

## The Mannose Receptor Mediates the Cellular Immune Response in Human Coccidioidomycosis

Neil M. Ampel,<sup>1,2,3\*</sup> Daniel K. Nelson,<sup>3</sup> Lijin Li,<sup>2</sup> Sara O. Dionne,<sup>2,4</sup> Douglas F. Lake,<sup>2,4</sup> Keira A. Simmons,<sup>5</sup> and Demosthenes Pappagianis<sup>5</sup>

Department of Medicine,<sup>1</sup> Department of Microbiology and Immunology,<sup>2</sup> and Arizona Cancer Center,<sup>4</sup> University of Arizona Health Sciences Center, and Medical and Research Services, Southern Arizona Veterans Affairs Medical Center,<sup>3</sup> Tucson, Arizona, and Department of Microbiology and Immunology, University of California at Davis, Davis, California<sup>5</sup>

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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 mannoses found on fungi and other pathogens (6), and has been shown to mediate the *in vitro* cellular immune response to fungal antigens (7). We used mannan, an MR ligand (11), and anti-CD206 ( $\alpha$ CD206), 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% CO<sub>2</sub>. For the first 30 min, mannan (from *Saccharomyces cerevisiae*; Sigma Chemical Company, St. Louis, Mo.) or  $\alpha$ CD206 (from clone 19.2; BD Biosciences Pharmingen, San Diego, Calif.) was added to wells. In some experiments, immunoglobulin G1 $\kappa$  (IgG1 $\kappa$ ) (no. 555748; BD Biosciences Pharmingen), the isotype of  $\alpha$ CD206, was used. Control wells received nothing. After 30 min, 10- $\mu$ g/ml T27K was added to cells and further incubated for 72 h. Adherent cells were removed and incubated with fluorescein isothiocyanate (FITC)-labeled  $\alpha$ CD206 or FITC-labeled IgG1 $\kappa$  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  $\alpha$ CD206 divided by the geometric MFI of cells stained with labeled isotype. Interleukin-2 (IL-2) and gamma interferon (IFN- $\gamma$ ) concentrations in harvested supernatant were measured using a flow cytometry-based immunoassay (CBA; BD Immunoscience, 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  $\times$  10<sup>6</sup> 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- $\gamma$  release ( $P = 0.05$ ) compared to incubation with T27K alone (Fig. 1). In another seven immune donors, incubation of PBMC with 20  $\mu$ g of  $\alpha$ CD206/ml and T27K also resulted in reduction in MR surface expression ( $P = 0.02$ ). Incubation with 20  $\mu$ g of IgG1 $\kappa$ /ml, the isotype of  $\alpha$ CD206, did not reduce MR expression (data not shown). Incubation of cells with  $\alpha$ CD206 and T27K decreased IL-2 concentrations significantly below those seen in control samples ( $P = 0.02$ ) and those for samples incubated with IgG1 $\kappa$  ( $P = 0.03$ ).

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-

\* Corresponding author. Mailing address: Medical Service (1-111), SAVAHCS, 3601 S. Sixth Ave., Tucson, AZ 85723. Phone: (520) 792-1450. Fax: (520) 629-1861. E-mail: nampel@email.arizona.edu.

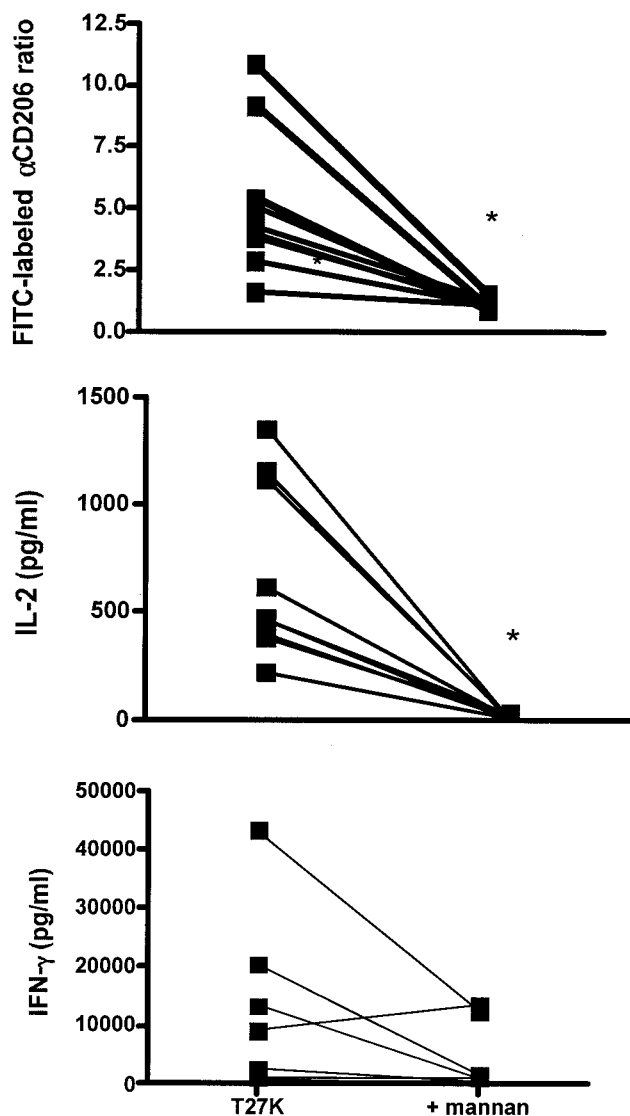


FIG. 1. Surface expression of MR, measured as the ratio of FITC-labeled  $\alpha$ CD206 to FITC-labeled isotype, on adherent PBMC and supernatant IL-2 and IFN- $\gamma$  concentrations after incubation for 72 h with 10  $\mu$ g of T27K/ml and with or without 3 mg of mannan/ml. Data represent results from samples of nine immune donors. \*,  $P = 0.02$ ; †,  $P = 0.05$ .

organisms play critical roles both in inducing the innate immune response and in modulating subsequent acquired immunity (6). 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 mannan and methyl- $\alpha$ -D-mannopyranoside resulted in diminished production of IL-2 by a T-cell hybridoma responsive to mannoprotein derived from *Cryptococcus neoformans* (7). Syme and colleagues demonstrated that blocking MR with an anti-MR antibody resulted in diminished phagocytosis of *C. neoformans* by

human dendritic cells (13). It is not clear from this study whether the addition of mannan or  $\alpha$ CD206 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 mannan and  $\alpha$ CD206 led to these moieties binding to surface MR, thus making it unavailable to T27K. The fact that mannan inhibited the association of FITC-dextran with cells suggests that it functionally blocked MR rather than simply interfering with  $\alpha$ CD206 binding, since pinocytosis of dextran is known to occur through MR (11).

Analysis of T27 revealed that mannose 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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