The Mannose Receptor Mediates the Cellular Immune Response in Human Coccidioidomycosis

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Mannose is the predominant monosaccharide in the coccidioidal antigen preparation T27K. Mannan and anti-CD206 antibody significantly decreased the surface expression of mannose receptor (MR) on adherent peripheral blood mononuclear cells and reduced the interleukin-2 (IL-2) release induced by T27K. These data suggest that MR mediates IL-2 release by T27K.

The mannose receptor (MR) is found on the surface of macrophages and dendritic cells, can bind terminal mannosyl residues on fungi and other pathogens (6), and has been shown to mediate the in vitro cellular immune response to fungal antigens (7). We used mannann, an MR ligand (11), and anti-CD206 (αCD206), a monoclonal antibody directed against MR (4), to block the surface expression of MR on adherent peripheral blood mononuclear cells (PBMC) and examined the effect of this on the cellular immune response induced by T27K, a glycosylated coccidioidal antigen preparation (1–3).

PBMC, derived from the blood of healthy human donors of known coccidioidal immunity, were resuspended in RPMI 1640 (GIBCO, Grand Island, Mich.) with 10% autologous serum, added to 35-mm flat-bottom wells (Falcon, Becton Dickinson Labware), and incubated at 37°C in 95% air–5% CO2. For the first 30 min, mannan (from Saccharomyces cerevisiae; Sigma Chemical Company, St. Louis, Mo.) or αCD206 (from clone 19.2; BD Biosciences Pharmingen, San Diego, Calif.) was added to wells. In some experiments, immunoglobulin G1 (IgG1) (no. 555748; BD Biosciences Pharmingen), the isotype of αCD206, was used. Control wells received nothing. After 30 min, 10-μg/ml T27K was added to cells and further incubated for 72 h. Adherent cells were removed and incubated with fluorescein isothiocyanate (FITC)-labeled αCD206 or FITC-labeled IgG1x for 30 min at 22°C in the dark. Cell viability just prior to flow cytometry was >90%, as determined by trypan blue exclusion. A gate was set around viable nonlymphocytes and 4,000 events were collected. MR surface expression was measured as the ratio of the geometric mean fluorescent intensity (MFI) of samples stained with FITC-labeled αCD206 divided by the geometric MFI of cells stained with labeled isotype. Interleukin-2 (IL-2) and gamma interferon (IFN-γ) concentrations in harvested supernatant were determined by using a flow cytometry-based immunoassay (CBA; BD Immunosciences, San Jose, Calif.) or by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn.).

Monosaccharide analysis of T27K was performed by the Glycotechnology Core Resource of the University of California at San Diego by using high-pH anion-exchange chromatography with pulsed amperometric detection after protein denaturation and desalting (A. Datta, personal communication). The Wilcoxon signed-rank test for paired data was employed for statistical analysis. All work was approved by the Human Subjects Protection Program of the University of Arizona.

To assess mannan blocking of MR, 3.0 × 106 PBMC in 2 ml of RPMI 1640 with 10% autologous serum were incubated for 72 h with or without 3 mg of mannan/ml. Subsequently, 1 mg of FITC-labeled dextran (Sigma)/ml was added for 1 h at either 37 or 4°C. After this, the adherent cells were removed using a sterile rubber scraper, resuspended in phosphate-buffered saline, and then analyzed by flow cytometry as described above. The geometric MFI in the FL-1 channel was used as a measure of cell association.

Mannose was the principal monosaccharide of T27K and was present at a concentration of 1,151 nM/mg. Other monosaccharides detected were glucose (375 nM/mg), galactose (163 nM/mg), N-acetylgalactosamine (58 nM/mg), and N-acetylglucosamine (24 nM/mg).

Among nine immune donors, incubation of PBMC with 3 mg of mannan/ml and T27K for 72 h resulted in significant reduction of MR surface expression on adherent PBMC (P = 0.01) as well as reduced IL-2 (P = 0.01) and IFN-γ release (P = 0.05) compared to incubation with T27K alone (Fig. 1). In another seven immune donors, incubation of PBMC with 20 μg of αCD206/ml and T27K also resulted in reduction in MR surface expression (P = 0.02). Incubation with 20 μg of IgG1x/ml, the isotype of αCD206, did not reduce MR expression (data not shown). Incubation of cells with αCD206 and T27K decreased IL-2 concentrations significantly below those seen in control samples (P = 0.02) and those for samples incubated with IgG1x (P = 0.03).

In five experiments, the median MFI of mononuclear cells after incubation at 37°C with FITC-dextran for 1 h was reduced from 825 (range, 574 to 1889) in control wells to 405 (272 to 741) when cells were incubated with mannan (P = 0.04). The median MFI was 403 (163 to 641) when cells were incubated with FITC-dextran at 4°C.

Pathogen-associated molecular patterns expressed on micro-
Organisms play critical roles both in inducing the innate immune response and in modulating subsequent acquired immunity. Among the ligands for these pathogen-associated molecular patterns, MR is of particular interest since it binds terminal mannoses present on fungi.

Several studies have shown that the immune response to fungal pathogens is mediated through MR. Mansour and colleagues demonstrated that blockade of MR with mannose and methyl-α-D-mannopyranoside resulted in diminished production of IL-2 by a T-cell hybridoma responsive to Cryptococcus neoforms (13). Syme and colleagues demonstrated that blocking MR with an anti-MR antibody resulted in diminished phagocytosis of C. neoforms by human dendritic cells (13). It is not clear from this study whether the addition of mannann or αCD206 simply blocked expression of MR on the cell surface through binding or whether it diminished production of MR. Our assumption is that preincubation of cells with mannann and αCD206 led to these moieties binding to surface MR, thus making it unavailable to T27K. The fact that mannann inhibited the association of FITC-dextran with cells suggests that it functionally blocked MR rather than simply interfering with αCD206 binding, since pinocytosis of dextran is known to occur through MR (11).

Analysis of T27 revealed that mannann is its predominant monosaccharide. Other studies (5, 8–10, 12, 14) have demonstrated that Coccidioides spp. contain significant amounts of mannose and 3-O-methylmannose. Based on this, it is clearly feasible that MR could bind T27K or even whole Coccidioides isolates and mediate a specific cellular immune response.

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