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CD30 Expression on Allergen- and Non-Allergen-Specific T Cell Lines and Its Role in Cytokine Production

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Abstract. Interest in CD30 has mainly focused on its ability to discriminate between T helper (Th)2 and Th1 subpopulations. The role of CD30 as the marker for Th2 cells is still controversial, which may be due to the fact that the expression and the role of CD30 is not fully understood. The data presented in this paper provides information on the expression and activity of CD30 in T cell lines specific to allergen or tuberculin-purified protein derivative (PPD) as the model of Th2 or Th1 responses, respectively. The results have shown that CD30 expression was the highest on T cells stimulated with antigen in the presence of interleukin (IL)-12 and it was present on both cell lines, regardless of antigen specificity. Activation of the CD30 receptor on $CD4^+$ T cells, however, showed differences in mRNA expression for IL-4 between these cells. IL-4 mRNA was induced by CD30 costimulation at the same level as was obtained with anti-CD28 agonistic antibodies in allergen-specific T cells. In PPD-specific T cells this effect was not observed. Additionally, there was no effect of anti-CD30 stimulation on IL-6 mRNA expression in any of the cell lines. Comparison of protein cytokine levels for IL-4 and interferon (IFN)-γ have shown that the highest production of IL-4 was obtained from allergen-specific T cells costimulated with anti-CD28. Although this effect was much lower in the case of CD30 costimulation, it was still above that of anti-CD3 activation alone. No effect of CD30 activation was observed in regard to IFN-γ mRNA or protein expression in any cell line. The results of the study showed that the CD30 receptor is not exclusively present on Th2 cells; however, its activity may promote a Th2-dependent reaction by modulating IL-4 production.

Key words: CD30; T cells; Th1; Th2.

Introduction

CD30 is a membrane glycoprotein of 120 kDa originally recognized by the monoclonal antobody Ki-1 on Reed-Sternberg cells of Hodgkin's lymphoma[7]. Currently it is used as a clinical marker of this disease[21]. CD30 belongs to the tumor necrosis factor receptor (TNFR) superfamily. It is expressed on $CD4^+$, $CD8^+$, and on virally transformed T cells. CD30 is activation-dependent, present on $CD45RO^+$ (memory/activated) T cells[8] and accompanied by the expression of p55 interleukin (IL)-2 receptor[2]. Exclusive expression of CD30 on T helper (Th)2 subpopulations of T cells is suggested by *in vivo* as well *in vitro* studies. *In vivo* observations include studies of CD30 expression on cells obtained from patients suffering from "Th2"- or "Th1"-dependent diseases. A significant number of T cells expressing CD30 is found in material samples obtained from patients who suffer from Th2 activation-dependent diseases such as: systemic sclerosis[16], Omenn's syndrom[4],

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and graft-versus-host disease[5]. No CD30 expression on the other hand, is observed on cells obtained from patients suffering from "Th1" activation-dependent diseases, especially those in which high levels of interferon (IFN)-γ are found. Among these are infections with *Helicobacter pylori*[3] and Crohn's disease[5]. The specificity of CD30 expression on Th2 cells is also suggested from a number of studies concerning allergy and asthma. The number of $CD30^+$ T cells obtained from bronchoalveolar lavage of asthmatic patients is high and consists of about half γδ T cell receptor (TCR) lymphocytes and 5–12% αβ TCR T cells[20]. The number of these cells obtained from the peripheral blood of allergic patients during the allergy season, although reported as very low or undetectable, increases after *in vitro* allergen stimulation. This increase correlates with symptomatic disease severity score and IL-4 levels. On the other hand, it inversely correlates with IFN-γ production and the IFN-γ : IL-4 ratio[6, 14, 15]. However, this exclusive expression of CD30 on Th2 subpopulations is controversial and questioned by many scientists[22]. $CD30^+$ T cells can produce concomitantly IL-5- and Th1-dependent cytokine IFN-γ[1], which together with CD30 expression can be induced by IL-12[2, 24]. The suggested existence of CD30 as a specific Th2 cell marker also contrasts with its discovery on *Mycobacterium tuberculosis*-stimulated cells and its dependence on the intracellular presence of IFN-γ[17]

The CD30 receptor is expressed on T cells upon their activation. Its presence on T cells is secondary to the TCR- and/or accessory molecule-mediated signals[8]. CD30 expression is missing from CD28-deficient, TCR-transgenic murine T cells, as well as from cytotoxic T lymphocyte antigen 4 (CTLA-4)-crosslinked wild-type T cells[10] or antibody-inhibited TNFR-related 2 (TR2), another member of the TNFR family[12]. CD30 expression is also dependent on signals delivered by cytokines. The highest expression of CD30 on T cells is found between 48 and 72 h after *in vitro* stimulation with anti-CD3 and recombinant IL-2[2, 8]. Among the modulators of CD30 expression are also IL-12 and IL-4. Activation of the CD30 receptor on human[2] and murine T cells[10] with agonistic antibodies provides co-signal for anti-CD3-induced primary and secondary proliferative responses. These responses may be indirectly affected by CD30-mediated increased production of IL-6, as has been shown for "T cell-like" Hodgkin's disease cells exposed to CD30L or stimulated with agonistic anti-CD30 antibody[11]. Agonistic antibodies against human CD30 receptor may also determine the development of T cell populations. The blockade of the CD30--CD30L interaction inhibits development of Th2 cells, lowers the production of IL-4 and IL-5, and shifts it toward a Th1 profile[10].

The goal of the present study was to investigate the expression of CD30 on allergen- or non-allergen-specific T cell lines and to assess what the activity of this receptor is. The results showed that CD30 is expressed on both Th1- and Th2-directed T cell lines and is affected by IL-12 costimulation. The activity of CD30, however, selects for the Th2 effect in the way it upregulates IL-4 mRNA and protein synthesis in allergen--specific $CD4^+$ T cells, but not those of purified protein derivative (PPD; Th1) specificity. Thus, although CD30 expression does not discriminate between Th1 or Th2 cells it may promote Th2 reactions by upregulating the production of IL-4.

Materials and Methods

Subjects. Subjects included both non-atopic (n=10) and atopic (n=8) donors. Non-atopic donors included 5 male and 5 female subjects with an average age of 35 years, characterized by negative skin tests and absence of symptoms to allergen. Atopic donors included 5 male and 3 female donors with an average age of 37. Atopic donors were characterized by positive skin tests, serum IgE>100 IU/ml, *in vitro* positive proliferation assays and stimulation on allergen presentation.

Cellular sources. Peripheral blood T cells were obtained from all donors. Blood was diluted 1:1 in phosphate buffered saline (PBS) and centrifuged over 1.077 g/ml Histopaque (Sigma, USA) at 400 × g for 30 min. Mononuclear cells aspirated from interphase were washed 3 times in PBS, resuspended in RPMI 1640 (Gibco/BRL) and placed into cultures at 2×10^6/ml.

T cell lines specific to allergen were established from atopic subjects with positive skin tests, and to non-allergen antigen (PPD) from healthy subjects according to the methods used in previous studies[18, 25]. The lines were obtained by stimulation of peripheral blood T cells (2×10^6/ml) every 12 to 14 days with a 1:3 to 1:5 ratio of irradiated autologous B cells as antigen-presenting cells (APCs) plus antigen at a concentration deemed optimal from the donor's previously established proliferation assay (usually 1–10 μg/ml). The lines were examined after 2 passages with antigen.

Reagents. Cells were cultured in RPMI 1640 media with 10% fetal calf serum (FCS), 200 U penicillin/ml, 200 μg streptomycin solution/ml, 4 mM L-glutamine, 50 μg/ml gentamicin, 0.1 mM nonessential amino acids, 10 mM HEPES and 1 mM sodium pyruvate (all Gibco/BRL, ALAB, Poland).

Allergens (Allergopharma, Poland) were used to generate allergen-specific T cell lines and PPD (Serum Staten Institute, Denmark) was used to obtain PPD-specific T cells. Other reagents were used as indicated in the description of methods.

T cell stimulation. Peripheral blood mononuclear cells (PBMCs) obtained from donors were the source from which the cell lines were established. These cells were also the object of investigations of "primary *in vitro* T cell activation" as indicated in the results. In this case, PBMCs were stimulated with antigen alone or together with the combination of the cytokines.

For "secondary T cell responses", T cell lines were established from PBMCs. They were obtained by stimulation of PBMCs with antigen alone for 10 days and subsequent restimulation with a 1:2 ratio of irradiated autologic B cells as APCs, allergen or PPD, and 10 U/ml of IL-2. Allergen or PPD was added at a concentration deemed optimal from the donor's previously established proliferation assay (usually 1–10 μg/ml). In total, 8 allergen-specific (4 *Dermatophagoides pteronyssinuss* – Der p 1, 2 cat allergen-Fel d 1, 2 rat allergen) and 10 PPD-specific T cell lines were established. The specific number of T cell lines used for each experiment is given in the figure legend.

For the final stimulation, T cell lines were set up at 1×10^6 cells/ml in culture media. Allergen extracts or PPD were added together with 10 U/ml of IL-2 and 2×10^6 cells/ml autologic B cells, prepared as described below, at a ratio of 1:2 as APCs and the cytokines IL-12 or IL-4. Each T cell line was analyzed for surface-receptor and cytokine expression at 72 h after stimulation with antigen alone or antigen and cytokines. The time of stimulation was chosen based on previous results, which have shown optimal CD30 expression on T cells 3 days after the stimulation[2, 23].

$CD4^+$ T cell stimulation. For polymerase chain reaction (PCR) and anti-CD30 stimulation studies from cell lines, $CD4^+$ T cells were selected by use of magnetic columns according to the negative selection method of Milteney. Cultures were prepared at 1×10^6 cells/ml in culture media. Allergen extracts or PPD were added together with 10 U/ml of IL-2 and 2×10^6 cells/ml autologic B cells, prepared as described below, at a ratio of 1:2 as APCs and the cytokine IL-12. Three days later, CD30 expression was estimated and the cells were used in anti-CD30 stimulation studies. $CD4^+$ T cells were stimulated with anti-CD3 (Pharmingen), anti-CD3+anti-CD28 (Pharmingen), anti-CD3+anti-CD30, or left unstimulated for 24 h at 37°C, 5%CO_2. For anti-CD30 stimulation, 48-well plates (MaxiSorp, Nunc, Denmark) were coated with 10 μg/ml of anti-CD30 antibodies (M44 clone, Immunex, Seatle, USA) and left overnight in carbonate buffer (pH = 9.5) at 4°C. The next day, 2×10^6 cells/well together with 1 μg/ml of anti-CD3, anti-CD3+anti-CD28, or without stimulator, were added to the uncoated wells. 2×10^6 cells were added to the anti-CD30 coated wells together with 1 μg/ml of anti-CD3.

Preparation of APCs. B cells were negatively selected by E-rosetting PBMCs and 1.077 g/ml Histopaque gradient centrifugation. To increase rosetting, sheep red blood cells were treated with 2-aminoethylisothiouronium hydrobromide solution. Before use in T cell stimulation, the B cells were gamma-irradiated at 3500 rads, and constituted the source of APCs for the T cell lines.

PCR analyses. From cells, total RNA was extracted (Qiagen, Syngen, Poland) and supernatants were collected. cDNA was synthesized from 1 μg of total RNA by oligo dT priming and MMLV reverse transcription (Clontech, Alexim, Poland). IL-4, and IFN-γ primers were obtained from Clontech. For IL-4 and IFN-γ PCR, the amount of cDNA was equilibrated based on the analysis of β-actin expression.

The quantity of PCR products was estimated by densitomeric analysis of gel electrophoretic separation of DNA.

Cell staining and flow cytometric analysis. Cells were stained by standard procedure with antibodies against CD4 and CD8 (PerCP-conjugated) and CD30 (FITC-conjugated) in PBS containing 2% FCS and 0.01% sodium azide. Control cells were incubated with irrelevant antibodies conjugated with FITC or PerCP to account for nonspecific binding. After 30 min of incubation at +4°C, the cells were washed 3 times and resuspended in 4% paraformaldehyde.

Flow cytometric analysis was performed on a FACScan Excalibur (Becton-Dickinson, Alexim, Poland). Cells were gated as shown in Fig. 1 and 5.

ELISA. IL-4 and IFN-γ levels in culture supernatants were measured by the standard ELISA procedure according to the manufacturer's (Biosource, Polaton, Poland) recommendations. Due to the expected high levels of cytokines in some samples, they were diluted 5 times with culture medium before measurement. The ELISA range of detection was 0.39–25 pg/ml with a sensitivity of 0.27 pg/ml for IL-4, and 15.6–1000 pg/ml with a sensitivity of < 4 pg/ml for IFN-γ.

Statistical analysis. Comparisons of CD30 expression and cytokine production were analyzed by the Mann-Whitney method to compare differences between the groups and the Wilcoxon matched pairs test utilizing GraphPad Prism software.

Results

CD30 expression

CD30 expression on T cells was estimated 3 days after the start of cell culture and on the 3rd day after the restimulation. Besides that of the antigen, the effects of the IL-12 and IL-4 cytokines were evaluated in this respect. Graphical presentations of the flow cytometric analyses are presented in Fig. 1 and 2. Stimulation of PBMCs for 3 days increased CD30 expression on T cells stimulated with allergen or PPD. Additionally, the frequency of cells expressing this receptor was enhanced when IL-4 or IL-12 was present in the culture medium (Fig. 3). CD30 expression was significantly higher on allergen-stimulated $CD4^+$ T cells than on PPD-stimulated cells or $CD3^+CD8^+$ cells. The T cells of secondary cultures had much higher percentages of cells expressing CD30 than cells from primary cultures. Stimulation of specific T cell lines with antigen alone was sufficient to induce CD30 expression, since addition of IL-12 or IL-4 did not cause further increase in the expression of this receptor. However, CD30 was still mostly present on $CD4^+$ T cells (Fig. 4) and the difference in this expression was no longer apparent between allergen- or PPD-specific T cell lines. In contrast to some reports, addition of CTLA-4-Ig fusion protein, which inhibits contact between CTLA-4 on T cells and CD80/CD86 on APCs had no effect on CD30 expression (data not shown). The expression of this receptor was also investigated upon stimulation with the antigen non-specific stimuli of anti-CD3 antibodies. The use of these antibodies alone does not induce CD30 expression on $CD4^+$ cells as specific antigen, but addition of a co-stimulator, anti-CD28 antibodies or IL-12, greatly enhanced this effect. However IL-4 was not so efficient (Fig. 5).

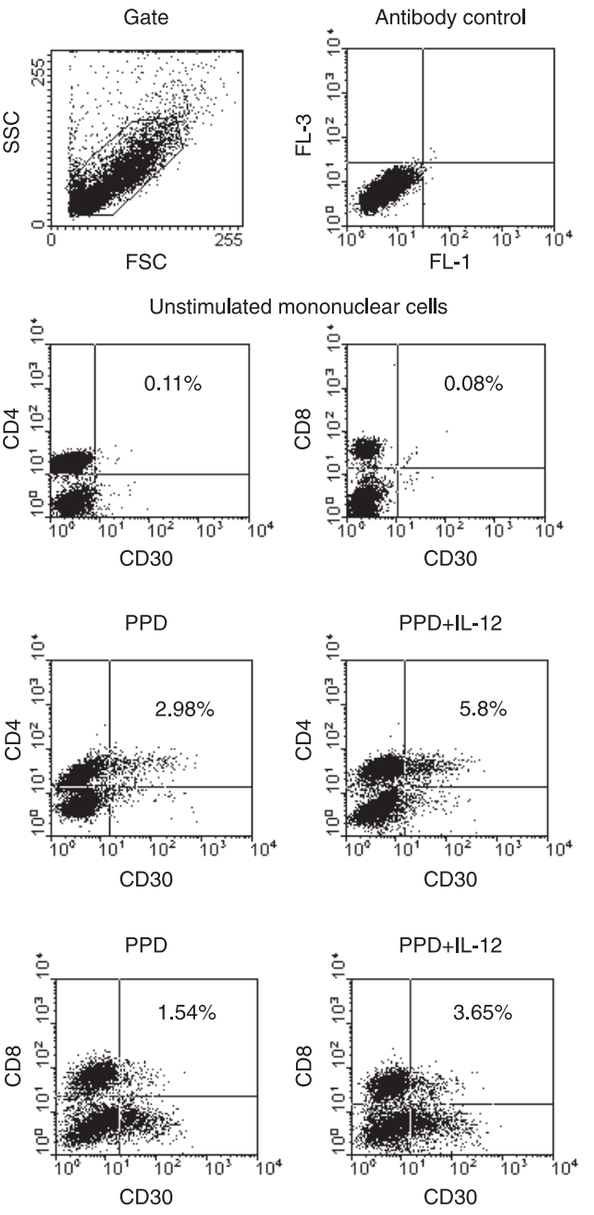

Fig. 1. Flow cytometry analyses of CD30 expression on PPD-stimulated PBMC's. Peripheral blood was drawn from healthy or allergic subjects. Mononuclear cells isolated from blood were seeded at 1×10^6 cells/ml of culture medium and incubated with the optimal concentration of antigen for 3 days. The graph represents the analysis of CD30 expression on CD4 and CD8 T cells of PPD-stimulated or unstimulated PBMCs from healthy subject and isotype control. The same analyses were undertaken for allergen-stimulated PBMCs

mRNA cytokine expression

mRNA expression analyses (Fig. 6) showed that stimulation of allergen-specific $CD4^+$ T cells with anti-CD30 antibodies increases the expression of IL-4 but not IFN-γ (Table 1). In comparison with the use of anti-CD3 antibodies alone, additional stimulation with anti-CD30 was as effective as co-stimulation with anti-CD28 antibodies. In contrast to IL-4 mRNA, the expression of IFN-γ was lower after addition of anti-CD30 or anti-CD28 co-stimulatory antibodies compared with using anti-CD3 antibodies alone.

The expressions of IL-4 or IFN-γ mRNA in PPD-specific $CD4^+$ T cells stimulated with anti-CD3 alone or in combination with anti-CD30 or CD28 antibodies did not show significant changes. Only cells stimulated with anti-CD3 and anti-CD30 showed decreased expression of IFN-γ (Table 1).

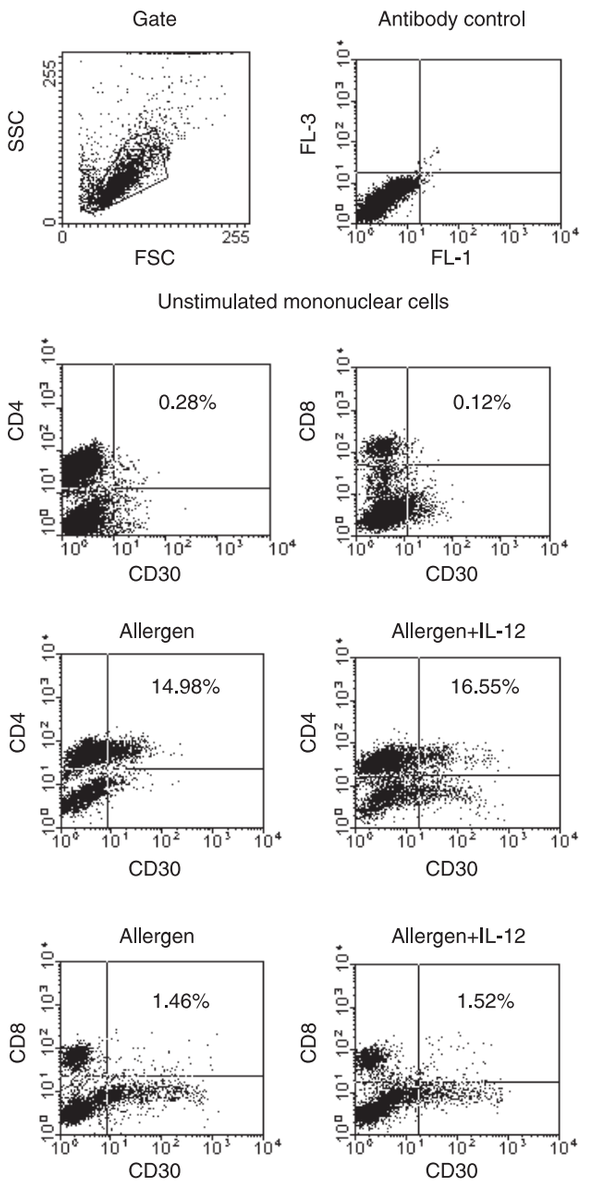

Fig. 2. Flow cytometry analyses of CD30 expression on allergen--specific T cells. CD30 expression was estimated on CD4 and CD8 cells 3 days after last restimulation with Ag and antigen-presenting cells in the presence of IL-12 or IL-4. The graph represents analysis of CD30 expression on allergen-restimulated CD4 or CD8 T cells form allergic subject or T cells not restimulated and isotype control. The same analyses were undertaken for PPD-specific T cells

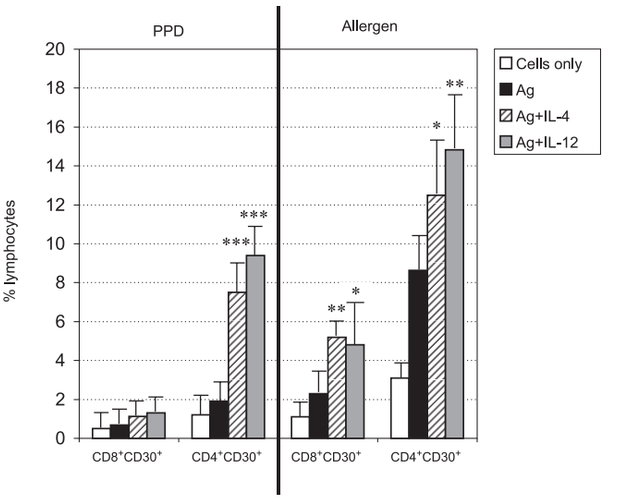

Fig. 3. CD30 expression on T cells 3 days after stimulation of PBMCs. Peripheral blood was drawn from healthy or allergic subjects. Mononuclear cells isolated from blood were seeded at 1×10^6 cells/ml of culture medium and incubated with the optimal concentration of antigen for 3 days. The percentage of $CD30^+$ CD4 or CD8 cells is averaged from the analysis of allergen-stimulated cells obtained from 8 atopic patients or PPD-stimulated cells from 9 healthy subjects. Significant increase of CD30 expression was found after addition of IL-12 or IL-4 in comparison to Ag stimulation alone. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

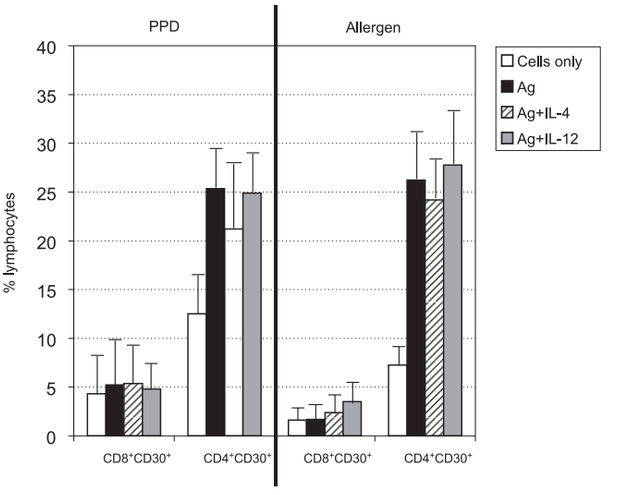

Fig. 4. CD30 expression 3 days after stimulation of cells of secondary cell cultures. From cultures of mononuclear cells after prolonged stimulation there were derived antigen specific T cells. CD30 expression was estimated on CD4 and CD8 cells 3 days after last restimulation with Ag and antigen-presenting cells in the presence of IL-12 or IL-4. Data presents estimations from 7 allergen-stimulated cell lines and 8 PPD-stimulated cell lines. Expression of CD30-induced by Ag and IL-12 or IL-4 was not significantly different from this induced by Ag alone

Cytokine levels in supernatant of $CD4^+$ allergen- or PPD-specific T cells

IL-4 and IFN-γ levels were measured in supernatants of $CD4^+$ allergen- or PPD-specific T cells. Al-

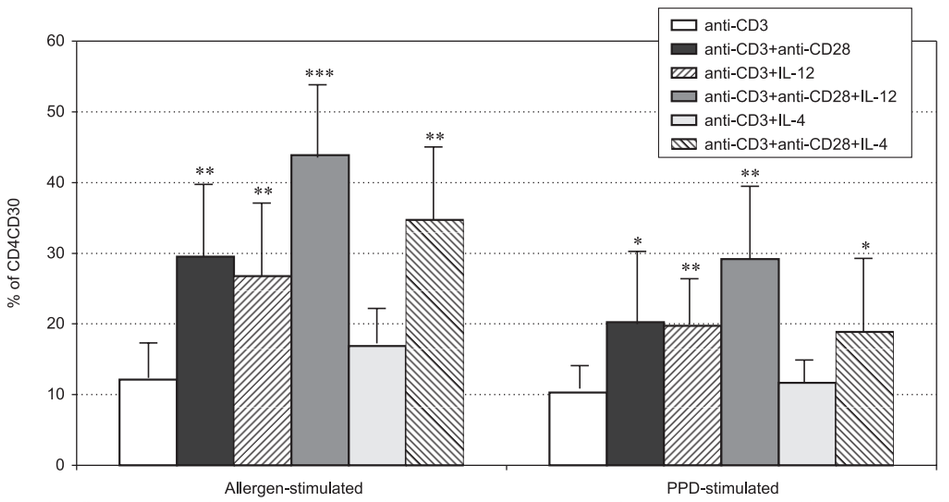

Fig. 5. CD30 expression 3 days after stimulation of cells of secondary cell cultures with antigen non-specific stimulator. CD30 expression was estimated on T cell lines (allergen- and PPD-specific) after 3 day stimulation with agonistic antibodies anti-CD3 and with costimulation with anti-CD28 antibodies in the presence of IL-12 or IL-4. Data presents estimations from 7 allergen-stimulated cell lines and 8 PPD-stimulated cell lines. Not significant changes were induced by addition of IL-4 to allergen- or PPD-specific T cells stimulated with anti-CD3. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

though concentrations of IL-4 are significantly higher in allergen- (Fig. 7) than in PPD-stimulated cells (Fig. 8), IFN-γ levels are not significantly different between these cells. In both allergen- and PPD-specific cells, stimulation with anti-CD3+anti-CD28 induced the highest cytokine production. However, this stimulation did not produce significantly higher levels of IFN-γ in PPD-specific $CD4^+$ cells compared with anti-CD3 stimulation alone. This could be due to the maximal production of this cytokine reached after the applied concentration of anti-CD3 antibodies alone. Stimulation of the CD30 receptor with agonistic antibodies in the presence of anti-CD3 has been shown to stimulate increased IL-4 production only in allergen-specific cells. The synthesis of IL-4 in these cells after CD30 stimulation was higher than in cells stimulated with anti-CD3 alone (131 vs 78 pg/ml). Stimulation of allergen-specific T cells with anti-CD3 and anti-CD30, however, induced significantly lower levels of IL-4 compared with the co-stimulation of CD28 (859 pg/ml). In the case of PPD-specific T cells, co-stimulation with anti-CD30 did not induce higher IL-4 production com-

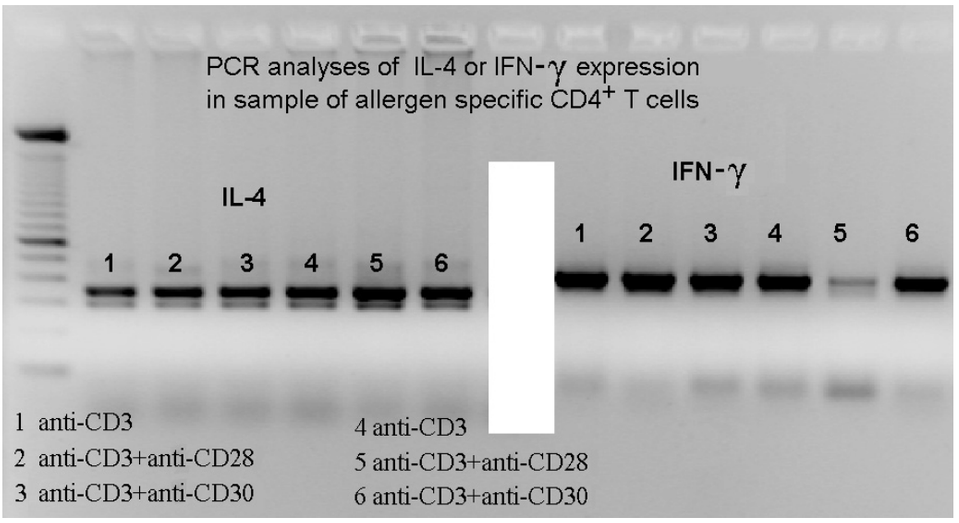

Fig. 6. Gel electrophoresis PCR product analyses. Figure presents example of gel electrophoresis analyses of PCR product of 2 samples of RNA isolated from $CD4^+$ T cells-specific for allergen

Table 1. Relative change of IL-4 mRNA and IFN-γ mRNA expression in $CD4^+$ cells of allergen- or PPD-stimulated T cells in comparison with control unstimulated samples

	Allergen-specific T cells (n=6)	PPD-specific T cells (n=7)
	IL-4 mRNA[a]	
Anti-CD3	2.2 (1.2–3.1)	1.7 (1.0–2.9)
Anti-CD3+anti-CD28	3.6 (2.0–5.5)*	1.6 (1.4–2.4)
Anti-CD3+anti-CD30	3.6 (2.9–4.8)*	1.4 (0.8–2.1)
	IFN-γ mRNA[a]	
Anti-CD3	1.4 (1.0–2.0)	1.6 (1.2–2.8)
Anti-CD3+anti-CD28	1.2 (0.8–1.6)	1.8 (1.0–3.4)
Anti-CD3+anti-CD30	0.9 (0.4–1.4)	1.0 (0.9–2.6)

From allergen- or PPD-specific T cell lines there were isolated $CD4^+$ T cells. These cells were stimulated for 24 h with agonistic antibodies against CD3 receptor or this receptor in the presence of agonistic anti-CD28 antibodies or anti-CD30. After that time RNA was isolated from cells and used for cytokine expression estimates.
* Significant at $p < 0.05$, [a] fold change in relation to control sample (range).

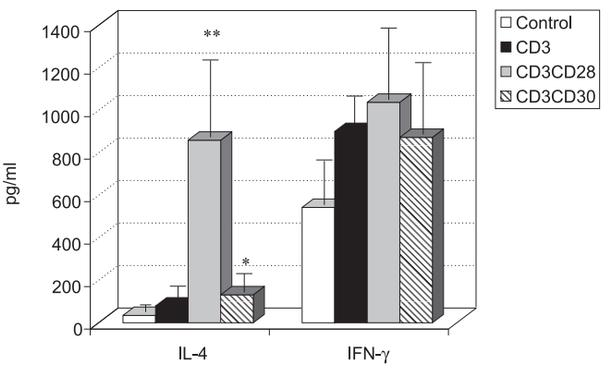

Fig. 7. Cytokine levels in supernatants of allergen-specific $CD4^+$ T cells. $CD4^+$ T cells isolated and stimulated the same way as for mRNA expression studies were used to estimate protein cytokine levels in supernatants. Stimulation of cells with anti-CD3+anti-CD28 or anti-CD3+anti-CD30 induced significantly higher levels of IL-4 in comparison with stimulation with anti-CD3 alone. No significant changes were observed for IFN-γ. * $p < 0.01$, ** $p < 0.001$

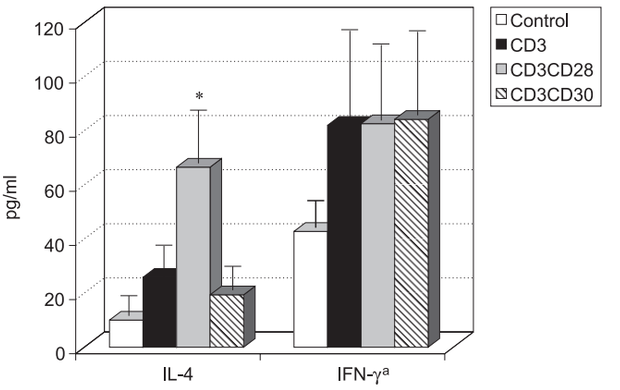

Fig. 8. Cytokine levels in supernatants of PPD-specific $CD4^+$ T cells. $CD4^+$ T cells isolated and stimulated the same way as for mRNA expression studies were used to estimate protein cytokine levels in supernatants. Only stimulation of cells with anti-CD3+anti--CD28 induced significantly higher levels of IL-4 in comparison with anti-CD3 stimulation alone. * $p < 0.001$, [a] concentrations of IFN-γ × 10

pared with anti-CD3 stimulation alone (19 vs 25 pg/ml). However, co-stimulation of CD28 was more effective and induced higher levels of IL-4 (66 pg/ml), but it was much lower than in allergen-specific T cells stimulated with any of the antibodies used or their combinations.

Discussion

Although a considerable amount of knowledge has been collected from different studies about CD30 expression and function in T cell-mediated reactions, its role is still largely unrecognized. *In vivo* observations show close correlation between the presence of $CD30^+$ T cells and Th2-like cytokine activation. A significant number of T cells expressing CD30 is found in material samples obtained from patients who suffer from diseases where high numbers of T cells that produce the Th2 pattern of cytokines can be found[4, 5, 16, 20]. No CD30 expression, on the other hand, is observed on cells obtained from patients suffering from "Th1" activation-dependent diseases[3, 5]. The specificity of CD30 expression on Th2 cells is also suggested from a number of studies concerning allergy and asthma. The number of $CD30^+$ T cells obtained from the peripheral

blood of allergic patients during the allergy season, although reported as very low or undetectable, increases after *in vitro* allergen stimulation[6, 14, 15]. Our study confirms these observations, but also shows that T cells from healthy subjects stimulated *in vitro* with nonallergic antigen can also express CD30. This result shows that CD30 is not specifically associated with Th2 type cells only, even though there were quantitative differences in the frequency of the CD30 expression between allergen- or PPD-stimulated PBMCs, which disappeared after prolonged antigen stimulation. Thus our study is in agreement with recently published study of CD30 T cells in rheumatoid synovitis that showed evidence for a lack of polarization of these cells toward a Th1 or Th2 profile[9]. It also agrees with the finding of a Th0 profile among $CD30^+$ T cells after *M. tuberculosis* stimulation[17]. Also, the study confirms the role of CD28 as a main costimulator of T cells for increased expression of CD30[10]. However, it does not confirm the inhibitory role of CTLA-4 shown by the same authors[10]. This discrepancy may be caused by a different experimental set-up but mainly by the differential way of blocking CTLA-4 receptor interaction. In the presented studies a fusion protein was used instead of blocking antibodies, thus inhibiting the accessory proteins on APCs rather than on T cells. Although the expression of the receptor may indicate the characteristic of the cells, whether they were stimulated or are resting, it does not directly show what its role for these cells is. The presented results show data which provide information on the activity of CD30 on $CD4^+$ T cells. It has been shown that CD30-receptor stimulation enhances CD3-stimulated IL-4 production from allergen- but not PPD-specific T cells. This effect was apparent in the IL-4 mRNA expression and cytokine level studies, but not in those of IL-6 mRNA or IFN-γ mRNA and protein. This study indicates that CD30 may be an important co-stimulator of IL-4 production, and may thus increase allergic, inflammatory reaction, and confirms observations made by others[19]. Although previous results have not shown correlation between the frequency of $CD30^+$ T cells and Th2 cytokine levels in cell supernatants[24], other studies show this correlation with the serum cytokine levels of allergic patients[14, 15]. The reason for this discrepancy may be related to the absence of polymorphonuclear cells or monocytes/macrophages bearing CD30L (CD153) in our cultures which can stimulate $CD30^+$ T cells and enhance cytokine production[19]. Our studies also suggest that CD30 expression itself cannot be regarded as a Th1/Th2 differentiation factor, but its activity can affect and propagate the Th2 cytokine pattern. This, however, depends not only on the presence of CD30L-bearing cells, but also on intracellular signaling that preferentially stimulates IL-4 in allergen- but not PPD-specific T cells. Our study adds important information to the importance of CD30--CD153 binding for Th2 cell propagation advanced by other authors who showed that the blockade of CD30--CD30L interaction inhibits the development of Th2 cells, lowers the production of IL-4 and IL-5, and shifts it toward a Th1 profile[10]. Together with recently published papers indicating its coexpression with chemokine receptors[13, 26] as a suggestive mechanism of transport, this study adds important information to the pathomechanisms of allergic reactions.

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