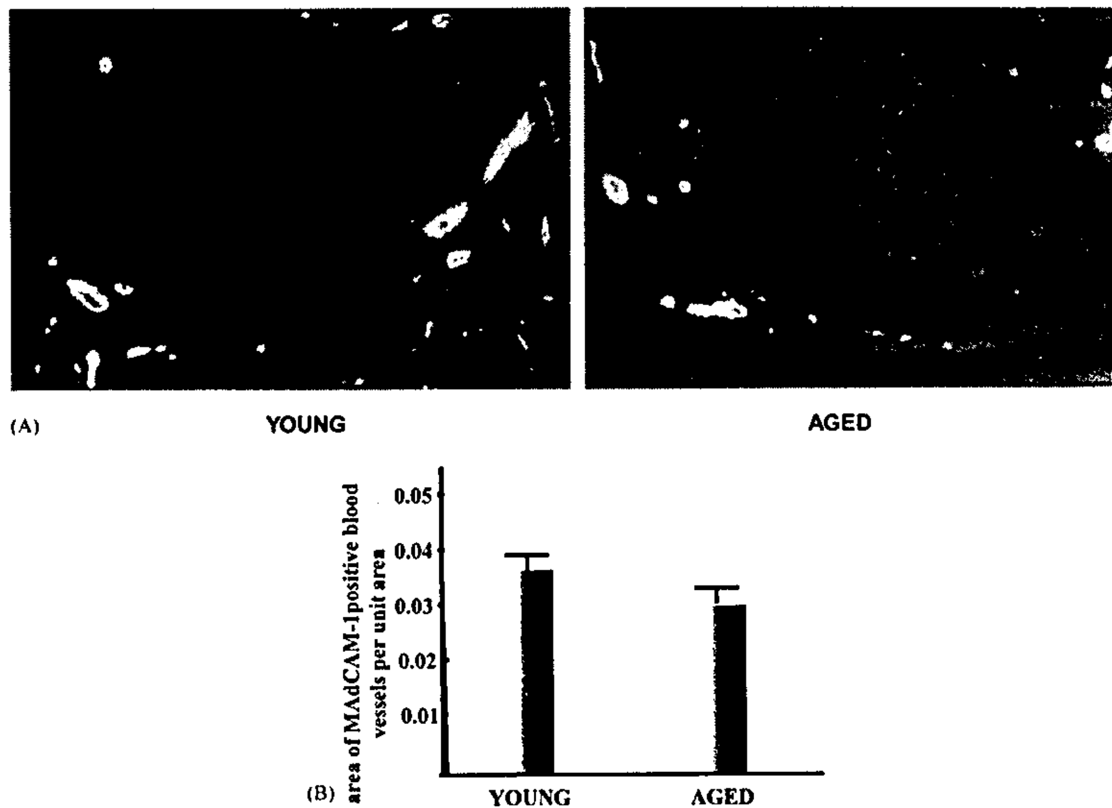

Fig. 3. (A) Representative images of MAdCAM-1 expression in Peyer's patches. The primary antibody used in the immunostaining was a monoclonal antibody directed against MAdCAM-1 (MECA367) (left panel, young adults; right panel, aged rats). MAdCAM-1 is expressed in the endothelial cells of postcapillary venules in Peyer's patches. (B) MAdCAM-1-positive vessels in the lamina propria and in the submucosa were determined as areas of positively stained vessels per unit area. Results are expressed as the mean ± S.E.M. of six animals; there were no significant differences between the young adult and the aged groups.

initial 10 min after transfer, whereas only a few of the T cells from aged donors transferred into aged rats adhered to PCVs during the same post transfer-interval. Nevertheless, the number of these cells gradually increased during the observation period.

We also determined whether or not there was an age-related shift in the migration of CD4 and CD8 T lymphocytes to Peyer's patches. Fig. 5 illustrates the relative numbers of cells sticking to the PCVs of Peyer's patches at 20 and 40 min after the transfer of 3×10^7 CD4- or CD8-negative lymphocytes (a) from young donors into young recipients or (b) from aged donors into aged recipients. In young animals, the numbers of either CD4- or CD8-negative adherent cells were always greater than that found in aged animals. Furthermore, CD8-negative cells always adhered more efficiently to the PCVs than did CD4-negative cells in either young and aged recipients. However, the inhibition of cell adhesion at 40 min post transfer was not statistically significant (for the CD4-negative or the CD8-negative populations, i.e. 86 and 89%, respectively). These data suggest that there is no apparent age-related shift in the adhesion of specific T lymphocyte subpopulations.

3.3. Migration of T lymphocytes after cross transfer

Fluorescent-labeled T lymphocytes from young or old donors were transferred into young and old recipient animals and lymphocyte kinetics were monitored. T cells from young donors transferred into young syngeneic rats exhibited the most efficient adherence to Peyer's patch PCVs (Fig. 6). The adherence of young donor cells to PCVs in old recipients was significantly lower, but was still greater than that found with cells from old donors transferred into old recipients. T cells from old donors did not adhere efficiently to PCVs in Peyer's patches of young syngeneic recipients, suggesting that the age-related decline is mainly due to changes in the donor lymphocytes. Nevertheless, changes in recipients are also involved because cells from old donors adhered more efficiently to PCV endothelium in young recipients in comparison to that in old rats.

3.4. Analysis of surface adhesion molecules on T lymphocytes

Expression of adhesion molecules (α4-integrin, β2-integrin and L-selectin) on the surface of T lymphocytes was

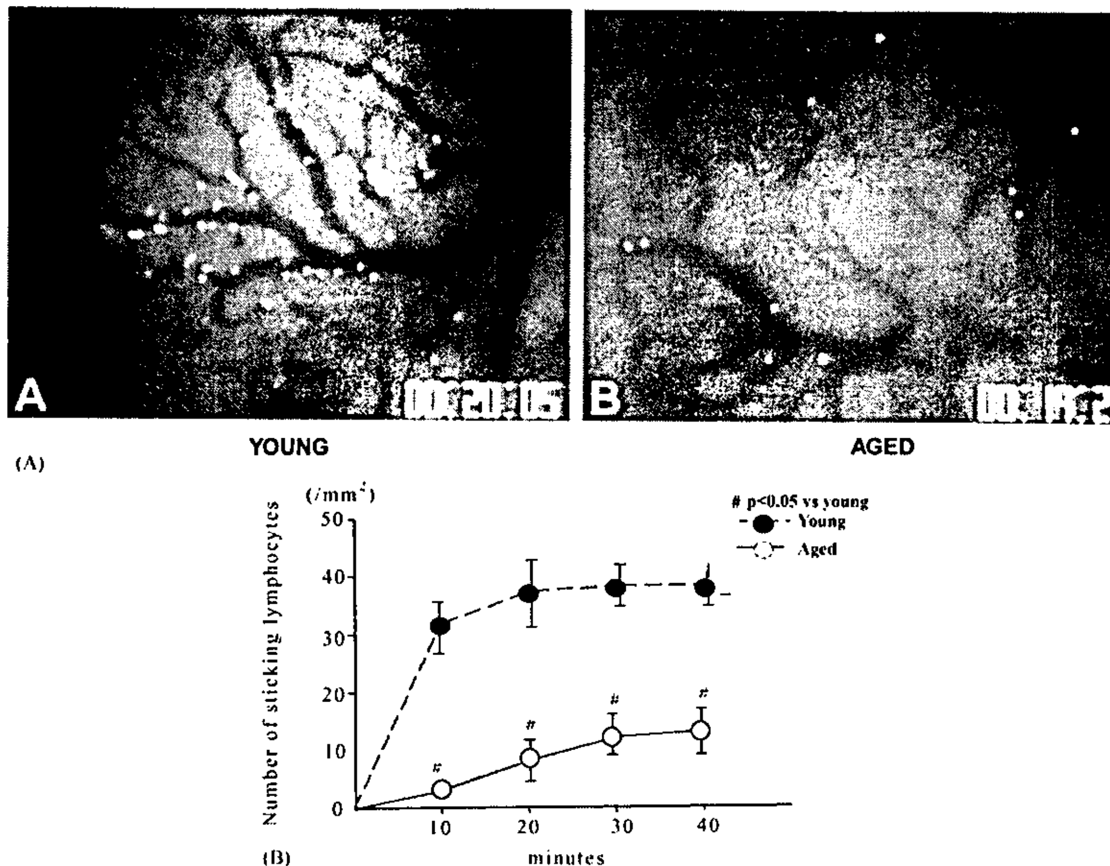

Fig. 4. (A) Representative photomicrographs showing the distribution of CFSE-labeled T lymphocytes in the postcapillary venules of Peyer's patches 20 min after infusion. (Left) Lymphocytes (3×10^7) from young adults were transferred into young animals. (Right) lymphocytes from aged rats were transferred into aged animals. (B) Time-course of changes in the number of T lymphocytes sticking to postcapillary venules in rat Peyer's patches and the difference between young adult and aged rats. Lymphocytes located inside or along venules were counted in the 1 mm^2 observation field. (#) $P < 0.05$ vs. young adult; data expressed as the mean ± S.E.M. for six separate animals.

determined using monoclonal antibodies. The populations of CD4- and CD8-positive lymphocytes in the intestinal lymph from both age groups were similar, i.e. ~55% CD4 and ~31.5% CD8 cells (data not shown). The cell surface expression of L-selectin was greater in young T cells in comparison to aged T cells, whereas the expressions of α4-integrin and CD18 did not differ significantly between young and aged T cells (Fig. 7).

4. Discussion

We demonstrated an age-associated decline in the migration of T lymphocytes to the PCVs in Peyer's patches. We also showed a significant decline in lymphocyte flux in the intestinal lymph of aged-rats, suggesting that the kinetics of T lymphocyte movement within Peyer's patches is inhibited with increasing age. Lymphocyte recirculation does not occur randomly, but is regulated by mechanisms inherent in lymphocyte–endothelial cell recognition (Butcher and Picker, 1996). Naive lymphocytes can migrate very efficiently from the blood into secondary lymphoid tissues (e.g. lymph nodes and Peyer's patches) by extravasating through the endothelium of specialized postcapillary or high endothelial venules (Girard and Springer, 1995). In contrast, memory and effector lymphocytes primarily access the extralymphoid immune effector sites (Butcher and Picker, 1996; Hokari et al., 1999; Mackey, 1993). Previously we reported that both CD4 and CD8 naive lymphocytes from the intestinal lymph recirculate through the intestine under physiological conditions, entering Peyer's patch PCVs and subsequently flowing out to the interfollicular zones (Hokari et al., 2001; Miura et al., 1995). This process enables naive cells to increase the frequency with which they encounter their cognate antigens. We suggest that there is an age-related impairment in the recirculation of naive T lymphocytes within Peyer's patches and that this may be related to the compromised gastrointestinal mucosal immune defense in the elderly.

We observed significant age-related impairments in both CD4 and CD8 lymphocyte migrations. The distribution of the CD8 population appears to be more readily suppressed

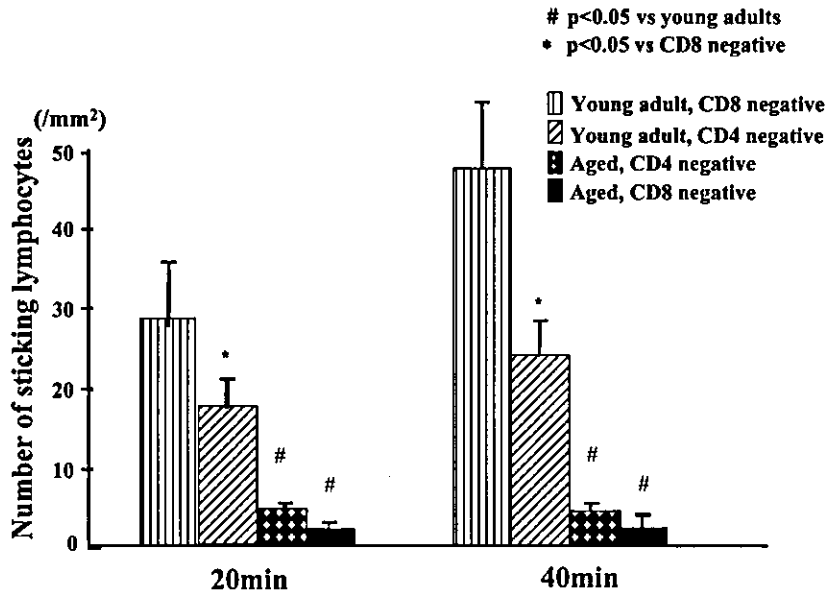

Fig. 5. The number of sticking lymphocytes to the postcapillary venules of Peyer's patches at 20 and 40 min post transfer of CD4- or CD8-negative cells into young and aged animals. Lymphocytes (3×10^7) from young adults were injected into different young animals and lymphocytes from aged rats were injected into the aged animals. (*) $P < 0.05$ vs. CD8-negative; (#) $P < 0.05$ vs. young adults; data expressed as the mean ± S.E.M. for six animals.

by ageing in comparison to the CD4 subpopulation. This finding is in agreement with the report by Kawanishi and Kiely (1987, 1989), who observed a decline in the T suppressor/cytotoxic cell subpopulation in the Peyer's patches of old mice. Several research groups have used flow cytometry to demonstrate the absence of age-related changes in the CD4 or CD8 subsets in Peyer's patches (Daniels et al., 1993; Fló and Massouh, 1997). The latter group also reported immunohistochemical evidence suggesting that ageing is accompanied by a shift in the distribution of CD8 cells from the interfollicular zone throughout the Peyer's patches (Daniels et al., 1993). The suppressor/cytotoxic T cell population may be more susceptible to age-related shifts in comparison to helper/inducer T cells in GALT. However, our in vivo data did not demonstrate a preferential decline in the CD8 lymphocyte–endothelial cell interaction in comparison

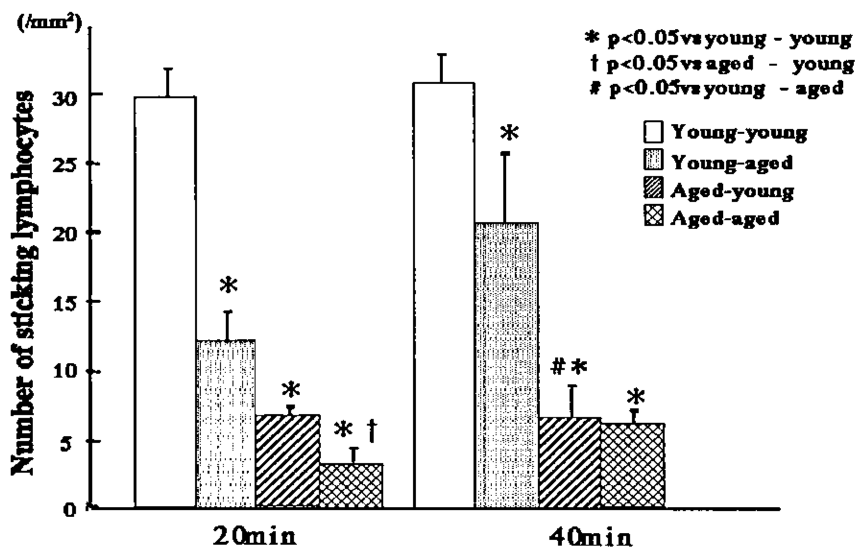

Fig. 6. The number of lymphocytes sticking to the postcapillary venules in Peyer's patches at 20 and 40 min after adoptive-transfer. The labeled T lymphocytes from young donors were transferred into old recipients, whereas the cells from old donors were transferred into young recipients. The kinetics of labeled T lymphocytes was compared among four different combinations, namely young–young, young–aged, aged–young, and aged–aged. (*) $P < 0.05$ vs. young–young; (#) $P < 0.05$ vs. young–aged; (†) $P < 0.05$ vs. aged–young; data expressed as the mean ± S.E.M. for four animals.

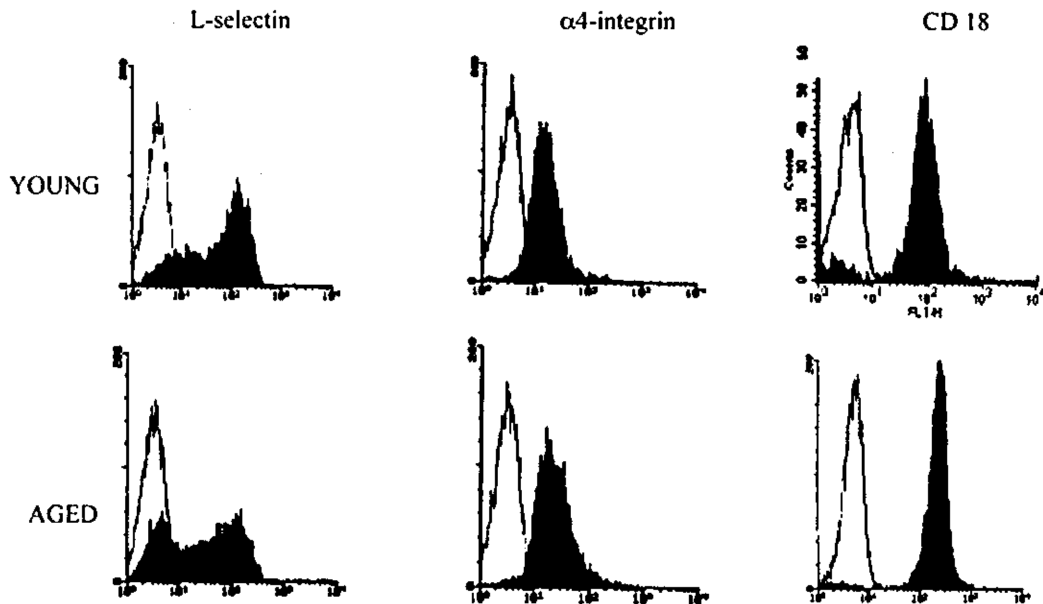

Fig. 7. The expression of adhesion molecules (L-selectin, α4-integrin, CD18) on T lymphocytes determined by flow cytometric analysis. Lymphocytes (2×10^5) were incubated with anti-rat monoclonal antibodies against L-selectin (HRL3, IgG1), α4-integrin (MRα4-1), and CD18 (WT.1). They were then incubated with 1 ml FITC-labeled anti-mouse IgG or anti-hamster IgG. Flow cytometric analysis was performed using FACSort (Becton Dickinson). Representative data from at least four individual measurements are shown.

to CD4 subsets. The subsequent migration processes into the follicular zones of Peyer's patches should be examined in the future investigations.

The impaired migration may reflect the altered expressions of homing receptors on lymphocytes or of their respective ligands on the vascular endothelial cells. We determined the expression of adhesion molecules on lymphocytes by flow cytometry and MAdCAM-1 expression on vascular endothelial cells by immunohistochemistry. We also performed adoptive-transfer studies using T lymphocytes isolated from young and old donors and young and old recipients. In mice, the α4/β7 heterodimer is the principal homing receptor that mediates lymphocyte binding to venules in the gut-associated lymphoid system via interaction with the endothelial addressin, MAdCAM-1 (Berlin et al., 1993; Briskin et al., 1993; Fujimori et al., 2002). Rather than one adhesion molecule with an exclusive function, studies have described several adhesion molecules with overlapping functions. For example, L-selectin and CD11a/CD18 also play a role in the interaction of lymphocytes and high endothelial venules in the gut wall (Arbones et al., 1994; Bargatze et al., 1995; Steeber et al., 1996). We provide evidence that ageing does not diminish the expression of either the lymphocyte integrin α4 or the vascular endothelial addressin, MAdCAM-1. Our data also suggest that ageing impairs the expression of L-selectin on lymphocytes. This is consistent with data reported by Steeber et al. (1996) suggesting that, (a) the expression of L-selectin is impaired by ageing and (b) this interferes with the migration of lymphocytes to secondary lymphoid tissues. Our results also demonstrated that T cells from old donors do not adhere to the endothelium of PCVs in Peyer's patches in young syngeneic recipients to the extent they did to the endothelium in old recipients. Furthermore, the number of adherent cells was significantly greater when T cells from young donors were used. These results strongly suggest the possibility that the age-related decline in T lymphocyte adhesion in Peyer's patches is due to changes in lymphocyte adhesion molecule expression, including decreased expression of L-selectin. Recently, Schmucker et al. (2000) reported that the proportion of α4β7-positive mononuclear cells in young rats was significantly higher than that measured in the blood of senescent animals. Therefore, a decline in the α4β7–MAdCAM-1 interaction in aged animals cannot be excluded, because (a) we did not test antibodies against α4β7 heterodimer epitope, and (b) L-selectin is known to facilitate the subsequent α4β7-MAdCAM-1 interaction in Peyer's patches (von Andrian and Mackay, 2000). Furthermore, age-related changes in recipient rats are not excluded because the adherence of T cells from young donors to PCV endothelium in old recipients is significantly lower in comparison to that in young recipients.

Thoreux et al. (2000) recently hypothesized that an age-related delay in the migration of IgA immunoblasts to the effector site, i.e. the intestinal mucosa, contributes to mucosal immunosenescence. Using lymphocyte adoptive-transfer, they demonstrated that factors intrinsic to both the donor cells and the recipients influence the migration of immunoblasts from the mesenteric lymph nodes to the effector site. Since we focused our studies on the altered migration of naive T cells in nonimmunized animals,

age-related changes in antigen-specific T cell migration remain to be elucidated. While the exact mechanism is unclear, our study is the first to report direct evidence of an age-related decline in the migration of naive T cells to the Peyer's patches in situ.

Acknowledgements

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ESTABLISHMENT AND ANALYSIS OF GERMFREE T CELL RECEPTOR TRANSGENIC MICE

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Abstract

Recent studies have shown that intestinal bacteria affect the intestinal immune system. In order to elucidate the effects of intestinal microflora on T-cell mediated immune responses of the intestine, we established germfree (GF) T cell receptor transgenic (TCR-Tg) mice. GF ovalbumin (OVA)-specific TCR-Tg mice were obtained from conventional (CV) TCR-Tg mice by hysterectomy. Cells from spleen, Peyer's patch (PP) and lamina propria (LP) were isolated from GF TCR-Tg mice, and the ratio of $CD4^{+}$ T cells was assessed by flow cytometry and the production of cytokines, in response to in vitro OVA stimulation, was measured by ELISA. The numbers of PPs were significantly lower in GF TCR-Tg mice compared with CV TCR-Tg mice and the ratios of $CD4^{+}$ T cells in PP and LP cells from GF TCR-Tg mice were decreased. When stimulated with OVA, PP cells from GF TCR-Tg mice secreted higher levels of interferon (IFN)-γ, interleukin (IL)-5 and IL-6, and LP cells from GF mice secreted higher levels of IFN-γ and IL-6 compared with CV mice. These results suggested that although the gut-associated lymphoid tissue is poorly developed in germfree mice, intestinal T cells developing under these conditions possessed an enhanced ability to secrete cytokines in response to antigenic stimulation. Our TCR-Tg system should be an informative system to evaluate the effect of intestinal microflora on antigen-specific T cell responses.

1. Introduction

Recent advances in intestinal flora research has demonstrated that intestinal bacteria affect the intestinal immune system. Of particular note are comparative studies between conventional and germfree animals that have shown that indigenous bacteria play crucial roles in the development of the gut-associated lymphoid tissue (GALT), the exclusion of microbial pathogens and the induction of oral tolerance. In germfree mice, B cell follicles of Peyer's patches (PPs) lack germinal centers

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and gut lumen lamina propria (LP) contain few IgA plasma cells (1, 2). The oral dose of *Listeria monocytogenes* required to kill germfree mice was $1\text{-}5\times10^2$ (3), which is far lower than that of conventional mice, and, in addition, oral tolerance was not induced in germfree mice (4). However, details of the effects upon intestinal immune responses that are caused by intestinal bacteria are not fully understood. Thus, we established germfree T cell receptor transgenic (GF TCR-Tg) mice in order to investigate the effect of intestinal microflora on T-cell mediated intestinal immune responses.

2. Materials and Methods

2.1 Mice

Conventional OVA23-3 TCR-Tg mice (CV TCR-Tg mice), transgenic for OVA 323-339 specific and I-A^d restricted TCR-$\alpha\beta$, on a BALB/c genetic background were originated from Professor Sonoko Habu of Tokai University. GF TCR-Tg mice were obtained from conventional OVA 23-3 mice by hysterectomy carried out under germfree condition and fosternursed by lactating germfree BALB/c mice in an isolator.

2.2 Preparation of spleen (SP), PP and LP cells

To obtain LP cells, murine small intestines were excised and the PPs were removed carefully. The intestine was reversed by using polyethylene tubing, and wiped carefully a few times with a paper towel. The intestine was cut into 4 pieces and placed in a 50 ml tube containing HBSS (–) (Hanks' balanced salt solution, Ca, Mg free, containing 5% FCS). This was incubated at 37°C with shaking for 30 min at 150 rpm (×3), the supernatant discarded by filtration with gauze, minced into 10 mm pieces and treated with collagenase (45 mg/45 ml HBSS (–) containing 5 U/ml DNase 1) in a 100 ml-flask with gentle stirring at 37°C for 20-30 min. After stirring, the preparation was filtrated with gauze, washed, suspended in HBSS (+) (containing Ca and Mg, and 5% FCS), filtrated by glass wool column and then washed by centrifugation at 4°C, 1,300 rpm for 5 min. The upper supernatant was removed and 3 ml of 100% Percoll added to the cells and filled to 10 ml with HBSS (30% Percoll), mixed, then centrifuged at 20°C, 1,800 rpm for 20 min and upper supernatant removed. Subsequently, 4.1 ml of 100% Percoll was added to 1 ml of the cell suspension, and filled to 10 ml with RPMI containing FCS (44% Percoll), then mixed. Two ml of 70% Percoll was injected into the bottom of the tube and was centrifuged at 20°C, 1,800 rpm for 20 min. The cells located at the interface of the 70% and 44% Percoll fractions were collected and washed with RPMI. Single cell suspensions of SP and PP cells were obtained by crushing with the organ with the end of a syringe.

2.3 Assessment of CD4$^+$ cells by flow cytometry

Approximately 1×10^6 cells were stained for 20 minutes with FITC-labeled anti-CD4 antibody and acquisition of data was performed on a LSR flow cytometer (BD Biosciences, San Jose, CA USA).

2.4 Cell culture for cytokine production

LP cells (5×10^5/ml), SP or PP cells (2.5×10^6/ml) were cultured in 200 μl of medium in 96-well culture plates containing 0.5 mg/ml of OVA. Supernatants were collected after 48 hours. Mitomycin C-treated BALB/c spleen cells (2×10^6/ml) were added as antigen presenting cells in LP cell cultures.

2.5 ELISA for cytokines

Cytokines in culture supernatants were measured by sandwich ELISA. Rat anti-mouse interleukin (IL)-4, IL-5, IL-6 and interferon (IFN)-γ monoclonal antibodies were used as the capture antibody, with biotinylated rat anti-mouse IL-4, IL-5, IL-6 and IFN-γ monoclonal antibodies, respectively, as the detection antibodies.

3. Results and Discussion

3.1 The number of PPs in GF TCR-Tg mice

GF TCR-Tg mice were obtained from conventional TCR-Tg mice by hysterectomy carried out under germ free conditions. The numbers of PPs from these GF TCR-Tg mice and CV TCR-Tg mice were counted and found to be significantly decreased in GF TCR-Tg mice compared with CV TCR-Tg mice (Fig. 1)

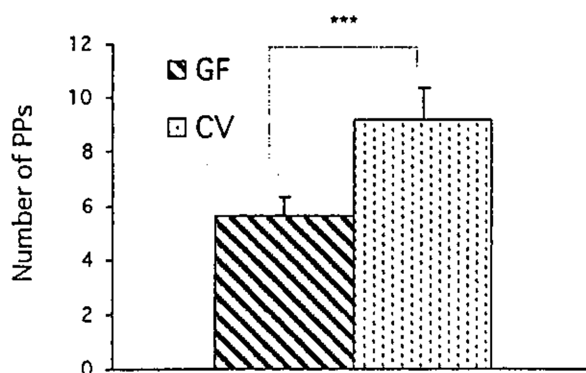

Fig. 1 The number of PPs in GF or CV TCR-Tg mice. The results are shown as mean ± SD of at least 4 experiments. ***, $p<0.001$.

3.2 Percentages of CD4$^+$ T cells in the lymphocyte fraction of SP, PP and LP of GF TCR-Tg mice

The percentages of CD4$^+$ T cells against the whole lymphocyte fraction of SP, PP and LP cells are shown in Fig. 2. The ratios of CD4$^+$ T cells were decreased in PP and LP of GF TCR-Tg mice compared with CV TCR-Tg mice. On the other hand, the ratios of CD4$^+$ T cells in SP were similar in GF and CV mice. The above results demonstrate that the GALT is poorly developed in GF TCR-Tg mice, as reported previously in other studies using germfree animals (1).

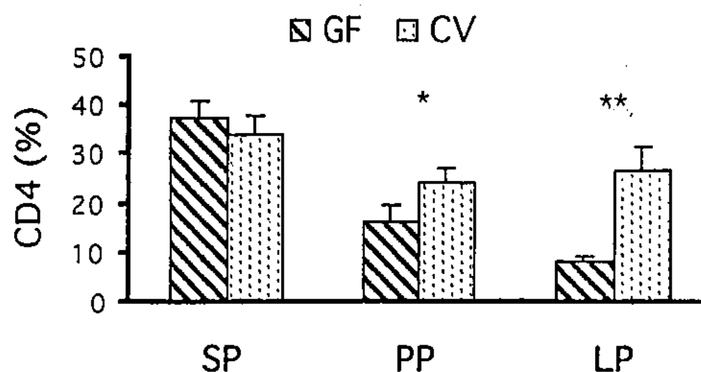

Fig. 2 Percentages of CD4$^+$ T cells in lymphocyte fraction of SP, PP and LP of GF TCR-Tg mice. The results were assessed by flow cytometry and shown as mean ± SD of 3 experiments. *, $p<0.05$; **, $p<0.005$.

3.3 Cytokine production of PP and LP cells of GF TCR-Tg mice

PP and LP cells from CV and GF mice were cultured with OVA and cytokine secretion was examined by ELISA. The results are summarized in Table 1. IL-6 and IFN-γ producing ability of PP and LP cells, and IL-5 of PP cells from GF mice, tended to be higher than CV mice. In particular, LP cells from GF mice secreted a large quantity of IL-6. These results showed that although the GALT is poorly developted in germ free conditions, intestinal T cells developing under these conditions possessed an enhanced ability to secrete cytokines in response to antigenic stimulation compared with those under conventional conditions. Therefore, continuous microbial stimulation may be involved in reducing the reactivity of intestinal T cells.

3.4 Conclusion

TCR-Tg mice are informative models for examining T cell-mediated immune responses to orally administrated protein antigens (5, 6). Our GF TCR-Tg system should be an additionally instructive resource for evaluating the effects of intestinal microflora on antigen-specific T cell responses to food proteins.

Table 1 Cytokine production of PP and LP cells in GF and CV TCR-Tg mice

		GF	CV
PP	IL-4	±	−
	IL-5	++	+
	IL-6	++	+
	IFN-γ	++	+
LP	IL-4	±	±
	IL-5	+	++
	IL-6	+++	+
	IFN-γ	++	+

Cytokines secreted in the culture supernatants were measured by ELISA. +++, ++, +, ±, − was judged based on the results of more than 3 experiments.

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Review

Modulation of Immune Functions by Foods

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Evidence is rapidly accumulating as to the beneficial effects of foods. However, it is not always clear whether the information is based on data evaluated impartially in a scientific fashion. Human research into whether foods modulate immune functions in either intervention studies or randomized controlled trials can be classified into three categories according to the physical state of subjects enrolled for investigation: (i) studies examining the effect of foods in healthy individuals; (ii) studies analyzing the effect of foods on patients with hypersensitivity; and (iii) studies checking the effect of foods on immunocompromized subjects, including patients who had undergone surgical resection of cancer and newborns. The systematization of reported studies has made it reasonable to conclude that foods are able to modulate immune functions manifesting as either innate immunity (phagocytic activity, NK cell activity) or acquired immunity (T cell response, antibody production). Moreover, improvement of immune functions by foods can normalize the physical state of allergic patients or cancer patients, and may reduce the risk of diseases in healthy individuals. Therefore, it is valuable to assess the immune-modulating abilities of foods by measuring at least one parameter of either innate or acquired immunity.

Keywords: amino acid – fatty acid – lactic acid bacteria – mineral – oligosaccharide – polyphenol – vitamin

Introduction

Foods contain various substances that can control the physiological functions of the body, and modulating immune responses is one of the most important functions of foods. Immune functions are indispensable for defending the body against attack by pathogens or cancer cells, and thus play a pivotal role in the maintenance of health. However, the immune functions are disturbed by malnutrition, aging, physical and mental stress or undesirable lifestyle. Therefore, the ingestion of foods with immune-modulating activities is considered an efficient way to prevent immune functions from declining and reduce the risk of infection or cancer.

In order to establish a diet capable of preserving immune functions, it is necessary to search and systematize reliable results on the immune-modulating effects of food-derived substances. To this end, we have selected reports that evaluated the immune-modulating abilities of foods in an intervention study or a randomized controlled trial. Thereafter, we classified these studies according to the physical state of their subjects into three categories: (i) studies examining immune parameters of healthy individuals whose immune functions are poorer than expected; (ii) studies analyzing immune parameters of patients with hypersensitivity; and (iii) studies checking immune parameters of immunocompromized subjects, including patients who had undergone surgical resection of cancer and newborns. We found that the measurement of at least one parameter representing either innate or acquired immunity was useful for evaluating the immune-modulating abilities of foods. This review summarizes the immune-modulating characteristics of foods that have been verified in human as well as animal studies. In addition, we briefly describe the pathways by which food-derived substances are absorbed into the body and the mechanisms through which food-derived substances exert their immune-modulating effects.

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Intestinal Transport of Foods

Molecules found in foods can be absorbed by multiple routes. Carbohydrates, proteins and lipids are broken down into monosaccharides, amino acids and fatty acids, respectively, by diverse hydrolases secreted in the gut lumen or associated with membranes of intestinal epithelial cells (IECs). These food components are actively transported via specific carrier molecules on the surface membrane of IECs and used as nutrients (Fig. 1). Vitamins and minerals in foods are also absorbed through IECs by passive diffusion or active transport using specific carrier molecules (Fig. 1). The main role of vitamins and minerals is to regulate the various physiological functions of cells.

Indigestible macromolecules such as rigid proteins are incorporated into Peyer's patches (PPs) developing throughout the intestine (1). PPs are organized lymphoid tissues that are covered by a particular epithelial layer, the follicle-associated envelope (FAE), and composed of both follicles rich in B cells and an interfollicular area filled with antigen-presenting cells and T cells. Horseradish peroxidase (HRP) given orally to mice was detectable in M cells (microfold cells) within the FAE (2). Moreover, a study using isolated intestinal loops from piglets has revealed that the absorptive rate of HRP was higher in the intestinal segments with PPs than without (3). These results demonstrate that M cells in FAE are the route for efficiently incorporating indigestible proteins (Fig. 1). Macromolecules incorporated into PPs are taken by antigen-presenting cells and induce antigen-specific immune responses. On the other hand, macromolecules with higher molecular size such as carrageenan (88 110 kDa) are hardly absorbed in the intestine.

Small molecules can be transported through IECs by endocytosis (Fig. 1). By contrast, food-derived substances are not usually transported between IECs (paracellular transport), because IECs closely connected by tight junctions do not usually allow food-derived substances to pass through. However, the barrier function of tight junctions is not stiff and breast milk-derived proteins can be transported without degradation between IECs in newborns (4) (Fig. 1). As the immune system in newborns is immature, it is reasonable that newborns incorporate breast milk-derived proteins including lactoferrin and maternal IgG to protect from infection. In contrast, dysfunction of the tight junction due to genetic defect is dangerous. Patients with food allergy have intestines with a

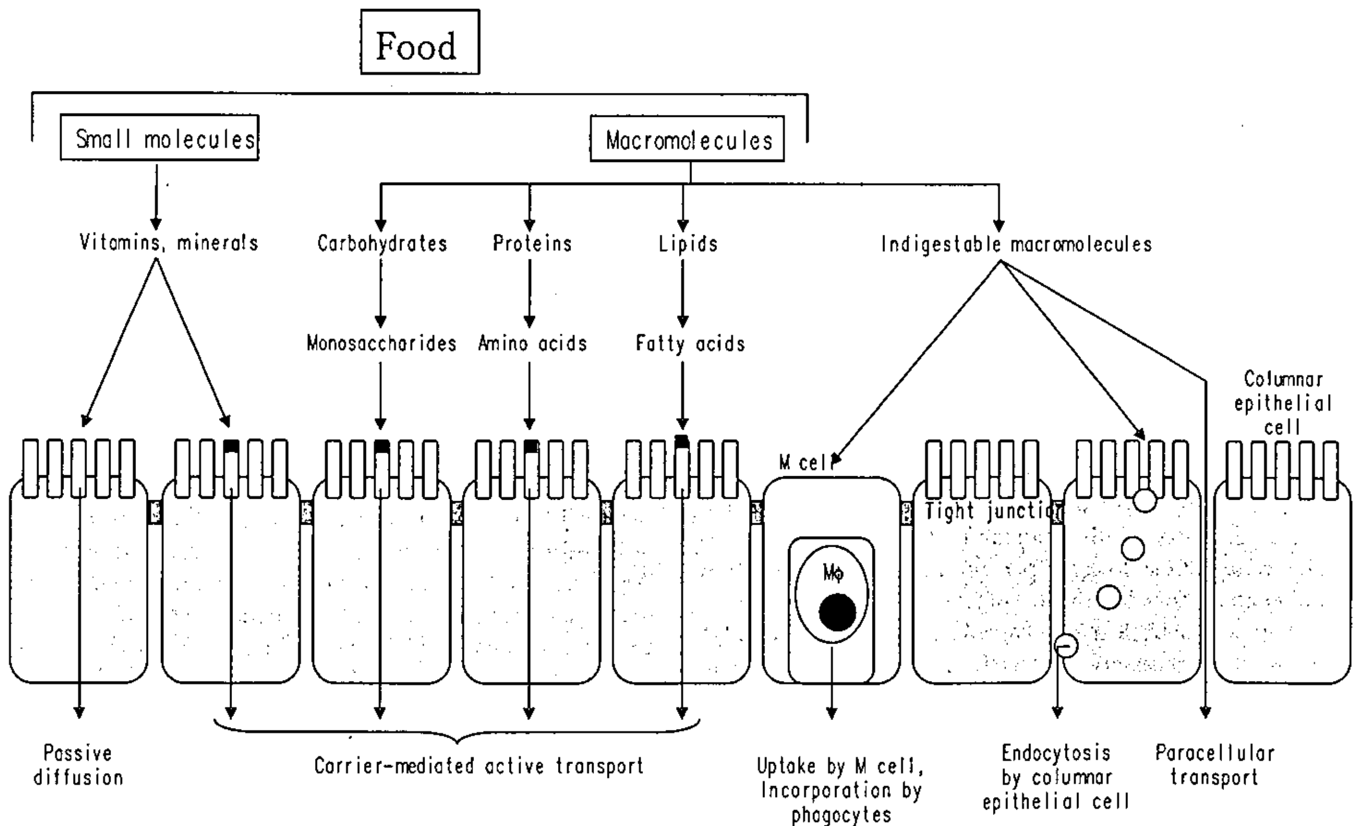

Figure 1. Mechanisms for transport of food-derived substances. Carbohydrates, proteins and lipids are digested by multiple hydrolases secreted into the gut lumen and associated with membranes of columnar epithelial cells. Specific carrier molecules transport monosaccharides, amino acids and fatty acids, and vitamins and minerals are incorporated by passive diffusion or transported by carrier proteins. In contrast, indigestable macromolecules are incorporated by M cells present in the follicle-associated envelope of Peyer's patches or through endocytosis by columnar epithelial cells. The incorporation of foods by diffusion through intercellular spaces between columnar epithelial cells does not occur except for in newborns or in subjects with a genetic deficiency in a barrier function.

reduced barrier function, and therefore an antigenic load in the gut lumen can be easily incorporated into the body (5).

The intestine and liver are important organs in terms of supply of nutrition and self-defense, and these organs are equipped with specialized immune systems. A huge number of IgA-producing cells and intraepithelial T lymphocytes (IELs) with unique physiological functions are colonized in the gut and play a pivotal role in defense against pathogens (6). Moreover, natural killer (NK) T cells are enriched in liver and highly effective in the eradication of tumor cells (7). Hazardous substances (pathogens, toxins and allergens) are recognized as antigens and activate the immune system, but most gut antigenic loads (food-derived molecules and indigenous intestinal bacteria) are harmless and the immune response to these antigens is suppressed in healthy humans (oral tolerance). In contrast, the immune system of patients with inflammatory bowel disease responds excessively to the indigenous intestinal bacteria, causing inflammation in the intestine (8).

It is of great interest that some substances in foods can open tight junctions between IECs. When the capsianoside contained in a sweet pepper was added to the apical side of a monolayer formed by the human IEC line Caco-2, the tight junctions transiently opened, followed by a drop of electric resistance between the apical and basal sides (9). While the tight junction basically acts as a barrier to pathogens or toxic substances in the intestine, the transient opening of tight junctions may be so important that antigens can be captured by dendritic cells in the intestinal lamina propria and immune responses to these antigens are efficiently evoked (10).

Regulation of Immune Functions by Foods

Immune-modulating abilities of foods have been investigated in a number of human studies. We tentatively classified these researches into three categories according to the state of immune system in subjects enrolled for investigation: (i) healthy individuals; (ii) patients with hypersensitivity; and (iii) subjects in immunocompromised state.

Foods Capable of Improving Immune Functions in Healthy Individuals

Immune functions are not stable and usually fluctuate within fixed limits. In addition, various endogenous and exogenous factors can influence immune functions. Corticosteroids suppress a broad range of immune functions efficiently and exhibit anti-inflammatory activity. Malnutrition, aging, stress and undesirable lifestyle are also factors lowering immune functions. The elderly exhibit higher susceptibility to infection than the young, and delayed type hypersensitivity (DTH), antigen-specific antibody production, the proliferative response of T cells and the relative proportion of T cells decline with aging (11–16). Many kinds of physical and mental stress also disturb immune functions. For instance, a surgical operation exhausts patients and is accompanied by a decline in their DTH, and caregivers of dementia patients show a decrease in NK cell activity, antigen-specific antibody production and T cell proliferation on account of depression (17,18). Moreover, NK cell activity deteriorates under not only mental stress after divorce but also physical stress of heavy exercise (19–24). It is widely known that systemic malnutrition associated with a deficiency of protein and energy causes a decline in immune functions and results in susceptibility to infection (25–28). A deficiency in vitamins and minerals induces an attenuation of immune functions including phagocytic activity, NK cell activity, DTH, antigen-specific antibody production, and the proliferative response of T cells (29,30). In addition, NK cell activity and the proliferative response of T cells decline in patients with chronic fatigue syndrome (31).

The deterioration of immune functions possibly causes loss of health. A higher risk of infection is closely linked with low NK cell activity, and increased risk of mortality in the elderly after pathogenic infection is correlated with a decline in DTH (32–35). Bodily dysfunctions in chronic fatigue syndrome patients are negatively correlated with the proliferative response of T cells (36).

These findings clearly demonstrate that immune functions in healthy individuals tend to be disturbed by various factors, and deterioration of health is closely connected with dysregulation of immune functions. On the other hand, it has been proposed that food-derived components can improve the immune functions in healthy individuals. Vitamins, minerals, and fatty acids enhance DTH (37–40), vitamins and minerals enforce antigen-specific antibody production (41–45) and vitamins, minerals and oligosaccharides increase T cells and augment their proliferative response (30,37,43,46–51). In addition, vitamins, minerals and lactic acid bacteria promote phagocytic activity and NK cell activity (30,43,52–61) (Fig. 2). The ingestion of these foods not only normalizes immune functions but also reduces the incidence of pathogenic infection (30,41,43,62–64).

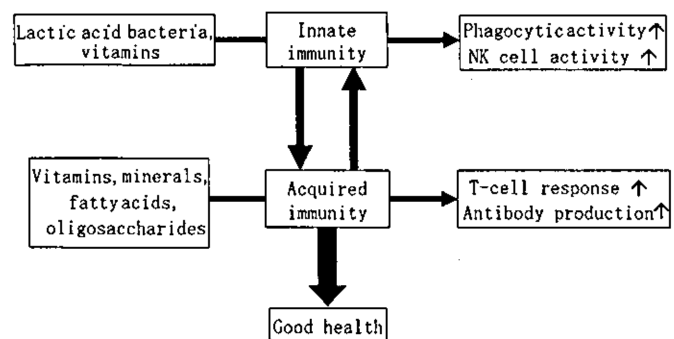

Figure 2. Modulation of immune functions by foods. The immune system is divided into innate immunity and acquired immunity, and food-derived substances can modulate either innate or acquired immunity. For example, probiotics such as lactic acid bacteria and some vitamins enhance phagocytic activity and natural killer (NK) cell activity (innate immunity), while vitamins, minerals, amino acids, fatty acids and oligosaccharides augment T cell responses and antibody production (acquired immunity). A balance of innate and acquired immunity is desirable for good health.

Table 1. Parameters available for evaluating the immune-modulating effects of foods in humans

Subjects	Beneficial changes to parameters induced by ingesting foods
Healthy individuals	Delayed type hypersensitivity ↑ (37–40), Antigen-specific antibody antibody production ↑ (41–45), Mitogen- or antigen-induced T cell proliferation and T cell number ↑ (46–51), NK cell activity and phagocytic activity ↑ (52–61)
Patients with hypersensitivity	Soluble CD4 level in serum ↓ (67), TNF-α level in feces ↓ (79), Inducible surface CD23 level ↓ (78), soluble IL-2R level in serum ↓ (67,78), Soluble VCAM level in serum ↓ (78), ECP level in feces or serum ↓ (67,86), IFN-γ production ↑ (81), TGF-β level ↓ (67), eosinophil number ↓ (81)
Subjects in immunocompromised state	Phagocytic activity ↑ (133), NK cell number ↑ (134,137), T cell number and IFN-γ level in serum ↑ (135), Delayed type hypersensitivity ↑ (133,135), NK cell activity ↑ (139), antigen-specific antibody production ↑ (140), T cell number and IgG level in serum ↑ (141)

Summarizing the results reported so far, it is reasonable to conclude that the effect of foods on immune functions can be evaluated in healthy subjects by measuring either parameters concerning innate immunity (phagocytic activity and NK cell activity) or parameters concerning acquired immunity (DTH, antigen-specific antibody production, the proliferative response of T cells and T cell number) (Table 1). Therefore, despite fears that immune functions may decline due to malnutrition, aging, stress or undesirable lifestyle, one can remain healthy and reduce the risk of infection or cancer by eating foods capable of enhancing phagocytic activity, NK cell activity, DTH, antigen-specific antibody production, the proliferative response of T cells and/or T cell numbers.

Foods Capable of Improving Clinical Symptoms in Patients With Hypersensitivity

Immune reactions are usually evoked in response to externally derived hazardous antigens. However, in patients with hypersensitivity represented by immediate type allergy, immune reaction to non-toxic antigens and sometimes to the body's own molecules is induced. The causes of hypersensitivity are mainly genetic, but environmental factors, including air pollution, dietary components and residential conditions, also play an important role. As clinical condition and immune parameters change concomitantly in allergic patients, it is possible to observe the effects of foods by measuring the immune parameters associated with allergic reactions.

Generation of pro-inflammatory cytokines and chemokines and expression of cell adhesion molecules are involved in the progression of allergic diseases including atopic dermatitis, pollinosis and allergic rhinitis. Levels of pro-inflammatory cytokines and chemokines increase and the expression of cell adhesion molecules is enhanced in allergic patients (65–70). Furthermore, eosinophils as well as mast cells secrete chemical mediators and worsen the clinical symptoms in the inflammatory areas (71,72). In order to establish an objective assessment of the clinical state of allergic patients, a skin test, the antigen-induced response and the SCORAD score have all been utilized (73,74).

When the immune parameters representing clinical symptoms characteristic of atopic dermatitis, pollinosis and allergic rhinitis normalize, the patients recover from allergic diseases (67,75). Therefore, normalization of these immune parameters by foods is helpful in that allergic patients recover their health and persons with a predisposition to allergies may avoid falling ill. Parietaria extract (76,77), herbal extract (78) and lactic acid bacteria (67,79–89) have been found to suppress allergic diseases in human subjects as well as animal models.

Based on findings reported to date, we conclude that the following immune parameters can be used to evaluate the effects of foods on the clinical symptoms of allergic patients: (i) parameters to directly assess clinical symptoms in allergic patients: skin test (75,82), skin-induced response (76), SCORAD score (74,78,90); (ii) parameters that vary in association with the clinical symptoms of allergic patients: TNF-α level (65,66,79,91), soluble CD4 level (67), soluble CD23 level or inducible surface CD23 level (68,78,92), soluble IL-2R level (67,68,93,94), soluble VCAM level (70,78), amount of granular protein in eosinophils (ECP, EPX) (67,86,95); (iii) parameters possibly involved in the clinical symptoms of allergic patients: IgG_4 level (75), IL-4/IFN-γ production (81,96), TGF-β level (67,97,98), eosinophil number (81,99) (Table 1).

An allergic reaction is a sequential immune response involving the processing and presentation of the allergen, activation of allergen-specific T and B cells, production of IgE against the allergen, and activation of mast cells and eosinophils triggered by the allergen. Therefore, food-derived materials could prevent allergy by counteracting at least one step in the cascade of allergic reactions. It has been reported that a variety of foods contain substances able to prevent an allergic reaction (100–102).

Foods Capable of Improving Immune Functions in Subjects in an Immunocompromised State

Cancer patients are usually immunosuppressed and at high risk of infection due to a reduction of immune functions. Therefore, foods capable of enhancing the immune responses of cancer patients with disturbed immune functions are valuable.

Invading pathogenic bacteria or viruses are captured and killed by phagocytes such as neutrophils and macrophages, and NK cells recognize and lyse infected cells. Activated NK cells and T cells produce huge amounts of IFN-γ, which further augments the anti-bacterial activity of macrophages (103–106).

Pathogens that have escaped capture by phagocytes or NK cells are incorporated and processed by professional antigen-presenting cells, which stimulate T cell clones expressing