J Vet Res 70, 2026
DOI:10.2478/jvetres-2026-0056

Yolkin promotes differentiation of RAW 264.7 macrophages to the pro-inflammatory phenotype capable of fulfilling efficient phagocytic function

Michał Zimecki[1]✉, Jolanta Artym[1], Maja Kocięba[2], Ewa Zaczyńska[3], Aleksandra Zambrowicz[4], Łukasz Bobak[4]

[1]Laboratory of Immunobiology, [2]Laboratory of Reproductive Immunology, Department of Experimental Therapy,
[3]Laboratory of Virology, Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 53-114 Wrocław, Poland
[4]Department of Functional Food Products Development, Wrocław University of Environmental and Life Sciences, 51-630 Wrocław, Poland
michal.zimecki@hirszfeld.pl

Received: March 3, 2026 *Accepted: October 26, 2026*

Abstract

Introduction: Yolkin, a protein isolated from hen egg yolk, is postulated to play a key role in embryo development. The aim of this work was to evaluate its differentiating effect on immature RAW 264.7 mouse macrophages by determining their pinocytic and phagocytic capabilities as well as cytokine production. **Material and Methods:** The pinocytosis of neutral red (NR) and phagocytosis of NR-labelled zymosan particles were measured spectrophotometrically, and the production of TNF α, IL-6, and IL-10 in the culture supernatants was evaluated by the immunoenzymatic method. **Results:** Cells of the RAW 264.7 line perform pinocytosis of NR and phagocytosis of NR-zymosan particles more efficiently when incubated with yolkin for 48 h than when in a control culture. Yolkin at a concentration of 10 µg/mL elicited production of TNF-α, IL-6 and IL-10, with a prevalent amount of TNF-α, thus leading to an M1 pro-inflammatory phenotype. **Conclusion:** Following incubation with yolkin, RAW 264.7 macrophages differentiate to an M1 phenotype. The results suggest the possibility of using yolkin as a nutraceutical or functional food ingredient to strengthen defences against infectious agents.

Keywords: yolkin, RAW 264.7 macrophage, phagocytosis, pinocytosis, TLR4.

Introduction

Phagocytosis is a process of innate immunity and consists of the engulfment and internalisation of large particles, microorganisms and apoptotic cells by several cell types (8). The most efficient phagocytic cells are short-lived neutrophils, playing a crucial role in antibacterial defence (11, 12). The B-1 subset of B-lymphocyte cells also has a phagocytic property (4, 9). Macrophages, residing in lungs (25) and lymphoid organs (13), are another important cell compartment, removing microorganisms and apoptotic cells from the organism. Pinocytosis is a form of endocytosis in which cells internalise extracellular fluid and dissolved solutes through the formation of membrane-bound vesicles. The function of pinocytosis may be also fulfilled by other cells than macrophages, such as dendritic cells by formation of active membrane protrusions (20, 24).

Cultures of RAW 264.7 macrophages represent a convenient model to study various aspects of phagocytosis. Studies have shown that the endocytic function in this model is stimulated

© 2026 M. Zimecki et al. This is an open access article distributed under the Creative Commons Attribution 4.0 License (CC BY 4.0)

by glycoproteins (40) and polysaccharides (6, 18, 28, 30, 33) as Toll-like receptor 4 (TLR4) ligands (26). The metabolic events resulting from interaction of the stimulatory ligands include enhanced phagocytosis (5, 18) associated with polarisation of macrophages to the M1 phenotype (40), stimulation of pro-inflammatory cytokines and inducible nitric oxide (NO) (7, 18), prostaglandin E2 and cyclooxygenase 2 (COX 2) (7) production. However, at the same time, superoxide dismutase (SOD) may be produced, which protects against oxidative damage (18).

Yolkin is a recently discovered egg yolk–derived glycoprotein with distinct immunoregulatory properties which render it suitable for application in prevention and therapy as a nutraceutical (35). Very recent studies by the present authors revealed protective actions of yolkin in mouse endotoxaemia (38) and in a homologous model of viral infection (unpublished). In targeting bone marrow–derived macrophages *via* TLR2/4, the protein activates mitogen-activated protein (MAP) kinases and production of TNF-α and type I interferons (IFNs) (14). In the model of RAW 264.7 mouse macrophages, yolkin induced expression of MAP kinases and COX 2 at both the mRNA and protein levels (37).

The accumulated data on the protective properties of yolkin in inflammation and infection prompted an investigation of the potential actions of yolkin on the pinocytic and phagocytic activity of macrophages, which play a key role in antimicrobial defence. The RAW 264.7 mouse macrophage model was chosen for the investigation as it is frequently used to study mechanisms of phagocytosis. More specifically, an evaluation was made of the capability of the RAW 264.7 cell to endocytose neutral red (NR) and NR-labelled zymosan particles in culture with yolkin. The accompanying production of TNF-α, IL-6, and IL-1 was also determined.

Material and Methods

Reagents. Neutral red (NR, N4638), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), zymosan A (Z4250), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and PBS, DMEM High Glucose (L0103-500), penicillin, streptomycin, and foetal calf serum (FCS) were from Biowest (Nuaillé, France). A TNF-α kit (cat. No. 430904), IL-6 kit (cat. No. 431304), and IL-10 kit (cat. No. 431414) were from BioLegend (San Diego, CA, USA).

Yolkin. Yolkin was isolated from egg yolk as originally described (22). It was prepared as three protein fractions with molecular weight below 45 kDa. Detailed characteristics of the protein were recently presented (21).

RAW 264.7 cell line. Cultures of RAW 264.7 mouse macrophages were conducted in high-glucose DMEM supplemented with 10% FCS and a penicillin/streptomycin solution. Upon cell transfer, the supernatants were removed, the cells in the flasks were washed with the DMEM and the flasks again filled with the medium. The adherent cells were then harvested from the flasks with a plastic cell scraper. The cell suspension was adjusted to an appropriate density (1 : 3), transferred to another flask and incubated at 37°C in an 8% CO_2 atmosphere.

Determination of RAW 264.7 viability. Volumes of 100 µL of RAW 264.7 cells with cell density of 3×10^4/well were pipetted into 96-well plates and incubated for 24 h at 37°C in an 8% CO_2 atmosphere. Then, yolkin at 20, 10, 5, 2, 1, 0.5, 0.25 and 0.125 µg/mL concentrations was distributed onto a monolayer cell culture, and the culture was incubated for 48 h at 37°C in an 8% CO_2 atmosphere. The control culture contained no yolkin. The quantitative and morphological changes were evaluated in an inverted microscope. The minimal concentration of yolkin causing degeneration of 50% of cells was defined as the 50% tissue culture cytotoxic dose. The analysed parameters of growth, cell morphology, and cell viability were determined by a colorimetric MTT test (10). In short, 25 µL from the stock solution (5 mg/mL) was added to each well at the end of the cell incubation time, and the plates were incubated for an additional 2 h in a cell culture incubator. Then, 85 µL of the extraction buffer (20% SDS with 50% dimethylformamide, pH 4.7) was added. After overnight incubation, the OD was measured at 550 nm with the reference wavelength of 630 nm (550/630 nm) in a spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT, USA). The experiment was performed in two replicates.

Induction of cytokine production by yolkin. Cultures of RAW 264.7 cells (2×10^4/well in DMEM with 5% FCS and antibiotics) were incubated overnight, after which yolkin was added to make final concentrations of 10–0.5 µg/mL. After 48 h incubation, the supernatants were collected and frozen at −80°C. Each experiment was performed in 6 or 7 replicates within a single assay (supernatants were pooled). The number of cells was estimated separately for each study group in a Bürker haemocytometer. The levels of TNF-α, IL-6 and IL-10 were determined using the appropriate ELISA kits as noted above. The lowest standard concentrations were 7.8 pg/mL for TNF-α and IL-6 and 31.3 pg/mL for IL-10. When the detected cytokine concentration was below the indicated value, the sample was classified as non-detectable and assigned a value of zero. The data were calculated using four-parameter logistic

regression with Assayfit Pro (Assay Cloud, Nijmegen, the Netherlands).

Pinocytosis. The studies were performed according to protocols in the literature (2, 3, 23) with some modifications. Neutral red was initially dissolved in DMSO (10 mg/mL) and subsequently diluted in DMEM containing 5% FCS to a concentration of 50 μg/mL. Cultures of RAW 264.7 cells were incubated with NR for 30 min at 37°C in an incubator. The control cultures included only NR in the medium and the other only the cells without NR. The cells were washed 4× with PBS. The cell pellet was re-suspended in 0.1 mL of acidic ethanol (1% v/v of glacial acetic acid and 50% ethanol in distilled water), and the culture plate was shaken for 10 min at 400 rpm. The absorbance was determined spectrophotometrically at 550/630 nm using the microtitre plate reader (Bio-Tek Instruments). The concentration of NR in each well was calculated based on a standard curve, and the reported NR values were normalised in relation to 10^6 cells. Each experiment was performed in seven replicates. The number of cells was estimated separately for each experiment group in the protocol with a preincubation step. In the experiment without preincubation, the cell numbers were determined only for the control group. The cell enumerations were performed in a Bürker haemocytometer in triplicate using three additional samples prepared exclusively for this purpose. Each summary graph presents a percentage in relation to the control value in the given experiment.

Phagocytosis of NR-labelled zymosan (NR-zymosan). These studies were also conducted according to protocols in the literature (2, 27, 29) with minor modifications. Neutral red was initially dissolved in PBS (0.2 mg/mL), and 3 mL was added to 3 mL of zymosan A in PBS (20 mg/3 mL). The suspension was subsequently incubated for 30 min at room temperature with occasional agitation. Next, the suspension was centrifuged at 1,000 × *g* for 10 min and washed with PBS, and the pellet was re-suspended in PBS. The NR-zymosan suspension was added to the RAW 264.7 culture at a density of 20×10^6 zymosan particles/well. The cells were incubated at 37°C for 3 h. The control cultures were also included as described above. The cultures were washed 5× with PBS. Further steps, including measurement of absorbance, the cell enumerations, and data presentation, are as described above, except that the plates were shaken for 30 min instead of 10 min.

Statistical analysis. The results are presented as mean values ±SD. The Brown–Forsyth test was used to determine the homogeneity of the variance between groups. When the variance was homogenous, one-way ANOVA was applied, followed by *post-hoc* comparisons with Tukey's test to evaluate the significance of the difference between groups. Nonparametric data were evaluated with the Kruskal–Wallis analysis of variance. Significance was defined as P-value < 0.05. Statistical analysis was performed using Statistica 7.0 for Windows (StatSoft, Tulsa, OK, USA).

Results

The effect of yolkin on cell viability. The yolkin concentration of 20 μg/mL resulted in a decrease in cell metabolism (Fig. 1), accompanied by distinct morphological changes such as spreading on the plastic surface (data not shown). Therefore, for evaluating the functional activities of RAW 264.7 cells, the highest concentration of yolkin was set at 10 μg/mL.

The effect of yolkin on NR pinocytosis. The effects of yolkin on NR pinocytosis are presented in Fig. 2. In the first protocol, RAW 264.7 cells (2×10^5/well) were cultured overnight, the cultures were treated with yolkin and NR, and no prior preincubation step of cells with yolkin was included. As shown, the applied experimental protocol did not reveal any effects of yolkin on NR pinocytosis (Fig. 2A). In the second protocol, yolkin was added to RAW 264.7 cells (2×10^4/well) which had been cultured overnight, the treated cells were preincubated for 48 h, and NR was added subsequently. The results show a significant stimulation of the pinocytic capacity of the cells with a maximum yolkin concentration of 10 μg/mL (Fig. 2B).

The effect of yolkin on phagocytosis of NR-zymosan particles. The effects of yolkin on phagocytosis of NR-zymosan particles are presented in Fig. 3. In the first protocol, RAW 264.7 cells (2×10^5 cells/well) were cultured overnight, after which yolkin and NR-zymosan were added. The results show no effect of yolkin on the phagocytosis (Fig. 3A). On the other hand, when the cells (2×10^4/well) were preincubated for 48 h with yolkin prior to NR-zymosan addition, a very significant stimulation of the phagocytosis with a maximal effect at 10 μg/mL of yolkin could be demonstrated (Fig. 3B).

The effect of yolkin on cytokine production by RAW 264.7 cells. These cells, incubated with polysaccharides or glycoproteins, were shown to produce cytokines with a prevailing Th1 profile (18, 40). For the present investigation, TNF-α, IL-6, and IL-10 were selected to evaluate production of Th1-profile cytokines by the cells following incubation with yolkin (Fig. 4). The results show a strong induction of TNF-α production after 48 h incubation with yolkin in a dose-dependent

manner (Fig. 4A). The production of IL-6 (Fig. 4B) and IL-10 (Fig. 4C) was rather modest in comparison with that of TNF-α. Of importance is that these results revealed the immunological status of the macrophages just before exposure of the cells to the phagocytic particles.

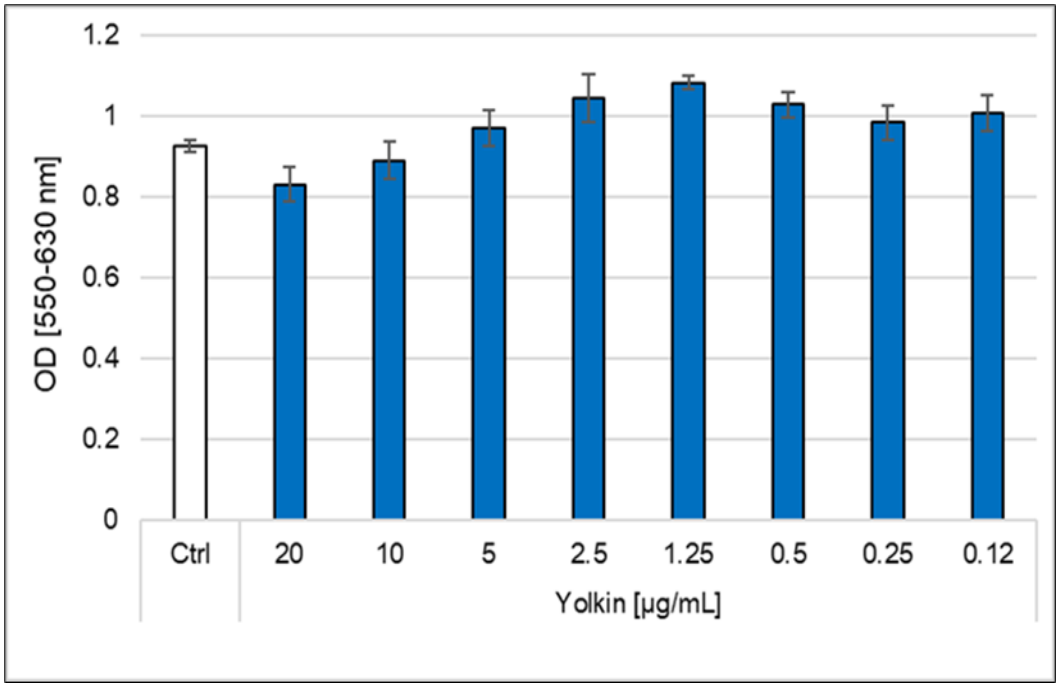


Fig. 1. The effects of yolkin on viability of RAW 264.7 cells. The results of one of the two separate experiments conducted are presented as the mean OD at 550/630 nm ±SD from five determinations for each concentration. Ctrl – control incubation of RAW 264.7 cells without yolkin

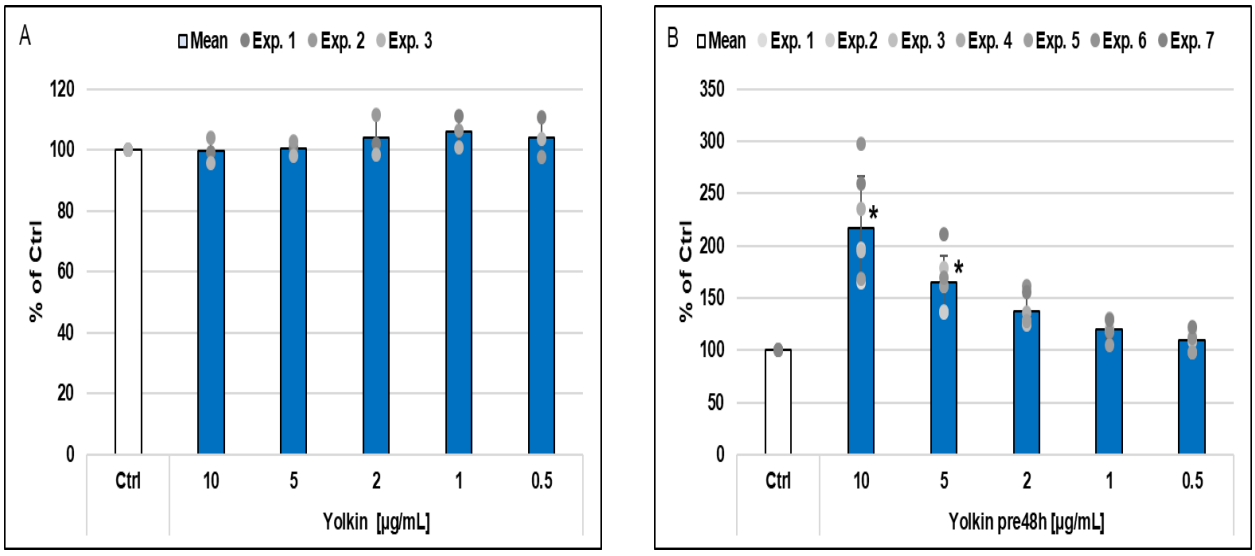


Fig. 2. The effects of yolkin on the capability of RAW 264.7 cells to internalise neutral red (NR). (A) Addition of yolkin and NR simultaneously to the culture (no preincubation step). The means ±SD from three replicates of the experiment are presented. (B) Addition of NR after preincubation with yolkin for 48 h. The means ±SD from seven replicates of the experiment are presented. * – Statistical significance (P-value < 0.05) compared to the control (RAW 264.7 cells in culture medium only); Ctrl – control incubation; pre48h – incubation with yolkin for 48 h

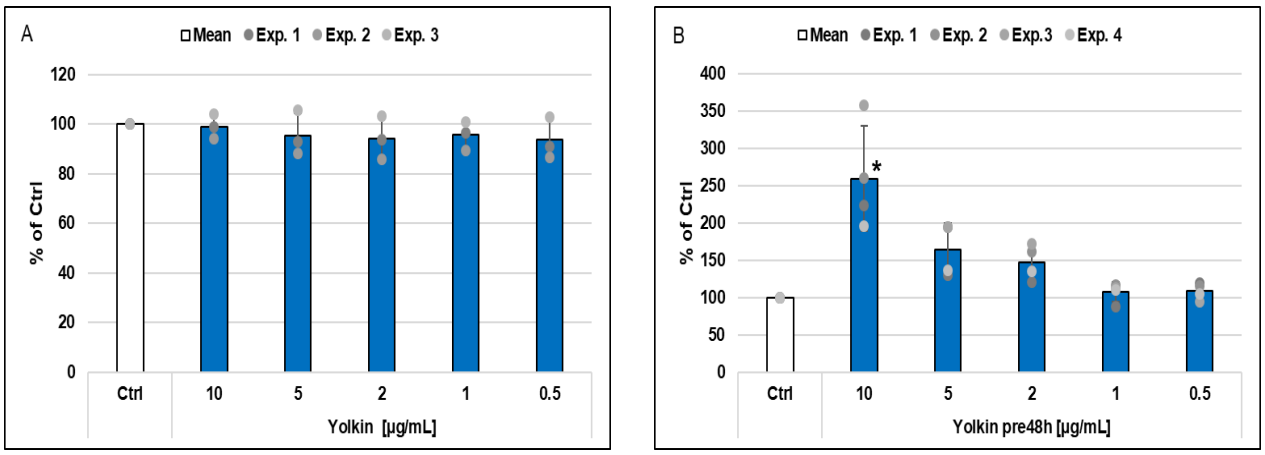


Fig. 3. The effects of yolkin on the capability of RAW 264.7 cells to engulf neutral red (NR)-stained zymosan particles. (A) Yolkin and NR-zymosan were added simultaneously to the culture (no preincubation step). The means ±SD from three replicates of the experiment are presented. (B) Addition of NR-zymosan after preincubation with yolkin for 48 h. The means ±SD from four replicates of the experiment are presented. * – statistical significance (P-value < 0.05) compared to the control (RAW 264.7 cells in culture medium only); Ctrl – control incubation; pre48h – incubation with yolkin for 48 h

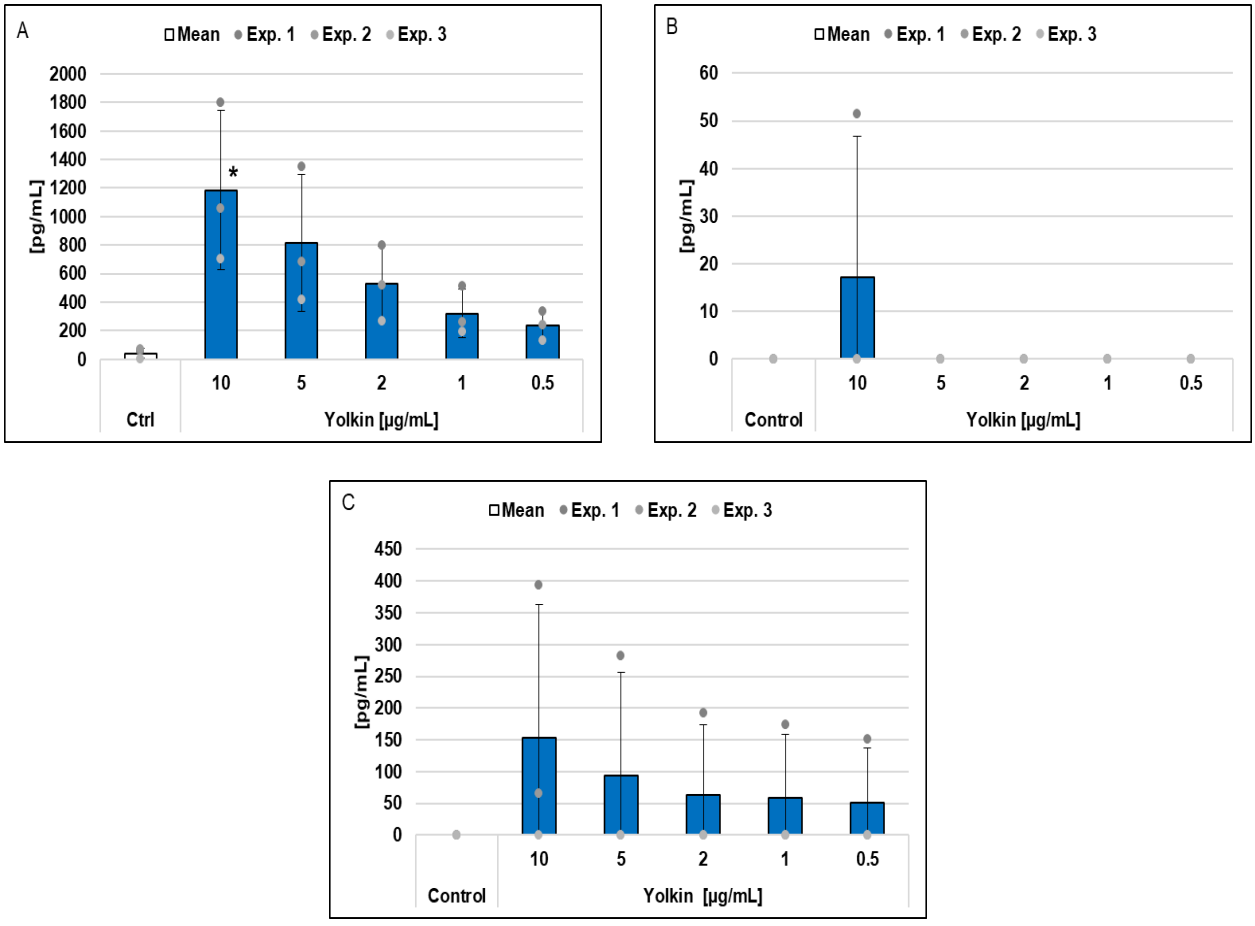


Fig. 4. The effect of incubation of RAW 264.7 cells for 48 h with yolkin on cytokine production. (A) Production of TNF α. (B) Production of IL-6. (C) Production of IL-10. A culture of RAW 264.7 cells was incubated overnight, yolkin was added and the cells incubated for 48 h. The results are presented as the mean ±SD from three independent experiments. Statistical significance is indicated by asterisks (* – P-value < 0.05) compared to the control (RAW 264.7 cells in culture medium only). Ctrl – control incubation

Discussion

In this work, we provided evidence that yolkin promotes the pinocytic and phagocytic functions of RAW 264.7 mouse macrophages. The stimulatory effects on pinocytosis and phagocytosis could resemble the action of yolkin on bone marrow–derived macrophages *via* TLR2 and TLR4 (15). Further, yolkin was a strong activator of pro-inflammatory cytokine production in mouse splenocyte cultures (37) and in bone marrow–derived macrophages (15). These events were correlated with a large increase in NFκB expression in RAW 264.7 cell cultures and upregulation of extracellular signal-regulated kinases 1/2 and c-Jun N-terminal kinase expression. In addition, yolkin strongly induced COX 2 expression and production in RAW 264.7 cultures (37).

The presented profiles of the secreted cytokines support the suggestion made by other authors (14) that yolkin directs polarisation of macrophages to the M1 phenotype. In this study, we demonstrated that following culture with yolkin, RAW 264.7 cells produce more TNF-α than IL-10. The TNF-α : IL-10 ratio was 7.8:1 after 48 h incubation. Following incubation with yolkin, the cytokine profile corresponded to the M1 macrophage phenotype capable of efficient pinocytosis and phagocytosis and characterised by that efficiency (36).

Additionally, the results indicate that activation of macrophages by yolkin could engage TLRs, as demonstrated for bone marrow–derived macrophages (15), since acquisition of the pinocytic ability was partially inhibited by anti-TLR2 and -TLR4 antibodies (data not shown). Taking into account the very complex glycosylation pattern of yolkin and the significant content of mannose (35), yolkin may also target RAW 264.7 cells *via* the mannose receptor (19) and sialoadhesin (31). More research is required to identify other yolkin receptors on relevant cells. Of note is that another key egg yolk protein – phosvitin – demonstrated similar actions in the RAW 264.7 model by enhancing phagocytosis and expression of pro-inflammatory mediators (17). The structure of its oligosaccharide also predisposes the protein to interact with these cells (32).

The activation of RAW 264.7 cells *via* TLR4, leading to the M1 phenotype and evidenced by the production of IL-2, TNF-α, and IFN, was also induced by a polysaccharide (18). Redirection of RAW 264.7 cells to the M1 phenotype was also induced by a glycoprotein, which stimulated pinocytic and phagocytic activity, and increased NO, TNF-α, IL-6, and chemokine production (36). Taken together, these alterations in the inflammatory mediators in macrophages induced by yolkin resemble those by which RAW 264.7 macrophage pinocytosis and phagocytosis are elicited by other glycoproteins and glycans (6, 7, 18, 36).

A finding of importance is that the registered stimulatory effects of yolkin on macrophage phagocytic function were accompanied by production of cytokines known to support antibacterial defence (11). Macrophages were also implicated in antibacterial defence in mice by another naturally occurring glycoprotein, lactoferrin (34), which exhibited a mechanism of action closely resembling that of yolkin (16). The protein was also shown to act in concert with specific bacteriophages in response to lethal bacterial infection (38) and to exhibit an adjuvant property when administered with BCG immunisation (39).

The ability of yolkin to enhance phagocytosis may also explain its strong stimulatory effect on the humoral immune response to sheep erythrocytes (21). In the mouse model used in that experiment, yolkin and an antigen (sheep red blood cells) were introduced by the same intraperitoneal route. Under these conditions, professional antigen-presenting cells, such as peritoneal macrophages and B-1 cells may be activated for enhanced phagocytosis, antigen degradation and presentation. We also speculate that the ability of yolkin to enhance phagocytosis may be applied to improve vaccine efficiency (1).

Conclusion

We demonstrated that yolkin stimulated the capability of cells to perform pinocytosis of NR and phagocytosis of NR-zymosan in a dose dependent manner, and to induce cytokine production of the Th1 profile. The results supplement the current knowledge on the immunotropic properties of yolkin, which may also play antibacterial and antiviral roles in developing embryos. Going forward, yolkin and other egg yolk–derived proteins may find use as nutraceuticals and ingredients of functional foods for boosting immunity to various infectious agents.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This research was funded by National Science Centre, Poland, No. DEC-2021/41/B/NZ6/01167. For the purpose of open access, the author has applied a CC-BY

public copyright license to any Author Accepted Manuscript (AAM) version arising from this submission.

Animal Rights Statement: None required.

CRediT Authorship Contribution Statement: Michał Zimecki: research concept and design, collection and assembly of data, data analysis and interpretation, writing the article, critical revision of the article, final approval of the article. **Jolanta Artym:** critical revision of the article, final approval of the article. **Maja Kocięba:** collection and assembly of data, data analysis and interpretation, critical revision of the article, final approval of the article. **Ewa Zaczyńska:** collection and assembly of data, data analysis and interpretation, critical revision of the article, final approval of the article. **Aleksandra Zambrowicz:** collection and assembly of data, final approval of the article. **Łukasz Bobak:** collection and assembly of data, final approval of the article.

References

1. Ajit J., Chen Q., Ung T., Rosenberger M., Kim, J., Solanki A., Shen J., Aaron P., Kahn E.: β-glucan induced trained immunity enhances antibody levels in a vaccination model in mice. PLoS One 2005, 20, e 0323376, doi: 10.1371/journal.pone.0323376.
2. Alves M.M.M., Arcanjo D.D.R., Carvalho R.C.V. de, Amorim L.V., Santos I.L. dos, Santos R.R.L., Figueiredo K.A., Figueiredo J.F.S., Sobrinho-Júnior E.P.C., Cruz L.P.L. da, Sousa V.C., Santos L.P. dos, Goncalves J.C.R., Carneiro S.M.P., Mendonça I.L. de, Carvalho F.A.A.: Methods of macrophages activation and their modulation for the prospection of new antileishmania drugs: a review. Bioscience J 2021, 37, e37077, doi: 10.14393/BJ-v37n0a2021-53770.
3. Antal P., Sipka S., Surányi P., Csipo I., Seres T., Maródi L., Szegedi G.: Flow cytometric assay of phagocytic activity of human neutrophils and monocytes in whole blood by neutral red uptake. Ann Hematol 1995, 70, 259265, doi: 10.1007/BF01784045.
4. Brito R.R.N., Toledo M.S., Labussiere G.M., Dupin T.V., Reis N.F.C., Perez E.C., Xander P.: B-1 cell response in immunity against parasites. Parasitol Res 2019, 118, 1343–1352, doi: 10.1007/s00436-019-06211-2.
5. Chen J., Liu T., Luo J., Chen Z., Yu Y., Sun H., Tan M., Sun Y., Li S., Li C., Ding Y.: HDAC6/Trim21 aggravates macrophage inflammatory response and titanium-induced osteolysis *via* GSDMD signaling pathway. J Immunol 2025, 214, 2357–2370, doi: 10.1093/jimmun/vkaf112.
6. Chen J., Yang Y., Xu Z., Li F., Yang M., Shi F., Lin L., Qin Z.: Characterization of effects of chitooligosaccharide monomer addition on immunomodulatory activity in macrophages. Food Res Int 2023, 163, 112268, doi: 10.1016/j.foodres.2022.112268.
7. Choi H.J., Park G.H., Choi J.W., Park S.J., Hwang J.H., Lee S.H., Kwon H.-Y., Choi M.Y., Jeong J.B.: Immunostimulatory activity of the aqueous extract from the leaves of *Sambucus racemosa* subsp. *pendula* through TLR4-dependent JNK activation in RAW264.7 cells. Biomed Rep 2024, 21, 133, doi: 10.3892/br.2024.1821.
8. Depierre M., Pompili C., Niedergang F.: Phagocytosis at a glance. J Cell Sci 2025, 38, jcs263833, doi: 10.1242/jcs.263833.
9. Gambero M., Teixeira D., Butin L., Ishimura M.E., Mariano M., Popi A.F., Longo-Maugéri I.M.: *Propionibacterium acnes* induces an adjuvant effect in B-1 cells and affects their phagocyte differentiation *via* a TLR2-mediated mechanism. Immunobiology 2016, 221, 1001–1011, doi: 10.1016/j.imbio.2016.03.003.
10. Hansen M.B., Nielsen S.E., Berg K.: Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods 1989, 119, 203–210, doi: 10.1016/0022-1759(89)90397-9.
11. Holub M., Cheng C-W., Mott S., Wintermeyer P., van Rooijen N., Gregory S.H.: Neutrophils sequestered in the liver suppress the proinflammatory response of Kupffer cells to systemic bacterial infection. J Immunol 2009, 183, 3309–3316, doi: 10.4049/jimmunol.0803041.
12. Hsu A.Y., Huang Q., Liu F., Balasubramanian A., Luo H.R.: Neutrophil death-more than meets the eye. Exp Hematol 2025, 150, 104857, doi: 10.1016/j.exphem.2025.104857.
13. Kashimura M.: Blood defence system – Proposal for a new concept of an immune system against blood borne pathogens comprising the liver, spleen and bone marrow. Scand J Immunol 2024, 99, e13363, doi: 10.1111/sji.13363.
14. Kazana W., Jakubczyk D., Pacyga-Prus K., Leszczyńska K., Górska S., Siednienko J., Macała J., Piechowiak G., Zabłocka A.: A novel mechanism of macrophage activation by the natural yolkin polypeptide complex from egg yolk. Int J Mol Sci 2022, 23, 3125, doi: 10.3390/ijms23063125.
15. Kazana W., Mitkiewicz M., Ochnik M., Sochocka M., Zambrowicz A., Piechowiak G, Macała J., Miernikiewicz P., Zabłocka A.: Yolkin isolated from hen 17 egg yolk as a natural immunoregulator, activating innate immune response in BMDM macrophages. Oxid Med

Cell Longev 2020, 2020, 5731021, doi: 10.1155/2020/5731021.
16. Kenzo K., Komine K.-i., Komine Y., Kuroishi T., Kozutsumi T., Kobayashi J., Ohta M., Kitamura H., Kumagai K.: Lactoferrin stimulates a *Staphylococcus aureus* killing activity of bovine phagocytes in the mammary gland. Microbiol Immunol 2002, 46, 187–194, doi: 10.1111/j.1348-0421.2002.tb02685.x.
17. Lee J.H., Moon S.H., Kim H.S., Park E., Ahn D.U., Paik H.D.: Immune-enhancing activity of phosvitin by stimulating the production of pro-inflammatory mediator. Poult Sci 2017, 96, 3872–3878, doi: 10.3382/ps/pex205.
18. Li Q., Zhao T., Mao G., Feng W., Chen Y., Zou T., Yang L., Qian J.-Y.: A Se-enriched *Grifola frondosa* polysaccharide induces macrophage activation by TLR4-mediated MAPK signaling pathway. Int J Biol Macromol 2023, 238, 124108, doi: 10.1016/j.ijbiomac.2023.124108.
19. Long L., Li M., Wang M., Liang B., Huang M., Yuan X., Wu X., Guo X., Li S., Liu Z., Liu W., Chen W., Wang W., Lyu Q., Li C.: Activation of mannose receptor C type 1 in macrophages improves renal fibrosis through mediating fibronectin endocytosis. Life Sci 2025, 371, 123593, doi: 10.1016/j.lfs.2025.123593.
20. Mylvaganam S., Freeman S.A., Grinstein S.: The cytoskeleton in phagocytosis and macropinocytosis. Curr Biol 2021, 31, R619–R632, doi: 10.1016/j.cub.2021.01.036.
21. Obmińska-Mrukowicz B., Szczypka M., Lis M., Pawlak A., Suszko-Pawłowska A., Sysak A, Zambrowicz A., Burster T., Kocięba M., Artym J., Zaczyńska E., Kochanowska I., Zimecki M.: Effects of yolkin on the immune response of mice and its plausible mechanism of action. Immunol Lett 2020, 220, 21–31, doi: 10.1016/j.imlet.2020.01.003.
22. Polanowski A., Sosnowska A., Zabłocka A., Janusz M., Trziszka T.: Immunologically active peptides that accompany hen egg yolk immunoglobulin Y: separation and identification. Biol Chem 2013, 394, 879–887, doi: 10.1515/hsz-2012-0337.
23. Rodrigues K.A.F., Amorim L.V., Dias C.N., Moraes D.F.C., Carneiro S.M.P., Carvalho F.A.A.: *Syzygium cumini* (L.) Skeels essential oil and its major constituent α-pinene exhibit anti-*Leishmania* activity through immunomodulation *in vitro*. J Ethnopharmacol 2015, 160, 32–40, doi: 10.1016/j.jep.2014.11.024.
24. Salloum G., Bresnick A.R., Backer J.M.: Macropinocytosis: mechanisms and regulation. Biochem J 2023, 480, 335–362, https://doi.org/10.1042/BCJ20210584.
25. Sandhu K.K., Scott A., Tatler A.L., Belchamber K.B.R., Cox M.J.: Macrophages and the microbiome in chronic obstructive pulmonary disease. Eur Respir Rev 2024, 33, 240053, doi: 10.1183/16000617.0053-2024.
26. Sigola L.B., Fuentes A.-L., Millis L.M., Vapenik J., Murira A.: Effects of Toll-like receptor ligands on RAW 264.7 macrophage morphology and zymosan phagocytosis. Tissue Cell 2016, 48, 389–396, doi: 10.1016/j.tice.2016.04.002.
27. Souza A.C., Alves M.M.M., Brito L.M., Oliveira L.G.C., Sobrinho-Júnior E.P.C., Costa I.C.G., Freitas S.D.L., Rodrigues K.A.F., Chaves M.H., Arcanjo D.D.R., Carvalho F.A.A.: *Platonia insignis* Mart., a Brazilian Amazonian plant: the stem barks extract and its main constituent lupeol exert antileishmanial effects involving macrophages activation. Evid-Based Complementary Altern Med 2017, 3126458, doi: 10.1155/2017/3126458.
28. Tian H., Ling N., Guo C., Gao M., Wang Z., Liu B., Sun Y., Chen Y., Ji C., Li. W.: Immunostimulatory activity of sea buckthorn polysaccharides via TLR2/4-mediated MAPK and NF-κB signaling pathways *in vitro* and *in vivo*. Int J Biol Macromol 2024, 283, 137678, doi: 10.1016/j.ijbiomac.2024.137678.
29. Weeks B.A., Keisler A.S., Myrvik Q.N., Warinner J.E.: Differential uptake of neutral red by macrophages from three species of estuarine fish. Dev Comp Immunol 1987, 11, 117–124, doi: 10.1016/0145-305x(87)90013-9.
30. Wen Y., Peng D., Li C., Hu X., Bi S., Song L., Peng B., Zhu J., Chen Y., Yu R.: A new polysaccharide isolated from *Morchella importuna* fruiting bodies and its immunoregulatory mechanism. Int J Biol Macromol 2019, 137, 8–19, doi: 10.1016/j.ijbiomac.2019.06.171.
31. Wu Y.; Lan C.; Ren D., Chen G.-Y.: Induction of siglec-1 by endotoxin tolerance suppresses the innate immune response by promoting TGF-β1 production. J Biol Chem 2016, 291, 12370–12382, doi: 10.1074/jbc.M116.721258.
32. Vogel H.J., Brockbank R.L.: Structure of the oligosaccharide of hen phosvitin as determined by two-dimensional 1H NMR of the intact glycoprotein. Biochemistry 1990, 29, 5574–5583, doi: 10.1021/bi00475a023.
33. Xie C., Lin X., Hu J., Wang S., Wu J., Xiong W., Wu L.: The polysaccharide from *Camellia oleifera* fruit shell enhances immune responses *via* activating MAPKs and NF-κB signaling pathways in RAW264.7 macrophages. Food Nutr Res 2022, 66, 8963, doi: 10.29219/fnr.v66.8963.
34. Zagulski T., Lipiński P., Zagulska A., Jarząbek Z.: Antibacterial system generated by lactoferrin in mice *in vivo* is primarily a killing system. Int J Exp Pathol 1998, 79, 117–123, doi: 10.1046/j.1365-2613.1998.00058.x.
35. Zambrowicz A., Kapczyńska K., Kania P., Nowak J.S., Kaszowska M., Szymczak-Kulus K., Kazana-Płuszka W., Piksa M., Górska S.,

Jakubczyk D., Macała J., Zabłocka A.: Unravelling the potential of yolkin for nutraceutical use: the origin, structure, and functional insights of a hen egg yolk polypeptide complex. Food Funct 2024, 15, 10746–10760, doi: 10.1039/d4fo03023k.
36. Zhao M., Qin S., Wang J., Zheng S., Ma X., Xu W.: Cirsii Herba glycoprotein promotes macrophage M1 polarization through MAPK and NF-κB signaling pathways via interaction with TLR4. Int J Biol Macromol 2025, 296, 139687, doi: 10.1016/j.ijbiomac.2025.139687.
37. Zimecki M., Artym J., Kochanowska I., Kocięba M., Zaczyńska E., Zambrowicz A., Bobak Ł.: Immunoregulatory effects of yolkin on cell proliferation, cytokine production and cell signaling. Braz J Poult Sci 2025, 27, 001–014, doi: 10.1590/1806-9061-2024-2027.
38. Zimecki M., Artym J., Kocięba M., Weber-Dąbrowska B., Lusiak-Szelachowska M., Górski A.: The concerted action of lactoferrin and bacteriophages in the clearance of bacteria in sublethally infected mice. Post Hig Med Dośw 2008, 62, 42–46.
39. Zimecki M., Kruzel M.L., Hwang S.-A., Wilk K.M., Actor J.K.: Lactoferrin as an adjuvant for the generation of delayed type hypersensitivity to orally administered antigen. Ann Clin Lab Sci 2021, 51, 359–367.