SUPPLEMENTARY INFORMATION

Supplementary Materials

Real time PCR detection of the PA receptors in cells

Tissue culture plates (6-wells) were seeded in triplicate with different cell lines (Jurkat, HeLa and Raw264.7) and total RNA was extracted with 500 μl of *Trizol* (Invitrogen) following the manufacture's protocol. The samples were treated with DNAse-free™ (Ambion) and RNA was transcribed with *Superscript III first strand* (Invitrogen). Possible residual RNA was degraded with RNase. Quantification standards for both human (Jurkat, HeLa) and mouse (Raw264.7) TEM8 and CMG2 were generated by subcloning coding regions of either receptors into pCRII-TOPO (Invitrogen) using the following pair of primers: TCCACCATATGTGCAGGAGA and AGATAGGCGCTGGACACAGT for human TEM8; AGGTTCGTTGGGGTGATAAA and TTGTCTGAGGAGGCTGGTG for human CMG2; GCTCAATGAGAAGCCCTTTG and AGCCGTCTGAACAGTGTGTG for mouse TEM8; GTTTTCTTCCCAAGCAACCA and CCGTCCGTCAAAGCAATTAT for mouse CMG2. Human GAPDH and mouse β-actin standards were a gift from M. De Bernard from VIMM Institute, Padova. For each sample, SybrGreen mix (Applied Biosystem) was added in the presence of primer pairs, each primer 500 nM. cDNA was used to 1/10 final dilution. Real time PCR was performed with a 96-well thermocycler, model 5700 from Applied Biosystems/ Perkin Elmer with the following protocol: 50°C for 2 min, 95° for 10 min, followed by 45 cycles at 95° for 15 s and 60°C for 1 min. Data were expressed as ratios of the mRNA of the PA receptors with respect to that of house-keeping genes, GAPDH for human samples and β-actin for mouse samples.

MAPKK3 cleavage by anthrax lethal factor.

1.5-2x10^4 HeLa cells were incubated with lethal factor 10 nM and PA 20 nM in appropriate complete medium at 37°C for different time periods in a 96-well plate. After removal of the culture medium, the cells were lysed, subjected to SDS-PAGE, and immunoblotted for the isoform 3 of MAPKK with a specific polyclonal antibody from Santa Cruz Biotechnology (USA). Samples were developed with ECL plus detection system (Amersham Biosciences) and chemiluminescence emission was detected with ChemiDoc™ XRS (Biorad). Images were elaborated with WCIFImageJ v1.35 (http://rsb.info.nih.gov/ij) and band intensities were quantified with the Quantity One® software from Biorad.