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Archives of Biochemistry and Biophysics 460 (2007) 100–106
ABB
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Activated α_2-macroglobulin induces cell proliferation and mitogen-activated protein kinase activation by LRP-1 in the J774 macrophage-derived cell line

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Received 27 November 2006, and in revised form 2 January 2007
Available online 23 January 2007

Abstract

The low-density lipoprotein receptor-related protein-1 (LRP-1) is an endocytic receptor of activated forms of the proteinase inhibitor α_2-macroglobulin (α_2M^*). It has been proposed that α_2M^* and LRP-1 modulate diverse cellular processes, including cell adhesion, proliferation, and migration, which are involved in inflammation and tumor progression. However, relatively little is known about the role of α_2M^*/LRP-1 interaction on these processes. In this work, we demonstrate that α_2M^* binding to LRP-1 induces cell proliferation and MAPK activation in the J774 macrophage-derived cell line, which were blocked by RAP, an antagonist of LRP-1-binding ligands, and by PD980059, a specific inhibitor for the Mek1-ERK1/2 pathway. In addition, we demonstrate that LPS, a bacterial product that it is known to down-regulate the LRP-1 expression on macrophage, abrogated the signaling activity triggered by α_2M^* on LPS-treated J774 cells. These results suggest that α_2M^*/LRP-1 interaction constitutes a key role in the macrophage functioning during inflammation and cancer.

Keywords: α-Macroglobulin; Endocytic receptor; LDL receptor; Proteinase inhibitor; Signal transduction

Alpha-2-macroglobulin (α_2M)[2] is a plasma proteinase inhibitor with broad specificity. Structurally, it is a tetramer composed of two non-covalently associated dimmers of disulfide-linked identical subunits (~180 kDa). α_2M contains a distinctive structure, characterized by a proteolysis-sensitive bait region sequence and an internal β-cysteinyl-γ-glutamyl thiol ester bond per subunit, which are susceptible to cleavage at the bait region by endopeptidases and to nucleophilic attack at thiol ester bonds by monoamines [1]. Consequently, α_2M undergoes an extensive conformation, alteration and compacting to become an activated form, designated α_2M^*, which recognizes the specific cell surface receptor, low density lipoprotein receptor-related protein-1 (LRP-1), a member of the LDL receptor gene family. However, α_2M^* is only recognized by LRP-1 and not by other LDL receptor members [2]. It has been demonstrated that α_2M^* is internalized by LRP-1 and, therefore, both molecules are implicated in the modulation of the extracellular activity of several serine- and metalloproteinases [3].

LRP-1 is a cell surface glycoprotein synthesized as a 600-kDa pro-receptor and post-translationally processed into 515- and 85-kDa chains that remain associated through non-covalent interactions [3]. LRP-1 is a typical scavenger receptor, which in addition to binding α_2M^*, also binds and internalizes multiple structurally and functionally diverse ligands including *Pseudomonas* exotoxin, lipoprotein lipase, apolipoprotein E-enriched lipoproteins, urokinase-, and

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[1] G.R. Bonacci and L.C. Cáceres contributed equally to this publication.
[2] *Abbreviations used:* α_2M, alpha-2-macroglobulin; LRP-1, low density lipoprotein receptor-related protein-1; RAP , receptor-associated protein; PIP, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; LPS, lipopolysaccharide; GST, glutathione *S*-transferase; FBS, fetal bovine serum; PMA, phorbol 12-myristate 13-acetate; NGF, nerve growth factor.

0003-9861/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2007.01.004

tissue-type plasminogen activator alone or in complexes with plasminogen inhibitors, tissue factor pathway inhibitor, lactoferrin, thrombospondin, and lipoprotein(a) [4]. These ligands do not compete with each other for binding, except for the receptor-associated protein (RAP), which blocks the binding of all known ligands to the receptor [3]. Although generally considered to be a scavenger receptor, increasing evidence indicates that LRP-1 has the potential to mediate cellular signaling [5–7]. The binding of lactoferrin, *Pseudomonas* exotoxin A, and lipoprotein lipase to LRP-1 on the macrophage cell surface triggers the hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) by phosphoinositide-specific phospholipase C (PLC), thus increasing cellular proliferation, intracellular inositol 1,4,5-trisphosphate (IP_3), and intracellular calcium levels [8].

Various signaling molecules such as hormones [9], growth factors [10], and matrix components [11] have been shown to alter LRP-1 levels in diverse cell types. Macrophage LRP-1 expression is subject to regulation by specific cytokines and bacterial products [12]. It has been reported that lipopolysaccharide (LPS) markedly decreased LRP-1 expression at the mRNA, antigen, and functional levels in macrophages [13,14], indicating that inducible changes in cellular expression of LRP-1 may represent a modification in the functional properties of macrophages against extracellular virulence factors of bacterial pathogens, such as *Pseudomonas* exotoxin A of *Pseudomonas aeruginosa* [13].

In the present study, we examined the effects of α_2M^* stimulation in the J774 macrophage-derived cell line. We found that α_2M^* binds to LRP-1 and increases cell proliferation and MAP kinase phosphorylation, which could be blocked by the pretreatment of J774 cells with RAP and LPS. These findings establish a novel mode of action elicited by the α_2M^*/LRP-1 interaction in macrophages, in which α_2M^* triggers intracellular signal transduction and cell proliferation via LRP-1.

Materials and methods

Materials

α_2M was purified from human plasma following a procedure previously described [15]. The native form of α_2M demonstrates proteinase inhibitory activity, but it is not recognized by LRP-1 [16]. The activated form (α_2M^*) was generated by incubating α_2M with 200 mM methylamine-HCl for 6 h at pH 8.2, as previously described [17]. Bacterial LPS (*Escherichia coli* 0127B8; protein content, <3.0%) was obtained from Sigma (St. Louis, MO). An expression construct, encoding RAP as a glutathione *S*-transferase (GST) fusion protein (GST-RAP), was provided by Dr. Joachim Herz (University of Texas Southwestern Medical Center, Dallas, TX). GST-RAP was expressed and purified as described before [18] and used without further modification. The primary antibodies used were as follows: antibodies against phosphospecific and total ERK1/2, phosphospecific and total Akt, phosphospecific c-jun, p38, and β-actin. These were purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA. Mouse anti-85-kDa chain of LRP-1 monoclonal antibody (clone 5A6) was generously provided by Dr. Dudley Strickland (University of Maryland School of Medicine, Rockville, MD). Secondary horseradish peroxidase-conjugated antibodies were obtained from Amersham Bioscience, Denmark.

Cell culture and methods

Mouse J774 macrophage-derived cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (50 U/ml), and streptomycin (50 μg/ml) at 5% CO_2, 95% humidity, and 37 °C. For Western blot analysis, J774 cells were lysated using 10 mM PBS, 150 mM NaCl, 1% Triton X-100, 0.5 % Sodium deoxycolate, 0.1% SDS, 0.2% NaN_3 and 0.1% NP40. Twenty micrograms of lysate were separated on 10% SDS–polyacrylamide gels and transferred to a nitrocellulose membrane (Amersham Bioscience, Denmark). Non-specific binding was blocked with 5% non-fat dry milk in Tris–HCl saline buffer containing 0.01% Tween 20 (TBS-T) for 60 min at room temperature. The membranes were incubated overnight with specific primary antibodies at 4 °C, washed three times with TBS-T, and then incubated with secondary horseradish peroxidase-conjugated antibodies for 1 h at room temperature. The specific bands were revealed by chemiluminescence reaction (Pierce, UK) and quantified by densitometric analysis using an image software (SCION Version free online).

Measurement of cell proliferation

Cell proliferation in J774 cells was measured essentially according to the method of Charlesworth and Rozengart [19]. Briefly, cultured cells (4×10^5 cells) were incubated in RPMI 1640 medium containing glutamine, penicillin, streptomycin, and 0.1% fatty acid free BSA for 2 h at 37 °C in a humidified CO_2 (5%) incubator as described above. The monolayers were washed three times with ice-cold Hank's balanced salt solution containing 10 mM Hepes (pH 7.4) and 3.5 mM $NaHCO_3$ (HHBSS), and a volume of RPMI medium was added. To each well, ^{3}H-thymidine (2 μCi/ml, 80 Ci/mmol) was added followed by the addition of various concentrations of α_2M^* (7, 20, and 60 nM) to the respective wells. The cells were incubated as above for 20 h. After incubation, the overlying media was removed and a volume of cold TCA (5%) was added to each well and left on ice for 30 min. The TCA was removed and cells were washed once more with TCA (5%) followed by washing three times with cold HHBSS. The cells were dissolved in 1 N NaOH, and radioactivity was determined on an aliquot of lysate by liquid scintillation counting. When the inhibitory effect on cell proliferation of GST-RAP (200 nM) and PD980059 (10 μM, Sigma, St. Louis, MO) was studied, these compounds were added 30 min prior to the addition of ligands and were present during the incubation period. For protein measurements, untreated cells, incubated identically, were washed and dissolved in 0.1 N NaOH, and protein was estimated according to Bradford [20].

Phosphorylation analysis of ERK1/2, c-jun, p38 and Akt

J774 cells (1×10^6 cells) were cultured in RPMI 1640 medium containing glutamine, penicillin, streptomycin, and 10% FBS, for 24 h at 37 °C in a humidified CO_2 (5%) incubator as described above. After aspirating the medium, fresh serum-free medium was added and cultured for 2 h. Then, the J774 cells were cultured with various concentrations of α_2M^* (7, 20, and 60 nM). Controlled time points were taken, and cell extracts were prepared and analyzed by SDS–polyacrylamide gel electrophoresis as described above. The membranes were probed, following the manufacturer's blotting instructions with phosphospecific antibodies, which recognize the phosphorylated state of ERK1/2, c-jun, p38 MAPK, and Akt, respectively. Treatments were normalized in parallel by assessment of protein loading, using phosphorylation-independent antibodies against total ERK1/2 and Akt. The membranes were then incubated with secondary horseradish peroxidase-conjugated antibodies. Then, the specific bands were revealed by chemiluminescence reaction and quantified by densitometric analysis as mentioned above. When the inhibitory effect on intracellular signaling of GST-RAP (200 nM) and PD980059 (10 μM) was studied, these compounds were added for 30 min in serum-free medium prior to the addition of ligands. To evaluate the effect of LPS on the LRP-1 down-regulation, J774 cells were cultured in RPMI 1640 medium containing glutamine, penicillin, streptomycin, and 10% FBS for 24 h at 37 °C in a humidified CO_2 (5%) incubator as described above. After aspirating the

medium, serum-free medium containing LPS (1 up to 100 ng/mL) was added and cultured for 24 h. Then, the cells were washed and stimulation with various concentrations of α_2M^* or phorbol 12-myristate 13-acetate (PMA: 100 μM) was performed in serum-free medium, with ERK1/2 phosphorylation being analyzed as described above. When the inhibitory effect on ERK1/2 activation of GST-RAP (200 nM) was studied in LPS-treated J774 cells, this compound was added for 30 min in serum-free medium prior to the addition of ligands.

Statistical treatment of data

Comparison of quantitative data was performed using the non-parametric Mann–Whitney *U* test.

Results

α_2M^* increases the cell proliferation of J774 cells

To evaluate the effect of α_2M^* on the cell proliferation, the incorporation of ^{3}H-thymidine into DNA was quantified in J774 cells stimulated for 24 h with various concentrations of α_2M^* (7, 20, and 60 nM). Fig. 1 shows that for concentrations from 20 nM, α_2M^* produced a significantly increased of ^{3}H-thymidine uptake, compared with buffer-treated J774 cells. Considering that α_2M^* binds LRP-1, but not other members of the LDL receptor family, and that its binding can be inhibited by RAP [3,21], we evaluated whether the effect of α_2M^* on cell proliferation is mediated by LRP-1 in J774 cells cultured in the presence of 200 nM GST-RAP. Fig. 1 shows that RAP significantly blocked the α_2M^*-induced cell proliferation, compared with controls.

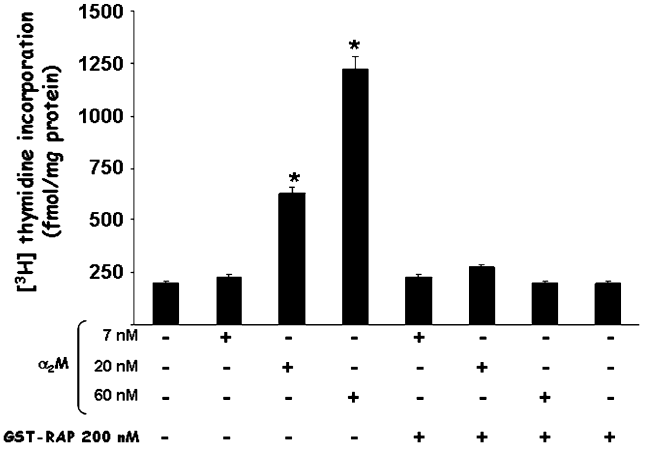

Fig. 1. Effect of α_2M^* on ^{3}H-thymidine incorporation into DNA and its modulation by GST-RAP in J774 cells. Cultured cells (4 x 10^5 cells) were incubated in RPMI 1640 medium containing glutamine, penicillin, streptomycin, and 0.1% fatty acid free BSA for 2 h at 37 °C. To each well ^{3}H-thymidine (2 μCi/ml, 80 Ci/mmol) was added followed by the addition of various concentrations of α_2M^* (7, 20, and 60 nM) to the respective wells and incubated as above for 20 h. The cells were dissolved in 1 N NaOH and radioactivity was determined on an aliquot of lysate by liquid scintillation counting. When the inhibitory effect of RAP on cell proliferation was studied, 200 nM GST-RAP was added 30 min prior to the addition of α_2M^* and this compound was present during the incubation period. The values are means ± SE from two independent experiments performed in quadruplicate and are expressed as fmol of ^{3}H-thymidine uptake/mg of protein. The symbol * denotes *p* values (<0.01) significantly different to controls. Experimental details are described in Materials and methods.

Although RAP is also a LRP-1 ligand, it did not induce cell proliferation when added alone to J774 cell cultures. Thus, we conclude that α_2M^*-increased J774 cell proliferation is mediated by LRP-1.

LRP-1 mediates the α_2M^*-induced intracellular signaling in J774 cells

In order to evaluate whether the α_2M^*-induced J774 cell proliferation is subsequent to the activation of intracellular signaling, we analyzed different mediators of signaling pathways, including ERK1/2, c-jun, p38, and Akt activation after the α_2M^* stimulation on J774 cells. Fig. 2 shows that 60 nM α_2M^* induces significant ERK1/2 phosphorylation from 10 min of incubation in J774 cells, increasing to up to ~6 times the control values. Under the same conditions, 60 nM α_2M^* also induced significant activation of c-jun from 10 min of stimulation, but it was lower than for ERK1/2 phosphorylation since it only increased to up to ~2 times the control values. On the other hand, 60 nM α_2M^* did not induce a significant activation of Akt or p38, compared to controls. In similar time-course experiments, we demonstrated that ERK1/2 phosphorylation also occurred with 20 nM, but not with 7 nM of α_2M^* (data not shown).

To evaluate whether the α_2M^* binding to LRP-1 triggers the activation of ERK1/2 phosphorylation in J774 cells, we used GST-RAP (200 nM) to inhibit the α_2M^*/LRP-1 interaction. Fig. 3 shows that the pretreatment of J774 cells with GST-RAP significantly blocked the ERK1/2 phosphorylation after α_2M^* stimulation. Thus, these results indicate that the α_2M^*/LRP-1 interaction triggers ERK1/2 phosphorylation in J774 cells.

It has been demonstrated that the promotion of cell proliferation by several growth factors and cytokines is determined by the activation of the Mek1-ERK1/2 pathways [22,23]. Herein, we evaluate the effect of PD980059, a specific inhibitor of Mek1, on the α_2M^*-induced ERK1/2 phosphorylation in J774 cells. Fig. 4a shows that PD980059 significantly blocked the ERK1/2 activation under α_2M^* stimulation as well as the PMA-induced ERK1/2 phosphorylation in control cells. In the same way, the pretreatment of J774 cells with PD980059 for 24 h significantly inhibited the α_2M^*-induced cell proliferation (Fig. 4b). These results taken together indicate that α_2M^* induces cell proliferation by MAPK-ERK1/2 activation via LRP-1 in J774 cells.

LRP-1 down-regulation abrogates the α_2M^*-induced MAPK-ERK1/2 phosphorylation

It has been reported that LPS down-regulates the LRP-1 expression in macrophages [13,14], which could be used as a tool to study the specific intracellular signaling activation of LRP-1 after α_2M^* stimulation in the J774-derived macrophage cell line. Herein, we evaluate by Western blot analysis the LRP-1 expression in pre-treated J774 cells

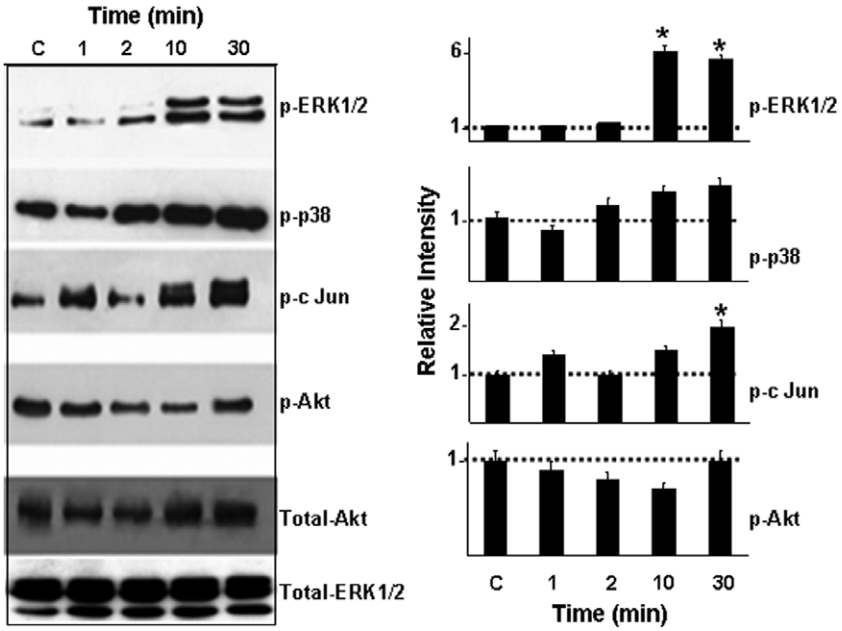

Fig. 2. α_2M^*-induced intracellular signaling in J774 cells. Total proteins were extracted from J774 cells treated for different times with 60 nM α_2M^*. After electrophoresis and electrotransfer to the nitrocellulose membrane, phosphorylated ERK1/2, c-jun, p38, and Akt were detected with primary antibodies and revealed with secondary horseradish peroxidase-conjugated antibodies. The protein loading control of total ERK1/2 and Akt are also shown. The peroxidase-conjugated antibodies were detected by enhanced chemiluminescence as described in Material and methods. The incubation times are indicated in the figure. The bars are the relative intensity of p-ERK1/2/total ERK1/2, c-jun/total ERK1/2, p38/total ERK1/2 and p-Akt/total Akt respect to controls (dotted line), representing means ± SE from triplicate experiments. The symbol ∗ denotes p values (<0.01) significantly different to controls.

with various concentrations of LPS (from 1 up to 100 ng/mL) for 24 h. Fig. 5a shows that LRP-1 is undetected in J774 cells after the treatment with 100 ng/mL. Then, using this model of LPS-treated J774 cells we determined the MAPK-ERK1/2 activation after α_2M^* stimulation. Fig. 5b shows that the α_2M^* induction of ERK1/2 phosphorylation was significantly abrogated in LPS-treated J774 cells with respect to the signaling activation observed in LPS-nontreated J774 cells. As a control, we observed that the treatment of J774 cells with LPS 100 ng/mL for 24 h had no effect on the ability of 100 μM PMA to induce ERK1/2 phosphorylation, compared to PMA-induced signaling activation in LPS-nontreated J774 cells. Thus, we can conclude that the MAPK-ERK1/2 activation induced by α_2M^* is mediated by LRP-1 in J774 macrophage cells.

Discussion

In this work, we demonstrate that the α_2M^*/LRP-1 interaction induces cell proliferation and MAPK activation in the J774 macrophage-derived cell line. Both the cell proliferation and intracellular signaling activity induced by α_2M^* were significantly blocked by RAP, an antagonist of LRP-1-binding ligands, and by PD980059, a specific inhibitor for the Mek1-ERK1/2 pathway. In addition, we also demonstrated that LPS, which is known to down-regulate the LRP-1 expression on macrophage [13,14], also abrogated the signaling activity triggered by α_2M^* on LPS-treated J774 cells.

Although generally considered to be an endocytic receptor, it has been demonstrated that LRP-1 can mediate intracellular signaling [3,7], and that different receptor-associated ligands activate transductional pathways, with sometimes opposing effects. The binding of apolipoprotein E to LRP-1 inhibits smooth muscle cell migration via protein kinase A activation [24]; conversely, LRP-1 mediates the migration-promoting activity of PAI-1 through activation of the Jak/Stat signaling pathway [25] . With respect to α_2M^*, it has been reported that the binding to LRP-1 promotes neurite outgrowth in primary cortical neurons by controlling the calcium influx via *N*-methyl-D-aspartate receptors [26–28]. Previous studies, from our laboratory and others, demonstrated that rat and human activated α-macroglobulins inhibit the nerve growth factor (NGF)-promoted neuritogenesis through LRP-1 in PC12 cells [17,29,30]. In this work, we demonstrate that α_2M^* induces cell proliferation through MAPK-ERK1/2 phosphorylation in J774 cells, which constitutes the first evidence about LRP-1-mediated signal transduction after α_2M^* stimulation in macrophage-derived cells. Both the α_2M^*-induced cell proliferation and the ERK1/2 phosphorylation were blocked by RAP, an inhibitor of the ligand binding to LRP-1. Although RAP can bind to other members of the LDL receptor family, α_2M^* only recognizes LRP-1 [3,21,31]. This indicates that the α_2M^*/LRP-1 interaction is involved in the activation of J774 cells.

Recently, it was reported that tPA, another LRP-1-associated ligand, promotes β-chain LRP-1 phosphorylation

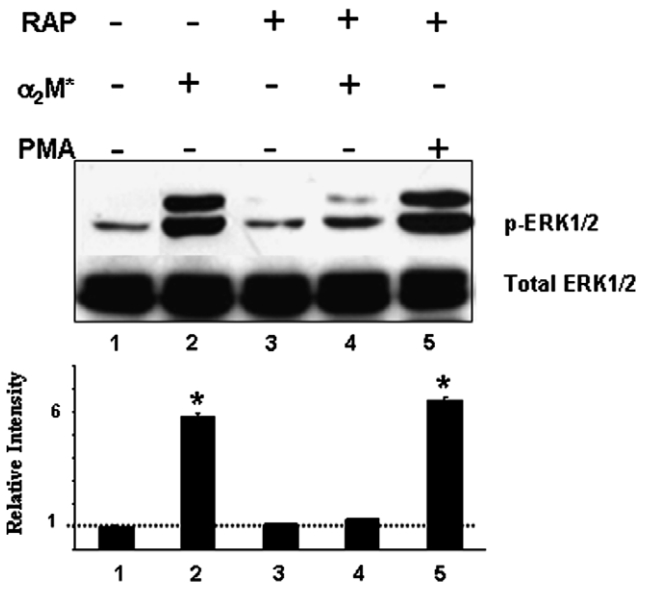

Fig. 3. Inhibitory effect of RAP on the α_2M^*-induced ERK1/2 phosphorylation in J774 cells. Total proteins were extracted from J774 cells treated with 60 nM α_2M^* for 10 min. Previous to α_2M^* stimulation, the cell cultures were incubated with 200 mM GST-RAP for 30 min in serum-free medium. After electrophoresis and electrotransfer to the nitrocellulose membrane, phosphorylated ERK1/2 was detected with primary antibody and revealed with secondary horseradish peroxidase-conjugated antibody. The protein loading control of total ERK1/2 is also shown. The peroxidase-conjugated antibodies were detected by enhanced chemiluminescence as described in Material and methods. PMA (100 μM) was used as a positive control of ERK1/2 phosphorylation, and previous its addition J774 cells were cultured in the presence of 200 mM GST-RAP for 30 min in serum-free medium as indicated above. The bars are the relative intensity of p-ERK1/2/total ERK1/2 respect to controls (dotted line), representing means ± SE from triplicate experiments. The symbol ∗ denotes p values (<0.01) significantly different to controls.

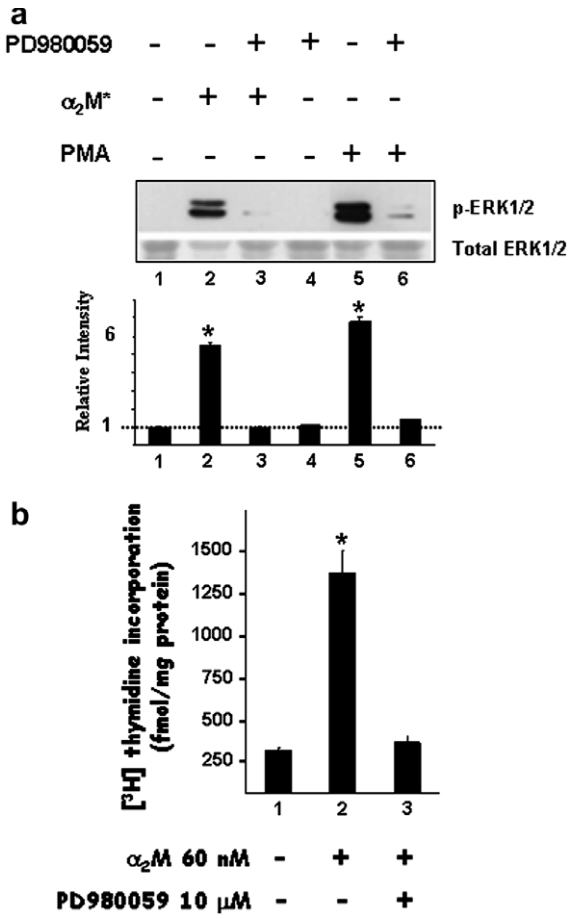

Fig. 4. (a) Inhibitory effect of PD980059 on the α_2M^*-induced ERK1/2 phosphorylation in J774 cells. Total proteins were extracted from J774 cells treated with 60 nM α_2M^* for 10 min. Previous to α_2M^* stimulation, the cell cultures were incubated with 10 μM PD980059 for 30 min in serum-free medium. After electrophoresis and electrotransfer to the nitrocellulose membrane, phosphorylated ERK1/2 was detected with primary antibody and revealed with secondary horseradish peroxidase-conjugated antibody. The protein loading control of total ERK1/2 is also shown. The peroxidase-conjugated antibodies were detected by enhanced chemiluminescence as described in Material and methods. PMA (100 μM) was used as a positive control of ERK1/2 phosphorylation, and previous its addition J774 cells were cultured in the presence of 10 μM PD980059 for 30 min in serum-free medium as indicated above. The PMA-induced ERK1/2 phosphorylation was completely blocked by the treatment of J774 cells with PD980059. The bars are the relative intensity of p-ERK1/2/total ERK1/2 respect to controls (dotted line), representing the mean ± SE from triplicate experiments. The symbol ∗ denotes p values (<0.01) significantly different to controls. (b) The inhibitory effect of PD980059 on the α_2M^*-induced proliferation in J774 cells. Cultured cells (4×10^5 cells) were incubated in RPMI 1640 medium containing glutamine, penicillin, streptomycin, and 0.1% fatty acid free BSA for 2 h at 37 °C. To each well ^{3}H-thymidine (2 μCi/ml, 80 Ci/mmol) was added followed by the addition of α_2M^* (60 nM) to the respective wells and incubated as above for 20 h. The cells were dissolved in 1 N NaOH and radioactivity was determined on an aliquot of lysate by liquid scintillation counting. When the inhibitory effect of PD980059 on cell proliferation was studied, this compound (10 μM) was added 30 min prior to the addition of α_2M^* and was present during the incubation period. The values are means ± SE from two independent experiments performed in quadruplicate and are expressed as fmol of ^{3}H-thymidine uptake/mg of protein. The symbol ∗ denotes p values (<0.01) significantly different to controls. Experimental details are described in Materials and methods.

and signal transduction via Mek1-ERK1/2 in the NRK-49F fibroblast-derived cell line [6]. Considering our results, further studies are required to determine whether Mek1-ERK1/2 activation induced by α_2M^* in J774 cells is also mediated by the β-chain LRP-1 phosphorylation in association with cytoplasmic adaptor proteins such as Shc proteins [4]. Another possibility of Mek1-ERK1/2 activation by α_2M^* in J774 cells could involve the calcium entry from external medium, mediated by calcium-permeable cationic channels expressed on the cell surface of macrophages such as L-type Ca^{2+} channels, which play a key role in cell proliferation and MAPK activation [32]. However, a combination of both mechanisms could also be considered. Therefore, we are currently performing new studies to investigate these possibilities.

There is evidence in the literature indicating that α_2M^* induces intracellular signaling via a chaperone protein, termed Grp-78, in peritoneal macrophages and the 1-LN prostate derived cell line, which is independent of LRP-1 [33,34]. Basically, it has been demonstrated that the α_2M^* interaction with Grp-78 activates proliferative and anti-apoptotic signaling, mediated by ERK1/2, PI3-kinase, MAPK-p38, and PI_3P followed by intracellular calcium

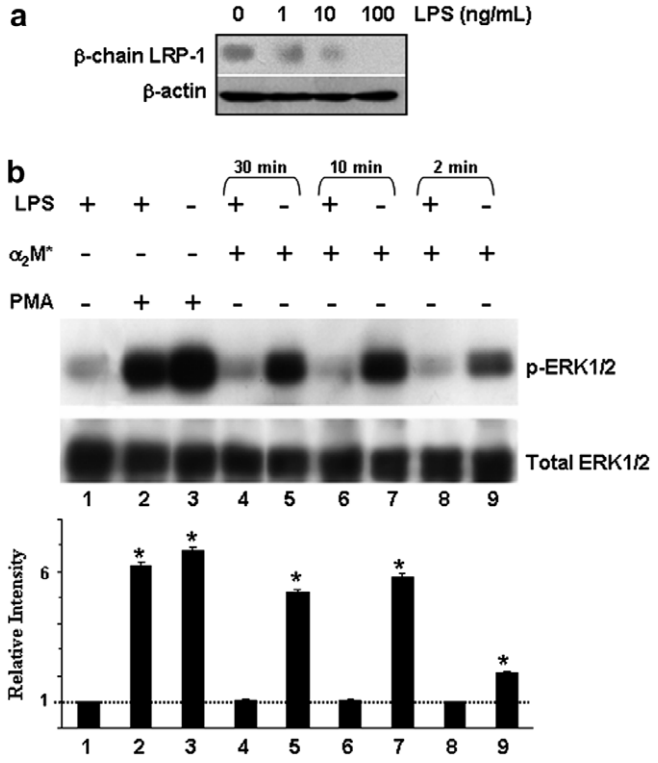

Fig. 5. (a) Analysis of LRP-1 expression in LPS-treated J774 cells. Total proteins were extracted from J774 cells treated for 24 h with serum-free medium, or LPS at the following concentrations: 1, 10, and 100 ng/mL. After electrophoresis and electrotransfer to the nitrocellulose membrane, LRP-1 was detected with mouse anti-85-kDa chain of LRP-1 monoclonal antibody (clone 5A6) and revealed with a secondary horseradish peroxidase-conjugated antibody. The protein loading control of β-actin is also shown. The peroxidase-conjugated antibodies were detected by enhanced chemiluminescence. (b) Western blot analysis of α_2M*-induced ERK1/2 phosphorylation in LPS-treated J774 cells. Total proteins were extracted from J774 cells treated for different times with 60 nM α_2M*. Previous to α_2M* stimulation, J774 cells were cultured in the presence of 100 ng/mL LPS for 24 h in serum-free medium. As a control, J774 cells were cultured in the absence of LPS for 24 h in serum-free medium and then stimulated for different times with 60 nM α_2M*. After electrophoresis and electrotransfer to the nitrocellulose membrane, phosphorylated ERK1/2 was detected with primary antibody, and revealed with secondary horseradish peroxidase-conjugated antibody. The protein loading control of total ERK1/2 is also shown. The peroxidase-conjugated antibodies were detected by enhanced chemiluminescence as described in Material and methods. The incubation times of α_2M* are indicated in the figure. PMA (100 μM) was used as a positive control of ERK1/2 phosphorylation, and previous its addition J774 cells were cultured in the presence or absence of 100 ng/mL LPS for 24 h in serum-free medium as indicated above. The PMA-induced ERK1/2 phosphorylation was unaffected by the treatment of J774 cells with LPS. The bars are the relative intensity of p-ERK1/2/total ERK1/2 respect to controls (dotted line), representing the mean ± SE from triplicate experiments. The symbol * denotes p values (<0.01) significantly different to controls.

mobilization [34–37]. Differentially, RAP, an antagonist of all known LRP-1 ligands, does not inhibit the binding of α_2M* to Grp-78 [8]. However, our results demonstrate that α_2M* promotes intracellular signaling in the J774 macrophage-derived cell line, characterized by the activation of MAPK-ERK1/2 and c-jun mediators; conversely, the activaction of other signaling pathway mediators, including Akt and p38, were not observed. In addition, both cell proliferation and MAPK-ERK1/2 signaling induced by α_2M* in J774 cells was completely inhibited by RAP. Perhaps Grp78 is not expressed in J774 cells, since we could not detect this protein by Western blot analysis from protein extracts of J774 cells (data not shown). Therefore, these results taken together clearly indicate that LRP-1, and not Grp-78, is mediating the α_2M*-induced signal transduction in the J774 macrophage-derived cell line. Clearly, further studies are needed to elucidate functional differences between Grp-78 and LRP-1.

Finally, and in order to confirm the specific role of LRP-1 in the α_2M*-induced ERK1/2 activation, we used LPS as a tool to supress the LRP-1 expression [12–14]. Our results demonstrate that the α_2M*-induced ERK1/2 phosphorylation was not detected in LPS-treated J774 cells, which clearly indicates that LRP-1 is mediating the MAPK activation by α_2M*.

In conclusion, we have demonstrated that the α_2M*/LRP-1 interaction induces cell proliferation and MAPK activation in the J774 macrophage derived cell line. These results could have implications for the macrophage activation during inflammatory stages, infectious diseases and cancer.

Acknowledgments

This work was supported in part by grants from SECyT (Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba), Agencia Nacional de Ciencia de la República Argentina (FONCyT: BID 1728/OC-AR PICT N° 05-13945), and CONICET (Consejo de Investigaciones Científicas y Tecnológicas de la República Argentina). G.R.B. is a Ph.D. fellow of SECyT-UNC and L.C.C. is a Ph.D. fellow of CONICET. M.C.S. and G.A.C. are members of the Research Career of CONICET. We are grateful to Dr. Paul David Hobson, native speaker, for checking the language of this manuscript.

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