SUPPLEMENT SECTION 1

Conventional production of gold nanoparticles. Gold nanoparticles at a size of approximately 7.1 nm (± 1.3) in diameter were produced in bi-distilled water (Milli-Q) with sodium borohydride as a reducing agent.[1] Since gold nanoparticles aggregate when resuspended in phosphate-buffered saline (PBS), the prepared nanoparticles were washed with Milli-Q water (Millipore, Bedford, MA) several times by centrifugation (16,000 g for 1 h) and incubated with 5 % human serum albumin (HSA, low endotoxin, Sigma, Sweden) for 0.5 h at room temperature for stabilization, and were then washed three more times with Milli-Q water.

Production of low-LPS gold nanoparticles. To produce gold nanoparticles with low LPS content the Milli-Q water was replaced in all mentioned steps by ultra-pure water (LPS < 0.25 ng/ml). Surrounding environment was controlled with PyroCLEAN™ (AlerCHEK, Maine, GB), as were the glassware before these were cleaned with aqua regia (HNO_3: HCl, 1:3). Afterwards glassware was not washed with tap water, but instead with Milli-Q and ultra-pure water. In addition, consistent use of gloves was introduced, as was the standard of always working inside fume hoods.

Characterization of gold nanoparticles. The particle size was determined with transmission electron microscopy (TEM; JEOL JEM-2000EX used at an acceleration voltage of 200 kV). A drop of the nanoparticle solution was placed onto a carbon coated copper grid and air dried at RT. The concentration of the gold nanoparticle solution was determined by atomic absorption spectroscopy (AAS; Varian, Australia), after the particles had been dissolved by aqua regia containing HCl (Sigma-Aldrich, St. Louis, MO, USA) and HNO_3 (Merck, Whitehouse Station, NJ, USA) at a ratio of 3:1. The solution was stored at 4 °C and used within a month.

LPS analysis. Supernatants of each batch of gold nanoparticles produced were controlled for lipopolysaccharide (LPS) concentrations by the endpoint chromogenic LAL test method (Limulus Amebocyte Lysate endochrome, Charles River Endosafe, Charleston, SC, USA) according to the manufacturer's instructions.

SUPPLEMENT SECTION 2

Generation of monocyte-derived dendritic cells. Human peripheral blood mononuclear cells (PBMCs) from healthy blood donors were separated from buffy coats (Karolinska University Hospital Blood Bank, Stockholm, Sweden) by standard gradient centrifugation with Ficoll Paque (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The PBMCs were harvested, and washed three times with PBS, followed by resuspension in MACS-buffer (80 μl/10^7 cells) containing 0.5 % BSA, 2 mM EDTA in PBS. Anti-CD14 microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany) were added according to the manufacturer's instructions. After 30 min at 4 °C, the $CD14^+$ cells were separated from the solution by autoMACS (Miltenyi Biotec), and analyzed by flow cytometry (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ, USA) to check for $CD14^+$ cell purity which was always above 97 %. This work was approved by the local ethics committee.

Immature MDDCs were generated mainly as described before[2] by culturing monocytes in RPMI 1640 medium (HyClone, Logan, UT, USA), supplemented with 25 μg/ml gentamicin (Gibco Invitrogen Corporation, Paisley, UK), 2 mM L-glutamine (Gibco Invitrogen Corporation), 100 IU/ml penicillin (Gibco Invitrogen Corporation), 100 μg/ml streptomycin (Gibco Invitrogen Corporation), 50 μM β-mercaptoethanol (KEBO-lab, Spånga, Sweden), 10 % fetal calf serum (Hyclone) and with the cytokines GM-CSF (550 IU/ml), and IL-4 (800 IU/ml), at a density of 4×10^5 cells/ml, in 37 °C with a 5 % CO_2 atmosphere. Both cytokines were from Biosource International (Camarillo, CA, USA). After 6 days, the cell surface molecules CD1a, CD14 and CD83 were analyzed by flow cytometry to confirm an immature phenotype with low CD83 expression (always below 4 %).[3]

Cell lines. L cells, a fibroblast cell line, and L cells transfected with CD40L[4] (kindly provided by Noemi Nagy at Microbiology and Tumor Biology Center, Karolinska Institutet), were cultured in similar medium as the MDDCs, but without cytokines. Before co-culturing with MDDCs, L cells were irradiated (8,000 rad) and cultured in 4 ml medium in 6-well plates overnight to adhere. Non-attached cells were washed away with PBS.

Immature MDDCs cultured with nanoparticles. MDDCs, at day 6 of culture, were transferred to 6 well plates (Becton Dickinson, San Jose, CA, USA) at a density of 4×10^5 cells/ml in 4 ml of complete culture medium. Gold nanoparticles (0.5, 5 or 50 μg/ml) were incubated with the cells for 6 or 24 h. As controls, untreated cells and cells treated with LPS

(0.1 μg/ml; L8274, *Escherichia coli*, serotype 026-B6, Sigma-Aldrich, Steinheim, Germany) were used. Polymyxin B (10 μM; Sigma-Aldrich), which inhibits the LPS-activated pathway by binding directly to LPS and forming a non-reactive complex,[5] was added as an extra control by itself or together with a 5 or 50 μg/ml gold nanoparticle solution.

SUPPLEMENT SECTION 3

Flow cytometry analysis. Cells were labeled with fluorescent phycoerythrin (PE) conjugated monoclonal antibodies (mAbs) specific for: CD1a (Coulter Corporation, Hialeah, FL, USA); and with fluorescein isothiocyanate (FITC) conjugated mouse monoclonal Abs specific for: CD40, CD80, CD83, CD86 and HLA-ABC, HLA-DR and CD14 (Becton Dickinson) according to the manufacturer's instructions. Control samples were labeled with isotype-matched antibodies conjugated with the same fluorochrome. Fluorescence was measured with a FACSCalibur flow cytometer (Becton Dickinson). Ten thousand cells were counted and analyzed by the program CellQuestPro. The M1 gate was set to 1 % in isotype control. When L cells were included in the experiment as control, all cells were double stained with either CD1a PE or HLA-DR FITC to be able to gate for MDDCs and to exclude the L cells from FACS analysis.

ELISpot. After pretreatment with 95 % ethanol, and washing with Milli-Q water and PBS, wells of MultiScreen-IP plates (Millipore, Bedford, MA, USA) were coated with 100 μl per well of monoclonal Abs to human IFN-α (MT-1) or total IL-12 (IL12-I) at 15 μg/ml in PBS overnight at 4 ˚C. PBMCs from buffy coats were diluted to 2×10^5 or 10^6 cells/ml in complete medium together with different additives where described. After wells were washed with PBS 6 times, 100 μl of the cell suspension was added in triplicates, and incubated at 37 ˚C for 20 h for IL-12 and 40 h for IFN-α. After another series of washings, captured cytokine was detected by adding 100 μl of biotinylated monoclonal Abs to human IFN-α (MT-2) or total IL-12 (IL12-II) at 1 μg/ml per well for 1 h (Abs kindly provided by Mabtech, Nacka, Sweden). Following washing with PBS and incubation with 100 μl of streptavidin-alkaline phosphate (Mabtech) for 60-90 min, substrate development was performed using BCIP/NBT (Bio-Rad, Hercules, CA, USA) for 15-60 min until dark spots emerged and stopped by washing with tap water. Spots were counted using a stereo microscope (Nikon, Surrey, UK) at ×40 magnitude. As controls, LPS, polymyxin B and CpG (CPG-ODN2216-class A, InvivoGen, Québec, Canada) were used.

Mixed lymphocyte reaction (MLR). PBMCs, depleted of $CD14^+$ cells as described in supplement section 2, were incubated in complete medium, in 96-well plates (Becton Dickinson), at a number of 1×10^5 cells/well. MDDCs, pre-incubated in medium (4×10^5 cells/ml), with low-or high-LPS gold nanoparticles (50 μg/ml) for 24 h, were added at different cell numbers (10^2, 10^3 and 10^4 MDDCs per well), to autologous or allogeneic CD14-

depleted PBMCs (resulting in a final volume of 200 μl). Phytohaemagglutinin (PHA, 10 μg/ml, Sigma-Aldrich) was used to ascertain that the PBMCs could proliferate. To detect the background proliferation, CD14-depleted PBMCs were incubated in medium alone. The cells were cultured in triplicates for 4 days and 1 μCi [^{3}H]-thymidine per well (specific activity 25 Ci/mmol (925 GBq/mmol), Amersham Biosciences, Buckinghamshire, UK) was added to the cultures for additional 18 h. The plates were kept at -20 ºC until [^{3}H]-thymidine incorporation was determined by scintillation counting.

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