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Effects of Oral Administration of 1α (OH)D_3 on Immunophenotypic Expression of Spleen Lymphocytes in Mice Infected with *Schistosoma mansoni*

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Abstract

Effects of oral administration of 1α (OH)D_3 were assessed by immunophenotypic analysis of spleen cells in mice infected with *Schistosoma mansoni*. During 7 to 11 weeks after infection, mice were given orally 0.1 or 1.0 μg/kg of 1α (OH)D_3 or medium solution only every other day. Flow-cytometric analysis of spleen lymphocytes was performed using FITC-conjugated monoclonal antibodies directed against pan T cells (Thy 1.2), helper/inducer (L_3T_4) or suppressor/cytotoxic (Lyt2) T cells or B cells (I-A^d). Pan T cell percentage appeared to be constant, whereas the ratio of helper/inducer T cells to suppressor/cytotoxic T cells and B cell percentage were depressed by the treatment. Although it is not clear whether the hormonal form of vitamin D_3 acts directly on activated lymphocytes or indirectly via modulation of other immune cells such as monocytes and macrophages in the present experimental system, our data suggest that the hormonal form of vitamin D_3 modulates immunological response of mice against *Schistosoma mansoni* infection.

Key words: Schistosomiasis. Flow-cytometry, T cell, B cell, T cell subsets

Introduction

The biologically active form of vitamin D_3, 1α, 25$(OH)_2D_3$, principally regulates Ca^{++} homeostasis by binding to specific receptors in bone, kidney and intestine. The cytosolic receptors for the hormonal form of vitamin D_3 have been detected also in monocytes and activated lymphocytes (Bhalla 983, Provvedini 1983), and further *in vitro* evidence has been reported in support of a possible role of this sterol in regulation of functions of immune cells (Bhalla 1984, Tsoukas 1984). *Schistosoma* (*s.*) *mansoni* infection elicits, in mice, immunogenic granulomas around parasite eggs trapped in the liver and intestine (Epstein 1979, Warren 1961). The hormonal form of vitamin D_3 may modulate granulomatous hypersensitivity in this parasite infection. To test a possible immunomodificatory role of the hormonal form of vitamin D_3, we have investigated the effects of oral administration of 1α (OH)D_3, a synthetic analogue of the hormonal form of vitamin D_3, on the population ratio of splenic cells in mice infected with *S. mansoni*. In this paper, we report that 1 (OH)D_3 treatment causes depression of ratio of helper/inducer T cells to suppressor/cytotoxic T cells and B cell population. The present data suggest that the hormonal form of vitamin D_3 modulates immunological response in mice infected with *S. mansoni* and offer an evidence for the *in vivo* immunomodificatory property of the hormonal form of vitamin D_3.

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Materials and Methods

Infection with *S. mansoni*

Each of 35 five-week-old female BALB/c mice (Charles River Japan, Atsugi) weighing about 25 gr was injected subcutaneously, as we reported previously (Nishimura 1982, 1985a, 1985b), in the back with 100 *cercariae* of the *Puerto Rican* strain of *S. mansoni* freshly released from the snails (*Biomphalaria glabrata*).

Administration of 1α $(OH)D_3$

Thirty mice infected with *S. mansoni* were separated into 3 groups. At 7 weeks after infection, 10 mice of each group were started to be given orally, using a plastic syringe with a metal gastric tube, 0.1 or 1.0 μg/kg of 1α $(OH)D_3$ (kindly provided by Teijin Co. Ltd., Tokyo; Lot No. 1507) or only medium solution every other day for 4 weeks. Fifteen age- and sex-matched mice without *S. mansoni* infection were separated into 3 groups (5 mice of each), then they had been treated with 1α $(OH)D_3$ in the same way as infected animals. 1α $(OH)D_3$, provided as a 0.1 mM solution in ethanol, had been protected from light and stored at $-20°C$, and before use, it was diluted with 0.02% Triton X-100 in distilled water to an appropriate concentration.

Determination of Serum Ca^{++} Concentration

Serum samples were taken from 5 mice of the 7th week postinfection (before starting oral administration of 1α $(OH)D_3$). Then, at 2 and 4 weeks of the treatment, 5 mice of each group were subjected to serum collection. Serum samples were taken also from mice without *S. mansoni* infection at 4 weeks of the treatment. Since individual sample (about 0.4 ml) was not enough for quantitative assay, the samples of each group were combined, and Ca^{++} concentration was measured using a commercially available kit (Iatroace Ca, Iatron, Tokyo, Japan).

Preparation of Spleen Cells

Immediately after taking blood samples, mice were killed to obtain spleens. Each spleen was teased in RPMI-1640 tissue culture medium. The released cells were washed in phosphate buffered saline (PBS), and adjusted to 1×10^7/ml. Cell viability of the final preparation was consistently more than 90% as determined by trypan blue exclusion.

Flow Cytometry

FITC-conjugated anti-Thy1.2, L_3T_4, Lyt_2, and $I\text{-}A^d$ monoclonal antibodies (Ortho Diagnostic Systems, Raritan, NJ) were used. Anti-Thy1.2 recognizes pan T cells, whereas $L_3T_4^+$ and Lyt_2^+ cells constitute reciprocal mature peripheral T cell subsets, which are generally associated with helper/inducer and suppressor/cytotoxic functions, respectively. The monoclonal antibody (5 μl) was added to the cell suspension (100 μl) and incubate at 4°C for 30 minutes. Two millilitters of PBS were then added, and the preparation was finally analyzed with a laminar flow cytometer (Ortho, Spectrum III).

Statistics

Data were expressed as mean $\pm$ SEM; Student's t test was used to compare the significance of differences.

Results

Serum Ca^{++} Concentration

As shown in Figure 1, serum Ca^{++} concentration of infected untreated control somewhat decreased at the 7th and 11th week after infection. In mice treated with the low dose (0.1 μg/kg/2 days) of 1α $(OH)D_3$, serum Ca^{++} concentration was kept at a level of 7th week postinfection during the observation period. In contrast, in mice treated with the high dose (1.0 μg/kg/2 days) of 1α $(OH)D_3$, serum Ca^{++} increased with time during the observation period. Circulating Ca^{++} levels in mice without *S. mansoni* infection at 4 weeks after the treatment were 8.7 mg/dl (negative control), 9.0 mg/dl (treated with the low dose of 1α $(OH)D_3$) and 11.3 mg/dl (treated with the high dose of 1α $(OH)D_3$).

Population Dynamics of Spleen Cells

As shown in Table 1, 1α $(OH)D_3$ treatment did not alter the percentage of pan T cells

throughout the observation period (during 7 to 11 weeks after infection). At 11 weeks after infection, the ratio of helper/inducer T cells to suppressor/cytotoxic T cells was significantly lower both in mice treated with the low dose (2.23 ± 0.10: $p < 0.025$) and the high dose (1.46 ± 0.07: $p < 0.001$) of 1α $(OH)D_3$ than that the negative control mice (3.07 ± 0.03). The percentage of B cells in mice treated with the high dose of 1α $(OH)D_3$ at 11th week postinfection was also significantly ($p < 0.05$) lower than that in mice without 1α $(OH)D_3$ treatment. Percentages of T and B cells and the ratio of helper/inducer T cells to suppressor/cytotoxic T cells did not show any significant change by the 4 weeks of 1α $(OH)D_3$ (Table 2).

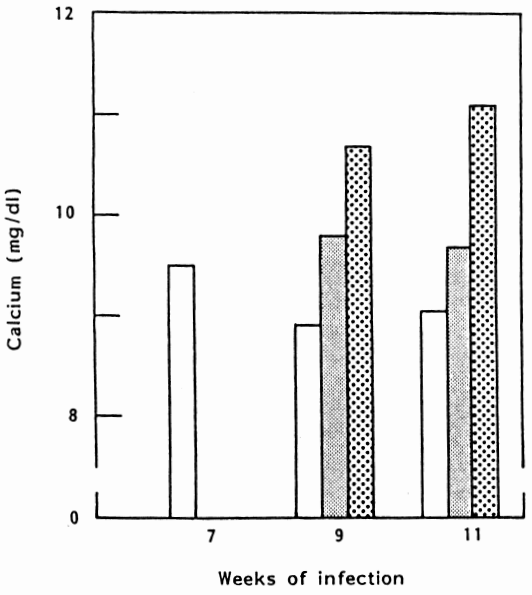

Fig. 1. Effects of oral administration of 1α $(OH)D_3$ on serum Ca^{++} concentration in mice infected with *Schistosoma mansoni*. ☐ negative control, ▒ low dose (0.1 μg/kg/2 days), ⁘ high dose (1.0 μg/kg/2 days).

Table 2. Effects of 1α $(OH)D_3$ Treatment (for 4 weeks) on Phenotypic Expressions of Spleen Cells in Noninfected Mice

Description (antibody)	Treatment	Percentage (Ratio) mean ± SEM
Pan T cell	N*	40.87 ± 9.25
(anti-Thy1.2)	L**	34.87 ± 6.02
	H***	22.87 ± 5.90
T helper•inducer/	N	(2.41 ± 0.72)
T suppressor•cytotoxic	L	(2.35 ± 0.67)
(anti-L_3T_4/anti-Lyt_2)	H	(2.31 ± 0.37)
B cell	N	30.13 ± 4.75
(anti-I-A^d)	L	29.20 ± 1.18
	H	27.40 ± 5.46

* negative control, ** low dose (0.1 μg/kg/2 days), *** high dose (1.0 μg/kg/2 days)

Table 1. Effects of 1α $(OH)D_3$ Treatment on Phenotypic Expressions of Spleen Cells in Mice Infected with *Schistosoma mansoni*

(antibody)	Treatment	Weeks of infection 7	9	11
Pan T cell	N*	31.00 ± 2.95†	23.20 ± 2.91	25.47 ± 2.37
(anti-Thy 1.2)	L**		25.47 ± 2.71	18.67 ± 3.38
	H***		24.33 ± 4.07	27.13 ± 5.05
T helper•inducer/				
T suppressor•cytotoxic	N	2.17 ± 0.14††	1.84 ± 0.09	3.07 ± 0.03
(anti-L_3T_4/anti-Lyt_2)	L		2.08 ± 0.06	2.23 ± 0.10 ¶
	H		2.09 ± 0.17	1.46 ± 0.07 ψ
B cell	N	28.73 ± 2.13†	47.20 ± 3.63	43.80 ± 0.58
(anti-I-A^d)	L		46.40 ± 3.80	31.87 ± 4.32
	H		38.67 ± 2.37	32.93 ± 3.77 ϕ

* negative control, ** low dose (0.1 μg/kg/2 days), *** high dose (1.0 μg/kg/2 days), † mean ± SEM %, †† mean ± SEM ratio

ϕ denotes significant difference ($p < 0.05$) from negative control, ¶ $p < 0.025$, ψ $p < 0.001$.

Comment

1 α $(OH)D_3$ is metabolized in the liver to 1 α, 25$(OH)_2D_3$, biologically the most active form of the compounds (Fukushima 1975). In murine schistosomiasis, a lowered metabolizing capacity for several drugs in the liver has been reported (Cha 1980). Therefore, to evaluate absorption and metabolization of orally administered 1 α $(OH)D_3$, we measured serum Ca^{++} concentration. Contrary to our expectation, however, 1 α $(OH)D_3$ treatment increased serum Ca^{++} concentration in mice infected with *S. mansoni* in a similar extent as in noninfected animals. From the view point of the effect on Ca^{++} metabolism, the present data indicate that granulomatous lesions in the intestine and liver induced by Schistosome eggs did not seem to give so much influence on the absorption or conversion of 1 α $(OH)D_3$. The present flow-cytometric analysis of splenic lymphocytes showed decrease of ratio of helper/inducer T cells to suppressor/cytotoxic T cells and B cell percentage in mice treated with 1 α $(OH)D_3$. Although further analysis on the population dynamics of spleen lymphocytes could not be made in the present study since quantitation of spleen cells has not been performed, the oral administration of 1 α $(OH)D_3$ actually affects the immunophenotypic expression of spleen cells. Such 1 α $(OH)D_3$-induced change in immunophenotypic expression of spleen cells has not been observed in noninfected animals. It is suggested that the hormonal form of vitamin D_3, as it does in vitro (Bhalla 1984, Tsoukas 1984), acts selectively to activated lymphocytes in vivo. Alternatively the effects of hormonal form of vitamin D_3 on lymphocytes may be mediated by other types of immune cells such as monocytes and macrophages, since these cells also possess receptors for the hormonal form of vitamin D_3 (Bhalla 1983, Provvedini 1983, Tsoukas 1984). Granulomas against parasite eggs in subacutely infected animals can be decreased in size by adoptive transfer of spleen cells from chronically infected animals (Colley 1976). The findings suggest a crucial role of lymphocytes for formation of schistosome egg granulomas. Further studies on the effect of the hormonal form of vitamin D_3 on hepatic granulomas of murine schistosomiasis are in progress in our laboratory.

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