Jaillon et al., http://www.jem.org/cgi/content/full/jem.20061301/DC1

SUPPLEMENTAL MATERIALS AND METHODS

Binding assay. Neutrophil-derived PTX3 (PMN-PTX3) was purified by immunochromatography from a PMN lysate (sepharose–protein G cross-linked with an anti-PTX3 mAb) as previously described (44); the purity of this preparation was ~90%. Binding of PTX3 to OmpA and C1q was performed essentially as previously described (18). In brief, 96-well plates (Nunc) were coated overnight at 4°C with 5 μg/ml KpOmpA or with 10 μg/ml C1q and blocked with 5% dry milk in washing buffer (PBS containing 0.05% Tween 20) for 2 h at room temperature before incubation for 30 min at 37°C with PMN-PTX3, PMN lysate, or recombinant PTX3 in washing buffer. After washing, plates were incubated with 1 μg/ml of a biotin-conjugated PTX3 affinity-purified rabbit IgG, followed by horseradish peroxidase–labeled streptavidin (Bio-Spa). Absorbance values were read at 450 nm after the addition of the chromogen substrate TMB and stop solution.

Binding of PMN-PTX3, obtained from a concentrated supernatant of PMN stimulated with *E. coli* LPS (200 ng/ml) and GM-CSF (50 ng/ml) for 16 h, to *A. fumigatus* conidia was performed as previously described (15) and analyzed by a flow cytometer (FACSCanto; BD Biosciences).

Analysis of PTX3, MPO, lactoferrin, and MMP-9 mRNA expression in HL60 cells and human bone marrow. For HL60 cells, total RNA isolation and cDNA synthesis were performed as described in Materials and methods. Bone marrow cDNA was obtained from CLONTECH Laboratories, Inc. Sequences of PTX3- and MPO-specific primers are described in Materials and methods. Lactoferrin and MMP-9 mRNA expression were evaluated using specific oligonucleotides (lactoferrin: 5′-ATCCCAACAAAGCAGTGACC-3′ and 5′-CAACGTCTCCAGCATTCTCA-3′; MMP-9: 5′-CATCGTCATCCAGTTTGGTG-3′ and 5′-GCCTTGGAAGATGAATGGAA-3′).

www.jem.org/cgi/doi/10.1084/jem.20061301