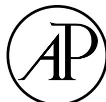

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Interleukin-2 is one of the targets of 1,25-dihydroxyvitamin D3 in the immune system☆

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Abstract

Interleukin (IL)-2 knockout (KO) mice, which spontaneously develop symptoms of inflammatory bowel disease similar to ulcerative colitis in humans, were made vitamin D deficient (D−) or vitamin D sufficient (D+) or were supplemented with 1,25-dihydroxyvitamin D_3 (1,25D3). 1,25-Dihydroxyvitamin D3 supplementation, but not vitamin D supplementation, reduced the early mortality of IL-2 KO mice. However, colitis severity was not different in D−, D+, or 1,25D3 IL-2 KO mice. Cells from D− IL-2 KO mice produced more interferon (IFN)-γ than cells from all other mice. Con A-induced proliferation was upregulated in IL-2 KO mice and downregulated in wildtype (WT) mice fed 1,25D3. All other measured immune responses in cells from IL-2 KO mice were unchanged by vitamin D status. In vitro addition of 1,25-dihydroxyvitamin D3 significantly reduced the production of IL-10 and IFN-γ in cells from D− and D+ WT mice. Conversely, IFN-γ and IL-10 production in cells from IL-2 KO mice were refractory to in vitro 1,25-dihydroxyvitamin D3 treatments. In the absence of IL-2, vitamin D was ineffective for suppressing colitis and ineffective for the in vitro downregulation of IL-10 or IFN-γ production. One target of 1,25-dihydroxyvitamin D3 in the immune system is the IL-2 gene. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: 1,25-dihydroxyvitamin D3; Interleukin-2; Ulcerative colitis; Vitamin D

The vitamin D receptor was discovered in resting and activated lymphocytes [1], suggesting a role of 1,25-dihydroxyvitamin D3 in immunoregulation. In vivo 1,25-dihydroxyvitamin D3 suppressed the development of various experimental autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE),[1] arthritis, and inflammatory bowel disease (IBD) [2–4]. Furthermore, experimentally induced vitamin D deficiency has been shown to increase the severity of autoimmune diseases including EAE and IBD [3,4]. Vitamin D status is a key factor that regulates the severity of autoimmune diseases.

IBDs are diseases characterized by deregulated immune responses, which result in inflammation of the gastrointestinal tract. $CD4^+$ T-cells and, in particular, T helper (Th) 1 cells, which produce interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, have been shown to play a central role in the development of IBD in humans and in experimental IBD [5,6]. $CD4^+$ T-cells are among the identified targets of 1,25-dihydroxyvitamin D3 in the immune system [3,7,8]. 1,25-Dihydroxyvitamin D3 decreased the production of IL-2, TNF-α, and IFN-γ [9–11] and inhibited T-cell proliferation [10]. The inhibition of T-cell proliferation caused by 1,25-dihydroxyvitamin D3 could be partially restored by the addition of exogenous IL-2 [9], which indicated a necessary role for IL-2. 1,25-Dihydroxyvitamin D3 is a T-cell inhibitor and inhibits the $CD4^+$ T-cells implicated in IBD pathology.

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[1] *Abbreviations used:* IL, interleukin; KO, knockout; D−, vitamin D deficient; D+, vitamin D sufficient; 1,25D3, supplemented with 1,25-dihydroxyvitamin D_3; EAE, experimental autoimmune encephalomyelitis; IFN, interferon; WT, wildtype; IBD, inflammatory bowel disease; TNF, tumor necrosis factor.

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In vivo vitamin D status has been shown to influence the development of experimental IBD in one animal model. IL-10 knockout (KO) mice spontaneously develop a form of IBD that resembles Crohn's disease in humans [4]. Vitamin D deficiency in IL-10 KO mice resulted in an accelerated form of IBD that eventually induced premature death [4]. Supplementation with 1,25-dihydroxyvitamin D3 blocked the progression of IBD and prevented death in the IL-10 KO mice [4]. A second form of IBD develops spontaneously in IL-2 KO mice. The inflammation of the gastrointestinal tract in IL-2 KO mice is restricted to the colon and resembles the human disease ulcerative colitis [12]. The effects of vitamin D status on the development of IBD in IL-2 KO mice were determined in the experiments outlined below.

IL-2 is an autocrine growth factor for T-cells. It is not known whether 1,25-dihydroxyvitamin D3 regulates T-cell proliferation and cytokine production independently of IL-2. Tsoukas et al. [1], showed that proliferation of lymphocytes activated with mitogen was inhibited by 1,25-dihydroxyvitamin D3 and the lymphocytes exhibited reduced IL-2 activity. Addition of exogenous IL-2 partially reversed the antiproliferative effects of 1,25-dihydroxyvitamin D3 [9], indicating that 1,25-dihydroxyvitamin D3 may be mediating its inhibitory effect through an IL-2-dependent pathway. Alroy et al. [13] showed that 1,25-dihydroxyvitamin D3 inhibited IL-2 transcription. The repression of IL-2 transcription was directly mediated by 1,25-dihydroxyvitamin D3 and was vitamin D receptor dependent [13]. IL-2 may be a necessary target for the regulation of the immune system by vitamin D.

IL-2 KO mice develop two distinct diseases, which can eventually result in the premature death of the animals [12]. IL-2 KO mice die within 6 weeks of age due to a disease, which induces lymphadenopathy, weight loss, and splenomegaly but not colitis [12]. The surviving IL-2 KO mice develop symptoms, which closely resemble ulcerative colitis in humans. IL-2 KO mice with IBD exhibit a Th1 pattern of cytokines with significant production of IFN-γ and TNF-α [14]. Conversely, Th2 cytokines such as IL-4 and IL-10 were reduced in IL-2 KO mice [14]. In vitro 1,25-dihydroxyvitamin D3 has been shown to regulate IL-2 production. Here we examined whether vitamin D status in vivo and 1,25-dihydroxyvitamin D3 in vitro regulates the development of colitis and T-cell function in cells and mice which are IL-2 deficient.

Materials and methods

Mice. Adult C57BL/6 IL-2 heterozygote (+/KO) breeding pairs were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were genotyped by isolating DNA from tail clippings and were identified as IL-2 KO, IL-2 +/KO, and wildtype (WT) by polymerase chain reaction with primers which spanned the site of the gene KO and a second set of primers specific for the neomycin insert. Only IL-2 KO and WT mice were used in this study. All of the procedures described were reviewed and approved by the Pennsylvania State University Institutional Animal Care and Use Committee on 1/25/99, IACUC No. 98118-A0.

Diet. Breeding females were fed a commercial mouse diet (No. 5105; Ralston Purina, Richmond, IN). For experiments, all mice were fed synthetic diets made in the laboratory [4]. Experimental diets were replaced every 2–3 days during the experiments. In the second week of gestation, pregnant females were selected and randomly distributed into three groups. The three groups differed only in the amount of vitamin D supplied in the diet. Group 1 was fed no vitamin D (D−), group 2 was fed 5 μg (200 IU) of vitamin D/day (D+), and group 3 was fed 0.005 μg (5 IU) of 1,25-dihydroxyvitamin D3/day (1,25D3). Feeding 1,25-dihydroxyvitamin D3 has been shown to be more effective than feeding vitamin D for halting the progression of experimental autoimmune diseases including IBD [2–4]. By feeding 1,25-dihydroxyvitamin D3 instead of vitamin D the body's need for processing vitamin D is bypassed and the active hormone is delivered directly to the site of inflammation in IBD. Starting pregnant dams on a vitamin D-deficient diet ensured that by 5 weeks of age the weanlings were vitamin D deficient [3]. Litters were weaned at 3 weeks of age and litters from all three groups were maintained on the same diet as their dams except that the fat content was reduced from 12 to 5%. Mice were housed under yellow light to prevent the synthesis of vitamin D in skin. After litters were weaned, dams were returned back to the breeding pool and fed a commercial diet.

1,25-Dihydroxyvitamin D3 supplementation. 1,25D3 weanlings were fed a diet supplemented with 0.005 μg of 1,25-dihydroxyvitamin D3/day for the first 3 weeks of life. At 4 weeks 1,25-dihydroxyvitamin D3 supplementation was increased to 0.010 μg (10 IU)/day and at 8 weeks increased again to 0.025 μg (25 IU)/day. Mice were sacrificed at 9–12 weeks of age.

Serum analysis. Mice were bled at the end of experiments to measure serum calcium. Blood was collected from the tail vein, and serum was extracted. Serum calcium levels were measured using calcium colometric kits (587-A) from Sigma Chemical (St. Louis, MO). Vitamin D deficiency was monitored by serum calcium analysis. Normal serum calcium levels for mice are 2.00–2.75 mmol/L. Vitamin D deficiency was established as values less than 1.27 mmol/L.

IBD severity. Mice were sacrificed at 9–12 weeks of age, and the body weights were recorded. A section of the large intestine was saved and sent to the Pennsylvania State Diagnostic Laboratory for sectioning and

staining with hematoxyalin and eosin. A minimum of four paraffin sections (4 μm) from each mouse were scored as described previously (4). The sections were blindly scored on scale of 0–5 for inflammation: 0, no inflammation; 1, few inflammatory cells; 2, mild inflammation; 3, abscess formation; 4, abscess formation with many inflammatory cells throughout; and 5, massive inflammation throughout the section.

Measurement of lymphocyte proliferation and cytokine production. Spleens were extracted under aseptic conditions, and cells were gently disrupted manually. Cells were placed in cell culture medium containing Hanks balanced salt solution (Sigma) supplemented with 1 mol/L of Hepes (Sigma) and 0.01 g/L gentomycin (Sigma). The cell suspensions were centrifuged at 1200 rpm for 5 min. Erythrocytes were lysed and the remaining cells were washed with Hanks buffer. The cells were resuspended in RPMI 1640 cell medium (Sigma) supplemented with 0.01 g/L gentomycin (Sigma), 200 mmol/L glutamine (Sigma), 5 mmol/L 2 mercaptoethanol (Sigma), and 10% fetal bovine serum (Hyclone, Hornby, Ontario, Canada). The viability of cells was determined using trypan blue exclusion and in all cases 90% or more of the cells were viable. T-cells were adjusted to a final density of 2×10^6 cells/well. T-cells were stimulated with 5 μg/ml of Con A or unstimulated controls. The in vitro vitamin D treatment was with 10 nmol/L of 1,25-dihydroxyvitamin D3 diluted in ethanol or ethanol only (control). Cells were cultured in 96-well plates for proliferation assays and 24-well plates for supernatants (Corning Costar, Corning, NY). After 72 h, supernatants were collected for enzyme-linked immnuosorbent assays (ELISAs). For proliferation assays, 0.4 μCi of [^{3}H]thymidine (ICN, Costa Mesa, CA) was added to each well and the cells were incubated for an additional 24 h. Radioactive thymidine incorporation was determined by liquid scintillation using a Beta plate Counter. Mouse IL-2, IL-4, IL-5, IL-10, TNF-α, and IFN-γ productions were detected by ELISA. All kits were from Pharmigen (San Diego, CA), and instructions were followed exactly as described. The ELISA detection limits were 25 pg/ml IL-2, 62 pg/ml IL-4, 312 pg/ml IL-5, 312 pg/ml IL-10, 62 pg/ml TNF-α, and 1000 pg/ml IFN-γ.

Statistical analysis. Groups of three to six age- and sex-matched C57BL/6 IL-2 KO and WT mice were used per experiment. Experiments were repeated as necessary and, where possible, values were reported as means from multiple experiments. In some cases, log-transformed data were used because these distributions were consistent with normality. The following dependent variables were log transformed: IL-10 and IFN-γ production and total body weight. Data were subjected to two-way ANOVA. The significance of differences across the six levels were compared using Scheffe's post hoc test. Differences between control treatment and in vitro addition of 1,25-dihydroxyvitamin D3 were compared by paired t test. A two-sample test for binomial proportions was used for analysis of the mortality among animals. Differences of $P < 0.05$ were considered statistically significant. Data were analyzed using PC-SAS (SAS, Cary, NC).

Results

Mortality of vitamin D-deficient and -sufficient and l,25D3-supplemented IL-2 KO mice. D− IL-2 KO mice began to die at 4 weeks of age with a mean age at death of 4.6 ± 0.3 weeks. D+ IL-2 KO mice also started dying at 4 weeks of age with a slightly longer time until death of 5.6 ± 0.4 weeks. Only two 1,25D3 IL-2 KO mice died, one at 5 weeks of age and one at 6 weeks of age. D−, D+, and 1,25D3 WT mice did not die during the course of the experiments (Table 1). Forty eight percent of the D−, 43% of the D+, and 17% of the 1,25D3 IL-2 KO mice were dead by 9 weeks of age (Table 1). 1,25-Dihydroxyvitamin D3 supplementation ($P = 0.05$), but not vitamin D supplementation ($P = 0.13$), significantly suppressed the mortality of the IL-2 KO mice (Table 1). The IL-2 KO mice did not develop diarrhea or IBD and the deaths in the IL-2 KO mice were from unknown causes (data not shown). In the IL-2 KO mice only 1,25-dihydroxyvitamin D3 treatment effectively reduced but did not eliminate the mortality of the mice.

IBD symptoms. Histopathology scores (colitis) were not dependent on vitamin D status in IL-2 KO mice (Table 2). At the end of the experiment D− IL-2 KO mice were significantly smaller than all other mice (Table 2, $P < 0.05$). As expected, the serum calcium levels of D− mice were significantly lower than those of D+ and 1,25D3 mice (Table 2, $P < 0.05$). The serum calcium levels of D+ IL-2 KO and WT mice were significantly higher than those of 1.25D3 IL-2 KO and WT mice (Table 2, $P < 0.05$), suggesting that the 1,25-dihydroxyvitamin D3 dose was not toxic. IBD symptoms were unaffected by vitamin D status.

Lymphocyte proliferation. Lymphocyte proliferation in response to Con A was significantly lower in cells from WT mice fed 1,25D3 compared to cells from

Table 1
Mortality of IL-2 KO and WT mice

Vitamin D status	IL-2 KO	WT
D−	10/23 (48%)	0/10 (0%)
D+	9/21 (43%)	0/10 (0%)
1,25D3	2/12 (17%)*	0/10 (0%)

Note. Values represent the number dead over total number (percentage).

* Value is significantly less than that of D− or D+ IL-2 KO mice ($P < 0.05$).

Table 2
Colitis severity in IL-2 KO and WT mice

Dietary treatment			
IL-2 KO	D−	D+	1,25D3
Total body weight (g)	12. 4[*a] (13.5–11.5)	21.1[b] (22.9–19.5)	19.5[b] (21.3–17.8)
Calcium (mmol/L)	1.09 ± 0.03[a]	2.50 ± 0.08[b]	2.11 ± 0.17[c]
Histology score	2.1 ± 0.2[a]	1.8 ± 0.1[a]	1.9 ± 0.1[a]
WT			
Total body weight (g)	19.3[b] (20.5–18.2)	22.0[b] (22.9–21.1)	20.1[b] (20.9–19.3)
Calcium (mmol/L)	1.26 ± 0.07[a]	2.26 ± 0.11[b]	1.85 ± 0.14[c]
Histology score	0[b]	0[b]	0[b]

[*] Values represent mean (range, for weights) or mean ± SE. All values were based on an n of 8–10 and analyzed by ANOVA ($P < 0.05$). Means with different superscripts were significantly different ($P < 0.05$).

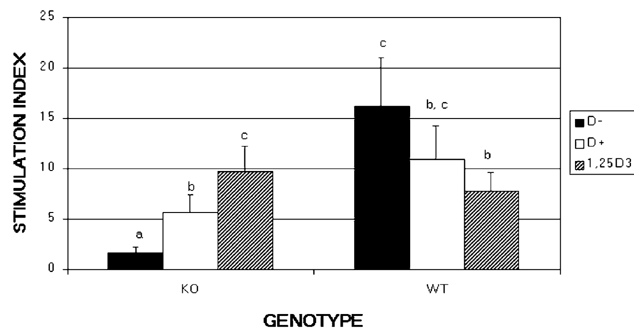

Fig. 1. Lymphocyte proliferation in cells from IL-2 KO and WT mice. 1,25D3 suppressed the proliferative capacity of T cells in WT mice. Vitamin D and 1,25D3 increased the proliferative capacity of IL-2 KO mice. Means with different superscripts are significantly different ($P < 0.05$). Stimulation index = CPM due to Con A stimulation/background CPM. Bars represent means + SE ($n = 4$–12). Data were analyzed by ANOVA.

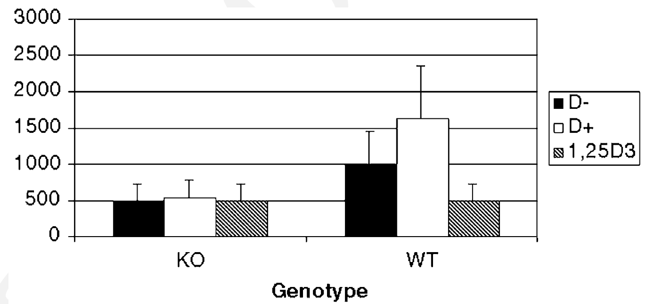

Fig. 2. IL-10 production as a function of vitamin D status. Diet did not significantly influence IL-10 production in cells from IL-2 KO mice or cells from WT mice. Bars represent means + SE ($n = 4$–10). Data were analyzed by ANOVA.

D− mice (Fig. 1). Interestingly, the lymphocyte proliferation to Con A was enhanced in cells from IL-2 KO mice fed l,25D3 (Fig. 1). Lymphocytes from D− IL-2 KO mice proliferated the least amount in response to Con A in vitro. Feeding IL-2 KO mice vitamin D and 1,25D3 increased Con A-induced lymphocyte proliferation by 2.5- and 6-fold, respectively (Fig. 1).

The addition of 1,25-dihydroxyvitamin D3 in vitro consistently decreased the Con A-induced proliferation of cells from both WT and IL-2 KO mice. Con A-induced proliferation of cells from WT mice was reduced by 26 ± 2% in the presence of 1,25-dihydroxyvitamin D3 (data not shown). Cells from IL-2 KO mice showed a 50 + 5% reduction in Con A proliferation in the presence of 1,25-dihydroxyvitamin D3 (data not shown). T-cell proliferation was inhibited by 1,25-dihydroxyvitamin D3 in lymphocytes from both WT and IL-2 KO mice.

Cytokine analysis. IL-4, IL-5, and TNF-α production were below detection levels in the supernatants of cells from all mice. IL-10 production was not significantly influenced by diet in cells from IL-2 KO and WT mice (Fig. 2). Cells from D− IL-2 KO mice produced significantly more IFN-γ than cells from all other diet groups in IL-2 KO and WT mice (Fig. 3). There were no other significant effects of diet on IFN-γ production. Overall, cells from IL-2 KO mice (pooled values from D−, D+, and 1,25D3 groups) produced significantly lower amounts of IL-10 (550 ± 201 pg/ml) than cells from WT mice (1020 ± 123 pg/ml; $P < 0.05$). Conversely, IFN-β production in cells from IL-2 KO mice (pooled values from D−, D+, and 1,25D3 groups) was higher (11, 358 ± 3596 pg/ml) than IFN-γ production in

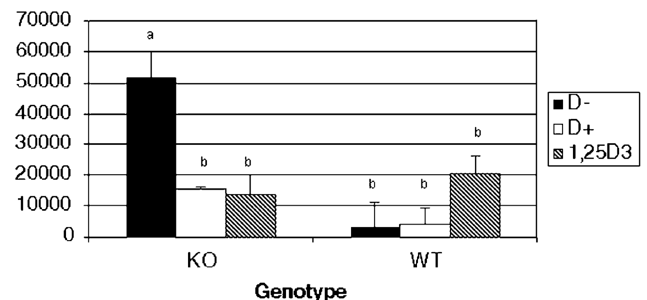

Fig. 3. IFN-γ production as a function of vitamin D status. Lymphocytes from D− IL-2 KO mice made significantly more IFN-γ then all other mice. Bars represent means + SE ($n = 4$–10). Means with different superscripts are significantly different ($P < 0.05$). Data were analyzed by ANOVA.

cells from WT mice (6003 ± 4322; $P = 0.09$). IL-2 production in cells from WT mice was unaffected by vitamin D status in vivo (data not shown).

In vitro addition of 1,25-dihydroxyvitamin D3 significantly reduced the production of IL-10 and IFN-γ in cells from D− and D+ WT mice compared to respective controls (Table 3, $P < 0.05$). Interestingly, in vitro addition of 1,25-dihydroxyvitamin D3 to cells from 1,25D3 WT mice had no effect on IFN-γ or IL-10 production (Table 3). 1,25-Dihydroxyvitamin D3 did not change the ability of cells from D−, D+, or 1,25D3 IL-2 KO mice to produce IL-10 or IFN-γ (data not shown). IL-2 production in cells from WT mice was 18 ± 3% lower in the presence of 1,25-dihydroxyvitamin D3 in vitro.

Discussion

The colitis which developed in IL-2 KO mice was unaffected by vitamin D status. Vitamin D deficiency accelerated the development of IBD in IL-10 KO mice. Moreover, colitis which developed in IL-10 KO mice was suppressed by 1,25D3 treatment [4]. Vitamin D deficiency did not accelerate the development of IBD symptoms in IL-2 KO mice. The difference between the effects of vitamin D status on IBD in IL-10 KO and that in IL-2 KO mice argues that one target of vitamin D in the immune system is IL-2. The inability of IL-2 KO mice to produce IL-2 resulted in a form of colitis, which was refractory to vitamin D status or 1,25D3 treatment.

1,25-Dihydroxyvitamin D3 treatment, but not vitamin D treatment, significantly reduced the early mortality of IL-2 KO mice. The deaths in IL-2 KO mice were of unknown cause and unrelated to the development of IBD. The dose of 1,25-dihydroxyvitamin D3 used to suppress mortality was fivefold higher than that used to suppress IBD mortality in IL-10 KO mice [4]. The ability of 1,25D3 to suppress the early mortality in IL-2 KO mice may provide the key to understanding the cause of death and warrants further investigation.

Vitamin D status differentially affected the ability of lymphocytes from IL-2 KO and WT mice to proliferate in response to Con A. 1,25D3 treatment suppressed Con A-induced lymphocyte proliferation in WT mice and enhanced Con A-induced lymphocyte proliferation in IL-2 KO mice. The opposing effects of 1,25D3 treatment in vivo must be a consequence of the ability/inability of the mice to produce IL-2. In IL-2 KO mice, feeding 1,25D3 induced the proliferative capacity of T-cells. Perhaps in the absence of IL-2, vitamin D is a survival factor for T-cells. In WT mice, where IL-2 production was possible, 1,25D3 suppressed the proliferative capacity of T cells probably by inhibiting IL-2-producing precursors in these cultures.

Consistent with previous research, in vitro addition of 1,25-dihydroxyvitamin D3 decreased Con A proliferation of cells from WT mice [9,15]. In cells from IL-2 KO mice the in vitro antiproliferative effects of 1,25-dihydroxyvitamin D3 were still apparent and twofold greater than those in cells from WT mice. Therefore, the antiproliferative effects of 1,25-dihydroxyvitamin D3 on T-cells must be independent of IL-2. The greater inhibition of IL-2 KO cells, compared to WT cells, may have been due to increased cell death when IL-2 was unavailable.

Vitamin D status did not affect the ability of cells from IL-2 KO and WT mice to produce IL-10. Cells from IL-2 KO mice produced significantly lower amounts of IL-10 than cells from WT mice. Conversely, IL-2 KO mice produced higher amounts of IFN-γ than WT mice. These data support results from other studies, which report that IL-2 KO mice exhibited decreased IL-10 and increased IFN-γ production [14].

Vitamin D deficiency increased the ability of cells from IL-2 KO mice to secrete IFN-γ. Vitamin D and 1,25-dihydroxyvitamin D3 in vivo reduced the production of IFN-γ in cells from IL-2 KO mice. The increased production of IFN-γ by lymphocytes from D− IL-2 KO mice probably reflects an increased precursor frequency of T cells secreting IFN-γ, since in vitro addition of 1,25-dihydroxyvitamin D3 had no effect on IFN-γ production in IL-2 KO cells. Increased IFN-γ secretion in D− IL-2 KO mice may be an indication of a more pronounced inflammatory response compared to all other mice. Perhaps if the mortality rates were lower in D− IL-2 KO

Table 3
In vitro effects of 1,25-Dihydroxyvitamin D3 on the production of IL-10 and IFN-γ in WT mice

Vitamin D status			
IL-10 (pg/ml)	D−	D+	1,25D3
Control	992[a,*] (1380–710)	1619 (2275–1152)	432 (507–368)
$1,25(OH)_2$ D_3	665* (925–478)	1152* (1754–757)	464 (472–445)
IFN-γ (pg/ml)			
Control	2164[a] (3428–1339)	3944 (4628–3361)	12332 (17854–8510)
$1,25(OH)_2$ D_3	1652* (2643–1032)	2980* (3568–2489)	9996 (14328–7259)

[a] Values represent geometric mean (range) ($n = 4$–10). Data were analyzed by paired t test.
* Significantly less than control counterpart.

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mice, these mice would eventually have developed more severe symptoms of IBD. WT mice do not spontaneously develop autoimmune disease and therefore vitamin D deficiency had no effect on IFN-γ production in WT mice.

In vitro addition of 1,25-dihydroxyvitamin D3 reduced the production of IL-10, IFN-γ, and IL-2 when the cells were from D− and D+ WT mice. Rigby et al. [9,11] also showed that in vitro addition of 1,25-dihyroxyvitamin D3 significantly inhibited the production of IFN-γ and IL-2. Decreased IL-10 production, following l,25-dihydroxyvitamin D3 addition, was unexpected and further studies will be conducted. Cells from l,25D3-fed WT mice were refractory to additional 1,25-dihydroxyvitamin D3 in vitro. l,25D3-fed WT mice may have been exposed to saturating amounts of 1,25-dihydroxyvitamin D3 in vivo and therefore further addition of 1,25-dihydroxyvitamin D3 in vitro was ineffective. The addition of 1,25-dihydroxyvitamin D3 to lymphocytes in vitro did not alter the IL-10 or IFN-γ production when the cells came from IL-2 KO mice. Our evidence suggests that IL-10 and IFN-γ may be indirectly regulated by 1,25-dihydroxyvitamin D3 and that the inhibition of these two cytokines by 1,25-dihydroxyvitamin D3 is dependent on a source of IL-2.

Vitamin D deficiency did not increase the severity of IBD in IL-2 KO mice. Furthermore the colitis, which developed in IL-2 KO mice, was refractory to 1,25D3 treatment. The ability of 1,25-dihydroxyvitamin D3 to inhibit the in vitro T-cell proliferation was independent of IL-2. Conversely, the in vitro 1,25-dihydroxyvitamin D3-mediated decrease in IFN-γ and IL-10 production required the ability to make IL-2. One likely target of vitamin D in the immune system is IL-2.

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