

SUPPLEMENTARY MATERIALS AND METHODS

Scanning Electron Microscopy (SEM)

For SEM observation, MDMs were fixed with 2.5% glutaraldehyde in 0.1 M Millonig's phosphate buffer (MPB) (to obtain 100 ml, mix 32 ml 2 M sodium-dihydrogen-orthophosphate, 14 ml 2 M di-sodium-hydrogen-orthophosphate, 50 ml DDW, pH 7.4) at 4°C for 1 hr. After washing in MPB, cells were post-fixed with 1% OsO₄ in the same buffer for 1 hr at 4°C and dehydrated using increasing acetone concentrations. The specimens were critical-point dried using liquid CO₂ and sputter-coated with gold before examination on a Stereoscan 240 scanning electron microscope (Cambridge Instr., Cambridge, UK). Quantitative assessment of the number of adhering conidia on cell surface was done, at 30 min post-challenge by analysing a minimum of 100 cells *per* sample; results were obtained as the mean values from three different experiments.

Evaluation of the phagocytic activity of human MDMs against fluorescent beads, by Confocal Laser Scanning Microscopy (CLSM)

The phagocytic activity of treated and untreated MDMs was tested by CLSM by adding to the cultures 2×10^7 beads/ml of yellow-green fluorescent polystyrene beads (\emptyset 1 μ m, at a ratio of at least 10 beads/cell), with excitation/emission wavelengths of approximately 495nm/515nm (Molecular Probes). After 30 min, cells were fixed with 4% paraformaldehyde, counter-stained with 1 μ g/ml propidium iodide (PI - Sigma-Aldrich) for nuclei visualization, and observed by the confocal microscope LEICA TCS SP5 (Leica Instruments, Heidelberg, Germany). A minimum of 200 cells *per* sample were observed, and the number of phagocytic MDMs (reported as percent of phagocytic cells), as well as the number of beads *per* cell, were counted. MDMs cultures subjected to beads addition and maintained at 0°C to block internalization, were used as a negative control of uptake

Quantitative Reverse Transcription Polymerase Chain Reaction (QPCR)

Serafino *et al.* “Thymosin α 1 activates complement receptor-mediated phagocytosis in human monocyte-derived macrophages”

SUPPLEMENTARY Materials and Methods and SUPPLEMENTARY Table

Total RNA was extracted using TRIzolTM Reagent (Invitrogen, Carlsbad, CA, USA). The purity, integrity and yield of RNA was monitored by micro-capillary electrophoresis (Bioanalyzer 2100, Agilent Technologies, Santa Clara, CA, USA) using the RNA 6000 LabChip kit. 1 μ g of total RNA was treated with DNase I Amplification Grade (Invitrogen) and reverse-transcribed using the SuperScriptTM III (Invitrogen). Quantitative PCR was performed in ABI PRISM 7000 light cycler (Applied Biosystems, Carlsbad, CA, USA) using SYBR Greener qPCR SuperMix for ABI Prism (Invitrogen). All primers were optimized for real-time RT-PCR amplification checking the generation of a single amplicon in a melting curve assay and the efficiency in a standard curve amplification (> 98% for each couple of primers). Sequences of primers used are reported in Supplementary Table 1. Quantitative RT-PCR sample value was normalized for the expression of GAPDH mRNA. The relative level for each gene was calculated using the $2^{-\Delta\Delta C_t}$ method [Livak and Schmittgen, 2001] and reported as arbitrary units. In all experiments each sample was analysed in triplicate.

Reference

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-(\Delta\Delta C(T))}$ method. *Methods* 2001; 25:402–408.