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Review Article

Vitamin D, innate immunity and upper respiratory tract infection

J Bartley

Abstract

Introduction: At the turn of the twentieth century, ultraviolet light was successfully used to treat tuberculosis of the skin. Upper respiratory tract infections had been inversely associated with sun exposure. During the last decade, basic scientific research demonstrated that vitamin D has an important anti-infective role.

Method: Review of the relevant literature on the influence of vitamin D on innate immunity and respiratory tract infection.

Results: Vitamin D is involved in the production of defensins and cathelicidin – antimicrobial peptides that provide a natural defence against potential microbiological pathogens. Vitamin D supplementation increases cathelicidin production. Low vitamin D levels are associated with an increased incidence of upper respiratory tract infections.

Conclusions: Vitamin D appears to play an important role in the regulation of innate immunity in the upper respiratory tract. Optimal vitamin D levels and appropriate dosing schedules have yet to be determined.

Key words: Vitamin D; LL-37; Antimicrobial Peptides; Innate Immunity; Chronic Rhinosinusitis; Respiratory Tract Infection; Cathelicidin; Defensin; Biofilm

Introduction

In 1903, the Danish doctor Niels Finsen was awarded the Nobel Prize for discovering that high intensity light produced from an electric arc lamp cured 95 per cent of patients with skin tuberculosis – a condition termed lupus vulgaris. By the 1920s, sun exposure was recognised as an effective treatment for pulmonary tuberculosis.[1] With the advent of penicillin and sulphanilamide, after the First World War, the idea that regular sun exposure might protect against infection was rapidly forgotten. However, over the last decade, research into the antimicrobial action of vitamin D has provided new insights into this historical intervention.[2] Recent laboratory[3–7] and epidemiological[8] evidence suggests that vitamin D plays an important role in both adaptive and innate immunity. Local innate defences, which rapidly recognise potential microbial pathogens, play an important role in preventing microbial colonisation that could lead to recurrent infection.[9,10]

This paper provides an overview of current knowledge on the contribution of vitamin D to innate immunity in the upper respiratory tract.

Mucosal defence and antimicrobial peptides

The upper respiratory tract is the primary site of contact for inhaled pathogens. Exposure to potential pathogens is common, and several protective mechanisms exist at mucosal surfaces. The first major physical defence covering the ciliated respiratory epithelium is a superficial gel layer of mucus which physically removes inhaled pathogens.[11,12] The second defence mechanisms are the antimicrobial peptides: defensins, cathelicidin, and larger antimicrobial proteins such as lysozyme, lactoferrin and secretory leukocyte protease inhibitor in the airway secretions.[13–15] The third defence mechanism is the initiation of the inflammatory response and the recruitment of phagocytic cells to any developing infection.[12]

Antimicrobial peptides, which are synthesised and released largely by epithelial cells and neutrophils, have a broad spectrum of antimicrobial activity against viruses, bacteria and fungi.[14,15] In contrast to many conventional antibiotics, these peptides appear to be bactericidal.[15] Bacteria have difficulty developing resistance to antimicrobial peptides, although some bacteria have developed mechanisms to evade their action.[2] The initial contact between an antimicrobial peptide and the microbe is thought to be electrostatic, as antimicrobial peptides are positively charged and most bacterial surfaces are negatively charged.[15,16] Antimicrobial peptides kill bacteria by inserting themselves into the cell membrane bilayers to form pores, by 'barrel-stave',

From the Department of Otolaryngology-Head and Neck Surgery, Counties-Manukau District Health Board, Auckland, New Zealand.
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'carpet' or 'toroidal pore' mechanisms. These pores disrupt cell membrane function. Recent evidence would suggest that antimicrobial peptides can also inhibit cell wall synthesis, nucleic acid synthesis, protein synthesis and enzymatic activity, and can disrupt mitochondrial membranes.[15–17] In response to infection, antimicrobial peptide production can also upregulate the signalling mechanisms that recruit phagocytic cells, assisting control of the infection.[12]

Two antimicrobial peptide defensin families have been identified in humans: α-defensins and β-defensins. In humans, neutrophils, nasal epithelial cells and intestinal Paneth cells produce α-defensins.[18–20] The epithelial cells of the lung, skin and gut produce β-defensins.[12] In epithelial cells, the expression of β-defensin-1 appears constitutive,[20,21] whereas expression of β-defensin-2, -3 and -4 is inducible.[12,22–24]

Cathelicidins are a diverse family of α-helical cationic antimicrobial peptides. They have been identified in multiple species. Whereas many species produce a variety of cathelicidins, humans make only one type of cathelicidin called hCAP-18. Cathelicidins are synthesised as prepropeptides and broken down by protease enzymes, releasing the C-terminal peptide, which has antibacterial activity.[25] The free C-terminal peptide of the human cathelicidin hCAP-18, released by protease enzymes is a peptide called LL-37. LL-37 had bactericidal activity. In the skin, the peptide LL-37 can be broken down into smaller peptides which may display a different spectrum of activity.[26] The cytotoxic concentrations of the antimicrobial peptide LL-37 are three to five times the concentrations needed to kill bacteria. Presumably, *in vivo* LL-37 concentrations are closely regulated in order to protect host cells from damage.[16] Cathelicidins are synergistic with both lysozyme and lactoferrin.[16,27] As well as having a broad spectrum of bactericidal antimicrobial activity, β-defensins and cathelicidins increase proinflammatory gene expression, are involved with epithelial proliferation and repair mechanisms, and modulate immune function via an effect on dendritic cell maturation.[12,21–23] Cathelicidin has been identified throughout the epithelium of the upper and lower respiratory tracts.[27–29]

Vitamin D influences the production of cathelicidin and β-defensin-2.[3–5,30,31] The vitamin D receptor genes are close to two genes that encode the antimicrobial peptides cathelicidin and β-defensin-2.[3] Vitamin D causes a small increase in cell manufacture of β-defensin-2. However, in a number of different cell types (including immune system cells and keratinocytes) vitamin D can cause a dramatic increase in cathelicidin production. Toll-like receptors, a family of evolutionarily ancient receptor proteins found on human immune cells, are germ line encoded receptors, which means that they are genetically determined.[9] To some extent, this allows the innate immune system to determine the nature of the infecting pathogen.[13] Toll-like receptors respond to the byproducts of bacterial cell walls by manufacturing both vitamin D receptor proteins and increasing cytochrome P450 CYP27B1, the enzyme that converts circulating 25-hydroxyvitamin D into biologically active 1,25-dihydroxyvitamin D.[7] This latter compound in turn interacts with the promoter of the gene for cathelicidin.[4] Adequate levels of 25-hydroxyvitamin D, the major circulating form of vitamin D, are necessary to activate cathelicidin production and to enhance macrophage function and innate immunity. Cathelicidin production would appear to be facilitated by vitamin D levels of up to 100 nmol/l.[5] Vitamin D supplementation also increases cathelicidin production.[7]

Biofilms and Cathelicidin

Bacteria are now recognised as existing in two forms: free-floating (i.e. planktonic) and in biofilms, sophisticated communities which adhere to both biological and non-biological surfaces. Many chronic infectious diseases appear to be caused by bacteria living in a biofilm state, including otitis media, tonsillitis and chronic rhinosinusitis.[32–34] Biofilms have been defined as 'structured communit[ies] of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface'.[35] In a biofilm state, the bacteria produce an extracellular matrix (often referred to as 'slime') which protects its inhabitants against environmental threats including 'biocides, antibiotics, antibodies, surfactants, bacteriophages and foraging predators such as free living amoebae and white blood cells'.[36] Bacteria within biofilms are difficult to culture and highly refractory to conventional antibiotic treatment. *In vitro*, such bacteria produce proteinases that can degrade antimicrobial peptides.[37,38] Whether this occurs *in vivo* has yet to be proven.[15] Antimicrobial peptides are also inactivated by products of inflammation, such as lipopolysaccharide from Gram-negative bacteria.[39,40] A large amount of research is currently being undertaken to assess techniques and drugs which could potentially prevent biofilm formation and/or break biofilms down.[41] *In vitro*, LL-37 the free C-terminal breakdown product of human cathelicidin appears able to both break down and to prevent development of *Pseudomonas aeruginosa* biofilms.[42,43]

Observational data

In 1926, Smiley noted a strong inverse association between sun exposure and upper respiratory tract infections, and theorised that 'disordered vitamine metabolism in the human ... directly due to a lack of solar radiation during the dark months of winter' might be responsible.[44] Low vitamin D levels are associated with an increased risk of upper and lower respiratory tract infection. Ricketts, caused by severe vitamin D deficiency in children, is associated with an increased risk of acute respiratory tract infection, particularly pneumonia.[45–49] Finnish army recruits with vitamin D levels of less than 40 nmol/l were found to be at increased risk of upper respiratory tract infection.[50] Two case–control studies have reported an association between serum 25-hydroxyvitamin D levels of less than 50 nmol/l and acute

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lower respiratory tract infection in children[51] and neonates.[52] Parents of Dutch children with the least sun exposure were twice as likely to report that their child had developed a cough, and three times more likely to report that their child had a runny nose, compared with parents of children with the most sun exposure.[53] Seasonal variation in influenza has also been linked to low vitamin D levels.[54] Vitamin D levels above 75 nmol/l are associated with a reduced incidence of upper respiratory tract infection.[8]

A high prevalence of vitamin D deficiency has been noted in patients attending a general otolaryngology clinic, but this was not specifically related to upper respiratory disease.[55] One study found that 50 per cent of children with otitis media with effusion had vitamin D levels of less than 50 nmol/l; there was no control group.[56] Several groups have reported that α- and β-defensins are produced by the sinonasal mucosa.[20,24] Cathelicidin mRNA is up-regulated in chronic rhinosinusitis patients, particularly those with eosinophilic mucus.[28,29] The influence of vitamin D on cathelicidin levels and the response to infection was not considered. If vitamin D levels are high, then increased cathelicidin levels will be seen initially and in response to infection. The converse also holds for low vitamin D levels.[5]

Interventional data

Most of the interventional data relating to treatment of upper respiratory tract infection is indirect.

Cod liver oil contains vitamin D as well as other nutrients that might be useful in the prevention or treatment of infection. In one study, 185 adults were given cod liver oil, and their prevalence of colds over four winter months was 44.9 per cent compared with 67.2 per cent in the control group.[57] A second study found that cod liver oil given to 1561 adults reduced the incidence of respiratory tract infections by 30 per cent.[58] Courses of suberythemal ultraviolet radiation administered twice a week for three years to 410 teenage athletes resulted in 50 per cent fewer respiratory viral infections and 300 per cent fewer days of absenteeism, compared with 446 non-irradiated athletes.[59]

In one interventional cohort study, 60 000 IU of vitamin D was given weekly for six weeks to children with recurrent respiratory tract infection; these children's incidence of such infection reduced to that of the control group.[60] In another cohort study, children were given 600 to 700 IU of vitamin D in cod liver oil daily together with a multivitamin supplement.[61] The children in the treatment group showed a 50 per cent reduction in the number of medical consultations for upper respiratory tract infections; there was no change in the control group.

In some studies in which vitamin D was given for skeletal health, a reduction in infection risk was also noted. In one randomised, controlled study assessing the influence of vitamin D supplementation on bone loss in 208 postmenopausal black (Afro-American) women, 7.7 per cent of women randomised to receive 800 to 2000 IU of vitamin D daily reported upper respiratory tract symptoms (i.e. colds or influenza symptoms), compared with 25.0 per cent of the control group, during three-year follow up.[62] Another randomised, controlled study of fracture prevention, in which patients were given 800 IU of vitamin D daily, found a statistically insignificant 10 per cent reduction in wintertime infection in the group receiving vitamin D; the type of infection was not specified.[63] In the latter three studies, it is unlikely that optimal anti-infective vitamin D levels were attained.

Conclusions

Antimicrobial peptides were previously thought to function as an initial, 'rapid response' defence system against infective threats, until adaptive immunity took over. However, it is now known that antimicrobial peptides also modulate innate and adaptive immune responses. Vitamin D appears to play an important role in the regulation of innate immunity in the upper respiratory tract. Chronic rhinosinusitis and otitis media with effusion could be related to low vitamin D levels. Currently, the optimum vitamin D serum concentration needed to prevent and/or treat respiratory tract infection is unknown.

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Address for correspondence:
Dr Jim Bartley,
10 Owens Rd,
Epsom, Auckland 1023,
New Zealand.

Fax: 649 631 0478
E-mail: jbartley@ihug.co.nz

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