ACTIVATION OF THE CELLULAR MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS ERK, P38 AND JNK DURING *TOXOPLASMA GONDII* INVASION

VALÈRE A.*, GARNOTEL R.***, VILLENA I.*, GUENOUNOU M.***, PINON J.M.* & AUBERT D.*

Summary:

Host cell invasion is essential for the pathogenicity of the obligate intracellular protozoan parasite *Toxoplasma gondii*. In the present study, we evaluated the ability of *T. gondii* tachyzoites to trigger phosphorylation of the different mitogen-activated protein kinases (MAPK) in human monocytic cells THP1. Kinetic experiments show that the peak of extracellular-signal-regulated kinase (ERK1/2), P38 and cJun-NH₂ terminal kinase (JNKs) phosphorylation occurs between 10 and 60 min. The use of specific inhibitors of ERK1/2, P38 and JNK1/2 phosphorylation indicates the specificity of MAPKs phosphorylation during invasion. Signaling through cellular and parasite mitogen-activated protein (MAP) kinase pathways appears to be critical for *T. gondii* invasion.

KEY WORDS : *Toxoplasma gondii*, human monocytic cell (THP1), MAP kinases, phosphorylation.

Résumé : CINÉTIQUE D'ACTIVATION DE LA VOIE DES MITOGEN-ACTIVATED PROTEIN KINASES CELLULAIRES ERK, P38 ET JNK DURANT L'INVASION PAR *TOXOPLASMA GONDII*

L'invasion d'une cellule hôte est essentielle pour la pathogénicité de *Toxoplasma gondii*, parasite protozoaire intra-cellulaire obligatoire. Dans cette étude, nous évaluons la capacité des tachyzoïtes de *T. gondii* à déclencher la phosphorylation des différentes mitogen-activated protein kinases (MAPK) dans une lignée de cellules myéломocytaires humaines THP1. Le pic de phosphorylation des extracellular-signal-regulated kinase (ERK1/2), P38 et cJun-NH₂ terminal kinase (JNKs) se produit entre 10 et 60 minutes et la spécificité de l'activation des MAPKs a pu être montrée par utilisation d'inhibiteurs spécifiques de ERKs, P38 et JNKs. Les voies de signalisation passant par les MAP kinases cellulaires, mais aussi parasitaires paraissent jouer un rôle critique dans les processus d'invasion de *T. gondii*.

MOTS CLÉS : *Toxoplasma gondii*, cellules monocytaires humaines (THP1), MAP kinases, phosphorylation.

T. gondii, the etiologic agent of toxoplasmosis is an ubiquitous protozoan parasite. Infection is generally asymptomatic, but congenital toxoplasmosis can have serious consequences and the disease can be life-threatening in immunocompromised patients. As an obligate intracellular parasite, *T. gondii* invades host cell by an active process. Macrophages play a key role in directing the host immune response to infection. Recruitment and stimulation of macrophages by cytokine and/or microbial products such as LPS result in the induction and release of several key immune effector molecules. They play a crucial role in the development of immunity against a number of intracellular pathogens including *Toxo-*

plasma gondii (Denkers & Gazzinelli, 1998). The mechanism by which *T. gondii* initially stimulates macrophages remains unknown. Recent studies indicate that different protozoan products [glycosylphosphatidylinositol (GPI) of *Trypanosoma cruzi*, *Plasmodium falciparum* and *Trypanosoma brucei* (Robert *et al.*, 2001; Schofield & Hackett, 1993; Tachado & Schofield, 1994); lipophosphoglycan (LPG) of *Leishmania* sp or soluble antigen extract of *T. gondii* tachyzoites (Li *et al.*, 1994)] have an essential role in triggering various macrophage functions, similar to the importance of LPS in infection with Gram negative bacteria (Robert & Gazzinelli, 2000). LPS has been reported to stimulate signal transduction through the MAPKs (Weinstein *et al.*, 1992). Belonging to the serine/threonine protein kinase family, MAP Kinases contribute to induce nuclear responses (Davis, 1993). The three main MAP kinases are extracellular-signal-regulated kinase (ERK), P38 and cJun-NH₂-terminal kinase (JNK). These signaling pathways are important in protozoan such as *Leishmania* (Becker & Jaffe, 1997), *Plasmodium* or *Theileria* (Shaw, 1996).

We previously described a new effector mechanism of interferon gamma (IFN- γ), the major cytokine of host resistance to *T. gondii* infection, mediated by phospholipase A₂ (PLA₂) (Gomez-Marin *et al.*, 1996). PLA₂

* Laboratoire de Parasitologie-Mycologie, EA 2070, IFR 53, UFR de Médecine, Université de Reims-Champagne-Ardenne, 51, rue Cognacq-Jay, 51095 Reims, France.

** Laboratoire de Biochimie et de Biologie Moléculaire, CNRS FRE 2534, IFR 53, UFR de Médecine, Université de Reims-Champagne-Ardenne, 51, rue Cognacq-Jay, 51095 Reims, France.

*** Laboratoire d'Immunologie, EA 2070, IFR 53, UFR de Pharmacie, Université de Reims-Champagne-Ardenne, 51, rue Cognacq-Jay, 51095 Reims, France.

Correspondence: Dominique Aubert, Laboratoire de Parasitologie-Mycologie, CHU Hôpital Maison-Blanche, 45, rue Cognacq-Jay, 51092 Reims, France.

Tel.: 33 (0)3 26 78 42 20 – Fax: 33 (0)3 26 78 73 28.

E-mail: daubert@chu-reims.fr

being a substrate for MAP kinases, we also observed a rise in the MAP kinase activity of infected THP1 cells, and showed that *T. gondii* possessed MAP kinase activity (Gomez-Marin *et al.*, 1996). In this study, we compared the kinetics of phosphorylation of the different members of the MAPK family. We show that *T. gondii* tachyzoites stimulate, with differential kinetics, the activation of ERK, P38 and JNK in THP1 human monocytic cells. The availability of specific inhibitors, allowed us to address the specificity of these activation during *T. gondii* invasion.

MATERIALS AND METHODS

IN VITRO INFECTION MODEL

T. gondii tachyzoites of the virulent, non cyst-forming RH strain were maintained by inoculation in the peritoneal cavity of female Swiss mice. Seventy-two hours after inoculation, tachyzoites were recovered by washing the peritoneal cavity with 5 ml of 0.9 % saline, penicillin 100 U/ml (Pharmacia, France), streptomycin 40 µg/ml (Pharmacia, France) and amoxicillin 80 µg/ml (Eurobio, France). Isolated tachyzoites were centrifuged at 2,000 g for 10 minutes and the pellets were resuspended in RPMI 1640 culture medium (Gibco Life Technologies, France) before being filtered through a 3 µm polycarbonate membrane (Nucleopore Inc, Cambridge, Mass., USA) yielding 99.9 % parasites free of host cells. Parasite viability was assessed by the trypan-blue exclusion test (0.4 % solution); samples were used when the viability exceeded 95 %. Human monocytic THP1 cells (American Type Cell Collection, Rockville, Maryland, USA), were grown in RPMI 1640 medium supplemented with 10 % decompartmented fetal calf serum (FCS; Boehringer, France), 2 mM L-glutamine (Gibco, France), penicillin (100 U/ml) and streptomycin (50 µg/ml) [Gibco, France], at 37°C in an atmosphere containing 5 % CO₂. Non adherent cells were distributed in 24-well plates (Nunc, Denmark) and inoculated with 500,000 tachyzoites per well; the parasite/ THP1 cell ratio was 1 (final volume: 1 ml).

TOTAL PROTEIN ASSAY

After six hours incubation, cells and tachyzoites were placed in lysis buffer (10 mM HEPES [Sigma, France], 150 mM NaCl, 1 mM sodium orthovanadate [Sigma, France], 10 % glycerol, 0.6 % Triton X 100, three anti-proteases: 0.2 mg/ml Pefabloc [Roth, Germany], 1 mM AEBSF [Uptima, France], and 10 µg/ml leupeptin [Sigma, France]) for 30 minutes, and were then centrifuged at 400 g for 15 minutes. Aliquots of supernatant (500 µl) containing the solubilized proteins were then concen-

trated by precipitation in 4 ml of absolute ethanol at - 20°C overnight. After centrifugation at 200 g for 10 minutes, precipitated proteins were determined according to the Bradford method (Bio-Rad Protein Micro Assay, France).

KINETICS OF HOST CELL MAP KINASE ACTIVATION (WESTERN BLOTTING)

Host cell MAP kinase (ERK, P38 and JNK) activation in the presence of viable *T. gondii* tachyzoites was detected in the total protein extract by Western blotting. The following primary antibodies recognizing the activated (phosphorylated) forms of the three MAP kinases were used: 1) murine monoclonal anti-phospho-p44/42 MAP kinase antibodies [(Thr202/ Tyr204) E10, (BioLabs, Ozymes, France) specific for human ERK1/2, at a dilution of 1/2,000]; 2) rabbit polyclonal antibodies against activated P38 pAb pTGPY [(Promega, France) specific for human P38 (approximately 48 kDa), at a dilution of 1/2,000]; and 3) rabbit polyclonal antibodies against activated JNK pAb pTPPY [(Promega, France) specific for human JNK (detection of JNK1 and JNK2 at approximately 46 kDa and 54 kDa), at a dilution of 1/2,000]. Comparable amounts of MAP Kinases are observed by probing parallel Western blots with antibodies at a dilution of 1/2,000 to unphosphorylated MAP Kinases [rabbit polyclonal P44/42 MAPK antibody (BioLabs, Ozymes, France); goat polyclonal P38 (Santa Cruz, Tebu, France); rabbit polyclonal JNK (BioLabs, Ozymes, France)]. According to the primary antibodies, two types of secondary antibodies were used: peroxidase-conjugated polyvalent anti-immunoglobulin (goat antimouse IgG, IgA and IgM), at a dilution of 1/10,000 (Sigma, France), or goat anti-rabbit IgG conjugated to alkaline phosphatase, at a dilution of 1/10,000 (Sigma, France). Non activated THP1 cells were used as negative control.

SPECIFICITY OF HOST CELL MAP KINASE ACTIVATION DURING *T. GONDII* INVASION

Selective inhibitors of the three MAP kinase families were used. PD 098059 (2'-amino-3'-methoxyflavone, Calbiochem, France), a non competitive inhibitor specific for the ERK family (Alessi *et al.*, 1995), inhibits the dephosphorylated form of MEK1/2 and thereby prevents MEK1/2 activation by Raf (without inhibiting the latter). SB 202190 [4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl) 1H-imidazole, Calbiochem, France] directly inhibits the activation of P38 family members (Manthey, 1998). SB 203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl) 1H-imidazole, Calbiochem, France], at the 50 µM concentration, inhibits JNK preferentially but also P38, as it only differs from the inhibitor SB 202190 by a methylsulfinyl group. THP1 cells were pretreated with a chosen concentration

of the different inhibitors (50 μ M) for 30 minutes before addition of *T. gondii*. The concentration of 50 μ M, preserving cell viability of at least 98 % (trypan blue exclusion test), was chosen in agreement with other studies (Roques & Vidal, 1999; Boden *et al.*, 2000; Kettritz *et al.*, 2001) to evaluate the specificity of MAPKs phosphorylation.

RESULTS

ACTIVATION OF HOST CELL ERK, JNK AND P38 PATHWAYS BY *T. GONDII* TACHYZOITES

We examined the kinetic of activation of THP1 cell ERK, JNK and P38 pathways by *T. gondii* tachyzoites. No variations of unphosphorylated MAP Kinase amount was observed (Figs 1-2-3), thus demonstrating that identical amounts of proteins were deposited. No basal level of ERKs, P38 or JNKs was detected in THP1 monocytic cells. As shown in Figures 1-2-3, tachyzoite infection leads to an earlier activation (five minutes) of the three MAP Kinase (ERKs, JNKs and P38). We observed that this effect was maximal at 60 minutes with ERKs (more important for ERK2) (Fig. 1) and P38 (Fig. 2). Both JNK isoforms were

Fig. 1. – Time-course of cellular unphosphorylated or phosphorylated [p] ERK1 and ERK2 MAPK during THP1 cell invasion by *T. gondii* tachyzoites. Total protein (5 μ g) were analysed by Western blotting using a polyclonal P42/44 MAPK (upper panel) and a monoclonal anti-phospho P44/42 MAPK (lower panel, pERKs). Negative Control (NC): non activated THP1 cells.

Fig. 2. – Time-course of cellular unphosphorylated or phosphorylated [p] P38 MAPK during THP1 cell invasion by *T. gondii* tachyzoites. Total protein (10 μ g) were analysed by Western blotting using a polyclonal P38 MAPK (upper panel) and a polyclonal anti-phospho P38 MAPK (lower panel, pP38). Negative Control (NC): non activated THP1 cells.

Fig. 3. – Time-course of cellular unphosphorylated or phosphorylated [p] JNK1 and JNK2 MAPK during THP1 cell invasion by *T. gondii* tachyzoites. Total protein (10 μ g) were analysed by Western blotting using a polyclonal JNK1/JNK2 MAPK (upper panel) and a polyclonal anti-phospho JNK1/JNK2 MAPK (lower panel, pJNKs). Negative Control (NC): non activated THP1 cells.

weakly activated with a maximum at 10 minutes (JNK1) and a decrease toward basal level after 60 minutes (Fig. 3). These results show that invasion by *T. gondii* stimulates all three MAPKs in human monocytic THP1 cells.

SPECIFIC EFFECT OF MAPK INHIBITORS ON ERK1/ERK2, P38 AND JNK1/JNK2 PHOSPHORYLATION

We determined the blocking effect of the specific inhibitors PD 098059, SB 202190 and SB 203580 on the three MAPKs activation in human monocytic cells activated by *T. gondii*. Host cell MAPKs activation in presence of viable *T. gondii* tachyzoites was detected in the total protein extract at 30 minutes (JNKs) or one hour (ERKs and P38) by Western blotting. We demonstrated that the three inhibitors prevent activation of host cell MAPKs during parasite infection (Fig. 4). Using specific inhibitors allowed us to address the specificity of these activations during *T. gondii* invasion.

DISCUSSION

While the different morphological steps of *T. gondii* invasion into host cells (recognition, attachment and internalization) are now well studied, the molecular mechanisms are still poorly described. Our present data have shown that cellular MAP Kinases (ERKs, P38, JNKs) are activated during the parasite invasion. Previous reports have shown that soluble antigen extract of *T. gondii* tachyzoites induced a pattern of protein-tyrosine phosphorylation similar to those with LPS (Li *et al.*, 1994) and that protein tyrosine phosphorylation and dephosphorylation are intracellular signaling mechanisms by which LPS can mediate some responses in macrophages (Weinstein *et al.*, 1993). Our findings correlate recent studies (Robert-Gangneux *et al.*, 2000; Roisin *et al.*, 2000), which also detected MAP kinase activity in *T. gondii* and charac-

Fig. 4. – Specific effects of MAPK inhibitors on cell MAPK activation by *T. gondii* using Western blotting. A single concentration of 50 μ M was used for each drug: PD 098059 [1], SB 202190 [2] and SB 203580 [3]. Five micrograms (ERK1/ERK2) or ten micrograms (P38, JNK1/JNK2) were analysed without (–) or with (+) inhibitor.

terized three MAP kinases in *T. gondii* lysates. Their molecular weights are respectively 47 kDa and 43 kDa (which were putatively identified as ERK1 and ERK2 homologs) and 80 kDa. Our studies support the importance of phosphorylation in the activation of signaling pathways in this parasite.

We demonstrated the ability of *T. gondii* tachyzoites to trigger phosphorylation of ERK1/2, P38 as JNK1/2 in human monocytic cells. All three classes of MAPKs are simultaneously activated by tachyzoites in THP1 cells with a maximum activation at 10 minutes (JNK) or 60 minutes (ERKs, P38) postcontact. However, the individual subtypes exhibit differential kinetics of activation in response to parasite. For example, while the activation of JNK1 and JNK2 rapidly decreases after 60 minutes, tachyzoite stimulation of ERK1/2 or P38 activation is longer sustained. We also demonstrated that the three inhibitors prevent activation of host cell MAPKs during parasite infection, showing the specificity of MAPKs phosphorylation during *T. gondii* invasion.

We have shown that activation of both cellular and parasitic MAP kinases was essential for parasite invasion

into monocytic cell (Gomez-Marin *et al.*, 1998). With other host cells model (murine fibroblastic cell line NIH-3T3), Robert-Gangneux *et al.* (2000) demonstrated that treatments with several compounds as PD 098059 led to a reduced tachyzoite invasion. MAP kinases phosphorylation linked to tachyzoite penetration or simple parasite-host cell contact, could trigger the PLA2 activation cascade in the host cell. This activation pathway, previously described in *Salmonella typhimurium* (Pace *et al.*, 1993), is very rapid. Moreover, our study of the kinetics of host cell MAP kinases demonstrated early activation (within five minutes) of ERKs and JNKs, in the presence of the parasite.

In other protozoan parasites, the importance of MAP kinases was recently emphasized. Thus, in *Leishmania mexicana*, a MAP kinase homologue (LMPK) has been described as necessary for the survival and development of the parasite within host cells, and involved in morphological differentiation during promastigote-amastigote transformation (Wiese, 1998). *Leishmania* lipophosphoglycans, which promote parasite survival, act by stimulating ERK MAPKs to inhibit macrophage IL-12 production (Feng *et al.*, 1999). In *Plasmodium falciparum* (Doerig *et al.*, 1996), a MAP kinase designated as Pf MAP has been characterized. Moreover, protein phosphorylation in *Plasmodium knowlesi* merozoites played an important role in their internalization into the erythrocytes (Ward *et al.*, 1994). The modulation of host cell protein kinase C activity has been observed in a number of parasitic infections, including *Leishmania donovani* (Descoteaux *et al.*, 1992), *Plasmodium falciparum* (Hall *et al.*, 1997) and *Toxoplasma gondii* (Grunvald *et al.*, 1996).

Our results imply the participation of the three studied MAP kinase pathways, ERKs, P38 and JNKs. The involvement of these three signaling pathways has been previously described in bacteria infection such as *Salmonella typhimurium* (Hobbie *et al.*, 1997) and *Listeria monocytogenes* (Tang *et al.*, 1998). There is growing evidence that modulation of host cell MAPK pathways during infection process may be part of opposed virulence strategies used by several pathogens. Thus, *Leishmania donovani* evade the activation of P38, JNK and ERK1/2 during infection of naive macrophages (Prive & Descoteaux, 2000). With *Listeria monocytogenes* (Tang *et al.*, 1998), the induction of MAPK pathways may be required for uptake by host epithelial cells. For *Salmonella typhimurium*, the activation of MAPK pathways may contribute to the acute inflammatory responses induced (Hobbie *et al.*, 1997).

In this study, we showed the kinetic of activation of THP1 cell MAPKs pathways by *T. gondii* tachyzoites. We demonstrated the ability of *T. gondii* to trigger an early and specific phosphorylation of cellular ERKs, P38 and JNKs.

ACKNOWLEDGEMENTS

This work was supported by Université de Reims Champagne-Ardenne, France. A. Valère was the recipient of a grant from Fondation Bayer Santé. We thank David Young for checking our English.

REFERENCES

- ALESSI D.R., CUENDA A., COHEN P., DUDLEY D.T. & SALTIEL A.R. PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase *in vitro* and *in vivo*. *The Journal of Biological Chemistry*, 1995, 270, 27489-27494.
- BECKER S. & JAFFE C.L. Effect of protein kinase inhibitors on the growth, morphology, and infectivity of *Leishmania* promastigotes. *Parasitology Research*, 1997, 83, 273-280.
- BODEN S.E., BERTSCHE T., AMMON H.P. & SAFAYHI H. MEK-1/2 inhibition prevents 5-lipoxygenase translocation in N-formylpeptide-challenged human neutrophils. *The International Journal of Biochemistry and Cell Biology*, 2000, 32, 1069-1074.
- DAVIS R.J. The mitogen-activated-protein kinase signal transduction pathway. *The Journal of Biological Chemistry*, 1993, 268, 14553-14556.
- DENKERS E.Y. & GAZZINELLI R.T. Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clinical Microbiology Reviews*, 1998, 11, 569-588.
- DESCOTEAUX A., MATLASHEWSKI G. & TURCO S.J. Inhibition of macrophage protein kinase C-mediated protein phosphorylation by *Leishmania donovani* lipophosphoglycan. *Journal of Immunology*, 1992, 149, 3008-3015.
- DOERIG C.M., PARZY D., LANGSLEY G., HORROCKS P., CARTER R. & DOERIG C.D. A MAP kinase homologue from the human malaria parasite, *Plasmodium falciparum*. *Gene*, 1996, 117, 1-6.
- FENG G.J., GOODRIDGE H.S., HARNETT M.M., WEI X.Q., NIKOLAEV A.V., HIGSON A.P. & LIEW F.Y. Extracellular signal-related kinase (ERK) and P38 mitogen-activated protein (MAP) kinases differentially regulate the lipopolysaccharide-mediated induction of inducible nitric oxide synthase and IL-12 in macrophages: *Leishmania* phosphoglycans subvert macrophage IL-12 production by targeting MAP kinase. *Journal of Immunology*, 1999, 163, 6403-6412.
- GRUNVALD E., CHIARAMONTE M., HIENY S., WYSOKA M., TRINCHIERI G., VOGEL S.N., GAZZINELLI R.T. & SHER A. Biochemical characterization and protein kinase C dependency of monokine-inducing activities of *Toxoplasma gondii*. *Infection and Immunity*, 1996, 64, 2010-2018.
- GOMEZ-MARIN J.E., BONHOMME A., GUENOUNOU M. & PINON J.M. Role of Interferon- γ against invasion by *Toxoplasma gondii* in a human monocytic cell line (THP1): involvement of the parasite's secretory phospholipase A₂. *Cellular Immunology*, 1996, 169, 218-225.
- GOMEZ-MARIN J.E., VALERE A., BONHOMME A., EL' BTAOURI H., ANTONICELLI F., BURLET H., AUBERT D., VILLENA I., GUENOUNOU M., HAYE B. & PINON J.M. Interferon- γ signal transduction during parasite infection: modulation of MAP kinases in the infection of human monocytes cells (THP1) by *Toxoplasma gondii*. *Parasite Immunology*, 1998, 20, 631-635.
- HALL B.S., DARAMOLA O.O., BARDEN G. & TARGETT G.A. Modulation of protein kinase C activity in *Plasmodium falciparum*-infected erythrocytes. *Blood*, 1997, 89, 1770-1778.
- HOBBIE S., CHEN L.M., DAVIS R.J. & GALAN J.E. Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by *Salmonella typhimurium* in cultured intestinal epithelial cells. *Journal of Immunology*, 1997, 159, 5550-5559.
- KETTRITZ R., SCHREIBER A., LUFT F.C. & HALLER H. Role of mitogen-activated protein kinases in activation of human neutrophils by antineutrophil cytoplasmic antibodies. *Journal of American Society of Nephrology*, 2001, 12, 37-46.
- LI Z.Y., MANTHEY C.L., PERERA P.Y., SHER A. & VOGEL S.N. *Toxoplasma gondii* soluble antigen induces a subset of lipopolysaccharide-inducible genes and tyrosine phosphoproteins in peritoneal macrophages. *Infection and Immunity*, 1994, 62, 3434-3440.
- MANTHEY C.L., WANG S.W., KINNEY S.D. & YAO Z. SB 202190, a selective inhibitor of P38 mitogen-activated protein kinase, is a powerful regulator of LPS-induced mRNAs in monocytes. *Journal of Leukocyte Biology*, 1998, 64, 409-417.
- PAGE J., HAYMAN M.J. & GALAN J.E. Signal transduction and invasion of epithelial cells by *Salmonella typhimurium*. *Cell*, 1993, 72, 505-514.
- PRIVE C. & DESCOTEAUX A. *Leishmania donovani* promastigotes evade the activation of mitogen-activated protein kinases P38, C-jun N-terminal kinase, and extracellular signal-regulated kinase-1/2 during infection of naive macrophage. *European Journal of Immunology*, 2000, 30, 2235-2244.
- ROBERT-GANGNEUX F., CREUZET C., DUPOUY-CAMET J. & ROISIN M.P. Involvement of the mitogen-activated protein (MAP) kinase signaling pathway in host cell invasion by *Toxoplasma gondii*. *Parasite*, 2000, 7, 95-101.
- ROISIN M.P., ROBERT-GANGNEUX F., CREUZET C. & DUPOUY-CAMET J. Biochemical characterization of mitogen-activated protein (MAP) kinase activity in *Toxoplasma gondii*. *Parasitology Research*, 2000, 86, 588-598.
- ROBERT C. & GAZZINELLI R.T. Signaling in immune system cells by glycosylphosphatidylinositol anchor and related structures derived from parasitic protozoa. *Current Opinion in Microbiology*, 2000, 3, 395-403.
- ROBERT C., ALMEIDA I.C., CLOSEL M., TRAVASSOS L.R., FERGUSSON M.A.J., COHEN P. & GAZZINELLI R.T. Requirement of mitogen-activated protein kinases and I κ B phosphorylation for induction of proinflammatory cytokines synthesis by macrophages indicates functional similarity of receptors triggered by glycosylphosphatidylinositol anchors from parasitic protozoa and bacteria lipopolysaccharide. *Journal of Immunology*, 2001, 166, 3423-3431.
- ROQUES M. & VIDAL H. A phosphatidylinositol 3-Kinase/p70 ribosomal S6 protein kinase pathway is required for the regulation by insulin of the p85 α regulatory subunit of phosphatidylinositol 3-kinase gene expression in human muscle cells. *The Journal of Biological Chemistry*, 1999, 274, 34005-34010.

- SCHOFIELD L. & HACKETT F. Signal transduction in host cells by glycosylphosphatidylinositol toxin of malaria parasites. *The Journal of Experimental Medicine*, 1993, 177, 145-153.
- SHAW M.K. *Theileria parva* sporozoite entry into bovine lymphocytes involves both parasite and host cell signal transduction processes. *Experimental Parasitology*, 1996, 84, 344-354.
- TACHADO S.D. & SCHOFIELD L. Glycosylphosphatidylinositol toxin of *Trypanosoma brucei* regulates IL-1 α and TNF- α expression in macrophages by protein tyrosine kinase mediated signal transduction. *Biochemical and Biophysical Research Communications*, 1994, 205, 984-991.
- TANG P., SUTHERLAND C.L., GOLD M.R. & FINLAY B.B. *Listeria monocytogenes* invasion of epithelial cells requires the MEK-1/ERK-2 mitogen-activated protein kinase pathway. *Infection and Immunity*, 1998, 66, 1106-1112.
- WARD G.E., FUJIOKA H., AIKAWA M. & MILLER L.H. Staurosporine inhibits invasion of erythrocytes by malarial merozoites. *Experimental Parasitology*, 1994, 79, 480-487.
- WEINSTEIN S.L., SANGHERA J.S., LEMKE K., DEFranco A.L. & PELECH S.L. Bacterial lipopolysaccharide induces tyrosine phosphorylation and activation of mitogen-activated protein kinases in macrophages. *The Journal of Biological Chemistry*, 1992, 267, 14955-14962.
- WEINSTEIN S.L., JUNE C.H. & DE FRANCO A.L. Lipopolysaccharide-induced protein tyrosine phosphorylation in human macrophage is mediated by CD14. *Journal of Immunology*, 1993, 151, 3829-3838.
- WIESE M. A mitogen-activated protein (MAP) kinase homologue of *Leishmania mexicana* is essential for parasite survival in the infected host. *The EMBO Journal*, 1998, 17, 2619-2628.

Reçu le 26 mars 2002

Accepté le 16 décembre 2002