

Cell surface characterization of T lymphocytes and allergen-specific T cell clones: Correlation of CD26 expression with T_{H1} subsets

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To evaluate the possibility of using surface molecules as markers for human T helper cell subsets, we studied the expression of surface molecules on T cell clones (TCCs) specific for the major birch pollen allergen, Bet v 1. No difference in the expression of the respective receptors for interferon- γ (IFN- γ), IL-4, IL-6, or IL-10 could be detected on T_H subsets, nor did CD25 expression (IL-2R α -chain) differ significantly. However, high expression of CD26 antigen (dipeptidyl peptidase IV) correlated with a T_{H1}/T_{H0}-like phenotype, whereas T_{H2}-like clones displayed a lower expression of CD26 antigen. Comparing cytokine production and CD26 expression simultaneously, we found a correlation between the IL-4/IFN- γ ratio and the density of CD26 per cell. We could show that the amount of IL-4 secretion, and not of IFN- γ secretion, was responsible for this correlation. To evaluate whether CD26 antigen expression is regulated by stimuli inducing a T_{H1}- or T_{H2}-like phenotype, peripheral blood mononuclear cells (PBMC) were cultured in the presence of IL-4, IFN- γ , and IL-12, respectively. Incubation with IL-4 led to T cells with a T_{H2}-like cytokine pattern and a significantly lower expression of CD26; IFN- γ and IL-12 led to a T_{H1} shift associated with an increased expression of CD26 on CD4⁺ T cells. By means of intracellular cytokine detection we analyzed expression of CD26 on CD4⁺ PBMC stimulated to produce IFN- γ or IL-4 on a single cell level. All activated, cytokine-producing CD4⁺ T cells expressed CD26, but the increase in CD26 expression was higher in cells producing IFN- γ . These data suggest that regulation of CD26 cell surface expression correlates with the production of T_{H1}-like cytokines. (*J Allergy Clin Immunol* 1997;100:348-55.)

Key words: T helper cell subsets, T cell clones, cell surface molecules, cytokine receptors, CD26, cytokine production

Abbreviations used

FITC:	Fluorescein-isothiocyanate
HBSS:	Hanks' balanced salt solution
INF- γ :	interferon γ
mAbs:	monoclonal antibodies
NK:	natural killer
T _{H1} , T _{H2} , T _{H0} :	T helper cells
TCCs:	T cell clones
PBMC:	Peripheral blood mononuclear cells
PE:	Phycoerythrin
PHA:	Phytohemagglutinin
PMA:	Phorbol 12-myristate 13-acetate

Human T cell responses have been subdivided into two functionally distinct subsets on the basis of their cytokine production profiles.¹⁻⁴ T_{H1} cells produce interferon- γ (IFN- γ) and IL-2 but little or no IL-4 or IL-5, whereas T_{H2} cells mainly produce IL-4 and IL-5. This concept was originally based on data from CD4⁺ cells, but evidence is increasing that similar subsets exist in the CD8⁺ population (T_{C1} and T_{C2} subsets).⁴⁻⁶

The main problem in studying the role of T_{H1} and T_{H2} subsets in different disease states is the lack of reliable cell surface markers for these cell subsets. Furthermore, individual human T cells and T cell clones (TCCs) display a remarkable diversity in their cytokine profiles.⁷⁻⁹ Collectively they form a continuous spectrum in which T_{H1} and T_{H2} cells may be only two of the possible extreme phenotypes. Recently, preferential expression of CD30 by CD4⁺ human T_{H2}-like cells has been reported.^{10, 11} Again, CD30 is not strictly limited to T_{H2} cells, but with these subsets the expression is stronger and more sustained. Human T_{H1} and T_{H2} cells differ not only in their lymphokine secretion profile, but also in their responsiveness to lymphokines.^{12, 13} Several reports suggest that IL-2, IL-4, IL-10, and IFN- γ regulate the differentiation of T cells into T_H subsets.^{13, 14} IL-4 is one of the most potent inducers of T_{H2} development and inhibits differentiation and function of T_{H1}-like cells, thereby favoring its own production.¹²⁻¹⁴ On the other hand, IFN- γ is an inducer of T_{H1}-like cells and these are characterized by IFN- γ production. In contrast to IL-4 and IFN- γ , IL-12 is a cytokine mainly produced by

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antigen-presenting cells (monocytes, macrophages, B cells). Due to its natural killer (NK) cell and T cell stimulating properties, IL-12 was originally termed NK cell stimulatory factor or cytotoxic lymphocyte maturation factor.¹⁵⁻¹⁷ IL-12 induces IFN- γ production by NK cells and T cells, initiates differentiation of T_{H1} cells,^{18, 19} and downregulates T_{H2} cell expansion.²⁰ Because IL-4, IFN- γ , and IL-12 are potent inducers of the T_{H1}- or T_{H2}-like phenotype, respectively, it is not unlikely that they influence the expression of surface molecules associated with a T_{H1}- or T_{H2}-like phenotype.

CD25 and CD26 are surface molecules correlating with T cell activation. CD25 is the α -chain of the IL-2R, and is expressed on activated cells including T cells, B cells, and monocytes.²¹ CD26 is a proteolytic enzyme (dipeptidyl peptidase IV) present on resting T cells, and T cell activation is accompanied by enhanced expression of CD26.²² Memory T cells have been shown to reside in the CD26⁺ T cell fraction. Recent developments indicate that CD26 is a multifunctional molecule that may play an important role in the regulation of immune functions and that is necessary for normal T cell function. CD26 is able to transmit an activating signal to the T cell and binds a variety of nonsubstrate ligands (including CD45 and the human immunodeficiency virus trans-activating [Tat] protein).^{23, 24} Recently, it has been suggested that CD26 expression correlates with the production of IFN- γ in granulomatous diseases.²⁵ Based on the concept that there might be a continuum within the CD4⁺ T cell population rather than several distinct populations, we looked for surface markers expressed on T cells in general, but which are likely to be expressed in different densities in distinct T cell subsets. Thus, in this study we attempted to characterize molecules that are mediating T_{H1}- or T_{H2}-like differentiation, and/or molecules that are induced after T cell activation.

We have analyzed the expression of receptors for cytokines (IFN- γ , IL-4, IL-6, and IL-10) and activation markers (CD25, CD26) on TCCs specific for the major birch pollen allergen, Bet v 1.²⁶ Furthermore, the cell surface density of CD26 was correlated with the amount of secreted IFN- γ and IL-4 in TCCs. Production of IFN- γ and IL-4 and expression of CD26 was studied on a single cell level on CD4⁺ lymphocytes from peripheral blood. The influence of IL-4, IFN- γ , and IL-12 on both parameters was investigated in peripheral blood mononuclear cells (PBMCs) of healthy and atopic donors. Our data suggest that expression and regulation of CD26 on CD4⁺ lymphocytes correlates with the production of a T_{H1}-like cytokine pattern.

METHODS

Subjects and allergen-specific T cell clones

PBMCs were obtained from 10 patients allergic to birch pollen. Type I allergy to birch pollen was assessed by typical case history, positive skin tests, and positive RAST scores. Furthermore, each patient's plasma was tested for the presence of Bet v 1-specific IgE by immunoblot analysis.²⁷ Ten healthy

volunteers without a history of atopic allergy were included in this study.

TCCs specific for Bet v 1 used in this study have been described previously.^{7, 8, 28} According to their cytokine production, TCCs were classified as T_{H1} or T_{H2} when the ratio of IL-4:IFN- γ production was <1:10 (T_{H1}) or >10:1 (T_{H2}), respectively. All other TCCs were designated T_{H0}. TCCs were restimulated either with 10 μ g/ml recombinant Bet v 1²⁹ or 1% phytohemagglutinin (PHA) vol/vol (Life Technologies, Grand Island, N.Y.) for 24 hours before analysis.

PBMC cultures

PBMCs were isolated from heparinized blood samples of healthy and atopic donors by Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells were analyzed on days 0, 7, and 14. After the initial examination on day 0, a quantity of 2×10^7 PBMCs were cultured in 20 ml Ultra Culture Medium (Bio Whittaker, Walkersville, Md.) supplemented with glutamine (2 mmol/L), gentamycin, and mercaptoethanol. Cultures were stimulated for the initial 3 days with 1% PHA vol/vol (Life Technologies) and either 100 U/ml IL-12 (gift from M. Gately, Hoffmann La-Roche, Nutley, N.J.), 1000 U/ml IFN- γ (Boehringer Mannheim, Mannheim, Germany), or 0.5 ng/ml IL-4 (Saxon Biochemicals, Hannover, Germany). On day 3, cells were washed and cultured in fresh medium with the same concentration of the respective cytokine as initially used, and IL-2 (20 U/ml, Genzyme, Boston, Mass.) was added. Cells were again analyzed on day 7. On day 10, 10 ml fresh medium was added to the 14-day cultures.

Cytokines (ELISA)

TCCs were stimulated with Bet v 1 or PHA (see above), and supernatants were collected after 24 hours of further incubation. IL-4 and IFN- γ levels in the supernatants were measured with sandwich ELISA techniques provided by Genzyme (IFN- γ) and Quantikine R&D Systems (IL-4) (Minneapolis, Minn.).

Flow cytometry and antibodies

Cells were harvested, washed, and resuspended in Hanks' balanced salt solution (HBSS) containing 0.2% bovine serum albumin and 0.3% NaN₃ and were preincubated for 20 minutes on ice with 10% human AB- serum. Thereafter, 5×10^5 cells were washed with HBSS and incubated with 50 μ l of monoclonal antibody (mAb) for 30 minutes on ice. After washing, the cells were treated with fluorescein-isothiocyanate (FITC)-labeled F(ab')₂ fragments of goat anti-mouse IgG antibodies from Caltag Laboratories (Medac, Hamburg, Germany) for 30 minutes on ice. Isotype-matched irrelevant mouse antibodies were used as a control. In bulk cultures, cells were washed and incubated with a phycoerythrin (PE)-labeled anti-CD4 or anti-CD8 mAb (Becton Dickinson, Mountain View, Calif.) for another 30 minutes on ice. After final washing three times, at least 10^4 cells were analyzed on a FACScan (Becton Dickinson). The mAbs recognizing the receptors for IL-4 (hIL-4R-M57), IL-6 (M91), IL-10 (B-R10 and B-R20), and IFN- γ (GIR-208) were obtained from the Fifth International Workshop on Leukocyte Differentiation Antigens. The mAbs against CD25 and CD26 were purchased from Immunotech (Marseille, France).

Intracellular cytokine detection

Flow cytometric assessment of T cell cytokine production was performed essentially according to the technique described by

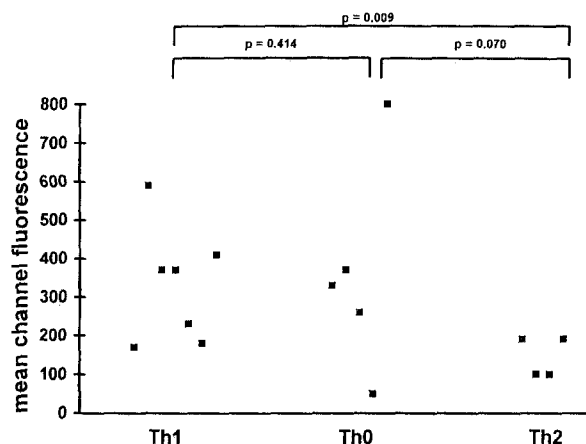


FIG. 1. Established Bet v 1-specific TCCs ($n = 16$) were classified as T_{H1} -, T_{H0} -, or T_{H2} -like as described in the Methods section. The clones were stimulated with rBet v 1 for 24 hours and subsequently stained for CD26. Data are presented as mean channel fluorescence. Expression of CD26 was significantly higher in T_{H1} - than in T_{H2} -like clones ($p = 0.009$), whereas no significant difference was found between T_{H1} : T_{H0} ($p = 0.414$) and T_{H0} : T_{H2} ($p = 0.070$).

Jung et al.³⁰ and modified according to Assenmacher et al.³¹ In brief, PBMCs were isolated and cultured as described above. On day 7, cells were stimulated with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1.25 μ M ionomycin in the presence of 2 μ M monensin (all from Sigma Chemical Co., St. Louis, Mo.) for 4 hours. Cells were then harvested, washed, and fixed with 2% formaldehyde. Cells were made permeable with 0.1% saponin in HBSS. Cytokine-specific mouse anti-human mAb (IFN- γ [4S.B3], FITC- or PE-labeled, and IL-4 [8D4-8], PE-labeled), as well as the respective isotype controls, were obtained from Pharmingen (San Diego, Calif.). The anti-CD4 mAb was labeled with PerCP (Becton Dickinson). Three-color staining was performed and cytokine production was analyzed in the CD4⁺ lymphocyte population. In some experiments, FITC-labeled anti-CD26 mAbs (Becton Dickinson) were combined with anti-CD4 mAbs and either anti-IFN- γ or anti-IL-4 mAbs.

Statistics

Data were analyzed according to Student's t test. The correlation between cytokine production and CD26 expression was analyzed by linear regression.

RESULTS

Expression of cell surface molecules on TCCs

Bet v 1-specific TCCs ($n = 16$) from atopic patients were stimulated with rBet v 1 (10 μ g/ml) for 24 hours and subsequently analyzed for the expression of receptors for IL-4, IL-6, IL-10, and IFN- γ , respectively. Clones had been characterized as T_{H0} -like ($n = 5$), T_{H1} -like ($n = 7$), or T_{H2} -like ($n = 4$) according to the ratio of their IL-4/IFN- γ production.⁷ Receptors for IL-6 and IFN- γ were expressed at rather high levels in all TCCs, with an average of 48% and 46%, respectively. On the other hand, IL-4R and IL-10R generally were expressed at lower levels (14% and 11%, respectively) and were undetectable in some cases. No significant

correlation between receptor expression and T_H subset could be found, neither with respect to the percentage of positive cells nor cell surface density. The same TCCs ($n = 16$) were also analyzed for the expression of CD25 and CD26. Whereas no correlation between CD25 expression and T_H subset could be observed, a significant difference in the amount of CD26 expression between T_{H1} - and T_{H2} -like phenotypes ($p = 0.009$) was detected (Fig. 1).

Correlation of CD26 expression and cytokine production on TCCs

To evaluate the association between cytokine production and CD26 expression, 36 Bet v 1-specific TCCs were stimulated with PHA for 24 hours. The ratio between IL-4 and IFN- γ production was compared with CD26 expression. As shown in Fig. 2, a low ratio of IL-4/IFN- γ was associated with high CD26 expression ($p = 0.012$). When absolute levels of IL-4 production were analyzed in comparison with CD26 expression (Fig. 2), the significance of a negative correlation was even higher ($p = 0.0002$), whereas no correlation was found between IFN- γ production and CD26 expression (Fig. 2).

CD26 expression on cytokine-producing cells

To determine CD26 expression on cells producing IFN- γ or IL-4 on a single cell level, PBMCs were stimulated for 4 hours with PMA and ionomycin in the presence of monensin, and subsequently stained for CD4, CD26, and the respective cytokine. Cells were gated as CD4⁺ lymphocytes. Fig. 3 shows dot plot representations of cells stained for IFN- γ (left side) and IL-4 (right side). Overlay histograms revealed that cells producing IFN- γ (lower left) as well as IL-4 (lower right) had elevated levels of CD26 as compared with the nonproducers. However, cells that produced IFN- γ displayed a more pronounced shift.

Cytokines regulate CD26 expression in CD4⁺ T cells

PBMCs from healthy and atopic donors ($n = 7$) were stimulated with PHA and cultured for up to 14 days with either IL-12 (100 U/ml), IFN- γ (1000 U/ml), IL-4 (0.5 ng/ml), or without any cytokine. Cells were double-stained for CD4 and for CD26 and CD25, respectively, and were gated as CD4⁺ lymphocytes. Fig. 4 shows that CD26 expression was significantly higher when IL-12 was present in the culture system ($p < 0.0001$ on day 7, $p = 0.0004$ on day 14). Only measurements taken on day 7 revealed significantly higher values of CD26 expression in cultures with IFN- γ ($p = 0.0164$). In contrast, cells cultured with IL-4 showed significantly lower CD26 expression as detected on day 7 ($p = 0.0101$) and day 14 ($p = 0.0062$). On day 7, cells generally displayed increased CD25 expression. However, differences in the expression of CD25 were significant only for cultures containing IL-12 as compared with control cultures ($p = 0.0013$). On day 14, CD25 expression had diminished to

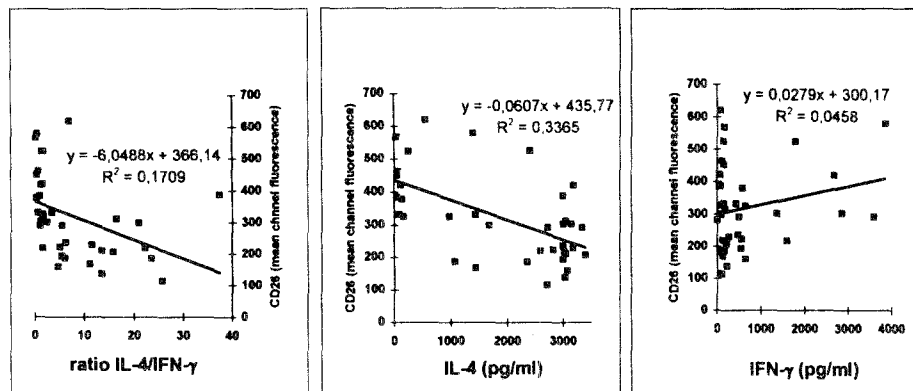


FIG. 2. Correlation between cytokine production and CD26 expression in Bet v 1-specific TCCs ($n = 36$). TCCs were stimulated for 24 hours with PHA and subsequently analyzed for CD26 expression. IL-4 and IFN- γ in the culture supernatants were determined by ELISA. Ratio of IL-4/IFN- γ production and CD26 expression (*left*) correlated significantly ($p = 0.0122$), as did as IL-4 production and CD26 expression ($p = 0.0002$). No correlation exists between IFN- γ production and CD26 expression.

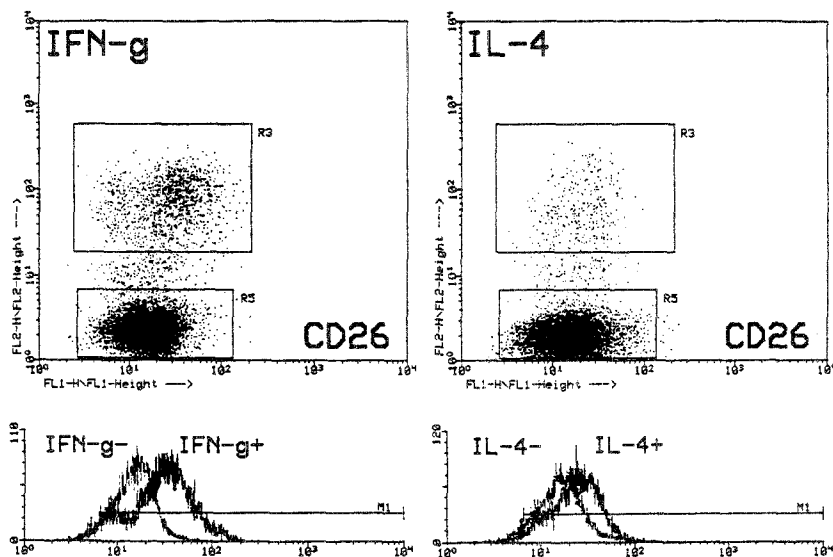


FIG. 3. CD26 expression in CD4⁺ lymphocytes producing IFN- γ or IL-4. PBMCs were stimulated for 4 hours with PMA and ionomycin in the presence of monensin, and subsequently stained for CD4, CD26, and the respective cytokine. Cells were gated as CD4⁺ lymphocytes. *Upper panels* show dot plot representations of cells stained for IFN- γ and CD26 (*left*), or IL-4 and CD26 (*right*). Cells were then gated as positive or negative for the respective cytokine. Overlay histograms for CD26 expression of those populations are shown in the *lower panels*.

the initial low levels, and did not correlate with CD26. CD25 expression in cultures with IL-12 was even lower than in control cultures, whereas it was marginally higher in IL-4 cultures compared with controls. In contrast, CD26 staining remained strongly positive and was significantly higher in cells treated with IL-12 than in untreated cells, and it was lower in the cells treated with IL-4 (see above).

In other experiments, cells were stained for CD8 and for CD26 or CD25, and were gated as CD8⁺ lymphocytes. Similar to CD4⁺ cells, culture with IL-4 led to a significantly lower expression of CD26. Culture with IL-12 resulted in a higher expression of CD26. With

respect to IFN- γ , no effects could be found for the CD8⁺ population. Table I shows the relative effects of IL-4, IFN- γ , and IL-12 on CD26 expression in comparison with the control culture for CD4⁺ and CD8⁺ T cells.

Regulation of cytokine profile by IFN- γ , IL-12, and IL-4 in CD4⁺ T cells

PBMCs from healthy donors ($n = 3$) and from atopic patients ($n = 3$) were cultured as described above until day 7, and restimulated for 4 hours with PMA/ionomycin in the presence of monensin. Cells were triple stained for CD4, IFN- γ , and IL-4 and were gated as CD4⁺ lymphocytes. A dot plot illustration from one represen-

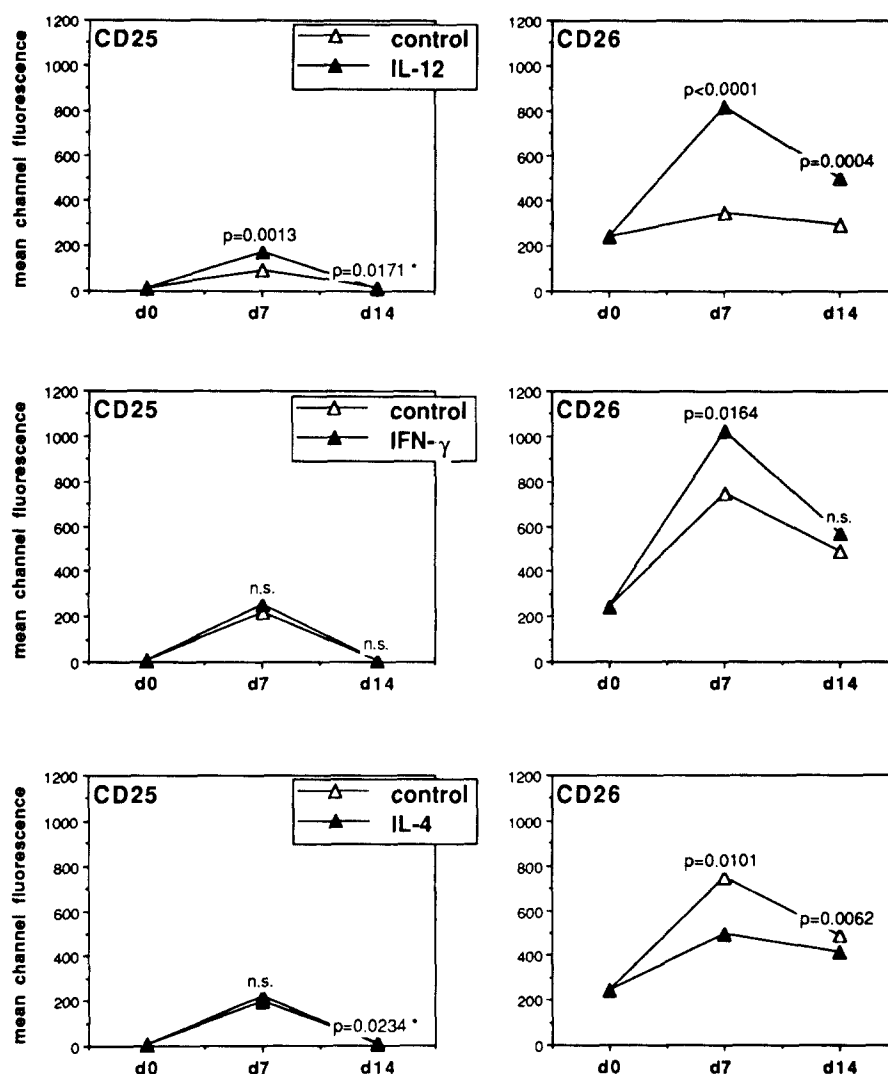


FIG. 4. Influence of cytokines on CD25 and CD26 expression on CD4⁺ T cells at different times. PHA-stimulated PBMCs from healthy and atopic donors were cultured for up to 14 days with or without the indicated cytokines. Data for IL-12 (100 U/ml, $n = 8$) are presented in the upper graphs, for IFN- γ (1000 U/ml, $n = 6$) in the middle graphs, and for IL-4 (0.5 ng/ml, $n = 6$) in the lower graphs. The statistical significance of the differences between control and cytokine-stimulated cultures is presented at the different time points. The asterisk indicates that the significant difference in CD25 expression was inverse to the difference in CD26 expression.

TABLE I. Relative effects of cytokines on CD26 expression

		IL-4	IFN- γ	IL-12
Day 7	CD4	$\times 0.64 (\pm 0.27)^*$	$\times 1.35 (\pm 0.26)^*$	$\times 2.36 (\pm 0.3)^*$
	CD8	$\times 0.67 (\pm 0.21)^*$	$\times 1.09 (\pm 0.21)$	$\times 1.63 (\pm 0.14)^*$
Day 14	CD4	$\times 0.86 (\pm 0.08)^*$	$\times 1.25 (\pm 0.53)$	$\times 1.71 (\pm 0.28)^*$
	CD8	$\times 0.86 (\pm 0.04)^*$	$\times 1.08 (\pm 0.44)$	$\times 1.18 (\pm 0.12)^*$

* $p < 0.05$ compared with controls.

tative experiment is shown in Fig. 5 and data from all experiments ($n = 6$) are shown in the respective quadrants as mean $\pm$ SD. The total percentages of CD4⁺ T cells producing IFN- γ ($4.33\% \pm 0.86\%$ in cultures with

medium alone) significantly increased in the culture with IFN- γ ($8.13\% \pm 3.12\%$, $p = 0.017$) and with IL-12 ($15.74\% \pm 5.3\%$, $p = 0.003$). Production of IL-4 ($2.13\% \pm 1.47\%$ in cultures with medium alone) only slightly

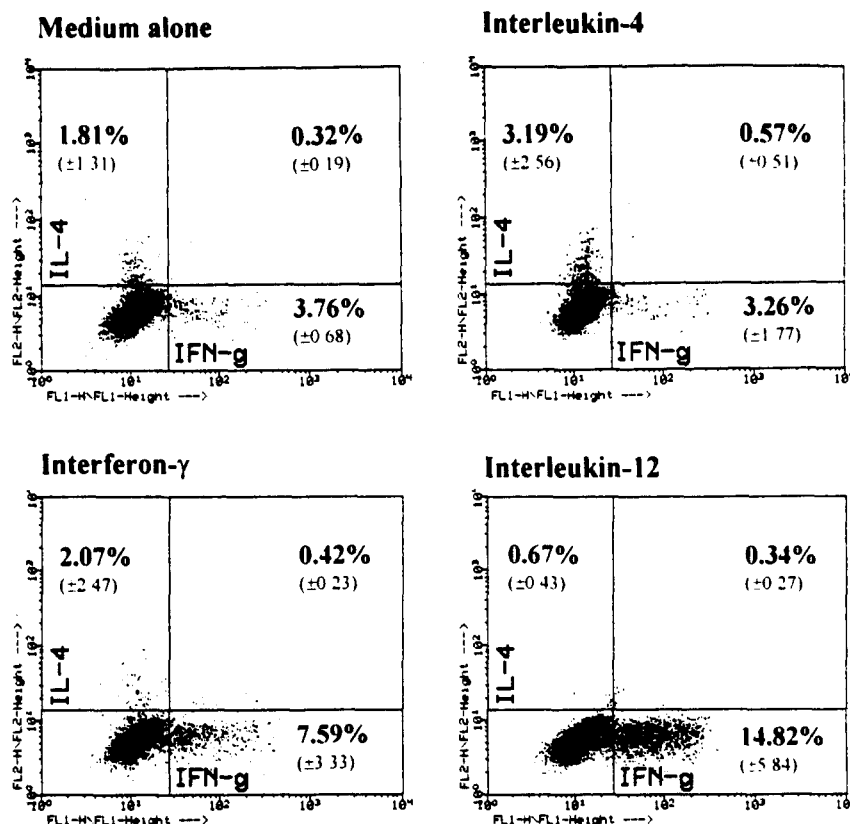


FIG. 5. Cytokine production of CD4⁺ T cells on day 7. PHA-stimulated PBMCs from healthy ($n = 3$) and atopic ($n = 3$) donors were cultured for 7 days with or without the indicated cytokines. After restimulation with PMA and ionomycin in the presence of monensin, percentages of cytokine cells were evaluated by intracellular cytokine detection. Dot plots from one representative experiment are shown. Mean ($\pm$ SD) values from six experiments are indicated in the respective quadrants. Cells were gated as CD4⁺ lymphocytes. In each dot plot populations of cells only producing IL-4 (and not IFN- γ) are presented in the upper left quadrant, and cells producing only IFN- γ (and not IL-4) are shown in the lower right quadrant. Populations producing both cytokines are presented in the upper right quadrants.

increased in the cultures with IL-4 ($3.75\% \pm 3.02\%$, $p = 0.041$) and decreased with IL-12 ($1.01\% \pm 0.68\%$, $p = 0.014$).

DISCUSSION

Our aim was to define a T cell surface marker that correlates with a T_H subset phenotype (T_{H1}⁺, T_{H2}⁺, or T_{H0}-like). Although cytokines such as IL-4 and IFN- γ are dominant factors that determine the differentiation of naive T cells into T_{H1}⁺ or T_{H2}-like cells,¹²⁻¹⁴ no differences could be detected in the expression of the respective cytokine receptors when established TCCs from patients with atopy were analyzed. In addition, the expression of other cytokine receptors, such as IL-2R, IL-6R, and IL-10R, did not reveal any correlation with T_H subsets either (data not shown). The effects of cytokines on T cell differentiation are apparently not mediated or accompanied by the up- or downregulation of the respective receptors. The observation that PBMCs of patients with atopic dermatitis show enhanced expression of IL-4 receptors³² may be due to the acute inflammatory process in those patients. Differentiated T

cells still may be able to respond to stimulating or inhibiting signals from cytokines, irrespective of their phenotype. However, different responses to cytokines by T_H subsets cannot be excluded, despite similar receptor expression. For example, it has been shown that in T_{H1} cells, the β -chain of the IFN- γ receptor (which is the signal transducing element) is downregulated and thus T_{H1} cells are rendered unresponsive to IFN- γ despite unaffected expression of the α -chain (CD130).³³

In contrast to the expression of cytokine receptors, a correlation between the high expression of the activation marker CD26 and a T_{H1}-like phenotype, and the low expression of CD26 and a T_{H2}-like phenotype, was observed (Fig. 1). Because classification of TCCs into T_H subsets is based on the ratio between IL-4 and IFN- γ production, we wondered whether the ratio might correlate directly to the cell surface density of CD26. As could be demonstrated, this was the case (Fig. 2). Moreover, we could elaborate that this correlation is due to the amount of IL-4 produced by the TCCs rather than to the level of IFN- γ production. Our experiments with T lymphocytes cultured under the influence of IL-12,

IFN- γ , or IL-4 indicated that cytokines involved in the differentiation of specific T_H subsets can influence expression of CD26. In our experiments with TCCs, we could demonstrate that the levels of IFN- γ production did not correlate with the expression of CD26. This is in accordance with our data from cultured PBMCs, in which IFN- γ was the less effective cytokine regarding CD26 expression (no significant difference on day 14, see Fig. 5), whereas IL-4 significantly downregulated CD26 on CD4 $^+$ T cells. This is also in accordance with our data concerning the correlation of high IL-4 production and low CD26 expression on TCCs (Fig. 2). Moreover, our data suggest that IL-12 directly increased CD26, and not via enhanced IFN- γ production. IL-12 significantly increased the frequency of CD4 $^+$ T cells producing IFN- γ and reduced IL-4 production. The enhanced expression of CD26 seen on day 7 appeared to be partially due to the activation-enhancing effect of IL-12. However, this was a temporary event, independent of its effects on T_{H1} -like differentiation and CD26 expression.

It is remarkable that regulation of CD26 expression by IL-4 and IL-12 on CD8 $^+$ and CD4 $^+$ T cells correlated. This is in line with reports about the potency of CD8 $^+$ cells to differentiate into T_{C1} and T_{C2} subsets and the role of IL-4, IFN- γ , and IL-12 in the induction of this dichotomy.³⁴⁻³⁶ On CD8 $^+$ T lymphocytes, IFN- γ was ineffective with respect to CD26 expression, which may reflect its weak effect on T_{C1} differentiation.³⁶

Similar effects of IL-4, IFN- γ , and IL-12 on CD26 regulation were found when cells from healthy and atopic donors were stimulated with the nonspecific mitogen PHA, which induces a polyclonal activation. This is not surprising, because atopy seems to be related to a pathologic immune response to a limited number of certain antigens, and the general mechanisms of T cell regulation are identical in patients with and without atopy.

Recently, a positive correlation of staining for IFN- γ and CD26 in skin biopsies from patients suffering from tuberculoid leprosy (the T_{H1} dominated outcome of this disease) was described, whereas no or very little reactivity for both parameters was found in cases of lepromatous, T_{H2} -like courses of leprosy.²⁵ Our data suggest that the low production of IL-4 (which is often accompanied by an increase of IFN- γ) may be associated with a high expression of CD26 and that all three parameters are most likely regulated by the level of IL-12 production.

Whether T_H subsets represent either discrete subpopulations or a continuum with polarized extremes is still a matter of discussion. The original concept may be oversimplified⁹ and the latter hypothesis may be closer to reality. This would implicate that we cannot expect to find discrete markers for T_H subsets. Recent data on human T cell expression of CD30 suggest that this might be a useful marker for activated T_{H2} cells.¹⁰ Again, CD30 expression was not strictly limited on T_{H2} cells, but it was stronger and more sustained. Costimulation via CD30 has been reported to result in preferential

development of T cell lines and clones showing a T_{H2} -like profile of cytokine secretion.¹⁰ However, this marker is not unequivocally accepted.³⁷

We could demonstrate that in vitro T_{H1} -like cytokine production correlates with higher CD26 expression. The combination of cell surface markers that are preferentially, although not exclusively, expressed on certain T_H subsets may increase the ability to define T_H subsets. However, the analysis of CD26 expression on T lymphocytes for the diagnosis of T_{H1} - or T_{H2} -like disease states in vivo is currently hampered by the ability to identify antigen-specific T cells in heterogeneous cell preparations.

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