Role of 1,25-dihydroxyvitamin D₃ in the generation of the acute-phase response in rats with talc-induced granulomatosis

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Abstract. Subcutaneous injection of nonspecific irritants such as magnesium silicate (talc) provokes granulomatous inflammation in the rat. Part of the acute phase response (APR) in these animals is the loss of trabecular bone at sites distant from the site of inflammation. To assess the possible involvement of vitamin D in the bone loss, we studied the development of the acute phase response in vitamin D-deprived rats. The serum APR provoked by subcutaneous inflammation in rachitic rats consisted of hypozincemia, hypercupremia, increased alkaline phosphatase activity and adrenocorticotropic hormone (ACTH) concentration, and was similar to that in control animals except for the absence of hypoferrremia. Control rats with talc-induced subcutaneous inflammation also had splenomegaly and decreased total and mononuclear peripheral blood cell counts, while subcutaneous inflammation did not induce spleen changes in rachitic rats. Subcutaneous inflammation induced the loss of trabecular bone and decreased the osteoblastic cell count in tibial metaphyses in control animals. Rachitic rats had abundant osteoid on trabecular surfaces, and the number of osteoblasts and osteoclasts was comparable to that of the controls. Subcutaneous inflammation did not affect any of the bone parameters in rachitic rats.

These results indicate that vitamin D plays an important role in the generation of the acute phase response during inflammation, particularly in the induction of spleen and bone cell changes. The discrepancy of the blood on one hand and bone and spleen indices of the APR on the other, indicate that there may be divergent pathways in the generation of the inflammatory response, some of which may be dependent on vitamin D.

Key words. Acute phase response; bone loss; inflammation; rickets; talc; vitamin D.

Vitamin D plays a role in the regulation of calcium metabolism. After modification in liver and kidney, 1,25(OH)₂D₃, the most potent form of vitamin D, acts primarily on cells in intestine, bone and kidney to increase intestinal calcium absorption and reabsorption in kidney, stimulate bone resorption and inhibit bone formation. 1,25(OH)₂D₃ has also been implicated in the regulation of a variety of cells of the immune system, including monocytes and lymphocytes. Moreover, activated monocytes and lymphocytes may produce the enzyme 1-alpha hydroxylase which converts 25(OH)D₃ into its active form, particularly in granulomatous disease, including sarcoidosis and tuberculosis. Clinical observations also linked vitamin D with blood cells. Children with vitamin D-deficient rickets have anemia and recurrent infections. Such patients have an impaired ability to react to nonspecific inflammatory stimuli and their peripheral leukocytes have decreased motility and an impaired capacity to phagocytize.

Subcutaneous injection of nonspecific irritants, such as magnesium silicate (talc), asbestos and agarose, provokes granulomatous inflammation in the rat. Talc has a particular clinical significance since its effects in humans were reported after accidental inhalation, contact with powdered surgical gloves and injection of crushed tablets in heroin addicts (for review see ref. 13). Part of the acute phase response (ARP) in rats with talc-induced inflammation is the loss of trabecular bone in tibial metaphysis distant to the inflammatory site. The cellular basis of the bone loss is a reduction of osteoblast number and activity and a retardation of longitudinal bone growth. In order to assess the role of vitamin D in the generation of the bone loss accompanying nonspecific inflammatory processes, we studied the development of the acute phase response and bone changes in vitamin D-deprived rats.

Materials and methods

Animals. Female Fisher rats, 8–10 weeks old, weighing 200 ± 10 g, were used in all experiments. Rachitic rats were produced by keeping pregnant mothers in conditions without ultraviolet (UV) light, and feeding them with a vitamin D-depleted diet (Altromin C1017, Lage, Germany; mineral content: Ca, 9.5 mg/kg, P, 7.5 mg/kg). Tap water was given ad lib. Pups were weaned at 22 days of age onto their mothers’ diet and housed in the conditions without UV-light. For the experiment, the animals were caged individually, were fed the vitamin D-depleted diet and drank tap water ad lib. Age-matched controls were kept under the same conditions.
Experimental conditions, except for a 12-hour light and dark cycle and a standard diet (Altromin C1000, Lage; mineral content: Ca, 9.5 mg/kg, P, 7.5 mg/kg). Subcutaneous inflammation was provoked by injections of 800 mg sterile talc (magnesium silicate, Mg₃H₂(SiO₃)₄; Merck, Darmstadt, Germany) suspended in 1 ml isotonic saline solution at four different locations on the back of the animal; inflammation sites were not located near the skeleton. Control animals received saline solution only. Weight gain and food intake were recorded daily. Since talc-injected rats consume less food than the controls, animals without subcutaneous inflammation were pair-fed with talc-injected ones to exclude possible effects of decreased food intake on bone growth. Rats were sacrificed seven days after talc injection. Blood was drawn from the abdominal aorta, and serum separated by centrifugation and stored at -20°C until analyzed. The spleen was dissected out and weighed.

**Histological analysis.** Tibial proximal ends were fixed in 70% ethanol, dehydrated in increasing ethanol concentrations, embedded undecalcified in methylmetacrylate, and cut lengthwise into two equal halves. Sections measuring 3 μm were cut from the resulting plane with a microtome (Reichert 1516, Heidelberg, Germany) and stained with modified Goldner’s stain. Bone morphometric parameters were measured at a magnification of 300× using the Zeiss II ocular grid (Zeiss, Jena, Germany) and semiautomatic image analyzer (Morphomat 10, Opton, Heidelberg, Germany). Trabecular bone and osteoid volume were measured as the percentage of total osseous space occupied by cancellous bone and osteoid, respectively, in the area 2 mm distal to the growth cartilage-metaphyseal junction (GCMJ), as described elsewhere. The numbers of osteoblasts and osteoclasts were counted within a 1 mm-wide trabecular area, with cortical bone forming lateral boundaries and the upper boundary 1.25 mm distal to the GCMJ. Results were expressed as number of cells per mm². The number of osteoblasts was decreased, while that of osteoclasts remained unaltered (table 1; p < 0.001 and p < 0.01 vs. control, respectively). Adrenocorticotropic hormone (ACTH) and osteocalcin concentrations were determined by radioimmunoassay (RIA) using commercial RIA kits, ACTH-PR and OSTK-PR, respectively (CIS, Gif-sur-Ivette, France). Concentration of corticosterone was also measured by a radioimmunoassay (RSL Inc., Carson, USA). Electrophoresis of serum proteins was performed on cellulose acetate gel membranes, and their relative amounts were determined by densitometry (14).

**Biochemical analysis of the serum.** Serum calcium, magnesium, iron, copper and zinc concentrations were determined by atomic absorption spectrometry (AAS, Pye Unicam, Cambridge, MA). Inorganic phosphate was measured using a Technicon Autoanalyzer, and alkaline phosphatase activity (table 1). Vitamin D deprivation induced a decrease in serum copper concentrations (table 1). The serum APR provoked by subcutaneous inflammation in rachitic rats was below the detection limit of the assay (data not shown), indicating a fully developed rickets. Rachitic animals weighed less than intact controls, but the difference was not significant (168.0 ± 9.7 g in rachitic vs. 178.0 ± 16.0 g in control rats). As shown in earlier studies, subcutaneous inflammation induced anorexia and weight loss in both rachitic and control rats (data not shown).

Control rats with subcutaneous inflammation had normal serum calcium and increased serum phosphate concentrations (table 1, ref. 11–15). Rachitic rats had hypophosphatemia and normal calcium concentration, while the concentration of magnesium was decreased (table 1). Subcutaneous inflammation abolished the effects of rickets on mineral concentrations, reverting hypophosphatemia and hypomagnesemia of rachitic animals into hyperphosphatemia and hypermagnesemia (table 1; p < 0.001 and p < 0.01 vs. control, respectively). A significant decrease in white blood cell (WBC) count was observed in control rats with subcutaneous inflammation. Mononuclear cell number was decreased, while the number of polymorphonuclears remained unchanged (table 1). Vitamin D deprivation did not have any effect on the peripheral WBC count, and subcutaneous inflammation in rachitic rats induced a decrease in total and mononuclear WBC similar to that in control animals (table 1). Spleen weight increased significantly as a consequence of subcutaneous inflammation (table 1). Rachitic animals weighed less than intact controls, but the difference was not significant (168.0 ± 9.7 g in rachitic vs. 178.0 ± 16.0 g in control rats). As shown in earlier studies, subcutaneous inflammation induced anorexia and weight loss in both rachitic and control rats (data not shown).

**Results**

The concentration of circulating 1,25(OH)₂D₃ in rachitic rats was below the detection limit of the assay (data not shown), indicating a fully developed rickets. Rachitic animals weighed less than intact controls, but the difference was not significant (168.0 ± 9.7 g in rachitic vs. 178.0 ± 16.0 g in control rats). As shown in earlier studies, subcutaneous inflammation induced anorexia and weight loss in both rachitic and control rats (data not shown).

Control rats with subcutaneous inflammation induced by talc injection presented a typical serum acute phase response: decreased zinc and iron concentrations, increased copper and ACTH concentrations (table 1), increased alpha₁-, alpha₂- and beta-globulin and decreased albumin and gama-globulin serum protein electrophoretic fractions (data not shown), and increased alkaline phosphatase activity (table 1). Vitamin D deprivation induced a decrease in serum copper concentration (table 1). The serum APR provoked by subcutaneous inflammation in rachitic rats was similar to that in control animals, except for the absence of hypoferremia (table 1).

Differential Blood Cell Count. Polymorphonuclear and mononuclear (lymphoeyctic and monocytic) fractions of the white blood cell compartments were determined by counting at least 100 cells per rat, after peripheral blood had been smeared on a glass slide, air-dried, and stained with May-Grünwald-Giemsa stain.