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Toxoplasma gondii: Expression of GRA1 gene in endoplasmic reticulum promotes both growth and adherence and modulates intracellular calcium release in macrophages

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ABSTRACT

In this study, effects of GRA1 organelle-targeted expression on macrophage functions were investigated. The recombinant plasmid pCMV/myc/ER-GRA1 was constructed and then was transfected into murine macrophage RAW264.7 by Lipofectamine, selected by resistance of G418. The selected mono-clone cell line was named ER-GRA1-RAW264.7. The expression of GRA1 was localized in ER of ER-GRA1-RAW264.7 cells by indirect immunofluorescence detection. GRA1 mRNA expression level in ER-GRA1-RAW264.7 cell was significantly enhanced with a concomitant increase in its growth and adherence activity. Fluorescence intensity of intracellular calcium in ER-GRA1-RAW264.7, ER-ctrl-RAW264.7 and RAW264.7 cells in the presence of 1 mmol/l arachidonic acid (AA) were assayed by confocal microscopy using calcium-sensitive dye, Fluo-3 AM. Cytoplasm $[Ca^{2+}]i$ peaked at about 18 s after AA treatment, and cytoplasm $[Ca^{2+}]i$ of RAW264.7 cell almost instantly stepped up after AA was added, and peaked in 3 s, with a minor cytoplasm $[Ca^{2+}]i$ vibration subsequently. These results demonstrated that the expression of GRA1 in ER of macrophages promotes both growth and adherence of macrophages and modulates the intracellular calcium release stimulated by AA.

1. Introduction

Toxoplasma gondii, an obligate intracellular protozoan parasite, generally resides in parasitophorous vacuole (PV) which resists typical phagosome lysosome fusion, and induces a mild asymptomatic infection in immune-competent individuals (Sibley et al., 1985). In humans, this relatively benign infection may activate under conditions of immune-suppression, resulting in toxoplasmosis encephalitis and other complications, and can also result in severe birth defects or abortion during the first trimester of pregnancy (Scorza et al., 2003; Calabrese et al., 2008).

T. gondii is highly specialized for regulation of protein secretion. *Toxoplasma* cells contain micronemes, rhoptries and dense-granule organelles that secreted a variety of proteins (MIC, ROP and GRA proteins) following invasion of the host cell, which may play an important role in the structure modifications of parasitophorous vacuole and have comparative strong immunogenicity in human body and the experimental infection (Achbarou et al., 1991a; Leriche and Dubremetz, 1991; Beckers et al., 1994; Cesbron-Delauw, 1994; Sibley et al., 1995; Da Gama et al., 2004; Magno et al., 2005; Mercier et al., 2005).

Functions of GRA proteins have been investigated, such as genetic information and protective immunity. For example, GRA3, GRA5, GRA7 and GRA8 are detected as PVM-associated proteins (Achbarou et al., 1991b; Lecordier et al., 1993; Bonhomme et al., 1998; Carey et al., 2000; Ahn et al., 2006; Kim et al., 2008). GRA1 (23 kDa) is a calcium binding protein uniformly distributed throughout the lumen of the vacuole (Cesbron-Delauw et al. 1989) and is associated with the network through peripheral interactions (Sibley et al., 1995). Recently, many investigators are studying the protective immunity induced by GRA1 (Scorza et al., 2003; Jongert et al., 2007, 2008). However, besides the genetic regulation and immunity modification of GRA1, the correlation between GRA1 and ER of host cells and the role of GRA1 of *T. gondii* in biological features in host cells remain unclear. In this study, to further understand GRA1 functions, we investigated the effects of GRA1 organelle-targeted expression on macrophage functions by stable transfection with the pShooter vector pCMV/myc/ER. The results demonstrate that the expression of GRA1 of *T. gondii* in endoplasmic reticulum promotes both growth and adherence, and modulates intracellular calcium release in macrophages.

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2. Materials and methods

2.1. Reagents

Taq DNA polymerase, alkaline phosphatase, restriction endonuclease (Xho I, Pst I, Sal I) and T4-DNA ligase were from BioLab company. Trizol® and One-Step RT-PCR kit were obtained from Invitrogen Life Technologies and all culture reagents were from Gibco-BRL. DNA Extract kit was from Watson BioTechnologies (Shanghai, China). Lysozyme solution and RNase A were provided by Qiagen. G418, liposome, *Escherichia coli* Top10 and plasmids pShooter organelle-targeted vector was purchased from Invitrogen Company (pCMV/myc/ER).

2.2. T. gondii tachyzoites

Male BALB/c mice, average weight 18–22 g, were purchased from Shanghai B&K experimental animal Limited. The RH strain used was obtained from animal facilities of our laboratory. The tachyzoites were cultured by intraperitoneal inoculation of male BALB/c mice. The parasites were harvested from mice by rinsing of the peritoneal cavity with 5 ml PBS (pH 7.2). In order to exclude a possible contamination, the tachyzoites were also sorting from the host cells by filtration through polycarbonate membrane filters (pore size 3 μm, Sigma) and centrifuged at 500 g for 10 min. Purity was monitored by light microscopy.

2.3. Cloning of the GRA1 gene

Total RNA of *T. gondii* was isolated as recommended by the manufacturer's instructions. After separation of RNA, reverse transcription of RNA and cDNA amplification were performed using Trizol®, in accordance with manufacturer's instructions. Briefly, cDNA synthesis was performed at 50 °C for 30 min. After 2 min of initial denaturation at 94 °C, the samples were subjected to 35 cycles of denaturation (94 °C, 15 s), annealing (56 °C, 30 s), and extension (72 °C, 1 min), followed by a final extension at 72 °C for 7 min. Total RNA (2 μg) obtained from the cells was reverse-transcribed using an oligo(dT) adaptor as a primer to produce cDNAs. The specific cDNA probe for the GRA1 gene was amplified by reverse transcription (RT)-polymerase chain reaction (PCR) using the selective primers and cloned in pShooter vector. The primers used were sense (GRA1-ERF): 5′ -AAA ACT GCA GGT GCG TGT GAG CGC TAT TG -3′ (Pst I); anti-sense (GRA1-ERR): 5′ -CCG CTC GAG CTC TCT CTC TCC TGT TAG G -3′ (Xho I), as described by Cesbron-Delauw et al. (1989). Band density of the amplified DNAs was measured after visualization on an UV transilluminator.

2.4. Construction of expression and transfer vectors

The pShooter vector pCMV/myc/ER was purchased from Invitrogen. The insert, tachyzoites GRA1 was amplified by PCR from cloned DNA fragments. Briefly, sense and anti-sense primers were designed to contain a corresponding restriction site. All plasmids were transformed into strain *E. coli TOP10* and propagated. Plasmid integrity was checked by agarose gel electrophoresis after digestion with restriction enzymes individually. The DNA concentration was determined by absorbance at 260 nm. The ER-targeted vectors that contain the ER retention signal were designated GRA1-ER. Confirmation of proper ligation was done by DNA sequencing.

2.5. Cell culture

Raw264.7 cells, a murine macrophage cell line (Shanghai Cell-Biological Institute, Shanghai, China), were cultured in RPMI1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Raw264.7 cells were plated at a density of 2–3 $\times$ 10^6/ml and pre-incubated for 24 h at 37 °C. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Medium was changed every 3–4 days and 0.25% trypsin was used for transfer of culture. For all experiments, cells were grown to 80–90% confluence and were subjected to not more than 20 cell passages. For cell proliferation assay, cells are treated in 96 well plates for trypan blue exclusive assay. Rate of growth of each cell line was calculated by counting the total number of living cells in duplicate wells every day for about 10 days.

2.6. Stable plasmid transfection

Cells were transfected using Lipofectamine according to the manufacturer's instructions (Invitrogen). Cells were plated 24 h before transfection at a density of 3 $\times$ 10^4 cells in 24-well plates. For stable transfection, 5 μl of Liopfectamine was mixed with 0.4 μg of P24 plasmid in 50 μl of serum-free medium. Cells were transfected by addition of RPMI1640 containing plasmid and Lipofectamine and then incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 16 h. After addition of 0.5 ml RPMI 1640 with 10% fetal bovine serum, cells were incubated for additional 48 h at 37 °C and 400 μg/ml of G-418 was added to select the resistant colonies.

2.7. Free calcium concentration measurement

Fluorescent emission spectra within living cells were recorded using a microspectrofluometer (RF-5301 PC, Shimadzu, Japan) as previously described (Toborek et al., 1997). Briefly, cells were washed with serum-free RPMI 1640 and loaded with 2.5 mmol/l Fura-2/AM for 80 min at 37 °C for each assay, Fura-2/AM-loaded cells were suspended to 1 $\times$ 10^8/ml BSS containing 0.2% BSA, vibrated at 37 °C and were analysed by a fluorospectrophotometer. $[Ca^{2+}]i$ was determined by Dual Wavelength Ratios. Super Ion Probe Software was used to determine the fluorescence emission spectra in the 300–450 nm range and the ratio of both emission intensities (I340/I380 nm).

Indirect immunofluorescence detection of localization of GRA1 in ER-GRA1-RAW264.7 cells.

Stable transfected macrophage cells (ER-GRA1-RAW264.7) were grown to 30% confluence on 22 mm glass coverslips in 6-well plates (Corning Incorporated, NY, USA) and transfected with pDsRed2-ER (Clontech). Forty-four hours later, cells were fixed by immune staining fix solution (Beyotime Institute of Biotechnology, Jiangsu, China) in 4 °C overnight. Then cells were washed in PBS and were put in 0.5% Triton X-100 for 10 min, then rehydrated in PBS for 20 min, and blocked in PBS containing 5% bovine serum albumin (BSA) and 2% ovine serum. Cells were further incubated with primary antibody (anti-myc antibody, Invitrogen) at 1:500 dilutions overnight. Then cells were washed in PBS for 5 min. Cells were then incubated in secondary antibody (anti-mouse IgG-FITC antibody) at 1:64 dilutions for 1.5 h. Finally cells were added 1 ml DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride, Sigma) diluted in PBS (final concentration 1.0 μg/ml). Cover slips were mounted with glycerol and visualized by a Zeiss LSM 510 confocal laser scanning microscope at 557 nm for pDsRed2-ER, 475 nm for FITC and 346 nm for DAPI with a 40× oil immersion lens.

2.8. Calcium imaging

Cells were labeled at 37 °C in dark for 30 min with Fluo-3 AM diluted in differentiation medium to a final concentration of 10 μM from a stock solution of 1 mM Fluo-3 AM and 20 mM pluronic F-127. Cells grown on cover slips were incubated in 1 ml of a solution containing 150 mM NaCl, 5 mM KCl, 2 mM CaCl 2,

1 mM MgCl2, 10 mM HEPES (*N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulphonic acid) and 4 mM glucose, pH 7.4 for an additional 15 min at room temperature. Individual experiments lasted less than 1 h after the addition of Fluo-3 AM. Images of calcium signal were obtained using an MRC-1024ES confocal laser scanning microscope (LSCM, BIO-RAD company) and OS/Z, WARP image processing software (IBM company). The same area of the cell was analyzed every time. Images were scanned at a frame rate of 0.3 s per frame and 30 frames were obtained to record a total of 9 s per stimulation. Image intensity within an outlined area was scanned in WARP and saved to a spreadsheet (Microsoft Excel 2000, Microsoft, Inc., and Redmond, WA). The three curves for each condition were averaged and normalized to the three measurements prior to stimulation. Concentration–response curves were performed at least five times for each condition.

2.9. Statistical analysis

Data were presented as mean ±SEM. All experiments were performed two or three times, and the results were analyzed by Student's. A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. GRA1 gene amplification and identification of recombinant plasmid

Total cellular RNA was isolated from *T. gondii* RH strain as PCR template and RT-PCR products were identified by 1% agarose gel electrophoresis. As shown in Fig. 1, one specific band appeared on 572 bp position, agreed with the expected length. Blank control without template didn't appear bands. Transformed clones ER-GRA1 were selected from selective antibiotics ampicillin LB plates. The plasmids were extracted by plasmid isolation kit. The specific band of digested band, agreed with expected length, was detected. The band amplified by PCR with recombinant plasmid DNA, agreed with the predicted size 572 bp (Fig. 2).

3.2. Selection of positive cell clone by stable transfection

Recombinant plasmids were stable transfected into RAW264.7 cells by sub-cellular vectors under normal culture medium with the specific selective antibiotics G418 (400 mg/ml). The selected positive mono-cloning cell was designated as ER-GRA1-RAW264.7. Mono-cloning RAW264.7 cells were kept growing with 400 mg/ml G418 in the next three weeks (Fig. 3).

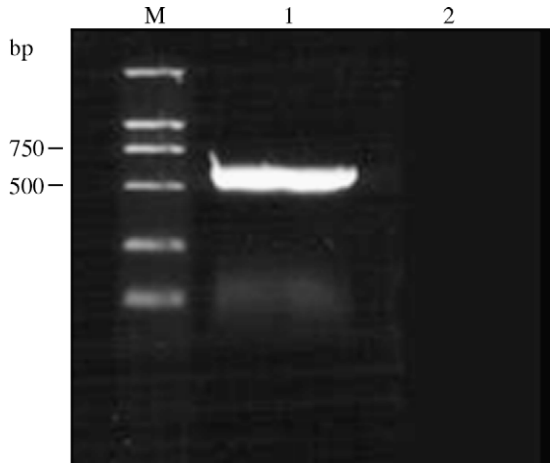


Fig. 1. Gel separation of GRA1 RT-PCR products amplified from *T. gondii* RH strain. Products were of the predicted sizes 572 bp. M, marker; 1, RH strain GRA1 gene from tachyzoites; 2, blank control.

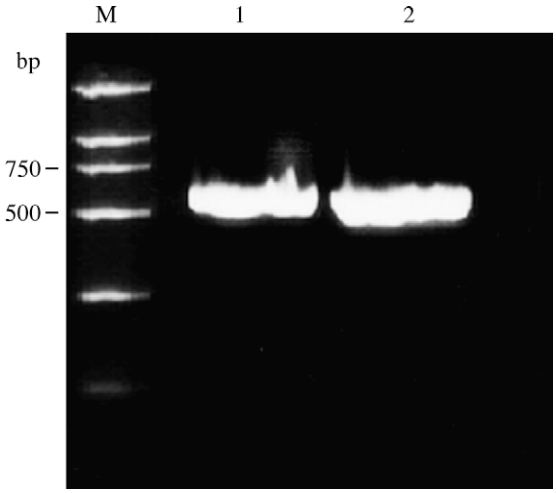


Fig. 2. Identification of recombinant plasmid pCMV/myc/cyto-GRA1 by 1% agarose gel electrophoresis for PCR amplified products. M, marker; 2, 3 – Amplified fragment from two pCMV/myc/cyto-GRA1 mono-clone.

3.3. Expression of GRA1 mRNA in the positive cell clone

GRA1 mRNA expression was detected by RT-PCR and then was analyzed by Syngene gel imaging system. As shown in Fig. 4, GRA1 mRNA expression level of four clones of ER-GRA1-RAW264.7 were 46.25 ± 2.46, 56.25 ± 2.75, 114.68 ± 4.67 and 49.24 ± 6.26, respectively. The highest expressed cell was picked out for the following experiments.

3.4. Localization of GRA1 in ER of ER-GRA1-RAW264.7 cells

To determine localization of GRA1 in ER-GRA1-RAW264.7 cells, pDsRed2-ER is designed for fluorescent labeling of the endoplasmic reticulum in living cells. As shown in Fig. 5, the fused myc tag GRA1 was labeled with mouse anti-myc antibody and anti-mouse IgG-FITC antibody and observed with laser scanning confocal microscope, which showed green and just distributed its light intensity similar to red obtained from pDsRed2-ER. Merged GRA1 and pDsRed2-ER co-localized and showed yellow, which confirmed the GRA1 localized on ER.

3.5. Effects of GRA1-ER-targeted expression on biological functions of macrophages

To investigate the effects of GRA1-ER-targeted expression on biological functions of macrophages, growth and adherence of macrophage RAW264.7, ER-GRA1-RAW264.7 and ER-ctrl-RAW264.7 were observed. In ER-GRA1-RAW264.7, more than half cells started to be adherent in 15–30 min after passage, some cells even were observed to spread out; 1 h later, 86.7% of cells showed adherence with spindle-shape partly; 4 h later, 97.3% of cells showed adherence and cells fully spread out, with spindle shape or oval shape; after 24 h, cells grew interlace. And the average time for showing adherence of macrophage RAW264.7 and ER-ctrl-RAW264.7 prolonged for 4–8 h, compared to ER-GRA1-RAW264.7.

The growth status of macrophage RAW264.7, ER-GRA1-RAW264.7 and ER-ctrl-RAW264.7 were observed and calculated, based on the average for three bottles of cells by every strain. As shown in Fig. 6, macrophage RAW264.7 cells grew slowly the first

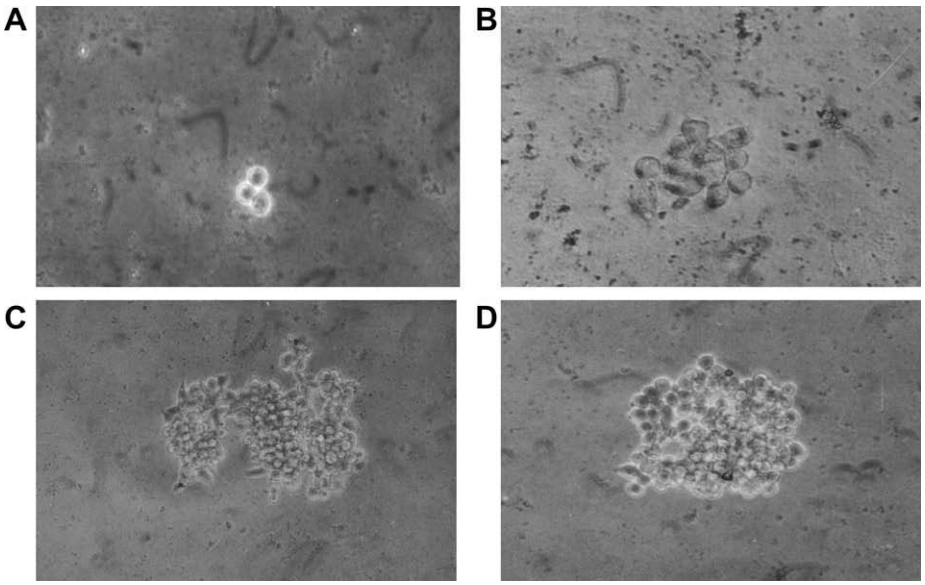


Fig. 3. Process of stable transfect ion selected by G418. Continual culture of selected mono-clone ER-GRA1 RAW264.7, from day 15 (A), day 16 (B), day 18 (C) and day 22 (D) after transfect ion. The morphology of RAW264.7 cells transfected by GRA1 gene was monitored using a phase-contrast microscope.

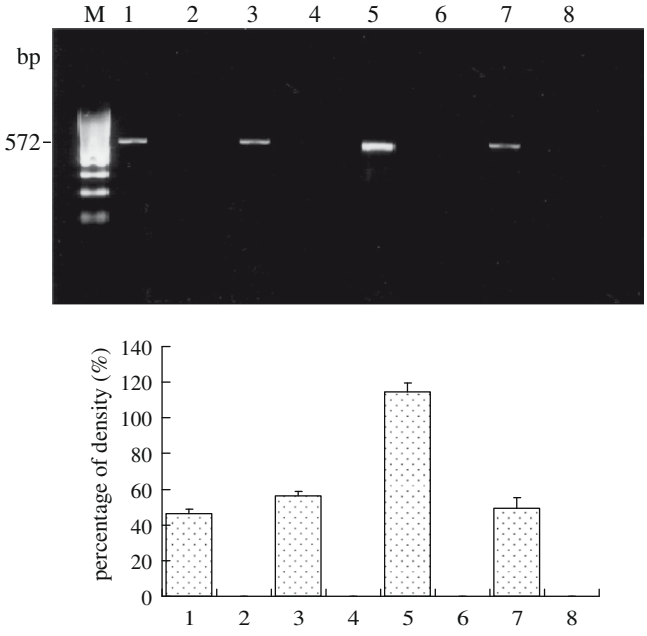


Fig. 4. GRA1 mRNA expression levels in macrophage by organelle-targed transfection. It was determined by RT-PCR analysis as described in "materials and methods". The densitometric analysis was reported in the histogram and revealed the quantitative levels of GRA1 expression. M, marker; 1, 3, 5, 7 – ER-GRA1; 2, 4, 6, 8 – blank vector ER-ctrl.

day after passage. Cells divided actively from the second day on, with logarithmic growth. Usually the divisional index peaked at the second and third day. When cells attained their saturation density, the growth of cells stagnated. The growth curve of cell appeared "creep-logarithmic-stagnation" mode. And ER-GRA1-RAW264.7 attained its division peak at the first day of passage, followed by saturation-stagnation-death state. The growth curve of ER-ctrl-RAW264.7 cells showed no difference with RAW264.7 cells. These data show that the expression of GRA1 in ER of RAW264.7 cell may promote the growth and adherence of macrophages.

3.6. Effects of GRA1-ER-targeted expression on intracellular calcium release of macrophages

It has been shown that GRA1 may function as a Ca^{2+} buffer modulating the Ca^{2+} concentration (Cesbron-Delauw et al., 1989). To determine whether GRA1 organelle-targeted expression affects the intracellular calcium release in macrophages, fluorescence intensity of intracellular calcium in ER-GRA1-RAW264.7, ER-ctrl-RAW264.7 and RAW264.7 cell in the presence of 1 mmol/l AA were assayed by confocal microscopy using calcium-sensitive dye, Fluo-3 AM. As shown in Fig. 7 and Fig. 8, calcium-response to arachidonic acid (AA) stimulation was in a time-dependent manner. No change in calcium levels appeared in control groups (data not shown). Cytoplasmic $[Ca^{2+}]i$ peaked at about 18 s in ER-GRA1-RAW264.7 cells and cytoplasmic $[Ca^{2+}]i$ in RAW264.7 cells almost instantly stepped up after added drug, and attained its peak at about 3 s, with a minor cytoplasmic $[Ca^{2+}]i$ vibration subsequently. These results demonstrate that the expression of GRA1 in ER of RAW264.7 cells is capable of modulating the intracellular calcium release stimulated by AA.

4. Discussion

Previous studies have suggested that GRA1 may be important in host-cell invasion, and it is as both diagnostic antigen and vaccine component in toxoplasmosis (Cesbron-Delauw et al., 1989; Ferrandiz et al., 2004; Jongert et al., 2007, 2008; Kato et al., 2005; Pietkiewicz et al., 2004; Scorza et al., 2003; Sibley et al., 1993). In this study, the effects of GRA1 organelle-targeted expression on biological function of macrophage were investigated to further understand GRA1 functions. The recombinant plasmid pCMV/myc/ER-GRA1 was constructed and then was transfected into murine macrophage RAW264.7 by polyvalent positive ion liposome Lipofectamine and selected by resistance of G418. The selected mono-clone cell lines were named ER-GRA1-RAW264.7. And we further confirmed that the expression of GRA1 was localized in ER of ER-GRA1-RAW264.7 cells by indirect immunofluorescence detection. We found that GRA1 mRNA expression level in ER-GRA1-RAW264.7 cell was significantly enhanced with a concomitant increase in its growth and adherence activity, suggesting that

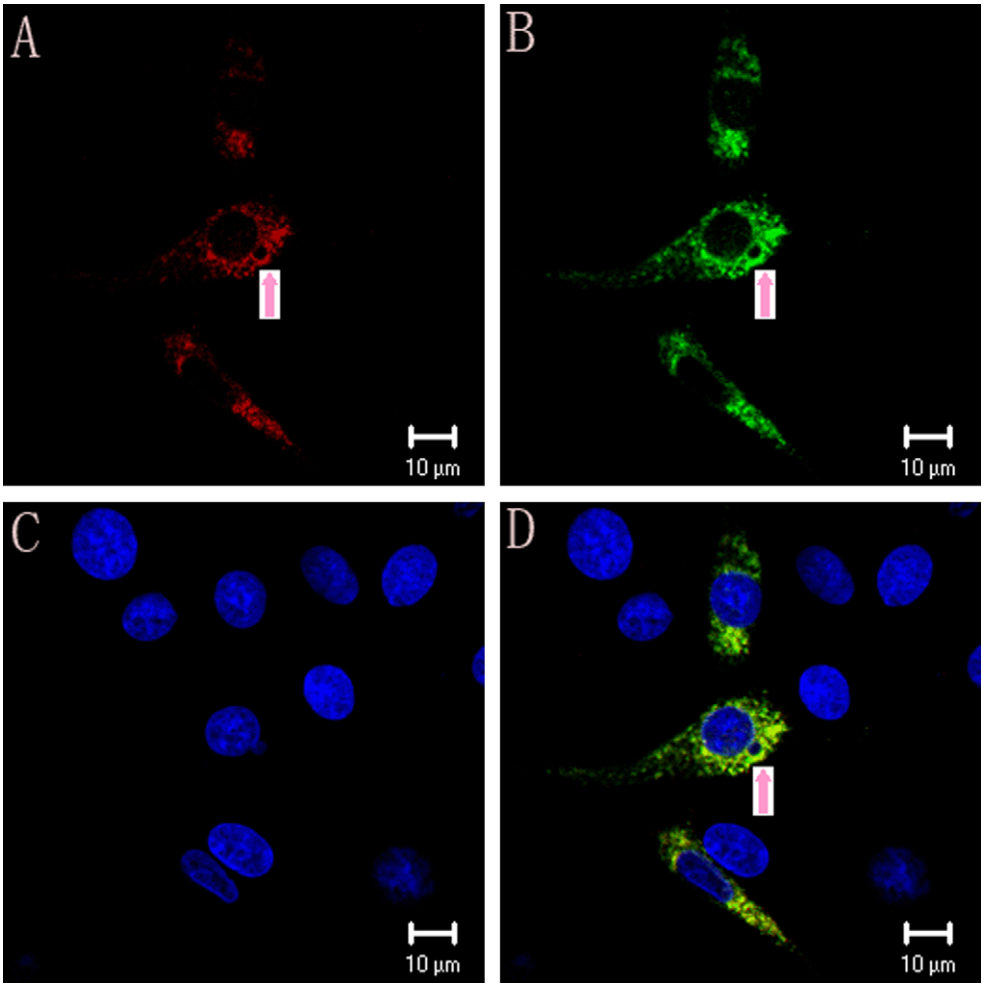


Fig. 5. After macrophage cells (ER-GRA1-RAW264.7) were transfected with pDsRed2-ER, which were labeled with mouse anti-myc antibody and anti-mouse IgG-FITC antibody. Confocal laser scanning was applied to observe the expression of GRA1 and co-localization (×400). (A) pDsRed2-ER showed red as indicated by arrow. (B) GRA1 conjugated FITC showed green as indicated by arrow. (C) DAPI dyed nuclei showed blue. (D) Merged pDsRed and GRA1 co-localized and showed yellow with blue nucleus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

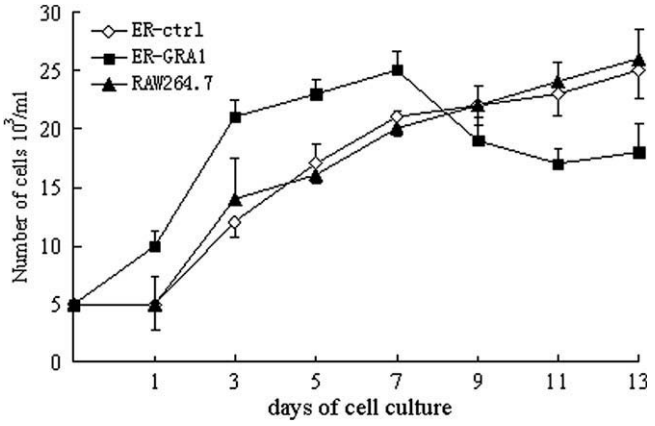
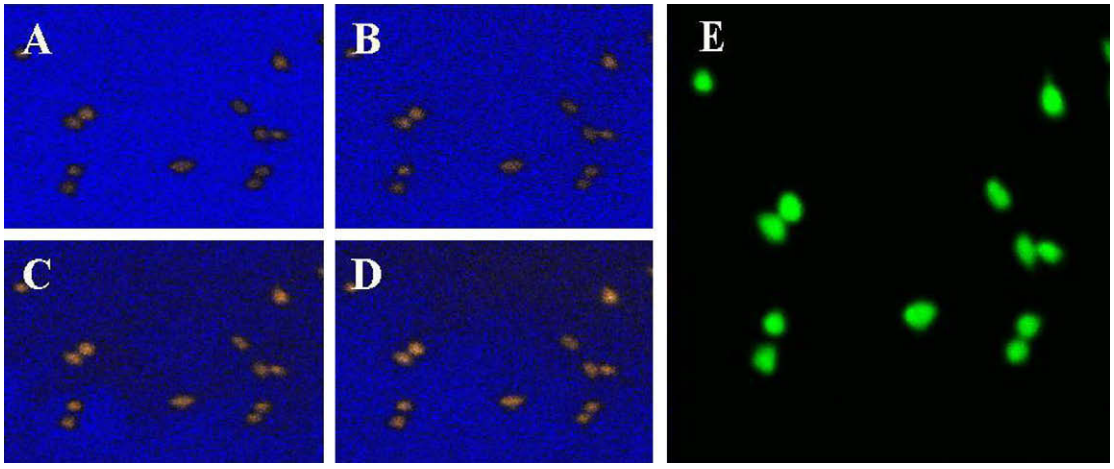


Fig. 6. Growth-curve of RAW264.7, ER-GRA1 and ER-ctrl cell. The growth of normal macrophage RAW264.7, ER-GRA1 and empty control vector transfected cells ER-ctrl were observed. ER-GRA1 cells were always cultured with 400 μg/ml of G-418. Three bottles cells of every strain were used to calculate average cell number.

the expression of GRA1 in ER of RAW264.7 cell is important for the growth and adherence of macrophages. Attach to, and invasion of a host cell is a crucial step in the establishment of infection and subsequent survival and proliferation of a protozoan (Sibley 2004). Previous studies have demonstrated that *T. gondii* is able to infect murine macrophages (Da Gama et al., 2004), monocytes (Courret et al., 2006) and dendritic cells (DCs) (Lambert et al., 2006) and were used as Trojan horses to disseminate throughout the host (Seipel et al., 2009). It indicated that the promotive effect of GRA1 on the growth and adhesion may contribute to the survivorship of *T. gondii* in macrophages and its dissemination throughout the host.

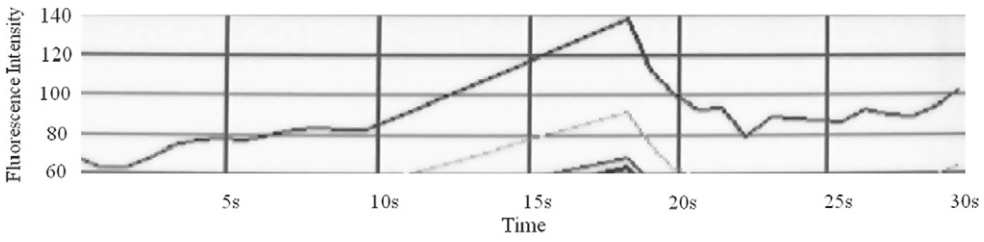
Calcium ions have long been known to play a key role in many cell events. Intracellular Ca^{2+} is an important second messenger in eukaryotic cells, mediating the cell's responses to external stimuli as well as playing a pivotal role in the control of many intracellular processes. Pezzella et al. (1997) have shown that the initiation of *T. gondii* tachyzoite into host cell is a Ca^{2+}-dependent process and that Ca^{2+} mobilization from intra-tachyzoite stores is essential for the success of parasite invasion (Pezzella et al., 1997). It has been reported that soluble *Toxoplasma* antigen (STAg) induces a transient calcium elevation in cells transfected with CCR5 (Aliberti et al. 2003) and longer-term imaging of macrophages exposed to live parasites reveals robust changes in intracellular calcium that derive from extracellular sources and this activity is independent of known pathways involved in the innate recognition of this organism (Masek et al., 2007). Recently, we have demonstrated



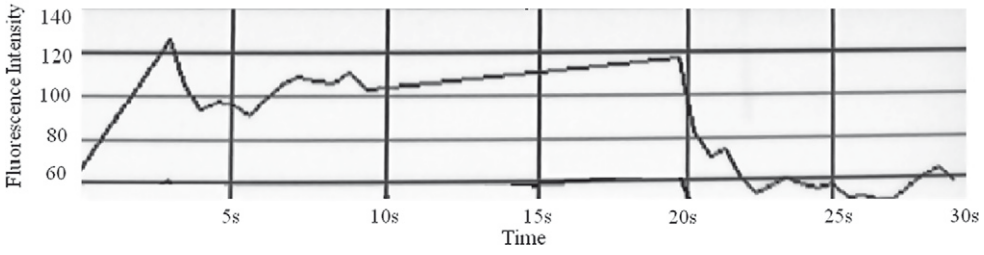
	0s	1s	2S	3S
Fluorescence Intensity (U)	65±3	65±3	79±2	102±1

Fig. 7. AA alters Fluo-3 fluorescence in RAW264.7 cells. RAW264.7 cells were labeled with Fluo-3 AM and stimulated with 1 mmol/l AA. Upper: Images were recorded every 1 s presented as a still image. A, 0 s; B, 1 s; C, 2 s; D, 3 s; E, light microscopy image. Lower, fluorescence intensity of intracellular calcium.

A



B



C

	Fluorescence Intensity (U)					
Time (s)	0	1	2	3	18	30
ER-GRA1	61±3	59±2	61±1	68±10	141±3*	101±16*
RAW264.7	65±3	79±2	102±1	131±3*	117±12	61±8

Fig. 8. Effect of AA on cytoplasmic free $[Ca^{2+}]i$. The upper was the effect of 1 mM AA on cytoplasmic $[Ca^{2+}]i$ of ER-GRA1 and the bottom was of RAW264.7. Each square on figure represent the time of 5 s. The drug added started from 0 s. The time for cytoplasmic $[Ca^{2+}]i$ peak attaining of ER-GRA1 (A) was about 18 s, and cytoplasmic $[Ca^{2+}]i$ of normal macrophage (B) almost instantly stepped up after added drug, and attained its peak in about 3 s, with a minor cytoplasmic $[Ca^{2+}]i$ vibration subsequently. Fluorescence intensity of two cell strains were shown in (C) $^{*}P < 0.05$.

that nitric oxide induces apoptosis in *T. gondii* tachyzoite via calcium signal transduction pathway (Peng et al., 2003a) and that the exogenous arachidonic acid (AA), a new second messenger, may enhance the rate of *T. gondii* infection via a calcium transduction pathway (Peng et al., 2003b). AA and its metabolites have already been proved to participate in a number of physiological and pathological physiological processes of toxoplasmosis. (Cornelly and Kierszenbaum, 1984; Radvin et al., 1985; Saffer et al., 1989; Saffer and Schwartzman, 1991; Peng et al., 2008).

It has been shown that GRA1 may function as a Ca^{2+} buffer modulating the Ca^{2+} concentration (Cesbron-Delauw et al., 1989). In this study, to further investigate the effect of GRA1-ER-targeted

expression on intracellular calcium release of macrophages, fluorescence intensity of intracellular calcium in ER-GRA1-RAW264.7, ER-ctrl-RAW264.7 and RAW264.7 cells in the presence of 1 mmol/l AA were assayed by confocal microscopy using calcium-sensitive dye, Fluo-3 AM. It has been shown that rising reaction of intracellular calcium in ER-GRA1-RAW264.7 cells was much slower than ER-ECV-RAW264.7 and RAW264.7 cell to AA stimulation, with buffer time prolonged to 18 s. The results indicated that the expression of GRA1 in ER of RAW264.7 cells is capable of modulating the intracellular calcium release stimulated by AA. However, the consequences and mechanism involved are still unknown. One possible mechanism is that GRA1 may act as a calcium ion buffer to facilitate invasion of macrophages by *T. gondii* which needs further studies to prove. An alternative explanation is that interaction of GRA1in ER components could perturb calcium handing by these organelles, leading to discharge in some cells. It is necessary in further studies to identify the molecules in ER binding to GRA1.

In conclusion, we have shown that the expression of GRA1 in ER of macrophages promotes both growth and adherence and modulates intracellular calcium release stimulated by AA. However, it is necessary in further studies to argue that modulation of macrophage functions in response to parasite molecules might affect the outcome of infection.

Acknowledgments

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