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# Involvement of MAP Kinases in Apoptosis of Macrophage Treated with *Trichomonas vaginalis*

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A primitive protozoan parasite *Trichomonas vaginalis* selectively activates the signal transduction pathways in macrophages (RAW264.7). This study evaluated the correlation of these signaling pathways and *T. vaginalis*-induced cell apoptosis. In macrophages infected with *T. vaginalis*, apoptosis was assessed on the basis of DNA fragmentation on agarose gel electrophoresis. Infection of macrophages with *T. vaginalis* induced tyrosine phosphorylation of several proteins. Infected cells with *T. vaginalis* were shown to associate with phosphorylation of the extracellular signal-regulated (ERK)1/2 kinase, p38, c-Jun N-terminal kinase (JNK) mitogen-activated protein (MAP) kinases on Western blot analysis. The present finding also demonstrated a link between the ERK1/2, JNK and p38 apoptotic pathways that was modulated by *T. vaginalis* infection.

**Key Words:** *Trichomonas vaginalis*, Tyrosine phosphorylation, MAP kinases, Apoptosis

*Trichomonas vaginalis* is a causative protozoan parasite in the urogenital tract infection of humans. Trichomoniasis is a sexually transmitted disease (STD) found w orldwide and associated with an increased risk for HIV-1 acquisition.[1,2] During pregnancy, trichomoniasis can lead to adverse outcomes such as preterm delivery of a low birth-weight infant.[3]

Macrophages play a key role in the primary immune responses to infection of many pathogens. Following inoculation into a mammalian host with promastigotes of *Leishmania* sp., the parasite must evade nonspecific defense mechanisms by entering macrophages through a receptor-mediated process.[4]

Several functions of macrophages, especially those induced in response to microbial stimuli, are known to be dependent on tyrosine phosphorylation processes.[5] Most eukaryotic surface receptors employ at least one of these highly conserved mitogen-activated protein (MAP) kinase cascades for signaling inside the cell. MAP kinases are important mediators involved in the intracellular network of interacting proteins that transduce extracellular signals to intracellular responses.[6] MAP kinases hold a central role in many host responses, including the mitogenic response to growth factors for which they are named, and are involved in the signal transduction of a wide variety of cellular responses including proliferation, differentiation, and apoptosis in all eukaryotic organisms.[7] *Leishmania* and other intracellular pathogens have evolved these mechanisms to modulate host signaling pathways in order to facilitate invasion and survival in the cells.[3] Survival of the intracellular protozoan parasite *Leishmania donovani* is correlated with activation of phosphotyrosine phosphatase, which in turn attenuates MAP kinase signaling.[4] ERK1/2 activation is involved in the uptake of *Listeria monocytogenes* by macrophages and this activation decreases in parallel with an overexpression of MAPK phosphatase (MKP)-1, a MEK-specific phosphatase.[8] The enteropathogenic bacterium *Yersinia enterocolitica* causes a down-regulation of SAPK/JNK, p38 MAP kinase, and ERK1/2 in macrophages by delivering of a set of bacterial outer proteins.[9] The tyrosine phosphorylation of

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three proteins in HeLa cells infected with *Escherichia coli* has been observed to induce ERK1/2 MAP kinase activation in T84 cells.[10,11]

This experiment was conducted to elucidate MAP kinases activation and apoptosis on the macrophages infected with *T. vaginalis*. Macrophages (RAW264.7) were distributed in a 60-mm dish with DMEM containing 10% fetal bovine serum, antibiotic at 37℃ in 5% $CO_2$ condition. Infection macrophages ($2\times10^6$-$3\times10^6$/dish) were incubated for the indicated time points with *T. vaginalis* at a parasite-to-macrophage ratio of 10:1. This ratio was chosen because it has been shown to promote optimal *T. vaginalis* interaction with this cells.[12] After incubation for various time periods, macrophages were washed three times with ice-cold PBS and immediately processed for analysis.[13] Protein tyrosine phosphorylation and MAP kinases activation in infected macrophages were induced and observed by immunoblot. DNA laddering of macrophages infected with *T. vaginalis* was analysed by agarose gel electrophoresis.

Phosphorylation kinetics by Western blotting revealed the appearance of tyrosine-phosphorylated protein in macrophages over time-course infection with *T. vaginalis*. *T. vaginalis* induced triggering of the tyrosine phosphorylation of several proteins in the macrophages. One major phosphorylated substrate of 46 kDa was identified in *T. vaginalis*-infected macrophages (Fig. 1).

The signal transduction pathways leading to MAP kinases activation were examined for alteration during the infection process. We measured the kinetics of MAP kinases phosphorylation in macrophages during the 0 to 120 min following infection with *T. vaginalis*. The activation of the three MAP kinases was assessed by detecting their dually phosphorylated forms with Western blot analysis using antibodies specific to their phosphorylated forms. Our results suggest that infection with *T. vaginalis* selectively affects the tyrosine phosphorylation of MAP kinases (Fig. 1, 2). Activated MAP kinase signaling pathways after infection with *T. vaginalis* stimulated mainly the ERK1/2 and we noted the absence of significant phosphorylation of p38 and SAPK/JNK was noted (Fig. 2). Time course observation of MAP kinases phosphorylation showed that reversed tyrosine phosphorylation and MAP kinases activation at 40 min after infection were remarkable. Interestingly, tyrosine phosphorylation of macrophages disappeared at 60min after infection with *T. vaginalis* and ERK1/2 was not activated in the cells, but p38 and SAPK/JNK activations were noted (Fig. 2). In this study, *T. vaginalis*-induced tyrosine phosphorylation and ERK1/2 activation was prompted, accompanied by the activation of p38 and diminished SAPK/JNK (Fig. 1 and 2). Different signal transduction pathways were demonstrated in activated host cells.[14] The role of both p38 and SAPK/JNK in cytokine production of immune cells are well established, but the involvement of individual MAP kinases in the

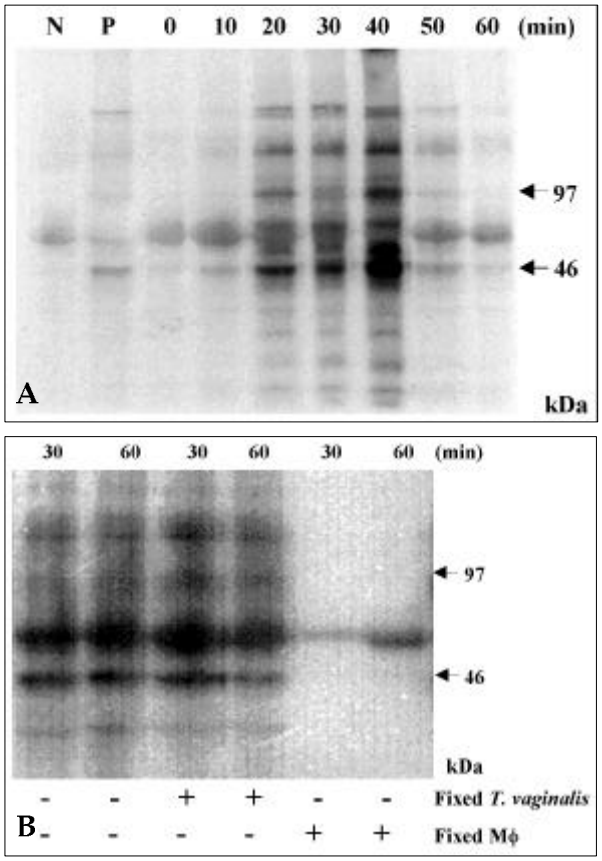


**Fig. 1.** Induction of protein tyrosine phosphorylation in macrophages infected with *T. vaginalis*. The macrophages were lysed, and the lysates were analyzed by immunoblot analysis, using the 4G10 anti-phosphotyrosine antibody. (A) Time-dependent induction of tyrosine phosphorylation in macrophages induced by *T. vaginalis* infection. (B) Fixed/unfixed macrophages were prepared with either fixed/unfixed *T. vaginalis* at a parasite:cell ratio of 10:1 for infection time (N: naive macrophages, P: LPS (100 ng/ml)-stimulated macrophages).

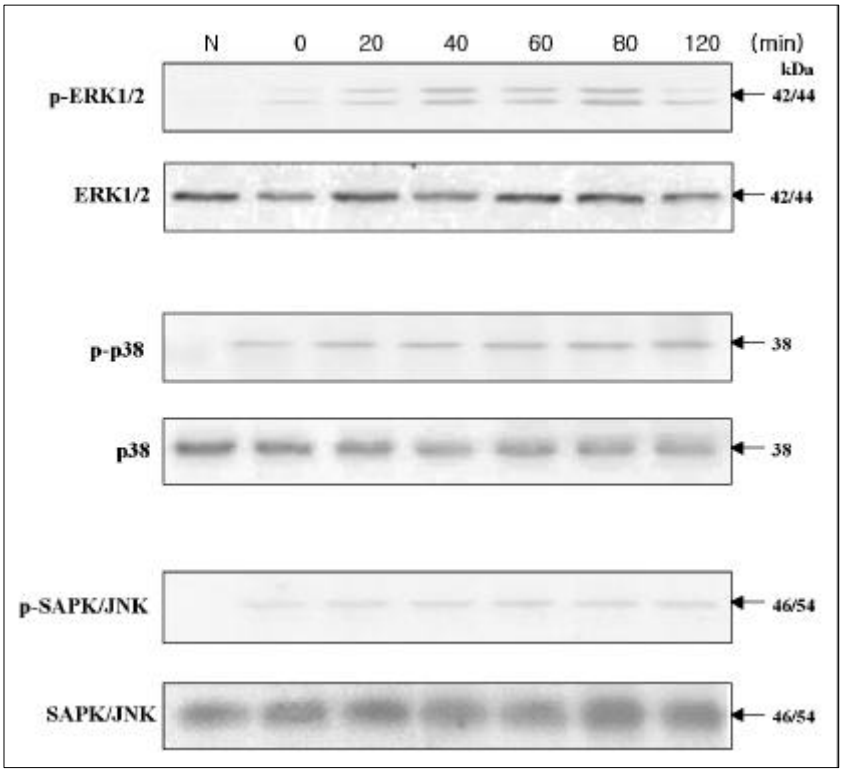


**Fig. 2.** Time course of *T. vaginalis* infection on the phosphorylation of MAP kinases in macrophages. Macrophages were infected with *T. vaginalis* (parasite:cell ratio of 10:1) and harvested at the indicated time (0-120 min). The macrophages were lysed, and the lysates were analyzed by immunoblot analysis, using antibodies to p-Erk1/2, p-p38, p- SAPK /JNK, Erk1/2, p38 and SAPK/ JNK (N: naive macrophages).

macrophages infected with *T. vaginalis* has not yet been clear proved.[15] ERK activation plays a central part in the response to hypotonic stress or to reactive oxygen species.[16] On the other hand, SAPK/JNK, a stress-activated member of the MAP kinase family, is constitutively active in a parasite infection. Surprisingly, p38, which is also a stress-activated protein kinase and is often together with SAPK/JNK, was found to be silent.[17]

When DNA from macrophages was extracted and analyzed by agarose gel electrophoresis, DNA fragmentation in macrophages infected with by *T. vaginalis* was observed at 60 min post-infection (Fig. 3). Internucleosomal DNA fragmentation is a characteristic morphology in apoptotic death.[8] This experiment suggested possible correlation between MAP kinases activation and apoptosis in macrophages infected *T. vaginalis*. The inhibition of ERK1/2 activation below a basal threshold level triggered apoptosis of HeLa cells that was induced by activation of p38 kinase, but not SAPK/JNK.[18] The inhibition of ERK activity and the concomitant activation of SAPK/JNK and p38 kinase improved in the apoptosis of nerve growth factor-deprived PC12 cells.[15] The ability of a cell

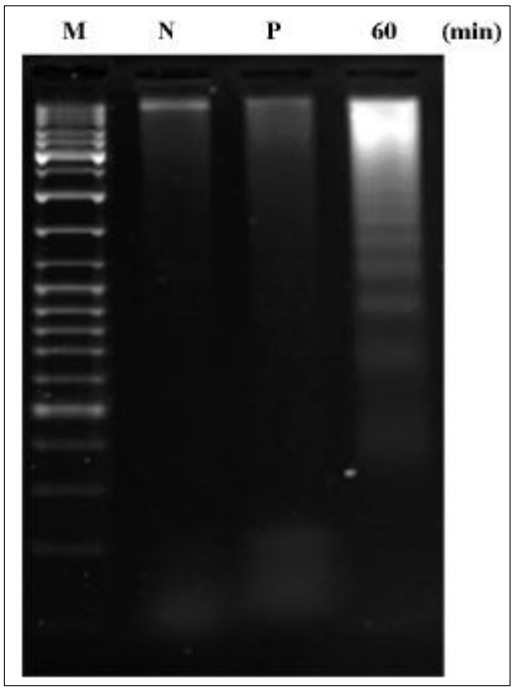


**Fig. 3.** Induction of internucleosomal DNA fragmentation in macrophages infected by *T. vaginalis*. Macrophages were infected with *T. vaginalis* for the indicated time (60 min), after which total DNA was extracted and analysed by agarose gel electrophoresis (M: DNA marker, N: naive macrophages, P: LPS (100 ng/ml)-stimulated macrophages).

to die or to survive appears to be dictated by a critical balance between the ERK and the SAPK/JNK and p38 pathways.[15] The involvement of SAPK/JNK and p38 kinase in apoptosis depend upon variety of stimuli and cell types.

By understanding how *T. vaginalis* parasites disturb the function of macrophages, specific molecules involved in the cross talk between parasites and host cells, as well as the effector of these cascades, may be identified, generating further candidate chemotherapy targets. Our results show that co-culture of *T. vaginalis* with macrophages leads to the activation of tyrosine phosphorylation and MAP kinases. Both p38 and SAPK/JNK pathways were required for *T. vaginalis*-induced activation of macrophages, and these two MAP kinase pathways played a role in the regulation of *T. vaginalis*-induced apoptosis.

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