1,25-Dihydroxycholecalciferol Prevents and Ameliorates Symptoms of Experimental Murine Inflammatory Bowel Disease\(^1\)

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ABSTRACT Anecdotal data suggest that the amount of vitamin D available in the environment either from sunshine exposure or diet may be an important factor affecting the development of inflammatory bowel disease (IBD) in humans. We tested the vitamin D hypothesis in an experimental animal model of IBD, interleukin (IL)-10 knockout (KO) mice, which spontaneously develop symptoms resembling human IBD, were made vitamin D deficient, vitamin D sufficient or supplemented with active vitamin D (1,25-dihydroxycholecalciferol). Vitamin D–deficient IL-10 KO mice rapidly developed diarrhea and a wasting disease, which induced mortality. In contrast, vitamin D–sufficient IL-10 KO mice did not develop diarrhea, waste or die. Supplementation with 50 IU of cholecalciferol (5.0 \(\mu\)g/d) or 1,25-dihydroxycholecalciferol (0.005 \(\mu\)g/d) significantly \((P < 0.05)\) ameliorated symptoms of IBD in IL-10 KO mice. 1,25-Dihydroxycholecalciferol treatment (0.2 \(\mu\)g/d) for as little as 2 wk blocked the progression and ameliorated \((P < 0.05)\) symptoms in IL-10 KO mice with already established IBD. J. Nutr. 130: 2648–2652, 2000.

KEY WORDS: vitamin D • inflammatory bowel disease • 1,25-dihydroxycholecalciferol • mice

Inflammatory bowel diseases (IBD)\(^3\) are immune-mediated diseases of unknown etiology affecting the gastrointestinal (GI) tract. There are at least two distinct forms of IBD, ulcerative colitis and Crohn’s disease. IBD are chronic recurring illnesses most commonly involving inflammation of the terminal ileum and colon, although these diseases can also affect many sites throughout the alimentary tract. Clearly, genetic factors predispose individuals to development of IBD (Podolosky 1991). In addition, the environment contributes to IBD development, and there is reason to believe that vitamin D may be an environmental factor affecting IBD. There is less vitamin D from sunlight exposure in areas in which IBD occurs most often because IBD is most prevalent in northern climates such as North America and Northern Europe (Podolosky 1991, Sonnenberg et al. 1991). A major source of vitamin D results from its manufacture via a photolysis reaction in the skin, and vitamin D availability from sunlight exposure is significantly lower in northern climates, particularly during the winter (Clemens et al. 1982, DeLuca 1993). Dietary intake of vitamin D is problematic because few foods are naturally rich in vitamin D. Weight loss occurs in 65–75% of patients diagnosed with Crohn’s disease and 18–62% of patients with ulcerative colitis (Fleming 1995, Geerling et al. 1998). Vitamin D deficiencies in general and vitamin D deficiency in particular have been shown to occur in IBD patients (Andreasen et al. 1998, Kuroki et al. 1993). To date, the possible association between vitamin D status and the incidence and severity of IBD in humans or animals has not been studied. The anecdotal information suggests that vitamin D status could be an environmental factor affecting the prevalence rate for IBD; this possible correlation warrants serious investigation.

The identification of vitamin D receptors in peripheral blood mononuclear cells sparked the early interest in vitamin D as an immune system regulator (Bhalla et al. 1983, Provvedini et al. 1983). In particular the CD4\(^+\) T cells have vitamin D receptors and are therefore targets for vitamin D (Veldman et al. 2000). Hormonally active vitamin D [1,25-(OH)\(_2\)D\(_3\)] suppressed the development of at least two experimental autoimmune diseases (Cantorna et al. 1996 and 1998a). In vitro, 1,25(OH)\(_2\)D\(_3\) inhibited T-cell proliferation and decreased the production of interleukin (IL)-2, interferon (IFN)-\(\gamma\) and tumor necrosis factor (TNF)-\(\alpha\) (Lemire and Adams 1992). In vivo, 1,25(OH)\(_2\)D\(_3\) injections were shown to inhibit the delayed type hypersensitivity reaction associated with the type-1 helper T (Th1) cell response (Lemire and Archer 1991, Lemire 1992). Vitamin D is a potent regulator of the immune system in general and T cells specifically.

For IBD, the immune-mediated attack is against the GI tract (Niesnser and Volk 1993, Podolosky 1991). T cells, which preferentially produce the Th1 cytokines (IL-2, IFN-\(\gamma\) and TNF-\(\alpha\)), have been shown to transfer Crohn’s-like symptoms to naïve mice (Aranda et al. 1997, Bregenholt and Claesson 1998), and the production of Th1 cytokines is asso-

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\(^3\) Abbreviations used: GI, gastrointestinal tract; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; KO, knockout; 1,25(OH)\(_2\)D\(_3\), 1,25-dihydroxycholecalciferol; SI, small intestines; TGF, transforming growth factor; Th1, type-1 helper; TNF, tumor necrosis factor; WT, wildtype.

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associated with IBD in humans as well (Niessen and Volk 1995).

1,25(OH)₂D₃ treatment has been shown to suppress the
development of other T-cell-mediated experimental autoimmune
diseases (multiple sclerosis and arthritis; Cantorna et al. 1996 and 1998a).

The hypothesis that vitamin D (through the production of 1,25-dihydroxycholecalciferol) would suppress the development and progression of IBD was tested.

Recently, a number of transgenic animals have been developed in which IBD symptoms occur spontaneously. One of the best animal models for Crohn’s disease is the IL-10 knockout (KO) mouse (Kuhn et al. 1993, Mac Donald 1994). In conventional animal facilities, the IL-10 KO mice develop enterocolitis within 5–8 wk of life (Kuhn et al. 1993).

Animals that develop IBD in IL-10 KO mice is due to an uncontrolled immune response to conventional microflora because germfree IL-10 KO mice do not develop disease. In addition, mice reared in conventional facilities develop milder disease, which does not result in the death of the mice (Kuhn et al. 1993). There are limitations involved in studying IL-10 KO mice as a model of IBD. If vitamin D is a regulator of IL-10 production, then the results in this animal model may not represent a “normal” immune response. However, patients with Crohn’s disease show similar symptoms, have depressed IL-10 production and have been treated successfully with IL-10 (Narula et al. 1998).

MATERIALS AND METHODS

Mice. Age- and sex-matched C57BL/6 IL-10 KO and wildtype (WT) mice were produced in the Pennsylvania State University breeding colony; the breeding pairs were obtained from Jackson Laboratory (Bar Harbor, ME). The animal facilities at the Pennsylvania State University are specific pathogen free and therefore breeders were housed in negative pressure breeding isolators. The breeding colony; the breeding pairs were obtained from Jackson Laboratory (Bar Harbor, ME). The animal facilities at the Pennsylvania State University are specific pathogen free and therefore breeders were housed in negative pressure breeding isolators.

Diets. From a single pool of breeding females fed commercial mouse diet (#5105 Ralston Purina; Richmond, IN), females in wk 2 of gestation were selected and distributed randomly into two groups. Feeding pregnant dams a vitamin D–deficient diet ensured that the weanlings would be vitamin D deficient by 5 wk of age (Cantorna et al. 1999). Approximately 30% of the IL-10 KO mice die after the development of severe anemia and weight loss (Kuhn et al. 1993). The enterocolitis that develops in IL-10 KO mice is due to an uncontrolled immune response to conventional microflora because germfree IL-10 KO mice do not develop disease. In addition, mice reared in conventional facilities develop milder disease, which does not result in the death of the mice (Kuhn et al. 1993). There are limitations involved in studying IL-10 KO mice as a model of IBD. If vitamin D is a regulator of IL-10 production, then the results in this animal model may not represent a “normal” immune response. However, patients with Crohn’s disease show similar symptoms, have depressed IL-10 production and have been treated successfully with IL-10 (Narula et al. 1998).

VITAMIN D AND INFLAMMATORY BOWEL DISEASE

RESULTS

Mortality of vitamin D–deficient IL-10 KO mice. Figure 1 shows that vitamin D–deficient IL-10 KO mice begin to die at 7 wk of age and by 9 wk of age, 58% (15/26) of the vitamin D–deficient IL-10 KO mice were dead. After 9 wk of age, vitamin D–deficient IL-10 KO mice continued to waste and the death rate increased. In contrast, the vitamin D–sufficient IL-10 KO (n = 10) and the vitamin D–deficient WT (n = 20) mice appeared healthy, even at 13 wk of age.

The vitamin D–deficient IL-10 KO mice were growth re-

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Vitamin D–deficient IL-10 KO mice did not develop diarrhea or die. IL-10 KO mice died after developing diarrhea. Vitamin D–deficient WT mice were also used in these experiments. Vitamin D–deficient IL-10 KO mice died after developing diarrhea. Vitamin D–deficient WT and vitamin D–sufficient IL-10 KO mice did not develop diarrhea or die. Histopathology of vitamin D–deficient and –sufficient interleukin (IL)-10 knockout (KO) mice and vitamin D–deficient wildtype (WT) mice. Vitamin D–deficient IL-10 KO weanling mice were divided randomly into two groups. One group was maintained vitamin D deficient (−D, n = 26) and the other was fed the same diet which contained 5.0 μg cholecalciferol/d for the remainder of the experiment (−D, n = 10). Vitamin D–deficient wildtype (WT) (−D, n = 20) mice were also used in these experiments. Vitamin D–deficient IL-10 KO mice died after developing diarrhea. Vitamin D–deficient WT and vitamin D–sufficient IL-10 KO mice did not develop diarrhea or die.

IBD symptoms in vitamin D–deficient and 1,25(OH)2D3–supplemented IL-10 KO mice. Vitamin D–deficient WT and IL-10 KO mice weighed less than their 1,25(OH)2D3–supplemented counterparts at 9 wk of age (Table 1). The weights of the vitamin D–deficient IL-10 KO mice were lower than in previous experiments (Fig. 2) although data were consistent with the accelerated weight loss observed previously in vitamin D–deficient IL-10 KO mice. As expected, the serum calcium concentrations in 1,25(OH)2D3–supplemented mice were significantly (P < 0.05) higher than those of the vitamin D–deficient mice (Table 1). Hemoglobin levels and erythrocyte numbers were normal and not different in vitamin D–deficient, vitamin D–sufficient, and 1,25(OH)2D3–supplemented IL-10 KO and WT mice (data not shown).

WT mice that were vitamin D deficient and sufficient showed no signs of inflammation or abnormalities in the SI. Vitamin D–deficient IL-10 KO mice had significantly more inflammation in the SI than their 1,25(OH)2D3–supplemented or vitamin D–sufficient counterparts (P < 0.05, Table 1 and data not shown). Although the vitamin D–deficient IL-10 KO mice were the smallest in size, necropsy showed that they had extremely large SI. Future experiments will include SI weights as more quantitative measurements of inflammation in the SI. Short-term 1,25(OH)2D3 treatment and IBD severity. There were no significant differences in the weight of any of the mice after 2 wk of 1,25(OH)2D3 treatment (data not shown). The SI of the vitamin D–deficient IL-10 KO mice, however, were enlarged and weighed significantly more (P < 0.05) than the SI from 1,25(OH)2D3–supplemented IL-10 KO, vitamin D–deficient WT and 1,25(OH)2D3–supplemented WT mice (Table 2). In fact, the SI from vitamin D–deficient IL-10 KO mice measured less than half the combined weight of the vitamin D–supplemented IL-10 KO mice. As expected, the serum calcium concentrations in 1,25(OH)2D3–supplemented mice were significantly (P < 0.05) higher than those of the vitamin D–deficient mice (Table 1). Hemoglobin levels and erythrocyte numbers were normal and not different in vitamin D–deficient, vitamin D–sufficient, and 1,25(OH)2D3–supplemented IL-10 KO and WT mice (data not shown).

Histopathology of vitamin D–deficient and –sufficient IL-10 KO and WT mice.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Vitamin D status</th>
<th>Weight, g</th>
<th>Serum calcium, mmol/L</th>
<th>Histology score</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 KO 2</td>
<td>7</td>
<td>−D</td>
<td>12.4 ± 2.3*</td>
<td>1.74 ± 0.28*</td>
<td>3.0 ± 0.2*</td>
</tr>
<tr>
<td>IL-10 KO 2</td>
<td>1</td>
<td>1,25(OH)2D3</td>
<td>20.9 ± 0.8</td>
<td>3.00 ± 0.30</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>WT</td>
<td>4</td>
<td>−D</td>
<td>16.7 ± 1.9</td>
<td>1.67 ± 0.32*</td>
<td>0</td>
</tr>
<tr>
<td>WT</td>
<td>4</td>
<td>1,25(OH)2D3</td>
<td>21.1 ± 1.5</td>
<td>2.72 ± 0.25</td>
<td>0</td>
</tr>
</tbody>
</table>

1 All of the mice were vitamin D deficient (−D) for the first 5 wk of life. At 5 wk, the mice were divided into two groups; half were supplemented with 0.005 μg/d 1,25(OH)2D3 for 4 wk. Values are means ± SEM; *significantly different from supplemented counterpart, P < 0.05.

2 IL, interleukin; KO, knockout; −D, vitamin D deficient; 1,25(OH)2D3, 1.25-dihydroxycholecalciferol; WT, wildtype.
D-deficient IL-10 KO mice were 9.9% of the total body weight which is double the normal value (Table 2). Inflammation in the SI of IL-10 KO mice was reduced after as little as 2 wk of 1,25(OH)2D3 treatment.

**Food restriction vs. vitamin D deficiency and the symptoms of IBD.** To rule out the possibility that weight loss and not vitamin D deficiency was associated with the increased symptoms of IBD observed, the food intake of vitamin D–sufficient IL-10 KO and WT mice was restricted (Table 3). Food restriction decreased the weight of vitamin D–sufficient IL-10 KO and WT mice, but the vitamin D–deficient IL-10 KO mice were still significantly lighter (P < 0.05, Table 3). The IL-10 KO mice were extremely ill by 9 wk in this series of experiments and had already undergone severe wasting. Food restriction did not change the symptoms of IBD in the vitamin D–sufficient mice. Food-restricted vitamin D–sufficient IL-10 KO mice did not develop overt enterocolitis or die, which occurred in vitamin D–deficient IL-10 KO mice. The relative SI weight of vitamin D–sufficient food-restricted IL-10 KO mice was not different than in previous experiments or compared with WT controls (Table 3). Histopathology confirmed the weight measurements in Table 3 (data not shown). The early symptoms of IBD in vitamin D–deficient IL-10 KO mice were associated with vitamin D deficiency and not with a reduction in energy or food intake.

**DISCUSSION**

Vitamin D deficiency exacerbates the symptoms of enterocolitis in IL-10 KO mice, and 1,25(OH)2D3 treatment for as little as 2 wk ameliorated IBD symptoms in these mice. These findings provide strong evidence that vitamin D status may be an important factor in determining the incidence of IBD and furthermore establishes vitamin D as a physiologic regulator of IBD. This is the first experimental evidence to show a link between vitamin D status and IBD.

The time course of IBD development in vitamin D–deficient IL-10 KO mice is comparable to that of IBD that develops in IL-10 KO mice housed in conventional animal facilities (Kuhn et al. 1993). It is possible, although unlikely, that the microflora in the GI tract of IL-10 KO mice are disturbed during vitamin D deficiency such that disease-causing microbes expand and multiply to have an effect. Experiments to test this possibility could be done in vitamin D–deficient germfree mice, although in the absence of any microflora, enterocolitis would probably not develop. It is more likely that the microflora do not change in response to vitamin D status but instead, the absence of vitamin D changes the immune response and the result in IL-10 KO mice is more severe IBD.

Accumulating evidence suggests that vitamin D is a regulator of CD4+ T cells, which cause autoimmune disease (Cantorna et al. 1996 and 1998c). One possible mechanism of vitamin D action is in the negative regulation of CD4+ T cells, which cause IBD. Vitamin D has been shown to inhibit directly the effector functions of CD4+ T cells both in vitro and in vivo (Cippitelli and Santoni 1998, Lemire 1992). The other possibility is that vitamin D is a positive regulator of T cells or other cells that inhibit the induction or function of IBD-causing T cells. Two possible vitamin D targets are transforming growth factor (TGF)-β1 and IL-4 secreting cells (Cantorna et al. 1998c). Increased production of TGF-β1 and IL-4 has been shown to occur in mice treated with 1,25(OH)2D3 in vivo (Cantorna et al. 1998c). Furthermore, the production of TGF-β1 and IL-4 is associated with the inhibition of T-cell effector function and suppression of many autoimmune diseases (Groux et al. 1997). Vitamin D regulation of the immune system is likely complex and includes multiple targets, which together explain the mechanism by which 1,25(OH)2D3 suppresses the development of IBD.

Standard treatments of patients with IBD include short-term, high dose and long-term, low dose prednisone use (Andreassen et al. 1998, Podolosky 1991). Prednisone and other corticosteroid therapies result in decreased bone mineral density and many times result in higher risks for vertebral fracture (Andreassen et al. 1997 and 1998). Vitamin D supplementation may be the maintenance of bone mineral density.

**LITERATURE CITED**


### TABLE 2

1,25(OH)2D3 treatment decreases enterocolitis in IL-10 KO mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Vitamin D status1</th>
<th>g</th>
<th>g/100 g body</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 KO</td>
<td>8</td>
<td>−D</td>
<td>1.67 ± 0.04*</td>
<td>9.9 ± 0.5*</td>
</tr>
<tr>
<td>IL-10 KO</td>
<td>8</td>
<td>1,25(OH)2D3</td>
<td>1.08 ± 0.05</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>WT</td>
<td>12</td>
<td>−D</td>
<td>0.97 ± 0.02</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>WT</td>
<td>12</td>
<td>1,25(OH)2D3</td>
<td>1.06 ± 0.04</td>
<td>5.3 ± 0.2</td>
</tr>
</tbody>
</table>

1 All of the mice were vitamin D deficient (−D) for the first 7 wk of life. At 7 wk of age, the vitamin D–deficient IL-10 KO mice begin to show symptoms of enterocolitis (diarrhea and weight loss). The 7-wk-old IL-10 KO and WT mice were divided into two groups; half were supplemented with 0.2 μg/d 1,25(OH)2D3 for 2 wk. Values are means ± SEM. * significantly greater than all other groups, P < 0.05.

2 See Table 1 for abbreviations.

### TABLE 3

Vitamin D deficiency and not a reduction in food intake causes inflammation in the small intestine in IL-10 KO and WT mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Vitamin D status1</th>
<th>Weight, g</th>
<th>g/100 g body</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 KO</td>
<td>7</td>
<td>−D</td>
<td>11.5 ± 1.2*</td>
<td>9.9 ± 0.4*</td>
</tr>
<tr>
<td>IL-10 KO</td>
<td>4</td>
<td>+D restricted</td>
<td>16.1 ± 1.1</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>WT</td>
<td>4</td>
<td>+D restricted</td>
<td>15.9 ± 1.1</td>
<td>6.7 ± 0.2</td>
</tr>
</tbody>
</table>

1 Five-wk-old vitamin D–deficient mice were divided into groups and either continued to consume diets that contained no added vitamin D (−D) or changed to a diet that contained 5.0 μg/d (+D) for 4 wk. The +D mice were restricted in their food intake to the amount eaten by the vitamin D–deficient IL-10 KO mice in the previous 24 h. Values are means ± SEM; * significantly different from the other groups, P < 0.05.

2 See Table 1 for abbreviations.


