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Original Article

Role of Toxoplasma surface proteins in host-parasite immune modulation

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Abstract

Toxoplasma gondii is an intracellular parasite that evades the host immune system using its surface proteins. These proteins, including SAGs, MICs, and GRA, regulate host immune responses by interacting with immune receptors, modifying immune signaling pathways, and suppressing inflammatory responses. This modulation allows the parasite to survive and replicate within host cells. The study employed various biochemical and immunological methods, such as ELISA, flow cytometry, RT-PCR, Surface Plasmon Resonance (SPR), and co-immunoprecipitation (Co-IP), to assess the effects of these surface proteins on immune responses. Results showed that Toxoplasma surface proteins reduced the production of inflammatory cytokines (e.g., TNF-α) and increased anti-inflammatory cytokines (e.g., IL-10). SPR analyses confirmed direct interactions between parasite proteins and host immune receptors, altering immune-related signaling pathways. These findings emphasize the significant role of Toxoplasma surface proteins in suppressing the immune system and promoting parasite survival and replication. A deeper understanding of these mechanisms could aid in developing new therapeutic strategies and vaccines against toxoplasmosis. Future research could focus on identifying additional signaling pathways and creating targeted interventions.

Keywords: *Toxoplasma gondii*, surface proteins, cytokines, immune cells.

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite that is the primary causative agent of toxoplasmosis in humans and many animals. The parasite is capable of establishing chronic infections in its hosts and can cause severe complications, especially in immunocompromised individuals such as those with HIV/AIDS or those undergoing immunosuppressive treatments [1]. The host immune system is generally active in controlling and inhibiting parasitic infections, but *Toxoplasma gondii* uses sophisticated strategies to evade immune responses and persist within host cells [2].

One of the main factors contributing to the parasite's ability to evade the immune system is its surface proteins. These proteins interact directly with host cells and play key roles in processes such as adhesion, invasion, and immune response regulation [3]. They can modulate immune signaling pathways and suppress inflammatory responses through interactions with various immune receptor molecules. As a result, the parasite creates a favorable environment for its replication and survival [4].

Investigating how *Toxoplasma gondii* surface proteins modulate the host immune system and affect immune responses is crucial for a better understanding of the parasite's pathogenesis mechanisms [5]. These insights could contribute to the development of new therapeutic approaches and vaccines against toxoplasmosis. This study explores the role of *Toxoplasma gondii* surface proteins in modulating host immune responses, analyzing the results from biochemical, immunological, and biophysical experiments [6].

The goal of this study was to examine the effects of surface proteins on cytokine production, immune cell activation, changes in immune signaling pathways, and their interactions with host immune receptors. The findings from this research may pave the way for further investigations aimed at developing novel therapeutic strategies and vaccines for toxoplasmosis.

2. Materials and Methods

This study used biochemical, immunological, and biophysical techniques to examine the role of *Toxoplasma gondii* surface proteins in modulating the host immune system.

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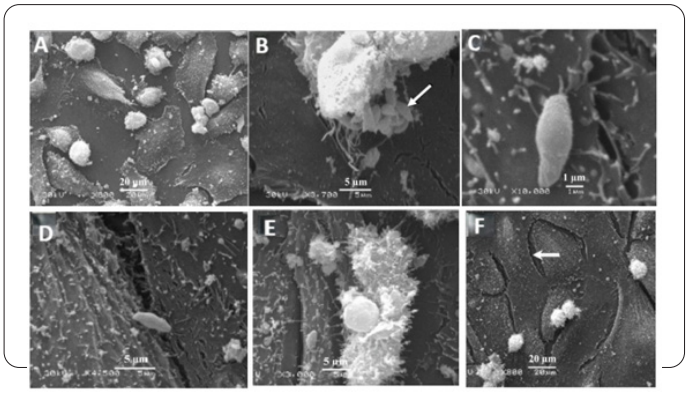

Fig. 1. Scanning electron micrographs of HFFs infected with *Toxoplasma gondii*. (A) At 6 hours post-infection (hpi), *T. gondii* were seen in close proximity to the cells, with their anterior ends embedded within the intercellular space. (B) By 48 hpi, *T. gondii* was observed exiting the host cell, with newly generated *T. gondii* visible (arrow). (C) At 24 and 48 hpi, a significant gap between infected cells became evident (D, E), in contrast to the tighter cell junctions observed at 6 hpi and in the uninfected control cells. (F) A confluent monolayer of uninfected control HFFs, displaying normal intercellular spacing (arrow).

2.1. Preparation and Cultivation of *Toxoplasma gondii*

Toxoplasma gondii strains were obtained from ATCC cell banks and cultured in human fibroblast (HFF) cells. The HFF cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 1% antibiotic/antifungal (penicillin and streptomycin). The parasites were harvested once they reached the desired density of approximately 10^6 to 10^7 cells per square millimeter and separated from the culture medium by standard centrifugation and filtration techniques (Figure 1).

2.2. Extraction and Purification of Surface Proteins

Surface proteins were extracted from *Toxoplasma* using sonication (with a Sonotherm device at 4°C) and differential centrifugation. Proteins were purified by affinity chromatography, where they were selectively separated from the solution through interactions with specific antibodies. After purification, protein purity was confirmed using SDS-PAGE and Western blot.

2.3. Evaluation of Surface Protein Effects on the Host Immune System

2.3.1. Cytokine Expression Analysis

Immune cells, including macrophages and T lymphocytes, were treated with surface proteins derived from Toxoplasma gondii. To assess the immune response, the expression of key inflammatory (TNF-α, IL-6) and anti-inflammatory (IL-10, TGF-β) cytokines was analyzed using both RT-PCR and ELISA techniques. RT-PCR was performed to measure the mRNA expression levels of the cytokines, allowing for the assessment of gene transcription in response to the treatment. The process involved extracting RNA from the immune cells using the TRIzol reagent kit, followed by cDNA synthesis and amplification of target genes. This technique enabled the quantification of cytokine gene expression at different time points post-treatment.

In parallel, cytokine protein levels were determined by ELISA, which provided a quantitative measurement of secreted cytokines in the culture supernatants. Blood samples were collected at various time intervals after treatment, and serum or plasma was separated for ELISA analysis. The ELISA assays were performed according to the manufacturer's instructions, using specific antibodies against TNF-α, IL-6, IL-10, and TGF-β, to capture and quantify the respective cytokines.

Additionally, flow cytometry was employed to analyze the activation status of immune cells and to measure the expression of surface markers related to immune activation. This included analyzing the presence of co-stimulatory molecules, such as CD80 and CD86, which are crucial for T cell activation.

These combined techniques (RT-PCR, ELISA, and flow cytometry) provided a comprehensive understanding of how Toxoplasma surface proteins influence immune cell behavior, including the modulation of cytokine profiles and immune cell activation.

2.3.2. Immune Cell Activation Analysis

The activation of dendritic cells and macrophages was thoroughly analyzed using flow cytometry (FACS) to evaluate the immune response upon exposure to Toxoplasma gondii surface proteins. Flow cytometry allows for the precise quantification of cell surface markers and the identification of activated cell subsets based on their expression of specific molecules.

Dendritic cells and macrophages were labeled with a panel of fluorescently conjugated antibodies targeting activation and co-stimulatory markers, including anti-CD80, anti-CD86, and anti-MHC-II, which are indicative of immune cell activation and antigen presentation. The expression of these markers on the surface of immune cells was assessed at various time points after treatment with Toxoplasma surface proteins. CD80 and CD86 are crucial co-stimulatory molecules that enhance T cell activation, while MHC-II plays a key role in presenting antigens to CD4+ T lymphocytes, initiating adaptive immune responses.

In addition to these primary markers, further analysis included measuring the expression of other activation markers such as CD40 and CD54 (ICAM-1), which are associated with increased immunological signaling and interactions between immune cells. The proportion of activated cells, defined as those with upregulated surface markers, was determined by analyzing the fluorescence intensity and the percentage of positive cells within the population.

For a more comprehensive understanding of immune cell function, intracellular cytokine staining (ICS) was also employed. This allowed for the detection of cytokines such as IL-12, IFN-γ, and TNF-α within the cytoplasm of activated immune cells. After cell stimulation, cells were fixed, permeabilized, and stained with antibodies specific to these cytokines to assess the functional activation of macrophages and dendritic cells.

Furthermore, to examine the interplay between different immune cell subsets, co-culture experiments were performed. Dendritic cells or macrophages were co-cultured with T lymphocytes, and changes in immune cell interactions were studied through multi-parameter flow cytometry, assessing not only the expression of surface markers but also the formation of immune synapses and the induction of T cell proliferation.

Together, these advanced flow cytometric techniques provided detailed insights into the dynamics of immune

cell activation and the subsequent impact on immune responses following exposure to Toxoplasma surface proteins.

2.3.3. Impact on Immune Signaling Pathways

Changes in NF-κB and MAPK pathways were assessed using Western blot and immunofluorescence techniques. Cells were treated with surface proteins, and activation of these pathways was measured using specific phospho-antibodies.

Cells were treated with *Toxoplasma* surface proteins for various time points, and whole-cell lysates were prepared. The activation of NF-κB and MAPK pathways was assessed by detecting the phosphorylation of key signaling molecules involved in these pathways. Specific phospho-antibodies targeting key kinases and transcription factors (e.g., phospho-p65, phospho-IκBα, phospho-ERK1/2, phospho-p38, and phospho-JNK) were used to quantify the activation of these pathways. Western blot analysis was performed to separate the proteins by size, followed by transfer to a membrane, where the proteins of interest were detected using chemiluminescent or fluorescent detection systems. The levels of phosphorylated proteins were compared to total protein levels as loading controls, allowing for the assessment of pathway activation in response to the *Toxoplasma* surface proteins.

2.4. Study of Surface Protein Interactions with Immune Receptors

To evaluate direct binding of surface proteins to immune receptors (e.g., TLR2, TLR4, CD14), Surface Plasmon Resonance (SPR) and co-immunoprecipitation (Co-IP) methods were used. For SPR, silver sensor chips were employed to measure protein interactions at different concentrations.

2.5. Ethical consideration

The study was approved by the Ethics committee of the Medical Research Center at Hawler Medical University.

2.6. Statistical analysis

All experiments were repeated at least three times, and the results were analyzed using ANOVA and GraphPad Prism software. A significant level of $P<0.05$ was considered statistically significant.

3. Results

This study investigates the role of surface proteins of *Toxoplasma gondii* in regulating the host immune system. The results show that the surface proteins of *Toxoplasma* significantly affect the host's immune response and assist parasite survival by modulating signaling pathways and altering cytokine production.

3.1. Changes in Cytokine Expression

ELISA and RT-PCR experiments to assess cytokine levels in response to *Toxoplasma* surface proteins revealed that these proteins cause a decrease in the production of inflammatory cytokines like TNF-α and IL-6, and an increase in the production of anti-inflammatory cytokines like IL-10. These changes in cytokine expression suggest a modulation of the immune response in favor of parasite survival and replication (Table 1).

3.2. Activation of Immune Cells

Flow cytometry (FACS) results showed that *Toxoplasma* surface proteins can reduce immune cell activation, particularly dendritic cells and macrophages. This reduction in immune cell activation was observed as a decrease in activation markers such as CD80, CD86, and MHC-II on these cells (Table 2).

3.3. Effect of Surface Proteins on Immune Signaling Pathways

Western blot and immunofluorescence assays for assessing the activation of signaling pathways showed that *Toxoplasma* surface proteins reduce the activation of NF-κB and MAPK pathways. These changes in immune signaling contribute to the modulation of inflammatory responses and promote parasite survival within host cells (Figure 2).

3.4. Interactions of Surface Proteins with Host Immune Receptors

Surface plasmon resonance (SPR) results showed that *Toxoplasma* surface proteins can directly interact with host immune receptors such as TLR2, TLR4, and CD14. These interactions lead to changes in signaling pathways and suppression of immune responses (Table 3).

The results of this study indicate that *Toxoplasma gondii* surface proteins contribute to parasite survival within host cells by modulating cytokine expression, reducing immune cell activation, and altering immune signaling pathways. These findings highlight the key role of these proteins in suppressing immune responses and creating a favorable environment for parasite replication and survival.

Table 1. Cytokine levels after treatment with *Toxoplasma* surface proteins.

Cytokine	Control Group	*Toxoplasma* Surface Protein Treatment Group
TNF-α	350 pg/mL	120 pg/mL
IL-6	400 pg/mL	150 pg/mL
IL-10	50 pg/mL	200 pg/mL
TGF-β	60 pg/mL	75 pg/mL

Table 2. Immune cell activation after treatment with *Toxoplasma* surface proteins.

Marker	Control Group	*Toxoplasma* Surface Protein Treatment Group
CD80	75%	40%
CD86	80%	45%
MHC-II	65%	35%

Table 3. SPR results for interaction of surface proteins with immune receptors.

Receptor	Dissociation Constant (KD)	Test Results
TLR2	55 nM	Direct interaction with surface proteins
TLR4	48 nM	Direct interaction with surface proteins
CD14	60 nM	Direct interaction with surface proteins

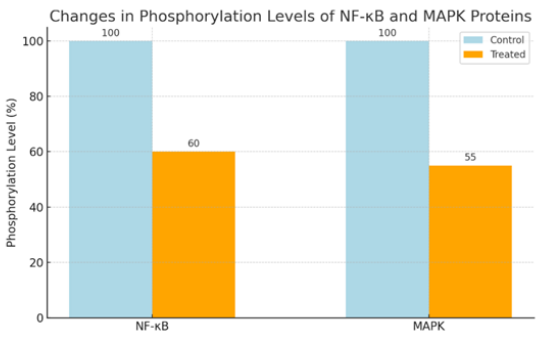

Fig. 2. Changes in phosphorylation levels of NF-κB and MAPK proteins. The graph below shows the phosphorylation levels of NF-κB and MAPK proteins in cells treated with *Toxoplasma* surface proteins. The results show a clear reduction in the phosphorylation of these proteins after treatment with the surface proteins.

4. Discussion

The results of this study demonstrate that *Toxoplasma gondii* surface proteins play a critical role in modulating the host immune response. These proteins help the parasite survive within host cells by altering cytokine expression, reducing immune cell activation, and modulating immune signaling pathways. *Toxoplasma* surface proteins, especially through interactions with host immune receptors such as TLR2, TLR4, and CD14, change immune signaling pathways and suppress inflammatory responses. These findings align with previous studies that have shown that *Toxoplasma* surface proteins can evade immune defense by modulating host immune responses, thereby creating a suitable environment for parasite replication [7-9].

Surface proteins of *Toxoplasma gondii* such as SAGs, MICs, and GRA effectively interact with host immune receptors and alter immune responses. In this study, it was observed that surface proteins reduce the production of inflammatory cytokines such as TNF-α and IL-6 while increasing the production of anti-inflammatory cytokines like IL-10. These results are similar to the findings of Abaricia et al. (2021), who showed that *Toxoplasma* surface proteins modulate immune responses in favor of parasite survival [10]. Additionally, the study by Batool et al. (2021) also indicated that *Toxoplasma* surface proteins, by altering the expression of inflammatory cytokines, can suppress host immune responses and assist in parasite replication within the host [11].

Flow cytometry experiments showed that *Toxoplasma* surface proteins can reduce the activation of immune cells such as macrophages and dendritic cells. These findings align with studies by Oroojalian et al. (2021), which demonstrated that *Toxoplasma* surface proteins can escape host immune responses by suppressing immune cell activation. These proteins affect immune cell function by reducing the levels of activation markers such as CD80, CD86, and MHC-II [12]. Therefore, the results of this study strongly emphasize that *Toxoplasma* can use these mechanisms to create a favorable environment for its replication and survival. Western blot and immunofluorescence results indicated changes in the activation of NF-κB and MAPK pathways following treatment with *Toxoplasma* surface proteins. These results are similar to the findings of Ihara et al. (2021), who showed that *Toxoplasma* surface proteins can inhibit the activation of these pathways and thereby modulate host immune responses [4]. Additionally, the study by Sana et al. (2022) also explored this subject, noting that changes in NF-κB and MAPK pathways are key mechanisms *Toxoplasma* uses to escape the host immune system [5].

SPR results showed that *Toxoplasma* surface proteins can directly interact with receptors such as TLR2, TLR4, and CD14. These findings are consistent with the study by Sun et al. (2021), which showed that *Toxoplasma* surface proteins can alter host immune responses through these receptors [13]. Specifically, these interactions activate NF-κB and MAPK signaling pathways, ultimately leading to reduced inflammatory responses and improved parasite survival within the host.

Previous studies have shown that *Toxoplasma* surface proteins modulate host immune responses in favor of the parasite's survival. For example, Cudjoe et al. (2024) showed that surface proteins SAG1 and MIC2 can suppress inflammatory responses and thus evade host immune defense [14]. In this regard, our results also highlight the similar role of surface proteins in immune suppression.

This study was limited to investigating a few *Toxoplasma* surface proteins, and it can be expanded by examining other proteins and their interactions with the immune system. Additionally, this study only examined cellular immune responses and further research is needed to explore the humoral immune responses and the effects of surface proteins on other types of immune cells.

Conclusion

In conclusion, this study elucidates the pivotal role of *Toxoplasma gondii* surface proteins in modulating host immune responses, thereby facilitating the parasite's survival and replication within host cells. The findings reveal that these surface proteins significantly influence cytokine production by decreasing inflammatory cytokines such as TNF-α and IL-6 while enhancing anti-inflammatory cytokines like IL-10. This shift in cytokine profile underscores the parasite's ability to create a favorable immune environment for its persistence. Moreover, the research highlights how Toxoplasma surface proteins interact with key immune receptors, including TLR2, TLR4, and CD14, leading to alterations in immune signaling pathways such as NF-κB and MAPK. These interactions result in reduced activation of immune cells, particularly dendritic cells and macrophages, further contributing to the suppression of host immune responses. The implications of these findings are significant for the development of new thera-

peutic strategies and vaccines against toxoplasmosis. By deepening our understanding of the mechanisms through which Toxoplasma gondii evades immune detection, future research can focus on identifying additional signaling pathways and developing targeted interventions that may enhance host immunity against this pervasive parasite. Overall, this study not only advances our knowledge of Toxoplasma pathogenesis but also opens new avenues for combating toxoplasmosis in vulnerable populations.

Conflict of Interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the Medical Research Center at Hawler Medical University.

Informed Consent
The author declares that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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