Vitamin A as an anti-inflammatory agent

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Vitamin A is necessary for normal differentiation of epithelial tissues, the visual process and reproduction, and is vital for the optimal maintenance and functioning of the innate and adaptive immune system. Vitamin A deficiency is one of the most profuse nutritional deficiencies worldwide. It is associated with increased susceptibility to infectious diseases in both man and animal models. Vitamin A also has a role as an anti-inflammatory agent. Supplementation with vitamin A has been found to be beneficial in a number of inflammatory conditions, including skin disorders such as acne vulgaris, broncho-pulmonary dysplasia and some forms of precancerous and cancer states. The present review suggests that vitamin A deficiency induces inflammation and aggravates existing inflammatory states. Supplementation with vitamin A in selected cases could ameliorate inflammation. The two main mechanisms which appear to be involved in the prevention of disease are the effects of vitamin A on the immune system and the effect on epithelial integrity.

Vitamin A: Inflammation

Background

Vitamin A (VA) plays a major role in vision, cell growth and maintenance of the integrity of epithelial cells, reproduction, the immune system and in modulation of various clinical and pathological conditions. These functions are mediated by different forms of the molecule. Retinol and retinal are both capable of maintaining normal vision and reproductive functions, whereas retinoic acid can substitute for either of these VA forms for normal growth and development.

The role of VA in the promotion of growth and differentiation of epithelial tissues makes it an important nutrient during development in the neonatal period and during infancy and childhood. VA deficiency (VAD) is undoubtedly the leading cause of childhood blindness in developing countries. Even at a subclinical level, VAD contributes to a great extent to increased childhood morbidity and mortality. More than 100 million preschool children live in areas endemic for VAD (UNICEF, 1997). There is also evidence that VAD exists to some extent in the developed countries. A necropsy study of American babies reported deficient liver VA concentrations ($< 0.07\,\mu$mol/g) in two-thirds of infants under 3 months of age and in one-quarter of 4–6-month-old infants (Olson *et al.* 1984).

The major factor contributing to VAD is inadequate intake, including breast-feeding and weaning diet. This factor is often aggravated by the high frequency of infections such as diarrhoea, respiratory diseases, measles and HIV (Feacham, 1987). It is not clear whether this synergism is a result of increased demands due to infection, decreased food intake or a redistribution of VA in tissues during infection. This last possibility lies behind the rationale justifying the administration of VA supplements to children with severe measles, irrespective of their previous VA status (Hussey & Klein, 1990). The synergism between VAD and infections is responsible for the excessive morbidity and mortality in children in the developing world, mostly in diseases affecting the respiratory and gastrointestinal tracts. It has been shown that children, even with mild VAD, are at an increased risk of respiratory disease and diarrhoea.

Human and experimental animal studies reveal that VA is required for optimal maintenance and functioning of the immune system. Studies in rats, mice and chicks have demonstrated lower antibody response to tetanus toxoid, rotavirus, diphtheria, pertusis, *Escherichia coli* and bacterial polysaccharide antigens in VAD animals as compared with non-VAD animals (Semba, 1994). Impaired cell-mediated immunity, a reduction in delayed hypersensitivity, has been reported in VAD, as well as decreased proliferation of T lymphocytes. Importantly, depressed levels of splenic

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natural killer cell activity and interferon titres have been observed in animals (Bowman *et al*. 1990). Peripheral blood mononuclear cells have low natural killer cell activity in children with acute measles. Decreased humeral immunity in VAD may be related to impaired T-helper cells.

The gastrointestinal system

VAD is associated with a reduction in the number of goblet cells per duodenal villus in mice (Ahmed *et al*. 1990). A decrease in luminal mucus and in other functional and morphological changes occurs in the rat gastrointestinal system (Reifen *et al*. 1998*b*). Gastrointestinal integrity is severely impaired during illness, but is responsive to additional VA. VAD aggravates the course of rota-virus infection via damage to the epithelial lining of both the small intestine and colon. These findings may elucidate the mechanism causing the more severe course of the disease in areas where VAD is endemic (R Reifen, A Mor and A Nyska, unpublished results). We have conducted two studies in which we tested the possibility that inflammation and its sequelae are (a) enhanced by VAD and (b) ameliorated by supplementation with VA in a rat model of induced colitis. To identify the possible effect of the nutrient on the acute and chronic disease state, assessments were conducted for two different time periods. A pair-fed group was included in the design to control for the confounding effects of lower food consumption on the outcome measurements (R Reifen, T Nur, K Ghebermeskel, G Zaiger, R Urizky and M Pines, unpublished results).

Consistent with our previous findings in rats and chicks (Uni *et al*. 1998), VAD was associated with a reduction in food consumption and weight gain. The mechanism for this adverse effect is not well understood. Nevertheless, we found that taste preference is impaired in VAD (Reifen *et al*. 1998*a*), and this factor may have an adverse effect on food consumption.

The most important finding of the investigation was the morphological and inflammatory changes in the colon of the VAD non-colitic rats. This phenomenon is characterised by shortened villi, infiltration of inflammatory cells and hyperplasia, fibrosis and activation of nuclear factor kappa B (NFκB), but was not manifested in the pair-fed group, which were VA sufficient. Hence, the phenomenon could not be attributed to an insufficiency of vital nutrients other than VA, or to low energy intake resulting from the reduction of food consumption. Although not as severe, the colon pathology of the VAD non-colitic rats was similar to that of the VAD and VA-sufficient colitic groups. The infiltration of inflammatory cells in the colon was markedly ameliorated in the VA-supplemented rats.

Consistent with our present findings, others have reported an inverse relationship between VA status and inflammatory response (Sauer *et al*. 1995; Swamidas *et al*. 1999). However, the mechanism involved is not well understood. The plasma malondealdehyde concentration of the VAD non-colitic rats was higher than that of the corresponding VA-sufficient and VA-supplemented non-colitic groups by 45 and 78% respectively, indicating that the VAD animals were under oxidative stress. It is possible that this oxidative load in the non-colitic VAD group may have resulted in

lipid peroxidation, activation of NFκB and the subsequent inflammatory cascade (chemotaxis and infiltration of inflammatory cells and collagen deposition in the colon). There is evidence that the antioxidant VA exerts an influence, in part, by inhibiting translocation of the transcription factor NFκB and interrupting the secretion of inflammatory cytokines (Horton *et al*. 2001).

There was also a marked increase in the activity of Cu/Zn superoxide dismutase after the induction of colitis in all our experimental groups. This increase was probably a physiological response to oxidative stress. Our data clearly demonstrate that VAD induces inflammation, fibrosis-increased collagen type 1 expression and NFκB activation, and morphological and histological changes in the colon. Colitis is amplified by VAD and ameliorated by supplementation with VA.

In a subsequent study (Nur *et al*. 2002) we investigated the molecular basis of the inflammatory processes in the colon caused by 2,4,6-trinitrobenzenesulfonate-mediated induction of colitis, and as induced by VAD. We employed the DNA micro-array technology, and compared the gene expression profiles in the colons of VAD, VA-supplemented and colitic rats. We only focused on genes which had shown a dramatic change in expression associated with VAD and colitis. This selection process yielded a number of genes whose expression was markedly up or down regulated in VAD and colitis. A similar pattern of differential gene expression was recorded in rats with induced colitis and VAD when compared with the VA-supplemented group.

The involvement of a number of the genes identified in our study to be down or up regulated has been previously associated with inflammatory conditions. For example, changes in the activity of the catabolic enzyme spermidine/spermine N1-acetyltransferase have been suggested to play a role in rheumatic arthritis (Furumitsu *et al*. 2000). Another gene of interest, whose involvement in VAD and in induced colitis was indicated by our findings, was ubiquitin. The involvement of the ubiquitin system in inflammatory processes is generally believed to occur through activation of the transcription factor NFκB, a heterodimeric protein that plays a pivotal role in immune and inflammatory responses (for review, see Verma *et al*. 1995). This DNA-binding protein is required for the *de novo* synthesis of numerous pro-inflammatory cytokines, chemokines, adhesion proteins and molecules critical for normal immuno-inflammatory function. Pro-inflammatory stimuli activate NFκB through tightly-regulated phosphorylation, ubiquitination and proteolysis of a physically-associated class of inhibitor molecules, NFκB inhibitory proteins (Karin & Ben-Neriah, 2000). The putative involvement of the ubiquitin system in pro-inflammatory processes in the colon of VAD as well as 2,4,6-trinitrobenzenesulfonate-induced colitic rats, as indicated in this study, requires further investigation. The simple observation of this gene's relative expression may not provide sufficient evidence to determine its physiological or pathological role in inflammatory processes in the colon. Micro-array technology provided us with a list of select genes that are similarly expressed in VAD and induced colitis. These findings can be used to generate hypothesis-driven mechanistic experiments to define VA function. These

findings confirmed our observation of the inflammatory processes elicited by VAD in the rat colon.

The respiratory tract

VA and its active metabolites are also important factors in promoting normal respiratory epithelial differentiation and growth (Biesalski & Stoftt, 1992). They exhibit a wide spectrum of activities, including anti-inflammatory properties. Children infected with respiratory syncytial virus had a low serum concentration of VA during acute illness, and these low levels were associated with more severe illness. This phenomenon could be attributed to the increased rate of VA utilization by the tissue damaged by the virus (Neuzil et al. 1994).

We have used the Brown Norway rat model of airway hypersensitivity, which can exhibit both early and late airway response (Eidlman et al. 1998). We hypothesized that allergen inhalation might interfere with cellular or systemic VA supply. To elucidate whether allergic bronchitis interferes with VA metabolism we measured the VA levels in the serum, the lung and the liver following repeated allergen challenge in a rat model and compared them with VA levels of normal rats (Shosayov et al. 2002).

We were able to demonstrate that recurrent allergen challenges result in a decrease in hepatic VA stores in sensitised rats.

VA intake was not different between the two groups, as measured by food intake and also reflected by body weight. The liver supplies VA to the serum and the various organs and maintains normal serum and tissue concentration as long as VA is not depleted. A decrease in hepatic VA stores might be a result of an increased demand of target tissues or decreased intake.

During acute and chronic inflammation the VA demand of tissues increases as a result of increased epithelialization (Biesalski & Stoftt, 1992). Several studies have shown the important role of VA in the respiratory and alveolar mucosa (Manna et al. 1995). The respiratory mucosa requires VA to ensure the proliferation and differentiation of mucus and ciliated cells. Recently, Baybutt et al. (2000) showed that VAD in rats leads to emphysematous lungs, reduced content of lung elastin (in areas of interstitial pneumonitis), decreased type II pneumocyte synthesis of surfactant and decreased ornithine decarboxylase activity in pneumocytes. Reduction in ornithine decarboxylase activity as a result of VAD results in a decreased proliferation of type II pneumocytes. Retinoids can inhibit the respiratory burst and degranulation of stimulated human polymorphonuclear leucocytes, probably through the mediation of transforming growth factor β-induced differentiation of tracheal epithelial cells into squamous cells (Jetten et al. 1986). The higher incidence of respiratory tract infections during measles may thus be attributed to the VAD.

VA supplementation could decrease an inflammatory response when rats are administered monocrotaline, a pro-inflammatory pneumotoxin (Swamidas et al. 1999). Furthermore, the role of VA in preventing inflammation is related to its interaction with leucocytes, particularly neutrophils, as VA reduces neutrophil superoxide production (Sharma et al. 1990) and VAD increases circulating leucocytes (Wiedermann et al. 1996). VAD could occur as a result of the increased proliferation during tissue repair and could accelerate the ongoing inflammation. Both tumour necrosis factor α and NO were higher in our study in the VAD group; these inflammatory markers increased when insufficient amounts of VA were available. These findings are in agreement with those of previous studies that have demonstrated an anti-inflammatory effect of VA and an increase in the inflammatory process during VAD (Sharma et al. 1990; Wiedermann et al. 1996). Our data are also consistent with those from a human study documenting an increased risk for chronic obstructive lung disease with decreased VA intake and an inverse relationship between plasma retinol status and the extent of airway obstruction assessed by forced expiratory volume (Morabia et al. 1990). Paiva et al. (1996) demonstrated lower plasma retinol levels in patients with moderate to severe chronic obstructive pulmonary disease. In addition, it was reported that high VA intake and high serum retinol levels were associated with lower prevalence of dyspnoea in patients with chronic obstructive pulmonary disease (Rautalahti et al. 1997).

Our study has shown that sensitization in a rat model leading to bronchial constriction, and thus mimicking asthma, increases depletion of liver VA and inflammation. We postulate that supplementation with VA during airway bronchial constriction may have some potential benefit by accelerating bronchial epithelial repair following asthmatic attacks, and consequently may reduce the sensitivity of the respiratory mucosa against inflammatory attacks. Further studies are in progress to elucidate the effects of retinoids on asthma in man and in an animal model.

Therapeutic potential of vitamin A as an anti-inflammatory agent

Does VA supplementation alleviate inflammation? Vitamin A has been reported to be beneficial in a number of conditions where inflammation is involved, including skin disorders, broncho-pulmonary dysplasia (BPD) and infectious disease.

In acne vulgaris, a condition known to respond to a topical application of all-trans retinoic acid (tretinoin), retinoic acid seems to increase the cellular turnover in the stratum corneum, making the epidermis very thin (Matskua, 1983). The formation of comedos (keratinous plugs) is thus prevented.

BPD is a life-threatening disease in neonates resulting from artificial ventilation and O_2 toxicity. Plasma retinol levels in babies with BPD have been reported to be lower than in those of premature babies without BPD. High doses of VA reduced the incidence of BPD from 85% in controls to 45% in the treated group. It has been suggested that low VA status interferes with the ability of the lung tissue to repair the tissue injury associated with the disorder (Shenai et al. 1987).

VA supplementation appears to reduce the severity of childhood pneumonia in developing countries (The Vitamin A and Pneumonia Working Group, 1995). We have observed favourable effects in a rat model of colitis and have been able to find suggestive evidence in a rat asthma model.

Conclusions

The greater understanding of VA activities stemming from research over the last two decades suggests that VA may be involved in vital functions, not only as a cofactor or as a structural component, but also through effects on gene expression. The way forward will be to delineate the mechanisms by which VA influences gene expression in the inflammatory process, for example through involvement in the immune system and alleviation of oxidative stress. VA may also be profitably exploited in terms of its effects in therapeutic interventions in inflammatory conditions.

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