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Cutting Edge: Vitamin D-Mediated Human Antimicrobial Activity against Mycobacterium tuberculosis Is Dependent on the Induction of Cathelicidin

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Host defense against intracellular pathogens depends upon innate and adaptive antimicrobial effector pathways. TLR2/1-activation of monocytes leads to the vitamin D-dependent production of cathelicidin and, at the same time, an antimicrobial activity against intracellular Mycobacterium tuberculosis. To determine whether induction of cathelicidin was required for the vitamin D-triggered antimicrobial activity, the human monocytic cell line THP-1 was infected with M. tuberculosis H37Ra and then activated with the active vitamin D hormone 1,25-dihydroxyvitamin D3 (1,25D3). 1,25D3 stimulation resulted in antimicrobial activity against intracellular M. tuberculosis and expression of cathelicidin mRNA and protein. Using small interfering RNA (siRNA) specific for cathelicidin, 1,25D3-induced cathelicidin mRNA and protein expressions were efficiently knocked down, whereas a nonspecific siRNA control had little effect. Finally, 1,25D3-induced antimicrobial activity was completely inhibited in the presence of siRNA against cathelicidin, instead leading to enhanced intracellular growth of mycobacteria. These data demonstrate that cathelicidin is required for the 1,25D3-triggered antimicrobial activity against intracellular M. tuberculosis. The Journal of Immunology, 2007, 179: 2060–2063.

The innate immune response rapidly recognizes microbiopathogens, serving as a first line of defense against the infection by mounting an antimicrobial response. For the intracellular pathogen Mycobacterium tuberculosis, a role for vitamin D in the antimicrobial activity of human monocytes and macrophages was first suggested by in vitro experiments in the laboratories of Rook in 1986 (1) and Crowle in 1987 (2). These experiments were performed by infecting human monocytes and macrophages with M. tuberculosis followed by addition of the active vitamin D3 hormone 1,25-dihydroxyvitamin D3 (1,25D3).3 1,25D3 triggered significant antimicrobial activity, yet there was no information on the downstream mediators of the antimicrobial response.

Recently, we demonstrated that the activation of monocytes with a TLR2/1 ligand, the M. tuberculosis-derived 19-kDa lipopeptide, triggered a vitamin D-dependent pathway leading to induction of the antimicrobial peptide cathelicidin (3) and antimicrobial activity against intracellular M. tuberculosis (3, 4). The TLR antimicrobial pathway was dependent on the presence of 25-hydroxyvitamin D3 (25D3), which was converted in monocytes and macrophages by the CYP27b1-hydroxylase to 1,25D3. Addition of exogenous 1,25D3 to monocytes and PBMCs triggered an antimicrobial activity against intracellular M. tuberculosis (3, 5), similar to findings in the Rook and Crowle studies (1, 2). Furthermore, 1,25D3-stimulated monocytes and PBMCs expressed cathelicidin mRNA and protein (3, 5), confirming the findings of Wang et al. (6). In the present study, we sought to determine whether or not the vitamin D-triggered induction of cathelicidin was required for the vitamin D-induced antimicrobial activity against intracellular M. tuberculosis.

Materials and Methods

Reagents and cells

THP-1 cells were purchased (American Type Culture Collection) and maintained in RPMI 1640 (Invitrogen Life Technologies) with 10% FCS (Omega Scientific). The M. tuberculosis H37Ra strain was a gift from Dr. J. Ernst (New York University School of Medicine, New York NY) and used as described below. 1,25D3 was purchased (BIOMOL International), stored at −80°C in amber tubes in small aliquots, and added to culture at concentrations of 10−7 M to 10−8 M for dose titration experiments or at 10−9 M, the concentration previously determined to be optimal for the provocation of THP-1 cells. [3H]Uracil was purchased (VWR International). The following Abs were purchased: α-cathelicidin (clone 1-1C12; Cell Sciences) and α-β-actin (clone mAbcam 8226; Abcam). Predesigned small interfering RNA (siRNA) oligos were purchased (siGLO, siCTRL, and siCath; Dharmacon), resuspended, and stored as recommended by the manufacturer. Cell Line Nucleofector kit V was purchased and used for siRNA transfections (Amaxa).

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3 Abbreviations used in this paper: 1,25D3, 1,25-dihydroxyvitamin D3; MOI, multiplicity of infection; qPCR, quantitative PCR; siRNA, small interfering RNA; siCath, siRNA specific for the cathelicidin gene; siCTRL, nonspecific siRNA control oligo.

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Results and Discussion

To ascertain the role of cathelicidin in 1,25D3-mediated antimicrobial activity against intracellular M. tuberculosis, we used siRNA technology to knock down cathelicidin gene transcripts. The human monocytic cell line THP-1 was selected because of its high efficiency of transfection with siRNAs and its subsequent viability after infection with M. tuberculosis. Furthermore, exposure of THP-1 cells to 1,25D3 is known to trigger an antimicrobial activity against intracellular M. tuberculosis (9).

For the present initial studies, THP-1 cells were infected with the avirulent M. tuberculosis H37Ra at an MOI of 1 and then treated with 1,25D3 for 3 days. Subsequently, the intracellular bacteria were harvested and tested for bacterial metabolism according to [3H]uracil uptake or viability according to CFU. The addition of 1,25D3 to the infected THP-1 cells resulted in a 55% decrease in [3H]uracil uptake by the bacterium (p < 0.01; Fig. 1a) and a 28% decrease in total CFU harvested (p < 0.05; Fig. 1b), consistent with previous results (9).

It should be noted that M tuberculosis H37Ra does not grow in THP-1 cells during the first 3 days of infection (data not shown) (10). The number of bacteria we observed after 3 days in the 1,25D3-treated cells was lower than the initial inoculums; therefore the activity of 1,25D3 is likely bactericidal rather than bacteriostatic. Although the percentage reduction of M. tuberculosis CFU was within an order of magnitude, M. tuberculosis infection in vivo can be produced by as few as 10–200 bacilli and is a slow process. In our experiments the time of in vitro assay was only 72 h, so a cumulative antimicrobial effect mediated by the vitamin D-cathelicidin innate immune pathway over time could have a profound effect on the number of bacilli during the course of infection.

Treatment of THP-1 cells with 1,25D3 up-regulated the cathelicidin mRNA expression in a dose-titratable manner from 48- to 140-fold induction (Fig. 2a). Cyp24 gene expression, a control vitamin D3 receptor downstream target gene, was similarly up-regulated, indicating that the vitamin D3 receptor was functional and activated (Fig. 2b). The up-regulation of the cathelicidin mRNA correlated with increased expression of the cathelicidin protein as detected by intracellular flow cytometry (Fig. 2c). These data indicate that the THP-1 cell line can be used for studying the relationship between 1,25D3-mediated antimicrobial activity and cathelicidin expression.

A standard molecular approach for knocking down gene expression in human cells is the use of siRNAs. THP-1 cells were transfected with a nonspecific, fluorescently tagged siRNA construct and assessed for transfection efficiency and viability by
flow cytometry and trypan blue exclusion, respectively. Following transfection, >99% of the cells were positive for intracellular siRNA and the cells were >99% viable (data not shown). Subsequently, THP-1 cells were transfected with either siRNAs specific for the cathelicidin gene (siCath) or a nonspecific control oligo (siCTRL) and then incubated with 1,25D₃ or medium for 18 h. Transfection of siCath into THP-1 cells almost completely knocked down the 1,25D₃-induced expression of cathelicidin mRNA, whereas siCTRL had little effect (Fig. 3a). The loss of cathelicidin mRNA expression also resulted in knockdown of cathelicidin at the protein level to that of the medium control as measured by intracellular flow cytometry (Fig. 3b). The knockdown was specific to cathelicidin, because neither siCath nor siCTRL affected 1,25D₃-induced Cyp24 mRNA levels (Fig. 3c). The β-actin levels did not change with either transfection or 1,25D₃ treatment, indicating that reduction of the global protein levels was not the cause for the loss of cathelicidin expression (Fig. 3d). These results demonstrate that the 1,25D₃-induced expression of cathelicidin can be efficiently knocked down in THP-1 cells while maintaining cell viability.

To address the role of cathelicidin in 1,25D₃-induced antimicrobial activity, siCath- and siCTRL-transfected as well as untransfected THP-1 cells were infected with H37Ra at an MOI of 1 for 18 h. The cells were then treated with medium alone or 1,25D₃ for 3 days and the intracellular bacteria were harvested and assayed for viability by CFU and [³H]uracil uptake. Treatment with 1,25D₃ reduced the viability of the intracellular bacteria by 30–50% in the untransfected and siCTRL transfected cells as measured by both [³H]uracil uptake (Fig. 4a) and CFU (Fig. 4b). The striking finding was that transfection with siCath completely blocked the 1,25D₃-mediated antimicrobial activity, resulting in a 140 and 170% reduction of 1,25D₃-induced antimicrobial activity according to [³H]uracil uptake (p < 0.05, siCath vs siCTRL; Fig. 4a) and CFU assay (p < 0.05, siCath vs siCTRL; Fig. 4b), respectively. These data suggest that cathelicidin is required for 1,25D₃-mediated antimicrobial activity against intracellular M. tuberculosis in human monocytes.

Although it has been known for 20 years that exposure of human monocytes and macrophages to 1,25D₃ results in antimicrobial activity against intracellular M. tuberculosis, the mechanism of this antimicrobial response has remained enigmatic. Potential mechanisms include activation of PI3K and

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Knockdown of 1,25D₃-induced cathelicidin via transfection of siRNA. a and b, siCath- and siCTRL-transfected as well as untransfected THP-1 cells were treated with 10⁻⁸ M 1,25D₃ for 18 h and measured for cathelicidin mRNA expression by qPCR (a) and protein levels by intracellular flow cytometry (b). c and d, Cyp24 mRNA expression is measured by qPCR (c) and β-actin levels are measured by intracellular flow cytometry (d). The dotted line, solid line, and the gray shaded region indicate the isotype control, medium-treated cells, and 1,25D₃ stimulated cells, respectively, for the cytometry experiments. Data shown are from one representative experiment of four individual experiments.

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** The effects of cathelicidin knockdown on 1,25D₃-mediated antimicrobial activity against intracellular M. tuberculosis. siCath- and siCTRL-transfected as well as untransfected THP-1 cells were infected with M. tuberculosis H37Ra at an MOI of 1 for 18 h and then treated with 10⁻⁸ M 1,25D₃ for 3 days. Following treatment, the intracellular bacteria were harvested and assayed for bacterial metabolism according to [³H]uracil uptake (a) and bacterial viability according to CFU (b). Data shown for [³H]uracil uptake represent the average bacterial viability percentage of six individual experiments (n = 6 ± SEM; *, p < 0.05). Bacterial viability is calculated as the 1,25D₃ value divided by the medium-treated value multiplied by 100. CFU data shown are the average of four individual experiments (n = 4 ± SEM). The data were normalized according to relative CFU proportionate to 10⁵ CFU in the medium-treated samples.
the subsequent generation of reactive oxygen intermediates (9), NO (11, 12), and the tryptophan aspartate-containing coat protein TACO (13, 14). The present data advance our understanding of the role of vitamin D in innate immunity, indicating that the induction and expression of the antimicrobial peptide cathelicidin is required for the 1,25D3-mediated antimicrobial activity against intracellular avirulent M. tuberculosis in the human monocyctic cell line THP-1. Nevertheless, additional experiments are needed to elucidate the role of cathelicidin as part of the TLR-mediated, vitamin D-dependent antimicrobial response against virulent M. tuberculosis in primary human monocytes and macrophages and the potential synergy of this pathway with other microbicidal effector pathways.

Progress in curtailing the human death rate from tuberculosis has been hampered by access to, the cost and effectiveness of current antibiotic regimes (15). Furthermore, the recent emergence of extensively drug resistant organisms is likely to have a global impact (16). Some of these problems could potentially be overcome by adding vitamin D to the treatment of tuberculosis, because a single oral dose increased the killing of mycobacteria in the blood of healthy donors (17), although the currently published studies on the effects of vitamin D supplementation are generally inadequate to evaluate the clinical efficacy of such established studies on the effects of vitamin D supplementation are generally inadequate to evaluate the clinical efficacy of such treatment (18). Knowledge of the basic innate immune defense pathways required for persistent infections.

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Disclosures
The authors have no financial conflict of interest.

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