Interactions of 1α,25-dihydroxyvitamin D3 with IL-12 and IL-4 on cytokine expression of human T lymphocytes

Ralf Thien, Karin Baier, Peter Pietschmann, MD, Meinrad Peterlik, PhD, MD, and Martin Willheim, MD *Vienna, Austria*

Background: 1α,25-Dihydroxyvitamin D3 (1α,25[OH]$_2$D$_3$) exerts its effects on the immune system, particularly through suppression of T helper/cytotoxic cell 1 (T_H/T_C1)–mediated reactions, although direct actions of 1α,25(OH)$_2$D$_3$ on human T lymphocytes have not yet been studied in detail.
Objective: We evaluated the effect of 1α,25(OH)$_2$D$_3$ on basal and cytokine-driven T-cell functions at the single-cell level.
Methods: We used 4-color flow cytometry for simultaneous detection of intracellular cytokines in CD4$^+$ and CD8$^+$ human PBMCs that had been cultured in the presence of 1α,25(OH)$_2$D$_3$ singly or in combination with either IL-12 or IL-4. According to the exploratory nature of these investigations, the Bonferroni correction was not applied for data analysis and presentation.
Results: 1α,25(OH)$_2$D$_3$ had little effect on T_H/T_C1 cytokines but significantly inhibited IL-12–induced IFN-γ production. Constitutive synthesis of T_H/T_C2-related cytokines was also only modestly affected by 1α,25(OH)$_2$D$_3$ alone. When T_H/T_C2 differentiation was induced by IL-4, 1α,25(OH)$_2$D$_3$ significantly expanded the percentages of IL-4$^+$ and IL-13$^+$ cells. However, the predominant effect of 1α,25(OH)$_2$D$_3$ on T lymphocytes, particularly in the presence of IL-4, was the induction of separate CD4$^+$ and CD8$^+$ subpopulations with almost exclusive expression of IL-6. This might be an important facet of the immunomodulatory action of 1α,25(OH)$_2$D$_3$ because IL-6 might act in parallel with 1α,25(OH)$_2$D$_3$ in modulation of T_H/T_C effector cell functions.
Conclusions: Our data imply that the specific actions of 1α,25(OH)$_2$D$_3$ on cytokine-stimulated T-cell functions could play a role in the prevention of T_H/T_C1-related autoimmune diseases but also predispose toward T_H/T_C2-mediated allergic reactions. (J Allergy Clin Immunol 2005;116:683-9.)

Key words: *Vitamin D, immune system, CD4, CD8, IL-2, IL-6, IL-13, IFN-γ, allergy, autoimmune disease*

Abbreviations used
1α,25(OH)$_2$D$_3$: 1α,25-Dihydroxyvitamin D3
PE: Phycoerythrin

T_H lymphocyte responses are mediated by 2 functionally distinct CD4$^+$ T-cell subsets, which can be distinguished on the basis of their different cytokine production profiles.[1-4] T_H1 cells produce IFN-γ and IL-2 but little or no IL-4 and IL-5, respectively, whereas T_H2 cells mainly produce IL-4, IL-5, and also IL-13. Corresponding cytokine production patterns were found also in 2 subsets of the CD8$^+$ T-lymphocyte population (ie, in T_C1 and T_C2 cells).[4-6]

The local cytokine environment plays an important role in the differentiation of T cells along the T_H1/T_C1 or T_H2/T_C2 pathway.[3,4] IL-4 is one of the most potent inducers of T_H2 development,[7-10] whereas IL-12, which is secreted mainly by antigen-presenting cells, initiates differentiation of T_H1 cells[9,10] and downregulates T_H2 cell expansion. The differentiation of CD8$^+$ lymphocytes into T_C1- and T_C2-type cells is triggered by the same agents that promote T_H1 and T_H2 development.[11,12]

The steroid hormone 1α,25-dihydroxyvitamin D3 (1α,25[OH]$_2$D$_3$) is the biologically active metabolite of vitamin D, which plays an important role in mineral homeostasis but has also been identified as a potent modulator of immune responses.[13-18] There is evidence that in animals 1α,25(OH)$_2$D$_3$ prevents the development of experimental autoimmune diseases, such as insulin-dependent diabetes mellitus, allergic encephalomyelitis, autoimmune thyroiditis, and inflammatory bowel disease.[19-24] Epidemiologic studies suggest that in human subjects high vitamin D intake reduces the risk, for example, for type I diabetes mellitus and rheumatoid arthritis.[25,26] It must be noted, however, that vitamin D, when given in early childhood, not only prevents rickets and juvenile diabetes[25] but at the same time seems to increase the risk of allergic diseases in later life.[27]

The preventive effect on autoimmune diseases can be explained by findings that 1α,25(OH)$_2$D$_3$, when added to cultures of murine or human PBMCs, suppresses the release of typical T_H1-type cytokines (ie, IL-2, IFN-γ, or TNF-α).[19,28-32] It is assumed that as a consequence of suppression of T_H1 cytokines by 1α,25(OH)$_2$D$_3$, the prevalence of T_H2-type immune reactions creates a condition that certainly favors the development of allergic diseases. However, it is unclear in which way 1α,25(OH)$_2$D$_3$

From the Department of Pathophysiology, Medical University of Vienna.
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Reprint requests: Martin Willheim, MD, Department of Pathophysiology, Waehringer Guertel 18-20, A-1090 Vienna, Austria. E-mail: martin.willheim@meduniwien.ac.at.
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modulates human T_H2 cell function because until now, respective studies were done only with murine PBMCs and, in addition, yielded rather conflicting results.[33,34]

To obtain more detailed information on the direct effects of $1\alpha,25(OH)_2D_3$ on the differentiation and function of individual T-lymphocyte subpopulations, we developed a protocol for evaluation of cytokine production at the single-cell level.[35-37] Using 4-color flow cytometric analysis, we were able to assess the intracellular content of 2 different cytokines simultaneously in both the $CD4^+$ and $CD8^+$ populations of cultured human PBMCs. In a previous study we could show that $1\alpha,25(OH)_2D_3$ induced a small but consistent reduction of T cells producing IL-2 and increased the percentage of T cells positive for IL-4 and IL-13.[36] Notably, $1\alpha,25(OH)_2D_3$ caused the appearance of a substantial T-cell subpopulation, which produced mainly IL-6 and could not be classified as T_H/T_C1 or as T_H/T_C2.[36,37] In the present study we assessed, for the first time, the interaction of $1\alpha,25(OH)_2D_3$ with IL-12 or IL-4, respectively, on coexpression profiles of IL-2, IFN-γ, IL-4, IL-13, and IL-6 in $CD4^+$ and $CD8^+$ cells to get deeper insight into the cooperative effects of $1\alpha,25(OH)_2D_3$ with T_H/T_C1- and T_H/T_C2-differentiating cytokines on specific effector functions of various T-lymphocyte subsets.

METHODS

Culture conditions

Human PBMCs were isolated from the heparinized blood of 6 healthy donors (3 male and 3 female subjects; age, 23-36 years) by means of Ficoll-Paque density gradient centrifugation. PBMCs were seeded at a density of 10^6/mL and cultured for up to 21 days in Ultra Culture Medium (Bio Whittaker, Walkersville, Md) supplemented with 2 mM L-glutamine (Sigma Bio Sciences, St Louis, Mo) and 170 mg/L gentamicin sulfate (Sigma Bio Sciences).

Cells from the same donor were treated in different ways (addition of different substances) under otherwise absolutely identical experimental conditions. For the first 3 days, aliquots of a PHA solution (Life Technologies, Grand Island, NY) were added to PBMC cultures to achieve a concentration of 1% vol/vol. Thereafter, IL-2 (20 U/mL; Roche Diagnostics GmbH, Basle, Switzerland) was added to maintain cell proliferation and viability. Cultures were exposed to $1\alpha,25(OH)_2D_3$ (10^{-8} M; a kind gift from Hoffmann La-Roche, Basle, Switzerland), IL-4 (500 U/mL; Genzyme, Boston, Mass), or IL-12 (200 U/mL; a generous gift from M. Gately, Hoffmann La-Roche, Nutley, NJ), as indicated. On days 7 and 14, cells were washed once, and fresh medium and treatments were added.

Intracellular cytokine detection

Expression of IFN-γ, IL-2, IL-4, IL-6, and IL-13 in the T-lymphocyte fraction of PBMCs was assessed by using the previously described 4-color flow cytometric technique.[35,36] On days 7, 14, or 21, cells were stimulated with 10 ng/mL phorbol 12-myristate 13-acetate and 1.25 μM ionomycin in the presence of 2.0 μM monensin (all from Sigma Bio Sciences). After 4 hours, cells were harvested, washed, and fixed with 2% formaldehyde.

Four-color fluorescence staining was performed with rat or mouse anti-human mAb and the respective isotype controls labeled with FITC, phycoerythrin (PE), peridinin chlorophyll protein, or allophycocyanin. Anti-CD4 (allophycocyanin) and anti-CD8 (peridinin chlorophyll protein) were purchased from Becton Dickinson (San Jose, Calif), and anti-IFN-γ (FITC), anti-IL-2 (PE), anti-IL-4 (PE), anti-IL-6 (PE), and anti-IL-13 (PE) were obtained from Pharmingen (San Diego, Calif).

For the study of coexpression of IL-6 with IL-2, IL-4, and IL-13, a FITC-labeled anti-IL-6 mAb (Pharmingen) was used. In this case a polyclonal FITC-labeled rabbit anti-rat IgG conjugate (STAR17B; Serotec, Oxford, United Kingdom) had to be used as a second-step reagent to reach sufficient signal intensity for IL-6. Percentages of $IL\text{-}6^+$ cells after optimal staining were similar with FITC and PE. Double-staining controls confirmed that the population detected by using both techniques was identical.

The gating strategy used to analyze cytokine (co)expression in $CD4^+$ and $CD8^+$ lymphocytes is illustrated in Fig 1. Cells were gated as lymphocytes by their light scatter characteristics and subsequently defined as $CD4^+$ and $CD8^+$. Cells fulfilling both criteria (lymphocytes and CD4 or CD8, respectively) were further analyzed for their cytokine production pattern.

Statistics

Data were analyzed with the 2-tailed paired Student *t* test. All groups showed a normal distribution according to the Kolmogorow-Smirnov test. Significance of difference was assumed at a P value of less than .05. According to the exploratory nature of these investigations, the Bonferroni correction was not applied for data analysis and presentation.

RESULTS

Effect of $1\alpha,25(OH)_2D_3$ on basal and IL-12–inducible T_H1/T_C1 differentiation

When PBMCs were cultured in the presence of 10^{-8} M $1\alpha,25(OH)_2D_3$, we, in accordance with our previously published data, observed a small but significant decrease in the percentage of $IL\text{-}2^+$ cells within the $CD4^+$ population at any time point tested (Fig 2). In $CD8^+$ lymphocytes significant inhibition of IL-2 production was only detected on day 14. An effect of $1\alpha,25(OH)_2D_3$ on IFN-γ production was detectable neither in $CD4^+$ nor in $CD8^+$ cells (Fig 2).

Incubation of PBMCs with IL-12 resulted in a consistent increase of $IL\text{-}2^+$ cells and an even more pronounced increase of $IFN\text{-}\gamma^+$ cells within both the CD4+ and the $CD8^+$ T-cell subsets (Fig 2). Simultaneous addition of $1\alpha,25(OH)_2D_3$ abolished the positive effect of IL-12 on the number of IL-2–producing $CD4^+$ T cells. Notably, $1\alpha,25(OH)_2D_3$ completely blocked the IL-12–related increase in the percentage of IFN-γ–positive lymphocytes within the $CD4^+$ and $CD8^+$ subset on day 7. Thereafter, $1\alpha,25(OH)_2D_3$-mediated inhibition gradually ceased toward the end of the culture period, although in $CD8^+$ cells a 50% reduction of IL-12–induced IFN-γ production was still noticed on day 21 (Fig 2).

Induction of T_H/T_C2-related cytokine production by $1\alpha,25(OH)_2D_3$ and IL-4

When cultures were treated with $1\alpha,25(OH)_2D_3$ alone, significance was only reached for the $IL\text{-}13^+$ $CD4^+$ population on day 21 (Fig 3).

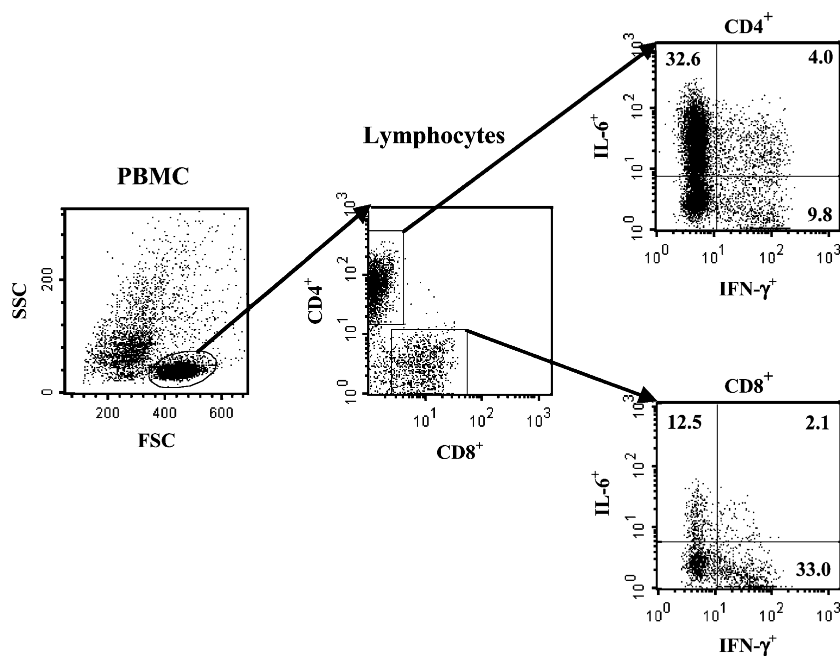

FIG 1. Intracellular detection of cytokines in human $CD4^+$ and $CD8^+$ peripheral blood lymphocytes by means of 4-color flow cytometry. Dot plots from one representative experiment (day 21) on the effect of $1\alpha,25(OH)_2D_3$ plus IL-4 on IL-6 and IFN-γ (co)expression are shown. Cells are gated as lymphocytes *(left panel)* and subsequently as $CD4^+$ or $CD8^+$ *(middle panel).* Dot plots of the *right panel* show specific cytokine-producing subpopulations of $CD4^+$ or $CD8^+$ lymphocytes, respectively. Quadrants represent the following: *upper left,* $IL-6^+/IFN-\gamma^-$ cells; *lower right,* $IL-6^-/IFN-\gamma^+$ cells; *upper right,* IL-6- and IFN-γ–coexpressing cells. Percentage values shown in each quadrant are mean values from 6 experiments. *SSC,* Side scatter; *FSC,* forward scatter.

Cultures treated with IL-4 alone showed significantly increased percentages of IL-4– and IL-13–producing cells within the $CD4^+$ population. Similar tendencies among the $CD8^+$ population commonly did not reach significance, except for IL-4–producing $CD8^+$ cells on day 21. Simultaneous addition of $1\alpha,25(OH)_2D_3$ and IL-4 dramatically increased the percentages of IL-4– and IL-13–producing CD4+ and $CD8^+$ cells, so that on day 21, more than 4 times as many T lymphocytes as in control cultures stained positively for IL-4 or IL-13, respectively (Fig 3, *A*).

Induction of an IL-6$^+$ T-cell subpopulation by $1\alpha,25(OH)_2D_3$ and IL-4

We observed another cooperative effect between $1\alpha,25(OH)_2D_3$ and IL-4 when we studied the induction of IL-6 production in $CD4^+$ and $CD8^+$ T lymphocytes (Fig 3, *B*). Although in control cultures both T-cell subsets remained virtually negative for IL-6, $1\alpha,25(OH)_2D_3$ induced an IL-6–producing population that steadily increased over time to 8.3% in $CD4^+$ lymphocytes on day 21. No significant increase was detectable in $CD8^+$ cells. $IL-6^+$ cells were rarely present in cultures treated with IL-4 alone, although a slight increase could be noticed in $CD4^+$ and $CD8^+$ subsets on day 7 (Fig 3, *B*).

However, the combination of $1\alpha,25(OH)_2D_3$ and IL-4 remarkably augmented the number of IL-6–producing cells (Fig 3, *B*). In both $CD4^+$ and $CD8^+$ T lymphocytes, IL-4 caused a 5-fold amplification of the percentage of $IL-6^+$ cells induced by $1\alpha,25(OH)_2D_3$ alone (Fig 3, *B*).

IL-12 neither induced IL-6 production in T lymphocytes nor modulated the effect of $1\alpha,25(OH)_2D_3$ thereon (data not shown).

Coexpression of IL-6 with IL-4 or IL-13

Because of the pronounced effect of $1\alpha,25(OH)_2D_3$ and IL-4 on T-lymphocyte differentiation in the T_H/T_C2 direction (Fig 3, *A*), we analyzed the effect of the steroid hormone and the cytokine on coexpression of IL-6 with each of the 2 T_H/T_C2-type cytokines, IL-4 and IL-13 (Fig 4).

The higher number of IL-6–producing cells in cultures treated with $1\alpha,25(OH)_2D_3$ and IL-4 was due to the expansion of IL-6 single-positive (ie, $IL-6^+/IL-4^-/IL-13^-$), as well as coexpressing (ie, $IL-6^+/IL-4^+$ or $IL-6^+/IL-13^+$), cells, respectively. In contrast, IL-4 and IL-13 single-positive (ie, $IL-6^-/IL-4^+$ or $IL-6^-/IL-13^+$) cells were only moderately expanded by the combination of $1\alpha,25(OH)_2D_3$ and IL-4 compared with $1\alpha,25(OH)_2D_3$ alone (Fig 4). Similar results were obtained for $CD8^+$ lymphocytes (data not shown).

$1\alpha,25(OH)_2D_3$ specifically modulates cytokine (co)expression of $CD4^+$ T lymphocytes

For further characterization of the $1\alpha,25(OH)_2D_3$ effect on the different pathways of cytokine-triggered T_H/T_C cell differentiation, we analyzed cytokine coexpression patterns in $CD4^+$ and $CD8^+$ PBMCs cultured for 21 days

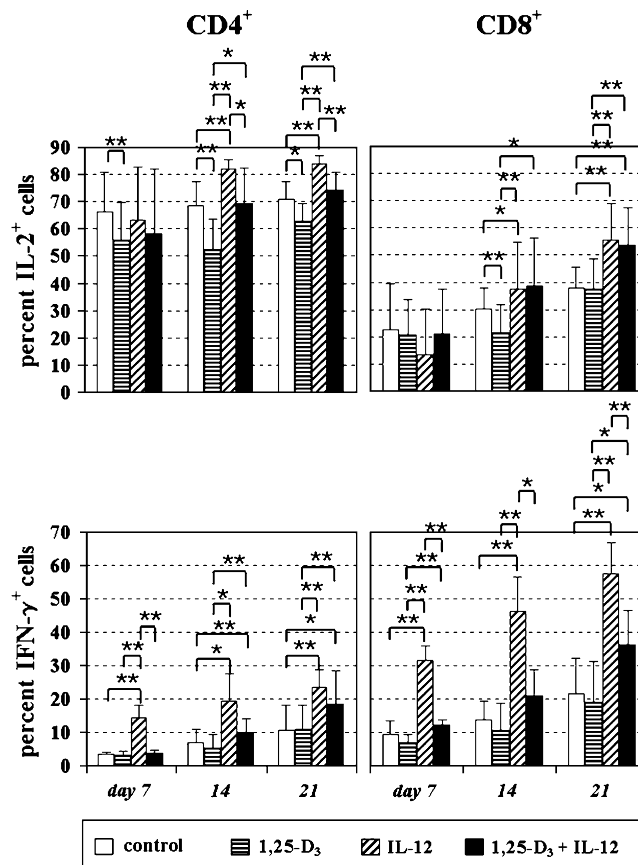

FIG 2. Effects of 1α,25(OH)$_2$D$_3$ and IL-12 on cytokine production by human CD4$^+$ and CD8$^+$ lymphocytes. Percentages of IL-2– and IFN-γ–producing cells were determined by means of 4-color flow cytometry on days 7, 14, or 21. Each *column* represents the mean ± SD from 6 experiments. *Asterisks* indicate statistically significant differences between groups (*$P < .05$, **$P < .01$).

(see Fig E1 in the Online Repository in the online version of this article at www.mosby.com/jaci).

IL-12 generated a typical T$_H$/T$_C$1 pattern inasmuch as a substantial fraction of CD4$^+$ cells were single positive for IFN-γ (Fig E1, *A*, lower right quadrants). A distinct percentage of IFN-γ–producing cells stained positively also for IL-4 or IL-13, respectively (Fig E1, *A*, upper right quadrants), whereas coexpression of IFN-γ with IL-6 was negligible.

Addition of 1α,25(OH)$_2$D$_3$ to IL-12–treated PBMC cultures reduced IL-12–induced IFN-γ expression. IL-6$^+$ cells, which emerged under the influence of 1α,25(OH)$_2$D$_3$, coexpressed IFN-γ, although only to a minor extent (Fig E1, *A*).

IL-4 induced a typical T$_H$/T$_C$2 phenotype characterized by a significant proportion of cells producing only IL-4 and IL-13 but not IFN-γ. In IL-4–treated cultures the number of IL-6$^+$ cells was low (Fig E1, *A*, upper left quadrants).

When PBMCs were cultured in the presence of IL-4 and 1α,25(OH)$_2$D$_3$, approximately one third of CD4$^+$ T cells exhibited a remarkable phenotype inasmuch as they showed positive immunostaining only for IL-6 (Fig E1, *A*, upper left quadrants). IL-6/IFN-γ–coexpressing cells only represented a small proportion of all IL-6–producing cells.

Addition of 1α,25(OH)$_2$D$_3$ expanded the populations of IL-4-induced single-positive (ie, IL-4$^+$/IFN-γ$^-$ or IL-13$^+$/IFN-γ$^-$) cells (Fig E1, *A*, upper left quadrants). The percentage of the IFN-γ single-positive cells and of those coexpressing IL-4 or IL-13 in IL-4–treated PBMC cultures was not changed by coculture with 1α,25(OH)$_2$D$_3$ (Fig E1, *A*).

1α,25(OH)$_2$D$_3$ specifically modulates cytokine (co)expression of CD8$^+$ T lymphocytes

In IL-12–treated PBMC cultures, the proportion of IFN-γ–positive cells was higher in the CD8$^+$ subset (see Fig E1, *B*, in the Online Repository in the online version of this article at www.mosby.com/jaci) than in the CD4$^+$ subset (Fig E1, *A*). Conversely, IL-4 seemed to induce lower percentages of CD8$^+$ cells producing IL-4 and IL-13 than of CD4$^+$ cells (Fig E1). Nevertheless, the regulatory effects of 1α,25(OH)$_2$D$_3$ on cytokine coexpression patterns were similar to those observed in CD4$^+$ cells. Again, in PBMC cultures treated with 1α,25(OH)$_2$D$_3$ plus IL-4, IL-6–producing cells were predominant, although less frequent than in the CD4$^+$ population (Fig E1).

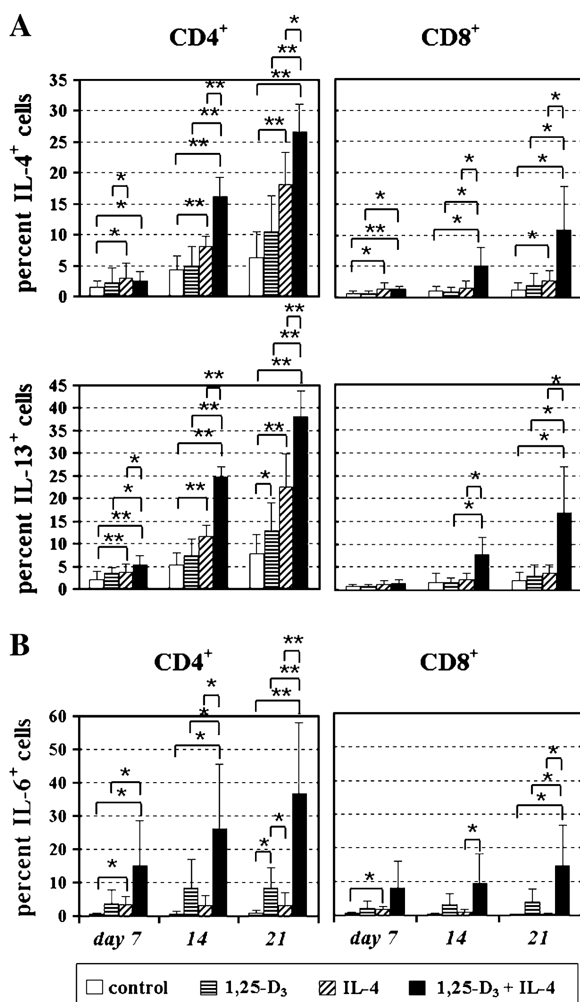

FIG 3. Effects of $1\alpha,25(OH)_2D_3$ and IL-4 on cytokine production by human $CD4^+$ and $CD8^+$ lymphocytes. Percentages of IL-4^+ and IL-13^+ **(A)** or IL-6–producing T cells **(B)** in cultured PBMCs were determined by means of 4-color flow cytometry on days 7, 14, or 21. Each *column* represents the mean ± SD from 6 experiments. *Asterisks* indicate statistically significant differences between indicated groups ($*P < .05$, $**P < .01$).

Mean percentages of IFN-γ single-positive $CD8^+$ lymphocytes were higher in PBMC cultures treated with IL-4 plus $1\alpha,25(OH)_2D_3$ when compared with those treated with IL-4 alone (Fig E1, *B*). However, this effect of $1\alpha,25(OH)_2D_3$, which was not observed in $CD4^+$ T lymphocytes (Fig E1, *A*), did not reach statistical significance. Remarkably, even when compared with control cultures, no decrease in the frequency of IFN-γ–producing T cells could be detected in cultures with simultaneous addition of IL-4 and $1\alpha,25(OH)_2D_3$ (data not shown).

DISCUSSION

Until now, it was assumed that the T_H1 subset of $CD4^+$ T cells is the classical target cell population for the immunomodulating action of $1\alpha,25(OH)_2D_3$, whereas only isolated effects of the hormone on $CD8^+$ lymphocytes had been reported.[28,29] We think it important to note that, consistent with previous reports from our laboratory,[36,37] the present study also clearly shows that $1\alpha,25(OH)_2D_3$ regulates cytokine production in $CD4^+$ and $CD8^+$ T cells in parallel. Statistical analyses were performed according to the exploratory nature of these investigations. However, even more stringent analysis with the Bonferroni correction, although reducing a number of the significant changes, did not negate any of the key results described.

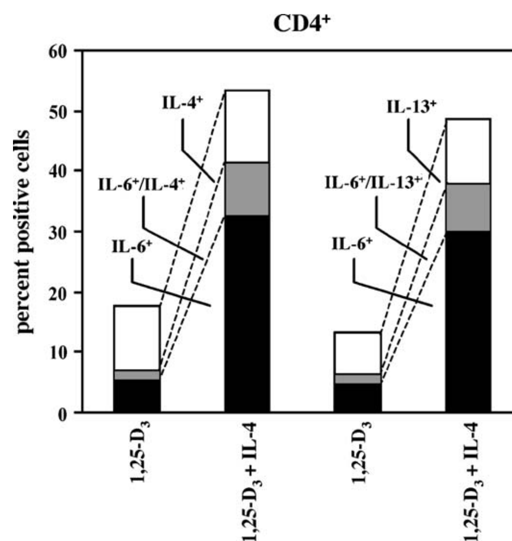

FIG 4. Coexpression of IL-6 with IL-4 or IL-13 in $CD4^+$ PBMCs. PHA-stimulated PBMCs from 3 healthy donors were cultured for 21 days with $1\alpha,25(OH)_2D_3$ alone or with $1\alpha,25(OH)_2D_3$ plus IL-4. *Filled columns*, Single-positive IL-6 *(IL-6^+)* cells; *shaded columns*, cells coexpressing IL-6 and IL-4 *(IL-6^+/IL-4^+)* or IL-13 *(IL-6^+/IL-13^+)*, respectively; *open columns*, single-positive IL-4 *(IL-4^+)* or IL-13 *(IL-13^+)* cells.

The immunomodulating effect of $1\alpha,25(OH)_2D_3$ is commonly attributed to its ability to suppress T_H1-associated cytokine production.[13,20,28,38] From the present study, it seems that constitutive production of T_H1 cytokines is only marginally influenced by $1\alpha,25(OH)_2D_3$ because it caused only a small reduction in the percentage of IL-2–producing, mainly $CD4^+$, cells and induced no consistent change in the IFN-γ–positive T-cell fraction. However, when T_H1 cytokines were induced by IL-12, the inhibitory effect of $1\alpha,25(OH)_2D_3$ was more visible on IFN-γ than on IL-2 production.

It has been suggested that suppression of IFN-γ production by $1\alpha,25(OH)_2D_3$ results from inhibition of IL-12 secretion from costimulatory cells, such as monocytes, dendritic cells, or B cells.[18,39] Results from our experiments in the present study clearly indicate that $1\alpha,25(OH)_2D_3$ also directly interferes with IL-12 action on $CD4^+$ and $CD8^+$ T cells. This is a novel facet of the suppressive action on T_H/T_C1 lymphocyte differentiation and function, by which $1\alpha,25(OH)_2D_3$ prevents the development of T_H1-mediated autoimmune diseases in

experimental animals[19-23] and most likely also in human subjects.[25,26]

Until now, it has been assumed that $1\alpha,25(OH)_2D_3$ interacts specifically with T_H/T_C1 cell function and has no effect on T_H/T_C2 cells. In the present study we confirmed previous data, showing that the effects of $1\alpha,25(OH)_2D_3$ on constitutive T_H/T_C2-related cytokine production in fact were rather modest (Fig 3). However, when T-cell differentiation into the T_H/T_C2 direction was promoted by addition of IL-4 to PBMC cultures, $1\alpha,25(OH)_2D_3$ provided a strong costimulatory signal for the induction of IL-4 and IL-13 production by T lymphocytes (Fig 3). Analysis of cytokine coexpression patterns revealed that $1\alpha,25(OH)_2D_3$ in combination with IL-4 expanded the populations of IL-4 and IL-13 single-positive/IFN-γ^- T cells (Fig E1 in the Online Repository in the online version of this article at www.mosby.com/jaci). No decrease in the frequency of IFN-γ–producing T cells could be detected in cultures with simultaneous addition of IL-4 and $1\alpha,25(OH)_2D_3$. Our data therefore strongly suggest that the prevalence of T_H/T_C2 over T_H/T_C1 cell functions in the presence of $1\alpha,25(OH)_2D_3$ is not only an indirect consequence from T_H/T_C1 suppression[28,29] but also due to a stimulatory effect of $1\alpha,25(OH)_2D_3$ on IL-4–driven T_H/T_C2 differentiation. It must be noted that expansion of both IL-4^+ and IL-13^+ cells is accompanied by coexpression of IL-6, a phenotype not detectable in T_H/T_C2 cells induced by IL-4 alone (Fig E1). It is also evident from Fig 4 that $1\alpha,25(OH)_2D_3$ plus IL-4 treatment induced no change in the percentages of IL-4^+/IL-6^- or, respectively, IL-13^+/Il-6^- T cells.

It should be emphasized that induction of a T-cell subpopulation with predominant expression of IL-6 (Figs 4 and E1) is the major effect of $1\alpha,25(OH)_2D_3$ on T lymphocytes. IL-4, which has negligible effects on IL-6 production by T lymphocytes (Fig 3), amplifies the effect of $1\alpha,25(OH)_2D_3$, so that between 30% and 40% of CD4^+ T cells in long-term PBMC cultures eventually become IL-6^+ (Fig 3, *A*).

We therefore wanted to know whether this was due to an independent effect of $1\alpha,25(OH)_2D_3$ or whether T_H/T_C2 cells under the influence of the hormone acquire the ability to produce IL-6. Results from flow cytometric analysis of cytokine coexpression patterns in CD4^+ and CD8^+ T lymphocytes (Figs 4 and E1) indicate that there is some overlap of IL-6 and T_H/T_C2-related cytokine expression. However, the majority of IL-6^+ T cells stained negative for typical T_H/T_C2 cytokines (ie, IL-4 and IL-13). Also, coexpression of IL-6 and T_H/T_C1-related cytokines was rarely observed.

Previous studies in the murine system have led to conflicting results regarding effects of $1\alpha,25(OH)_2D_3$ on naive T cells.[33,34] In a recent study we have shown that $1\alpha,25(OH)_2D_3$ is able to suppress IL-12-triggered T_H/T_C1 differentiation, as well as IL-4–triggered T_H/T_C2 differentiation, of naive T cells from human cord blood,[37] whereas it induces, particularly in the presence of IL-4, a similar IL-6–producing phenotype as in cells from adult donors. This indicates that $1\alpha,25(OH)_2D_3$ exerts substantially different effects on immature and naive T cells and on a more mature T-cell population in the peripheral blood of adults that consists, to a significant extent, of cells with a memory phenotype.

Taken together, our data provide strong evidence that $1\alpha,25(OH)_2D_3$ causes the appearance of a novel T-cell subpopulation, which typically produces IL-6 only and thus does not fit into the T_H/T_C1–T_H/T_C2 dichotomy. With respect to the latter, Diehl and Rincon[40] have summarized the evidence that IL-6 also regulates T_H functions in a dual way: IL-6 inhibits T_H1 differentiation through an indirect effect on IFN-γ signaling. At the same time, IL-6 augments T_H2 differentiation by upregulation of IL-4 mRNA expression in T lymphocytes. Therefore we hypothesize that induction of an IL-6–producing T-lymphocyte subpopulation might be an important facet of the immunomodulating role of $1\alpha,25(OH)_2D_3$ because IL-6 could enhance the 2 key actions of $1\alpha,25(OH)_2D_3$ on T_H/T_C effector cells (ie, suppression of basal and IL-12–induced T_H/T_C1 functions, as well as stimulation of IL-4–dependent T_H/T_C2 differentiation).

Predominance of T_H/T_C2 over T_H/T_C1 functions induced by $1\alpha,25(OH)_2D_3$ has been observed recently also *in vivo*. Matheu et al[41] reported that in a murine model of pulmonary eosinophilic inflammation, vitamin D treatment augmented allergen-induced T-cell proliferation along with T_H2 cytokine (IL-4 and IL-13) and IgE production. Thus it seems most likely that active expansion of T_H/T_C2 effector cell populations by $1\alpha,25(OH)_2D_3$, as observed in the present study, is underlying the observation made in epidemiologic studies, namely that vitamin D prophylaxis predisposes toward development of allergic diseases in later life.[27]

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Basic and clinical immunology

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