Impaired Autologous Mixed-Lymphocyte Reaction of Peripheral Blood Lymphocytes in Adult Periodontitis

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The autologous mixed-lymphocyte reactions (AMLR) of peripheral blood lymphocytes from 80 patients with adult periodontitis were examined. Some but not all patients showed clearly low AMLR responses; 31 of 80 subjects (39%) showing consistently low responses in AMLR (less than the mean −2 standard deviations of the healthy control group values) were designated low-AMLR patients, whereas the 42 patients (53%) who showed normal AMLR responses were designated normal-AMLR patients. However, there were no significant differences in the clinical parameters between these two groups of patients. The phenotypic analysis of T-cell fractions revealed a lower percentage of CD45RA-positive cells in CD4-positive cells (CD4$^+$ CD45RA$^+$ T cells) in the low-AMLR patients than those in normal-AMLR patients and healthy control subjects. No significant differences were demonstrated between the two groups in terms of the proportion of CD4-positive and CD8-positive cells in the T-cell fractions or in the expression of human leukocyte antigen DR of the monocytes and B cells in the non-T-cell fractions. In the low-AMLR patients, the allogeneic MLR was found to be normal, but the interleukin 2 production in the AMLR was found to be significantly depressed. The depressed AMLR responses and the lower percentage of CD4$^+$ CD45RA$^+$ T cells in the low-AMLR patients were found to be normalized following the periodontal therapy. These results might reflect changes in regulatory T-cell function induced by development of periodontal diseases.

Immunological mechanisms have been implicated in the pathogenesis of human periodontal diseases. Advanced periodontitis is recognized as a B-cell-rich lesion that includes immunoglobulin G-producing plasma cells (14). However, it has been reported that not a few T cells were also present subjacent to the pocket epithelium in conjunction with the plasma cells (21, 31). Our previous study revealed that the proportion of CD4-positive (CD4$^+$) T cells decreased and that the proportion of CD8-positive (CD8$^+$) T cells increased in the inflamed human gingiva (20). These histopathological findings suggested that periodontal lesions might be induced by the immunoregulatory imbalance of T cells, since T cells and T-cell-derived factors have been recognized to play a central role in the immunoregulatory network at both local and systemic levels.

The autologous mixed-lymphocyte reaction (AMLR), which represents an immunologic response of the T-cell reaction to surface major histocompatibility complex class II antigens expressed on non-T cells, has been thought to reflect an autoregulatory immune mechanism (10, 22). In several studies of subjects with periodontal diseases, the spontaneous proliferative response (SPR), as a measure of AMLR, of peripheral blood lymphocytes has been demonstrated. These reports have shown the depression of the SPR in young subjects with advanced periodontitis (41) and generalized juvenile periodontitis (35) and in both young and older subjects with periodontal diseases (3). However, the nature of the AMLR in periodontal diseases is still poorly understood and undefined.

The present study was designed to examine further the nature of AMLR in adult periodontitis and any phenotypic and functional alterations in the peripheral blood monocytes and lymphocytes in these patients. The phenotypic analyses

were performed in both responder and stimulator fractions in the AMLR by means of the direct immunofluorescence technique using antibodies to CD36, human lymphocyte antigen DR (HLA-DR), CD4, CD8, and CD45RA. To characterize the functional alterations, interleukin 2 (IL-2) production in AMLR and allogeneic MLR was evaluated by using the lymphocytes from the same patient population.

MATERIALS AND METHODS

Subjects. The 80 participating patients (mean age, 37.1 ± 0.9 years), 40 men and 40 women, suffering from adult periodontitis were selected from the Osaka University Dental Hospital. All patients completed medical and dental histories and had thorough clinical and radiographic dental examinations. On the basis of these findings, the patients had been assigned a diagnosis of adult periodontitis by using previously published criteria (24). As the clinical parameters, gingival index (13), plaque index (32), the percentage of alveolar bone destruction (30), and the number of affected teeth showing more than 30% bone loss on radiographs (11) were recorded. Following subject selection with a screening examination, each participant was given plaque control instructions and supra- and subgingival scaling and root planing as the initial periodontal treatment and received flap surgery with or without osseous reductions. The 25 control subjects (mean age, 34.4 ± 1.4 years), 17 men and 8 women, who had clinically healthy gingiva were selected for this study in a similar fashion.

Preparation of T-cell and non-T-cell fractions. Heparinized peripheral blood was obtained from the patients by venipuncture. Mononuclear cells were isolated by Percoll density gradient centrifugation ($\rho = 1.077$) (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and washed with Hank's balanced salt solution (Sigma Chemical Co., St. Louis, Mo.). T-cell and non-T-cell fractions were separated by rosetting

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with neuraminidase (Behringwerke AG, Marburg, Germany)-pretreated sheep erythrocytes.

AMLR. The AMLR assay was performed by using a modification of the method described by Sakane et al. (28). In brief, lymphocytes were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), 5×10^{-5} M 2-mercaptoethanol, and 10% (vol/vol) heat-inactivated fetal calf serum (Flow Laboratories, Irving, Scotland) in 96-well round-bottomed microtiter plates (Corning Glass Works, Corning, N.Y.). Stimulator cells (non-T-cell fraction) were pretreated with mitomycin C (50 μg/ml) (Kyowa Hakko, Tokyo, Japan) at 37°C for 30 min. T cells (10^5) were cultured with mitomycin-pretreated non-T cells (10^5) at 37°C for 7 days in a humidified 5% CO_2 atmosphere. Cultures were pulsed for the final 20 h of incubation with [^{3}H]thymidine (^{3}H-TdR; 18.5 kBq per well). The results were expressed as the net ^{3}H-TdR uptake (disintegrations per minute) (i.e., disintegrations per minute in AMLR culture minus that in T-cell culture). T-cell control values corresponding to the peak AMLR ranged from 126 to 6,431 dpm (1,717 ± 199 [mean ± standard error of the mean {SEM}; $n = 80$]), and corresponding non-T-cell control values ranged from 92 to 1,472 dpm (560 ± 48, mean ± SEM; $n = 80$).

Phenotypic analysis of peripheral blood mononuclear cells. Immunofluorescence analysis of cell surface antigens was conducted by the direct immunofluorescence technique using the following antibodies: fluorescein isothiocyanate (FITC)-conjugated OKT3 (FITC-OKT3), FITC-OKT4, FITC-OKT8, FITC-OKM5, phycoerythrin-conjugated OKDR (Ortho Diagnostic Systems Inc., Raritan, N.J.), and phycoerythrin-conjugated 2H4 (Coulter Immunology, Hialeah, Fla.). Cells were incubated with a single monoclonal antibody or with selective pairs of monoclonal antibodies (FITC conjugated and phycoerythrin conjugated) for 20 min at room temperature. After being washed twice with phosphate-buffered saline, the cells were analyzed by using a flow cytometer (FCS-1X; Japan Spectroscopic Co., Tokyo, Japan). Positive cells were determined by setting a threshold with reference to a relevant negative control.

IL-2 production in the AMLR and the allogeneic MLR. The IL-2 activity in the AMLR culture supernatant was determined on the basis of the ability to support the growth, as measured by ^{3}H-TdR incorporation, of the IL-2-dependent T-cell line CTLL-2 (5). Our preliminary experiments indicated that IL-2 activity of the AMLR culture supernatant peaked on day 3 of culture. Accordingly, in this study, day 3 culture supernatants were used for the evaluation of IL-2 production in the AMLR. The allogeneic MLR were carried out as described above, but the stimulator cells were prepared from the peripheral blood of the healthy control.

Statistical analysis. Data were analyzed by either the multiple comparison method (ANOVA) or the paired t-test. The P values were corrected by Dunn's method.

RESULTS

The distribution of the AMLR responses of the patients is illustrated in Fig. 1. Compared with the control subjects, different individual periodontitis patients gave various AMLR responses, ranging from 51 to 59,485 dpm, although the mean AMLR response of the patients (17,607 ± 1,428 dpm, mean ± SEM) was significantly lower than that of the control (24,980 ± 1,332 dpm) ($P < 0.05$).

In the patient group, it was found that some subjects showed significantly low AMLR responses, whereas some
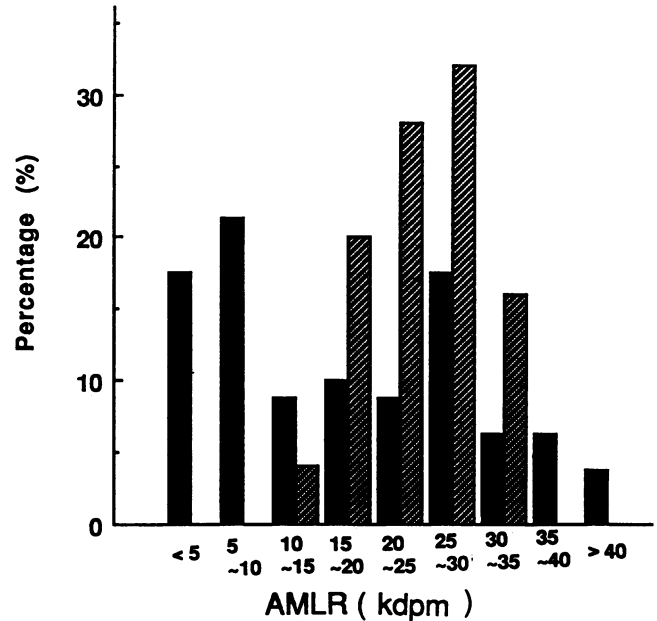

FIG. 1. Distribution of the AMLR responses of the patients with adult periodontitis. T-cell and non-T-cell fractions were separated from peripheral blood mononuclear cells of 80 adult periodontitis patients and 25 healthy control subjects by the method of sheep erythrocyte-rosette formation. The T-cell fraction (1×10^5) was incubated with the MMC-pretreated non-T-cell fraction (1×10^5) for 7 days at 37°C. The AMLR was expressed as the net incorporation of ^{3}H-TdR for last 20 h of culture (disintegrations per minute). ■, percentage of the patients in each range of AMLR values; ▨, percentage of the control subjects.

showed normal AMLR responses. Although we examined the responses over several days and at different times of the day, consistent results in the AMLR responses of an individual subject were obtained in both groups. The patients (31 of 80 subjects [39%]) showing consistently low AMLR responses (less than the mean −2 standard deviations [SD] of the healthy control group) were designated low-AMLR patients in this study, whereas the 42 patients (53%) who showed normal AMLR responses (from mean −2 SD to mean +2 SD of the healthy control group) were designated normal-AMLR patients.

The kinetics of the AMLR responses revealed that the peak AMLR responses in low- and normal-AMLR patients occurred coincidentally, showing an increase from day 5 to a peak at day 7, followed by a decrease through day 9 to day 11 (Fig. 2).

The mean age and the clinical parameters, including the mean probing pocket depth, gingival index, plaque index, mean percentage of alveolar bone loss, and percentage of the teeth affected (teeth with >30% bone loss), of the low- and normal-AMLR patients before periodontal treatment are shown in Table 1. The statistical analysis by mean age and the clinical parameters used in this study revealed no significant differences between low- and normal-AMLR patients. Furthermore, no significant correlation was found between AMLR and any clinical parameter in either patient group (data not shown).

To determine whether the AMLR response of the individual patient changes along with the conventional periodontal treatment, we examined the AMLR responses of both low- and normal-AMLR patients just after the initial periodontal
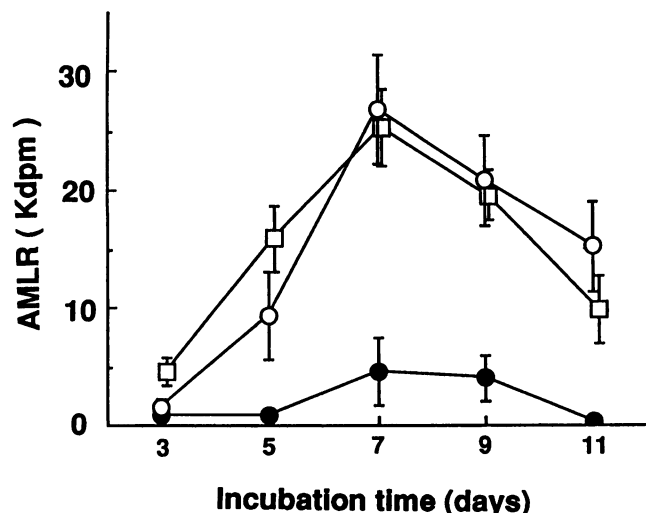
FIG. 2. Kinetics of the AMLR responses of low- and normal-AMLR patients. T-cell and MMC-pretreated non-T-cell fractions from low-AMLR patients (●), normal-AMLR patients (O), and healthy control subjects (□) were incubated for 3 to 11 days. All cultures were pulse-labelled with ^{3}H-TdR 20 h before harvest, and the AMLR was expressed as the net ^{3}H-TdR uptake.

FIG. 3. Effect of periodontal treatments on the AMLR response and the percentage of CD4$^+$ CD45RA$^+$ T cells in the patients with adult periodontitis. The AMLR responses of both low-AMLR patients (●) and normal-AMLR patients (O) were examined just after the initial periodontal treatment and during the maintenance phase (6 months after the initial periodontal treatment). The phenotypic analysis of the peripheral blood mononuclear cells was also carried out, and the changes during the periodontal treatments in the percentages of CD4$^+$ CD45RA$^+$ T cells in the low-AMLR patients (■) and normal-AMLR patients (□) are illustrated. Before treatment, a significantly lower AMLR response (*; $P < 0.01$) and percentage of CD4$^+$ CD45RA$^+$ T cells (**; $P < 0.05$) was observed in low-AMLR patients than that in normal-AMLR patients.

treatment and in the maintenance phase (6 months after the initial periodontal treatment) (Fig. 3). In low-AMLR patients, the AMLR response increased after the initial periodontal treatment and reached appreciable normal levels at the maintenance-phase examination. In contrast, the AMLR response of normal-AMLR patients remained unchanged. However, the periodontal treatment resulted in significant improvements of the clinical parameters in both low- and normal-AMLR patients (Fig. 4).

The phenotypic analyses of non-T-cell and T-cell fractions from both low- and normal-AMLR patients as well as those from healthy controls are summarized in Tables 2 and 3, respectively. In the studies with the non-T-cell fraction, no significant differences were observed in terms of the expression of HLA-DR on the monocytes (CD36-positive HLA-DR-positive [CD36$^+$ HLA-DR$^+$] cells) and B cells (CD36$^-$ HLA-DR$^+$ cells) among the three groups.

On the other hand, the phenotypic analysis of the T-cell fraction revealed that the percentage of CD45RA-positive cells in CD4-positive cells (CD4$^+$ CD45RA$^+$ T cells) in low-AMLR patients (23.3 ± 2.3%) was significantly lower than that in normal-AMLR patients (33.3 ± 3.4%; $P < 0.05$) and healthy control subjects (35.6 ± 2.5%; $P < 0.05$). There were no significant differences in the percentages of CD4-positive and CD8-positive cells or in the CD4/CD8 cell ratio between low- and normal-AMLR patients. A typical staining
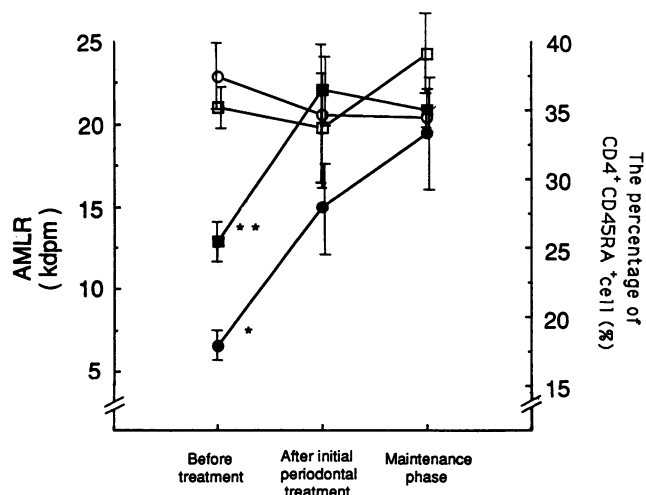
profile of the T-cell fraction from a low-AMLR patient and a normal-AMLR patient is displayed in Fig. 5. All of the results for the normal-AMLR patients shown in Tables 2 and 3 were not significantly different from those of healthy control subjects.

The percentages of CD4$^+$ CD45RA$^+$ T cells varied along with the periodontal treatment in a manner similar to AMLR responses in either patient group. Figure 3 also illustrates the changes with the periodontal treatment in the percentages of CD4$^+$ CD45RA$^+$ T cells of low- and normal-AMLR patients. In low-AMLR patients, the percentage of CD4$^+$ CD45RA$^+$ T cells increased and reached normal levels during the periodontal treatment, whereas the percentage of CD4$^+$ CD45RA$^+$ T cells in normal-AMLR patients did not change essentially until the maintenance phase.

Since earlier studies suggested that in the course of AMLR, CD4$^+$ cells could produce IL-2, a finding which supports the proliferative response of CD4$^+$ cells and probably CD8$^+$ cells as well (26, 33, 37), we next determined whether the low AMLR response in the low-AMLR patients

TABLE 1. Clinical parameters[a] of the patients with adult periodontitis showing low or normal AMLR responses

Subject group	Age (yrs)	Gingival index	Plaque index	Pocket depth (mm)	Alveolar bone loss (%)	Teeth with >30% bone loss (%)	AMLR (dpm)
Low-AMLR patients ($n = 31$)	36.6 ± 1.5	1.27 ± 0.10	1.06 ± 0.12	4.46 ± 0.18	35.6 ± 1.9	47.1 ± 3.8	5,653 ± 571[b]
Normal-AMLR patients ($n = 42$)	36.6 ± 1.2	1.09 ± 0.08	1.00 ± 0.09	4.45 ± 0.21	33.0 ± 2.1	49.2 ± 4.8	22,035 ± 1099
Healthy subjects ($n = 25$)	33.4 ± 1.4	0.10 ± 0.05	0.15 ± 0.02	1.75 ± 0.09			24,958 ± 1,333

[a] Values given are means ± SEM.
[b] Significantly lower response than those in normal-AMLR patients and in healthy controls ($P < 0.01$).

TABLE 2. Ratios[a] of monocytes ($CD36^+$ $HLA\text{-}DR^+$) and B cells ($CD36^-$ $HLA\text{-}DR^+$) in the non-T-cell fractions

Subject group	$CD36^+$ $HLA\text{-}DR^+$ (%)	$CD36^-$ $HLA\text{-}DR^+$ (%)	AMLR (dpm)
Low-AMLR patients ($n = 11$)	37.8 ± 3.7	12.2 ± 1.4	$6,022 \pm 805^b$
Normal-AMLR patients ($n = 11$)	40.8 ± 4.5	15.1 ± 2.1	$25,735 \pm 2,230$
Healthy subjects ($n = 10$)	36.6 ± 5.8	11.5 ± 0.9	$26,238 \pm 446$

[a] Values given are means $\pm$ SEM.
[b] Significantly lower response than those in normal-AMLR patients and in healthy controls ($P < 0.01$).

was associated with the diminished production of IL-2 in AMLR. Table 4 shows the mean IL-2 activities of day 3 AMLR culture supernatants from low- and normal-AMLR patients. On the basis of this assay, only low-AMLR patients revealed low levels of IL-2 production; normal-AMLR patients and healthy controls showed normal IL-2 production in AMLR.

Allogeneic MLR of the T-cell fraction from low- and normal-AMLR patients were conducted by using the non-T-cell fractions from the healthy control subjects (Fig. 6). In contrast to AMLR, the T-cell fraction from both low- and normal-AMLR patients had normal allogeneic MLR.

DISCUSSION

The results demonstrated that the peripheral blood lymphocytes of some of the patients with adult periodontitis showed consistently low AMLR responses. Although the peripheral blood lymphocyte fractions from the same individual were collected on several different days and at different times of the day in preliminary experiments, the AMLR response of an individual subject was found to be essentially consistent. Moreover, kinetic studies of AMLR revealed that peak responses occurred coincidentally. Therefore, we further examined the nature of AMLR, comparing low-AMLR patients to the normal-AMLR patients and healthy controls. Similar observations with AMLR responses of peripheral blood lymphocytes were reported in the studies with Sjögren's syndrome (15, 29), Hodgkin's disease (7), acute infectious mononucleosis (16), and chronic active liver diseases (42). In these diseases, it was demonstrated that some but not all patients showed significantly low AMLR responses. In systemic lupus erythematosus (SLE), it was reported that the patients with active SLE showed markedly diminished AMLR responses, whereas the patients with inactive SLE showed a slight reduction in AMLR responses (1, 9, 28, 34).

In periodontal diseases, by using SPR as a measure of AMLR in most studies, the depression of the SPR was demonstrated in some types of periodontal diseases although the abnormalities in AMLR in relation to the types of periodontal diseases were still controversial. Tew et al. (41)

and Suzuki et al. (35) demonstrated the depression of the SPR only in young periodontitis patients (20 to 30 years) and in patients with generalized juvenile periodontitis, respectively. Osterberg et al. (23), however, reported that the patients with adult periodontitis but not with juvenile or rapidly progressive periodontitis showed a depressed SPR. In all of these studies, however, the distribution of individual AMLR responses was not clear and the phenotypic and/or functional alterations in the peripheral blood mononuclear cells in relation to AMLR responses were undefined. Therefore, the nature of AMLR in periodontal disease is still poorly understood. In this study, we examined the distribution of AMLR responses in 80 adult periodontitis patients and found that some but not all patients showed low AMLR responses. Since the patients in both groups were clinically diagnosed with adult periodontitis, it was not surprising that there were no significant differences in the mean age and the clinical parameters between low- and normal-AMLR patients.

The present results, however, indicated that periodontal treatment had modulated the AMLR responses in low-AMLR patients but not in normal-AMLR patients, although significant improvements in the clinical parameters were observed in both patient groups. The AMLR response in the low-AMLR patients increased after the initial periodontal treatment, and it appeared to restore normal responsiveness through the maintenance phase (Fig. 3). These findings are in accordance with the results of Tew et al. (41) and Osterberg et al. (23), who found that the SPR was increased to normal levels following the completion of the periodontal treatments. It should be mentioned here that Evans et al. (8) reported that a complete return to normal levels of SPR did not occur immediately after the initial periodontal treatment. Since AMLR has been suggested to be a possible indicator of immunoregulation in inflammatory diseases, the depressed AMLR responses in the low-AMLR patients might reflect changes in regulatory T-cell function induced by some state(s) of the periodontal diseases.

The AMLR is a proliferative response of T cells stimulated in vitro by autologous non-T cells in the absence of any antigen, although the immunological mechanisms have not yet been completely defined. In the AMLR, T cells are

TABLE 3. Phenotypic analysis[a] of T-cell fractions of the patients showing low or normal AMLR responses

Subject group	$CD4^+$ (%)	$CD8^+$ (%)	$CD45RA^+$ (%)	$CD4^+$ $CD45RA^+$ (%)[b]	$CD4^+/CD8^+$	AMLR (dpm)
Low-AMLR patients ($n = 11$)	39.5 ± 2.3	32.2 ± 2.7	38.2 ± 2.8	23.3 ± 2.3^c	1.33 ± 0.15	$5,213 \pm 774^d$
Normal-AMLR patients ($n = 11$)	41.8 ± 2.3	29.8 ± 2.0	44.9 ± 2.7	33.3 ± 3.4	1.48 ± 0.15	$22,699 \pm 2,786$
Healthy subjects ($n = 10$)	41.8 ± 3.4	32.2 ± 2.4	46.1 ± 2.8	35.6 ± 2.5	1.49 ± 0.19	$19,906 \pm 2,344$

[a] Values given are means $\pm$ SEM.
[b] The percentage in $CD4^+$ cells.
[c] Significantly lower than those in normal-AMLR patients and healthy controls ($P < 0.05$).
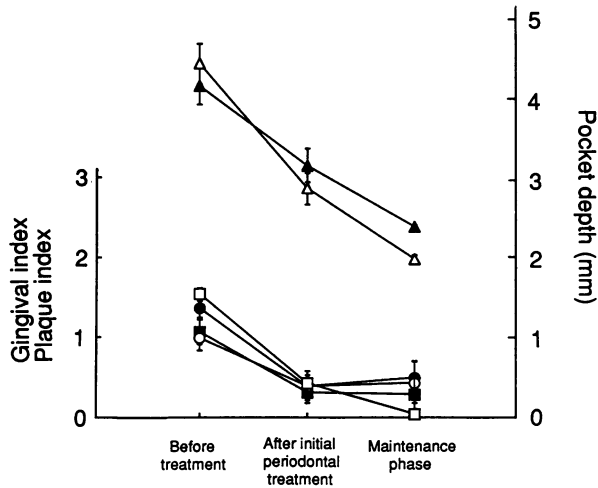[d] Significantly lower than those in normal-AMLR patients and healthy controls ($P < 0.01$).

FIG. 4. Effect of the periodontal treatment on the clinical parameters in the patients with adult periodontitis. The mean probing pocket depth, gingival index, and plaque index of both low-AMLR patients (△, ○, and □, respectively) and normal-AMLR patients (▲, ●, and ■, respectively) were examined just after the initial periodontal treatment and during the maintenance phase. During the periodontal treatments, significant improvements in all the clinical parameters tested were observed in both low- and normal-AMLR patients. However, no significant differences were found between the two patient groups with respect to any clinical parameter.

induced to proliferate by major histocompatibility complex class II antigens (HLA-DR) presented by non-T cells, mainly monocytes and B cells (2, 6, 12, 25). Phenotypically, no significant differences in the expression of HLA-DR in monocytes and B cells between the two patient groups were observed (Table 2). Therefore, it is unlikely that low AMLR responses could be attributable to an alteration in the expression of the major histocompatibility complex class II antigens on the non-T-cell fraction in the low-AMLR patients.

Recently, it has become evident that the $CD4^+$ lymphocytes can be further subdivided into functionally and phenotypically distinct populations of cells on the basis of their expression of CD45RA (18, 27, 38). The $CD4^+$ $CD45RA^+$ cells have been demonstrated to proliferate preferentially in response to autologous non-T cells (17, 39). It was also demonstrated that $CD4^+$ $CD45RA^-$ cells as well as $CD4^+$

TABLE 4. Production of IL-2[a] in the AMLR supernatants from adult periodontitis patients showing low or normal AMLR responses

Subject group	IL-2 production (dpm)	(S.I.)[b]	AMLR (dpm)
Low-AMLR patients (n = 12)	780 ± 174[c]	2.14	7,102 ± 899[d]
Normal-AMLR patients (n = 14)	1,394 ± 277	6.15	24,610 ± 2,751
Healthy subjects (n = 12)	2,089 ± 670	7.97	26,347 ± 4,119

[a] Values given (ranges) are means ± SEM.
[b] Ratio of IL-2 activity in the supernatant of coculture (T cells + non-T-cells) to that of single culture (T cells alone).
[c] Significantly lower than those in normal-AMLR patients and in healthy controls (P < 0.05).
[d] Significantly lower than those in normal-AMLR patients and in healthy controls (P < 0.01).
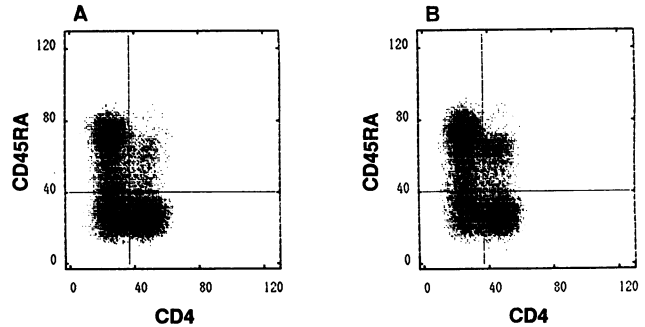

FIG. 5. Typical staining profile of the T-cell fraction from a low-AMLR patient (A) and a normal-AMLR patient (B). Cells were incubated with FITC-OKT4 and phycoerythrin-conjugated 2H4 for 20 min at room temperature. After being washed twice with phosphate-buffered saline, the cells were analyzed by the flow cytometer. The percentages of $CD4^+$ $CD45RA^+$ T cells of the low-AMLR and the normal-AMLR patients were 25.1 and 33.0%, respectively. The AMLR responses of these patients were 7,175 and 24,193 dpm, respectively.

$CD45RA^+$ cells could proliferate in response to allogeneic non-T cells (17, 18). In the present study, the phenotypic analysis of the T-cell fraction revealed that the percentage of $CD4^+$ $CD45RA^+$ T cells in low-AMLR patients was significantly lower than those in normal-AMLR patients and healthy control subjects. Furthermore, the allogeneic MLR of the T-cell fraction from low-AMLR patients was found to be normal (Fig. 6). The present findings on the phenotypic analysis of the T-cell fraction and the results in the allogeneic MLR in low-AMLR patients are in accordance with the results presented by Morimoto and coworkers (17, 18, 39) and strongly suggested that the low responsiveness in low-AMLR patients could be attributable to the reduction in the percentage of the $CD4^+$ $CD45RA^+$ T-cell population.

Recent studies regarding $CD4^+$ subsets defined with anti-CD45RA monoclonal antibody have revealed that $CD4^+$ $CD45RA^-$ cells exhibit a "memory" proliferative response to soluble recall antigen and provide help for antibody
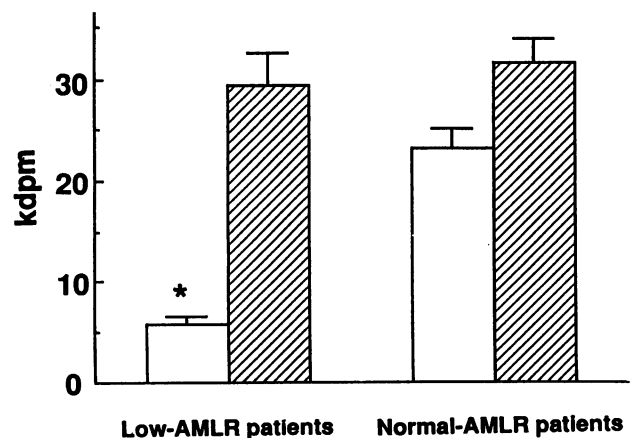

FIG. 6. AMLR and allogeneic MLR of the T-cell fractions from low- and normal-AMLR patients. T-cell fractions (1 × 10^5) were incubated with autologous (□) or allogeneic (▨) non-T-cell fractions (1 × 10^5) for 7 days. The allogeneic non-T-cell fractions used in this study were prepared from the healthy controls. A significantly lower AMLR response was observed in low-AMLR patients than that in normal-AMLR patients (*; P < 0.01).

production (18, 40), whereas CD4$^+$ CD45RA$^+$ cells have little helper function and seem to induce suppressor capability (18). Thus, Tedder and coworkers proposed that the CD4$^+$ CD45RA$^-$ helper cells were "memory" T cells derived from CD4$^+$ CD45RA$^+$ "naive" T-cell precursors and that the phenotypic and functional characteristics of these CD4$^+$ subsets were manifestations of their maturational stages (4, 40). In this study, the percentages of CD4$^+$ CD45RA$^+$ T cells as well as the AMLR response increased during the periodontal treatment in low-AMLR patients. A likely explanation for this finding may be that before treatment CD4$^+$ cells were actively differentiating into memory cell populations by the continuous stimulation of the local periodontopathic antigens and that the periodontal treatment decreased active stimulation, resulting in the gradual restoration of CD4$^+$ CD45RA$^+$ subsets to normal levels. However, further experiments will be required, since 42 of 80 patients with adult periodontitis in this study showed consistently normal AMLR responses and percentages of CD4$^+$ CD45RA$^+$ cells before and after periodontal treatments.

Low levels of IL-2 production in the AMLR were found only in low-AMLR patients (Table 4). Earlier studies demonstrated that CD4$^+$ cells could produce IL-2, which may support T-cell proliferation in the AMLR (25, 26, 33, 37, 42). However, Suzuki et al. (36) suggested that IL-3 but not IL-2 could induce proliferation of the responder cells in the AMLR. The present finding with IL-2 production in AMLR is in accordance with the results reported by Oen et al. (19). They found that some patients with juvenile rheumatoid arthritis had both a low AMLR and low levels of IL-2 production in the AMLR and suggested that the reduction of AMLR in these patients might be due to a decrease in IL-2 production and a decreased responsiveness to IL-2 as well. Takada et al. (37) also demonstrated depressed IL-2 production in the patients with SLE who showed low AMLR responses. Since CD4$^+$ CD45RA$^+$ cells have been demonstrated to proliferate preferentially in response to autologous non-T cells, it is likely that CD4$^+$ CD45RA$^+$ cells mainly produce IL-2 in the AMLR, which in turn induces proliferation of the cells responding to the factor. However, further studies will be required, since the possibility that IL-2 production was normal but was consumed in low-AMLR patients was not ruled out in this study.

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