Lineage-specific Effects of 1,25-Dihydroxyvitamin D$_3$ on the Development of Effector CD4 T Cells

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Vitamin D deficiency is implicated in autoimmune disease. We therefore evaluated the effects of 1α,25-dihydroxyvitamin D$_3$ (1,25-D$_3$), the active form of vitamin D, on the development of T helper 1 (Th1), Th17, and Th9 cells, which are implicated in the pathogenesis of different types of autoimmunity. 1,25-D$_3$ compromised the development of Th17 and Th9 cells, including IL-22-expressing cells while simultaneously increasing the frequency of IL-10-competent cells. Relative to Th1 and Th9 cells, the effects of 1,25-D$_3$ on Th1 cells were modest, reflecting the significantly reduced levels of the receptor for vitamin D in this lineage. The use of cells deficient in IL-10 or antibodies that block IL-10 signaling abolished the inhibitory effect of 1,25-D$_3$ on Th9 cells but had no effect on inhibition of Th17 cell frequencies. Thus, the induction of IL-10 in cultures of Th9 cells is an important mechanism by which 1,25-D$_3$ compromises Th9 development but does not explain inhibition of Th17 cells. A survey of select representatives of the Th17 transcriptome revealed that the levels of mRNA that encode RORγt, IL-17A, IL-17F, IL-23R, and IL-22, were reduced by 1,25-D$_3$, whereas IL-21 and aryl hydrocarbon receptor mRNA remained unchanged. These data suggest that vitamin D deficiency may promote autoimmunity by favoring the inordinate production of Th17 and Th9 cells at the expense of regulatory IL-10-producing T cells.

Accumulating evidence indicates that clinically relevant vitamin D deficiency may be widespread throughout the human population (1). Insofar as exposure to sunshine provides much more vitamin D than can be obtained from dietary sources (1), this deficiency follows from the relatively recent transition in employment practices from hunting, gathering, and farming to the indoor activities characteristic of industrialized economies. The racial disparity in vitamin D status that is seen in the United States (2) along with the seasonal fluctuation of vitamin D levels measured in the United Kingdom (3) and elsewhere (4) highlights the contribution made by sunlight to vitamin D status.

A large body of epidemiological and animal data suggests that vitamin D deficiency promotes autoinflammatory disease. The incidence of several human autoimmune diseases has been reported to correlate with increased geographical latitude, low vitamin D intake, and low vitamin D status (1). Systemically administered 1α,25-dihydroxyvitamin D$_3$ (1,25-D$_3$),$^2$ the active form of vitamin D, protects mice against many experimental forms of autoimmune disease (5–9), and deficiency in the vitamin D receptor (VDR) aggravates inflammatory bowel disease in the CD45RB transfer and IL-10-null models (10). Topically applied vitamin D analogs are effective against human psoriasis (1), and a recent report suggests that VDR is a “master regulator” of mouse skin inflammation (11).

The contributions made by CD4 T cells to the pathogenesis of many autoimmune diseases were historically ascribed to the activity of the Th1 subset. These cells express IFN-γ and develop from naïve precursors by activation in the presence of IL-12 and by induction or activation of the transcription factors T-bet, STAT1 and STAT4 (12). More recently, a third effector T cell subset, Th17, has been linked to immunopathology in some forms of autoimmunity previously thought to be Th1-mediated (12). Th17 cells express IL-17A, IL-17F and IL-22 and develop in response to TGF-β1 and IL-6 and by the induction or activation of the transcription factors STAT3, RORγt and RORα, with IL-21 and IL-23 providing subsequent reinforcement of this lineage (12). IL-1β enhances the development of Th17 cells (13), whereas IL-23 enhances the development of cells that express both IL-17A and IL-22 when TGF-β1 is limiting (14–16). Still more recently, a subset of IL-9-expressing T cells (i.e. “Th9” cells) has been implicated in autoimmunity (17–19). The development of these cells is directed by TGF-β1 and IL-4 (19–21) and is increased in the presence of IL-17E (known also as IL-25) (22). In light of recent insights into the pathogenicity of Th17 and Th9 cells and the longstanding appreciation of the protective effects of vitamin D, we reevaluated the impact of vitamin D signaling on the development of CD4 effector T cells.

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$^1$ The abbreviations used are: 1,25-D$_3$, 1α,25-dihydroxyvitamin D$_3$; EAE, experimental autoimmune encephalomyelitis; ROR, retinoic acid-related orphan receptor; VDR, vitamin D receptor; APC, antigen-presenting cells; OVA, ovalbumin; IL-23R, IL-23 receptor.

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**EXPERIMENTAL PROCEDURES**

*Mice—Vdr-deficient mice were obtained as a generous gift from Dr. Shigeaki Kato (University of Tokyo, Tokyo, Japan) and were fed a rescue diet high in lactose (20%), calcium (2%), and phosphorous (1.25%) (TD.96348; Harlan Teklad, Madison, WI). Rorc<sup>-/-</sup> (C57BL/6) and/or C4d4<sup>lo</sup> fraction after surface staining with FITC-labeled anti-CD25 (clone 7D4; BD Pharmingen) and phycoerythrin-labeled anti-CD62L (clone MEL-14; BD Pharmingen) and/or FITC-labeled anti-CD44 (clone IM7; eBioscience) or by magnetic-activated cell sorting (Miltenyi) for proliferation studies. Cells were labeled with carboxyfluorescein diacette succinimidyl ester, according to the manufacturer’s instructions (CellTrace, Invitrogen). A stock solution of 1,25-D<sub>3</sub> (Sigma) dissolved in ethanol to a concentration of 10<sup>−5</sup> or 6 days after activation, T cell expander beads were excluded from analysis based on their prominent autofluorescence in unused channels. Data were acquired on a BD LSRII running FACSdiva software and subsequently analyzed with FlowJo software (Tree Star, Inc.).

**Gene Expression Analyses—**cDNA was prepared from T cells as described previously (25). Real-time PCR was performed as described previously (25) and employed the following additional primers: IL-21 sense primer (5'-GGGA-GGAAAGAAAAGAGACAT-3'), and antisense primer (5'-AAATCTTTGGTGTCCTTTTCTCA-3'), RORα sense primer (5'-GCGGCGTAAAGGATGTATTTT-3'), and antisense primer (5'-TCCACAGATCTTGCATGGAATAA-3'), IL-22 sense primer (5'-CAGGAGGTGTGCTCTTCTTCT-3'), and antisense primer (5'-TGGTGCCTACCGCTGATGT-3'), IL-23R sense primer (5'-TCCCCTCCTTGTCCACCAA-3'), and antisense primer (5'-TTCTGGATTTGCTGGAGATGTT-3'), Foxp3 sense primer (5'-TGCAGACCCCTTTCACCT-3'), and antisense primer (5'-AGTGTTCTCTGCTCCTCCG-3'), and TGF-β sense primer (5'-CGGAGACGCTTGGATACCAA-3') and antisense primer (5'-TGTACAGCTGTCGCAACACA-3'). Quantitative analysis of VDR mRNA was performed by TaqMan PCR using VDR sense primer (5'-CCCATATGCCGACCT-3') and antisense primer (5'-TGGAGATAGCTCCTCATGTACT-3'), probe (5'-FAM-CCCTTGGCGACGACACTTCC-3') and antisense primer (5'-ACTCCCAATCCACTTTCATAGG-3'), β2 microglobulin sense primer (5'-CCTGCAGAGTTAACGATGCAGC-3') and antisense primer, (TGTCCGTGCACTGACATC-3'), and probe (5'-Texas Red-TGGCCGAGCCCAAGACCCG-3').

**Statistical Analyses—**Statistical significance was calculated by two-tailed unpaired t test for the data shown in Fig. 2B and by two- or one-tailed paired t test for the matched comparisons of solvent-treated versus 1,25-D<sub>3</sub>-treated samples in Fig. 5, D and E, respectively. All p values ≤0.05 are considered significant.

**RESULTS**

1,25-D<sub>3</sub>, Compromises the Development of Th17 and Th9 Cells but Not Th1 Cells—The recent description of the Th17 lineage along with the putative role played by vitamin D in limiting autoimmunity prompted us to explore the effects of 1,25-D<sub>3</sub> on the development of effector CD4 T cell subsets. To compare the influence of 1,25-D<sub>3</sub> on the development of Th1 and Th17 cells, we activated naïve, OVA-specific CD4 T cells with irradiated splenic feeder cells and OVA peptide (OVA) under Th1- and Th17-polarizing conditions. At the time cultures were initiated, parallel samples were exposed to different concentrations of 1,25-D<sub>3</sub> or to the corresponding concentration of vehicle alone. 1,25-D<sub>3</sub> significantly reduced the yield of IL-17A<sup>+</sup> cells in Th17-polarized cultures, with
maximal inhibition at a concentration of 10 nm, but did not influence the frequency of IFN-γ+ cells in the Th1-polarized cultures (Fig. 1A).

Antigen-presenting cells (APCs) respond to 1,25-D3 (1, 26, 27). To determine whether the inhibitory effect of 1,25-D3 on the yield of IL-17A+ cells was dependent on the presence of APCs, naïve CD4 T cells were activated with immobilized anti-CD3 and anti-CD28 under conditions that favor the development of cells that express IL-17A (B), IL-22 (C), or IL-9 (D), and exposed to 10 nm 1,25-D3 or vehicle alone. Plots show live cells. TNF-α (30 ng/ml) was added as indicated (D). A–D, results are representative of two independent experiments.

Recently, Th9 cells have been implicated in experimental autoimmune encephalomyelitis (EAE) (17, 18) and colitis (19). To study the effect of 1,25-D3 on Th9 cells, we first sought to optimize the development of this subset and found that 1,25-D3 reduced their frequency, similarly to the effect observed for IL-17 (Fig. 1C).

Th17 cells. We found that 1,25-D3 promotes the development of IL-10+ cells in cultures of Th9 and Th17 cells but has negligible effects on cultures of Th1 cells (Fig. 2A). Given the relative lack of effect that 1,25-D3 has on the endpoints that we measured in Th1 cells (Figs. 1A and 2A), we sought to establish whether or not these cells might respond to 1,25-D3 in ways not yet measured. We, therefore, used quantitative real-time PCR to assess the expression of VDR mRNA in Th1 cells relative to Th9 and Th17 cells and found that Th1 cells express 40-fold less VDR mRNA than do the other two subsets (Fig. 2B). Thus, Th1 cells are relatively refractory to 1,25-D3 due to limited expression of VDR.

1,25-D3-mediated Inhibition of Th9 Cells, but Not Th17 Cells, Depends on Induction of IL-10—Our data suggest that the protective effects of vitamin D against autoimmunity may relate more to vitamin D signaling in Th17 and Th9 cells than to signaling in Th1 cells. We, therefore, focused our attention on mechanistic aspects of 1,25-D3-mediated suppression of Th17 and Th9 cells. Because IL-10 has been reported to inhibit the development of these cells (31, 32), we investigated the possibility that 1,25-D3 inhibits these subsets by the induction of IL-10 that we observed in the preceding experiments. We, therefore, tested the effect of 1,25-D3 on development of Th17 and Th9 cells in the presence or absence of antibodies that neutralize IL-10 and its receptor. As shown in Fig. 3A, IL-10 blockade prevented 1,25-D3 from inhibiting the development of Th9 cells but had no effect on Th17 cells (Fig. 3A). To investigate this by a second approach, we tested the effect of 1,25-D3 on the development of Th9 and Th17 cells from naïve precursors derived from mice deficient in IL-10 and from wild-type mice. In parallel with results shown in Fig. 3A, the use of cells that are deficient in IL-10 abolished 1,25-D3-mediated inhibition of Th9 cells, whereas the inhibition of Th17 cells was unabated (Fig. 3B). Indeed, in the absence of IL-10, 1,25-D3 may actually enhance Th9 development (Fig. 3, A and B). Given the ability of the IL-10 blockade to enhance Th9 cell, but not Th17 cell frequencies (Fig. 3A), we also tested the effect of exogenously added IL-10 on the develop-
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Inhibitory Effect of 1,25-D₃ on Developing Th17 Cells—Having established the mechanism by which 1,25-D₃ inhibits Th9 development, we next examined the effects of 1,25-D₃ on Th17 cell development in more detail. LPS-stimulated dendritic cells activate vitamin D by expression of CYP27B1 (26, 27) and also produce proinflammatory cytokines that augment Th17 cell development (33), we investigate the influence of 1,25-D₃ on Th17 cell development under the conditions examined.

Various Th17-promoting Conditions Fail to Override the Inhibitory Effect of 1,25-D₃ on Developing Th17 Cells—Having established the mechanism by which 1,25-D₃ inhibits Th9 development, we next examined the effects of 1,25-D₃ on Th17 cell development in more detail. LPS-stimulated dendritic cells activate vitamin D by expression of CYP27B1 (26, 27) and also produce proinflammatory cytokines that augment Th17 cell development (33). These putatively counter-vailing effects of LPS stimulation on Th17 development suggest the prospect of a dominance hierarchy among 1,25-D₃ and proinflammatory signals. We, therefore, tested whether these signals might desensitize developing Th17 cells to the inhibitory effect of 1,25-D₃. We found, however, that 1,25-D₃ reduced the frequency of IL-17A⁺ cells to a similar extent irrespective of the presence of IL-1β, TNF-α, or IL-23 (Fig. 4A and B). As has been reported (14), IL-23 does not enhance Th17 development when cells are cultured in 5 ng/ml TGF-β1 (supplemental Fig. S2), thereby necessitating the use of reduced TGF-β1 concentration (Fig. 4B).

Because the magnitude of TCR activation can influence CD4 T cell lineage development (34), we investigated the influence of T cell activation strength and found that the yield of IL-17A⁺ cells positively correlated with the strength of activation whether stimulated with antigen and APCs (supplemental Fig. S3) or under APC-free conditions using anti-CD3/CD28 beads (Fig. 4C). However, the magnitude of 1,25-D₃-mediated inhibition was unaffected by the strength of T cell activation (Fig. 4C).

Selective Effects of 1,25-D₃ on the Th17 Transcriptome—We next investigated mechanistic aspects of 1,25-D₃-mediated inhibition of Th17 cells. Polarization of these cells is enhanced by expression of IL-21 (35) and inhibited by expression of IL-2 (36), T-bet (24), STAT1 (24), and STAT4 (24). Effects on expression of these genes or function of the gene products may account for 1,25-D₃-mediated inhibition of Th17 cell development as emphasized by the ability of 1,25-D₃ to prolong STAT1 signaling in THP-1 cells (37) and modulate IL-2 signaling in human lymphocytes (27). In agreement with a previous report that IL-2 inhibits Th17 polarization (36), we found that enhanced Th17 induction resulted from the combined use of antibodies that neutralize IL-2 and its receptor (Fig. 5A). However, stringent blockade of IL-2 signaling did not prevent 1,25-D₃ from inhibiting Th17 development (Fig. 5A). We also tested the effect of 1,25-D₃ on cells obtained from mice deficient in the four other candidate genes implicated in inhibition or promotion of Th17 development and found that 1,25-D₃-mediated inhibition occurred despite the absence of T-bet, STAT1, STAT4, or IL-21 (supplemental Fig. S4).

1,25-D₃ regulates cell cycle progression in a variety of cells (1). This suggested that 1,25-D₃ might reduce the frequency of IL-17A⁺ cells by selectively compromising the proliferation of those cells relative to the IL-17A⁻ cells. We, therefore, monitored the proliferation of these cell subsets under conditions that lead to the development of Th17 cells. Although in some experiments the addition of 1,25-D₃ modestly...
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inhibited the relative expansion of IL-17A+ cells, in most experiments the inhibitory effect of 1,25-D3 on the frequency of IL-17A+ cells occurred without altering proliferation (Fig. 5B).

Alternate receptors for 1,25-D3 have been proposed but are controversial (38). We, therefore, investigated the role of the classical receptor in mediating the inhibitory effect of 1,25-D3 by polarizing naïve cells that were isolated from VDR-deficient (Vdr−/−) and wild-type (Vdr+/+) littermates. As shown in Fig. 5C, 1,25-D3-mediated inhibition of Th17 cells is dependent on the classical VDR. Furthermore, the inhibitory effect occurs in the absence of effects on Th17 cell viability. Thus, 1,25-D3-mediated reduction in the frequency of IL-17A+ cells does not depend on a selective impairment of their proliferation or viability.

The classical VDR influences cell signaling in part by protein-protein interactions. Notably, the signaling pathways of TGF-β1 and vitamin D converge via a direct physical interaction between Smad3 and VDR (39). We, therefore, speculated that 1,25-D3 might counteract the effects of TGF-β1 on Th17 cell development. We found, however, that the magnitude of the inhibitory effect of 1,25-D3 on Th17 cells was not a function of the concentration of TGF-β1 (supplemental Fig. S5).

Importantly, TGF-β1 has dose-dependent effects on Th17 cell development, as it promotes these cells at lower concentrations (12) but inhibits them at higher concentrations by inducing Foxp3 (14). T cells produce TGF-β1 (40), suggesting the possibility that 1,25-D3 inhibits Th17 development by leading to high endogenous expression of TGF-β1. However, 1,25-D3 did not enhance expression of mRNA that encodes TGF-β1 or Foxp3 (supplemental Fig. S6A). Furthermore, 1,25-D3 neither enhanced iTreg development (supplemental Fig. S6B) nor lost its ability to suppress Th17 cell development in the presence of saturating concentrations of exogenous TGF-β1 (supplemental Fig. S6C). Although this indicates that induction of TGF-β1 as a direct effect of 1,25-D3 on T cells does not contribute to suppression of Th17 cells, this does not rule out indirect effects. Specifically, 1,25-D3 inhibits human Th2 responses in the presence of dendritic cells, an effect that is dependent on enhanced TGF-β1 signaling and which may be due to 1,25-D3-mediated repression of OX40L in dendritic cells (41).

Because VDR and RORα share several binding partners (42, 43) and because forced expression of RORα has been reported to partially override the activation of the osteocalcin promoter by 1,25-D3 (44), we hypothesized that the reduction of IL-17A+ cell frequency might reflect antagonism of RORα but not RORγt. We, therefore, isolated naïve CD4 T cells from mice deficient in RORα (Rorα−/−), RORγt (Rorc−/−), or WT littermates and assessed the effect of 1,25-D3 on their ability to develop into Th17 cells. We found that, as with WT T cells, 1,25-D3 reduced the frequency of IL-17A+ cells and increased the frequency of IL-10− cells irrespective of which orphan receptor was absent (supplemental Fig. S7, A and B). Interestingly, the absence of either orphan receptor not only reduced the frequency of IL-17A+ cells (RORγt much more so than RORα), but also, unexpectedly, increased the frequency of IL-10− cells. Thus, the development of IL-10− cells in Th17-skewing conditions does not depend on expression of these transcription factors and may even be inhibited by them.

Because the classical VDR is a ligand-activated transcription factor, we next surveyed the effects of 1,25-D3 on the expression of select representatives of the Th17 transcriptome. 1,25-D3 inhibited the development of IL-22-competent cells (Fig. 1C), an effect that could reflect either direct regulation of IL-22 expression or, rather, interference of signaling pathways upstream of IL-22 expression. We, therefore, first polarized Th17 cells in the presence of IL-6, IL-1β, IL-23, and an intermediate concentration of TGF-β1. Cells were harvested at
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48 h, and mRNA levels were evaluated by quantitative RT-PCR. 1,25-D₃ inhibited the expression of transcripts that encode RORαt, RORγt, IL-17A, IL-17F, IL-23R, and IL-22 but not IL-21 (Fig. 5D). The failure of 1,25-D₃ to reduce levels of mRNA for IL-21, a cytokine that promotes the Th17 developmental program in an autocrine manner (35), is consistent with the ability of 1,25-D₃ to inhibit Th17 cell polarization of naïve precursors obtained from mice deficient in IL-21 (supplemental Fig. S4). Although signaling through IL-23R and aryl hydrocarbon receptor promotes expression of IL-22 (15, 16, 45), suppression of AHR mRNA by 1,25-D₃ was not statistically significant (Fig. 5D), suggesting that the inhibition of IL-23R mRNA may play a more prominent role in the suppression of IL-22.

RORγt and IL-23 operate in a reinforcing signaling network, where induction of RORγt enhances IL-23R expression, activation of which further up-regulates RORγt. This led us to question whether suppression of RORγt by 1,25-D₃ occurs late in Th17 development by compromising IL-23 signaling or, rather, is an earlier event. We, therefore, polarized Th17 cells in the traditional manner, in which IL-23 signaling is precluded by the addition of TGF-β1 to a concentration of 5 ng/ml and by withholding exogenous IL-1β and IL-23. As before, we found that 1,25-D₃ inhibited the expression of mRNA that encodes RORγt, IL-17A, and IL-17F but not of IL-21 mRNA (Fig. 5E). The reduced expression of RORαt was not deemed statistically significant under these conditions (Fig. 5E). Thus, 1,25-D₃ impairs Th17 development, at least in part, through inhibition of the key transcription factor, RORγt, both in the presence and absence of IL-23 signaling, indicating a proximal impairment of the Th17 developmental pathway.

DISCUSSION

The association of vitamin D deficiency with autoimmunity prompted us to assess the impact that 1,25-D₃ has on the development of Th1, Th9, and Th17 cells in the absence of other, potentially 1,25-D₃-responsive cells. We find that 1,25-D₃ compromises the development of Th9 and Th17 cells while simultaneously increasing the development of IL-10⁺ cells. The absence of IL-10 signaling completely abolished the inhibitory effect of 1,25-D₃ on Th9 cells but had no effect on 1,25-D₃-mediated inhibition of Th17 cells (Fig. 3). Indeed, we find that IL-10 suppresses the development of Th9 cells but not Th17 cells. Thus, the induction of IL-10 by 1,25-D₃ during the development of Th9 and Th17 cells is the mechanism by which 1,25-D₃ suppresses development of the former but does not explain suppression of the latter.

1,25-D₃ inhibits Th17 development by activating VDR (Fig. 5C), a transcription factor that regulates gene expression in a variety of cells. Whereas it was recently reported that 1,25-D₃ blocks IL-17A expression without reducing expression of RORγt-encoding mRNA (46), our data consistently reflect suppression of this transcript, in agreement with reported reduction of RORγt⁺ cells in mice with autoimmune uveitis treated with active vitamin D (46). By reducing the expression of RORγt mRNA, 1,25-D₃ may be said to compromise Th17 developmental programming, but the effects are not uniform across the Th17 transcriptome (Fig. 5, D and E). Although 1,25-D₃ inhibited expression of IL-23R in cultures that contained a non-suppressive concentration of TGF-β1 (Fig. 5D), the reduction of RORγt does not depend on altered IL-23 signaling, as suppression of these orphan receptors also occurs in the absence of IL-23 and the presence of IL-23R-repressing concentrations of TGF-β1 (Fig. 5E). As with IL-23, IL-21 reinforces Th17 development that is initiated by TGF-β1 and IL-6 (47–49). However, we show that IL-21 deficiency does not prevent the 1,25-D₃-mediated inhibition of IL-17A⁻ cell development (supplemental Fig. S4), nor does 1,25-D₃ alter IL-21 expression (Fig. 5, D and E). Because induction of IL-21 by IL-6 and STAT3 is undiminished by the absence of RORγt (48, 49), the preservation of IL-21 expression in the presence of 1,25-D₃ implies that 1,25-D₃-mediated suppression of RORγt does not reflect a general disruption of IL-6 or STAT3 signaling.

Relative to Th9 and Th17 cells, Th1 cells were resistant to effects of 1,25-D₃ (Fig. 1 and 2A), a finding reflected by significantly lowered expression of VDR mRNA (Fig. 2B). These data contrast with previous reports that 1,25-D₃ inhibits Th1 cell development in vitro (50–52) but are in accord with the report that the Th1 response of mice challenged with Leishmania major is unaffected by global VDR competence (53). The basis for these discrepancies is unclear. However, we found that high concentrations of 1,25-D₃ (i.e., 100 nm) can compromise the viability of developing Th1 cells (data not shown). Because non-viable CD4 cells stain positively for vital dyes is essential to eliminate the potentially confounding analytical consequences of either random or 1,25-D₃-mediated variations in cell viability.

Vitamin D signaling has been linked to immune tolerance by several lines of evidence. Human autoimmunity may correlate with winter (54), distance from the equator (54, 55), and industrialization (56), all of which influence vitamin D status, a parameter often found to be low in patients (54, 57). VDR (10) and exogenously administered 1,25-D₃ (5–9) limit disease in several mouse models of autoimmunity. Although we cannot as yet rule out model-specific induction of VDR in Th1 cells, our data indicate that direct effects of 1,25-D₃ on Th17 and Th9 cells are likely to be more important in limiting autoimmunity than are direct effects on Th1 cells (Figs. 1 and 2). Investigations of the role played by Th9 cells in autoimmunity are too preliminary to permit predictions with confidence regarding the relevance of our findings to autoimmunity. Nonetheless, IL-9 has been reported to aggravate EAE (17, 18) and colitis (19), diseases that are ameliorated by IL-10. Our data, therefore, suggest diversion of developing CD4 T cells away from the production of IL-9 in favor of IL-10 as a heretofore unrecognized mechanism by which vitamin D may limit autoimmunity.
Although considerably more is understood about Th17 cells and their pathogenicity, not all reports are consistent with an exclusively proinflammatory role for these cells. Th17 cells that are exposed to IL-23 promote EAE, whereas cells that develop in the presence of TGF-β1 and IL-6 are not pathogenic and protect against EAE when cotransferred with IL-23-treated cells (58). Furthermore, in the CD45RB model of colitis, adoptive transfer of cells that are unable to produce, or respond to IL-17A led, relative to wild-type cells, to more inflammation in recipient mice, not less (59). However, RORγt promotes acanthosis (15, 28), thereby raising the prospect that 1,25-D3 plays an important role in mitigating autoimmune disease mediated by this effector subset, although additional work will be needed to establish the manner in which these findings relate to the pathophysiology of specific autoimmune models. The suppression of IL-22-expressing cells (Figs. 1C and 5D) is especially intriguing in this regard. Topically applied analogs of 1,25-D3 are effective in the treatment of psoriasis (1), an effect that is attributed to 1,25-D3-mediated terminal differentiation of keratinocytes (63). However, IL-22 promotes acanthosis (15, 28), thereby raising the prospect that the therapeutic effect of vitamin D in psoriasis reflects in part the suppression of IL-22 production by the immune system.

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