Control of T cell activation by vitamin D

To the Editor:

We read with great interest the paper by von Essen *et al.* published in the April 2010 issue of *Nature Immunology*[1]. The authors show a critical difference in the expression of the T cell antigen receptor (TCR) pathway signaling molecule PLC-γ1 between naive $CD4^+$ T cells and *in vitro*–primed $CD4^+$ T cells. However, the suggested role of vitamin D in the induction of PLC-γ1 expression is disputable.

First, von Essen *et al.* demonstrate that expression of the vitamin D receptor (VDR) precedes PLC-γ1 expression after activation of freshly isolated $CD4^+$ T cells and propose a causal relationship between these events. Such a relationship is unlikely, however, as neither the ligand of the VDR, 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), nor its precursor, 25-hydroxyvitamin D ($25(OH)D_3$), is reported to be present in the culture system.

Second, two normocalcemic analogs (ZK191784 and ZK203278) are erroneously used as VDR antagonists. Both analogs have been developed as therapeutic alternatives to $1,25(OH)_2D_3$ because of their lack of effects on calcium homeostasis and their ability to retain the immunomodulatory potential of active vitamin D. Indeed, these molecules are comparable to $1,25(OH)_2D_3$ in their ability to inhibit proliferation and cytokine production of peripheral blood mononuclear cells[2,3]. The claim that these compounds are VDR antagonists is based on a study with a structurally distinct analog (ZK159222), which indeed has antagonistic immunomodulatory effects[4].

Third, the conclusions of the experiments using ketocanozole to block the hydroxylase CYP27B1 (which is required for intracellular conversion of the inactive vitamin D precursor $25(OH)D_3$ into $1,25\ (OH)_2D_3$) are premature. Ketoconazole is not a specific inhibitor of CYP27B1 and, moreover, inhibits the proliferation of T cells[5]. Additionally, $1,25(OH)_2D_3$ and $25(OH)D_3$ are used in equal concentrations here, despite the over 100-fold greater potency of $1,25(OH)_2D_3$ to inhibit the proliferation of $CD4^+$ T cells[6].

Finally, the lower T cell proliferation in vitamin D–deficient patients on chronic dialysis relative to that of healthy control subjects might be due to factors other than poor vitamin D status itself. In addition, the promotion of the proliferation of $CD4^+$ T cells after exposure to $1,25(OH)_2D_3$ is in contrast to numerous other published observations[2,6,7].

In summary, von Essen *et al.* show that PLC-γ1 is upregulated after TCR triggering in a culture system in the presumed absence of $1,25(OH)_2D_3$ and that the addition of VDR agonists inhibits T cell proliferation and PLC-γ1 expression. The conclusion that vitamin D stimulates the activation of human T cells by controlling the expression of VDR and PLC-γ1 is therefore questionable. Alternatively, as PLC-γ1 is expressed after T cell activation, the observed downregulation of PLC-γ1 in T cells exposed to VDR agonists can be explained by the inhibitory effects of $1,25(OH)_2D_3$ on T cell activation.

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COMPETING FINANCIAL INTERESTS
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Geisler replies:

The induction of VDR and PLC-γ1 takes place in both $CD4^+$ T cells and $CD8^+$ T cells after TCR stimulation[1] and not only in $CD4^+$ cells, as stated by Smolders *et al.* above. As for the availability of $25(OH)D_3$ and $1,25(OH)_2D_3$ in the T cells in our experimental setup, it is known that the concentrations of $25(OH)D_3$ and $1,25(OH)_2D_3$ in serum are around 100 nM and 80 pM, respectively. Most $25(OH)D_3$ and $1,25(OH)_2D_3$ in serum is bound to the vitamin D–binding protein DBP[2]. We now have data that demonstrate endocytosis of DBP by T cells (data not shown). The intracellular concentrations of $25(OH)D_3$ and $1,25(OH)_2D_3$ in freshly isolated T cells are therefore most likely equal to their concentrations in serum. In addition, according to the manufacturer, X-VIVO 15 medium contains human serum albumin and thus most probably also contains DBP[3]; both of these are sources of $25(OH)D_3$ and $1,25(OH)_2D_3$.

It is known that ligand-bound VDR can upregulate or downregulate several hundred genes[4]. VDR analogs affect VDR and VDR target genes differently than does $1,25(OH)_2D_3$. In accordance with that, the applied VDR analogs work as VDR antagonist on some of the VDR target genes (for example, genes involved in differentiation of the HL-60 human leukemia cell line[5] and genes involved in calcium homeostasis[5,6]).

Ketoconazole belongs to the large family of azoles, which inhibit P450 enzymes, including CYP27B1. Azoles are regularly used as CYP27B1 inhibitors[7,8]. We have shown that $1,25(OH)_2D_3$, but not $25(OH)D_3$, can reverse the effect of ketoconazole. It is thus reasonable to conclude that the effect is caused by inhibition of CYP27B1. The fact that ketoconazole inhibits T cell proliferation[9] is indeed in accordance with our study.