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Control of scavenger receptor-mediated endocytosis by novel ligands of different length

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Abstract

The scavenger receptor recognized as a multiligand family of receptors falls in the group that is internalised through endocytosis. In this report we used several recombinant fragments of the tapeworm protein paramyosin, known to form filamentous dimers that bind collagenous structures as ligands of different length for the class A type I scavenger receptor (SR-AI). While native CHO cells are unresponsive to any of the recombinant fragments, it is shown that CHO cells transfected with this receptor efficiently internalise recombinant fragments that correspond to two thirds of the full-length paramyosin. In contrast, recombinant products corresponding to one-third of the full-length paramyiosin are not internalised. It is also shown that important molecules in the organization of the coated pit, are enriched when the two-thirds long paramyosin fragments were bound and internalised through the SR-AI. Moreover, internalisation of these fragments trigger a classical apoptotic pathway shown by the presence of TUNEL positive cells and the appearance of apoptotic bodies. We report paramyosin as a new ligand for the scavenger receptor and provide evidence supporting the notion that these receptors upon the formation of arrays with length-specific molecules, not only trigger endocytosis but also seem to regulate the synthesis of molecules involved in the organization of coated pits. (Mol Cell Biochem **271:** 123–132, 2005)

Key words: membrane receptors, lipoproteins, endocytosis, paramyosin, cytoskeleton, pattern-formation

Introduction

The family of proteins known as scavenger receptors was originally identified by Brown and Goldstein studying the endocytosis of lipoproteins [1]. These receptors trigger the endocytosis of chemically modified low-density lipoprotein (LDL), culminating in the transformation of macrophages into foam cells characteristic of atherosclerotic plaques [2, 3]. Scavenger receptors are internalised through endocytosis when bound to a remarkable variety of ligands ranging from chemically modified lipoproteins [4, 5], platelet secretory proteoglycans [6–8] to molecules involved in cell adhesion and bacterial pathogenesis [9]. Moreover, scavenger receptor knockout mice show increased susceptibility to infections with *Listeria monocytogenes* and herpes simplex virus type-1 [10]. Gram-positive bacteria are also endocytoced via these scavenger receptors [11].

Five classes of scavenger receptors have been recognized on the basis of structural homologies and ligand-binding properties [4, 12]. Class A corresponds to transmembrane homotrimeric glycoproteins where SR-AI and SR-AII are alternative transcripts from the same gene with a cysteine rich domain in the carboxyl-terminal region of SR-AI. One striking feature of SR-A is the 72 aa collagenous domain resulting from GXY amino acid repeats including a conserved sequence (KGQKGEEKGS) that forms a triple

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helix, and the 22 amino acid region at the carboxyl end containing the site for binding of acetylated LDL [13]. Strong electrostatic interactions between three K/E residues (intramolecular ion pairs) and K/Q residues mostly stabilize the triple-helical conformation (by hydrogen bonding). SR-AI and SR-AII also show a 26 aa transmembrane region and a 50 aa cytoplasmic domain that does not contain any of the internalisation signals present in similar cytoplasmic tails of different membrane proteins [14]. Different cytosolic domain sequences are known to trigger internalisation, like the highly degenerated tyrosine-based and leucine-based signals [15], through poorly studied lock and key recognition events based on specific protein–protein interactions between receptor tails and cytoskeletal components [16].

The dynamics in the organization of receptors during endocytosis is still largely undefined. However, reports describing the formation of receptor complexes have been described [17, 18]. It is believed that organized arrays of receptors might be assembled through interaction with intracellular-linking proteins [16, 19, 20]. Previous work on the binding and internalization of β-very low-density lipoproteins (β-VLDL) through the apo-B/E-(LDL) receptor, led us to propose that molecules affecting the lateral motion of scavenger receptors, also affect the formation and consolidation of specific arrays that determine the internalization of β-VLDL particles [21]. In this report, we continue analysing whether SR-AI requires the formation of arrays on the plasma membrane in order to trigger internalization. We used novel ligands for the SR-AI that consisted of recombinant fragments of the filamentous, α-helical, protein of about 100 kDa known as paramyosin, present in the muscle of invertebrates including the parasitic tapeworm *Taenia solium* [22, 23]. *T. solium* paramyosin (TPmy) binds polymeric collagen and can be isolated from crude extracts of invertebrates by a one-step affinity procedure. It inhibits the complement cascade at the level of C1, possibly through binding of C1q [22], and shows a complex quaternary structure including a domain with collagenous triple-helical structure and ligand-binding properties similar to SR-AI [24].

TPmy is reported here as a new ligand for the scavenger receptor. It is also shown that SR-AI mediated endocytosis induced by recombinant length specific TPmy also regulates the synthesis of molecules involved in the organization of the coated pit and triggers apoptosis.

Materials and methods

Preparation of human lipoproteins

LDL (d = 1.019−1.063 g/ml) and lipoprotein deficient serum (d > 1.215 g/ml) were obtained by sequential density gradient ultracentrifugation from normal human plasma, and acetylated LDL (AcLDL) prepared as previously described [6].

Recombinant fragments of TPmy

A series of fragments derived from the full-length coding sequence of TPmy [23] were recombinantly expressed and purified by affinity chromatography as described elsewhere [25]. The DNA constructs were named as follows: TPmy VW2-1 (268 aa), for the amino end third of the molecule; TPmy VW3-3 (283 aa), for the central third; TPmy VW 4-1 (312 aa), for the carboxyl end third; TPmy VW5-3 (551 aa), for the two thirds of the amino end and TPmy VW6-1 (595 aa) for the two-third carboxyl end (Fig. 1A and B). The recombinant proteins were dialyzed against 0.5 M NaCl, pH 7.3, and the protein concentration was determined using the BCA protein assay (PIERCE Laboratories, Rockford, IL). The full-length TPmy was not used in this study because of aggregation problems encountered when using cell culture media.

Cell culture

The CHO-SR cell line used in this study consists of CHO cells stably transfected with the bovine class A type I scavenger receptor grown in 45% Dulbecco's modified Eagle's medium (DMEM), 45% F12 medium supplemented with 10% fetal bovine serum (FBS), and geneticin (50 μg/ml) at 37 °C using 5% CO_2. Control CHO cells were cultivated in the same medium but containing penicillin (50 U/ml)/streptomycin (50 μg/ml). All cell culture reagents were purchased from Gibco BRL (Germany).

Internalization assays with FITC-labelled lipoproteins and paramyosin fragments

AcLDL and recombinant paramyosin fragments were conjugated to FITC. Two-hundred micrograms of protein were incubated with 5 mM FITC for 5 h at room temperature and excess FITC removed by elution through G-25 columns. CHO and CHO-SR cells were incubated for 5 h at 37 °C with different FITC-paramyosin fragments or with FITC-AcLDL in 45% DMEM/45% F12 medium without fetal bovine serum. Where indicated, AcLDL or fucoidin (Sigma Chemical Co) were added to the incubation medium as competitors. After incubation, the cells were washed five times with PBS containing 2 mg/ml BSA and once with PBS before dissolution in 0.1 N NaOH. Fluorescence was evaluated on an ISS-PC1 fluorometer using an excitation wavelength of 480 nm and emission scans between 495–580 nm. Fluorescence

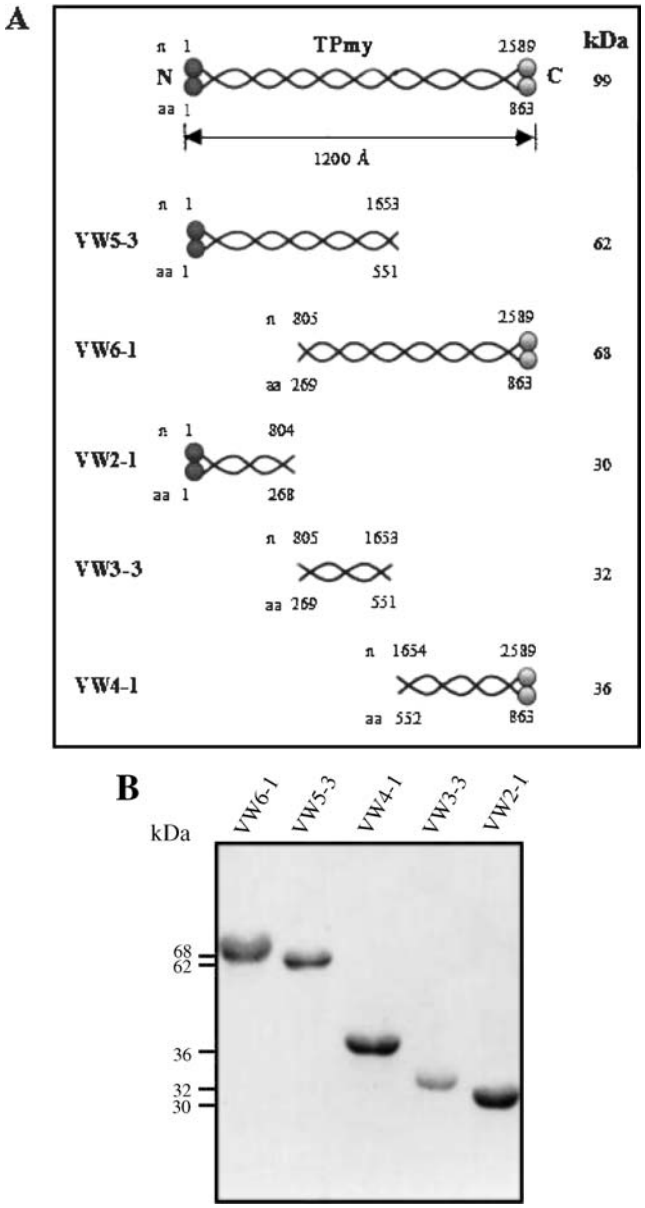

Fig. 1. TPmy recombinant fragments. (A) Schematic representation of the five recombinant fragments of TPmy employed in this study, indicating their nucleotide sequence (n), amino-acid sequence (aa), and molecular weight (kDa). (B) SDS-PAGE of the five TPmy recombinant fragments used. The kilo Dalton values for each one of the fragments is slightly higher since a tail of 46 aa (±5,000 mw) needed for the affinity purification of the recombinant products is still present in these molecules.

measurements were calculated using standard fluorescence, dividing maximal fluorescence values by the percentage of protein labelling efficiency, taking into account the molar extinction values for both FITC and TPmy. When the non-labelled paramyosin fragments were used in order to stimulate cells previous their lysis, the following procedure was used. CHO-SR cells were incubated for 1 h at 37 °C in medium without fetal bovine serum prepared as described before. TPmy VW5-3 or VW2-1 (15 μg/ml) was added and incubation was carried out for 4 h at 37 °C. After incubation, the cells were washed with PBS and harvested. For competition assays with fucoidin and TPmy VW5-3, CHO-SR cells were incubated as previously described. Proteins isolated from CHO-SR cells were washed in cold PBS and lyzed for 30 min at 4 °C in lysis buffer containing 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 0.5% NP40, 1% Triton X-100, 10 mM Tris, pH 7.4, 0.2 mM sodium orthovanadate, 10 mM benzamidine, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 250 μM PMSF. The lyzed cells were centrifuged and the supernatant recovered. The TPmy VW5-3 was localized as described further on using western blot analysis. Protein concentration was determined using the BCA protein assay.

Subcellular fractionation

CHO-SR cells previously incubated with or without TPmy VW5-3 for 4 h at 37 °C were rinsed with ice-cold PBS and harvested in lysis buffer as described before without NP40 and Triton X-100. Cells were harvested by cell scraping and sonicated for 1 min and then centrifuged at 1500 *g* for 10 min at 4 °C. The pellet was eliminated and the supernatant further centrifuged at 100 000 *g* for 45 min at 4 °C. The cytosol supernatant fraction was retained and the pellet was solubilised in lysis buffer containing 1% Triton X-100 and 0.5% NP40 and further sonicated. The suspension was centrifuged at 15 000 *g* for 15 min at 4 °C. The resulting supernatant was collected as the membrane fraction. Equal amounts of protein were boiled for 5 min in sodiumdodecylsulfate (SDS)-sample buffer and subjected to SDS-PAGE for subsequent immunoblot analysis.

Western blots analysis

Protein concentration was determined using the BCA protein assay, and samples (10 μg/lane) from the total protein fraction and samples from the sub-cellular fractionation were separated by SDS-PAGE (10%) and electrotransferred overnight to nitrocellulose membranes (BioRad) at 4 °C and 80 mA in a transfer buffer containing 150 mM glycine, 25 mM Tris-HCl pH 8.8 and 20% methanol. The membranes were blocked at 4 °C overnight with blocking buffer (5% nonfat milk in Tris-buffered saline (TBS) pH, 7.6 with 1% Tween 20). The following antibodies each diluted 500-fold were used: goat polyclonal anti-heavy chain of clathrin, β2 and α2-adaptins, and a rabbit polyclonal anti-Eps15, all purchased from Santa Cruz Biotechnology. A rat anti-mouse anti-CD204 (scavenger receptor) monoclonal antibody was obtained from Serotec, Raleigh, NC. A porcine antibody against TPmy

paramyosin was manufactured by our group. The secondary antibodies; donkey anti-goat IgG and goat anti-rabbit IgG were purchased from Santa Cruz Biotechnology (both conjugated to horseradish peroxidase), goat anti-rat horseradish peroxidase-mouse from Serotec, Raleigh, NC, and a rabbit anti-porcine horseradish peroxidase from Zymed laboratories, Inc. All secondary antibodies were used employing 5000-fold dilutions and incubated for 1 h at 37 °C in blocking buffer. The membranes were washed with TBS-Tween 1%, and HRP activity was detected using a western-light chemiluminescence protocol (Amersham Pharmacia Biotech, UK). The percentage of band intensity was measured using the LaserPix software (multi-image BioRad Pic files) instrument where film background was employed as a zero or basal value, and 100% intensity the band with the highest signal. This resulted to be the band obtained when measuring protein eps15 in VW5-3 stimulated CHO-SR cells.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL)

CHO-SR cells (1×10^6) were treated with TPmy VW5-3 (30 μg/ml) for 24 h and rinsed with ice-cold PBS. Briefly, cells were fixed with 1% (w/v) paraformaldehyde in PBS on ice for 15 min. The cells were washed with ice-cold PBS twice and stored at −20 °C in 70% (v/v) ethanol prior to performing the TUNEL assays. Apoptotic cell death was assessed using the APO-BrdU TUNEL Assay Kit (Molecular Probes), and cells were treated according to the supplier's instructions. Cells with the DNA strands cleaved were labelled with BrdUTP and terminal deoxinucleotidyl transferase (TdT) in the DNA-labelling solution at 22–24 °C overnight. At the end of incubation time, the cells were rinsed twice with rinse buffer provided by the Kit, and the BrdU was detected using an Alexa Flour 488 dye-labelled anti-BrdU monoclonal antibody. Cells were viewed under fluorescence microscopy at ×100 magnification, using an excitation wavelength of 488 and an emission wavelength of 494. Viability was also analysed by flow cytometry.

Results

Internalization of FITC-labelled lipoprotein and paramyosin fragments carried out by the CHO-SR cell line

In order to explore if TPmy recombinant fragments with ability to bind collagen-like structures, triggered SR-AI mediated endocytosis, the five fragments described in our study were evaluated using internalization assays. Control experiments showed that CHO-SR cells expressing the class A type I scavenger receptor, efficiently internalise FITC-labelled AcLDL employed as an internalization control. Internalization was blocked by the presence of excess AcLDL or fucoidin in the incubation medium, but not by LDL (Fig. 2A). Similar internalization assays of FITC-labelled recombinant fragments of TPmy showed that fragments VW5-3 and VW6-1 were efficiently endocytoced, whereas the shorter ones VW4-1, VW2-1 and VW3-3 were not (Fig. 2B). Scavenger receptor molecules mediate endocytosis of FITC-labelled VW5-3 and VW6-1 fragments, since CHO cells that do not express this receptor were unresponsive to any of the TPmy recombinant fragments tested. Furthermore, the highly negative molecule fucoidin efficiently competed with the two active fragments of TPmy (Figs. 2C, D and Fig. 3A), in agreement to previous reports studying the binding and internalization of several molecules carried out by the scavenger receptor [2]. These results demonstrated that endocytosis of the recombinant fragments of TPmy was length-dependent and mediated by the scavenger receptor most probably through binding to its collagenous stalk. This phenomenon presents the possibility that TPmy VW5-3 and VW6-1 induced the arrangement of receptors at optimal distances from each other that in turn might have stimulated the formation of coated pits and accelerated endocytosis, whereas the shorter TPmy fragments, VW4-1, VW2-1 and VW3-3, did not. Using the incubation conditions described, it was not possible to evaluate the internalization characteristics of the full-length TPmy, because of serious aggregation problems encountered during the assays.

Localization of endocytic proteins in CHO-SR cells

In order to determine if the extracellular events induced by the TPmy fragments employed as ligands for the scavenger receptor were indeed reflected on any of the intracellular events associated to endocytosis, as a first approach, we investigated the levels of expression of several proteins involved in the organization of coated pits. Using Western blot analysis carried out on total protein fractions obtained from CHO-SR cells, we found that clathrin, β-adaptin, α-adaptin, eps15, and the scavenger receptor itself, showed a modest increase in their level of expression during the internalization of the TPmy VW5-3 fragment with respect to basal conditions (Fig. 3B). As a semiquantitative way to express these changes, Fig. 3C shows the percentage increase of band intensity as shown in Fig. 3B, taking the basal value as background and the band with the highest value as 100% intensity. Although protein eps15 was taken as the 100% intensity band, it becomes evident that β-adaptin, α-adaptin and the scavenger receptor itself show the best response to TPmy fragment VW5-3. When total protein fractions were separated into membrane/cytoskeleton and cytosol fractions, these differences not only became clearer, but we had the opportunity to differentiate between protein mainly associated with the

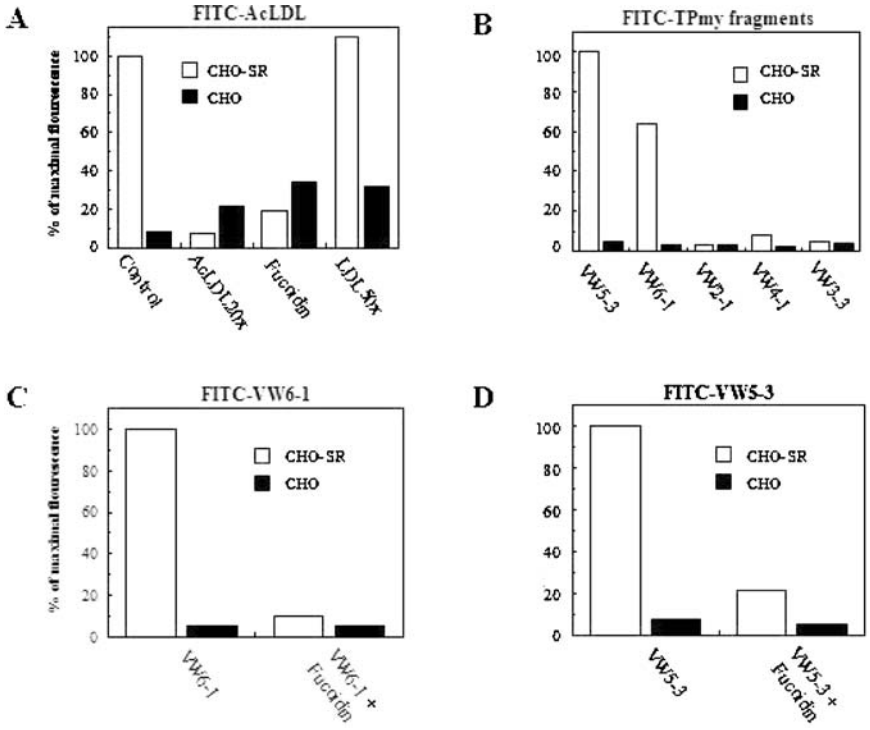

Fig. 2. Internalization of FITC-AcLDL and FITC-TPmy by CHO and CHO-SR cells. (A) Control experiments employing cells incubated for 5 h with FITC-labelled AcLDL (10 μg/ml), 200 μg/ml of AcLDL and fucoidan, and 500 μg/ml of LDL were used as competitors when added to the incubation medium. (B) Internalization of the FITC-labelled TPmy recombinant fragments (15 μg/ml) by cells incubated for 5 h at 37 °C. Internalization of the FITC-labelled fragments: (C) VW5-3, and (D) VW6-1, showing the inhibition rendered by fucoidan. Data represent the mean of three independent experiments using triplicates.

membrane/cytoskeleton or protein located in the cytosolic fraction (Fig. 3D). Clathrin was found associated almost exclusively with the cytosolic fraction whereas β-adaptin, α-adaptin and eps15 with the membrane/cytoskeleton fraction. The scavenger receptor was found in both fractions.

Cell death provoked by the TPmy fragment VW5-3 in CHO-SR cells

During the internalization process of TPmy VW5-3 by CHO-SR cells, using transmission electron microscopy important morphological changes were observed in the plasma membrane, nucleus and mitochondria, as well as the appearance of crystal like structures in the nucleus and in general an increase in cell size (data not shown). Looking further into the possibility that these changes might have been associated to apoptotic damage, confocal microscopy showed the presence of typical apoptotic bodies when internalization of TPmy VW5-3 was carried out for 24 h (Fig. 4). These experiments clearly showed a change in size between CHO-SR cells internalising TPmy VW5-3 through the SR-AI, and CHO control cells most probably internalising a basal amount of TPmy VW5-3 through the SR-BI receptor known to the present in this cell type. Basal internalization through the SR-BI seem to account for less than 10% of the total TPmy found inside CHO-SR cells shown suggested in the experiment presented in Fig. 2.

Furthermore, using flow cytometry searching for TUNEL positive cells, we substantiated the viability loss and the fact that cells internalising TPmy VW5-3 might be entering a classical apoptotic pathway (Fig. 5). As shown in Fig. 5A, although increasing concentrations of TPmy VW5-3 caused an elevated cell death rate, we observed that the highest concentrations employed seemed to have promoted a tendency for the cells to recover. We believe this is not the case since a decrease in cell death might be associated to the fact that, as shown in Fig. 4, the use of high TPmy VW5-3 concentrations promotes cell/cell phagocytosis as shown by the presence of multiple apoptotic nuclei. Therefore, the flow cytometry apparatus might have taken multinucleated cells as single events with the concomitant loss of individual positive cells. Employing the highest value of TUNEL positive cells, we carried out the viability analysis of CHO-SR cells. This analysis showed that a control experiment kept 95% of viable cells with only 5% of cells undergoing apoptosis. On the other hand, TPmy VW5-3 treated cells only showed 76% viability and 24% of cells undergoing apoptotic cell death (Fig. 5B).

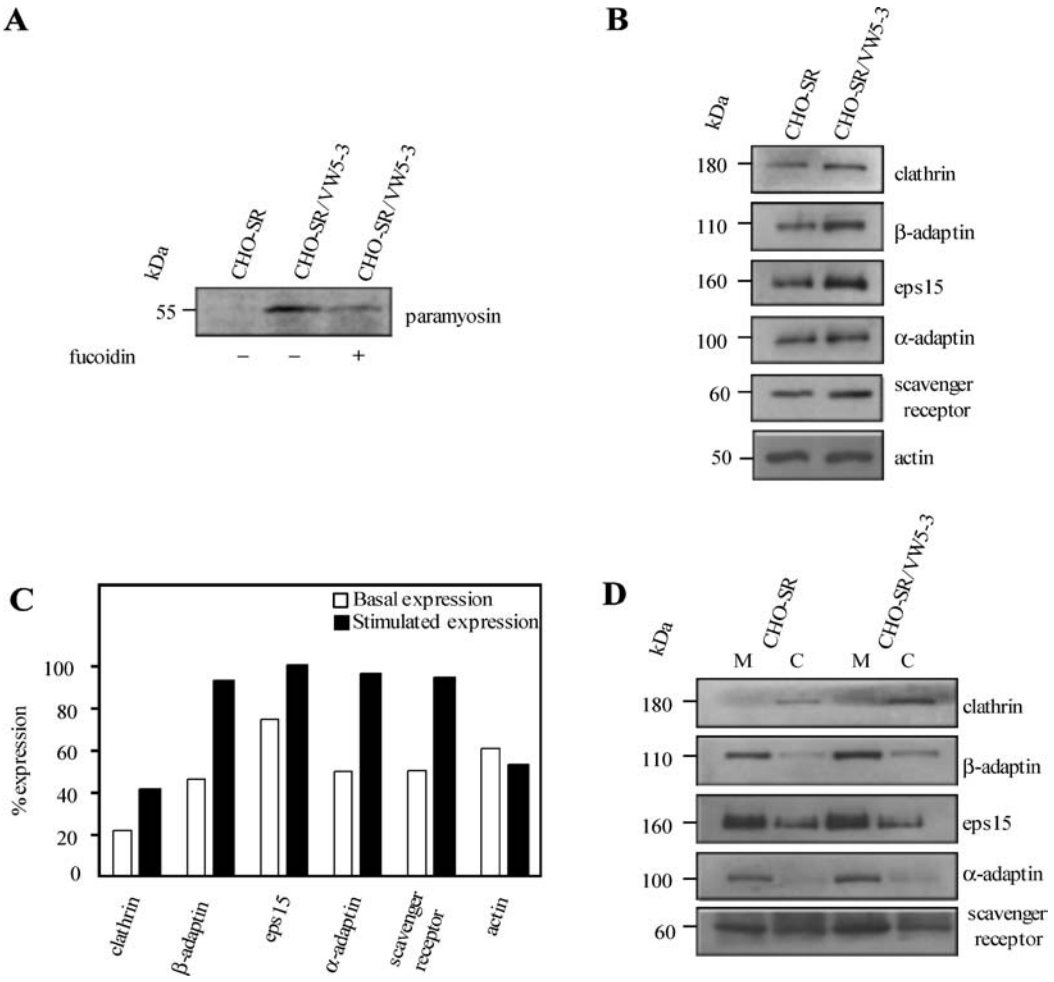

Fig. 3. Western blot analysis of endocytic proteins from CHO-SR cells incubated with TPmy VW5-3. (A) Total protein fractions (30 μg) from CHO-SR cells previously incubated with or without TPmy VW5-3 in the presence or the absence of fucoidin were immunoblotted in order to detect the localization of TPmy VW5-3, using an anti-TPmy VW5-3 antibody. (B) Total protein fractions (10 μg) from CHO-SR cells previously incubated in the absence or the presence of TPmy VW5-3. The specific endocytosis related proteins shown in the figure were visualized using specific antibodies against each one of them. (C) Protein quantitation as shown in B. Percentage increments in band intensity taking film background as the basal value and the band with highest intensity as 100% (eps15 in VW5-3 stimulated CHO-SR cells). (D) Subcellular protein fractions (10 μg) (membrane/cytoskeleton, M; cytosolic, C) from CHO-SR cells previously incubated in the absence or the presence of TPmy VW5-3 were analysed as in B.

Discussion

Natural and recombinant *T. solium* paramyosin have been reported to bind type I collagen, a property that can be exploited for its isolation from a crude extract of this parasite through a one-step affinity procedure [26]. TPmy also binds C1q and blocks function of the complement cascade [22]. The feature shared by collagen type I and C1q is the presence of triple helical collagenous structures. Although a number of aspects on the binding of paramyosin to collagen type I or C1q are not clear, we proposed that long-range periodicities of charged surface residues along this coiled-coil filamentous protein, might be responsible. Thus, the initial idea underlying our assays with recombinant fragments of *T. solium* paramyosin was to determine if it could also interact with the SR-AI, which also posses a triple helical collagenous domain. The way to evaluate endocytosis of TPmy was carried out by measuring the internalization of FITC-labelled recombinant TPmy fragments of different lengths, by CHO cells that were stably transfected with the SR-AI. Our results indicated that fragments corresponding to two thirds of the full-length protein (VW5-3 and VW6-1) induced endocytosis, whereas fragments that correspond to only one third of the full-length protein, coming from the amino end, the middle section and the carboxyl end (VW4-1, VW3-3, VW2-1) were all inactive. Interestingly, VW5-3 and VW6-1 only

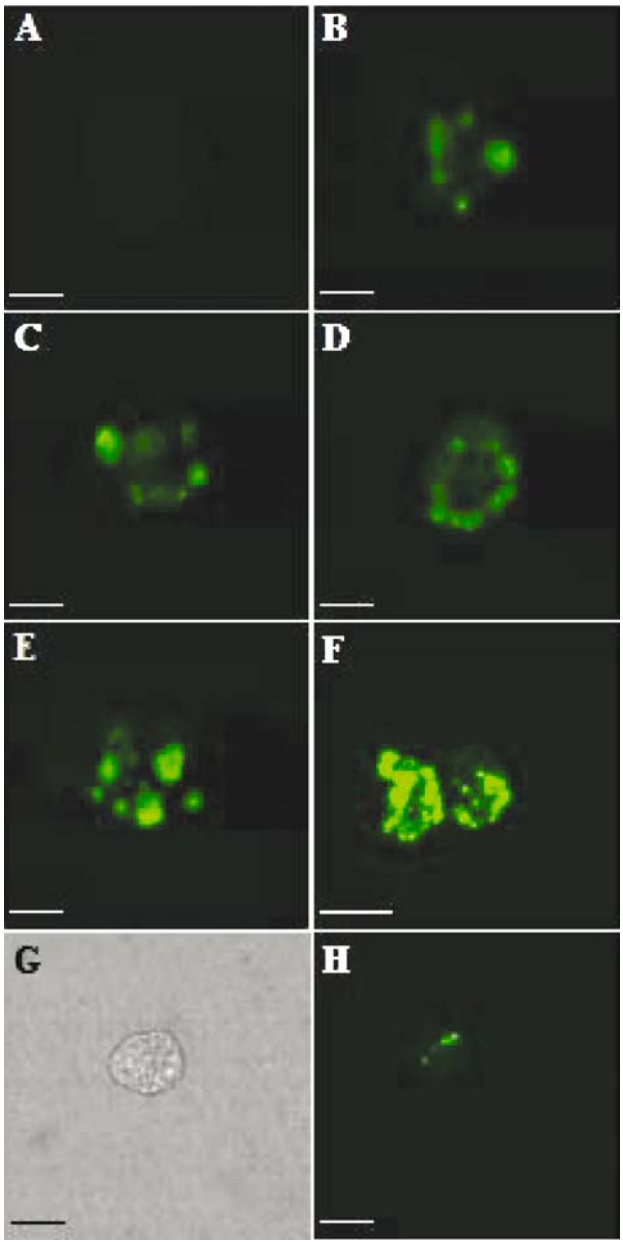

Fig. 4. Apoptotic cell death in CHO-SR cells treated with TPmy VW5-3. CHO-SR cells treated with increasing amount of TPmy VW5-3 (0, A; 10, B; 20, C; 30, D; 40, E and 50 μg/ml, F) were subjected to confocal fluorescence microscopy. Detection of BrdU associated to apoptotic bodies was achieved using an Alexa Fluor 488 dye-labelled anti-BrdU monoclonal antibody. Scale bars, 5 μm (B, C, D, E) and 10 μm (F). CHO control cells were treated with 30 μg/ml TPmy (G, H). Phase contrast image (G) and confocal fluorescence image (H).

share one third corresponding to the middle portion of TPmy, suggesting that the relevant feature for activity is their length and not their location on the complete protein. The activity of these fragments is mostly specific for the SR-AI, since non-transfected CHO cells naturally containing the SR-BI, seem to present a basal internalization activity counting for less than 10% of the total TPmy internalization shown in CHO-SRAI transfected cells. Taken together, these results suggest that a physical arrangement between SRAI type receptors at the cell surface maintaining critical distances from each other, might be a factor that promotes internalization.

Clathrin and a group of heterotetrameric proteins known as adapters are the main components of coated pits; AP-1 restricted to the coated pit in the Golgi region and AP-2 associated with the cell membrane [27]. AP-2 is a heterotetrameric complex that binds clathrin lattices and functions as an adaptor by linking receptors [14, 28, 29]. The α-subunit apparently interacts directly with dynamin, a GTPase that participates in the formation of the budding of clathrin-coated vesicles [30, 31]. This subunit has been also associated to several factors including Eps15, a protein that binds to the epidermal growth factor receptor tyrosine kinase [32, 33], and Epsin that seems to assist clathrin-mediated endocytosis [32]. The β-2 chain of AP-2 has been also proposed to bind the heavy chain of clathrin, as well as to protein CALM involved in the regulation of clathrin recruitment in the membrane [34–36]. The μ2-chain is suspected to interact equally well with the leucine and the YXXθ internalization signals present in several cytoplasmic tails of membrane receptors [27, 37, 38]. The internalization signal FXNPXY contained at the cytoplasmic tail of the LDL receptor, apparently interacts directly with clathrin [39], and it is not dependent on adapter proteins. Although this might be considered a regulatory mechanism for the association of AP-2 with clathrin, the binding sites of AP-2 with several membrane components, often taken for granted, are still unclear [29].

Several authors have proposed that formation of protein microdomains on the cell surface regulate processes such as the sorting of receptors in polarized cells [14], signal transduction [40], and the propagation of conformational changes of receptors across the membrane [41]. For instance, it has been suggested that clustering of glycosylphosphatidylinositol-anchored membrane proteins bearing the same ectodomains is explained through the establishment of protein arrays as “platforms” or “rafts” stabilized by cholesterol [42, 43]. However, the evidence related to the formation of organized structures on the cell membrane with concomitant physiological responses, still remains fragmentary. In the case of apoptosis, it has been suggested that this phenomenon can be triggered in response to changes in cell shape, where mechanical forces in the cytoskeleton play an apparent control [44]. It has been also reported that cells with extended shape divide actively, whereas cells competing for space in the same culture dish tend to adopt a round shape and die [45]. This evidence seems to support our previous proposal that special arrangements of “networking proteins” on the cell membrane, such as single-crossing receptors that

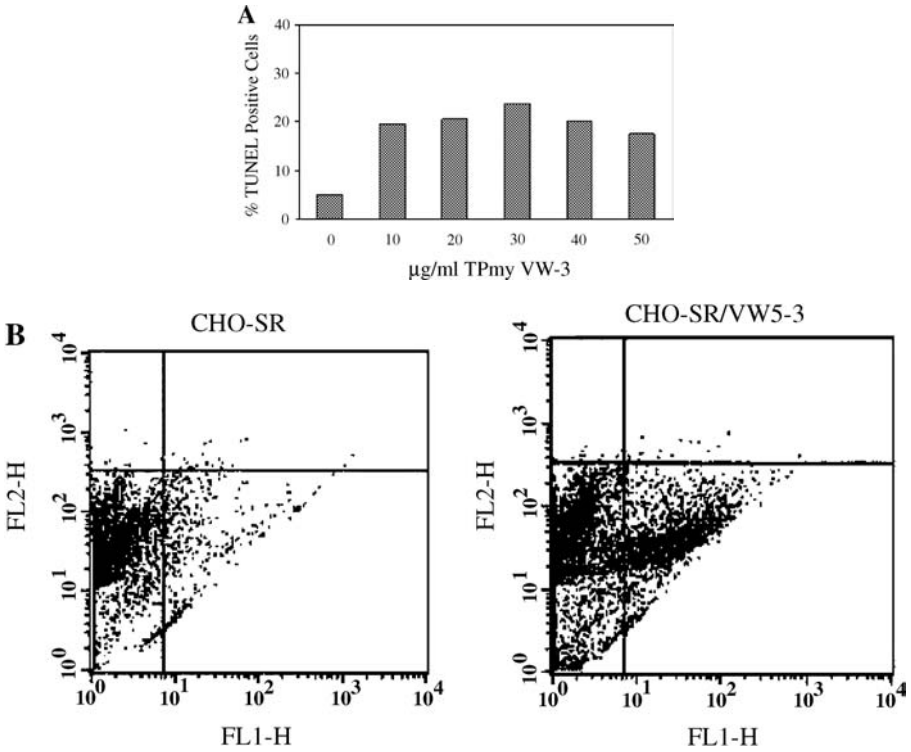

Fig. 5. Viability loss of CHO-SR cells caused by TPmy VW5-3. (A) Percentage of TUNEL-positive cells determined by flow cytometry 24 h after incubation with different amount of TPmy VW5-3 (average result of two independent experiments). (B) Flow cytometry of CHO-SR control cells (left panel) and CHO-SR cells treated with TPmy VW5-3 (30 μg/ml) for 24 h (right panel). Representative images of three independent experiments.

extend from the extracellular space to the cytoskeleton, can be reflected on intracellular face responses [21]. It is conceivable that specific arrays of receptors on the cell membrane result in the transduction of mechanical signals into physiological responses through cytoplasmic protein–protein recognition.

Our results are consistent with the fact that during internalization of TPmy fragment VW5-3 and only after the rearrangement of receptors at the all surface, their cytoplasm tails optimally interact with cytoskeleton components, a triggering mechanism that optimises endocytosis and allows the optimal expression of specific coated pit components. Moreover we present evidence that internalization of TPmy might be considered a stressful condition, as previously shown by us with the internalization of several other ligands through the scavenger receptor type A [46], since CHO-SR cells treated with increasing concentrations of TPmy VW5-3 tend to lower viability through the process of apoptotic cell death. This possibility is substantiated by increased percentages of tunel positive cells directly related to the presence of cell apoptotic vesicula and multinucleated cells.

Since transduction of the signal employed by receptors such as the LDL receptor and the scavenger receptor is not easily explained by conventional mechanisms, we believe that specific arrangement of receptors might be relevant. Therefore, based on the present results we propose that ligand-controlled dynamic arrays of receptors at the cell surface not only stimulate internalization but also trigger the recruitment and regulation of specific cytosolic molecules associated to the formation of the coated pit promoting in this specific case an apoptotic event.

Our study also identifies the scavenger receptor as a potential binding site for TPmy and provides a route to further characterize the role of paramyosins in several host-parasite relationships. Since paramyosin binding and uptake by the scavenger receptor may participate in the processing and/or presentation to B or T cells [44], this phenomenon may be also of relevance in the study of the immune response shown by infected hosts.

Acknowledgments

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