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Particulate beta-glucan induces early and late phagosomal maturation in murine macrophages

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1. ABSTRACT

Beta-glucans are carbohydrates (glucose polymers) found in the cell walls of fungi, yeast, algae, lichens, and plants such as oats and barley. Beta-glucans bind to glucan receptor on phagocytic cells and modify these cells to become “immunologically active” by generating a variety of innate immune responses. Particulate beta-glucan has been specifically shown to engage dectin-1 receptor, which leads to the recruitment and activation of nicotinamide adenine dinucleotide phosphate oxidase-2 (NOX-2) and release of antimicrobial reactive oxygen species (ROS). Here, we report that yeast-derived beta-glucan particles (YDGP) of a specific size are easily phagocytosed by macrophages and induce the production of ROS. Furthermore, the present work also demonstrates that phagosomal maturation, appearance of acidic vesicular compartments (AVO), and light chain protein-3 (LC3) accumulation within murine macrophages, occur at early and delayed time points after the phagocytic uptake of YDGP. These data suggest that particulate glucans can be exploited to trigger autophagy within phagocytes and, thereby, have implications in antimicrobial therapy.

2. INTRODUCTION

Beta-glucans, the major component of cell walls in various organisms such as yeast, mushrooms, *Candida*, barley, and oats are polymeric carbohydrates with glucose units containing beta-(1, 3) linkages and occasional beta-(1, 6) branches (1-2). Beta-glucans enhance the innate immune system and are pharmacologically classified as biological response modifiers (2). The beta-1, 3/1, 6 glucan derived from the cell walls of Baker’s yeast (*Saccharomyces cerevisiae*) is reported to possess greater biological activity than the 1, 3/1, 4 counterparts. Such glucan preparations are hollow and porous 2–4 μm particles, which allow encapsulation and targeting of proteins (3), DNA (4), siRNA (5), and small drug molecules (6, 7). These particles have been shown to enhance the efficacy of immunotherapy in mouse mammary carcinoma models, by inducing a protective Th1 cellular response (IL-12 and IFN-γ) (8). Beta-glucans are known to act as a pathogen associated molecular pattern (PAMP) and are specifically recognized by Dectin-1, a C-type lectin pattern recognition receptor (PRR), present on the phagocytes such as macrophages, dendritic cells, and neutrophils.

Receptor-mediated endocytosis by FcγR, TLRs, and Dectin-1 receptors among others, involves the assembly of NOX2 on the emerging phagosomal membrane. The NOX2 components include two transmembrane flavocytochrome b proteins, $gp91^{phox}$ and $p22^{phox}$, which form the catalytic core of the enzyme. Generation of reactive oxygen species (ROS) requires the translocation of cytosolic proteins, $p47^{phox}$, $p40^{phox}$, $p67^{phox}$, and a small G protein, Rac, to the transmembrane $gp91^{phox}/p22^{phox}$ complex. NOX2 activation generates superoxide by transfer of electrons from cytosolic NADPH to oxygen in the forming/nascent phagosome lumen (9). Superoxide is an extremely reactive radical that leads to the generation of various other ROS species within the phagosome and can directly eradicate microbes (10, 11). NOX-2 generated ROS molecules induce rapid translocation of autophagic protein LC3 to PRR-engaged phagosomes (12).

Phagosomal maturation, a process involving the recruitment and fusion of intracellular proteins is essential for the removal of fungal pathogens by the phagocytes. The role of Dectin-1 receptor in fungal recognition, Th17 responses, and phagosomal maturation has been recently defined (13). The fusion of phagosomes with late endosomes, and consequently with lysosomes, leads to the formation of an acidic compartment called the phagolysosome. Progressive phagosomal acidification leads to the degradation and killing of internalized pathogens within the phagocytes, so that further bacterial invasion is prevented. The progressive acidification of phagosomal compartment, by fusion with late endosomes, is a prerequisite for the process of phagosome–lysosome fusion (14). The phagosome–lysosome fusion also facilitates antigen presentation, and is thus, an important event in the elicitation of innate immune response. Moreover, previous studies have demonstrated that the engagement of PRRs during phagocytosis activates autophagy, phagosomal maturation, and microbial killing (15). Thus, a clear link exists between receptor-mediated phagocytosis and autophagy.

Autophagy is a multi-step, evolutionarily conserved, degradative cellular pathway in which mis-folded proteins, damaged organelles, and intracellular pathogens are sequestered inside a double-membrane 'phagophore' that further transforms into 'autophagosome'. These autophagosomes target the engulfed material for lysosomal degradation (16). Autophagy induction has, therefore, been identified as an innate host defense strategy that overcomes phago-lysosome maturation block and, thereby, targets, degrades, and eliminates the intracellular bacteria (17, 18). Autophagy activation has been shown to be associated with the killing of microbes, such as *Mycobacterium tuberculosis* (*M. tb.)* (19), *Aspergillus fumigatus* (20), *Salmonella typhimurium* (12). It acts by removing the pathogens from the cytosol, limits their escape from phagosomes, and promotes phagosomal maturation and antigen presentation (21-23). Several recent studies suggest that the phagocytosis and innate signaling pathways intersect to mount appropriate immune responses that lead to microbial restriction and autophagy induction (22, 13).

LC3 is a highly conserved, microtubule-associated protein that was previously considered as a specific marker of autophagy. Recently it has been shown to play a key role in a comparable, but distinct, innate immune pathway named as LC3 associated phagocytosis (LAP) that facilitates faster LC3 recruitment and degradation of the phagocytosed cargo (22). Dectin-1 mediated phagocytosis of particulate beta-glucan leads to LAP and triggers rapid lysosomal maturation within the phagocytes in a NOX2- and ATG5-dependent manner (15,22-24).

Our laboratory has been involved in investigations on YDGPs as vehicles for therapeutic intervention against bacterial diseases, particularly tuberculosis (TB). A major challenge in treating infections by intracellular pathogens is the induction of the classical immune activation within an otherwise infected immunosuppressed host macrophage (25) that normally act as a first line of defense. During the course of this study, we examined ROS generation, phagosomal maturation, and autophagy induction at early and late exposure of YDGP within the macrophage. Our results indicate that the internalization of YDGP induces significant ROS production at 30 min and 24 h. At both these time points, a higher lysosomal and AVO accumulation was observed, indicating the activation of rapid and delayed type of phagosomal maturation. We also observed an accumulation of LC3-II protein at both these time points, indicating autophagy activation within macrophage by the YDGP uptake.

3. MATERIALS AND METHODS

3.1. Cell culture and maintenance

Mouse macrophage cell line (J774A.1) was procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were grown and maintained in DMEM medium supplmented with 10% FBS and 1X solution of 10,000 U penicillin, 10 mg streptomycin, and 25 µg amphotericin B per ml of 0.9% normal saline in a humidified atmosphere of 5% CO_2 at 37°C The cell culture media and supplements were obtained from Sigma Chemical Corp., MO, USA.

3.2. Reagents and solutions

Dry yeast (*Saccharomyces cerevisae*) was purchased from a nearby authentic bakery (Polo Enterprises, Lucknow). Other reagents used were anti LC3-II antibody (Sigma), Alexa Fluor 568 labeled goat

anti-Rabbit IgG secondary antibody (Sigma), DCFH-DA (Sigma), nitroblue tetrazolium (NBT; HIMEDIA, Mumbai, India), acridine orange (HIMEDIA), Rhodamine (THOMAS BAKER). All the chemicals and solvents were of analytical grade.

3.3. Preparation and characterization of YDGP

Beta-glucan microparticles (MP) were prepared from dry yeast by chemical extraction method followed by sonication and spray drying techniques (6, 26). Particle size distribution was determined by a laser-based size analyzer (Mastersizer 2000, Malvern Instruments, UK) at the Pharmaceutics Division, Central Drug Research Institute, Lucknow. Morphological characterization of YDGP was performed by scanning electron microscopy (SEM) at Birbal Sahni Institute of Palaeobotany, Lucknow.

3.4. Particle counting, cell cytotoxicity, and phagocytosis assay

Flow cytometry was performed for the counting of fluorescent dye (Rhodamine)-tagged YDGP. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay, a colorimetric assay, was used to determine the effect of YDGP exposure on macrophage viability. Macrophage were incubated for 24 h in the presence of different number of particles (1:2, 1:5, 1:10, and 1:20), after which the cells were further incubated with 10 µl MTT (10.4 mg/ml) at 37˚C in humidified 5% CO_2 for 4 h to convert water soluble MTT to insoluble formazan, which occurs only in viable cells. After 4 h, 100 µl of 50% dimethylformamide with 20% SDS was added to each well to solubilize the formazan, and the plates were incubated at 37˚C overnight. The absorbance was then measured at 595 nm with background subtraction at 655 nm.

Cells (1×10^6 per well) were seeded in a six well plate and exposed to rhodamine-tagged YDGP (10 YDGP per cell) for phagocytosis assay. After 15 min exposure, the cells were washed twice with PBS and visualized under a fluorescent microscope (Nikon Eclipse Ti-S) with a red filter.

3.5. Nitro blue tetrazolium (NBT) reduction and DCFH-DA assays for ROS measurement

ROS generation by macrophage in response to YDGP administration was assessed using DCF-DA followed by quantitative fluorometric measurements and by NBT assay.

In the NBT assay, cells (5×10^5 per well) were incubated with YDGP and 0.3% NBT. After 2 hours incubation in 5% CO_2 at 37°C, the cells were washed twice with PBS and images of cells were captured by a phase contrast microscope (Nikon eclipse Ti-S). In DCFH-DA assay, cells (0.1×10^6 cells/well) were seeded in a 96-well plate and loaded with 10 µM DCFH-DA solution for 15 min. Thereafter, these cells were exposed to YDGP for different time periods. The fluorescence levels, indicating the intracellular ROS production, were measured using a fluorescent multi-well microplate reader (Synergy HT BIO-TEK) with excitation at 485 nm and emission at 530 nm.

3.6. Staining of YDGP exposed cells with LysoTracker-G dye

After YDGP exposure for 30 min, 4 h, and 24 h, the cells were washed with PBS and stained with LysoTracker-G (50 nM). After 15 min of incubation at 37°C, the cells were washed with PBS and visualized under a fluorescent microscope.

3.7. Detection of acidic vesicular organelles (AVOs) by acridine orange staining

YDGP-exposed cultured cells were incubated with AO (1 µg/ml) for 15 min to detect acidic vesicular organelles (AVOs) and were visualized after illumination with blue light (488 nm). In AO-stained cells, the acidic compartments were seen to fluoresce bright red, the intensity of the fluorescence being proportional to the degree of acidity. The nucleolus and cytoplasm emitted green fluorescence (520 nm) upon excitation with the same wavelength of light. Photographs were obtained with an upright fluorescent microscope at 20X magnification. The macrophage cultures were induced to undergo autophagy by 24 h of rapamycin treatment and were treated as positive control.

3.8. Immunofluorescence assay for the detection of autophagic protein (LC3-II)

For LC3-II detection, the cells were exposed with YDGP for 30 min and 24 h, and then washed with PBS. The cells were fixed with 4% paraformaldehyde and incubated with PBS containing 0.02% Triton X-100 and 0.1% BSA to block the nonspecific binding sites. These cells were incubated overnight at 4°C with primary antibody (anti-LC3-II) and diluted 1:100 in PBS containing 0.2% Triton X-100 and 0.1 % BSA. The cells were washed twice with PBS and incubated with a secondary antibody for 2 h on a rocker. The cells were washed thrice with PBS for 5 min and the nuclei were counter-stained with hoechst. Thereafter, the cells were visualized under an upright fluorescent microscope. The macrophage cultures were induced to undergo autophagy by 24 h of rapamycin treatment (positive control).

3.9. Statistical analysis

Statistical analyses were performed using Microsoft Excel and Instat software. P values were calculated using two-tailed two-sample equal variance Dunnett's *t* test (compared the data from YDGP-exposed and control cells). The cell viability and ROS estimation data have been expressed as means ± SE of the values from three replicate experiments.

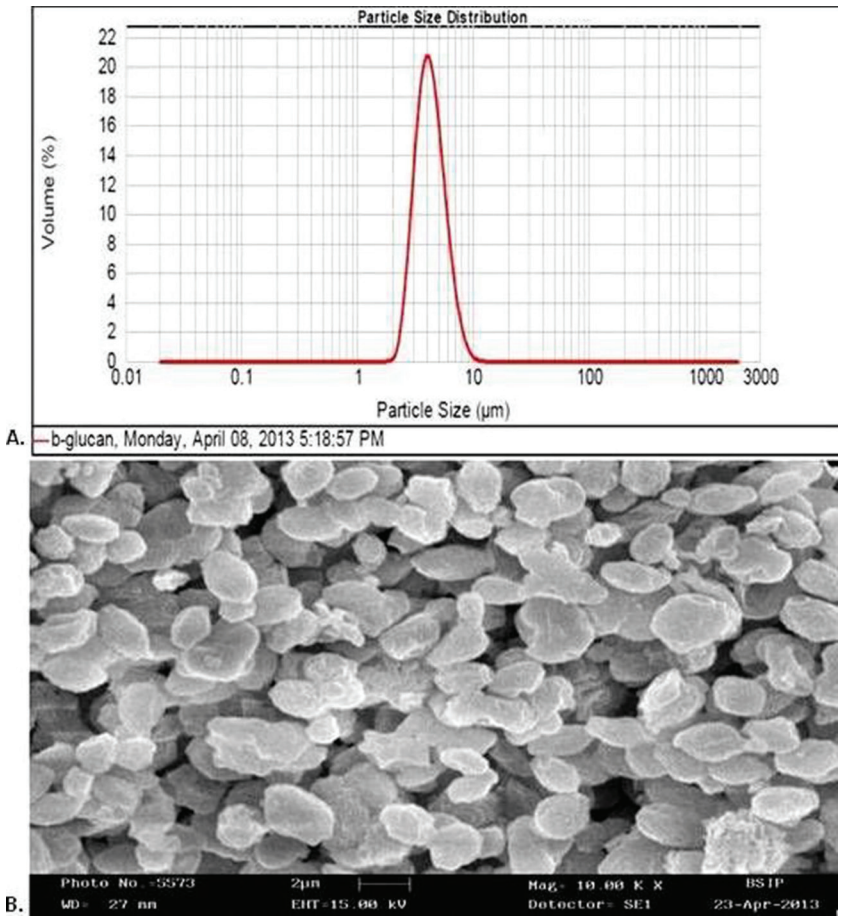

Figure 1. Characterization of YDGP. (A). Graphical representation of size distribution of YDGP by Malvern Master Sizer. The plot of particle size versus volume (%) shows the particle size in the range of 2 to 10 µm (B). Scanning Electron Micrograph of YDGP formulation, showing uniform 2–4 µm particles.

4. RESULTS

4.1. Phagocytosis of the formulated YDGP by macrophage

The characteristics of particulate glucan synthesized from yeast as described earlier are shown in Figure 1. Size characterization by laser scattering (Figure 1A.) revealed that more than 75% particles were 2–5 µm in size. The SEM image showed a near spherical morphology of YDGP (Figure 1B) and confirmed the particle size. FACS studies indicated the presence of an average number of 1.68×10^{8} rhodamine-tagged fluorescent particles (Rd-YDGP) per mg dry weight of the YDGP formulation (Figure 2A). On the basis of this approximation, macrophage were exposed to different ratios of YDGP per cell and it was found that 10 particles per cell (amounting to 10 µg/ml) was the highest concentration of YDGP that did not show any significant cytotoxicity after 24 hour exposure ($P>0.0.5$). Beyond this, the cells showed significant cytotoxicity *($P<0.0.1$)* in comparison to the control cells (Figure 2B). A cell: particle ratio of 1:10 was thus selected for the subsequent experiments in this study. Macrophage were exposed to Rd-YDGP, visualized under a fluorescent microscope, and were found to emanate bright red fluorescence (Figure 2C). The bright field images of YDGP-exposed cells (Figure 2D) showed conspicuous granulation within the cytoplasm in comparison to the unexposed control cells, indicating the uptake of YDGP by macrophage within 5 min of exposure.

4.2. Generation of ROS upon YDGP uptake by murine macrophage

NBT reduction assay was also used as a probe for ROS generation. YDGP administration led to the formation of a dark purple formazan precipitate within macrophage, indicating appreciable ROS production (Figure 3A). Intracellular ROS generation was assessed at different time points by the DCFH-DA assay, which showed significant ROS generation after a 5 min exposure with YDGP ($P<0.0.01$). It was observed that after 30 min and 24 h exposure, significantly high amounts of ROS (Figure 3B) were generated (the increase being

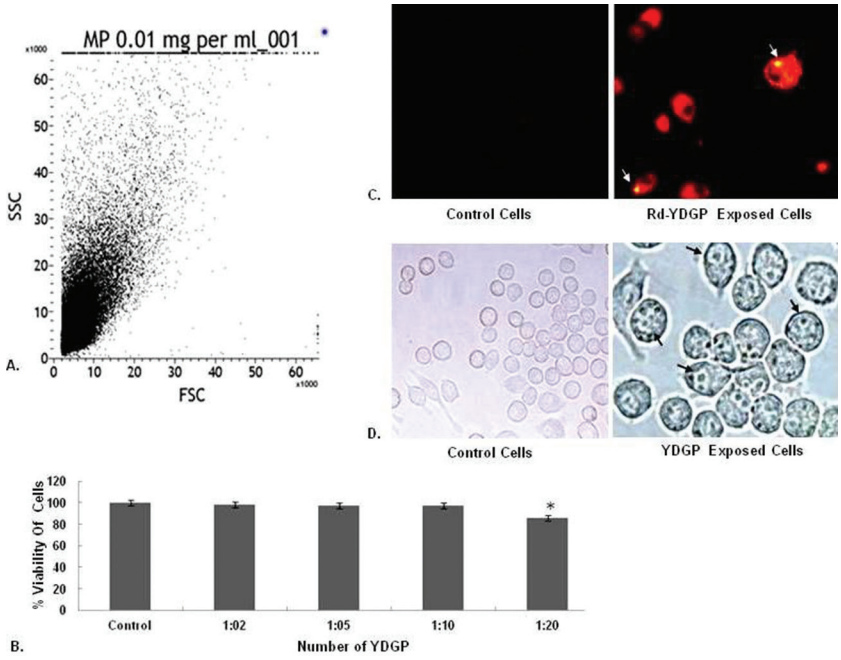

Figure 2. *In vitro* phagocytic uptake of YDGP by J774 cells (murine macrophage cell line). (A). Dot plot of fluorescent YDGP (Rd-YDGP) showing size and granularity. The average number of particles estimated by flow cytometry was found to be 1.68 × 10^6 per mg of YDGP formulation. (B). MTT assay to examine macrophage viability after 24-h exposure to YDGP, at various ratio(s) of YDGP per cell. '*' indicates significance by paired *t*-test at *P*≤0.0.5, on comparing cell viability in YDGP-exposed group with the unexposed control cells. (C). Fluorescent image(s) of Rd-YDGP exposed cells at 20X magnification. White arrows show the internalized Rd-YDGP within macrophage. (D). Bright field image(s) of YDGP-exposed macrophage at 40X magnification. Black arrows show the phagocytosed YDGP within the cells.

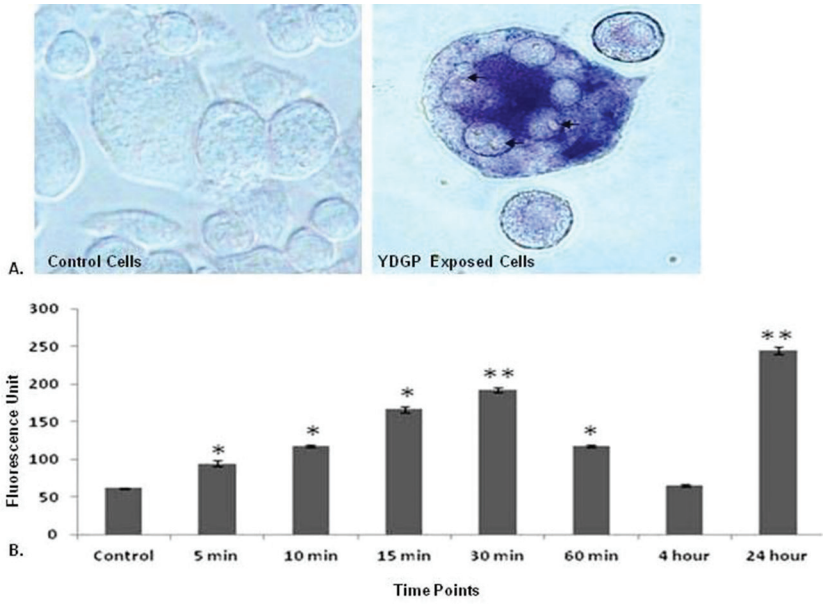

Figure 3. Detection and quantitation of ROS generation by NBT reduction and DCFH-DA assay. *(A).* Microscopic visualization of dark purple formazan crystals formed within YDGP exposed cells, indicating ROS generation within macrophage. White arrows show the formazan precipitate within the YDGP exposed cells. *(B).* Quantitative analysis of ROS generation within the YDGP-exposed cells by DCFH-DA assay at various time points after particle uptake. '*' indicates significance by paired *t*-test at *P*≤0.0.5, on comparing ROS levels in YDGP-exposed group with the unexposed control cells. Of these, two time points showing highest ROS generation ('**') were selected for further experiments in the study.

significant at P<0.0.001 and P<0.0.001, respectively). On the basis of these data, further experiments were subsequently performed after 30 min and 24 h of YDGP exposure.

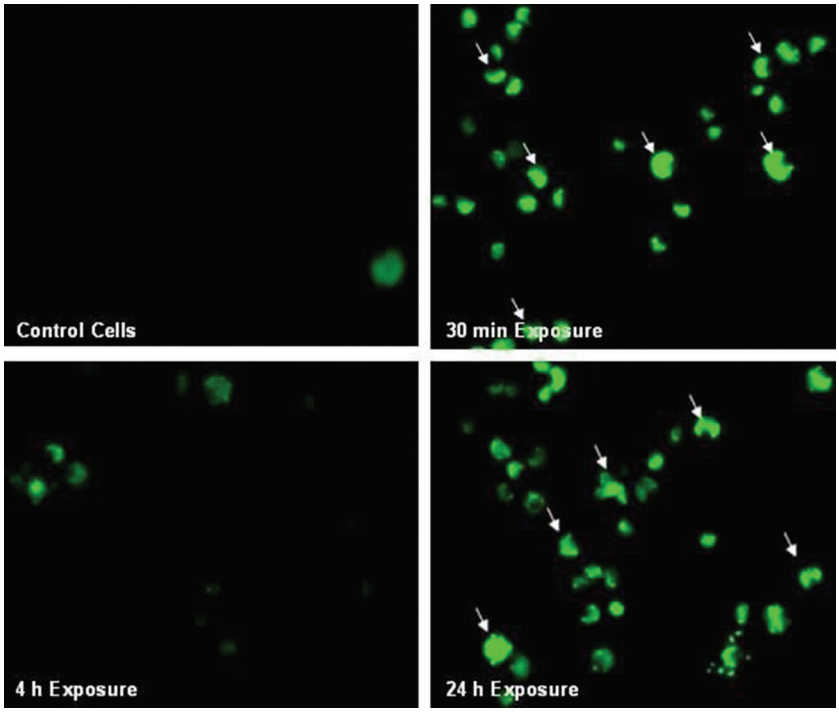

Figure 4. Time course of lysosomal activity upon YDGP exposure. YDGP-exposed cells were labeled for lysosomal compartments and phagosomal acidification upon lysosome fusion was monitored using LysoTracker-G. The control cells showed no visible green fluorescence, whereas at 30-min and 24-h exposure to YDGP, a conspicuous green fluorescence was observed, indicating vital lysosomal activity at these time points. At 4 h, slight lysosomal activity was observed upon exposure to YDGP. Arrows show phagosomal maturation within the YDGP-exposed macrophage, preloaded with LysoTracker G.

4.3. Accumulation of lysosomes and formation of acidic vesicular organelles (AVOs) within macrophage upon YDPG uptake

After internalization of YDGP, macrophage were incubated for 30 min, 4 h, and 24 h and stained with an acidic lysosomal tracking dye LysoTracker-G. We observed a rapid increase in green fluorescence intensity after 30 min exposure of YDGP, indicating the accumulation of lysosomes. After 4 h exposure to YDGP, very slight accumulation of lysosomes was observed, whereas 24 h post-exposure, a conspicuous increase in the fluorescence was observed, indicating the activation of phagolysosomal maturation (Figure 4). To detect the appearance of AVOs, which is characteristic of autophagy, we performed vital staining of macrophage with AO after YDGP exposure at 30 min and 24 h (Figure 5). AO is a weak base that moves freely across biological membranes, when uncharged. Its protonated form accumulates in AVOs, where it forms aggregates that fluoresce bright red (27). Concentrated dye in the acidic vesicles, was seen to fluoresce bright red, whereas cytoplasm and nucleolus showed green fluorescence. The majority of unexposed, control cells exhibited mainly green fluorescence with minimal or no red fluorescence. The YDGP exposed cells exhibited bright red fluorescence at 30 min and 24 h post YDGP exposure, pointing towards the induction of autophagy at these time points. The rapamycin treated cells were used as a positive control for autophagy induction (Figure 5).

4.4. YDGP internalization recruits autophagy protein LC3-II to phagosomes

Similar to our observations with the AO staining, the immunofluorescence studies also showed LC3-II accumulation after 30 min as well as after 24 h of exposure to YDGP; this was comparable to the LC3-II accumulation in the rapamycin treated cells (Figure 6). The red fluorescence in both 30 min and 24 h exposed cells indicated the accumulation of LC3-II protein and the blue fluorescence denoted the cell nuclei. However, one can visualize LC3–II accumulation within the cells, as bright pink fluorescence obtained in the merged panels. This observation confirms that autophagy was activated at both the time points as a consequence of YDGP exposure.

5. DISCUSSION

Phagocytosis of particulate material has been previously shown to stimulate the phagocyte, inducing macrophage activation and anti-microbial responses (26, 28, 29). The homogenous preparations of YDGP in the size range of 2–10 µm (Figure 1A, B) were rapidly phagocytosed by the macrophage (Figure 2C, D) and led to rapid induction of ROS within

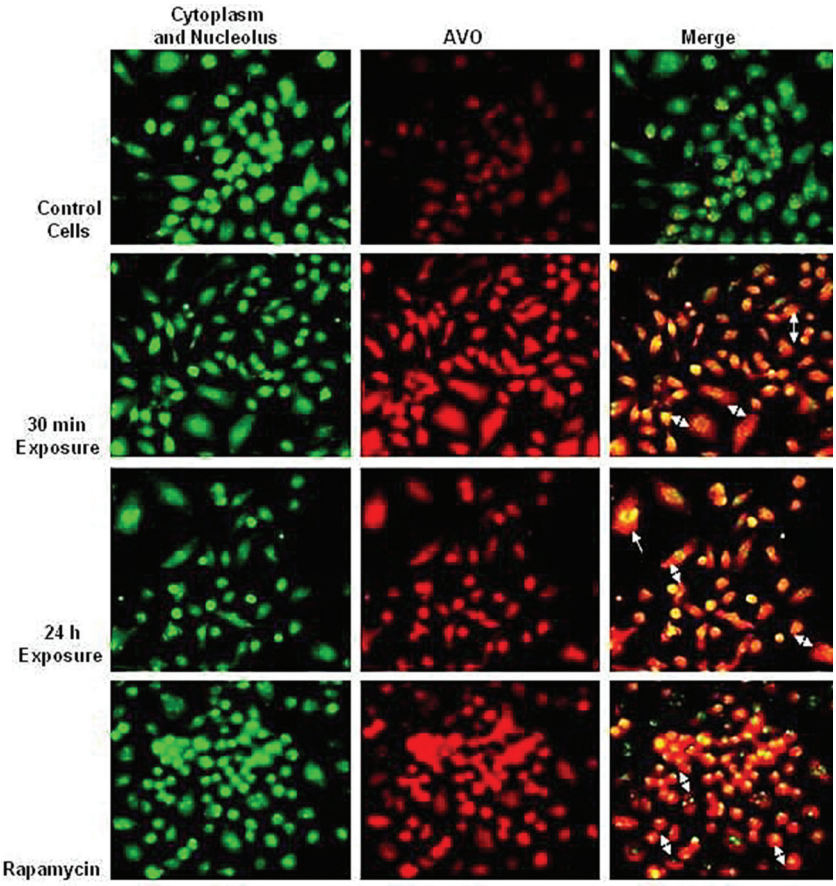

Figure 5. Detection of acidic vesicular organelle (AVO) formation upon YDGP exposure. The control cells showed no acidic organelles whereas the YDGP exposed cells showed observable (red color) AVO formation at the two time points. Rapamycin treatment served as a positive control. Arrows show the red fluorescence, indicating the formation of AVO within the YDGP-exposed cells and the positive control cells.

5 min of exposure (Figure 2B). Maximum and significant levels of ROS were generated at 30 min and 24 h of exposure, indicating macrophage activation by YDGP exposure at both of these time points. Since NOX-2 is the only NADPH oxidase expressed in phagocytes (12), the ROS generation by macrophage upon exposure to YDGP can be attributed to be mainly mediated by NOX-2 activation at both the time points showing maximum ROS generation. NOX-2 activation is a key event occurring as a consequence of particle internalization and is associated with the assembly of catalytic subunits on the nascent phagosomal membrane, leading to the production of ROS. ROS are highly unstable molecules that may directly eradicate microbes and also activate lysosomal degradation (10, 30). Vigorous lysosomal activity was induced by YDGP suggesting rapid phagosomal maturation within the cells that increased with the time of exposure (Figure 4). Our earlier work on PLA microparticle (MP) targeting to lung macrophage has shown the interaction of golgi vesicles with phagosomal membranes and the release of their contents into phagosomes containing MP (28). Rapid phagosomal maturation and its fusion with lysosomes are proficiently inhibited by both virulent and attenuated *M. tb.* (31), and phagolysosome fusion is a positive sign of an effective host response (39). Enhancement of oxidative radical production clearly indicates classical activation of macrophages as a consequence of treatment with the microparticles. The importance of 'stimulating the phagocyte' for a favorable therapeutic outcome has been established in our previous studies (28).

The increased detection of AVOs *viz.* autophagic vacuoles and lysosomes indicates enhanced phagosomal acidification upon exposure to YDGP at both the early and late time points (Figure 5). This is most likely to be mediated by an increased incorporation and retention of the vacuolar type proton-transporting ATPase ($V\text{-}H^+\text{-}ATPase$) subunits from lysosomes into the phagosomal membranes (33). It has been shown that pH within the zymosan-containing phagosomes in murine bone marrow-derived macrophages drops to about pH 4.7 (31). The ability of host cells to lower the phagosomal pH contributes to several bactericidal mechanisms. Higher concentration of protons is directly toxic to most of the microorganisms. Additionally, lower

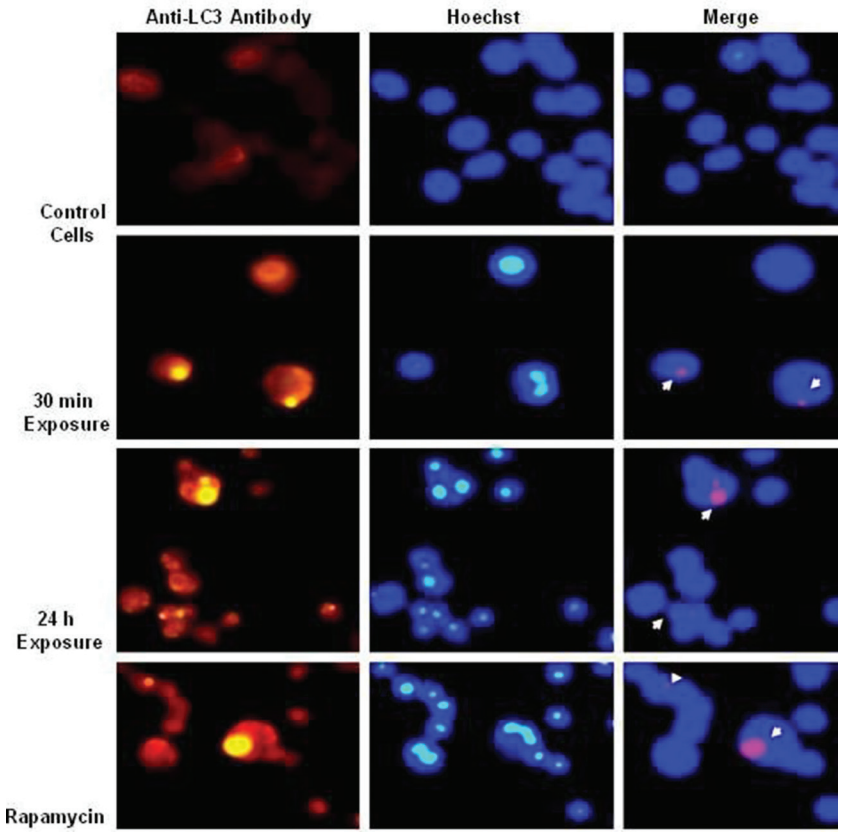

Figure 6. Immunofluorescence assay for the detection of autophagy-specific protein LC3-II at 30 min and 24 h of exposure to YDGP. The control cells showed no accumulation of LC3 whereas the YDGP exposed macrophage showed LC3-II accumulation at 30 min and 24 h of exposure. Arrows indicate the accumulation of lapidated LC3-II protein (pink fluorescence) in YDGP exposed cells at both the time points. Rapamycin-treated cells served as positive control.

pH promotes the generation of hydrogen peroxide and provides optimal conditions for the activity of hydrolytic enzymes such as cathepsin A (32).

NOX2-generated ROS molecules are reportedly required for the translocation of LC3 to phagosomes that eventually fuse with lysosomes and degrade the cargo or invading pathogens (12). LC3 is an active partner in phagosome maturation process and directly correlates with autophagosome numbers (34). Endogenous LC3 expression at protein level (Figure 6) confirmed autophagy induction upon YDGP exposure at early as well as delayed time points. This is expected because the NADPH complex that gets internalized from PM during phagosome formation serves as the major source of ROS in phagocytes under normal physiological conditions. It is well known that apart from NADPH oxidases, mitochondrial complex-1 is also reported to be a major source of ROS, and thus, plays an important role in canonical autophagy activation during starvation and stress conditions (40). It remains to be established whether ROS production mediated by NOX-2 or mitochondria serves as the main player in autophagy induction by YDGP. Nevertheless, it is encouraging to observe that the particles induce autophagy, in addition to the appropriate innate responses, pointing towards their 'positive' and 'synergistic' roles in macrophage activation and bacterial killing. However, whether the YDGP induced phagosomal maturation and autophagy activation is able to target the intracellular pathogenic bacteria or whether the autophagy pathways become subverted during infection, remains to be seen.

The uptake of particulate glucan by macrophage was seen to rapidly trigger the translocation of LC3 to YDGP containing phagosomes and promote phagosomal maturation at both the early and late time points. The results of the present study are in agreement with those reported in the last few years, and show a direct connection between phagocytosis and autophagy induction (12). The induction of autophagy by YDGP is consistent with other reports showing rapid LC3 recruitment by various forms of glucans, as zymosan, that serve as ligands for TLR and Dectin-1 receptors (PRRs) (23, 12). In addition, our data show that YDGP signaling can activate phagosomal maturation and autophagy induction within macrophage at two different time points.

The selection of the early and delayed time points based on those showing highest, significant levels of ROS upon YDGP administration, allowed us to observe rapid phagosomal maturation and autophagy induction within macrophage, at both the time points. On the basis of the time points of LC3 recruitment to phagosomes and AVO accumulation, we speculate the responses to be due to the induction of 'LAP' within the macrophage at 30 min and because of 'macroautophagy' at 24 h after the YDGP exposure. Triggering the canonical autophagy allows the host to survey the cytosolic pathogens that may have escaped the rapid phagosomal maturation. These autophagic pathways are expected to redirect microbes to lysosome for their degradation and, thus, might contribute to bacterial killing. However, more extensive investigations are required so as to validate this observation. In either case, the induction of phagosomal maturation by YDGP at different time points would be expected to have implications in triggering autophagic disposal of intracellular microbes and protein aggregates involved in pathological disorders.

Our observations, therefore, have therapeutic implications in terms of cellular activation and autophagy induction *via* YDGP, leading to induction of appropriate innate immune responses within these phagocytic cells. YDGP loaded with rifampicin, a first line anti-TB drug has been demonstrated to have better antimycobacterial activity as compared to soluble drug (6). Isoniazid and pyrazinamide (anti TB pro-drugs) have been reported to induce autophagy and phagosomal maturation in *M. tb.* infected cells (37). Therefore, such autophagy inducing agents can be incorporated in macrophage targeting vehicles to achieve a higher therapeutic efficacy than that obtained with drugs alone (38).

In the present study, we demonstrate for the first time that YDGP can activate phagosomal maturation and autophagy induction within macrophage at two different time points. We, therefore, conclude that YDGP in the size range of 2–10 μm are easily taken up by macrophage and generate significant ROS. At 30 min and 24 h exposure, maximum significant level of ROS was generated along with higher vesicular acidification and lysosomal accumulation. We show induction of autophagy by particulate glucan within murine macrophage at two different time points. Further studies are currently underway to delineate molecular mechanisms involved in autophagy induction by YDGP at these time points and its dependence on ROS. This study, therefore, provides evidence that YDGP uptake mediated signaling serves as a common underlying event that regulates phagosome maturation and autophagy induction at early and delayed time points within murine cells macrophage.

6. ACKNOWLEDGEMENTS

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Abbreviations: NOX-2: nicotinamide adenine dinucleotide phosphate oxidase-2; ROS: reactive oxygen species; YDGP: yeast derived glucan particles; Rd-YDGP: rhodamine-tagged YDGP; MP: microparticles; AVO: acidic vesicular compartment; AO: acridine orange; PAMP: pathogen associated molecular pattern; PRR: pattern recognition receptor, LC3: light chain protein-3; LAP: LC3 associated phagocytosis; TLR: toll like receptor; TB: tuberculosis

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