Murine T cell lines can change CD25 expression patterns as well as proliferation and cell death patterns in response to interleukin 2

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Abstract

T cell subpopulations were obtained from F12.5 and B245/270D T cell lines during long-term culture. Two altered F12.5 subpopulations proliferated more intensively than the original clone. These two subpopulations of F12.5 constantly expressed CD25 (interleukin 2 receptor α chain) at a high level and exogenously added interleukin 2 (IL-2) enhanced cell death for one of these subpopulations. However, the original clone expressed CD25 only after activation and IL-2 inhibited cell death of the original clone. On the other hand, the altered B245/270D subpopulation lost the antigen-specific proliferation ability. This altered cell line under the stimulation culture did not express CD25 even after activation, although the original line expressed CD25. However, the expression pattern of CD25 on the altered cell line at resting state was induced similar to that of the original one. These results suggest that an expression pattern of CD25 can be changed during long-term cultures, accompanied with alteration in response to proliferation and cell death.

Abbreviations: APC – antigen-presenting cells; bCII – bovine type II collagen; bCII245–270 – a peptide of residues 245–270 of bCII; β -Lg – β -lactoglobulin; CIA – collagen-induced arthritis; Con A – concanavalin A; ELISA – enzyme-linked immunosorbent assay; FCS – fetal cow's serum; FITC – fluorescein isothiocyanate; HBSS – Hanks' balanced salt solution; IL-2 – interleukin 2; IL-2R – IL-2 receptor; TCR – T cell receptor.

Introduction

T cells secrete interleukin 2 (IL-2) and express a high affinity form of IL-2 receptor (IL-2R) on their surfaces, when these cells are activated through the T cell receptor (TCR)/CD3. The high affinity form of IL-2R consists of α , β and γ chains. The α chain (CD25) is expressed on T cell surfaces only after the cells are activated, whereas the other two chains are constantly expressed (Taniguchi and Minami, 1993). IL-2 induces proliferation of T cells (Lipkowitz et al., 1984; Seder and Paul, 1994). However, IL-2 has also been reported to exert negative modulation of T cells. Cloned T cells cultured in the presence of IL-2 undergo apoptosis by subsequently provided TCR

stimulation (Lenardo, 1991). Mice deficient for the IL-2 or IL-2R β gene lack crucial negative immunoregulatory functions and develop autoimmune diseases similar to inflammatory bowel disease (Sadlack et al., 1993; Suzuki et al., 1995). Molecular mechanisms for such different effects of IL-2 on T cells have not been clarified yet.

In this study we examine expression patterns of CD25 on T cells using subpopulations derived from established T cell clones and lines. F12.5 is a CD4⁺ Th0 type T cell clone which responds specifically to β -lactoglobulin-derived peptide of residues 119–139 presented by I-A^b and produces IL-2 (Totsuka et al., 1997). Some subpopulations with an increased ability in proliferation appeared, after F12.5 was maintained

for a long time. On the other hand, we also obtained altered subpopulations from lined T cells specific for a peptide (bCII245-270) of residues 245–270 of bovine type II collagen (bCII) which contains an immunodominant T cell determinant for H-2^d DBA/1 mice. It has been suggested that T cells specific for bCII245-270 are involved in the induction and development of autoimmune collagen-induced arthritis (CIA) in those mice (Brand et al., 1994). We repeatedly found that T cells specific for bCII245-270 lost their proliferation ability after a long-term (12 weeks or more) stimulation culture with antigen and that these altered cells displayed a different pattern of change in the proliferation ability from that of the F12.5 cells. In this study, we show different responses in proliferation and cell death of each T cell subpopulation, depending upon expression patterns of CD25.

Materials and methods

Mice

Female C57BL/6NJcl (Japan Clea, Tokyo, Japan) and DBA/1JNCrj mice (Charles River Japan, Yokohama, Japan) (both 6 weeks old) were housed in our animal facility. Experimental procedures in this study met the guidelines for the use of laboratory animals at the University of Tokyo.

Antigen

β -Lactoglobulin (β -Lg) was purified from cow's milk. The genomic type for β -Lg was AA. A crude fraction of β -Lg was isolated from skimmed milk according to the method of Achaffenburg and Drewry (Achaffenburg and Drewry, 1957). The crude β -Lg was applied on a DEAE-Sephacel column (Pharmacia Biotech, Uppsala, Sweden) and the β -Lg fraction was eluted by using a linear gradient of NaCl from 0 to 0.5 M in the buffer of 0.05 M imidazole buffer, pH 6.7 including 200 mg l⁻¹ NaN₃. This fraction was dialyzed with deionized water, lyophilized, and used as a purified protein.

The peptide of bCII245-270 (ATGPLGPKGQT-GEPGIAGFKGEQGP) was synthesized with a 403A peptide synthesizer (Applied Biosystems, Foster City, CA) by F-moc chemistry. The released and deprotected peptides were subsequently purified by reversed-phase HPLC with a C18 SG 120 Å column (Shiseido, Tokyo, Japan). The amino acid sequence

was confirmed by a PSQ-1 protein sequencer (Shimadzu, Kyoto, Japan). Proline was substituted for hydroxyproline found in the native equivalent.

T cell lines

F12.5, a clone specific for residues 119–133 derived from β -Lg plus I-A^b was established by Totsuka et al. (1997) from C57BL/6 mice.

T cells specific for bCII245–270 were acquired from DBA/1J mice which had been immunized with bCII245-270 (10 μ g) plus adjuvant complete H37Ra (Difco Laboratories, Detroit, MI) by injection in the hind footpads. Mice were sacrificed 10 days later, and the draining lymph nodes were removed and suspended in cRPMI, which is a RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal cow's serum (FCS; Cansera, Ontario, Canada), 100 U ml⁻¹ penicillin (Banyu Pharmaceutical, Tokyo, Japan), 100 μ g ml⁻¹ streptomycin (Meiji Seika Kaisha, Tokyo, Japan), 2×10^{-5} M 2-mercaptoethanol, and 1% L-glutamine. The lymph node cells were cultured in the presence of 10 μ g ml⁻¹ bCII245-270 for four days and subsequently 4×10^6 cells ml⁻¹ of antigen-presenting cells (APC) were added to the culture. After adding the APC, the cells were stimulated with bCII245-270 plus APC every 10 days. The method for maintaining lined cells and preparing APC is described below.

Culture of T cell lines

Fresh cRPMI containing 10% of the conditioned medium and 2.5×10^6 cells ml⁻¹ APC with or without antigen was added every 10 days to 1.25×10^5 cells ml⁻¹ F12.5 or bCII245-270-specific T cells. The antigen was 50 μ g ml⁻¹ β -Lg for F12.5 and 10 μ g ml⁻¹ bCII245-270 for its specific T cells. In this study, we considered cultures with antigen as the stimulation culture, and those without antigen as the resting culture.

Syngenic spleen cells irradiated at 3000 rad were used as APC. The conditioned medium was culture supernatant of rat spleen cells stimulated with 5 μ g ml⁻¹ concanavalin A (Con A; SIGMA, St. Louis, MO) for 48 h and neutralized with α -methylmannoside (SIGMA).

Proliferation assay

We used T cell lines under the stimulation culture 10 days after the last stimulation with antigen. As-

says were conducted by culturing 1×10^5 cells ml^{-1} T cells, 2×10^6 cells ml^{-1} APC and antigen ($50 \mu\text{g ml}^{-1}$ β -Lg or $10 \mu\text{g ml}^{-1}$ bCII245-270) in cRPMI for 72 h. In other experiments, T cells were cultured with APC and recombinant human IL-2 (provided by Takeda Chemical Industries, Osaka, Japan) for 48 h. T cell lines under the stimulation culture were also incubated at 1.5×10^6 cells ml^{-1} in the presence of $10 \mu\text{g ml}^{-1}$ Con A for 48 h. The cells were pulsed with 1 μCi per well [^3H]-thymidine (ICN Pharmaceuticals, Costa Mesa, CA) during the last 24 h of the culture. DNA from the cells was collected onto glass fiber filters with a cell harvester, and the radioactivity was measured with a beta counter. For some cultures, the supernatant was collected for measurement of IL-2 after 24 h.

Enzyme-linked immunosorbent assay (ELISA) for IL-2

A certain amount of secreted IL-2 was measured by the two-site ELISA method. Briefly, 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated with anti-IL-2 capture antibody (clone JES6-1A12, PharMingen, San Diego, CA), incubated overnight at 4°C , and then blocked with 3% bovine serum albumin. Samples and standards were incubated overnight at 4°C , and, after washing with phosphate buffered saline containing 0.05% Tween 20, biotinylated anti-IL-2 detection antibody (clone JES6-5H4, PharMingen) was added and the plates were incubated for 2 h at room temperature. Streptavidin-conjugated alkaline phosphatase (Zymed, South San Francisco, CA) was then added, the plates were incubated for 30 min, and the color reaction was developed with p-nitrophenylphosphate disodium salt. The reaction was stopped by addition of 5 N NaOH and the absorbance was read at 405 nm.

Analysis of CD25 expression on T cells

One million cells ml^{-1} T cell clones or lines were incubated with $10 \mu\text{g ml}^{-1}$ Con A for 24 h. After washing with Hanks' balanced salt solution containing 5% FCS and 0.1% NaN_3 (5% FCS/HBSS), the collected cells were stained with 2 μg per 1×10^6 cells fluorescein isothiocyanate (FITC)-conjugated anti-CD25 antibody (clone 7D4, PharMingen) at 4°C for 30 min. After washing, the cells were subsequently resuspended in 1 ml of 5% FCS/HBSS and analyzed on a flow cytometer (FACSsort; Becton Dickinson Immunocytometer, San Jose, CA). Dead cells were detected

by staining with propidium iodide (SIGMA) at a concentration of $500 \mu\text{g ml}^{-1}$.

Analysis of dead cells by staining with annexin V

Viable cells of T cell lines were isolated from those under the resting culture using Mouse Sodium Metarizozate Ficoll (Japan Immunoresearch Laboratories, Gunma, Japan), and were incubated at 2.5×10^5 cells ml^{-1} in the presence of 2.25×10^6 cells ml^{-1} APC, antigen plus APC, or 2.5 ng ml^{-1} IL-2 plus APC for several hours. These T cells were also incubated at 2.5×10^6 cells ml^{-1} in the presence of $10 \mu\text{g ml}^{-1}$ Con A for several hours. In this assay, we used syngenic irradiated spleen cells as APC from which T cells were eliminated by magnetic cell sorting with anti-Thy-1.2 magnetic beads (Miltenyi Biotec, Bergish Gladbach, Germany). After washing with 5% FCS/HBSS, the collected cells were stained with 2 μg per 1×10^6 cells of biotin-conjugated anti-CD3 antibody (clone 145-2C11, PharMingen) at 4°C for 15 min. After washing, the cells were subsequently stained with FITC-conjugated annexin V (Boehringer Mannheim, Mannheim, Germany) and 2 μg per 1×10^6 cells of phycoerythrin-conjugated streptavidin (Gibco BRL, Gaithersburg, MD) in the incubation buffer (10 mM HEPES containing 140 mM NaCl and 5 mM CaCl_2 , pH 7.4) at 4°C for 15 min. After resuspending the cells in the incubation buffer, CD3^+ cells were analyzed for annexin V reactivity on a flow cytometer.

Results

Three subpopulations of F12.5 showed distinct proliferative responses to the antigen

The incorporation of [^3H]-thymidine was analyzed for three subpopulations of F12.5 harvested on day 10 after the last stimulation with β -Lg and APC (Figure 1). In this study, three subpopulations of F12.5A, F12.5B and F12.5C derived from F12.5 were used. F12.5B and F12.5C were obtained during the long-term culture of F12.5A. Figure 1 indicates that F12.5A cells proliferated with the antigen plus APC, but not with APC alone. F12.5B cells proliferated more intensively, compared with F12.5A. Although F12.5B cells proliferated more strongly in the presence of the antigen, these cells also proliferated in the absence of the antigen. On the other hand, F12.5C

Figure 1. [^3H]-thymidine incorporation into cells of subpopulations of F12.5 in the presence or absence of antigen. Ten days after the last stimulation with antigen, F12.5A, F12.5B or F12.5C cells (1×10^5 cells ml^{-1}) plus APC (2×10^6 cells ml^{-1}) were incubated with or without antigen ($50 \mu\text{g ml}^{-1}$ β -Lg) for 72 h. Data represent mean $\pm$ SD.

cells lost antigen-specific proliferation reactivity, proliferating to a similar extent in both the presence and absence of the antigen.

F12.5B and F12.5C expressed high levels of CD25 before stimulation with Con A

The expression level of CD25 on the cell surface of each subpopulation of F12.5 was analyzed on a flow cytometer before and after 24 h-stimulation with Con A in the absence of APC (Table 1). The expression of CD25 on F12.5A cells was faint before stimulation with Con A, and slightly increased by the stimulation. On the other hand, F12.5B and F12.5C cells expressed CD25 at a high level both before and after Con A-stimulation. The expression of CD25 on F12.5B cells was slightly increased by the stimulation, while little difference was found in the expression level of CD25 on F12.5C cells before and after stimulation with Con A.

Response of F12.5A to added IL-2 and Con A was different from that of F12.5B and F12.5C

The incorporation of [^3H]-thymidine into the cells of each subpopulation of F12.5 in the presence of IL-2 with APC or Con A without APC for the last 24 h, during a 48 h-incubation, was analyzed. As Figure 2 shows, the proliferation of F12.5A cells was

Table 1. The expression level of CD25 on F12.5 subpopulations before and after Con A-stimulation

	Condition of culture	Stimulation with Con A	Relative median
F12.5A	Stimulation culture	Before	1.32
		After	4.25
	Resting culture	Before	1.23
		After	3.85
F12.5B	Stimulation culture	Before	42.1
		After	47.0
	Resting culture	Before	28.4
		After	39.2
F12.5C	Stimulation culture	Before	36.5
		After	30.5
	Resting culture	Before	23.7
		After	22.1

Each subpopulation of F12.5 was cultured with Con A in the absence of APC for 24 h. The expression level of CD25 on those cells was analyzed on a flow cytometer. The expression level of CD25 on those cells before Con A-stimulation was also examined.

The relative median was calculated as follows: (the median of fluorescence intensity when anti-CD25 antibody was added)/(the median of fluorescence intensity when no antibody was added).

dependent on the concentrations of exogenously added IL-2. On the other hand, F12.5B and F12.5C cells intensively proliferated regardless of added IL-2. Figure 3A shows that the proliferation of F12.5A cells was enhanced by Con A. However, Con A remarkably decreased proliferative responses of F12.5B and F12.5C cells, which intensively proliferated without Con A-stimulation. F12.5A cells secreted IL-2 into the culture supernatant when stimulated with Con A (Figure 3B). On the other hand, IL-2 was scarcely detected in the culture supernatants of F12.5B and F12.5C cells incubated both with and without Con A.

IL-2 increased sensitivity of F12.5B to cell death

A percentage of dead cells were examined for each subpopulation of F12.5 stimulated with the antigen plus APC, with IL-2 plus APC, with APC alone or with Con A alone for several hours of culture (Figure 4). Incubation with the antigen plus APC had no influence on the amount of dead cells, when compared only with APC (Figures 4A and B). An order of a percentage of dead cells incubated in the presence of APC or the antigen plus APC was as follows: F12.5A > F12.5C > F12.5B. When cultured with 2.5 ng ml^{-1} IL-2 plus APC, the amount of dead F12.5A cells decreased, compared with those cultured without IL-2 added (Figures 4A and C). The addition of IL-2 increased the sensitivity of F12.5B to cell death, but did not influence the amount of dead cells of F12.5C. Culture with Con A induced cell death of F12.5A at a similar level to that with APC, whereas dead cells were scarcely found for F12.5B and F12.5C incubated with Con A (Figures 4A and D).

T cell lines specific for bCII245-270 lost their antigen-specific proliferation ability after a long-term culture

Independently repeated studies showed that T cell lines specific for bCII245-270 markedly decreased their proliferation ability after culture for 12 weeks or more with antigenic stimulation every 10 days. Antigen-specific proliferation of those T cell lines cultured for different periods was analyzed 10 days after the last stimulation (Figure 5). In this study, two T cell lines were distinguished on the basis of culture periods before assay. One lineage of B245/270D9.1 was obtained by culture for four to eight weeks and another B245/270D4.3 for 12 weeks or more. B245/270D9.1 cells proliferated in response to antigenic stimulation, although those cells also proliferated minimally in the

Table 2. The expression level of CD25 on subpopulations of bCII245-270-specific T cell lines before and after Con A-stimulation

	Condition of culture	Stimulation with Con A	Relative median
B245/270D9.1	Stimulation culture	Before	2.29
		After	6.49
	Resting culture	Before	2.05
		After	4.07
B245/270D4.3	Stimulation culture	Before	1.39
		After	1.50
	Resting culture	Before	1.58
		After	12.0

Cells were incubated similarly to those in Table I. The relative median was defined as described in Table I.

absence of antigen. On the other hand, B245/270D4.3 cells lost antigen-specific proliferation ability. Those cells proliferated more widely than B245/270D9.1 when cultured without antigen. However, a proliferative response of B245/270D4.3 was not increased by stimulation with antigen and it was at a lower level than that of B245/270D9.1.

T cell lines specific for bCII245-270 displayed different patterns of CD25-expression between subpopulations and between different culture conditions

The expression level of CD25 on cell surface was analyzed for B245/270D4.3 and B245/270D9.1 before and after 24 h-stimulation with Con A in the absence of APC (Table II). The expression of CD25 on the surface of B245/270D9.1 was strongly enhanced by Con A-stimulation. No difference in the expression level of CD25 on B245/270D9.1 cells between the stimulation and resting cultures existed. On the other hand, B245/270D4.3 under the stimulation culture expressed CD25 at a low level both before and after Con A-stimulation. However, the expression of CD25 on the surface of B245/270D4.3 under the resting culture was induced by Con A-stimulation.

Antigenic stimulation increased sensitivity to cell death of B245/270D9.1

A percentage of dead cells were examined for B245/270D9.1 stimulated with antigen, with IL-2 or with Con A for several hours (Figure 6). Stimulation

Figure 2. [³H]-thymidine incorporation into F12.5 cells cultured in the presence of IL-2. Ten days after the last stimulation with antigen, F12.5 cells (1×10^5 cells ml⁻¹) plus APC (2×10^6 cells ml⁻¹) were incubated with various concentrations of recombinant human IL-2 for 48 h. Data represent mean $\pm$ SD.

with the antigen plus APC increased the amount of dead cells more intensively, compared with that cultured only with APC. When incubated without APC, but with Con A for 15 h, dead cells appeared at the same level as those incubated with APC only, although the percentage of the dead cells was smaller for Con A-stimulated cells when stimulated for 9 h. The percentage of the dead cells in B245/270D9.1 cells incubated with 2.5 ng ml^{-1} IL-2 plus APC for 9 h was almost the same as for those cultured without IL-2.

Discussion

We obtained distinct subpopulations of F12.5B and F12.5C derived from a parent line of F12.5 (called F12.5A in this study) after a long-term culture with the antigen plus APC. Our *in vitro* study clearly indicated that during culture, lined T cells can change proliferation ability, expression level of CD25, and susceptibility to cell death in response to the antigen, to IL-2, and to mitogen. The results indicated that F12.5 subpopulations were categorized into three types: (1) F12.5A is a type of a subpopulation with the ability of an antigen-specific proliferative response (Figure 1). However, cell death was moderately induced by stimulation with Con A, antigen plus APC, or APC alone (Figures 4A, B and D). Expression of

CD25 on F12.5A cells was induced upon stimulation with Con A and proliferation of these cells was dependent on the concentrations of exogenously added IL-2 (Table I and Figure 2). IL-2 inhibited cell death of those cells (Figures 4A and C). (2) F12.5B is a type of subpopulation still possessing the ability of antigen-specific proliferation (Figure 1). Cell death was minimally induced, when cells were stimulated with APC alone, with antigen plus APC or with Con A (Figures 4A, B and D). CD25 was constantly expressed at a high level and its expression was further increased by stimulation with Con A (Table I). F12.5B cells proliferated irrespective of the concentrations of exogenously added IL-2 (Figure 2). However, cell death was induced by stimulation with 2.5 ng ml^{-1} IL-2 (Figures 4A and C). (3) F12.5C is a type of subpopulation similar to F12.5B. However, F12.5C completely lost the antigen-specific proliferation ability (Figure 1). F12.5C was more sensitive to cell death than F12.5B upon stimulation with the antigen plus APC, or with APC alone (Figures 4A and B). On the contrary, cell death was neither induced nor inhibited by stimulation with 2.5 ng ml^{-1} IL-2 (Figures 4A and C). F12.5B and F12.5C each exhibited a single peak of CD25-expression (data not shown), while F12.5A did not express CD25 before stimulation with Con A. These data suggest that the F12.5B subpopulation was not a mixture of F12.5A and F12.5C cells.

Figure 3. [³H]-thymidine incorporation into F12.5 cells stimulated with Con A (A) and IL-2 secretion of these cells (B). Ten days after the last stimulation with antigen, F12.5 cells (1.5×10^6 cells ml⁻¹) of each subpopulation were incubated with $10 \mu\text{g ml}^{-1}$ Con A for 48 h. Culture supernatants were collected at 24 h, and IL-2 secreted into these supernatants was measured by ELISA. Data represent mean $\pm$ SD.

IL-2 plays an important role in 'activation-induced cell death' caused by an antigen (Lenardo, 1991). In this study we showed that stimulation with IL-2 and APC in the absence of an antigen could induce cell

death for a T cell subpopulation of F12.5B. Many reports have proposed the existence of regulatory T cell subpopulations which constantly express CD25 (Sakaguchi, 2000). However, those regulatory T cells

Figure 4. Cell death of subpopulations of F12.5. Live F12.5 cells at 2.5×10^5 cells ml⁻¹ were incubated with A) 2.25×10^6 cells ml⁻¹ APC, B) antigen and APC, or C) 2.5 ng ml⁻¹ IL-2 and APC for several hours. These T cells were also incubated at 2.5×10^6 cells ml⁻¹ with D) $10 \mu\text{g ml}^{-1}$ Con A for several hours. Dead F12.5 cells were detected as CD3⁺, and as annexin V⁺ on a flow cytometer.

Figure 5. [³H]-thymidine incorporation into each subpopulation of bCII245-270-specific T cell lines. Ten days after the last stimulation with antigen, B245/270D4.3 or B245/270D9.1 cells (1×10^5 cells ml⁻¹) plus APC (2×10^6 cells ml⁻¹) were incubated with or without antigen ($10 \mu\text{g ml}^{-1}$ bCII245-270) for 72 h. Data represent mean ± SD.

Figure 6. Sensitivity of B245/270D9.1 to cell death. Cells were incubated similarly to those in Figure 4. Dead B245/270D9.1 cells were detected as CD3⁺, and as annexin V⁺.

scarcely proliferate with or without stimulation by an anti-CD3 antibody (Thornton and Shevach, 1998). Therefore, the F12.5B and F12.5C cells which showed a great proliferative ability are unique and distinct from CD25⁺ regulatory T cells. As noted elsewhere, IL-2 has the dual effect of both activation and down-regulation. However, the molecular mechanisms for such opposite effects of IL-2 on T cells have not been clarified yet. We expect that F12.5B can be used for further analyzing the mechanism of cell death induced by IL-2.

Although IL-2 was little detected in the culture supernatants of F12.5B or F12.5C (Figure 3B), these cells proliferated at a high level in the absence of antigen. It is likely that autocrine IL-2 secreted by those F12.5B or F12.5C cells were enough to fully activate those cells, although IL-2 produced by those cells was exhausted through augmented expression of the high affinity form of IL-2R and not detected.

Intensive proliferation of F12.5B and F12.5C was inhibited by incubation with Con A for 48 h (Figure 3A) although both F12.5 subpopulations greatly proliferated by incubation without Con A. Nevertheless, cell death of those cells was also inhibited with Con A-stimulation for 15 h or less (Figure 4D). These data indicate that the cell death of Con A-stimulated F12.5B and F12.5C occurred in 15 h or later. It is likely that although signaling through Con A can inhibit cell death, IL-2 which was secreted by F12.5B in

response to Con A can contribute to cell death. It has been reported that in response to macrophage, IL-2 is secreted at 4 h after stimulation and its amount reaches maximum at 12 h (Lipkowitz et al., 1984). We found that added IL-2 induced cell death of F12.5B at 15 h or later (Figure 4C). At a later phase, the inducing effect of IL-2 on cell death may be more dominant than the inhibition effect of Con A.

B245/270D4.3 lost the antigen-specific proliferation ability after long-term culture. Those altered cells showed a decreased expression of CD25 on cell surfaces under the stimulation culture (Table II). These results suggest that decreased expression of CD25 lowered the proliferation ability of B245/270D4.3 in response to the antigen. However, expression of CD25 on B245/270D4.3 at a resting state was promoted in a similar manner to that of B245/270D9.1 stimulated with Con A. B245/270D9.1 cells changed into B245/270D4.3 after repeated stimulation cultures and the altered B245/270D4.3 cells did not express CD25 under the stimulation culture. Exogenously added IL-2 induced proliferation of B245/270D9.1 (data not shown). The inability of B245/270D4.3 to bind to IL-2 through its high affinity receptor is considered to be a fatal factor for survival.

Although in vitro T cells specific for bCII245-270 could not be cultured for a long time, these cells may be involved in the development of CIA in vivo (Brand et al., 1994). IL-15 is abundant in the synovial tis-

sues of juvenile rheumatoid arthritis patients (Scola et al., 2002). IL-15 and IL-2 have the common effect of inducing proliferation and differentiation of T cells (Wilkinson and Liew, 1995) except for the induction of apoptosis by IL-2. Proliferation of CD8 T cells derived from aged mice, in which autoimmune responses occur quite often, is enhanced by IL-15, although IL-2 inhibits the activation of those CD8 T cells (Ku et al., 2001). Even if splenocytes did not strongly stimulate T cells specific for bCII245-270, IL-15 may contribute to the activation of those cells in the joints and also to the progression of CIA.

This study revealed that the increased or attenuated expression of CD25 on the surface of the F12.5 or B245/270D T cell lines reflects a change in the proliferation of these cells. A change in the expression levels of CD25 also affected the sensitivity of the F12.5 cells to cell death induced by IL-2. Further study is needed to know what causes the opposite change in expression of CD25 observed in the F12.5 and B245/270 T cell lines.

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