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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*. 1992*a*,*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*.

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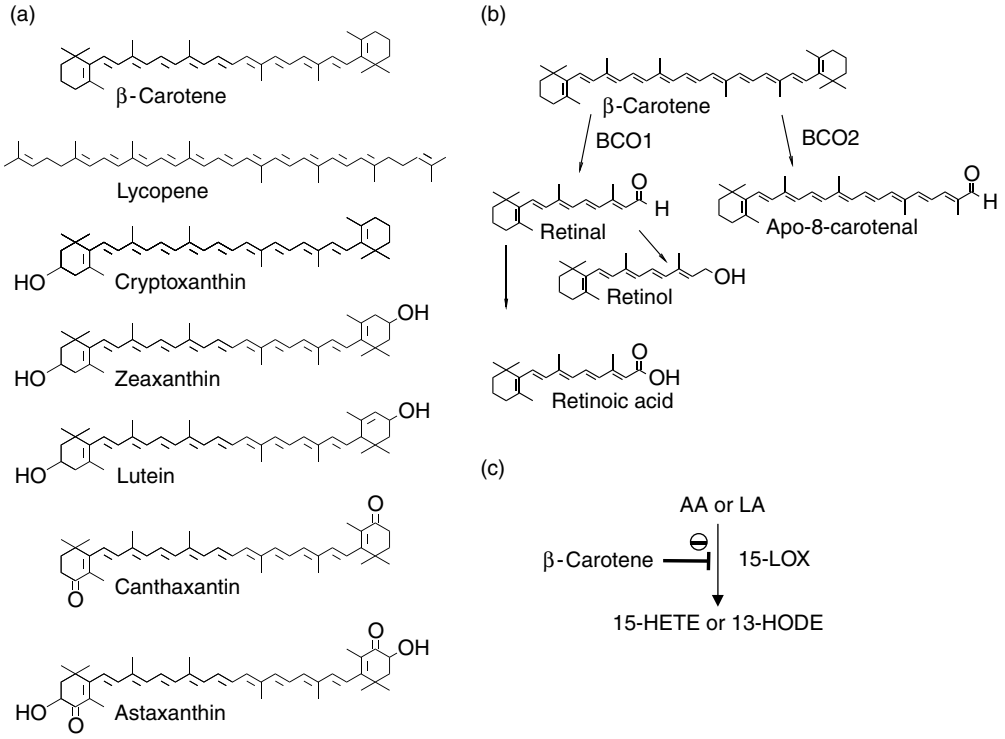

Fig. 1. (a) The structural formulas of various nutritionally-relevant carotenoids. (b) Metabolic activation and degradation pathways via β-carotene oxygenases (BCO) of β-carotene. (c) 15-Lipoxygenase (15-LOX) inhibitory pathways of β-carotene in the conversion of the fatty acids arachidonic acid (AA) and linoleic acid (LA) to 15-hydroxyeicosatetraenoic acid (15-HETE) and 13-hydroxyoctadecadienoic acid (13-HODE).

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 Cα (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-carotenoic 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-carotenoic acids have been undertaken, but it is known that apo-carotenals and apo-carotenoic 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

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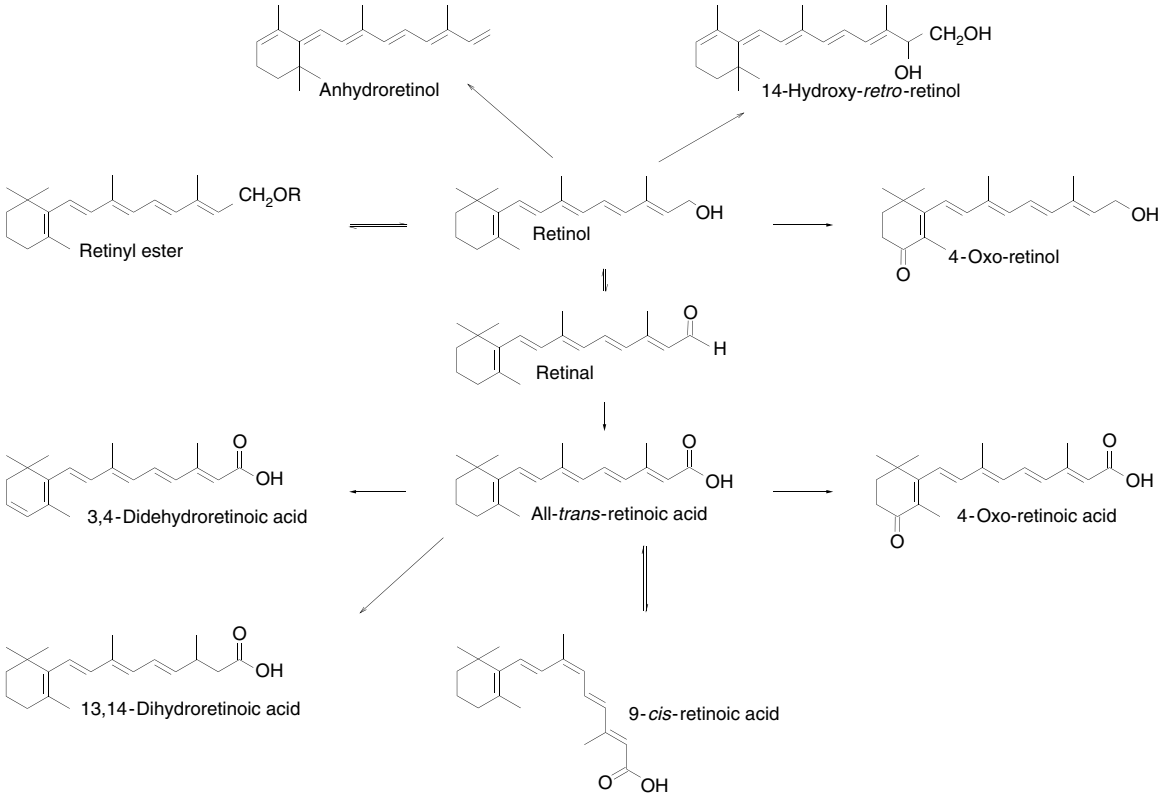

Fig. 2. Structural formulas and interrelationships between the various retinoids.

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)).

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

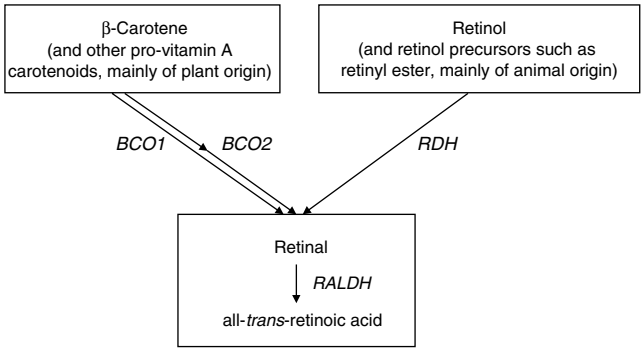

Fig. 3. Biologically-relevant pathways originating from provitamin A carotenoids and vitamin A for the generation of all-*trans*-retinoic acid. BCO, β-carotene oxygenase; RDH, retinol dehydrogenase; RALDH, retinaldehyde dehydrogenase.

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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*. 1996*a*,*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 retinoic 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).

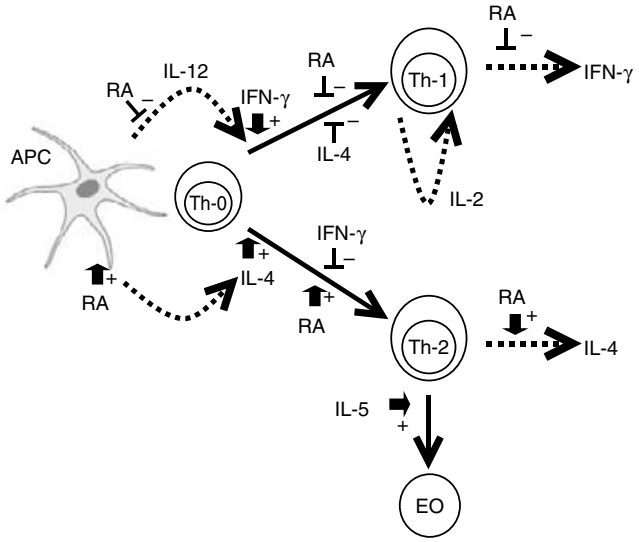

Fig. 4. Retinoic acid (RA)-modified pathways for T-helper (Th) 1 and Th2 regulation. APC, antigen-presenting cell; IFN, interferon; EO, eosinophil; +, ⬆, promoted; –, ⊥, inhibited; ⇢, effects of cytokines.

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 (Abbas *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

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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 (Szegezdi *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 Fritzsche, T Nemeth, E Szegezdi, T Perlmann 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, canthaxantin 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; Jyonouchi *et al*. 1994) the non-provitamin A carotenoids canthaxantin, 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* (Halevy & Sklan, 1987). It is suggested that the production of prostaglandin E_2, an immunosuppressive mediator, is down regulated (Halevy & 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 carotene-regulated processes in immune development will be discussed.

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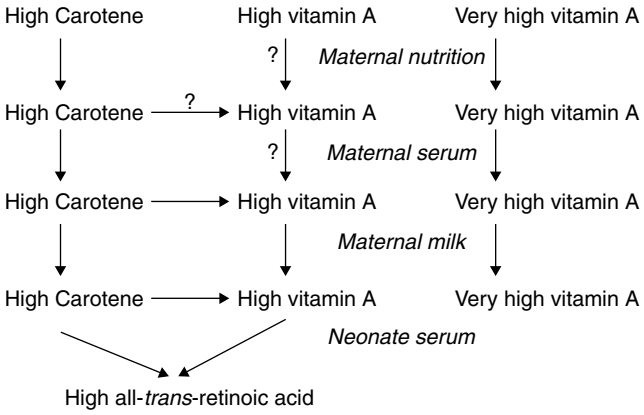

Fig. 5. Simplified and schematic effects of human relevant mechanisms in the transfer of β-carotene and/or vitamin A to breast milk and subsequently to the child.

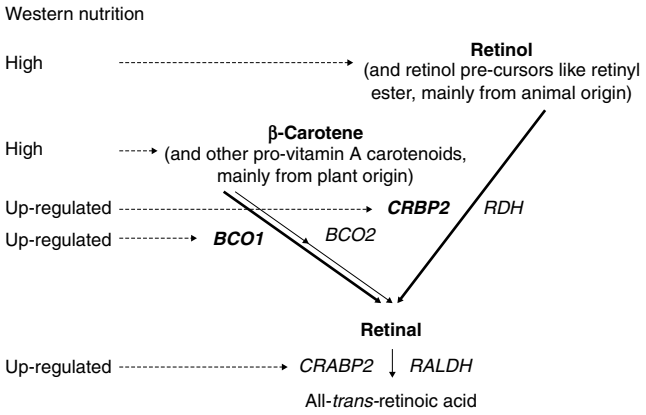

Fig. 6. Influence of Western nutrition on some of the factors involved in carotenoid and retinoid metabolism to all-*trans*-retinoic acid. RAR, retinoic acid receptor; BCO, β-carotene oxygenase; RALDH, retinaldehyde dehydrogenase; CRBP, cellular retinol-binding protein; RDH, retinol dehydrogenase.

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). Vitamin A appears in milk mainly as retinyl esters (Vahlquist & Nilsson, 1979). Later studies (Davila *et al*. 1985) in rats 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*. 2001*a*) have established that chylomicrons contribute at least one-third of the vitamin A in milk in rats fed at the higher level of vitamin A, while chylomicrons 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*. 2001*b*). 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 (Piantedosi *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

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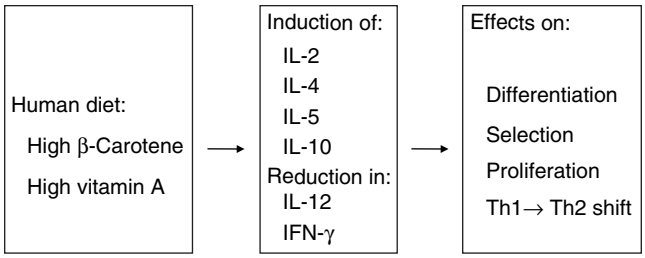

Fig. 7. Influence of the human diet, via reduction in, and induction of, cytokine release, on some of the factors involved in postnatal immune development. IFN, interferon; Th, T-helper.

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 the thymus and spleen has been observed (West *et al*. 1989); on the other hand, retinoids at higher concentrations are toxic and cause involution of lymphoid organs, in particular the thymus (Makori *et al*. 2002). A recent study (I Kiss, R Rühl, B Fritzsche, T Nemeth, E Szegezdi, 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*. 1996*a*,*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

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

Abbas AK, Murphy KM & Sher A (1996) Functional diversity of helper T lymphocytes. *Nature* **383**, 787–793.

Achkar CC, Derguini F, Blumberg B, Langston A, Levin AA, Speck J *et al.* (1996) 4-Oxoretinol, a new natural ligand and transactivator of the retinoic acid receptors. *Proceedings of the National Academy of Sciences USA* **93**, 4879–4884.

Al-Delaimy WK, van Kappel AL, Ferrari P, Slimani N, Steghens JP, Bingham S *et al.* (2004) Plasma levels of six carotenoids in nine European countries: report from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutrition* **7**, 713–722.

Allen LH & Haskell M (2002) Estimating the potential for vitamin A toxicity in women and young children. *Journal of Nutrition* **132**, 2907S–2919S.

Allenby G, Bocquel MT, Saunders M, Kazmer S, Speck J, Rosenberger M *et al.* (1993) Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. *Proceedings of the National Academy of Sciences USA* **90**, 30–34.

Altucci L & Gronemeyer H (2001) Nuclear receptors in cell life and death. *Trends in Endocrinology and Metabolism* **12**, 460–468.

Arnhold T, Tzimas G, Wittfoht W, Plonait S & Nau H (1996) Identification of 9-cis-retinoic acid, 9,13-di-cis-retinoic acid,

and 14-hydroxy-4,14-retro-retinol in human plasma after liver consumption. *Life Sciences* **59**, PL169–PL177.
Ballow M, Wang W & Xiang S (1996*a*) Modulation of B-cell immunoglobulin synthesis by retinoic acid. *Clinical Immunology and Immunopathology* **80**, S73–S81.
Ballow M, Xiang S, Wang W & Brodsky L (1996*b*) The effects of retinoic acid on immunoglobulin synthesis: role of interleukin 6. *Journal of Clinical Immunology* **16**, 171–179.
Bar-Natan R, Lomnitski L, Sofer Y, Segman S, Neeman I & Grossman S (1996) Interaction between beta-carotene and lipoxygenase in human skin. *International Journal of Biochemistry and Cell Biology* **28**, 935–941.
Baron JM, Heise R, Blaner WS, Neis M, Joussen S, Dreuw A, Marquardt Y, Saurat JH, Merk HF, Bickers DR & Jugert FK (2005) Retinoic acid and its 4-oxo metabolites are functionally active in human skin cells in vitro. *Journal of Investigative Dermatology* **125**, 143–153.
Barua AB & Olson JA (2000) Beta-carotene is converted primarily to retinoids in rats in vivo. *Journal of Nutrition* **130**, 1996–2001.
Berggren Soderlund M, Fex G & Nilsson-Ehle P (2003) Decreasing serum concentrations of all-trans, 13-cis retinoic acids and retinol during fasting and caloric restriction. *Journal of Internal Medicine* **253**, 375–380.
Blomhoff HK (2004) Vitamin A regulates proliferation and apoptosis of human T- and B-cells. *Biochemistry Society Transactions* **32**, 982–984.
Bonilla S, Redonnet A, Noel-Suberville C, Pallet V, Garcin H & Higueret P (2000) High-fat diets affect the expression of nuclear retinoic acid receptor in rat liver. *British Journal of Nutrition* **83**, 665–671.
Boulanger A, McLemore P, Copeland NG, Gilbert DJ, Jenkins NA, Yu SS, Gentleman S & Redmond TM (2003) Identification of beta-carotene 15, 15′-monooxygenase as a peroxisome proliferator-activated receptor target gene. *FASEB Journal* **17**, 1304–1306.
Boyd RL, Tucek CL, Godfrey DI, Izon DJ, Wilson TJ, Davidson NJ, Bean AG, Ladyman HM, Ritter MA & Hugo P (1993) The thymic microenvironment. *Immunology Today* **14**, 445–459.
Buck J, Derguini F, Levi E, Nakanishi K & Hämmerling U (1991) Intracellular signaling by 14-hydroxy-4,14-retro-retinol. *Science* **254**, 1654–1656.
Buck J, Grun F, Derguini F, Chen Y, Kimura S, Noy N & Hammerling U (1993) Anhydroretinol: a naturally occurring inhibitor of lymphocyte physiology. *Journal of Experimental Medicine* **178**, 675–680.
Canfield LM, Clandinin MT, Davies DP, Fernandez MC, Jackson J, Hawkes J *et al.* (2003) Multinational study of major breast milk carotenoids of healthy mothers. *European Journal of Nutrition* **42**, 133–141.
Canfield LM, Giuliano AR, Neilson EM, Blashil BM, Graver EJ & Yap HH (1998) Kinetics of the response of milk and serum beta-carotene to daily beta-carotene supplementation in healthy, lactating women. *American Journal of Clinical Nutrition* **67**, 276–283.
Canfield LM, Giuliano AR, Neilson EM, Yap HH, Graver EJ, Cui HA & Blashill BM (1997) β-Carotene in breast milk and serum is increased after a single β-carotene dose. *American Journal of Clinical Nutrition* **66**, 52–61.
Canfield LM, Taren DL, Kaminsky RG & Mahal Z (1999) Short-term beta-carotene supplementation of lactating mothers consuming diets low in vitamin A. *Journal of Nutritional Biochemistry* **10**, 532–538.
Cantorna MT, Nashold FE & Hayes CE (1994) In vitamin A deficiency multiple mechanisms establish a regulatory T helper cell imbalance with excess Th1 and insufficient Th2 function. *Journal of Immunology* **152**, 1515–1522.
Cantrell A & Truscott TG (2004) Carotenoids and radicals; interaction with other nutrients. In *Carotenoids in Health and Disease*, pp. 31–52 [NI Krinsky, ST Mayne and H Sies, editors]. New York: Marcel Dekker.
Cariati R, Zancai P, Quaia M, Cutrona G, Giannini F, Rizzo S, Boiocchi M & Dolcetti R (2000) Retinoic acid induces persistent, RARalpha-mediated anti-proliferative responses in Epstein-Barr virus-immortalized b lymphoblasts carrying an activated C-MYC oncogene but not in Burkitt's lymphoma cell lines. *International Journal of Cancer* **86**, 375–384.
Carman JA, Smith SM & Hayes CE (1989) Characterization of a helper T lymphocyte defect in vitamin A-deficient mice. *Journal of Immunology* **142**, 388–393.
Chew BP & Park JS (2004) Carotenoid action on the immune response. *Journal of Nutrition* **134**, 257S–261S.
Davila ME, Norris L, Cleary MP & Ross AC (1985) Vitamin A during lactation: relationship of maternal diet to milk vitamin A content and to the vitamin A status of lactating rats and their pups. *Journal of Nutrition* **115**, 1033–1041.
De Luca LM (1991) Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. *FASEB Journal* **5**, 2924–2933.
DeCicco KL, Youngdahl JD & Ross AC (2001) All-trans-retinoic acid and polyriboinosinic: polyribocytidylic acid in combination potentiate specific antibody production and cell-mediated immunity. *Immunology* **104**, 341–348.
DeCicco KL, Zolfaghari R, Li N & Ross AC (2000) Retinoic acid and polyriboinosinic acid act synergistically to enhance the antibody response to tetanus toxoid during vitamin A deficiency: possible involvement of interleukin-2 receptor-beta, signal transducer and activator of transcription-1, and interferon regulatory factor-1. *Journal of Infectious Diseases* **182**, Suppl. 1, S29–S36.
Derguini F, Nakanishi K, Hämmerling U & Buck J (1994) Intracellular signaling activity of synthetic (14R)-, (14S)-, and (14RS)-14-hydroxy-4,14-retro-retinol. *Biochemistry* **33**, 623–628.
Dillehay DL, Li W, Kalin J, Walia AS & Lamon EW (1987) In vitro effects of retinoids on murine thymus-dependent and thymus-independent mitogenesis. *Cellular Immunology* **107**, 130–137.
Di-Poi N, Tan NS, Michalik L, Wahli W & Desvergne B (2002) Antiapoptotic role of PPARbeta in keratinocytes via transcriptional control of the Akt1 signaling pathway. *Molecular Cell* **10**, 721–733.
Duester G (2000) Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. *European Journal of Biochemistry* **267**, 4315–4324.
During A, Nagao A & Terao J (1998) Beta-carotene 15,15′-dioxygenase activity and cellular retinol-binding protein type II level are enhanced by dietary unsaturated triacylglycerols in rat intestines. *Journal of Nutrition* **128**, 1614–1619.
Ertesvag A, Engedal N, Naderi S & Blomhoff HK (2002) Retinoic acid stimulates the cell cycle machinery in normal T cells: involvement of retinoic acid receptor-mediated IL-2 secretion. *Journal of Immunology* **169**, 5555–5563.
Foerster M, Sass JO, Rühl R & Nau H (1996) Comparative studies on effects of all-trans-retinoic acid and all-trans-retinol-β-D-glucuronide on the development of foetal mouse thymus in an organ culture system. *Toxicology In Vitro* **10**, 7–15.
Ford ES (2000) Variations in serum carotenoid concentrations among United States adults by ethnicity and sex. *Ethnicity and Disease* **10**, 208–217.
Fritsche J, Stonehouse TJ, Katz DR, Andreesen R & Kreutz M (2000) Expression of retinoid receptors during human monocyte differentiation in vitro. *Biochemical and Biophysical Research Communications* **270**, 17–22.

Fryburg DA, Mark RJ, Griffith BP, Askenase PW & Patterson TF (1995) The effect of supplemental beta-carotene on immunologic indices in patients with AIDS: a pilot study. *Yale Journal of Biology and Medicine* **68**, 19–23.

Garbe A, Buck J & Hämmerling U (1992) Retinoids are important cofactors in T cell activation. *Journal of Experimental Medicine* **176**, 109–117.

Garcia AL, Rühl R, Herz U, Koebnick C, Schweigert FJ & Worm M (2003) Retinoid- and carotenoid-enriched diets influence the ontogenesis of the immune system in mice. *Immunology* **110**, 180–187.

Geissmann F, Revy P, Brousse N, Lepelletier Y, Folli C, Durandy A, Chambon P & Dy M (2003) Retinoids regulate survival and antigen presentation by immature dendritic cells. *Journal of Experimental Medicine* **198**, 623–634.

Ghebremeskel K, Burns L, Costeloe K, Burden TJ, Harbige L, Thomas B & Temple E (1999) Plasma vitamin A and E in preterm babies fed on breast milk or formula milk with or without long-chain polyunsaturated fatty acids. *International Journal for Vitamin and Nutrition Research* **69**, 83–91.

Gossage C, Deyhim M, Moser-Veillon PB, Douglas LW & Kramer TR (2000) Effect of beta-carotene supplementation and lactation on carotenoid metabolism and mitogenic T lymphocyte proliferation. *American Journal of Clinical Nutrition* **71**, 950–955.

Gossage CP, Deyhim M, Yamini S, Douglass LW & Moser-Veillon PB (2002) Carotenoid composition of human milk during the first month postpartum and the response to beta-carotene supplementation. *American Journal of Clinical Nutrition* **76**, 193–197.

Green MH, Green JB, Akohoue SA & Kelley SK (2001*a*) Vitamin A intake affects the contribution of chylomicrons vs. retinol-binding protein to milk vitamin A in lactating rats. *Journal of Nutrition* **131**, 1279–1282.

Green MH, Snyder RW, Akohoue SA & Green JB (2001*b*) Increased rat mammary tissue vitamin A associated with increased vitamin A intake during lactation is maintained after lactation. *Journal of Nutrition* **131**, 1544–1547.

Guzman JJ & Caren LD (1991) Effects of prenatal and postnatal exposure to vitamin A on the development of the murine immune system. *Life Sciences* **49**, 1455–1462.

Halevy O & Sklan D (1987) Inhibition of arachidonic acid oxidation by beta-carotene, retinol and alpha-tocopherol. *Biochimica et Biophysica Acta* **918**, 304–307.

Hayes CE, Nashold FE, Enrique Gomez F & Hoag KA (1999) Retinoids and immunity. In *Retinoids: The Biochemical and Molecular Basis of Vitamin A and Retinoid Action*, pp. 599–610 [H Nau and WS Blaner, editors]. Berlin: Springer.

Heinonen M (1991) Food groups as the source of retinoids, carotenoids, and vitamin A in Finland. *International Journal for Vitamin and Nutrition Research* **61**, 3–9.

Hellemans K, Rombouts K, Quartier E, Dittie AS, Knorr A, Michalik L, Rogiers V, Schuit F, Wahli W & Geerts A (2003) PPARbeta regulates vitamin A metabolism-related gene expression in hepatic stellate cells undergoing activation. *Journal of Lipid Research* **44**, 280–295.

Hellenbrand WB, Bauer G, Boeing H, Seidler A & Robra BP (2000) Diet in residents of East and West Germany in 1991–1992 as ascertained by a retrospective food frequency questionnaire. *Sozial- und Präventivmedizin* **45**, 13–24.

Hoag KA, Nashold FE, Goverman J & Hayes CE (2002) Retinoic acid enhances the T helper 2 cell development that is essential for robust antibody responses through its action on antigen-presenting cells. *Journal of Nutrition* **132**, 3736–3739.

Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, Witztum JL, Funk CD, Conrad D & Glass CK (1999) Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature* **400**, 378–382.

Hughes DA (2004) Carotenoids and immune responses. In *Carotenoids in Health and Disease*, pp. 503–517 [NI Krinsky, ST Mayne and H Sies, editors]. New York: Marcel Dekker.

Imam A, Hoyos B, Swenson C, Levi E, Chua R, Viriya E & Hämmerling U (2001) Retinoids as ligands and coactivators of protein kinase C alpha. *FASEB Journal* **15**, 28–30.

Ito Y, Shimizu H, Yoshimura T, Ross RK, Kabuto M, Takatsuka N, Tokui N, Suzuki K & Shinohara R (1999) Serum concentrations of carotenoids, alpha-tocopherol, fatty acids, and lipid peroxides among Japanese in Japan, and Japanese and Caucasians in the US. *International Journal for Vitamin and Nutrition Research* **69**, 385–395.

Iwata M, Eshima Y & Kagechika H (2003) Retinoic acids exert direct effects on T cells to suppress Th1 development and enhance Th2 development via retinoic acid receptors. *International Immunology* **15**, 1017–1025.

Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C & Song SY (2004) Retinoic acid imprints gut-homing specificity on T cells. *Immunity* **21**, 527–538.

Jiang XL, Everson MP & Lamon EW (1993) A mechanism of retinoid potentiation of murine T-cell responses: early upregulation of interleukin-2 receptors. *International Journal of Immunopharmacology* **15**, 309–317.

Jyonouchi H, Zhang L, Gross M & Tomita Y (1994) Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. *Nutrition and Cancer* **21**, 47–58.

Kang BY, Chung SW, Kim SH, Kang SN, Choe YK & Kim TS (2000) Retinoid-mediated inhibition of interleukin-12 production in mouse macrophages suppresses Th1 cytokine profile in CD4(+) T cells. *British Journal of Pharmacology* **130**, 581–586.

Khachik F, Beecher GR, Goli MB & Lusby WR (1992*a*) Separation and quantitation of carotenoids in foods. *Methods in Enzymology* **213**, 347–359.

Khachik F, Beecher GR, Goli MB, Lusby WR & Daitch CE (1992*b*) Separation and quantification of carotenoids in human plasma. *Methods in Enzymology* **213**, 205–219.

Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao DY & Katz NB (2002) Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Experimental Biology and Medicine* **227**, 845–851.

Khachik F, Spangler CJ, Smith JC Jr, Canfield LM, Steck A & Pfander H (1997) Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Analytical Chemistry* **69**, 1873–1881.

Kiefer C, Hessel S, Lampert JM, Vogt K, Lederer MO, Breithaupt DE & von Lintig J (2001) Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A. *Journal of Biological Chemistry* **276**, 14110–14116.

Kreutz M, Fritsche J, Andreesen R & Krause SW (1998) Regulation of cellular retinoic acid binding protein (CRABP II) during human monocyte differentiation in vitro. *Biochemical and Biophysical Research Communications* **248**, 830–834.

Landen WO Jr, Hines DM, Hamill TW, Martin JI, Young ER, Eitenmiller RR & Soliman AG (1985) Vitamin A and vitamin E content of infant formulas produced in the United States. *Journal of the Association of Official Analytical Chemists* **68**, 509–511.

Lee CM, Boileau AC, Boileau TW, Williams AW, Swanson KS, Heintz KA & Erdman JW (1999) Review of animal models in carotenoid research. *Journal of Nutrition* **129**, 2271–2277.

Lotan R (2003) Receptor-independent induction of apoptosis by synthetic retinoids. *Journal of Biological Regulators and Homeostatic Agents* **17**, 13–28.

Ma Y, Chen Q & Ross AC (2005) Retinoic acid and polyriboinosinic:polyribocytidylic acid stimulate robust anti-tetanus antibody production while differentially regulating type 1/type 2 cytokines and lymphocyte populations. *Journal of Immunology* **174**, 7961–7969.

Makori N, Peterson PE, Lantz K & Hendrickx AG (2002) Exposure of cynomolgus monkey embryos to retinoic acid causes thymic defects: effects on peripheral lymphoid organ development. *Journal of Medical Primatology* **31**, 91–97.

Malek TR (2003) The main function of IL-2 is to promote the development of T regulatory cells. *Journal of Leukocyte Biology* **74**, 961–965.

Mallia AK, Smith JE & Goodman DW (1975) Metabolism of retinol-binding protein and vitamin A during hypervitaminosis A in the rat. *Journal of Lipid Research* **16**, 180–188.

Mensink G (2002) *Beiträge zur Gesundheitsberichtserstattung des Bundes; Was essen wir heute? Ernährungsverhalten in Deutschland (Contributions to the Health Report Refunding of the Federation What Do We Eat Today? Nutritional Behaviour in Germany)*. ISBN 3-89606-132-1. Berlin: Mercedes-Druck.

Miller SC & Kearney SL (1998) Effect of in vivo administration of all trans-retinoic acid on the hemopoietic cell populations of the spleen and bone marrow: profound strain differences between A/J and C57BL/6J mice. *Laboratory Animal Science* **48**, 74–80.

Mohty M, Morbelli S, Isnardon D, Sainty D, Arnoulet C, Gaugler B & Olive D (2003) All-trans retinoic acid skews monocyte differentiation into interleukin-12-secreting dendritic-like cells. *British Journal of Haematology* **122**, 829–836.

Moise AR, Kuksa V, Blaner WS, Baehr W & Palczewski K (2005) Metabolism and transactivation activity of 13,14-dihydroretinoic acid. *Journal of Biological Chemistry* **280**, 27815–27825.

Moise AR, Kuksa V, Imanishi Y & Palczewski K (2004) Identification of all-trans-retinol : all-trans-13,14-dihydroretinol saturase. *Journal of Biological Chemistry* **279**, 50230–50242.

Na SY, Kang BY, Chung SW, Han SJ, Ma X, Trinchieri G, Im SY, Lee JW & Kim TS (1999) Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid X receptor and NFkappaB. *Journal of Biological Chemistry* **274**, 7674–7680.

Naderi S & Blomhoff HK (1999) Retinoic acid prevents phosphorylation of pRB in normal human B lymphocytes: regulation of cyclin E, cyclin A, and p21(Cip1). *Blood* **94**, 1348–1358.

Napoli JL (1999) Interactions of retinoid binding proteins and enzymes in retinoid metabolism. *Biochimica et Biophysica Acta* **1440**, 139–162.

Neuhouser ML, Rock CL, Eldridge AL, Kristal AR, Patterson RE, Cooper DA, Neumark-Sztainer D, Cheskin LJ & Thornquist MD (2001) Serum concentrations of retinol, alpha-tocopherol and the carotenoids are influenced by diet, race and obesity in a sample of healthy adolescents. *Journal of Nutrition* **131**, 2184–2191.

O'Connell MJ, Chua R, Hoyos B, Buck J, Chen Y, Derguini F & Hämmerling U (1996) Retro-retinoids in regulated cell growth and death. *Journal of Experimental Medicine* **184**, 549–555.

Olafsdottir AS, Wagner KH, Thorsdottir I & Elmadfa I (2001) Fat-soluble vitamins in the maternal diet, influence of cod liver oil supplementation and impact of the maternal diet on human milk composition. *Annals of Nutrition and Metabolism* **45**, 265–272.

Olmedilla B, Granado F, Southon S, Wright AJ, Blanco I, Gil-Martinez E, Berg H, Corridan B, Roussel AM, Chopra M & Thurnham DI (2001) Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from five European countries. *British Journal of Nutrition* **85**, 227–238.

O'Neill ME, Carroll Y, Corridan B, Olmedilla B, Granado F, Blanco I *et al.* (2001) A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *British Journal of Nutrition* **85**, 499–507.

Piantedosi R, Ghyselinck N, Blaner WS & Vogel S (2005) Cellular retinol-binding protein type III is needed for retinoid incorporation into milk. *Journal of Biological Chemistry* **280**, 24286–24292.

Prabhala RH, Maxey V, Hicks MJ & Watson RR (1989) Enhancement of the expression of activation markers on human peripheral blood mononuclear cells by in vitro culture with retinoids and carotenoids. *Journal of Leukocyte Biology* **45**, 249–254.

Redmond TM, Gentleman S, Duncan T, Yu S, Wiggert B, Gantt E & Cunningham FX Jr (2001) Identification, expression, and substrate specificity of a mammalian beta-carotene 15,15′-dioxygenase. *Journal of Biological Chemistry* **276**, 6560–6565.

Reifen R (2002) Vitamin A as an anti-inflammatory agent. *Proceedings of the Nutrition Society* **61**, 397–400.

Ribaya-Mercado JD (2002) Influence of dietary fat on beta-carotene absorption and bioconversion into vitamin A. *Nutrition Reviews* **60**, 104–110.

Ridge JP, Fuchs EJ & Matzinger P (1996) Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* **271**, 1723–1726.

Ross AC, Davila ME & Cleary MP (1985) Fatty acids and retinyl esters of rat milk: effects of diet and duration of lactation. *Journal of Nutrition* **115**, 1488–1497.

Rühl R, Garcia A, Schweigert FJ & Worm M (2004) Modulation of cytokine production by low and high retinoid diets in ovalbumin-sensitized mice. *International Journal for Vitamin and Nutrition Research* **74**, 279–284.

Rühl R, Hamscher G, Garcia AL, Nau H & Schweigert FJ (2005) Identification of 14-hydroxy-retro-retinol and 4-hydroxy-retinol as endogenous retinoids in rats throughout neonatal development. *Life Sciences* **76**, 1613–1622.

Rühl R, Schweigert FJ, Wahn U & Grüber C (2006) Serum carotenoids in children of different ethnic origin from Berlin, Germany. *Proceedings of the German Nutrition Society* **8**, 65.

Santos MS, Meydani SN, Leka L, Wu D, Fotouhi N, Meydani M, Hennekens CH & Gaziano JM (1996) Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. *American Journal of Clinical Nutrition* **64**, 772–777.

Schweigert FJ, Bathe K, Chen F, Buscher U & Dudenhausen JW (2004) Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions. *European Journal of Nutrition* **43**, 39–44.

Schweigert FJ, Baumane A, Buchholz I & Schoon HA (2000) Plasma and tissue concentrations of beta-carotene and vitamin A in rats fed beta-carotene in various fats of plant and animal origin. *Journal of Environmental Pathology, Toxicology and Oncology* **19**, 87–93.

Semba RD (1994) Vitamin A, immunity, and infection. *Clinical Infectious Diseases* **19**, 489–499.

Sen J (2001) Signal transduction in thymus development. *Cellular and Molecular Biology (Noisy-le-Grand)* **47**, 197–215.

Shaw N, Elholm M & Noy N (2003) Retinoic acid is a high affinity selective ligand for the peroxisome proliferator-activated receptor beta/delta. *Journal of Biological Chemistry* **278**, 41589–41592.

Sidell N, Famatiga E & Golub SH (1981) Augmentation of human thymocyte proliferative responses by retinoic acid. *Experimental Cell Biology* **49**, 239–245.

Siegel EM, Craft NE, Roe DJ, Duarte-Franco E, Villa LL, Franco EL & Giuliano AR (2004) Temporal variation and identification of factors associated with endogenous retinoic acid isomers in serum from Brazilian women. *Cancer Epidemiology, Biomarkers and Prevention* **13**, 1693–1703.

Smith SM, Levy NS & Hayes CE (1987) Impaired immunity in vitamin A-deficient mice. *Journal of Nutrition* **117**, 857–865.

Sommerburg O, Meissner K, Nelle M, Lenhartz H & Leichsenring M (2000) Carotenoid supply in breast-fed and formula-fed neonates. *European Journal of Pediatrics* **159**, 86–90.

Stephensen CB (2001) Vitamin A, infection, and immune function. *Annual Review of Nutrition* **21**, 167–192.

Stephensen CB, Jiang X & Freytag T (2004) Vitamin A deficiency increases the in vivo development of IL-10-positive Th2 cells and decreases development of Th1 cells in mice. *Journal of Nutrition* **134**, 2660–2666.

Stephensen CB, Rasooly R, Jiang X, Ceddia MA, Weaver CT, Chandraratna RA & Bucy RP (2002) Vitamin A enhances in vitro Th2 development via retinoid X receptor pathway. *Journal of Immunology* **168**, 4495–4503.

Szegezdi E, Kiss I, Simon A, Blasko B, Reichert U, Michel S, Sandor M, Fesus L & Szondy Z (2003) Ligation of retinoic acid receptor alpha regulates negative selection of thymocytes by inhibiting both DNA binding of nur77 and synthesis of bim. *Journal of Immunology* **170**, 3577–3584.

Szondy Z, Reichert U & Fesus L (1998) Retinoic acids regulate apoptosis of T lymphocytes through an interplay between RAR and RXR receptors. *Cell Death and Differentiation* **5**, 4–10.

Takase S, Suruga K & Goda T (2000) Regulation of vitamin A metabolism-related gene expression. *British Journal of Nutrition* **84**, Suppl. 2, S217–S221.

Takase S, Tanaka K, Suruga K, Kitagawa M, Igarashi M & Goda T (1998) Dietary fatty acids are possible key determinants of cellular retinol-binding protein II gene expression. *American Journal of Physiology* **274**, G626–G632.

Tan NS, Michalik L, Desvergne B & Wahli W (2003) Peroxisome proliferator-activated receptor (PPAR)-beta as a target for wound healing drugs: what is possible? *American Journal of Clinical Dermatology* **4**, 523–530.

Tan NS, Michalik L, Desvergne B & Wahli W (2005) Multiple expression control mechanisms of peroxisome proliferator-activated receptors and their target genes. *Journal of Steroid Biochemistry and Molecular Biology* **93**, 99–105.

Tan NS, Michalik L, Noy N, Yasmin R, Pacot C, Heim M, Fluhmann B, Desvergne B & Wahli W (2001) Critical roles of PPAR beta/delta in keratinocyte response to inflammation. *Genes and Development* **15**, 3263–3277.

Theocharis S, Margeli A, Vielh P & Kouraklis G (2004) Peroxisome proliferator-activated receptor-gamma ligands as cell-cycle modulators. *Cancer Treatment Reviews* **30**, 545–554.

Thürmann PA, Steffen J, Zwernemann C, Aebischer CP, Cohn W, Wendt G & Schalch W (2002) Plasma concentration response to drinks containing beta-carotene as carrot juice or formulated as a water dispersible powder. *European Journal of Nutrition* **41**, 228–235.

Tibaduiza EC, Fleet JC, Russell RM & Krinsky NI (2002) Excentric cleavage products of beta-carotene inhibit estrogen receptor positive and negative breast tumor cell growth in vitro and inhibit activator protein-1-mediated transcriptional activation. *Journal of Nutrition* **132**, 1368–1375.

Tokuyama H, Tokuyama Y & Nakanishi K (1995) Retinoids inhibit IL-4-dependent IgE and IgG1 production by LPS-stimulated murine splenic B cells. *Cellular Immunology* **162**, 153–158.

Tokuyama Y & Tokuyama H (1996) Retinoids as Ig isotype-switch modulators. The role of retinoids in directing isotype switching to IgA and IgG1 (IgE) in association with IL-4 and IL-5. *Cellular Immunol* **170**, 230–234.

Toth B, Ludanyi K, Kiss I, Reichert U, Michel S, Fesus L & Szondy Z (2004) Retinoids induce Fas(CD95) ligand cell surface expression via RARgamma and nur77 in T cells. *European Journal of Immunology* **34**, 827–836.

Vahlquist A & Nilsson S (1979) Mechanisms for vitamin A transfer from blood to milk in rhesus monkeys. *Journal of Nutrition* **109**, 1456–1463.

Vakiani E & Buck J (1999) Retro-retinoids: Metabolism and action. In *Retinoids: The Biochemical and Molecular Basis of Vitamin A and Retinoid Action*, pp. 97–115 [H Nau and WS Blaner, editor]. Berlin: Springer.

van het Hof KH, West CE, Weststrate JA & Hautvast JG (2000) Dietary factors that affect the bioavailability of carotenoids. *Journal of Nutrition* **130**, 503–506.

van Vliet T, Boelsma E, de Vries AJ & van den Berg H (2001) Retinoic acid metabolites in plasma are higher after intake of liver paste compared with a vitamin A supplement in women. *Journal of Nutrition* **131**, 3197–3203.

Vogel S, Piantedosi R, O'Byrne SM, Kako Y, Quadro L, Gottesman ME, Goldberg IJ & Blaner WS (2002) Retinol-binding protein-deficient mice: biochemical basis for impaired vision. *Biochemistry* **41**, 15360–15368.

von Lintig J & Vogt K (2000) Filling the gap in vitamin A research. Molecular identification of an enzyme cleaving beta-carotene to retinal. *Journal of Biological Chemistry* **275**, 11915–11920.

von Lintig J & Wyss A (2001) Molecular analysis of vitamin A formation: cloning and characterization of beta-carotene 15,15′-dioxygenases. *Archives of Biochemistry and Biophysics* **385**, 47–52.

Wang W & Ballow M (1993) The effects of retinoic acid on in vitro immunoglobulin synthesis by cord blood and adult peripheral blood mononuclear cells. *Cellular Immunology* **148**, 291–300.

Wang XD (1994) Review: absorption and metabolism of beta-carotene. *Journal of the American College of Nutrition* **13**, 314–325.

Wang XD, Russell RM, Liu C, Stickel F, Smith DE & Krinsky NI (1996) Beta-oxidation in rabbit liver in vitro and in the perfused ferret liver contributes to retinoic acid biosynthesis from beta-apocarotenoic acids. *Journal of Biological Chemistry* **271**, 26490–26498.

Watzl B, Bub A, Brandstetter BR & Rechkemmer G (1999) Modulation of human T-lymphocyte functions by the consumption of carotenoid-rich vegetables. *British Journal of Nutrition* **82**, 383–389.

West KP Jr, Howard GR & Sommer A (1989) Vitamin A and infection: public health implications. *Annual Review of Nutrition* **9**, 63–86.

Worm M, Herz U, Krah JM, Renz H & Henz BM (2001) Effects of retinoids on in vitro and in vivo IgE production. *International Archives of Allergy and Immunology* **124**, 233–236.

Worm M, Krah JM, Manz RA & Henz BM (1998) Retinoic acid inhibits CD40+ interleukin-4-mediated IgE production in vitro. *Blood* **92**, 1713–1720.

Yagi J, Uchida T, Kuroda K & Uchiyama T (1997) Influence of retinoic acid on the differentiation pathway of T cells in the thymus. *Cellular Immunology* **181**, 153–162.