

## Commentary

### Crohn's disease & osteoimmunology - Is vitamin D the cross-talk co-ordinator?

In this issue Joseph *et al*<sup>1</sup> report the 25(OH) vitamin D [25(OH)D] levels in Crohn's disease (CD) and their association with sun exposure and disease activity. The authors<sup>1</sup> have measured the 25(OH)D levels in patients with Crohn's disease and equal number of age matched patients with irritable bowel syndrome and correlated the disease activity (assessed by Bradshaw scores) and duration of exposure to sunlight. The study<sup>1</sup> also documents low body mass index, haemoglobin, serum albumin and less physical activity in patients with Crohn's disease. Patients with Crohn's disease had lower serum 25(OH)D levels than the controls. Quantum of sunlight exposure correlated positively with serum 25(OH)D levels, negatively with disease activity. Those with jejunal involvement had lower serum 25(OH)D levels compared with patients who did not.

Recent advances in understanding the pathophysiology of Crohn's disease have revealed intriguing observations, namely the so-called north-south gradient of Crohn's disease<sup>2</sup>. In a genetically predisposed individual, Crohn's disease results due to dysregulated response of mucosal immune system to intraluminal antigens of bacterial origin. The normal state of mucosal immune system is one of inhibited immune response to luminal antigens and suppression of gut inflammation (*immune tolerance*). The mechanism whereby sunlight exposure may exert a beneficial effect on intestinal inflammation may involve vitamin D. Sunlight and vitamin D might protect against Crohn's disease by downregulating the T helper-1 ( $T_H1$ ) driven immune response<sup>3</sup>.

#### Vitamin D and immunology of Crohn's disease

Studies have shown that 1,25-dihydroxy vitamin D [ $1,25(OH)_2D$ ] not only modulates the production of cytokines such as interleukin-2 (IL-2) but also

suppresses the  $T_H$  lymphocyte proliferation<sup>4</sup>. Activation of naive  $T_H$  lymphocytes by antigen results in generation of pluripotent  $T_H0$  lymphocytes. These synthesize a broad spectrum of cytokines IL-2, IL-4, IL-10 and interferon- $\gamma$  (IFN- $\gamma$ ). The proliferating pluripotent  $T_H0$  lymphocytes differentiate into two major types of CD4+ T cells:  $T_H1$  [IL-2, IFN- $\gamma$ , tumour necrosis factor (TNF)] and  $T_H2$  (IL-3, IL-4, IL-5, IL-10) which respectively support cell mediated and humoral immunity. The immunoregulatory property of  $1,25(OH)_2D$  is in its ability to inhibit expression of  $T_H1$  cytokines and augmenting  $T_H2$  cytokines acting either directly via effects on lymphocytes or indirectly via effects on antigen presenting cells (APCs)<sup>5</sup>. In Crohn's disease, suppression of inflammation is altered leading to uncontrolled inflammation.  $T_H1$  cells induce transmural granulomatous inflammation resembling Crohn's disease and the  $T_H2$  cells appear to induce superficial mucosal inflammation resembling ulcerative colitis. Crohn's disease has  $T_H1$  type cytokine profile known to be associated with upregulation of IL-12<sup>2</sup>.

There is mounting evidence indicating that  $1,25(OH)_2D$  has a central role in regulation of immune response. Vitamin D receptors (VDR) are widely expressed in CD4+ and CD8+ T-lymphocytes, APCs, such as macrophages and dendritic cells. The active form of vitamin D,  $1,25(OH)_2D$ , inhibits dendritic cell differentiation and maturation. The T-cell differentiation is then skewed away from  $T_H1$  cell type towards  $T_H2$  cell type<sup>6</sup>. In experimental studies it has been shown that  $1,25(OH)_2D$  suppressed gut hormone receptors  $\alpha4\beta7$ , an integrin adhesion molecule expressed by most leukocytes, and chemokine receptor 9 (CCR9), a highly specific receptor expressed by T-cells that migrate selectively to the digestive tract. Both the molecules are implicated in CD and are targeted by novel anti-CD drugs<sup>7</sup>.

A recent study has indicated that  $1,25(\text{OH})_2\text{D}$  downregulates adaptive as well as innate immune responses, including toll-like receptor (TLR) signalling and antimicrobial effector pathways<sup>8</sup>. Under physiological conditions vitamin D helps to maintain intestinal homeostasis by dampening excessive adaptive immune response and boosting innate immunity. Absorption of dietary vitamin D occurs in small intestine. Patients with anatomical involvement of Crohn's disease in upper gastrointestinal tract should be considered for risk of malabsorption of vitamin D. Hypovitaminosis D is more common in these subjects. Vitamin D deficiency may thus trigger intestinal inflammation particularly relevant in the context of Crohn's disease.

### Vitamin D and osteoimmunology

The differentiation of osteoclast is regulated by macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor- $\kappa\text{B}$  ligand (RANKL) a TNF family cytokine. RANKL is expressed not only by bone forming osteoblasts that support osteoclastogenesis in bone forming tissue, but also by activated T-cells indicating osteoclastic bone resorption is influenced by immune system. It is unexpected for both the biologists and immunologists that osteoclast differentiating factor (RANKL) expressed by osteoblasts is the same molecule expressed by T-cells to stimulate dendritic cells (DCs).

Characterization of function of RANKL, and its receptors namely, receptor activator of nuclear factor- $\kappa\text{B}$  (RANK) and osteoprotegerin (OPG) has

contributed significantly to osteoimmunology with respect to interplay between active immunity and maintenance of bone homeostasis. RANKL-RANK-OPG axis has diverse physiological functions. When osteoclastogenesis is stimulated, osteoblast or stromal support cells express more RANKL than OPG, which facilitates binding of RANKL to RANK. This is a critical signal for the terminal differentiation of osteoclasts from precursor cells. This binding also stimulates and maintains resorption activity in mature cells. Thus stromal and osteoblast cells regulate bone resorption (osteoblast induced osteoclast differentiation). The OPG is secreted by TNF superfamily member and is a potent inhibitor of osteoclasts formation and a decoy receptor of RANKL. Thus RANKL is a potent stimulator of both formation of osteoclasts from precursor cells and bone resorbing activity of mature osteoclasts (Fig.).

When osteoclastogenesis is stimulated, osteoblasts or stromal support cells express relatively more RANKL than OPG. Formation of mature osteoclasts is significantly enhanced by co-stimulatory molecules of osteoclasts precursor cells. In inflammatory states both B- and T- cells also produced RANKL. Local production of proinflammatory cytokines IL-1, IL-6, TNF and RANKL by inflamed tissues can lead on to osteoclastogenesis. Also, RANKL is not limited to bone cells. These are important cytokines for activity/viability of macrophages and dendritic cells. The TLR activation can enhance osteoblast-mediated osteoclast differentiation by inducing RANKL and TNF- $\alpha$  on osteoblasts.

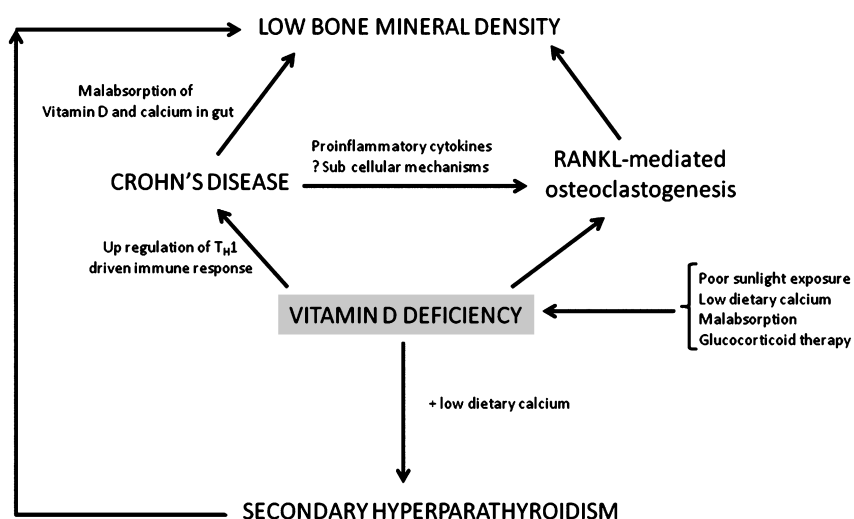


Fig. Schematic representation of the hypothetical interaction of cross-talk between vitamin D Crohn's disease and osteoimmunology. RANK, receptor activator of nuclear factor- $\kappa\text{B}$ .

A variety of cytokines also regulate osteoblastic cells. TNF- $\alpha$  and IFN- $\gamma$  inhibit collagen synthesis in osteoblasts. One study<sup>9</sup> found that CD has elevated levels of OPG which is derived from the site of inflammation and inversely correlates with bone loss. In another study CD patients have elevated levels of both OPG and RANKL<sup>10</sup>. Studies in the supernatants of cultured colonic biopsies in patients with CD have shown that vitamin D deficiency induces RANKL-mediated osteoclastogenesis and bone loss<sup>11</sup>. Proinflammatory cytokines such as TNF, IL-1 and IL-6 on osteoclast have adverse effect on bone mineral density in Crohn's disease. Malabsorption of calcium, vitamin D and smoking are major factors of metabolic bone disease in Crohn's disease. The secondary hyperparathyroidism set due to low dietary calcium and vitamin D deficiency also demineralises the bone.

Glucocorticoid therapy associated with treatment of Crohn's disease worsens hypovitaminosis D and bone mineral density (BMD). Active Crohn's disease is associated with anorexia, decreased nutritional intake due to fear of lactose intolerance leading to avoidance of dairy foods. Further Crohn's disease is associated with physical inactivity leading to reduced sunlight exposure compromising the serum vitamin D level. The observations in the present study<sup>1</sup> also bring forth the same point.

The present study also highlights the high prevalence of vitamin D deficiency and insufficiency (91%) in patients with Crohn's disease which is higher than the general population (79%) observed by the study. Interestingly the proportion of vitamin D deficiency [25(OH)D levels < 20 ng/ml] is higher than general population (79 vs 50%). These data suggest that patients with Crohn's disease in the study region (12° 56' Northern Latitude) are more at risk of metabolic bone disease due to Crohn's disease. Major limitation of the study is the absence of data on dietary calcium intake and resorption parameters of bone (PTH and  $\beta$ -CrossLaps). The authors have extrapolated the data from milk intake and fraction excretion of phosphorus which has its own limitations. While low serum vitamin D levels may tilt the immunological balance in Crohn's disease, the disease process *per se* worsens the absorption of calcium and vitamin D from the gut and may set a vicious cycle.

Sunlight and vitamin D might protect against Crohn's disease by downregulating the T<sub>H</sub> 1 cells-driven immune response. Exposure to UV radiation

is immunosuppressive. Mechanism of heliotherapy (Ultra Violet-B rays) induced immune suppression may include induction of various T<sub>H</sub> 2 cytokines such as IL-4 and IL-10<sup>12</sup>. Vitamin D may be the co-ordinator of the cross-talk between immunological system of the gut and various subcellular events of bone formation. More light might be thrown by the newly developing field of osteoimmunology in future. It is intriguing to understand how distinct functions are achieved with similar mechanisms.

Before these data are translated to clinical benefits, normalization of vitamin D levels by enrichment of food and heliotherapy along with supplementation of dietary calcium could help in improving bone health and also Crohn's disease to some extent. Should we evaluate for bone disease in patients with Crohn's disease at diagnosis is a question which need to be addressed to. The role of addition of vitamin D to therapeutic armamentarium of Crohn's disease might benefit the disease as well as improve BMD in patients with Crohn's disease. Interventional studies will be of more help and newer therapeutic modalities might emerge targeting some of these subcellular mechanisms.

**C.V. Harinarayan**

Department of Endocrinology &

Metabolism

Sri Venkateswara Institute of Medical Sciences

Tirupati 517 507, Andhra Pradesh, India

cvhari5endo@rediffmail.com

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