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Uptake of vitamin A in macrophages from physiologic transport proteins: Role of retinol-binding protein and chylomicron remnants

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Vitamin A plays an important role in reducing infectious disease morbidity and mortality by enhancing immunity, an effect that is partly mediated by macrophages. Thus, knowing how these cells take up vitamin A is important. The results in the present study demonstrate that J774 macrophages efficiently take up chylomicron remnant retinyl esters and retinol-binding protein (retinol-RBP) bound retinol by specific and saturable mechanisms. The binding of ^{125}I-RBP to plasma membrane vesicles demonstrated that the macrophage receptor had a similar binding affinity, as was discovered previously for other cells. The B_{max} for the macrophages was smaller than the values reported for placenta, bone marrow, and kidney, but larger than that reported for liver. The J774 cells also bound and took up [^{3}H]retinol-RBP. Approximately 50 to 60% of the uptake may compete with excess unlabeled retinol-RBP and approximately 30 to 40% with excess transtyrethin. Following the uptake of [^{3}H]retinol-RBP, an extensive esterification occurred: After 5 hours of incubation, 77.8 ± 3.9% (SD; n = 3) of the cellular radioactivity was recovered as retinyl esters. The J774 cells also demonstrated saturable binding of chylomicron remnant [^{3}H]retinyl esters, and a continuous uptake at 37°C followed by an extensive hydrolysis of the retinyl esters. Binding could be inhibited by approximately 50% by excess unlabeled low density lipoprotein (LDL). In addition, lipoprotein lipase increased the binding of chylomicron remnant [^{3}H]retinyl esters by approximately 30% and the uptake of chylomicron remnant [^{3}H]retinyl ester by more than 300%. Furthermore, because sodium chlorate reduced binding with 40% and uptake with 55%, the results suggest that proteoglycans are involved in the uptake. Thus, the results suggest that both LDL receptor and LDL-related protein are involved in the uptake of chylomicron remnant [^{3}H]retinyl ester in macrophages. (J. Nutr. Biochem. 10:345–352, 1999)

Keywords: retinoids; vitamin A; chylomicron remnants; retinol-binding protein; macrophages; J774 cells; lipoprotein lipase; proteoglycans

Introduction

Vitamin A plays important roles in the regulation of immune function because vitamin A deficiency compromises immunity.[1–3] The link between clinical vitamin A deficiency and infectious disease morbidity and mortality has been known since the beginning of this century from the classical work of Wolbach and Howe,[4] Green and Mellanby,[5] and Lassen.[6] More recently, community- and hospital-based clinical trials show that vitamin A supplementation reduces child mortality by 20 to 30%,[3] and vitamin A capsule distribution is recognized as one of the most cost-effective interventions to improve public health in large areas of the world.[7]

The basis for the use of vitamin A supplementation to reduce infectious disease morbidity and mortality is its role in enhancing immunity. This effect on immunity is mediated by a complicated crosstalk that involves fundamental aspects of several cell types including mucosal immunity, skin barrier function, hematopoiesis, first line defense by

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neutrophils, natural cytotoxicity by natural killer cells, and the function of monocytes/macrophages, Langerhans cells, and T and B lymphocytes.[1–3] The cells of the monocytes/macrophages lineage play a central role in immunity as antigen-presenting cells and as generators of oxygen and nitrogen radicals. Vitamin A metabolites influence both the differentiation of monocytes to macrophages and the function of differentiated macrophages.[1–3]

Therefore, it is interesting to study the mechanism by which macrophages accumulate vitamin A from plasma. In plasma, vitamin A is present as retinol bound to retinol-binding protein (RBP) and as retinyl esters in chylomicrons and their remnants:[8–10] The concentration of retinol bound to RBP is under strict homeostatic control and is close to between 1 and 3 μM irrespective of recent dietary intake. When the liver store is depleted, as it is in vitamin A deficiency, the plasma concentration of retinol-RBP also is reduced. Several other factors including inflammation, certain synthetic retinoids, iron deficiency, and kidney failure also may alter the homeostatic control of the plasma retinol-RBP concentration.[8–10] However, retinyl esters in chylomicrons and their remnants are directly related to dietary intake over the last 5 to 10 hours. The postprandial concentration of retinyl esters in chylomicrons and their remnants is normally in the submicromolar range, but may rise to several micromolars following the intake of supplements containing large amounts of vitamin A.

Although parenchymal cells in the liver remove the majority of chylomicron remnant retinyl esters from plasma, other cell types that are exposed to the lipoproteins and are expressing their receptors may accumulate considerable amounts of vitamin A by this route.[11–18] It has been demonstrated that bone marrow[11,13,14] and peripheral blood cells[14,18] can take up intestinally derived vitamin A in vivo, that chylomicron remnant retinyl esters are taken up by human leukemia cell lines[15–17] and in B lymphocytes *in vitro*,[12] and that J774 macrophages take up chylomicron remnants in vitro.[19]

The cell surface receptor for RBP is expressed in relatively large amounts in placenta, retina pigment epithelial cells, bone marrow, kidney cortex, choroid plexus, and undifferentiated keratinocytes and in lower amounts in small intestine, spleen, liver, and lung.[20] Saturable uptake of retinol-RBP also has been demonstrated in B lymphocytes.[12]

Because the uptake of vitamin A by macrophages has not been characterized either *in vivo* or *in vitro*, we studied binding and uptake of vitamin A associated with their physiologic plasma transport proteins (i.e., RBP and chylomicron remnants). The macrophage cell line J774 was used as a model. Although we used an in vitro system in this study, with all its limitations, we have tried to make the in vitro conditions reflect the in vivo situation. Thus, we added retinol or retinyl esters in physiologic concentrations and as part of the physiologic plasma carrier proteins rather than dissolved in an organic solvent. The results demonstrate that the cells efficiently take up chylomicron remnant retinyl esters as well as retinol-RBP by specific and saturable uptake mechanisms.

Materials and methods

Chemicals and animals

Fetal calf serum and phosphate buffered isotonic saline (PBS) were obtained from Gibco Limited (Paisly, UK); Dulbecco's modified Eagle's medium (DMEM) tissue culture medium and gentamicin sulfate were obtained from Bio Whittaker (Wakesville, MD USA). Retinol, KBr, and essentially fatty acid free albumin were from Sigma Chemical Co. (St. Louis, MO USA). Acitretin [all-trans-9-(4-metoxy-2,3,6-trimetylphenyl)-3,7-dimetyl-2,4,6,8-nonatetraenoic acid] was kindly provided from Hoffman La Roche (Basel, Switzerland). Temgesic was obtained from Reckitt & Colman Ltd (Hull, UK).

[11,12-^{3}H]Retinol (50 Ci/mmol) was purchased from DuPont-New England Nuclear (Boston, MA USA). Lipoprotein lipase (LPL) from bovine milk was kindly provided from Thomas Olivecorna and Gunilla Bengtsson-Olivecrona (Umeå, Sweden). Sodium chlorate was obtained from Fluca (Buchs, Switzerland). Dibutylphthalate and dinoylphthalate were obtained from Merck (Darmstadt, Germany). Human TTR was purchased from Scigen Ltd (Sittingbourne, UK). Iodobeads were purchased from Pierce (Rockford, IL USA) and Na[^{125}I] from Dupont-New England Nuclear.

Male Wistar rats (270–350 g) were fed an ordinary pelleted diet (EWOS Sverige AB, Sødertelje, Sweden) that contained 3.6 mg retinol/kg.

Cells

The murine macrophage cell line J774A.1 and the human hepatoma cell line HepG2 were obtained from American Type Culture Collection (Rockville, MD USA). The cells were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum from Sigma, 2 mM L-glutamine, and 0.06 mg/mL gentamicin sulphate from BioWhittaker. Cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. For experiments, cells were seeded at a density of 2×10^6 cells/mL, and grown to confluence. Viability was assessed by microscopy, trypan blue dye exclusion, and a cytotoxicity detection kit (Boehringer Mannheim, Mannheim, Germany) based on measuring lactate dehydrogenase (LDH) released from the cells during incubation. Low LDH leakage indicated high viability. The concentration of endogenous retinoids in the J774 cells was less than 20 nM.

For the incubations with retinol-RBP, cells first were washed three times with PBS before incubation with DMEM containing [^{3}H]retinol-RBP. After incubation, cells were washed three times with ice-cold PBS. Then 1 mL 0.1N NaOH was added to each well, cell lysates were collected, and aliquots were taken for protein determinations. Lysates were extracted three times with hexane, and cell-associated radioactivity was determined in a Packard 1900 TR liquid scintillation analyzer (Packard Instruments, Meriden, CT USA).

For the incubations with chylomicron remnant [^{3}H]retinyl esters, cells were washed three times with PBS before the addition of DMEM containing 10% lipoprotein deficient serum (LPDS) and 20 to 50 μL chylomicron remnant [^{3}H]retinyl esters per milliliter (200,000 dpm and approximately 1.5 mM triacylglycerol and 2.2 μM retinyl ester) was added to each well. After the indicated time points, the medium was removed and washed six times with ice-cold PBS before 1 mL 0.1 M NaOH or 1 mL ethanol was added to each dish. Ethanol was used when cell lysates were applied to high performance liquid chromatography (HPLC) analysis and NaOH was used when radioactivity was determined in lysates. Ethanol lysates were harvested by a cell lifter, sonicated for 20 seconds, and extracted with hexane.

Preparation of [^{3}H]retinol-RBP

HepG2 cells were incubated for 12 to 16 hours in serum-free DMEM, followed by incubation with the same medium supplemented with [^{3}H]retinol (0.01 μCi/mL, 0.3 μM final concentration, dissolved in ethanol) overnight. Medium was then collected and RBP isolated by TTR affinity chromatography as previously described.[20,21]

Isolation of RBP from human plasma

Human RBP was purified by a modification of the procedure described by Vahlquist *et al*.:[21] An additional gel-filtration step (Sephadex G75) was included for the final RBP preparation to obtain a pure protein,[20] as determined by polyacrylamide gel electrophoresis and Commasie blue staining.[22] RBP was iodinated using the Iodobead method as described in the instructions from the manufacturer. The iodinated RBP was used within 4 weeks after labeling.

Membrane vesicle receptor binding assay

Cultured J774 cells were harvested by a cell lifter and pelleted by centrifugation at 200 × *g* for 10 minutes. The cell pellet was resuspended in an ice-cold hypotonic 20 mM Tris-HCl buffer, pH 7.5, containing 4 mM $MgCl_2$ and homogenized with 10 strokes in a Dounce homogenizer. The suspension was homogenized further by aspiration through a 25-gauge needle and gentle stirring on ice for 1 hour. The subsequent centrifugation procedure for isolation of membrane vesicles was identical to that described for isolation of membrane vesicles from organs.[20] The purity of the final preparation was assessed by measuring the 5′-nucleotidase, which increased more than 20-fold over that of the initial homogenates.[20,23]

The assay of ^{125}I-RBP binding to membrane vesicles was performed by using an oil centrifugation method adapted from that described by Sivaprasadarao and Findlay.[23] Fifty microliter aliquots of membrane vesicles were incubated with 50 μL of ^{125}I-RBP (approximately 100,000 dpm and 2 nM RBP) in a 20 mM sodium phosphate buffer, pH 7.4, containing 0.1% ovalbumin and 150 mM NaCl. Following incubation at room temperature for 15 minutes, the membranes were centrifuged for 2 minutes at 12,500 × *g*, and 200 μL precooled phthalate oil mixture (three parts of dibutyl phthalate and two parts of dinonyl phthalate) was added. Membrane bound ^{125}I-RBP was separated from unbound ^{125}I-RBP by centrifugation for 1 minute at 12,500 × *g*. Three parallel incubations in the presence or absence of 2 μM unlabeled RBP were carried out to determine specific and nonspecific binding. The tubes were then frozen in solid CO_2, and the bottoms of the tubes containing the pellets were cut off and counted in a Packard gammacounter. All incubations were done with membrane vesicles containing 250 μg protein. The Scatchard and correlation analysis was done using Fig. P, Biosoft (Cambridge, UK).

HPLC analysis of retinol and retinyl esters

Cell homogenates were extracted three times with hexane.[24] The samples were dissolved in methyl-tert-butyl-ether:methanol (1:1). Retinol and retinyl esters were separated on a 5-μm Supelcosil LC 8 (250 × 4.6 mm) column (Supelco Inc., Bellefonte, PA USA) with a mobile phase of acetonitrile:water:methyl-tert-butyl-ether in a gradient starting with 96:4:0, changing linearly to 20:2:78 (v/v/v) in 22 minutes at a flow rate of 1 mL/min. The mobile phase was degassed continuously with helium. Retinol and retinyl esters were detected by absorbance at 326 nm. [^{3}H]Retinoid radioactivity of column fractions was measured by liquid scintillation.

Preparation of lymph

Intestinal lymph was obtained from lymph fistula in Wistar rats (mean weight 300 g).[25] Anesthesia of rats was invoked by injecting a mixture of Dormicum/Hypnorm 1:1 subcutaneusely. One milliliter of groundnut oil containing 24 μmol retinyl palmitate (Afi A Diagnosticum, Nycomed, Oslo, Norway), 250 μCi [11,12-^{3}H]retinol (50 Ci/mmol), and 7 mL 10 mM taurocholic acid was mixed and sonicated over 40 seconds and injected with an infusion pump through a duodenal catheter during the first 4 hours after surgery.[26] Intestinal lymph was obtained from lymph fistula in Wistar rats while the rats were kept in a Bollman restraining cage.[25] Temgesic was given subcutaneously for pain relief after surgery.

Chylomicron remnants

Chylomicron remnants were made from rat lymph by incubation with postheparin plasma and bovine serum albumin (BSA).[15] Each milliliter contained 0.6 mL of 20% essentially fatty acid free serum albumin in PBS, pH 7.4, 0.2 mL postheparin plasma, and 0.2 mL lymph (10–15 μmol triacylglycerol). The tubes were incubated for 90 minutes at 37°C in a shaking water bath. Adding KBr to a density of 1.019 g/mL inactivated the LPL activity. Chylomicron remnants were isolated by ultracentrifugation (103,000 × *g* for 20 hours), dialyzed against 20 mM phosphate buffer containing 0.9% NaCl and 2 mM EDTA at 4°C for 24 hours, and finally sterile filtered. Mean content of retinyl esters, which were determined as described above, was approximately 250 μM in the final preparation. Mean content of triacylglycerol was approximately 35 mM in the final preparation.

Postheparin plasma was made by an intravenous injection of 150 μL heparin (100 I.E./mL) per 100 g body weight. The rat was exsanguinated after 10 minutes and plasma isolated by centrifugation (15 minutes at 3,500 rpm).

Preparation of lipoprotein deficient serum

Human LPDS was prepared from blood collected from normal fasting subjects. Serum was adjusted to a density of 1.23 g/mL and centrifuged for 48 hours at 40,000 rpm. The lipoprotein containing supernatant was removed and the LPDS dialyzed against 20 mM phosphate buffer containing 0.85% NaCl and 2 mM EDTA for 24 hours and stored in the freezer at −20°C.

Triacyglycerol and protein measurement

An enzymatic kit from bioMérieux (Morcy I'Etoile, France) was used to determine of triacylglycerol. Protein was quantified using BSA protein assay reagent from Pierce (Rockford, IL USA.).

Results and discussion

Expression of RBP receptors on J774 macrophages

The promyelocytic cell line HL60 cells and monoblastic cell line U937 cells are macrophage/monocyte precursor cells that are efficiently regulated by retinoids with respect to cell growth and differentiation. These precursor macrophages previously have been demonstrated to take up retinol via cell-surface receptors for RBP and utilize the retinol taken up in the regulation of differentiation and proliferation.[16,27] Because no information is available on the existence of RBP receptors on macrophages, we examined whether J774 macrophages also accumulate retinol by a specific uptake mechanism involving RBP. Plasma membrane vesicles

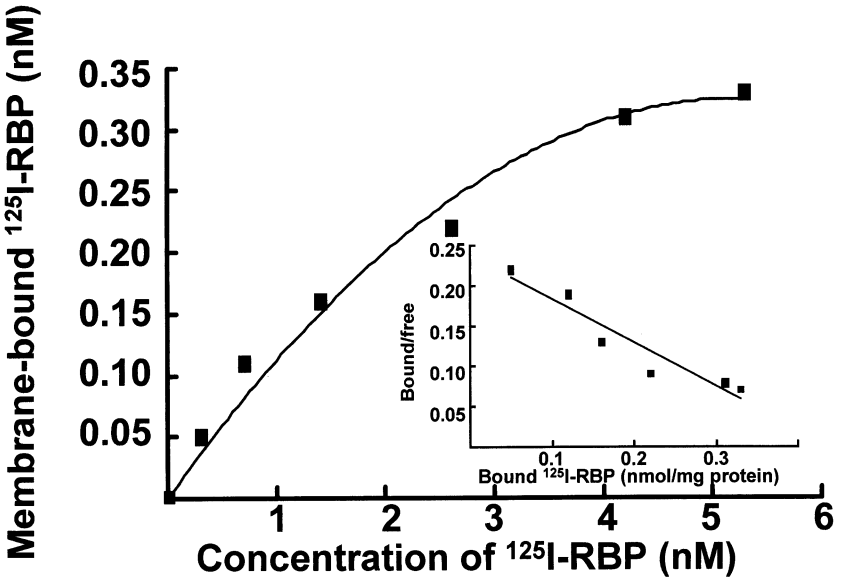

Figure 1 Binding of ^{125}I-retinol-binding protein (RBP) to J774 plasma membrane vesicles. Membrane preparations (1 mg/mL) were incubated with various concentrations of ^{125}I-RBP at 20°C for 60 minutes, and binding was determined as described in Materials and methods. The data were plotted according to Scatchard (insert). The values represent means of triplicates.

were prepared and the binding of ^{125}I-RBP was tested in an established RBP receptor assay. Membrane vesicles were incubated with various concentrations of ^{125}I-RBP at 20°C for 60 minutes and bound ^{125}I-RBP was separated from unbound by centrifugation using oil as described in Materials and methods. The binding curve is presented in *Figure 1*. When these data were plotted according to Scatchard (insert, *Figure 1*), it was evident that a specific binding was obtained. The binding had a K_d of 1.9×10^{-9} M, and the B_{max} was 480 fmol/mg protein. The values obtained are in agreement with earlier published data for the high affinity binding of RBP in a similar receptor-binding assay.[21,23] The B_{max} has smaller than the values reported for placenta, bone marrow, and kidney, but larger than that reported for liver.[21,23]

Binding and uptake of retinol-RBP in J774 macrophages

We then incubated J774 macrophages with trace amounts of [^{3}H]retinol-RBP at 4°C and 37°C, and studied cell association of [^{3}H]retinol after different periods of time. As shown in *Figure 2A*, the binding at 4°C was relatively slow. After 120 minutes, between 1 and 2% of the added radioactivity was cell-associated per milligram of protein. At 37°C the accumulation of radioactivity appeared much faster, resulting in between 5 and 7% cell-associated radioactivity per milligram of protein after the same incubation period. The uptake at 37°C was rapid the first hour, and slower for the next hour.

To test whether the uptake at 37°C of retinol-RBP was saturated, we incubated J774 cells with tracer amounts of [^{3}H]retinol-RBP in the presence and absence of excess (2 μM) cold retinol-RBP (*Figure 2A*). These experiments demonstrated that 50 to 60% of the uptake of [^{3}H]retinol-RBP could compete with excess ligand (*Figure 2B*), indicating that the uptake is saturable.

Because it is known that TTR binds reversibly to the entrance of the retinol barrel of the RBP molecule,[28] we also tested whether TTR could compete with the uptake. J774 cells were incubated with trace amounts of [^{3}H]retinol-RBP at 37°C in the absence or presence of TTR (2 μM). As demonstrated in *Figure 2B*, uptake of [^{3}H]retinol-RBP was reduced by approximately 30 to 40% in the presence of excess TTR.

Esterification of retinol following uptake of retinol-RBP

The initial metabolic conversion following cellular uptake of retinol-RBP includes oxidation to retinol and retinoic acid as well aw esterification of retinol with long chain fatty acids. Therefore, to test whether the cells have the ability to metabolize retinol taken up from RBP, we incubated the cells with [^{3}H]retinol-RBP for 4 hours at 37°C. During this incubation, extensive esterification occurred. Initially, no retinyl esters were detected in the cells, whereas following 4 hours of incubation 77.8 ± 3.9% (SD; $n = 3$) and 8.1 ± 0.6% (SD; $n = 3$) of the cellular radioactivity was recovered as retinyl esters and retinol, respectively.

These results demonstrated that J774 macrophages express RBP receptors that are able to internalize retinol-RBP in a saturable manner. As in most other cells,[1–3] the uptake was reduced by TTR, suggesting that it is the free and not the TTR bound retinol-RBP that delivers retinol to the cells. The results also showed that retinol taken up by the macrophages from retinol-RBP complex is metabolized to retinyl esters as is the case for several other cell types.[29–31]

Binding and uptake of chylomicron remnant retinyl esters in J774 macrophages

In the postabsorptive phase, retinol is present in plasma as retinyl esters in chylomicrons and their remnants. After intake of a vitamin A rich meal, the plasma concentration of these retinyl esters may be in the micromolar range;[16] that is, at a concentration similar to that of retinol-RBP. Binding of chylomicron remnant [^{3}H]retinyl esters to J774 macro-

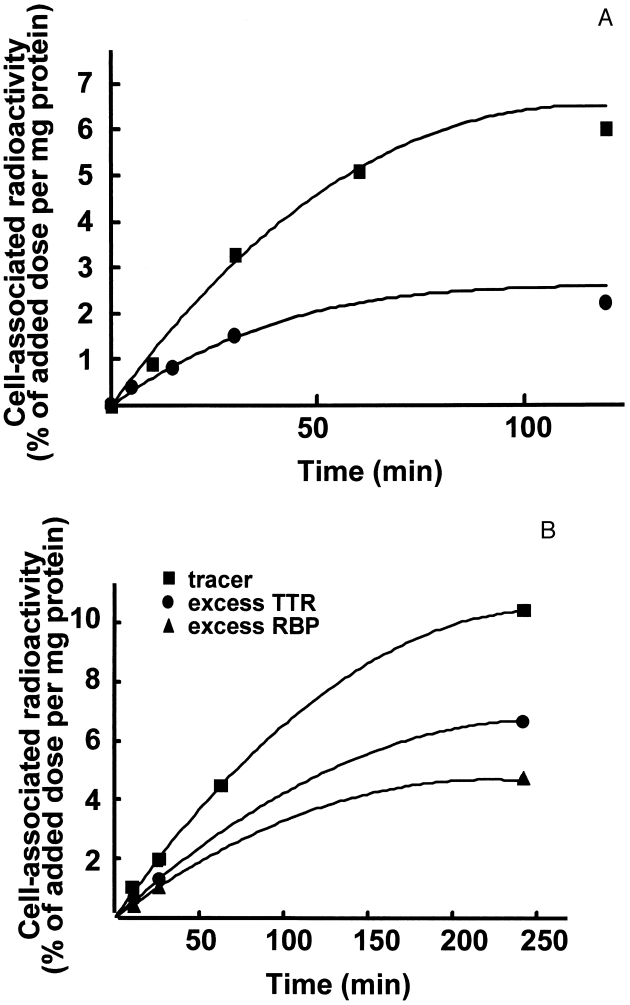

Figure 2 Binding and uptake of [^{3}H]retinol-rentinol-binding protein (RBP) by J774 cells. (*A*) J774 cells were incubated with 0.1 μM [^{3}H]retinol-RBP (12–20,000 dpm/well) for various periods of time at 4°C (●) and 37°C (■), and cell-associated radioactivity was determined. The values represent means of triplicates. (*B*) J774 cells were incubated with 0.1 μM [^{3}H]retinol-RBP (12–20,000 dpm/well) at 37°C in the absence (■) and presence of excess (2 μM) unlabeled retinol-RBP (▲) and TTR (●), and cell-associated radioactivity was determined. The values represent means of triplicates.

phages was tested at 4°C. The cells first were incubated with chylomicron remnant (final concentrations 1.5 mM triacylglycerol and 2.2 μM retinyl esters) for various periods of time. The results, which are presented in *Figure 3A*, revealed an initially rapid binding, followed by a slower rate of accumulation. After 1 hour, approximately 1% of the radioactivity added to the incubation mixture was bound per milligram of cell protein. In parallel dishes, cells were incubated at 4°C with various concentrations of chylomicron remnant [^{3}H]retinyl esters for 1 hour. This experiment (*Figure 3B*) demonstrates that the binding is saturable.

The cells then were incubated with chylomicron remnant-[^{3}H]retinyl esters at 37°C (*Figure 3C*). During the entire incubation period, cell-associated radioactivity accumulated continuously. After 5 hours, approximately 7% of the added radioactivity was associated with the macrophages per milligram of cell protein.

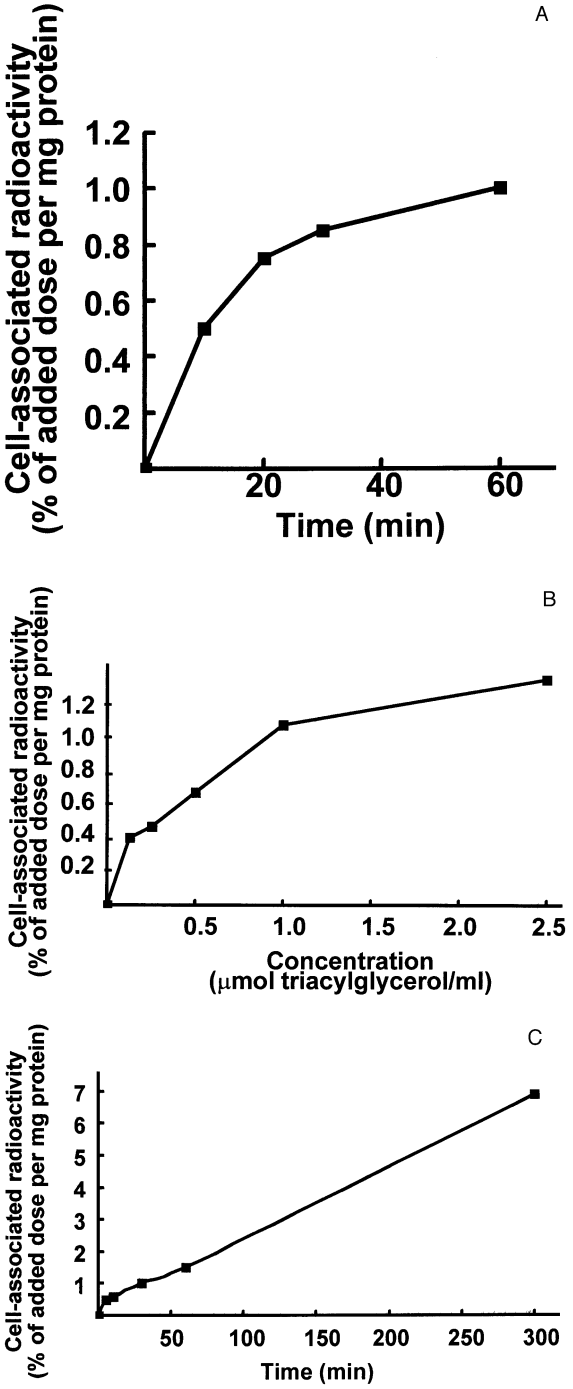

Figure 3 Binding and uptake of chylomicron remnant [^{3}H]retinyl esters. (*A*) Confluent layers of J774 cells in 30-mm dishes were washed with ice-cold phosphate buffered saline and incubated at 4°C with Dulbecco's modified Eagle's medium (DMEM) containing chylomicron remnant [^{3}H]retinyl esters (200,000 dpm and approximately 1.5 mM triacylglycerol and 2.2 μM retinyl ester). After the indicated time, medium was removed and cell-associated radioactivity was determined. Values represent mean of six parallel incubations. (*B*) Cells were incubated as in *A*, but with various concentrations of chylomicron remnant [^{3}H]retinyl esters (0.2–2.5 mM triacylglycerol). Values represent mean of six parallel incubations. (*C*) J774 cells were incubated at 37°C with DMEM containing chylomicron remnant [^{3}H]retinyl esters (200,000 dpm and approximately 1.5 mM triacylglycerol and 2.2 μM retinyl ester). After the indicated time, medium was removed and cell-associated radioactivity determined. Values represent mean of three parallel incubations.

To test whether uptake of retinyl esters was dependent on the concentration of retinyl esters in the chylomicron remnant particles, uptake of chylomicron remnants containing different concentrations of retinyl ester but with the same amounts triacylglycerol was studied. However, these results demonstrated that the kinetics of the chylomicron remnant uptake was unchanged irrespective of the retinyl ester concentration (data not shown).

Effects of LDL, LPL, and proteoglycans on the binding and uptake of chylomicron remnant retinyl esters

Uptake of chylomicron remnants by various cells have been reported to be inhibited by LDL and increased by LPL[32–38] and proteoglycans.[34,35] The data suggest that the LPL molecule binds to heparan-sulfate proteoglycans on the cell surface and to the remnant particle, thus linking the particle to cell surface and enhancing the binding of the lipoprotein to its receptor. Therefore, we tested whether these components also affected binding and uptake of chylomicron remnant [^{3}H]retinyl esters by the J774 cells. First, cells were incubated for 1 hour at 4°C with [^{3}H]retinyl ester labeled chylomicron remnants and 5 or 20 time excess unlabeled chylomicron remnants or excess unlabeled LDL. The results (*Figure 4A*) demonstrated that there was a concentration dependent inhibition by unlabeled chylomicron remnant. A 20-fold excess of chylomicron remnants reduced uptake by approximately 80%. LDL was also able to reduce binding substantially (i.e., by approximately 50%). Furthermore, LPL (1 μg/mL) increased the binding of chylomicron remnant [^{3}H]retinyl esters by approximately 30% (*Figure 4A*). The uptake at 37°C was increased even further by LPL. The addition of 1 μg LPL per milliliter increased the uptake of chylomicron remnant [^{3}H]retinyl ester from approximately 5 to 16% of the added dose (*Figure 4B*).

Proteoglycan synthesis is inhibited when cells are incubated with sodium chlorate in sulphur-free medium.[39] Therefore, J774 cells were pretreated (20 hours) with sodium chlorate before the binding of chylomicron remnant [^{3}H]retinyl ester was tested. After 1 hour at 4°C, 2.50 ± 0.16% (SD; $n = 3$) of the added radioactivity was bound per milligram of protein to the control cells compared with 1.47 ± 0.20% (SD; $n = 3$) of the sodium chlorate treated cells (*Figure 4A*). When cells were incubated at 37°C for 5 hours, 7.9 ± 1.45% (SD; $n = 3$) of the added dose was bound to the control cell per milligram of protein compared with 4.43 ± 0.61% (SD; $n = 3$) of the sodium chlorate treated cells. These results indicate that proteoglycans and LPL are important for the uptake of chylomicron remnant retinyl esters.

Results from a number of laboratories have indicated that several types of receptors are involved in uptake of chylomicron remnants, including the LDL receptor,[40,41] the LDL receptor related protein (LRP),[42,43] and the very low density lipoprotein receptor.[44] In addition, phagocytosis also has been suggested as an uptake mechanism in macrophages.[45] Macrophages express both LDL receptor and LRP, and probably another as yet unidentified lipoprotein receptor mediating chylomicron remnant uptake.[42,43] Our results also suggest that several are involved in the uptake of chylomicron remnant [^{3}H]retinyl ester in macrophages: The observation that LDL to some extent is able to compete with the binding suggests an involvement of the LDL receptor. Furthermore, the observation that binding and uptake is inhibited by sodium chlorate and stimulated by LPL suggests the involvement of LRP.

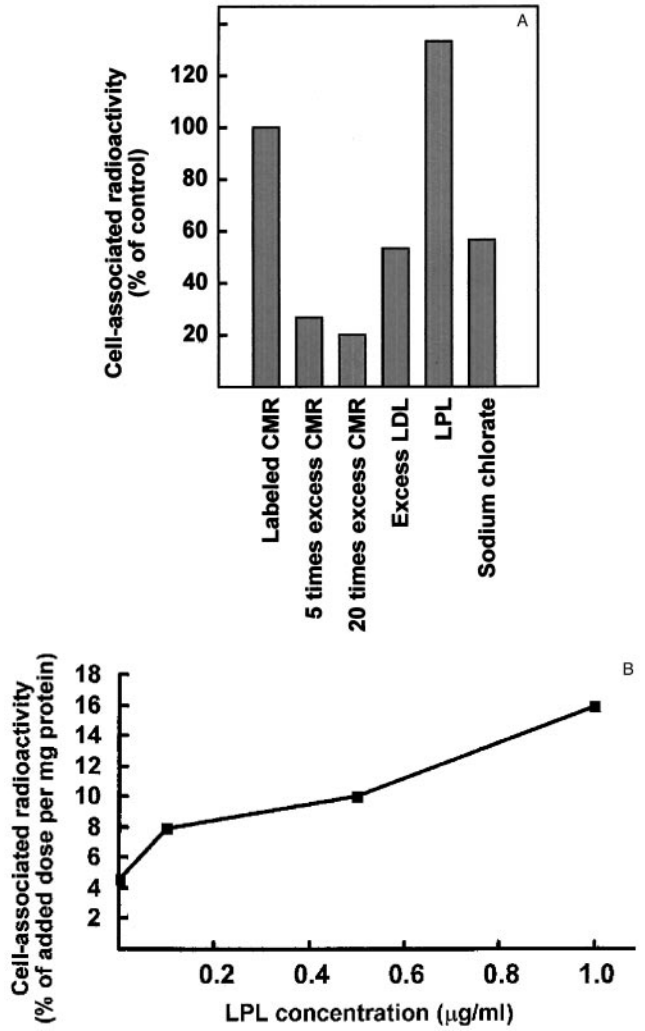

Figure 4 Influence of excess chylomicron remnants, low density lipoproteins (LDL), lipoprotein lipase (LPL) and sodium chlorate on binding and uptake of chylomicron remnant retinyl esters (CMR). (*A*) J774 cells were incubated for 1 hour at 4°C with Dulbecco's modified Eagle's medium (DMEM) containing chylomicron remnant [^{3}H]retinyl esters (200,000 dpm and approximately 1.5 mM triacylglycerol and 2.2 μM retinyl ester) in the absence or presence of 5 or 20 times excess CMR, LDL (500 mg protein/mL), LPL (0.1 μg/mL), or sodium chlorate (20 mM). Values represent mean of three parallel incubations. (*B*) J774 cells were incubated for 1 hour at 37°C with DMEM containing chylomicron remnant [^{3}H]retinyl esters (200,000 dpm and approximately 1.5 mM triacylglycerol and 2.2 μM retinyl ester) in the absence or presence of various concentrations of LPL as indicated. Values represent mean of three parallel incubations.

Cellular hydrolysis of chylomicron remnant retinyl esters

The chylomicron remnant used in these experiments contained 96 ± 4% (SD; $n = 3$) of the radioactivity in retinyl

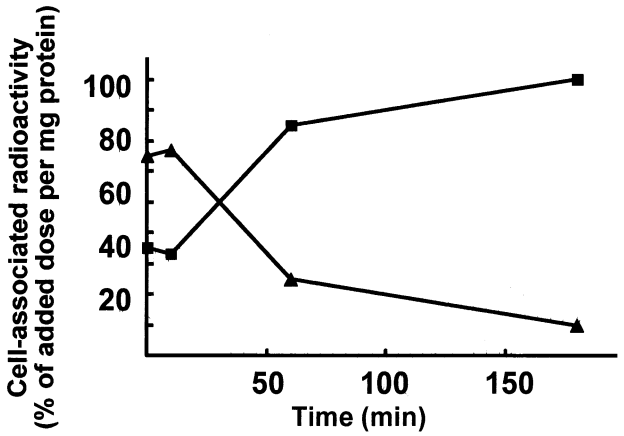

Figure 5 Hydrolysis of chylomicron remnant retinyl esters by J774 cells. Confluent layers of J774 cells in 30-mm dishes were washed with ice-cold phosphate buffered saline (PBS) and incubated at 4°C with Dulbecco's modified Eagle's medium (DMEM) containing (200,000 dpm and approximately 1.5 mM triacylglycerol and 2.2 μM retinyl ester). After 1 hour the medium was removed and the cells were washed six times with ice-cold PBS before 1 mL DMEM with 10% lipoprotein deficient serum (37°C) was added to each dish. The cells were further incubated at 37°C for the indicated periods of time, and radioactivity in the retinol (■) and retinyl ester (▲) fractions was determined. Values represent mean of three parallel incubations.

ester and the rest in the retinol moiety. The initial metabolic conversion following cellular uptake of retinyl esters is the hydrolysis to retinol and free fatty acids. Therefore, to test whether J774 cells have the ability to perform this catalytic step, we incubated the cells with chylomicron remnant [^{3}H]retinyl esters for 1 hour at 4°C to obtain binding predominantly to the cell surface (i.e., little internalization). The cells then were washed and further incubated at 37°C (*Figure 5*). During this incubation at 37°C, an extensive hydrolysis of retinyl esters took place. After 3 hours of incubation, the percentage of cell-associated radioactivity in the retinyl ester moiety was reduced to approximately 10%, whereas the majority could be recovered in the retinol fraction.

Conclusion

Vitamin A plays an important role in reducing infectious disease morbidity and mortality by enhancing immunity. This effect is partly mediated by effects of vitamin A on the monocyte/macrophage cell lineage. Thus, knowing how these cells take up vitamin A is important. In plasma vitamin A is almost exclusively present as retinol bound to RBP and as retinyl esters in chylomicrons and their remnants. The results in the present study demonstrated that macrophages efficiently take up chylomicron remnant retinyl esters and retinol-RBP by specific and saturable mechanisms.

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