Online Supplemental Data

CHITIN PARTICLES ARE MULTIFACETED IMMUNE ADJUVANTS

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Preparation of Chitin Particles

Chitin fragments were generated as previously described (6, 7, 21, 22). In brief, chitin powder (Sigma Chemical Co., St. Louis, MO) was suspended in sterile Phosphate Buffer Saline (PBS 1X: Gibco, Grand Island, NY). Chitin of different sizes was generated using sonication or by digestion with chitin-specific recombinant acidic mammalian chitinase (rAMCase, a gift from Dr. A. Coyle, MedImmune Inc.). In the former, chitin was sonicated at 25% output power three times for 5 minutes with a Branson sonicator (Sonifier 450, Branson Ultrasonics, Danbury, CT). In the latter, chitin was incubated for up to 72 hours with rAMcase (37°C, pH 3.0) or vehicle control. The suspensions were then filtered with 70µm and 40µm sterile cell strainers (BD Biosciences, Bedford, MA). Following centrifugation (2800 x *g*, 10 min), chitin pellets (40-70µm) were suspended in the desired volume of sterile PBS and autoclaved. Particle sizes and size distribution were evaluated by flow cytometry by comparing the chitin to latex bead controls (42.0 and 75µm in diameter; Polysciences, Warrington, PA). Prior to utilization, the chitin particles were concentrated by speed vacuuming. Endotoxin levels were below the limits of detection in a *Limulus* Amebocyte Lysate assay (Sigma).

Cell Preparation and Cell Culture

Bone-marrow dendritic cells

Bone-marrow cells were extracted from the legs of mice with cell culture medium and cultured for 3 days in RPMI 1640 (Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal calf serum (FCS; Atlanta Biologicals, Lawrenceville, GA), 1% L-glutamine, 50U/ml penicillin, 50µg/ml streptomycin (all reagents from Invitrogen Corp.) and 1% granulocyte macrophage-colony stimulating factor (GM-CSF) obtained from filtered supernatant of J558L cell culture.

Fresh medium was added to the cells for an additional 3 days. Bone-marrow dendritic cells (BMDCs) were analyzed for the expression of CD11c by flow cytometry (FACScalibur, BD Biosciences, San Jose, CA).

CD4+ T cells

Mice were immunized with two intra-peritoneal (i.p.) injections of a mixture containing either 500µg/ml of chitin particles and 1mg/ml of OVA (Sigma) in 200µl of sterile PBS 1X, or with 200µl of an aluminum hydroxide (Alum, 2mg/ml) solution and 1mg/ml of OVA. Fourteen days later, primed CD4+ T cells were isolated from splenocytes using the EasySep mouse CD4+ T cell enrichment kit (StemCell Technologies, Vancouver, Canada) according to the manufacturer's instructions. These cell preparations were consistently >96% pure as assessed by flow cytometry using an APC-conjugated CD4 antibody (BD Biosciences). T cells were incubated with carboxyfluorescein diacetate succinimidyl ester. The reaction was stopped by adding fetal calf serum and washed intensively.