

Conversion of the CD4⁺ T cell profile from T_{H2}-dominant type to T_{H1}-dominant type after varicella-zoster virus infection in atopic dermatitis

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Skin lesions of atopic dermatitis were examined for cytokine expression by reverse transcription-polymerase chain reaction. The profile of mRNA for various cytokines revealed that both T_{H1} and T_{H2} types of CD4⁺ T cells, probably including T_{H0} type, infiltrate into the skin lesion. We observed that atopic skin lesions improved after varicella infection. In such lesions, expression of T_{H1} type cytokines predominated. The peripheral blood T cells from atopic patients exhibited a differentiation into T_{H2} type cells upon in vitro stimulation with mite antigen. In contrast they differentiated into T_{H1} type cells upon stimulation by varicella antigen. Since IL-12 has been reported to switch the in vitro recall response of allergen-specific T cells of atopic donors from a T_{H2}- to a T_{H1}-like phenotype, we examined its local production in varicella lesions. IL-12 p35 and p40 mRNA were expressed in fresh lesions. Peripheral blood mononuclear cells from atopic patients expressed p40 mRNA upon in vitro stimulation with live varicella zoster virus, but they did not show p40 mRNA without stimulation. This finding suggested that in atopic skin lesions containing the virus, IL-12 was produced and the cell type was changed to T_{H1} type-predominance. These results suggested that patients with atopic dermatitis always have highly reactive CD4⁺ T cells infiltrating into their skin, and that the switch to T_{H1} or T_{H2} dominance is related to whether the lesion is improved or exacerbated. (*J Allergy Clin Immunol* 1997;100:274-82.)

Key words: Atopic dermatitis, varicella, IL-12, cytokines, T_{H1}/T_{H2}

Atopic dermatitis (AD) is characterized by eczema with itching, with repeated exacerbation and remission. Many cases of AD frequently show a high level of IgE antibody production.¹ It appears that AD has been regarded as a CD4⁺ T cell-mediated disease because immunohistologic examination of skin lesions revealed

Abbreviations used

AD:	Atopic dermatitis
ELISPOT:	Enzyme-linked immunospot
IFN- γ :	Interferon-gamma
mAb:	Monoclonal antibody
PBMCs:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
RT-PCR:	Reverse-transcription polymerase chain reaction
VZV:	Varicella zoster virus

the infiltration of CD4⁺ T cells^{2,3} and because immunosuppressive agents such as cyclosporine and tacrolimus are markedly effective.^{4,5}

Since the existence of functionally distinct subsets of CD4⁺ T cells, T_{H1}/T_{H2}, was revealed by Mosmann et al.,⁶ hyperfunction of T_{H2}-type cells⁷⁻⁹ in AD patients has been reported, and allergen-specific T_{H2}-like clones have been established from skin lesions^{10,11} or peripheral blood lymphocytes of patients.¹² Based on these findings, AD is considered to be a disease mediated by relatively hyperfunctioning T_{H2}-type cells caused by an imbalance of T_{H1}/T_{H2}.^{13,14} However, the mechanism for repeated exacerbation and T_{H1}/T_{H2} imbalance still remains unclear.

We have observed that many cases of refractory AD did improve several months after viral infections associated with eruption such as varicella and Kaposi's varicelliform eruption. Varicella shows more severe manifestations in children with AD than in those without AD.^{1,15} This finding implied to us that there may be some immunologic change in the AD lesion after varicella. In order to determine this possibility, we examined the changes in CD4⁺ T cell subsets at sites of AD exanthema and investigated the factors relating to the remission of AD after infection.

METHODS

Skin biopsy specimens and peripheral blood mononuclear cells (PBMCs)

Skin biopsy specimens were obtained after informed consent from ten patients with AD, from three of these ten patients

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0091-6749/97 \$5.00 + 0 1/1/81773

whose AD skin lesions improved after contracting varicella, and from ten patients with varicella who had no history of atopic disease. After being embedded in Tissue-Tek 4583 OCT medium (Miles, Elkhart, Ind.), specimens were snap frozen in liquid nitrogen and stored at -80°C . Peripheral blood was obtained from patients with AD and normal controls who had no personal history of atopic disease. PBMCs were isolated on a Ficoll-Paque (Pharmacia Biotechnology AB, Uppsala, Sweden) gradient.

RNA isolation and cDNA synthesis

Total RNA was extracted from each biopsy specimen by the method of Chomczynski and Sacchi¹⁶ with some modification. To facilitate the rapid lysis of the cells isolated from tissues, 50 pieces of 4 μm cryostat sections were suspended in 4 mol/L guanidinium isothiocyanate buffer. The samples were treated with DNase (Promega Co., Madison, Wis.) for 60 minutes at 37°C . RNase inhibitor (Boehringer Mannheim Co., Indianapolis, Ind.) was present during all enzymatic manipulations of RNA. cDNA was synthesized from oligo-dT (Pharmacia) primed RNA by incubation at 43°C with Moloney murine leukemia virus (MMLV) reverse transcriptase (Gibco BRL, Life Technologies, Inc., Gaithersburg, M.D.) and 0.5 mmol/L deoxyribonucleoside triphosphate (dNTP) (Pharmacia) for 1 hour.

Amplification of cDNA by polymerase chain reaction (PCR)

PCR was performed as previously described.^{17,18} The PCR reaction mixture consisted of 10 mmol/L of Tris hydrochloride, pH 9.0, 50 mmol/L of potassium chloride, 2.5 mM of magnesium chloride, 0.1% (W/V) gelatin, 0.2 mmol/L of dNTPs, 25 pM of forward and reverse (5' and 3' oligonucleotide) primers, and 2.5 U of *Taq* polymerase (Promega). cDNA was amplified by PCR by means of oligonucleotide primers specific for β -actin, CD3 δ , IL-2, interferon (IFN)- γ , IL-4, IL-5 and p35 and p40 chains of IL-12. A DNA thermal cycler 480 (Perkin-Elmer Cetus, Norwalk, Conn.) was used for 35 cycles of denaturation at 94°C for 1 minute and annealing/extension at 55°C (in the case of IL-2 and IFN- γ) or 65°C for 2 minutes (in the case of β -actin, CD3 δ , IL-4, IL-5, and IL-12 p35 and p40).

The sequences of cytokine specific primer pairs were as follows: β -actin; 5'-GTGGGGCGCCCCAGGCACCA-3' and 5'-CTCCTTAATGTACGCACGATTTC-3', CD3 δ ; 5'-CTGGA-CCTGGGAAAACGCATC and 5'-GGTGGCTGTACTGAG-CATCATC, IL-2; 5'-ACTCACCAGGATGCTCACAT and 5'-AGGTAATCCATCTGTTCAGA, IFN- γ ; 5'-AGTTATA-TCTTGGCTTTTCA and 5'-ACCGAATAATTAGTCAGCTT, IL-4; 5'-CTTCCCCCTCTGTTCCT and 5'-TTCCTGTC-GAGCCGTTTCAG, IL-5; 5'-ATGAGGAT GCTTCTG-CATTTG and 5'-TCAACTTTCTATTATCCACTCG GTGT-TCATTAC, IL-12 p35; 5'-CCTCAGTTTGGCCAGAAACC and 5'-GGTCTTTCTGGAGGCCAGGC, IL-12 p40; 5'-CCAA-CAACTTGCAGCTGAAG and 5'-TGGGTCTATTCCGTTGT-GTC.

The PCR product was subjected to electrophoresis on 1.5% agarose gel and visualized under ultraviolet illumination after being stained with ethidium bromide.

Radioactive hybridization of the PCR product

To confirm the specificity of amplification of p35 and p40 mRNA, the PCR product was transferred to Hybond-N nylon membranes (Amersham International Plc., Amersham, U.K.) and hybridized with a radioactive oligonucleotide probe com-

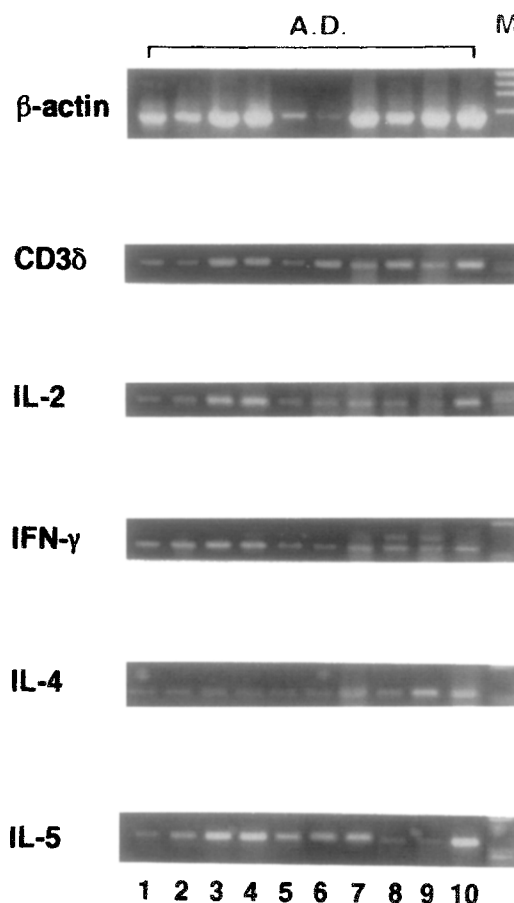


FIG. 1. Cytokine profiles of AD lesions. Erythematous skin lesions of AD were examined for cytokine mRNA expression by means of RT-PCR. Total RNA extracted from each biopsy specimen was reverse transcribed to cDNA. cDNA for β -actin, CD3 δ , IL-2, IFN- γ , IL-4, and IL-5 was amplified by PCR. DNA size markers (ϕ X174/*Hae*III digest) are shown in last lane on right in both panels. Predicted sizes of PCR products for β -actin, CD3 δ , IL-2, IFN- γ , IL-4, and IL-5 were 548, 316, 266, 356, 317, and 405 bp, respectively.

plementary to sequences internal to those recognized by the P35- and p40-specific primers. The sequences of the probes for IL-12 p35 and p40 were ACTCTAGAATTTTACCCITG-CACT and TGC GTTCAGGTCAGGGCAAGAGC, respectively. The probe was labeled at the 5' end with T4 polynucleotide kinase (Boehringer) and [^{32}P] γ adenosine triphosphate (Amersham). Blots were hybridized with the probe for 16 hours at 65°C , washed for 15 minutes with $2\times$ SSC and 0.1% SDS at 50°C , then exposed to Kodak X-OMAT film at -80°C using intensifying screens.

Enzyme-linked immunospot (ELISPOT) assay

To enumerate IL-4, IL-5, and IFN- γ -producing cells, an ELISPOT assay was carried out as described by Kawamura et al.¹⁹ with some modification. PBMCs were suspended in complete medium that consisted of Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco) supplemented with 10% fetal calf serum (Gibco), 50 μg of gentamicin/ml, 5×10^{-5} mol/L 2-ME, 5 gm of HEPES/L, and 2 gm of sodium bicarbonate/L. PBMCs (2×10^6 cells/ml) were cultured in 24-well flat-bottomed plates (Costar, Cambridge, Mass.) with or with-

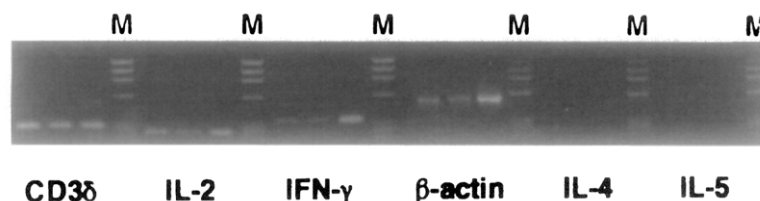


FIG. 2. Cytokine profiles in atopic skin lesions after these patients contracted varicella. Atopic skin lesions subsequently improved. Total RNA extracted from each biopsy specimen was reverse transcribed to cDNA. cDNAs for β -actin, CD3 δ , IL-2, IFN- γ , IL-4, and IL-5 were amplified by PCR. Predicted sizes of PCR products for β -actin, CD3 δ , IL-2, IFN- γ , IL-4, and IL-5 were 548, 316, 266, 356, 317, and 405 bp, respectively. Expression patterns of cytokines before contracting varicella are shown in lanes 1 to 3 of Fig. 1.

out antigen at 37°C for 48 hours. Mite antigen (10 μ g/ml *D. Pteronyssinus*, Greer Lab. Inc., Lenoir, N.C.) or varicella zoster virus (VZV, 100 PFU/ml; Varicella Vaccine Live Attenuated "Biken," The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan) were used as stimulation antigens.

After being washed with RPMI 1640 medium, the cells were resuspended in complete medium and placed at concentrations of 5×10^2 to 5×10^5 cells/100 μ l per well in a nylon-based 96-well plate (MAIPS 4510; Millipore Co. Ltd., Tokyo, Japan) which had been coated with specific monoclonal antibody (mAb) and blocked with complete medium. Coating with each mAb was done by the following concentrations: 15 μ g/ml solution of mouse antihuman IL-4 mAb (Genzyme, Cambridge, Mass.), 10 μ g/ml solution of mouse antihuman IL-5 mAb (Genzyme), and 10 μ g/ml solution of mouse antihuman IFN- γ mAb (Genzyme). After a further incubation for 24 hours, the plates were thoroughly washed with phosphate-buffered saline solution (PBS) 0.05% Tween-20 and incubated with rabbit antihuman IL-4 polyclonal antibody (1/700, Genzyme), rabbit antihuman IL-5 polyclonal antibody (1/100, Genzyme), or rabbit antihuman IFN- γ polyclonal antibody (1/250, Genzyme) for 90 minutes. The plates were repeatedly washed and incubated with peroxidase-conjugated goat antirabbit IgG (1/800, Cappel Organon Teknika Co., Durham, N.C.) for 90 minutes. After four washings with PBS- 0.05% Tween 20, spots representing IL-4, IL-5, or IFN- γ -producing cells were developed by the addition of 100 μ l of 3-amino-9-ethylcarbazole (0.27 mg/ml) in 0.1 mol/L phosphate-citrate buffer, pH 5.0. The spots were counted under a dissecting microscope.

Induction of IL-12 p40 mRNA expression of PBMCs by VZV antigen stimulation

PBMCs (2×10^6 cells/ml in complete medium) obtained from atopic or nonatopic donors were incubated in 24-well flat-bottomed plates with or without 100 PFU/ml VZV for 5 hours. The cells were then washed with PBS, and total RNA was extracted. IL-12 p40 mRNA expression was examined by using reverse transcription (RT)-PCR.

Statistics

Statistical significance of the data was determined by Student's *t* test, and *p* < 0.05 was taken as significant.

RESULTS

Cytokine profiles in AD lesions

Lesions from the acute phase of AD were examined for cytokine expression by RT-PCR. We used mRNA for β -actin and CD3 δ as the markers for all total cellular RNA and T cells, respectively. The levels of mRNA

expression reflected individual differences. Because the band intensity of PCR products for β -actin were different from sample to sample, the amount of PCR product for each cytokine cannot be compared directly by its intensity. Nevertheless, this result suggested that cytokines derived from both T_{H1} and T_{H2} type CD4⁺ T cells, probably including T_{H0} type, infiltrate into atopic skin lesions (Fig. 1).

Alteration of cytokine profiles in atopic lesions after contracting varicella

We observed that acute atopic skin lesions frequently improve when AD patients contract varicella. Usually, 7 to 10 days after the appearance of vesicles of varicella, acute erythematous lesions of AD turned to slightly pigmented macules with a few scales. In specimens obtained from such lesions, IL-4 and IL-5 mRNA expression were no longer detected and expression of T_{H1} type cytokines predominated with the cycles used (Fig. 2). Expression patterns of cytokines of these three patients before infection are shown in lanes 1 to 3 in Fig. 1. It was difficult to compare the amount of PCR product in Fig. 2 with that in Fig. 1. However, the absence of IL-4 and IL-5 mRNA expression was clear and we have confirmed their absence even after 50 cycles of amplification (data not shown). This result suggested that the shift to T_{H1} predominance may take place in AD lesions after varicella infection.

Enumeration of IL-4-, IL-5- and IFN- γ -producing cells by ELISPOT assay

The cytokine expression patterns of atopic skin lesions varied depending upon clinical improvement or exacerbation. Differentiation patterns of CD4⁺ T cell subsets after in vitro stimulation with different antigens were determined with PBMCs from three patients with AD that showed improvement after varicella and from three healthy volunteers as controls. ELISPOT assay was used to enumerate IL-4-, IL-5- and IFN- γ -producing cells. In many cases, as well known in clinical dermatology, atopic exacerbated lesions are induced by in vivo local application of mite antigens. In atopic patients examined in the present study, a prick test and a RAST with mite (*D. Pteronyssinus*) antigen showed a strongly positive result. As shown in Fig. 3, A, the peripheral blood T cells from atopic patients differentiated into IL-4- and IL-5-

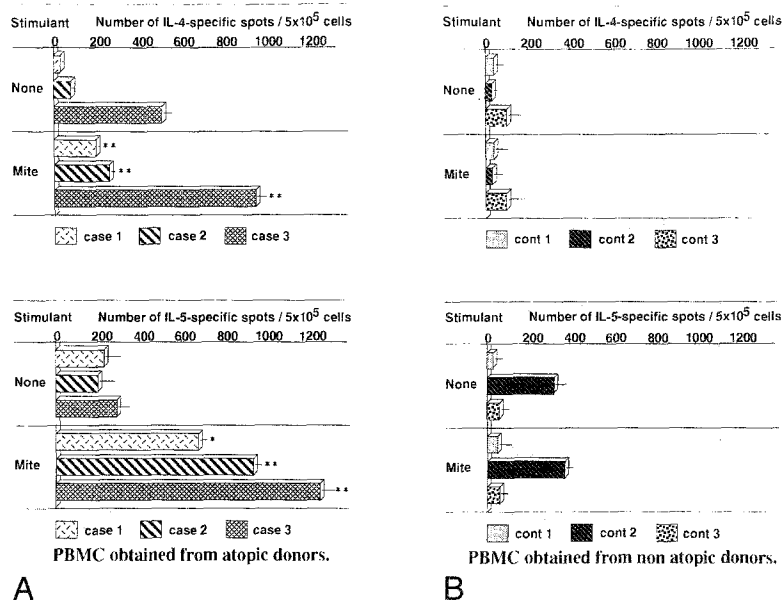


FIG. 3. Enumeration of IL-4- and IL-5-producing cells by ELISPOT assay. PBMCs obtained from **A**, three AD patients showing improvement after varicella (*cases 1 to 3*) and from **B**, three healthy volunteers as controls (*cont 1 to 3*) were stimulated with 10 μ g mite antigen/ml for 48 hours. After cells were washed, serial dilutions of cell suspension were transferred to nylon-based plates that had been coated with anti-IL-4 or anti-IL-5 mAb, and further incubated for 24 hours. Plates were then washed and incubated with anti-IL-4 or anti-IL-5 polyclonal antibodies. Plates were again washed and incubated with peroxidase-conjugated 2nd antibody. Spots representing IL-4- or IL-5-producing cells were visualized by adding 3-amino-9-ethylcarbazole in 0.1 mol/L phosphate-citrate buffer. Data are expressed as mean of three wells $\pm$ SD. * $p < 0.05$, ** $p < 0.001$ p -value compared with control (*none*) every individual case.

producing cells upon in vitro stimulation with mite antigen, while such a differentiation was not observed in T cells from nonatopic donors (Fig. 3, *B*). In contrast, as shown in Fig. 4, the peripheral blood T cells from atopic patients did not show the differentiation into IL-4- and IL-5-producing cells upon in vitro stimulation with VZV antigens. Instead, peripheral blood T cells from such atopic patients differentiated into IFN- γ -producing cells upon stimulation with VZV antigens, and these differentiations were increased by mixing the mite and VZV antigens (Fig. 5). Such differentiation into IFN- γ -producing cells was not induced by stimulation with mite antigen.

Detection of mRNA for IL-12 p35 and p40 in varicella lesions

This experiment was conducted to determine the mechanisms involved in a switch to T_{H1} predominance after contracting varicella. IL-12 has been reported to switch the in vitro recall response of allergen-specific T cells of atopic donors from T_{H2} - to T_{H1} -like phenotype.²⁰ Skin biopsy specimens were obtained from ten patients with varicella who had no personal history of atopic disease, and we examined the varicella lesions for IL-12 mRNA expression by RT-PCR. Our IL-12 primers occasionally amplified an extra band; therefore southern blot analysis was used to detect IL-12 p35- or p40-specific mRNAs. As shown by hybridization, expression of IL-12 p35 mRNA was observed in all of ten patients

with varicella lesions (Fig. 6). IL-12 p40 mRNA was detected in varicella lesions of eight patients (lanes 1 to 8) whose specimens were obtained from lesions within 4 days after the appearance of vesicles. The remaining two specimens (lanes 9 and 10) were obtained 7 to 10 days after the appearance of vesicles.

Induction of IL-12 p40 mRNA expression by PBMC by means of VZV antigen stimulation

The expression of IL-12 mRNA was observed in nonatopic varicella lesions. We next examined whether VZV antigen could induce IL-12 in patients with AD by using p40 mRNA expression as an indicator for recent stimulation with VZV antigen. PBMCs were obtained from five patients with AD and from five normal controls who had no personal history of atopic disease. As shown in Fig. 7, *A*, the PBMCs from atopic patients expressed IL-12 p40 mRNA upon in vitro stimulation with VZV antigen, whereas the PBMCs from normal controls expressed IL-12 p40 mRNA spontaneously (Fig. 7, *B*). By using cells from cases in which a sufficient number was available, we have determined IL-12 (p70) levels in the culture supernatant with ELISA. The mean values for the IL-12 p70 protein levels of nonstimulated condition were 4.5 pg/ml ($n = 4$) in normal controls and less than 4 pg/ml ($n = 2$) in AD. Mean values for the p70 levels of stimulation with VZV antigen were 8.2 pg/ml ($n = 4$) in normal controls and 5.6 pg/ml ($n = 2$) in AD.

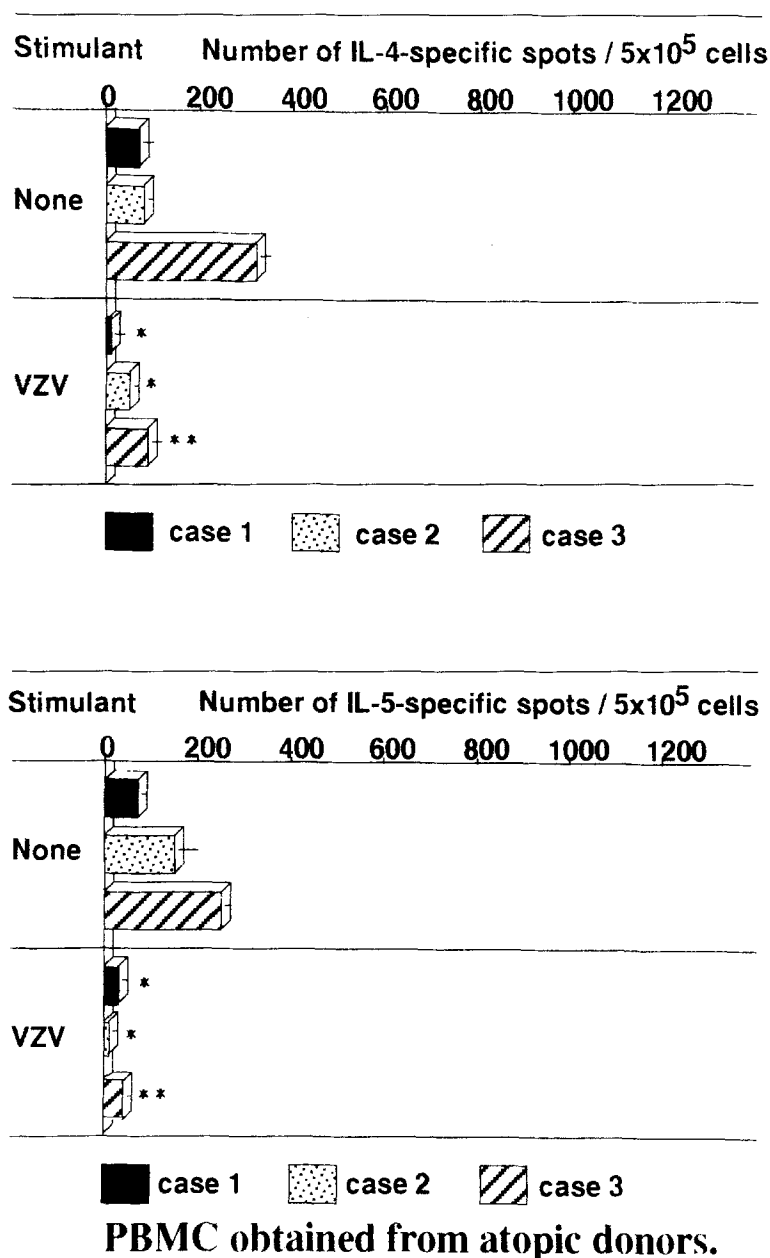


FIG. 4. Enumeration of IL-4- and IL-5-producing cells by ELISPOT assay. PBMCs obtained from three AD patients showing improvement after varicella (cases 1 to 3) were stimulated with 100 PFU VZV/ml for 48 hours, and IL-4- and IL-5-producing cells were counted as described for Fig. 3. Data are expressed as mean of three wells $\pm$ SD. * $p < 0.05$, ** $p < 0.001$ p -value compared with control (none) every individual case.

DISCUSSION

We clinically observed an improvement of severe atopic skin lesions several months after the onset of varicella. A similar finding was reported previously by Lacour.¹⁴ The improved state continued for at least 2 to 3 months, up to 5 to 6 months. None of the patients, however, showed complete healing during this period. These phenomena were observed after viral infections associated with eruption such as varicella, measles, and Kaposi's varicelliform eruption without secondary infec-

tion, but were not observed in any other viral infections without eruption such as influenza. All of our patients were adults with varicella, whose tuberculin reactions were positive. Therefore, cellular immunologic competence during varicella was determined by the tuberculin reaction. The reaction did not change to negative during varicella (data not shown). It is generally known that cellular immunity is affected during the course of infections with a variety of viruses. From these facts, the above phenomenon (no change in the tuberculin reac-

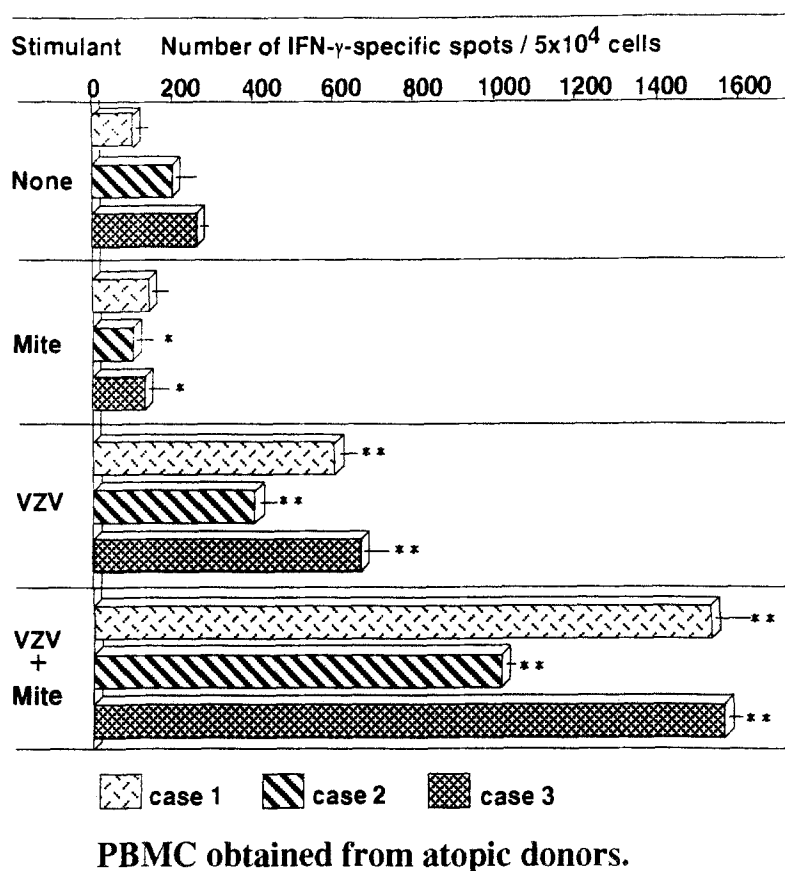


FIG. 5. Enumeration of IFN- γ -producing cells by ELISPOT assay. PBMCs obtained from three AD patients showing improvement after varicella (cases 1 to 3) were stimulated with 10 μ g/ml mite antigen or 100 PFU/ml VZV or mixing both for 48 hours. IFN- γ producing cells were counted as described for Fig. 3. Data are expressed as mean of three wells $\pm$ SD. * p < 0.05, ** p < 0.001 p -value compared with control (*none*) every individual case.

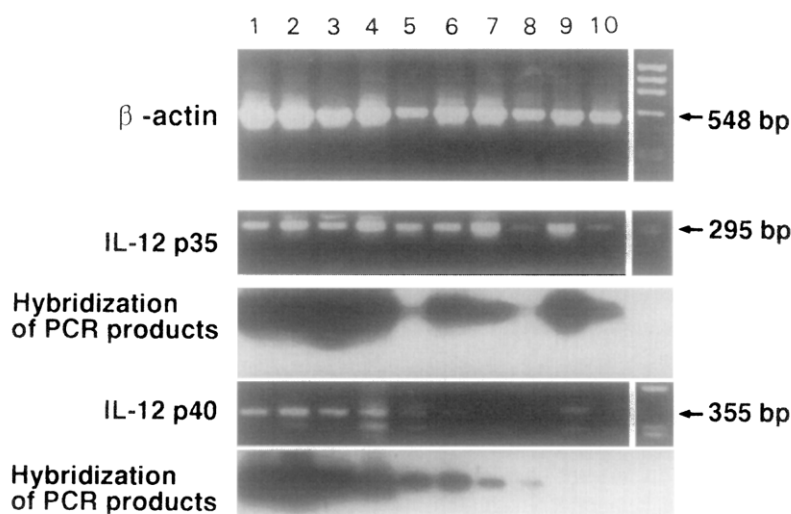


FIG. 6. PCR detection of mRNA for IL-12 p35 and p40 in varicella lesions. Skin biopsy specimens were obtained from lesions within 4 days after appearance of vesicles (*lanes 1 to 8*) and from crusting lesions 7 to 10 days after appearance of vesicles (*lanes 9 and 10*). RNA from each varicella lesion was reverse transcribed into cDNA and amplified by PCR with primers specific for β -actin and IL-12 p35 and p40. DNA size markers (ϕ X174/*Hae*III digest) are shown in last lane on right. PCR products for IL-12 p35 and p40 cDNA were transferred to nylon membranes and hybridized to an internal ³²P-labeled probe.

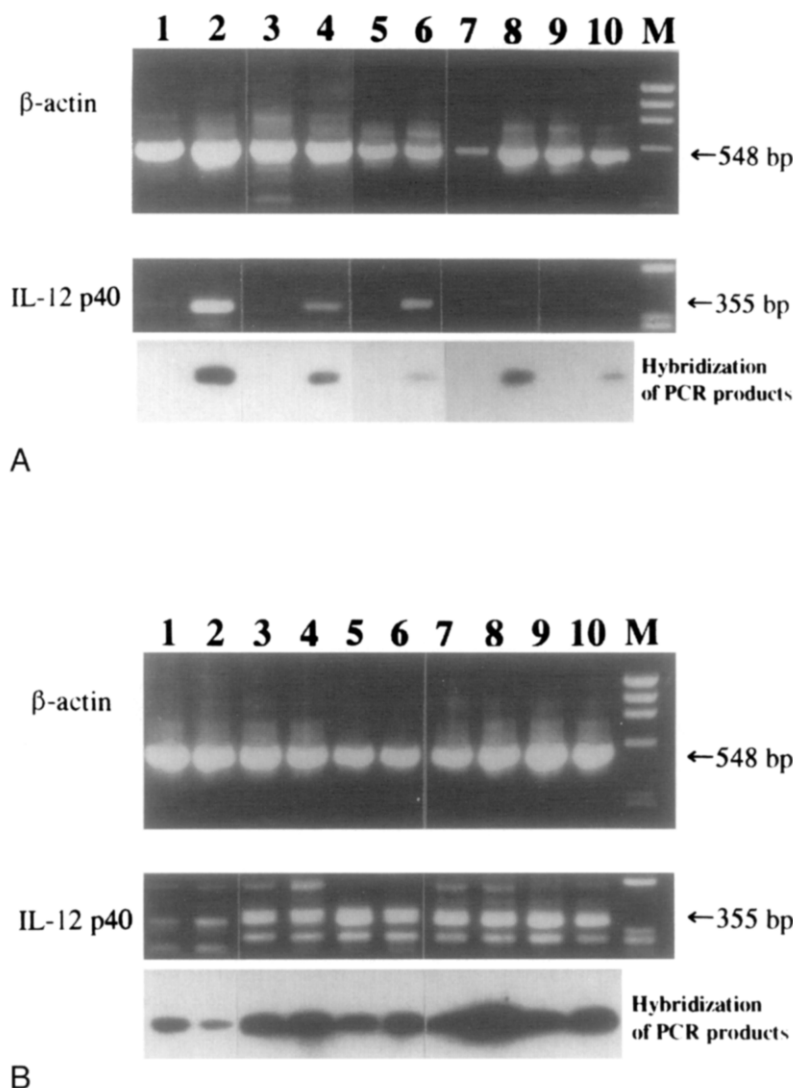


FIG. 7. IL-12 p40 mRNA production induced by VZV. PBMCs from atopic or nonatopic donors were cocultured for 5 hours in medium alone or medium containing VZV. RNA was reverse transcribed into cDNA and amplified by PCR with primers specific for β -actin and IL-12 p40. DNA size markers (ϕ X174/*HaeIII* digest) are shown in last lane on right. PCR products for IL-12 p40 cDNA were transferred to nylon membranes and hybridized to an internal ^{32} P-labeled probe. Even-numbered lanes show results for VZV-stimulated cells. Odd-numbered lanes, show results for nonstimulated cells. **A**, PBMCs from atopic donors. **B**, PBMCs from nonatopic donors.

tion during varicella) was considered to be unique for some kinds of viral infections including varicella.

Mosmann et al.^{6,21} reported that murine CD4⁺ T cells can be divided into two functionally different subsets, T_{H1} and T_{H2}. Since then, the roles of T cell subsets and cytokines in bacterial, parasitic, protozoal, and viral infections have been clarified in murine models of infections.²²⁻²⁸ Del Prete et al.²⁹ and Romagnani³⁰ showed that CD4⁺ T cells of the peripheral blood are divided into T_{H1} and T_{H2} subsets according to the difference in the stimulation antigen in human beings as well. The significance of T_{H1} and T_{H2} subsets in human morbid conditions and the involvement in human diseases has been investigated in leprosy, leishmaniasis,

allergic conjunctivitis, and AD.^{8,9,31-34} With regard to leprosy and leishmaniasis, the pattern of mRNA expression of cytokines at the local lesion indicated that the T_{H2} subset was selectively activated in lepromatous-type leprosy, whereas the T_{H1} subset was activated in tuberculoid-type leprosy and localized cutaneous leishmaniasis.^{31,32} However, in patients with allergic conjunctivitis and AD, cloning of allergen-specific T cells indicated that T_{H2} subset was selectively activated.^{8,33,34}

In the present study, we have investigated the pattern of local expression of cytokines in erythematous skin lesions of AD. The results indicated infiltration of both subsets of T_{H1} and T_{H2}, probably including also T_{H0}, into the lesions. The pattern of local expression of

cytokines at the AD site showing an improvement following the onset of varicella was T_{H1} dominant, suggesting switching into $CD4^+$ T cell subsets. To clarify the relationship between improvement of AD lesions after the onset of varicella and the switching of $CD4^+$ T cell subsets, we determined the difference in the pattern of differentiation of $CD4^+$ T cell subsets against antigen stimulation by ELISPOT assay. The lymphocytes from AD patients differentiated into T_{H2} -type cells after stimulation with mite antigen, an antigen responsible for AD lesions, but differentiated into T_{H1} -type cells after stimulation with VZV. These results suggested that $CD4^+$ T cells show variable differentiation according to the type of antigen. Differentiation into T_{H1} -type cells was increased by culturing with both antigens simultaneously, suggesting differentiation of cells other than varicella reactive T cells.

In recent years, it has become clear that IL-12 is a cytokine inducing T cell differentiation into T_{H1} -type cells. IL-12 is believed to act on T cells and NK cells to facilitate the IFN- γ production and to induce differentiation into T_{H1} -type.³⁵⁻³⁷ It is a disulfide-linked heterodimeric cytokine composed of two subunits of molecular masses of 40KD (p40) and 35KD (p35),^{38,39} and the p70 heterodimer is the only functional molecule. Since p70 mRNA cannot be detected by present methodology, we have determined the p40 mRNA level to quantify IL-12 expression. The local expression of IL-12 at the lesion site has been investigated for tuberculous and lepromatous lesions by Zhang et al.⁴⁰ and Sieling et al.,⁴¹ respectively. The level of IL-12 at the lesions determined by bioactivity has been reported to be consistent with the level of mRNA expression of p40. Expression of p35 was detected not only at the fresh varicella lesions but also at the site of a crusting varicella lesion that developed at least 1 week after the appearance of a vesicle, whereas p40 was detected at the site of a fresh lesion alone which contained VZV. Therefore, using expression of p40 as the indicator, we investigated the possibility that IL-12 is induced by stimulation with VZV in patients with AD. It became clear that IL-12 is indeed induced by in vitro stimulation with the virus in such patients as well. On the other hand, nonatopic healthy persons showed a spontaneous induction of IL-12 p40 under the stimulation-free condition. When the heterodimeric p70 protein was measured by ELISA, it was found to be increased by stimulation with VZV.

Manetti et al.²⁰ cultured lymphocytes from an AD patient in the presence and absence of IL-12 and found an induction of allergen-specific T_{H2} clone in the absence of IL-12 and of a T_{H1} -type clone in the presence of IL-12. They concluded that T_{H0} cells mature and differentiate into IFN- γ -producing cells by virtue of IL-12 regardless of the antigen specificity. With respect to the improvement of AD that we observed after the occurrence of varicella, a similar mechanism may have been involved in vivo. In other words, multiple vesicles containing VZV may have developed along with the occurrence of varicella, and AD may have improved because

of the T_{H1} -dominant differentiation of skin-infiltrating T cells as a result of local production of IL-12 by stimulation with VZV in the vesicles. In addition, the induced IL-12 may also have suppressed IgE production and proliferation of T_{H2} cells through the increased IFN- γ production and suppressed IL-4.⁴²⁻⁴⁴ The IL-12 may also have suppressed the increase in eosinophils and mast cells at the site of lesions.⁴⁵

The importance of IL-12 as a costimulatory factor for T_{H1} clones might be relevant to the T_{H1}/T_{H2} imbalance that occurs in various disease states.^{22,23} Some studies using peripheral lymphocytes from AD patients have shown spontaneous expression of IL-4 mRNA⁷ and reduced IFN- γ secretion.⁴⁶⁻⁴⁸ We showed spontaneous expression of IL-12 p40 in PBMCs from nonatopic healthy persons under the stimulation-free condition and the expression of IL-12 p40 could be induced by stimulation with VZV in AD patients, in spite of the absence of spontaneous expression of IL-12 p40 in these patients. This is an interesting finding in a view of the imbalance of T_{H1}/T_{H2} in AD patients.

The findings shown in this study suggested that patients with AD always have highly reactive $CD4^+$ T cells infiltrating into the skin lesion, and that the switch to T_{H1} or T_{H2} dominance is related to the outcome of the lesion: improvement or exacerbation.

We thank Dr. Masahiro Yamamura (Okayama University) for help in designing the cytokine primers and probes, Dr. Yasuko Yogi (National Institute for Leprosy Research, Tokyo) for excellent technical advice, and Yuko Hamada for technical assistance.

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