Immunomodulatory Effects of Vitamin D on Innate and Adaptive Immune Responses to Streptococcus pneumoniae

Marie Olliver,1 Laura Spelmink,1 Jeffni Hiew,1 Ulf Meyer-Hoffert,4 Birgitta Henriques-Normark,1,2,a and Peter Bergman2,3,a

1Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, 2Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden, and 3Department of Medicine, Center for Infectious Medicine, Karolinska University Hospital, Huddinge, Stockholm, Sweden; and 4Department of Dermatology, University Hospital Schleswig-Holstein, Kiel, Germany

Background. Streptococcus pneumoniae forms part of the normal nasopharyngeal flora but can also cause a broad spectrum of inflammatory diseases. Vitamin D has potent effects on human immunity, including induction of antimicrobial peptides and suppression of T-cell proliferation, but its ability to modulate the immune response to pneumococci is unknown.

Methods. Monocyte-derived dendritic cells (DCs) were stimulated with pneumococcal peptidoglycan (PGN) in the presence or absence of vitamin D. Expression of maturation markers, cytokines, pattern recognition receptors, and antimicrobial peptides were measured with flow cytometry, enzyme-linked immunosorbent assay and quantitative polymerase chain reaction. Stimulated DCs were cocultured with autologous T-helper cells, and concentrations of T-helper (Th) 1−, Th17−, and regulatory T-cell–related cytokines were measured with enzyme-linked immunosorbent assay.

Results. Vitamin D enhanced DC maturation and expression of the migration marker C-C chemokine receptor type 7 (CCR7) in PGN-stimulated cells. It also enhanced expression of key pattern recognition receptors (Toll-like receptor 2, Nucleotide-binding oligomerization domain-containing protein 2 [Nod2]) and induced a synergistic up-regulation of the inflammatory mediator IL-1β and the β-defensin Human Beta Defensin 3 (hBD-3). Furthermore, vitamin D skewed the DC-mediated T-helper response to PGN from an inflammatory Th1/Th17 phenotype toward a regulatory T-cell phenotype.

Conclusion. Vitamin D modulates key elements of innate immunity while dampening adaptive immune responses in DCs after pneumococcal challenge, which may have implications for prevention and treatment of pneumococcus-induced inflammation.

Keywords. Vitamin D; immune modulation; Streptococcus pneumoniae; dendritic cell; antimicrobial peptides.

Invasive infections caused by the human-specific bacterium Streptococcus pneumoniae remain a serious problem worldwide. Its main ecological niche is the nasopharynx of healthy children, where it forms part of the normal flora. However, nasopharyngeal carriage is a precursor to pneumococcal disease, and protection relies on successful regulation of colonization in the nasopharynx. Local immune responses play an important role in preventing the pneumococcus from invading mucosal tissues and spread systemically. Thus, inflammatory reactions may be beneficial because they help to eradicate invading organisms; however, excessive inflammation may result in disease symptoms such as tissue damage and septic shock.

Vitamin D signaling has emerged as a key regulator of immunity in humans. The inactive storage form of vitamin D, 25-hydroxyvitamin D3 (25[OH]D3), is present in the circulation and is activated by the converting enzyme 1-alpha hydroxylase (Cyp27B1), which yields the active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25-[OH]2D3). Responsiveness to vitamin D depends on the activity of this enzyme but also on

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*B. H. N. and P. B. contributed equally to this work.

Correspondence: Birgitta Henriques-Normark, Microbial Pathogenesis, Gustaf V:s Forskningsinstitut, Karolinska Hospital, 171 76 Stockholm, Sweden (birgitta.henriques@ki.se).

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expression of the nuclear vitamin D receptor, which binds to specific vitamin D response elements in the promoters of approximately 200 target genes in the human genome [1]. Cyp27B1 and vitamin D receptor are expressed in a number of different immune cells, among these dendritic cells (DCs) and activated T cells [2−4]. Thus, vitamin D−mediated effects on the adaptive immune system may be exerted either directly on T cells or indirectly through modulation of DC responses. Vitamin D has been shown to inhibit the differentiation of monocytes into DCs in vitro [5, 6] and drive the development of DCs with tolerogenic properties [7−9]. It has also been reported to inhibit CD4+ T-cell production of interferon (IFN) γ [10] and interleukin 17 (IL-17) [11] and induce regulatory T-cell responses [12]. In addition, vitamin D is a potent inducer of antimicrobial peptides (AMPs) in macrophages and epithelial cells [13].

Both innate and adaptive immunity are important in the host defense against pneumococci. DCs serve as a major link between the innate and adaptive immune system, and signals provided to DCs, through their pattern recognition receptors (PRRs), affect the cytokine milieu in which naive T-helper cells differentiate. Although T-helper (Th) 1 and Th17 cells have been shown to be important in the protection against pneumococcal colonization and disease [14−16], little is known about the mechanisms through which pneumococci trigger T-helper responses in the human host.

Several studies have shown a strong correlation between low levels of 25(OH)D3 in serum and the risk for respiratory tract infections (reviewed in [17]), and recently a randomized controlled intervention study showed that vitamin D supplementation could reduce the symptoms in respiratory tract infections as well as the consumption of antibiotics among patients with antibody deficiency [18]. However, despite data in support of a beneficial role for vitamin D in human immunity, there is a lack of mechanistic insight about the effects of vitamin D on human immune cells. In the current study, we sought to decipher the potential role played by vitamin D in modulating immune responses in DCs after pneumococcal challenge.

MATERIALS AND METHODS

Reagents
Muramyl dipeptide (MDP) was purchased from Invivogen. Cytochalasin D and fluorescein isothiocyanate were from Sigma. Calcitriol (1,25−(OH)2D3, the hormonally active form of vitamin D) and calcidiol (25(OH)D3, the proform of calcitriol), from Tocris Bioscience (Bristol), were dissolved in ethanol and stored at −20°C. Itraconazole was from Santa Cruz Biotechnology.

Strains
The serotype 4 S. pneumoniae strain T4 (TIGR4; ATCC BAA-334) [19] was used in this study as well as its isogenic mutant deficient in the capsule (T4R) [20]. Pneumococci were grown overnight on blood agar plates at 37°C. Colonies were inoculated into C + Y medium (Karolinska University Laboratories) and grown to mid-log phase (optical density at 620 nm, 0.5). The cultures were pelleted and resuspended in Iscove’s Modified Dulbecco’s Medium (IMDM) (Sigma) before infection of cells (multiplicity of infection [MOI], 1). The MOI was confirmed by viable count on blood agar plates.

Peptidoglycan Preparation
Insoluble peptidoglycan (PGN) was prepared from the unencapsulated and autolysin-deficient S. pneumoniae strain T4RΔlytA [20], as reported elsewhere [21].

Preparation of Monocyte-Derived DCs
Buffy coats were obtained from healthy volunteers, provided by Karolinska University Hospital. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque PLUS (GE Healthcare) gradient centrifugation and transferred to a large plastic culture flask. Cells were incubated at 37°C and monocytes were allowed to adhere. Nonadherent cells were washed off after 2 hours and the adherent monocytes were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal calf serum (FCS) and 2 mM L-glutamine (from Invitrogen) in the presence of 65 ng/mL human recombinant granulocyte-macrophage colony-stimulating factor and 65 ng/mL human recombinant interleukin 4 (PeproTech). On day 3, cells were given fresh media and cytokines (ratio 1:1), and the culture was continued until day 6. The DC cultures were assessed for surface marker expression (CD11c+, CD1a+, and CD14−) with fluorescently labeled antibodies (BD Biosciences) before use.

Isolation of CD4+ Naive and Memory T Cells
Nonadherent PBMCs that were washed from the flask with adherent monocytes were resuspended in 10% dimethyl sulfoxide in FCS and frozen at −80°C until T cells were isolated and used for coculture with autologous DCs. Vials of frozen nonadherent PBMCs were quickly thawed in a 37°C water bath, and CD4+ memory and CD4+ naive T cells were prepared with EasySep human memory CD4+ T-cell enrichment kit and EasySep human naive CD4+ T-cell enrichment kit (StemCell Technologies), respectively. T cells were resuspended in IMDM (Sigma), supplemented with 10% heat-inactivated FCS and 2 mM L-glutamine (Invitrogen). The isolated cells were stained with fluorescently labeled antibodies against CD3, CD4, CD45RO, and CD45RA (BD Biosciences), and analyzed by flow cytometry. The purity of the cells was >97% CD4+CD45RO−CD45RA− (memory CD4+ T cells) and >95% CD4+CD45RO−CD45RA+ (naive CD4+ T cells).

Stimulation of DCs
DCs (5 × 105) were stimulated with live pneumococci (MOI, 1), pneumococcal PGN (1 µg/mL, unless otherwise specified) and
MDP (5 µg/mL) in 96-well flat-bottom plates. Each treatment for each donor was done in triplicate wells. For live pneumococcal stimulations, 100 µg/mL gentamicin (Invitrogen) was added after 30 minutes to kill extracellular bacteria. In some experiments, DCs were preincubated with cytochalasin D (1 µg/mL), vitamin D (1,25-(OH)2D3; 100 nmol/L), the proform of vitamin D, (25(OH)D3; 500 nmol/L) or itraconazole (10 µmol/L). Stimulated cells were incubated at 37°C in a humidified atmosphere with 5% CO2. At 24 hours after infection, cells were analyzed for gene and protein expression or cocultured with autologous CD4+ T cells.

**DC–T-Cell Cocultures**
At 24 hours after infection of DCs, autologous memory or naive CD4+ T cells were added in a 1:10 ratio (DC to T cells) and incubation at 37°C was continued. Supernatants were collected 5 days later for analysis of cytokines with enzyme-linked immunosorbent assay (ELISA).

**Flow Cytometry Analysis of DC Markers**
The DCs were stimulated as described above and at 24 hours, cells were harvested and stained with fluorescently labeled antibodies to human CD86, CD80, major histocompatibility complex (MHC) class II and C-C chemokine receptor type 7 (CCR7) (BD Biosciences) and analyzed with flow cytometry (FACSort; BD Biosciences). At least 5000 cells were counted.

**PGN Uptake Assay**
Pneumococcal PGN was labeled with fluorescein isothiocyanate solution for 30 minutes on ice and washed in phosphate-buffered saline before it was added to DCs. In some wells, DCs were preincubated for 2 hours with cytochalasin D (1 µg/mL). After 1 hour, cells were washed in PBS, and uptake of PGN by DCs was analyzed by flow cytometry. To quench any extracellular PGN, trypan blue was added to the samples before flow cytometry analysis.

**Bacterial Uptake Assay**
DCs were pretreated with vitamin D or vehicle (ethanol) and incubated at 37°C for 24 hours. Subsequently, DCs were stimulated with pneumococci (MOI, 50), and at 2 hours after infection, gentamicin (200 µg/mL) was added to the cultures for 1 hour to kill extracellular bacteria. Cells were washed with PBS and lysed with 0.1% saponin in PBS. Serial dilutions of intracellular bacteria were plated on blood agar for enumeration of viable bacteria.

**Cytokine Detection by ELISA**
DC supernatants were assayed for interleukin 1β (IL-1β), and coculture supernatants were assayed for IFN-γ, IL-17, and IL-10, using Ready-SET-Go! ELISA kits from eBioscience.

**RNA Isolation, cDNA Synthesis and Real-time Quantitative Polymerase Chain Reaction**
Total cellular RNA was extracted from stimulated DCs using RNeasy Kit (Qiagen). The concentration and purity of isolated RNA was determined spectrophotometrically with Nanodrop ND 1000. Complementary DNA (cDNA) was synthesized from isolated RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Real-time polymerase chain reaction (PCR) was performed using Power SYBR Green PCR master mix (Applied Biosystems). Reverse-transcription PCR reactions were performed with an initial step at 50°C for 2 minutes and an activation step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 55°C for 1 minute. Pre-designed primer mixes containing forward and reverse primer for the specific reverse-transcription PCR target were purchased from Qiagen (QuantiTect Primer Assay). The following primer assays were used: Nucleotide-binding oligomerization domain-containing protein 2 (Nod2) (Hs_NOD2_1_SG), Toll-like receptor 2 (Hs_TLR2_1_SG), TLR4 (Hs_TLR4_2_SG), IL-1β (Hs_IL1B_1_SG), Human Beta Defensin 3 (hBD-3) (Hs_DEFBI03B_2_SG), and γ-actin (Hs_ACTG1_1_SG). Each primer pair was validated for specificity by performing dissociation curve analysis of the PCR product to ensure the absence of primer dimers. For each sample, the messenger RNA expression level was normalized to the level of γ-actin and relative expression of Nod2, TLR2, TLR4, IL-1β, and hBD-3 was determined with the delta delta cycle (δδCT) threshold method. Each PCR run included a no-template control. All samples were assayed in triplicates.

**Antimicrobial Peptide**
The hBD-3 was synthesized recombinantly by cloning cDNA encoding the 45 amino acids containing the natural form of hBD-3 into the pET-30c expression vector (Novagen), which contains an N-terminal His tag sequence allowing purification of the fusion protein with a nickel affinity column. The recombinant peptide revealed the identical mass of the natural peptide upon mass spectrometric analyses. In addition, reversed-phase (C18) high-performance liquid chromatography of recombinant hBD-3 demonstrated an elution profile identical to that of the natural hBD-3, indicating the correct folding of the recombinant hBD-3.

**Antimicrobial Assay**
Test isolates were grown in tryptic soy broth at 37°C, washed in 10 mmol/L sodium phosphate buffer (pH, 7.4), and adjusted to 10^4–10^5 bacteria per milliliter. A 100-µL volume of the bacterial suspension was mixed with 10 µL of hBD-3 (final concentration, 0.0125–100 µg/mL) and incubated at 37°C for 2 hours. To determine the number of colony-forming units, serial dilutions were plated and colony counts were performed the following day. Results are given as Minimum Bactericidal Concentration (MBC) (≥99.9% killing) and Lethal Dose 90% (LD90).
Statistical Analysis
Statistical analysis was performed using GraphPad Prism 4.0 software. Paired (2-sided) $t$ tests were conducted on data, and differences were considered significant at $P < .05$. Data are presented as means ± standard errors of the mean for $\geq 3$ donors, unless otherwise specified.

RESULTS

Vitamin D Enhancement of DC Maturation in Response to Pneumococci
The maturation of DCs is characterized by up-regulation of MHC and the costimulatory molecules CD80 and CD86. We recently demonstrated that live pneumococci induce maturation of human DCs [22]. To further investigate the mechanisms by which pneumococci trigger DC maturation, we first tested the effect of capsular expression by comparing the encapsulated wild type strain T4 with the unencapsulated isogenic mutant T4R. This experiment revealed that only T4R induces significant maturation of DCs (Figure 1A), suggesting that the polysaccharide capsule, which renders the pneumococcus resistant to phagocytosis, is an important determinant to prevent DC maturation. Next, we examined the ability of pneumococcus-derived polymeric cell wall PGN to induce DC maturation. PGN triggered a significant increase in CD86 and MHC class II (Figure 1A). By contrast, stimulation with the monomeric PGN fragment MDP did not result in up-regulation of CD86 and MHC class II (Figure 1A). Internalization of PGN was completely inhibited by cytochalasin D (Figure 1B), demonstrating that uptake of PGN requires actin polymerization. Nevertheless, PGN-induced CD86 expression was only partly inhibited by cytochalasin D (Figure 1C), suggesting that both intracellular and extracellular receptors may be involved in the recognition of PGN.

Given the well-known immunomodulatory effects of vitamin D, we sought to determine its potential impact on DCs infected with pneumococci. When DCs were stimulated with T4 and T4R in the presence of active vitamin D (1,25-(OH)$_2$D$_3$; 100 nmol/L), expression of CD86 was significantly up-regulated (Figure 1D). Vitamin D did not affect CD86 expression in unstimulated cells (Figure 1D). Moreover, addition of vitamin D enhanced DC expression of CD86 and CD80 in PGN-stimulated cells (Figure 1D and data not shown). To relate these findings to human physiology, we investigated whether 25(OH)D$_3$, the inactive proform, could induce maturation of DCs. Interestingly, this resulted in a small up-regulation of CD86, which was blocked by itraconazole, an inhibitor of Cyp27B1 (Figure 1E).

Moreover, we observed that vitamin D enhanced the expression of CCR7 in response to PGN (Figure 1F). Up-regulation of CCR7 initiates the migration of DCs to draining lymph nodes where they present antigens to T cells. This process is associated with loss of phagocytic capacity, and to test whether the ability of DCs to internalize pneumococci was affected by vitamin D exposure, we examined pneumococcal uptake by DCs that had been preincubated with vehicle or vitamin D. Notably, uptake of T4R was significantly inhibited by vitamin D (Figure 1G), indicating that vitamin D-mediated enhanced maturation of DCs renders the cells less phagocytic.

Synergistic Up-regulation of Key Elements of the Innate Immune System
Nod2 and TLR2 are important receptors for recognition of gram-positive bacteria such as pneumococci. Interestingly, gene expression analysis of DCs treated with vitamin D showed a strong up-regulation of Nod2 and TLR2, whereas no difference was observed in TLR4 expression (Figure 2A–C). Moreover, exposure of DCs to both vitamin D and PGN led to an enhanced up-regulation of Nod2 and TLR2 but not TLR4 (Figure 2A–C). These observations suggest that vitamin D has the capacity to elevate the immune response to pneumococci by up-regulating key PRRs in DCs.

To further examine the immunomodulatory effects of vitamin D, we investigated its influence on the production of the proinflammatory cytokine IL-1β. Vitamin D strongly enhanced both gene and protein expression of IL-1β in PGN-stimulated DCs (Figure 2D and 2E). A compilation of results from multiple donors showed that after 24 hours of stimulation, gene expression was increased 100-fold (Figure 2D), and protein expression was increased 7-fold (Figure 2E). To study whether vitamin D also influences the induction of AMPs, we measured the expression of the β-defensin hBD-3, because β-defensins have been shown to be induced in DCs in response to bacterial stimulation [23]. Expression of hBD-3 was up-regulated 10-fold in vitamin D-exposed cells, and stimulation of cells with PGN in the presence of vitamin D led to a significant and synergistic up-regulation of hBD-3 (Figure 2F). Interestingly, hBD-3 had significant bactericidal activity against T4 and T4R, with a Minimum Bactericidal Concentration (MBC) of 3.125 µg/mL and a Lethal Dose 90% (LD$_{90}$) value of 0.780 µg/mL for both strains. Together, these experiments suggest that vitamin D treatment of human DCs induces an increased innate immune response to pneumococci.

Vitamin D Suppression of Th1/Th17 Responses and Amplification of Regulatory T-Cell Response to Pneumococcal PGN
CD4$^+$ T cells have been shown to be important in the protection against pneumococcal colonization and disease in murine infection models. Therefore, we analyzed DC-mediated T-helper cytokine responses to pneumococci. PGN-stimulated DCs were cocultured with autologous CD45RA$^-$ naive or CD45RO$^+$ memory T-helper cells for 5 days, and the production of Th1 (IFN-γ$^+$), Th17 (IL-17$^+$), and regulatory T-cell (IL-10$^+$)-associated
cytokines was measured. We found that naive T-helper cells, cocultured with PGN-stimulated DCs, produced minimal IFN-γ and no IL-17 or L-10 (data not shown), whereas memory T-helper cells showed an up-regulation in all 3 cytokines (Figure 3A–C), although the amount varied considerably between donors.

Our previous results suggested that the ability of vitamin D to act on DCs is likely to affect the DC-mediated adaptive immune response to pneumococci, and thus we next studied the effect of vitamin D on the subsequent T-helper response. Exogenous vitamin D abrogated IFN-γ and IL-17 production, whereas IL-10 production was enhanced (Figure 3D–F).
compilation of the results from 5 donors showed that IFN-γ and IL-17 were dampened by 63% and 75%, respectively, whereas IL-10 production increased 2.6-fold (Figure 3G). Thus, vitamin D has the capacity to modulate the host response to pneumococci from an inflammatory Th1/Th17 response toward an IL-10–regulated anti-inflammatory response.

DISCUSSION

Vitamin D is known to affect the immune system at several levels, with the net effect of strengthening mucosal immunity and suppressing the adaptive immune response [24]. Vitamin D is synthesized in the skin by ultraviolet B light and the lowest vitamin D levels occur during the dark time of the year. Interestingly, the seasonal variation in vitamin D serum levels is reciprocally correlated to the incidence of invasive pneumococcal disease [25]. However, even though epidemiological data suggest a beneficial role for vitamin D in pneumococcal infections, interventional trials are missing and few studies address vitamin D–mediated immune responses in the context of pneumococcal infections.

Because DC responses have been shown to be affected by vitamin D, we studied the role played by vitamin D in DCs challenged with pneumococci and pneumococcal cell wall PGN. We showed that PGN increases maturation of human DCs, as measured by CD86, CD80 and MHC class II surface expression. By contrast, stimulation of DCs with the PGN monomer MDP did not result in DC maturation. Intact polymeric PGN was recently shown to be a better stimulator of human innate immune cells than MDP, and the polymeric nature of PGN was required for efficient phagocytosis and lysosomal degradation [26]. Similarly, we noted that pneumococcal PGN was efficiently phagocytosed by the DCs and that cytochalasin D–inhibited internalization of PGN fragments dampened DC maturation. Furthermore, we demonstrated that addition of exogenous vitamin D leads to an enhanced maturation of DCs, as well as an up-regulation of CCR7, a surface marker for DC migration to the lymph nodes. Notably, the up-regulation in CD86, CD80, and CCR7 expression was paralleled by reduced cellular uptake of intact pneumococci. Taken together, these results indicate that vitamin D promotes a mature, migratory and nonphagocytic phenotype in DCs.

Given the importance of DC receptors in recognizing and responding to microbial products, we investigated the expression of key PRRs in pneumococcal infection by quantitative PCR. We observed that vitamin D enhanced the expression of Nod2 and TLR2 but not TLR4 in PGN-stimulated DCs. Thus, vitamin D seems to elevate the sensing capacity of DCs, which could be important in the context of pneumococcal and other gram-positive bacterial infections. One downstream event of PRR activation is the expression of AMPs, which may kill bacteria directly but can also activate immune cells via TLRs [27].
The β-defensin hBD-3 has been shown to be up-regulated in DCs in response to bacterial stimulation [23], but the role of vitamin D has previously not been explored. We found that the gene expression of hBD-3 was strongly up-regulated in DCs stimulated with PGN in the presence of vitamin D. In addition, we demonstrate that hBD-3 has high antibacterial activity against pneumococci. hBD-3 has been described as an "endogenous adjuvant" by virtue of its capacity to activate and recruit antigen-presenting cells via TLRs and C-C chemokine receptor type 2 (CCR2) [28, 29]. Thus, our data suggest that vitamin D activates an alert system in DCs, based on PRRs and AMPs, which in concert can recognize and respond to a microbial assault. This activation coincided with an enhanced production of IL-1β, a pro-inflammatory mediator that has been shown to play a major role in resistance to pneumococcal infection in mice [30–32].

Several investigators have presented data supporting a role for T-helper cells in reducing pneumococcal carriage and disease in murine models [14–16, 33]. However, little is known about T-helper cell responses to pneumococci in the human system. In the current study, we demonstrated that DCs stimulated with pneumococcal PGN promote IFN-γ, IL-17 and IL-10 production in cocultured memory, but not naive, CD4+ T cells. Exogenous addition of vitamin D skewed the DC-mediated T-helper response from an inflammatory Th1/Th17 phenotype to a regulatory T-cell phenotype. These observations are in line with other reports of vitamin D-mediated inhibition of Th1 and Th17 cytokine production and induction of IL-10 production [11, 12, 34–36]. Given the immunomodulatory effects of vitamin D on adaptive immune responses in our in vitro system, we speculate that vitamin D may have a role in suppressing the inflammatory response triggered in the host during pneumococcal disease.

In conclusion, our data show that the pneumococcal cell wall component PGN influences DC functions such as maturation and migration to lymph nodes, and we suggest that several innate immune responses could be strengthened by treatment with vitamin D. Vitamin D seems to regulate the immune balance in response to pneumococci by inducing innate immune responses with potential effects on mucosal immunity and by mitigating inflammation caused by the adaptive immune response. A thorough understanding of innate and adaptive immune responses induced by pneumococci is essential for the development of novel therapeutic strategies, including regulation of immune responses and inflammation, and there is a need to further elucidate the role of DC-mediated cytokine responses in the delicate balance between protective immunity and immunopathology. Our findings suggest that vitamin D might be useful as an immunomodulatory drug, targeting the inflammatory response caused by pneumococci. Further studies—both experimental and clinical—that explore the impact of vitamin D on human immune responses to pneumococcal disease.

Figure 3. Vitamin D suppresses pneumococcus-driven inflammatory T-cell responses. A–C, Dendritic cells (DCs) were stimulated with pneumococcal peptidoglycan (PGN) for 24 hours; they were then cocultured with CD4+ memory T cells for 5 days. Coculture supernatants were analyzed for interferon (IFN) γ (A), interleukin 17 (IL-17; B), and interleukin 10 (IL-10; C) production. Data shown represent means for 5–10 donors. D–G, DCs were stimulated with PGN in the presence or absence of vitamin D and cocultured with CD4+ memory T cells for 5 days. Supernatants were analyzed for T-helper cytokine production. Data shown in D–F are means ± standard deviations for 1 donor and are representative of 5 donors. Data shown in G represent mean±standard error of the mean) fold change in cytokine production by cells stimulated in the presence of vitamin D relative to vehicle (5 donors). *P<.05.
disease pathology associated with Th1- or Th17-mediated inflammation are warranted.

Notes

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