Symposium on ‘Nutritional influences on developmental immunology’

Effects of dietary retinoids and carotenoids on immune development*

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Carotenoids and retinoids are groups of nutritionally-relevant compounds present in many foods of plant origin (carotenoids) and animal origin (mainly retinoids). Their levels in human subjects vary depending on the diversity and amount of the individual’s nutrient intake. Some carotenoids and retinoids have been investigated for their effects on the immune system both in vitro and in vivo. It has been shown that retinoids have the potential to mediate or induce proliferative and differentiating effects on several immune-competent cells, and various carotenoids are known to be inducers of immune function. The immune-modulating effects of retinoids have been well documented, while the effects of carotenoids on the immune system have not been investigated as extensively, because little is known about their molecular mechanism of action. The present review will mainly focus on the molecular mechanism of action of retinoids and particularly carotenoids, their nutritional origin and intake, their transfer from the maternal diet to the child and their effects or potential effects on the developing immune system.

Carotenoids: Retinol: Retinoic acid: Immune development: Allergy

Carotenoids and retinoids in human nutrition

Carotenoids and retinoids are groups of compounds that are of nutritional relevance in man. Their levels in the human body vary according to dietary intake and the type of diet (e.g. ‘Western’ diet) and the nutritional intake of the individual (Khachik et al., 1992a, b, 2002, 1997; Ford, 2000; Olmedilla et al., 2001; O’Neill et al., 2001; Al-Delaimy et al., 2004).

Retinoids occur mainly in animal-derived foods such as dairy and meat products and eggs in the form of retinol and retinyl esters (Heinonen, 1991). Carotenoids are present as: α- or β-carotene (Fig. 1(a)) in vegetables and fruits with an yellow–orange colour such as carrots, sweet potatoes (Ipomoea batatas), apricots (Prunus armeniaca, Armeniaca vulgaris), mangoes (Mangifera indica) and pumpkin (Cucurbita maxima); and as a food colorant (exclusively beta-carotene) in lemonades, butter, margarine, soup powders, pasta, dairy products (cheese, ice cream, yoghurt, custard) and drinks containing high amounts of vitamins A, C and E; β-cryptoxanthin (Fig. 1(a)) in papaya (Carica papaya), oranges, peaches (Prunus persica), vegetables (chilli and peppers); lycopene (Fig. 1(a)) in tomatoes, processed tomato products (ketchup, tomato soup, tomato sauce); lutein and zeaxanthin (Fig. 1(a)) in vegetables such as lettuce, cabbage, beans, broccoli, spinach (Spinacia oleracea), maize and squash (Cucurbita spp.).

In Western societies the retinoid levels of individuals are high (Olafsdottir et al., 2001; Allen & Haskell, 2002; Mensink, 2002), even up to 100% higher in relation to some recommended reference values for the dietary intake of retinol (Mensink, 2002). As a result of homeostatic mechanisms serum retinol concentrations are relatively stable over a range of intakes. For carotenoids the levels vary; in individuals in Western societies α- and β-carotene and lycopene levels are higher, while those of carotenoids such as lutein and zeaxanthin are much lower (Ito et al. *The other papers from this symposium were published in Proceedings of the Nutrition Society (2006) 65, 311–325.

Abbreviations: IFN-γ, interferon-γ; RAR, retinoic acid receptors; RBP, retinol-binding protein; RXR, retinoid-X receptors; Th, T-helper.

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1999; Ford, 2000; Neuhouser et al. 2001; Rühl et al. 2006). Carotenoid levels are higher in migrants to Western countries, e.g. Japanese-, Mexican- and African-Americans in the USA (Ito et al. 1999; Ford, 2000; Neuhouser et al. 2001) and children of Turkish origin in Germany (Rühl et al. 2006).

Mechanism of action of retinoids and carotenoids

Mechanism of action of retinoids

Retinoic acids in their all-trans or 9-cis configuration are highly-potent activators of the retinoic acid receptors (RAR) and the retinoid-X receptors (RXR). By activation of these nuclear receptors retinoic acids can influence the transcription of various retinoid-response genes (De Luca, 1991). In addition to the retinoic acids several other retinoids, such as 13,14-dihydroretinoic acid (Moise et al. 2004, 2005), 3,4-didehydroretinoic acids (Allenby et al. 1993), 4-oxo-retinol (Achkar et al. 1996) and also 4-oxo-retinoic acids (Baron et al. 2005), have been found to be potent activators of RAR (Fig. 2).

Another important pathway relevant to the immune system involves retinoids with a retro-structure such as anhydroretinol (4,5-didehydro-15,5-retro-deoxyretinol, AR) and 14-hydroxy-retro-retinol (14-HRR) (Fig. 2). 14-HRR has been shown to be a crucial factor for lymphocyte proliferation and AR is a factor responsible for the induction of apoptotic effects (Buck et al. 1991, 1993; Derguini et al. 1994; O’Connell et al. 1996). How these effects are mediated is still not clear, but one mechanism for 14-HRR is that it activates protein kinase Ca (Imam et al. 2001).

Mechanism of action of carotenoids

The exact mechanism of action of carotenoids has been only partially elucidated, with the focus mainly on β-carotene in the majority of investigations. Two mammalian enzymes have been identified so far, the cyclic cleavage enzyme 15,15′-β-carotene oxygenase 1 (BCO1) (von Lintig & Vogt, 2000; Redmond et al. 2001; von Lintig & Wyss, 2001) and the acyclic cleavage enzyme 2 (BCO2) (Kiefer et al. 2001; Fig. 1(b)). BCO1 divides β-carotene into two units of retinal, which can be either oxidised to retinoic acid or reduced to retinol (Redmond et al. 2001; von Lintig & Wyss, 2001), while BCO2 has been shown to transform β-carotene into apo-8-carotenal (Kiefer et al. 2001; Fig. 1(b)). It is not known whether apo-carotenals occur endogenously in mammals, but it has been shown that apo-8-carotenal can be oxidised to apo-8-carotenolic acid or degraded to other short-chain apo-carotenals via the β-oxidation pathway (Wang et al. 1996; Barua & Olson, 2000; for review, see Wang, 1994). Few investigations of the biological activity of apo-carotenals and apo-carotenolic acids have been undertaken, but it is known that apo-carotenals and apo-carotenolic acids are only weak activators of the retinoid receptors RAR and RXR (Tibaduiza et al. 2002).

In addition to their nuclear receptor-mediated effects carotenoids also exhibit antioxidant activity by quenching...
radicals such as singlet oxygen (for review, see Cantrell & Truscott, 2004), and via these antioxidant effects carotenoids may inhibit radical- or peroxide-mediated biological effects such as fatty acid metabolism by lipoxygenase-mediated pathways (Bar-Natan et al. 1996). Thereby, carotenoids may mediate gene activation via metabolism of the PUFA linoleic acid and arachidonic acid to their hydroxy-metabolites 15-hydroxyeicosatetraenoic acid (15-HETE) and 13-hydroxyoctadecadienoic acid (13-HODE), which are highly potent activators of PPAR (Huang et al. 1999; Fig. 1(c)).

Fig. 2. Structural formulas and interrelationships between the various retinoids.

Regulation of carotenoids and retinoid concentrations in man

Two major pathways have been described for the regulation of the concentration of the active retinoid and RAR activator all-trans-retinoic acid (Fig. 3):

(a) organ-specific targetted temporal and spatial synthesis by retinaldehyde dehydrogenase (RALDH) isoforms (for review, see Duester, 2000). In reproductive tissues such as embryo, uterus, ovaries and testes retinoic acid is synthesised via controlled spatial and temporal expression of RALDH isoforms. In the adult retinoic acid synthesis mainly occurs in continually-differentiating tissues such as skin, hair and the immune and intestinal systems (for review, see Napoli 1999);

(b) a second means of regulation is non-specific regulation via nutrient bioavailability. High intakes of vitamin A, mainly in the form of retinyl esters, lead to increased concentrations of retinyl esters, retinol and retinoic acids (Arnhold et al. 1996; van Vliet et al. 2001). High vitamin A intakes also lead to long-term increases in retinoic acid concentrations (Siegel et al. 2004). In addition, provitamin A carotenoids such as β-carotene, α-carotene and β-cryptoxanthin are mainly stored in organs such as the liver and adipose tissue, and their release into the serum seems to be non-homeostatically regulated. This finding implies that high consumption of provitamin A carotenoids potentially leads to high organ concentrations and high serum concentrations of...
these carotenoids and, further, higher serum concentrations of all-trans-retinoic acid. For example, human subjects supplemented with β-carotene have been shown to have increased concentrations of all-trans-retinoic acid (Thürmann et al. 2002).

Carotenoids and retinoids and the immune system

Retinoic acid has been shown to mediate various processes of the immune system. The main interactions can be divided into three categories: proliferating and differentiating effects; regulation of apoptosis; alteration of regulation of genes relevant to the immune response.

Differentiating and proliferating effects of retinoids on immune competent cells

Retinoids and their differentiating and proliferating effects on lymphocytes. Several studies (Sidell et al. 1981; Dillehay et al. 1987; Garbe et al. 1992; Jiang et al. 1993) have reported that all-trans-retinoic acid stimulates proliferation of T-lymphoid cells such as thymocytes (Sidell et al. 1981; Dillehay et al. 1987) and murine spleenic T-cells (Garbe et al. 1992; Jiang et al. 1993). In particular, the lymphocyte response to mitogens is highly retinoid dependent (Wang & Ballow, 1993; Ballow et al. 1996a,b). Furthermore, the differentiating effects on human peripheral blood T-cells are mediated by physiologically-relevant concentrations of all-trans-retinoic acid, particularly when the cells are co-stimulated with agents such as phorbol 12-myristate 13-acetate and phytohaemagglutinin (Ertesvag et al. 2002). The suggested mechanism for these differentiating effects is that retinoic acid enhances phorbol 12-myristate 13-acetate-induced phosphorylation of the tumour suppressor protein pRB (Ertesvag et al. 2002). Further studies (Malek, 2003) have shown that the proliferative effects of retinoic acid on lymphocytes are mediated via alteration of IL-2 production. IL-2 is produced on stimulation of T-cells, and at the same time as IL-2 receptors are expressed. IL-2 mRNA levels are rapidly enhanced after phorbol 12-myristate 13-acetate or phytohaemagglutinin treatment and co-treatment with all-trans-retinoic acid (Blomhoff, 2004). No retinoic acid-response elements have been found in the IL-2 promoter, although potential candidates for retinoid acid regulation are being investigated (for review, see Blomhoff, 2004).

In contrast to T-cells, the proliferation of B-cells and B-cell precursors is inhibited by physiological levels of all-trans-retinoic acid (Worm et al. 1998; Cariati et al. 2000). The inhibition of the cell-cycle machinery has been found to be the mechanism of this inhibition (Naderi & Blomhoff, 1999). Moreover, all-trans-retinoic acid increases the antibody response in retinoic acid-treated rats and mice (DeCicco et al. 2000, 2001; Ma et al. 2005).

Several studies (Buck et al. 1991; Derguini et al. 1994; O’Connell et al. 1996; Vakiani & Buck, 1999) have revealed that the retro-retinoids 14-HRR and AR play important roles in lymphocyte proliferation, signalling and activation, and these effects are not mediated via RAR/RXR receptor pathways. AR may induce rapid cell death in T-cells, while 14-HRR is required for normal lymphocyte proliferation (O’Connell et al. 1996). The proposed mechanism for 14-HRR is that these retinoids act as ligands and co-activators of protein kinase Cα (Imam et al. 2001).

Retinoids and T-helper cell 1 – T-helper cell 2 balance.

Probably the most important aspect of the role of retinoic acid in relation to the immune system is its effect on the T-helper (Th) 1–Th2 balance (Cantorna et al. 1994; Tokuyama et al. 1995; Tokuyama & Tokuyama, 1996; Hoag et al. 2002; Rühl et al. 2004). Several research groups (Tokuyama et al. 1995; Worm et al. 1998, 2001; Hoag et al. 2002; Stephensen et al. 2002; Iwata et al. 2003) have reported that in in vitro models as well as in in vivo models retinoic acid exerts a direct effect on T-cells, suppressing Th1 development and enhancing Th2 development via an RAR-mediated response. In vitamin A-deficient mice, in particular, there is definite evidence for a Th2 defect (Carman et al. 1989). There are few Th2 cells and the addition of retinyl acetate restores Th2 cell numbers. Thus, vitamin A-deficient mice have an insufficiency of Th2 cells to drive B-cell proliferation and differentiation. Excessive interferon-γ (IFN-γ) synthesis may partly account for this Th2 cell insufficiency, because IFN-γ inhibits Th2 cell development (Abbás et al. 1996), as summarised in Fig. 4. Various aspects of vitamin A deficiency that relate to the immune response are summarised in a review by Hayes et al. (1999).

How all these effects are mediated has not been established, but it has been shown that retinoic acid regulates the expression of IFN-γ, IL-2, IL-10 and IL-12 production (Cantorna et al. 1994; Tokuyama & Tokuyama, 1996; Stephensen et al. 2002, 2004; Iwata et al. 2003; Rühl et al. 2004).

Retinoids and myeloid cell differentiation. Several studies have also shown important effects of retinoic acid during human monocyte differentiation (Kreutz et al. 1998; Fritsche et al. 2000). The initial data relating to myeloid
cell differentiation were obtained from vitamin A-deficient animals, in which a marked increase in the total number of macrophages in secondary lymphoid organs was observed (Smith et al. 1987). However, retinoic acid treatment is associated with a decrease in the number of monocytes found in bone marrow and spleen (Miller & Kearney, 1998). All-trans-retinoic acid has been shown to skew monocyte differentiation into IL-12-secreting dendritic-like cells (Mohty et al. 2003), although retinoic acid inhibits IL-12 production in primary macrophages in vitro (Na et al. 1999; Kang et al. 2000). IL-12 produced by macrophages, acting as antigen-presenting cells, later promotes the development of Th1 cells, which themselves produce IFN-γ. This IFN-γ production can lead to increased macrophage activation (Fig. 4; for review, see Stephensen, 2001). In summary, these data indicate that vitamin A deficiency enhances macrophage-mediated inflammation by increasing production of IL-12 and IFN-γ, but impairs the ability of macrophages to ingest and kill bacteria.

Dendritic cells are also a target of retinoic acid, which regulates the survival and antigen presentation by immature dendritic cells, as well as the maturation of immature dendritic cells to mature dendritic cells (Geissmann et al. 2003). Dendritic cells from the gut-associated lymphoid organs produce retinoic acid from retinol, revealing a role of retinoic acid in the imprinting of gut-homing specificity on T-cells (Iwata et al. 2004).

Retinoids and apoptotic effects

An important function of endogenous retinoids is the induction and inhibition of apoptotic effects (for review, see Szondy et al. 1998). Retinoids induce apoptosis of immune-competent cells during back regulation of immune reactions (see p. 464) and during thymic selection (Foerster et al. 1996; Yagi et al. 1997; Szondy et al. 1998). In various cell lines it has been shown that apoptosis is a major effect induced by retinoids (for review, see Altucci & Gronemeyer, 2001). The apoptotic effects of retinoids are mainly induced via RAR/RXR-mediated effects (Szondy et al. 1998), other nuclear receptors such as PPAR (Theocharis et al. 2004) and Nur77 (Szegedy et al. 2003; Toth et al. 2004), and also via non-nuclear receptor-mediated effects (for review, see Lotan, 2003).

Retinoids and thymic selection. During postnatal development thymic selection of T-cells is an important factor in the development of the immune system (for review, see Boyd et al. 1993). Apoptosis induction via distinct signalling pathways shapes the subsequent T-cell repertoire (for review, see Szondy et al. 1998). Retinoids as well as glucocorticoids are involved in regulating positive selection of T-cells as well as negative selection of T-cells. Two subgroups of the RAR receptor are involved in inducing opposite effects during thymic selection: RARγ induces apoptosis of T-cells; RARα prevents both RARγ-induced proliferation and T-cell receptor-mediated cell death (Szondy et al. 1998). Studies by the author’s group (I Kiss, R Rühl, B Fritzschke, T Nemeth, E Szegedy, T Perllmann and Z Szondy, under review) have shown that retinoic acid synthesis, retinoic acid-response gene up-regulation and thymic cellularity are highest when the T-cell selection process is most active, as reflected by the high rate of apoptosis.

Retinoids and back regulation of immune responses. After an inflammatory response the immune system has to be back regulated to ‘normal’, and the results of several studies emphasise that retinoids also play a key role in this process. These effects have been shown to be mediated via the PPARβ- (also known as PPARδ) RXR receptor heterodimer during wound healing (Tan et al. 2001, 2003; Di-Poi et al. 2002). PPARβ/δ-mediated transcription may be activated via: (a) PPARβ/δ agonists (for review, see Tan et al. 2005); (b) RXR agonists such as 9-cis-retinoic acid (Tan et al. 2005); (c) all-trans-retinoic acid (Shaw et al. 2003).

Carotenoids and immune responses

Several effects of carotenoids are thought to be mediated by their metabolism to vitamin A and subsequent mediation of RAR/RXR-response pathways. Surprisingly, even non-provitamin A carotenoids such as lutein, canthaxanthin and lycopene exhibit marked effects on the immune system (for summary, see Chew & Park, 2004; Hughes, 2004). In general, carotenoids modulate T-cell proliferation, e.g. β-carotene potentiates the increase in CD4+ cells and is suggested to be an immuno-enhancing agent in the management of HIV infections (Fryburg et al. 1995). Various supplementation studies with carotenoids in man (Watzl et al. 1999) have found that enriching the diet with β-carotene (by carrot juice), lycopene (by tomato juice) or lutein (by spinach powder) to some extent mediates T-cell proliferation. In cell-culture experiments (Prabhala et al. 1989; Iyonouchi et al. 1994) the non-provitamin A carotenoids canthaxanthin, astaxanthin and lutein have been shown to enhance T-cell proliferation.

Natural killer cell activity seems to be another important target of carotenoid action; supplementation of subjects with β-carotene enhances their natural killer cell activity as compared with subjects of a similar age given placebo treatment (Santos et al. 1996).

A proposed mechanism for carotene-mediated immunostimulation is related to its ability to suppress the generation of arachidonic acid cascade products in vitro (Haley & Sklan, 1987). It is suggested that the production of prostaglandin E2, an immunosuppressive mediator, is down regulated (Haley & Sklan, 1987). This effect is possibly mediated via cyclooxygenase inhibition comparable with the lipoxygenase inhibition mechanism (Bar-Natan et al. 1996; Fig. 1(c)).

Carotenoids and retinoids and postnatal development

The previous discussion has focused mainly on how carotenoids and retinoids act at the molecular level and the type of processes in the immune response that are affected. The following sections will focus on the supply of nutritionally-relevant retinoids and carotenoids during the period of immune development in man and the role of vitamin A and carotenoids during this period. In particular, the impact of ‘Western’ nutrition on these vitamin A and carotenoregulated processes in immune development will be discussed.
Vitamin A and carotenoid transfer to milk and subsequently to the child

Retinol is the predominant retinoid in human serum and is mainly transported by the retinol-binding protein (RBP), although after high-vitamin A supplementation retinyl esters are incorporated and transported in lipoproteins (Mallia et al. 1975). The transport of vitamin A from the maternal serum to the milk may be mediated via a number of possible transfer mechanisms. Vahlquist & Nilsson (1979) have shown that RBP-mediated transfer is the most important source of milk vitamin A, providing a constant supply of the vitamin, while plasma lipoproteins become important in transport during increased intakes of vitamin A. The relative inefficiency of lipoprotein-mediated transfer may help to protect the offspring from ingestion of toxic levels of milk vitamin A in the case of maternal hypervitaminosis A (Vahlquist & Nilsson, 1979). Later studies (Davila et al. 1997) have shown that increased ingestion of vitamin A is not associated with increased maternal serum vitamin A concentrations. However, the liver vitamin A concentrations of the dams, their milk vitamin A concentrations and the liver vitamin A concentrations of their 14-d-old pups are higher when dams are fed higher-vitamin A (30 retinol equivalents/kg) diets during lactation. These findings indicate that the transfer of vitamin A in milk from mother to offspring and the vitamin A status of the dams and their suckling pups are influenced by maternal vitamin A intake during lactation (Fig. 5). More recent studies (Green et al. 2001a) have established that chylo- microns contribute at least one-third of the vitamin A in milk in rats fed at the higher level of vitamin A, while chylothorax from rats fed at the lower level of vitamin A contain negligible amounts of vitamin A. In the animals fed the lower level of vitamin A holoRBP is able to deliver vitamin A to the lactating mammary tissue, since vitamin A is present even if rats are fed a vitamin A-free diet (Green et al. 2001b). Experiments with RBP-/- mice (Vogel et al. 2002) have shown that there is some variability in milk vitamin A levels with time, although there are no overall differences through the weaning period. The importance of postprandial vitamin A for retinyl ester incorporation into the mammary tissue and subsequently into the milk also involves the cellular RBP (CRBP) 3. In CRBP3-/- mice less vitamin A, particularly in the form of retinyl esters, is incorporated into the milk (Plantedosi et al. 2005).

β-Carotene transport has not been investigated as extensively as the vitamin A transport mechanisms. A study in healthy lactating women (Schweigert et al. 2004) has suggested that lipoprotein transport of carotenoids from serum to the milk is responsible for carotenoid transfer. β-Carotene levels in the maternal plasma and breast milk are increased after single-dose β-carotene supplementation, but retinol concentrations are not affected (Canfield et al. 1997). Long-term supplementation with 30 mg β-carotene/d is associated with a small but non-significant increase in human breast milk levels (Gossage et al. 2002), while a study (Canfield et al. 1998) that provided 60 and 210 mg β-carotene/d has reported increases in both serum and milk retinol and β-carotene. Maternal β-carotene supplementation seems to be an important factor in the supply of vitamin A to the infant; in addition to increasing the levels of vitamin A in breast milk, maternal β-carotene supplementation also increases β-carotene in breast milk and can thereby supply retinol for the nursing infant (Canfield et al. 1999). However, it has not been established where the bioconversion of the milk-derived β-carotene to retinol takes place (for summary, see Fig. 5).

Whether this β-carotene or vitamin A can be converted to bioactive all-trans-retinoic acid in young mammals is not known, although a recent study (Rühl et al. 2005) has shown that all-trans-retinoic acid is not present in the serum of rat pups aged 3 and 11 d, while other bioactive vitamin A metabolites are present at high concentrations.

Influence of Western nutrition on carotenoids and retinoid transfer in human subjects

Fig. 6 summarises how ‘Western’ nutrition, which is high in dietary fat, vitamin A and β-carotene, mediates
metabolism to the active vitamin A metabolite all-trans-retinoic acid. Not only is the dietary intake of vitamin A (Olafsdottir et al. 2001; Allen & Haskell, 2002; Mensink, 2002) and β-carotene (Hellenbrand et al. 2000) high, but also the high levels of dietary fat may increase various factors responsible for improved absorption and subsequent bioactivation of β-carotene and vitamin A to all-trans-retinoic acid.

A high-fat diet up regulates the expression of RAR (Bonilla et al. 2000), CRPB 1 and 2, which are responsible for retinol uptake (During et al. 1998; Takase et al. 1998, 2000; Hellemans et al. 2003), and BCO1 (During et al. 1998; Boulanger et al. 2003). In addition, high intakes of fat and β-carotene result in increased β-carotene levels in several organs and increased levels of vitamin A in the serum and various organs (Schweigert et al. 2000; van het Hof et al. 2000; Ribaya-Mercado, 2002). In contrast, a diet low in energy reduces serum concentrations of retinoic acid and retinol (Berggren Soderlund et al. 2003).

**Effects of carotenoids and retinoids on immune development**

Immune development after birth in man involves three major processes in which retinoids are involved: the differentiation of immune-competent cells; thymic selection; the proliferation and expansion of lymphocytes (summarised in Fig. 7).

During thymic selection T-cells develop in the thymus through a series of stages defined by the expression of the cell-surface markers CD4 and CD8. The development of the human thymus starts before birth and ceases during puberty with involution of the thymus (Sen, 2001). Vitamin A deficiency is known to be accompanied by immune deficiency and a susceptibility to a wide range of infectious diseases (for review, see Reifen, 2002; Semba, 1994). In vitamin A-deficient animals a marked atrophy of lymphoid organs, in particular the thymus (Makori et al. 2002). A recent study (I Kiss, R Rühl, B Fritzschke, T Nemeth, E Szegedi, T Perlmann and Z Szondy, under review) has shown that retinoic acid-synthesising enzymes peak at the same time as RAR response, when thymic cellularity is highest and the T-cell selection process, as indicated by a high rate of apoptosis, is most effective. It can be concluded that thymic selection is a direct target of retinoic acid during thymocyte development, and it can be postulated that high maternal dietary intake of vitamin A or provitamin A carotenoids may modify thymic selection processes. Until now there has been no research on the effects of carotenoids on thymic selection processes.

The second important process in which retinoids are involved is the proliferation of lymphocyte populations; the lymphocyte response to mitogens, in particular, is retinoid dependent (Wang & Ballow, 1993; Ballow et al. 1996a,b). In a recent study (Garcia et al. 2003) of pregnant mice fed a basal (control) diet or different retinoid- and carotenoid-enriched (4500 retinol equivalents/kg) diets from day 1 of conception the percentage and total numbers of splenic mononuclear cells were determined on days 1, 3, 5, 7, 14, 21 and 65 of pregnancy. Increases were observed in the early days of pregnancy (3 and 5) with vitamin A (retinyl palmitate) supplementation, while β-carotene supplementation was found to mainly increase CD3+ cell numbers from day 5 to day 14. At day 7 increases were found in CD4:CD8 after vitamin A supplementation and in T-cell:B-cell after vitamin A and β-carotene supplementation. In general, IgG levels were not found to be altered by the different diets. These results confirm that supplementation with vitamin A and β-carotene affects immune cell functions during ontogenesis. However, maternal vitamin A supplementation via intraperitoneal injections has been shown to increase serum IgM and Th2-specific IgG1 levels in the progeny (Guzman & Caren, 1991). Furthermore, in a human supplementation study (Gossage et al. 2000), which investigated the effects of β-carotene supplementation during early lactation (days 4–32 post partum) on circulating carotenoids and the T-cell proliferative response to phytohaemagglutinin, it has been found that neither lactation nor β-carotene supplementation affects T-cell proliferation.

Recently, a study (R Rühl, A Hänel, A Garcia, U Herz, FJ Schweigert and M Worm, under review) has been performed in mice in which vitamin A-supplemented (30 mg/d) or vitamin A-free diets were fed to the dams throughout lactation and directly to the pups after weaning with or without ovalbumin sensitisation. It was found that vitamin A supplementation decreases splenic T-cell and B-cell numbers and also enhances IL-4 production and specific IgE after sensitisation. By contrast, the mice fed the vitamin A-free diet were found to show no alteration in lymphocyte cell numbers, a slightly increased IL-4 production and no decrease in specific IgE levels. Together these findings show that the severity of allergic sensitisation depends on the vitamin A content of the maternal diet during lactation. In addition, vitamin A strongly enhances immune responses only after mitogen stimulation, while in the absence of immune stimulation vitamin A only has marginal effects.

The Th1—Th2 switch is another important process that is affected by vitamin A during postnatal development. Initial evidence is available from a study (Guzman & Caren, 1991) that describes increased Th2-specific IgG1 concentrations after vitamin A supplementation and from a recent study (R Rühl, A Hänel, A Garcia, U Herz, FJ Schweigert and M Worm, under review) showing enhanced IL-4 secretion and increased specific IgE

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**Fig. 7.** Influence of the human diet, via reduction in, and induction of, cytokine release, on some of the factors involved in postnatal immune development. IFN, interferon; Th, T-helper.
levels after a vitamin A-supplemented diet and ovalbumin sensitisation. Whether these outcomes are mediated by lymphocyte-mediated effects, or via antigen-presenting cell-mediated effects, is as yet unknown. Previous studies have shown that, as antigen-presenting cells, dendritic cells are the early regulators of the Th1–Th2 response (Ridge et al. 1996).

The effects of carotenoids are quite difficult to investigate because of the pronounced differences in carotenoid absorption, kinetics and metabolism between man and rodent laboratory animals (for review, see Lee et al. 1999). A collaborative study (Rühl et al. 2006) has shown that carotenoids with provitamin A activity or non-provitamin A activity are present at different levels in children of different ethnicity and with a different risk of allergic sensitisation. Provitamin A carotenoids are low in children of Turkish origin who live in Germany and have low cultural adaptation to the German lifestyle, but they are higher in well-adapted Turkish children and are highest in German children. On the other hand, the levels of non-provitamin A carotenoids are high in Turkish children and low in German children. This study suggests that in man different levels of carotenoids may be associated with an increased prevalence of allergic diseases; whether these differences in carotenoid distribution between different groups is associated with the bioactive vitamin A metabolite all-trans-retinoic acid is under investigation.

**Human breast milk v. milk formulas**

In addition to a different pattern of proteins, milk formulas also have a different profile of carotenoids and retinoids. Breast milk mainly contains retinyl esters conjugated with different fatty acids such as palmitic, stearic, oleic and linoleic acids (Ross et al. 1985; Piantedosi et al. 2005), as well as retinol (Olafsdottir et al. 2001), while milk formulas contain only retinyl palmitate or retinyl acetate as the vitamin A source (Landen et al. 1985). However, the plasma vitamin A concentrations for the formula-fed baby and the breast-fed baby are comparable (Ghebremeskel et al. 1999). Breast milk contains the whole spectrum of carotenoids present in the human diet and serum (Khachik et al. 1997; Canfield et al. 2003; Schweigert et al. 2004), while milk formulas contain no carotenoids, very low concentrations of carotenoids or a limited variety of carotenoids (Sommerburg et al. 2000).

The importance of these different retinoid and carotenoid patterns in relation to the development of the immune system in Western countries is hard to predict, particularly as many aspects of the function of carotenoids remain elusive.

**Perspectives**

Various factors in the developing immune system may be altered by carotenoids and retinoids. The present review focuses on the interrelationship between physiological mechanisms and retinoids, and how dietary carotenoids and retinoids can modify immune responses.

The main focus is the dietary intake in Western societies, which tends to be high in vitamin A, provitamin A carotenoids, fat and cholesterol. The effects of these different factors have been investigated in both in vitro and in vivo studies. It has proved difficult to correlate and predict the effects in the human situation for the following major reasons:

(a) non-nutritionally-relevant concentrations used in various in vitro and in vivo studies are difficult to compare with the human situation;

(b) in human diets factors such as high fat, vitamin A, provitamin A carotenoids and cholesterol mainly occur together and their additive effects are difficult to predict and to correlate with the human situation;

(c) the use of milk formulas instead of breast milk may influence the development of the immune system.

In general, retinoids are involved in various pathways important in the ontogenesis of the immune system. However, until now there has been no direct evidence that carotenoids are nutritionally-relevant key factors in this process. A diet high in carotenoids and retinoids seems to have only a marginal influence on the development of the immune system, but under mitogen stimulation retinoids can strongly trigger and shift immune responses. A series of in vivo studies in rodents and supplementation studies in human subjects are needed to further evaluate the immune-modulating potential of carotenoids and retinoids, particularly during postnatal immune development.

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**References**


