

Original article

CD30 expression on circulating memory CD4⁺ T cells as a Th2-dominated situation in patients with atopic dermatitis

Th2 clones have been reported to express CD30 preferentially, but whether T cells producing Th2-type cytokines may favor CD30 expression in the *in vivo* state remains unknown. We investigated the expression of CD30 on circulating T cells in atopic dermatitis (AD) as a Th2-dominated disorder. Peripheral blood mononuclear cells were prepared from 51 AD patients and 14 nonatopic controls, and their phenotypes were analyzed with flow cytometry. Cytokine production by stimulated CD4⁺ T cells was also assessed by the single-cell-staining method. Flow cytometric analysis clearly revealed that CD30⁺ T cells were identifiable in the blood of AD patients with greater frequency compared to controls. The important finding was that CD30 expression was restricted to a small but substantial population of memory (CD45RO⁺) CD4⁺ T cells, but not CD8⁺ ones. In AD patients, it was demonstrated that the percentages of CD30⁺ cells within CD45RO⁺ CD4⁺ T cells correlated well with the disease severity, serum IgE levels, peripheral eosinophil counts, and tendency toward Th2-dominant cytokine pattern as determined by the ratio of interleukin-4 to interferon-gamma production. This study suggests that CD30 expression in circulating T cells might serve as an *in vivo* marker for the Th2-dominated condition.

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It is widely accepted that after activation, naive CD4⁺ T cells differentiate into two major effector subsets (Th1 and Th2) defined by the pattern of cytokines they produce. Th1 effector cells produce interferon-gamma (IFN- γ) and lymphotoxin, and control cell-mediated immune responses, whereas Th2 cells secrete interleukin (IL)-4, IL-5, and IL-13, which are involved in responses dominated by IgE production and eosinophil generation (1). The differentiation process of CD4⁺ T cells appears to be determined by the antigenic nature of activation and the cytokines present during the initiation of the T-cell response. The antigen-primed effector cells primarily develop in the secondary lymphoid organs, migrate into the circulating blood pool, and are eventually recruited to local inflammatory sites, where they exert individual effector functions. This current concept of immune regulation has led to the notion that the deviation to Th2-dominated responses in the host is involved in the pathogenesis of allergic diseases (2). Atopic dermatitis (AD) is a chronic inflammatory skin disease, often associated with increased levels of serum IgE and eosinophilia. Seemingly supporting the Th2

cell-predominance in AD, a number of studies have shown that allergen-specific T-cell clones of the Th2 type are obtainable from the peripheral blood (3), as well as the skin lesions, of AD patients (4).

It has been demonstrated that expression of CD30, a member of the tumor necrosis factor receptor superfamily (5), is closely associated with production of Th2-type cytokines in both CD4⁺ (6) and CD8⁺ T-cell clones (7). Furthermore, the interaction between CD30 and its ligand has been found to promote the *in vitro* development of antigen-specific CD4⁺ T cells into the Th2-type of T-cell phenotype (8). Although the association of expression of CD30 with the Th2-type response is deduced from the *in vitro* studies, additional evidence for *in vivo* involvement of CD30⁺ cells in allergic diseases has appeared from immunohistochemical findings of CD30 expression on CD4⁺ cells infiltrating into AD skin lesions (9). In addition, increased levels of soluble CD30 (sCD30), which is proteolytically released from CD30-expressing cells (10), have been observed in the serum of AD patients (11). However, the presence of identifiable T cells

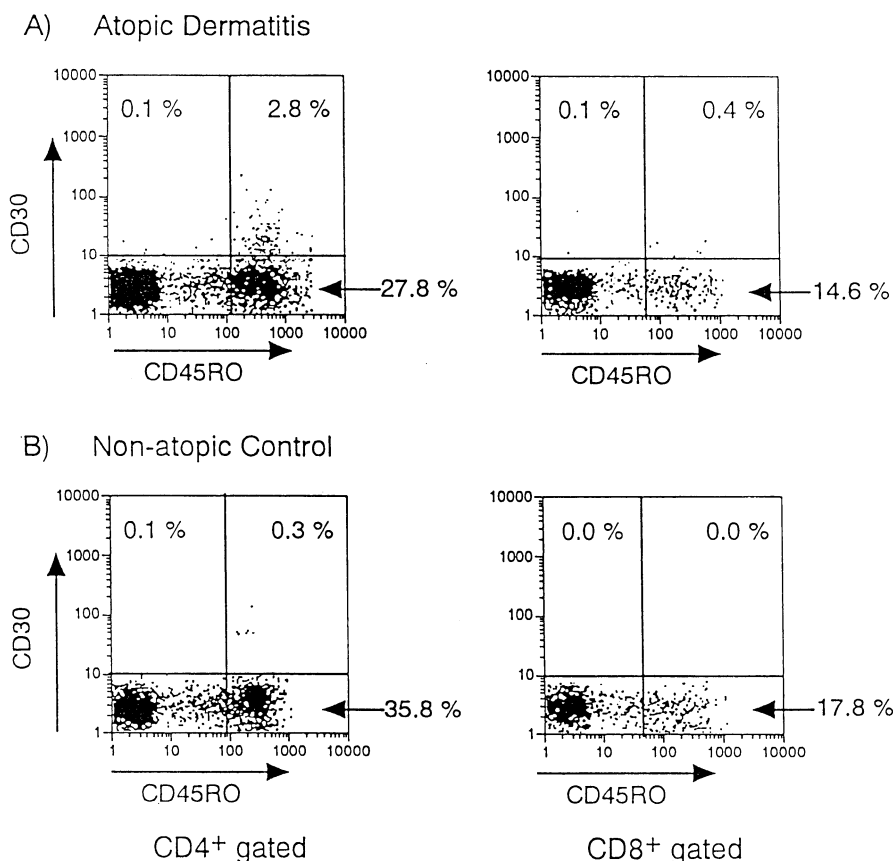


Figure 1. Flow cytometric detection of CD30-positive cells in peripheral blood cells. Freshly isolated PBMCs were stained with anti-CD30, anti-CD45RO, and anti-CD4 or anti-CD8 mAbs. Relation between CD30 and CD45RO expressed on CD4⁺ or CD8⁺ T cells was analyzed by flow cytometer. Staining of two representatives of AD (A) and nonatopic control (B) is shown.

expressing CD30 in the blood of AD patients remains to be determined. In this study, we examined whether a fraction of circulating T cells in AD as a Th2-dominated disorder might express CD30 as a function of the disease. We will show that CD30 can be expressed on some of the memory or antigen-primed (CD45RO⁺) CD4⁺ T cells in the peripheral blood, which seems to participate actively in the Th2-type response *in vivo*.

Material and methods

Subjects

Fifty-one patients (age: 0–27 years, mean 8.6 years) with AD diagnosed according to Hanifin & Rajka's criteria (12) were enrolled for this study. According to Rajka & Langeland's scoring criteria (13), the patients were subdivided into three groups: mild ($n=19$), moderate ($n=22$), and severe ($n=10$). Fourteen sex- and age-matched healthy donors without history of atopy were used as control. The study was conducted according to the ethical standards of Toyama Medical and Pharmaceutical University (Japan), which require informed consent from each subject.

Reagents

The following mAbs were purchased from Dako Japan (Kyoto, Japan): FITC-conjugated anti-CD30 (Ber-H2), PE-Cy5-conjugated anti-CD4 (MT310), PE-Cy5-conjugated anti-CD8 (DK25), PE-conjugated CD45RO (UCHL-1), and PE-conjugated CD20 (B-Ly1). PE-conjugated anti-CD16 mAb (3G8) was obtained from Immunotech (Marseille, France). Anti-CXCR3 (1C6, mouse IgG1) mAb was generously provided by Leukosite (Cambridge, MA, USA). Anti-CCR4 mAb (KM2160, mouse IgG1), a kind gift from Dr K. Matsushima (University of Tokyo, Tokyo, Japan), has been established and characterized as described previously (14). PE-conjugated antimouse IgG1 Ab was obtained from Southern Biotechnology Associates (Birmingham, AL, USA). Chemicals for intracellular cytokine staining and mAbs against IL-4 (8D4-8) and IFN- γ (4S.B3) were purchased from Beckton Dickinson (Franklin Lakes, NJ, USA). Other chemicals were obtained from Sigma (St Louis, MO, USA).

Flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were

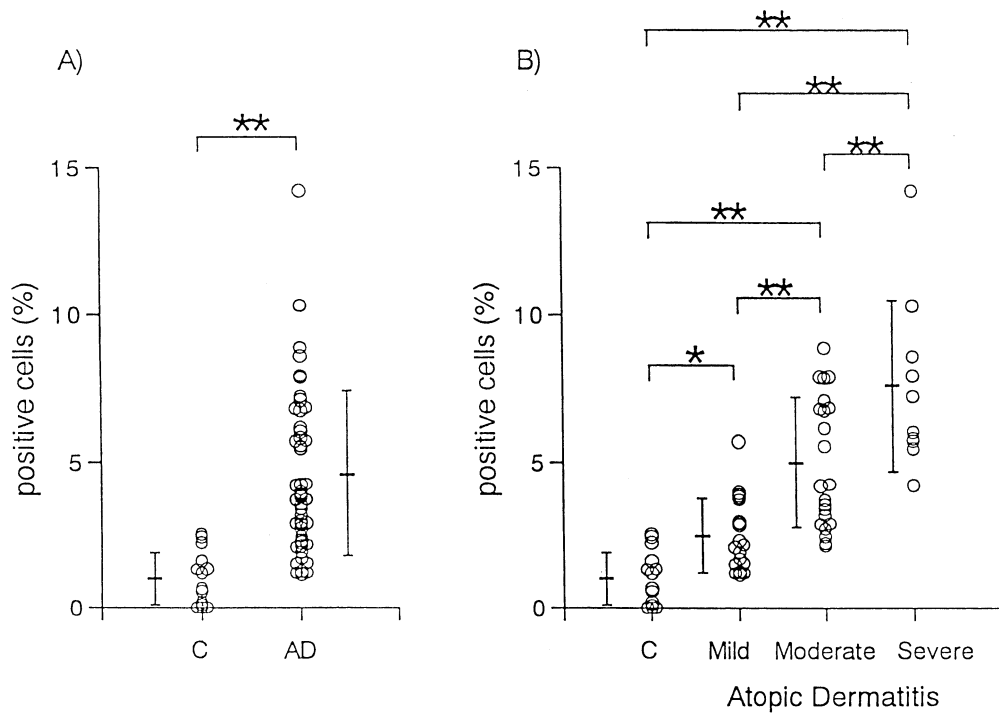


Figure 2. CD30 expression on T cells in AD. Mean frequency of CD30⁺ cells in CD4⁺ CD45RO⁺ T cells in AD patients and nonatopic controls (A), and in each grade of AD (B). According to Rajka & Langeland's scoring criteria, patients were subdivided into three groups: mild ($n=19$), moderate ($n=22$), and severe ($n=10$). Data points are from individual subjects. Horizontal bars represent average of each group, and error bars indicate standard deviation. * $P<0.05$; ** $P<0.001$.

isolated from heparinized venous blood using density gradient centrifugation with Histopaque-1077. After isolation, the cells were stained with FITC-conjugated anti-CD30 mAb together with PE-conjugated anti-CD45RO mAb and PE-Cy5-conjugated anti-CD4 or

anti-CD8 mAb for 30 min at 4°C. CD4- or CD8-positive cells were gated, and these positive cells were subsequently analyzed for the expression of CD30 and CD45RO. In some experiments, the isolated cells were stained with FITC-conjugated

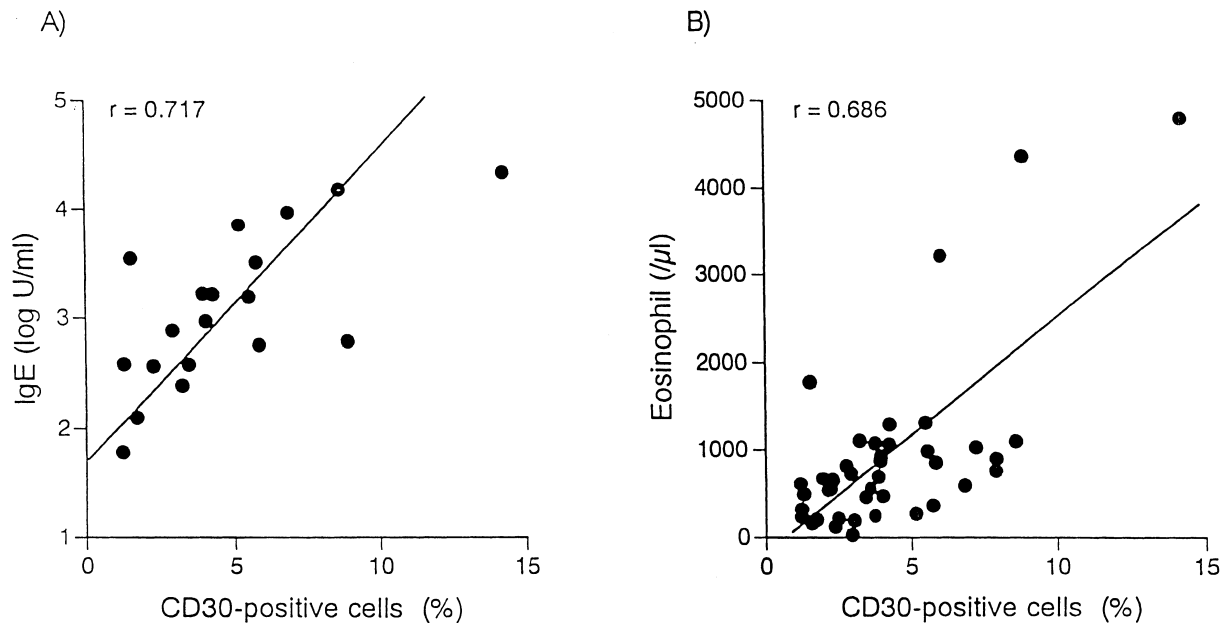


Figure 3. Correlations between CD30-positive rates in CD4⁺ CD45RO⁺ T-cell population and serum IgE levels or peripheral eosinophil counts. Serum IgE levels ($n=18$) and peripheral eosinophil counts ($n=42$) were assessed at same time that CD30 expression was measured in AD patients.

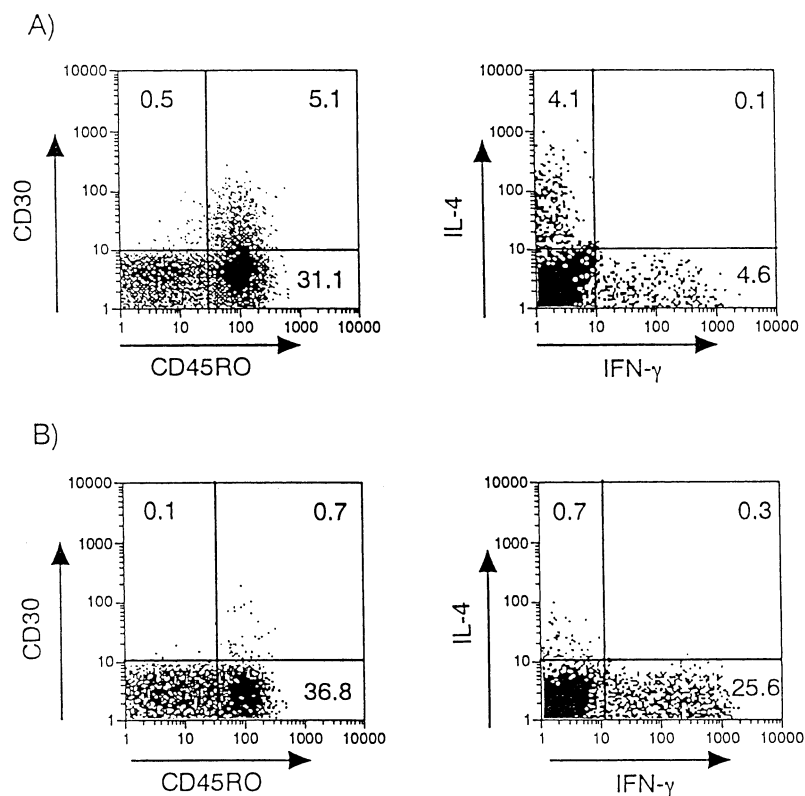


Figure 4. Intracellular cytokine expression by single-cell-staining method. Cells were stimulated with PMA and ionomycin for 4 h in presence of Brefeldin A; then intracellular cytokine production of IFN- γ and IL-4 in CD4⁺ T cells was analyzed by flow cytometer. CD30 expression in CD4⁺ T-cell population was determined on freshly isolated cells before stimulation. Staining of two representatives of relatively higher (A) and lower (B) CD30 expression cases is shown.

anti-CD30 mAb together with PE-conjugated anti-CD16 or anti-CD20 mAb. The samples were analyzed on an EPICS XL-MCL (Beckman Coulter KK, Japan). Some AD patients were selected at random to assess serum IgE levels ($n=18$), peripheral eosinophil counts ($n=42$), and intracellular cytokine production ($n=27$) at the same time that CD30 expression was evaluated. Recently, preferential expression of some chemokine receptors with Th1 or Th2 cells has been reported. Th1 cells predominantly express CXCR3 (15), and Th2 cells selectively express CCR4 (14). We therefore examined coexpression of CD30 and these chemokine receptors on CD4⁺ T cells in the peripheral blood of five AD patients whose CD30 expression was relatively high. Isolated cells were incubated with the mAb against CXCR3 or CCR4, and then this step was followed by a second incubation with goat PE-conjugated anti-mouse IgG1 Ab. After saturation of nonspecific binding sites with 5% normal mouse serum, the cells were stained with FITC-conjugated anti-CD30 and PE-Cy5-conjugated anti-CD4 mAbs.

Cytokine detection at single cell level

Intracellular cytokines were detected by flow cytometry by the method of Waldrop et al. (16) with the

FastImmune[™] system (Becton Dickinson). Briefly, whole blood cells were stimulated with 2.5 ng/ml of PMA (phorbol 12-myristate 13-acetate) and 1 μ g/ml of ionomycin in the presence of 10 μ g/ml of Brefeldin A. After incubation for 4 h at 37°C in a humidified atmosphere of 7% CO₂ in air, the cells were washed and then surface-stained with PerCP-conjugated anti-CD4 mAb for 15 min. After washing, the cells were cultured in lysing solution for 5 min at room temperature. After further washing, the cells were resuspended in permeabilizing solution, and incubated for 10 min at room temperature. The cells were rinsed again, and then stained for intracellular cytokines with FITC-conjugated anti-IFN- γ mAb and PE-conjugated anti-IL-4 mAb for 30 min. CD4-positive cells were gated; these positive cells were subsequently analyzed for the expression of both cytokines.

Statistical analysis

Results are shown as mean $\pm$ SD. One-way ANOVA was used to determine the significance in differences between groups of patients and control subjects. Linear regression analysis was used to determine whether a relationship existed among frequency of CD30⁺ cells, serum IgE level, peripheral eosinophil

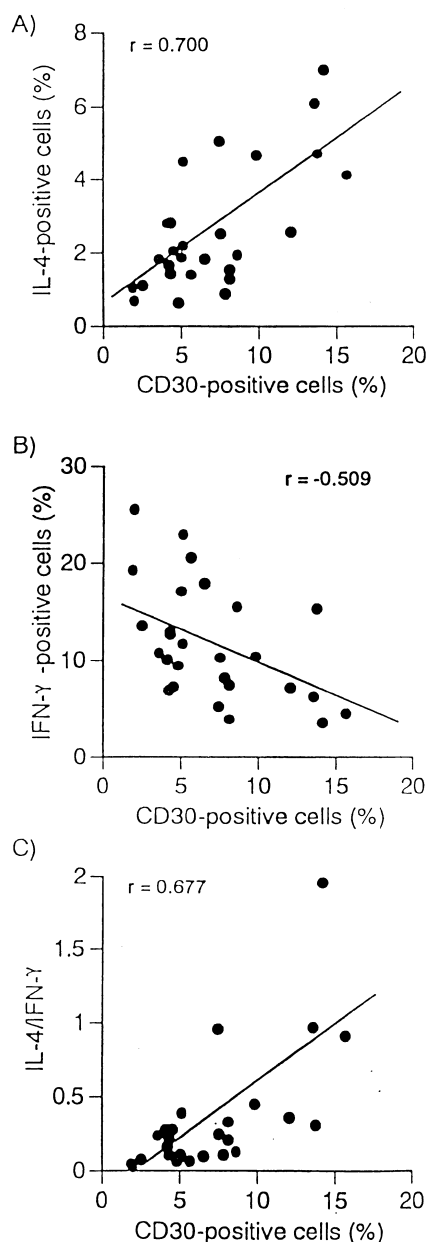


Figure 5. Relationship between intracellular cytokine expression and frequency of CD30⁺ cells in CD4⁺ CD45RO⁺ T-cell population. Intracellular cytokines were detected in randomly selected AD patients ($n=27$) at same time that CD30 expression was measured. Frequency of IL-4⁺ cells (A) and IFN- γ ⁺ cells (B) in CD4⁺ T-cell population is shown. For each patient, IL-4/IFN- γ ratio was calculated (C).

count, and percentage of intracellular cytokine-producing cells. P values of <0.05 were considered significant.

Results

Expression of CD30 was evaluated in PBMCs by three-color flow cytometric analysis (Fig. 1). A substantial number of CD30-positive cells could be detected in the CD4⁺ CD45RO⁺ population of the patients with AD.

In contrast, a few cells expressed CD30 in the CD4⁺ CD45RO⁺ population of nonatopic controls. A few cells were CD30-positive in the CD8⁺ CD45RO⁺ populations of both atopic and nonatopic subjects. In the CD16⁺ or CD20⁺ populations of both groups, the CD30 molecule was hardly detected (data not shown).

Frequency of CD30 expression was calculated as percentage of CD30⁺ cells in the CD4⁺ CD45RO⁺ lymphocyte population. The mean frequency of CD30⁺ cells in AD patients was significantly higher than that in nonatopic controls (4.6 ± 2.8 vs $1.0 \pm 0.9\%$, $P < 0.01$) (Fig. 2A). Therefore, we determined whether there was a correlation between the frequency of CD30⁺ cells and clinical findings of AD. The increase in CD30 expression was still significant when the patients were subdivided into three groups by their severity. Significantly, even mild AD patients had a significantly higher frequency of CD30 expression than nonatopic subjects. In addition, the more severe a patient's AD, the greater the percentage of CD4⁺ CD45RO⁺ cells expressing CD30 ($P < 0.01$ by ANOVA) (Fig. 2B). Furthermore, frequency of CD30⁺ cells significantly correlated with serum IgE levels and peripheral eosinophil counts in AD patients ($r = 0.717$ and $r = 0.686$, respectively) (Fig. 3).

Helper T cells are divided into two subgroups by profile of cytokine synthesis. Intracellular cytokine expression of CD4⁺ cells was evaluated by the single-cell-staining method at the same time that CD30 expression was assayed. Fig. 4 shows the representative cases. Intracellular IL-4 expression was prominent in the subject whose CD30 expression rate was relatively high, whereas the percentage of IFN- γ positively stained cell was high in the patient with relatively low CD30 expression. Fig. 5 shows the relationship between the frequency of CD30⁺ cells in CD4⁺ CD45RO⁺ T cells and the percentage of CD4⁺ T cells staining positively for the cytokines IFN- γ and IL-4 after 4-h stimulation with PMA and ionomycin in the presence of Brefeldin A. There was a significantly positive correlation between CD30 expression rate and the percentage of IL-4-producing cells ($r = 0.700$, $P < 0.0001$) (Fig. 5A). In contrast, a significantly inverse correlation was found between CD30 expression rate and the percentage of IFN- γ -producing cells ($r = -0.509$, $P < 0.01$) (Fig. 5B). For each patient, we calculated an IL-4/IFN- γ ratio to take account of the balance of Th2 activity (IL-4) and Th1 activity (IFN- γ) and found a significantly positive correlation between the percentage of CD4⁺ cells expressing CD30 and the IL-4/IFN- γ ratio ($r = 0.677$, $P < 0.0001$) (Fig. 5C). We also asked whether CD30 molecules were detected on CCR4⁺ T cells, which have been considered to be expressed preferentially on Th2 cells. As shown in Fig. 6, CD30⁺ cells were almost entirely included in the CCR4⁺ CD4⁺ T-cell population. In contrast, CXCR3 as a marker of Th1 was not detected in CD30⁺ population.

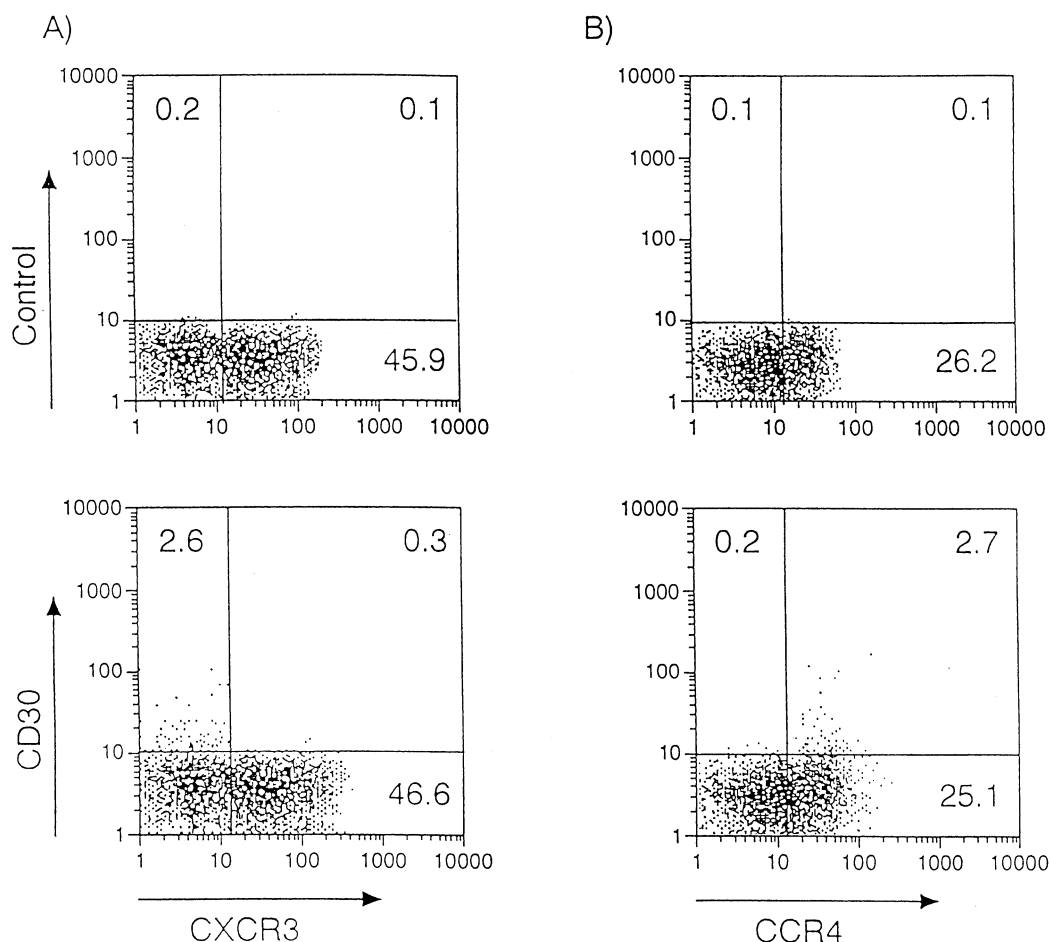


Figure 6. Coexpression of CD30 molecule on chemokine receptor, CXCR3 (A) and CCR4 (B). Freshly isolated PBMCs were stained with anti-CD30, anti-CD4, and anti-CXCR3 or anti-CCR4 mAbs. Relation between CD30 and chemokine receptors expressed on CD4⁺ T cells was analyzed by flow cytometer. Similar results were obtained from four different AD samples.

Discussion

The number of circulating CD30⁺ T cells has been considered too low to be detected by flow cytometry in normal and atopic subjects (17–19). In the present study, we demonstrated a small but clearly detectable fraction of CD4⁺ T cells coexpressing CD30 and CD45RO in the peripheral blood. Interestingly, the frequency of CD30⁺ cells in the CD45RO⁺ CD4⁺ population of AD patients was higher than in nonatopic controls. Although imbalance of memory helper T (CD45RO⁺ CD4⁺) cells in peripheral blood of patients with allergic diseases has been reported, the results are inconsistent (20, 21). In this study, the proportion of CD45RO⁺ CD4⁺ cells did not differ between AD patients and controls, and no significant correlation was found between the frequency of CD30⁺ cells and the percentage of CD45RO⁺ CD4⁺ cells (data not shown). Moreover, CD30 expression was not augmented in patients with psoriasis, another inflammatory skin disease (22) (data not shown). These results indicate that increased expression of CD30 in AD patients is not due to elevated percentage of memory

helper T cells, and that CD30 cannot be considered a nonspecific activation marker on T cells. The frequency of CD30⁺ T cells in AD patients correlated with their clinical findings including the severity of AD, serum IgE level, and peripheral blood eosinophil count. Recently, serum sCD30 levels have been determined in various kinds of diseases in which a pathogenic role of Th2 cells has been suggested (17, 23, 24). In AD, elevated sCD30 levels have been reported, but the relationship between sCD30 and clinical findings is controversial (11, 25, 26). Therefore, we measured serum sCD30 levels in some patients ($n=27$) with a commercially available ELISA kit (DAKO) at the same time that CD30 expression was assayed. Although serum sCD30 levels of AD patients tended to be higher than those of nonatopic subjects, there was no significant correlation between sCD30 level and clinical findings. Furthermore, no relationship between sCD30 level and the frequency of CD30⁺ cells was found (data not shown). These results suggest that level of circulating CD30⁺ T cells rather than serum sCD30 level might be a more useful marker for evaluation of disease activity in AD.

Regarding the function of CD30, *in vitro* studies have shown its effects on both cell death and cell activation (27–29). Emerging data in CD30-deficient mice (30) and transgenic mice overexpressing CD30 (31) indicate that CD30 has an important role in thymic negative selection by means of programmed cell death. In contrast, a function of CD30 in the *in vivo* response of Th2 cells is largely unknown. It is widely accepted that the balance between Th1 and Th2 cells must determine the outcome of physiologic and pathologic immune responses, including allergic diseases (2), and that Th2-type cells play a key pathogenetic role in AD (32). Th2 cells synthesize IL-4 and IL-5, but not IFN- γ . In the present study, we determined the Th1/Th2 balance by assaying intracellular cytokine production of peripheral CD4⁺ cells after 4-h nonspecific stimulation, and demonstrated that the frequency of CD30⁺ T cells correlated with the ratio of IL-4/IFN- γ . Leonard et al. evaluated CD30 expression on CD4⁺ T cells and cytokine release of PBMCs of atopic asthmatics (33). After 10-day incubation with mite allergen, an inverse correlation between the percentage of CD4⁺ T cells expressing CD30 and both IFN- γ production and IFN- γ /IL-4 ratio was found. Furthermore, we recently demonstrated that CCR4⁺ CD4⁺ T cells in the peripheral blood were more increased in AD patients

than in normal persons, whereas CXCR3⁺ CD4⁺ T cells were present in a lower frequency in AD patients than in normal persons. These CCR4⁺ and CXCR3⁺ cells isolated from the peripheral blood synthesized Th2- and Th1-type cytokines, respectively (34). In this study, CD30⁺ T cells coexpressed CCR4, not CXCR3. Taken together, these findings show that CD30⁺ T cells may be associated with an *in vivo* Th2-dominated condition. However, the present study could not show directly that CD30⁺ cells truly synthesize Th2-type cytokines. To determine whether the CD30 molecule itself is a true marker for Th2 cells, determination of the cytokine profile of CD30⁺ T cells is necessary. This is the subject of further work and obviously is of great interest in view of the controversy about CD30 expression and T-cell cytokine phenotype (35, 36).

Acknowledgments

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References

1. MOSMANN TR, SAD S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996;**17**:138–146.
2. UMETSU DT, DE KRUYFF RH. TH1 and TH2 CD4⁺ cells in human allergic diseases. *J Allergy Clin Immunol* 1997;**100**:1–6.
3. KÄGI MK, WÜTHRICH B, MONTANO E, BARANDUN J, BLASER K, WALKER C. Differential cytokine profiles in peripheral blood lymphocyte supernatants and skin biopsies from patients with different forms of atopic dermatitis, psoriasis and normal individuals. *Int Arch Allergy Immunol* 1994;**103**:332–340.
4. TENGVALL LINDER M, JOHANSSON C, ZARGARI A, et al. Detection of *Pityrosporum orbiculare* reactive T cells from skin and blood in atopic dermatitis and characterization of their cytokine profiles. *Clin Exp Allergy* 1996;**26**:1286–1297.
5. BEUTLER B, VAN HUFFEL C. Unraveling function in the TNF ligand and receptor families. *Science* 1994;**264**:667–668.
6. DEL PRETE G, DE CARLI M, ALMERIGOGNA F, et al. Preferential expression of CD30 by human CD4⁺ T cells producing Th2-type cytokines. *FASEB J* 1995;**9**:81–86.
7. MANETTI R, ANNUNZIATO F, BIAGIOTTI R, et al. CD30 expression by CD8⁺ T cells producing type 2 helper cytokines. Evidence for large numbers of CD8⁺ CD30⁺ T cell clones in human immunodeficiency virus infection. *J Exp Med* 1994;**180**:2407–2411.
8. DEL PRETE G, DE CARLI M, D'ELIOS MM, et al. CD30-mediated signaling promotes the development of human T helper type 2-like T cells. *J Exp Med* 1995;**182**:1655–1661.
9. DUMMER W, ROSE C, BROCKER EB. Expression of CD30 on T helper cells in the inflammatory infiltrate of acute atopic dermatitis but not of allergic contact dermatitis. *Arch Dermatol Res* 1998;**290**:598–602.
10. JOSIMOVIC-ALASEVIC O, DÜRKOP H, SCHWARTING REB, STEIN H, DIAMANTSTEIN T. Ki-1 (CD30) antigen is released by Ki-1-positive tumor cells *in vitro* and *in vivo*. I. Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay. *Eur J Immunol* 1989;**19**:157–162.
11. BENGTSSON A, HOLM L, BÄCK O, FRANSSON J, SCHEYNIUS A. Elevated serum levels of soluble CD30 in patients with atopic dermatitis. *Clin Exp Immunol* 1997;**109**:533–537.
12. HANIFIN JM, RAJKA G. Diagnostic features of atopic dermatitis. *Acta Dermatovener (Stockh) Suppl* 1980;**92**:44–47.
13. RAJKA G, LANGELAND T. Grading of the severity of atopic dermatitis. *Acta Derm Venereol (Stockh) Suppl* 1989;**144**:13–14.
14. IMAI T, NAGIRA M, TAKAGI S, et al. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 1999;**11**:81–88.
15. QIN S, ROTTMAN JB, MYERS P, et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest* 1998;**101**:746–754.

16. WALDROP SL, PITCHER CJ, PETERSON DM, MAINO VC, PICKER LJ. Determination of antigen-specific memory/effector CD4⁺ T cell frequencies by flow cytometry: evidence for a novel, antigen-specific homeostatic mechanism in HIV-associated immunodeficiency. *J Clin Invest* 1997;**99**:1739–1750.
17. CHILOSI M, FACCHETTI F, NOTARANGELO LD, et al. CD30 cell expression and abnormal soluble CD30 serum accumulation in Omenn's syndrome: evidence for a T helper 2-mediated condition. *Eur J Immunol* 1996;**26**:329–334.
18. ELLIS TM, SIMMS PE, SLIVNICK DJ, JACK HM, FISHER RI. CD30 is a signal-transducing molecule that defines a subset of human activated CD45RO⁺ T cells. *J Immunol* 1993;**151**:2380–2389.
19. LEONARD C, TORMEY V, FAUL J, BURKE CM, POULTER LW. Allergen-induced CD30 expression on T cells of atopic asthmatics. *Clin Exp Allergy* 1997;**27**:780–786.
20. GEMOU-ENGESAEATH V, BUSH A, KAY AB, HAMID Q, CORRIGAN CJ. Inhaled glucocorticoid therapy of childhood asthma is associated with reduced peripheral blood T cell activation and "Th2-type" cytokine mRNA expression. *Pediatrics* 1997;**99**:695–703.
21. MATSUYAMA T, URANO K, OHKIDO M, et al. The quantitative and qualitative defects of CD4⁺ CD45RO⁺ memory-type T cells are involved in the abnormality of TH1 immunity in atopic dermatitis patients. *Clin Exp Allergy* 1999;**29**:687–694.
22. CHRISTOPHERS E. The immunopathology of psoriasis. *Int Arch Allergy Immunol* 1996;**110**:199–206.
23. CALIGARIS-CAPPIO F, BERTERO MT, CONVERSO M, et al. Circulating levels of soluble CD30, a marker of cells producing Th2-type cytokines, are increased in patients with systemic lupus erythematosus and correlate with disease activity. *Clin Exp Rheumatol* 1995;**13**:339–343.
24. PIZZOLO G, VINANTE F, MOROSATO L, et al. High serum level of the soluble form of CD30 molecule in the early phase of HIV-1 infection as an independent predictor of progression to AIDS. *AIDS* 1994;**8**:741–745.
25. DUMMER W, BRÖCKER EB, BASTIAN BC. Elevated serum levels of soluble CD30 are associated with atopic dermatitis, but not with respiratory atopic disorders and allergic contact dermatitis. *Br J Dermatol* 1997;**137**:185–187.
26. FREZZOLINI A, PARADISI M, RUFFELLI M, CADONI S, DE PITA O. Soluble CD30 in pediatric patients with atopic dermatitis. *Allergy* 1997;**52**:106–109.
27. TELFORD WG, NAM SY, PODACK ER, MILLER RA. CD30-regulated apoptosis in murine CD8 T cells after cessation of TCR signals. *Cell Immunol* 1997;**182**:125–136.
28. GILFILLAN MC, NOEL PJ, PODACK ER, REINER SL, THOMPSON CB. Expression of the costimulatory receptor CD30 is regulated by both CD28 and cytokines. *J Immunol* 1998;**160**:2180–2187.
29. LEE SY, CHOI Y. TRAF-interacting protein (TRIP): a novel component of the tumor necrosis factor receptor (TNFR)- and CD30-TRAF signaling complexes that inhibits TRAF2-mediated NF-kappaB activation. *J Exp Med* 1997;**185**:1275–1285.
30. AMAKAWA R, HAKEM A, KUNDIG TM, et al. Impaired negative selection of T cells in Hodgkin's disease antigen CD30-deficient mice. *Cell* 1996;**84**:551–562.
31. CHIARLE R, PODDA A, PROLLA G, PODACK ER, THORBECKE GJ, INGHIRAMI G. CD30 overexpression enhances negative selection in the thymus and mediates programmed cell death via a Bcl-2-sensitive pathway. *J Immunol* 1999;**163**:194–205.
32. GREWE M, BRUIJNZEEL-KOOMEN CA, SCHÖPF E, et al. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 1998;**19**:359–361.
33. LEONARD C, TORMEY V, BURKE C, POULTER LW. Allergen-induced cytokine production in atopic disease and its relationship to disease severity. *Am J Respir Cell Mol Biol* 1997;**17**:368–375.
34. YAMAMOTO J, ADACHI Y, ONOUE Y, et al. Differential expression of the chemokine receptors by the Th1- and Th2-type effector populations within circulating CD4⁺ T cells. *J Leukoc Biol* 2000 (in press).
35. SPINOZZI F, AGEA E, PIATTONI S, FALINI B, GRIGNANI F, BERTOTTO A. Lack of correlation between membrane CD30 expression and cytokine secretion pattern in allergen-primed naive cord blood T-cell lines and clones. *Scand J Immunol* 1997;**45**:417–422.
36. OKUMURA M, HIDAKA Y, MATSUZUKA F, et al. CD30 expression and interleukin-4 and interferon-gamma production of intrathyroidal lymphocytes in Graves' disease. *Thyroid* 1999;**9**:333–339.