SUPPLEMENTARY MATERIALS AND METHODS

Media, buffers, TLR ligands and recombinant cytokines and chemokines

Complete medium was composed of RPMI Glutamax 1640 (Life Technologies) supplemented with 10% of fetal bovine serum (FBS) (HyClone), antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin; Life Technologies) and 5×10^{-5} M β2-mercaptoethanol (Life Technologies). MACS buffer consisted of PBS (Life Technologies) supplemented with 0.5% of FBS and 2.5 mM ethylenediamine tetra-acetic acid (EDTA) (Life Technologies). FACS buffer was composed of PBS supplemented with 1% of FBS.

The TLR-9 ligand CpG2216 was custom synthesized by Sigma and used at 10 μg/mL.

The recombinant cytokines and chemokines were purchased from R&D systems (BioTechne).

Tumor cell lines

TC-1 tumor cells expressing the oncoproteins E6 and E7 from human papilloma virus HPV-16, and derived from primary mouse lung epithelial cells [1], were obtained from the American Type Culture Collection (ATCC; LGC Promochem) and were cultured in complete medium supplemented with 0.4 mg/mL geneticin G418 (Life Technologies) and 0.2 mg/mL of hygromycin B (Roche), at 37°C and 5% CO_2.

B16-OVA tumor cells, which are B16 melanoma cells transfected with OVA antigen were kindly provided by L. Rosthein and L. Sigal (University of Massachusetts, Worcester, MA). They were cultured in complete medium supplemented with 2 mg/mL geneticin G418 (Life Technologies) and 60 μg/mL hygromycin B (Roche).

For the engraftment of tumor cells, 5×10^5 TC-1 and 2.5×10^5 B16-OVA tumor cells were suspended in 200 μL of sterile PBS and were injected subcutaneously into the shaved right flank of mice. The tumor size was measured every 2-3 days using a digital caliper (Mitutoyo).

Flow cytometry analysis

The following mAbs were used: anti-B220 (clone RA3-6B2), anti-CD3 (clone 500A2), anti-CD11b (clone M1/70), anti-CD11c (clone N418), anti-CD27 (clone LG.7F9 or LG.3A10), anti-CD317 (clone 927), anti-CD45.2 (clone 104), anti-CD49b (clone DX5), anti-CD69 (clone H1.2F3), anti-CD80 (clone 16-10A1), anti-CD86 (clone GL1), anti-F4/80 (clone BM8), anti-Gr1 (clone RB6-8C5), anti-H2-K^b (clone AF6-88.5.5.3), anti-I-A^b (clone AF6-120.1), anti-ICOS-L (clone HK5.3), anti-Ly6C (clone HK1.4), anti-Ly6G (clone 1A8), anti-NK1.1 (clone

PK136), anti-OX40-L (clone RM134L), anti-PDC-TREM (clone 4A6), anti-PDL-1 (clone MIH5) and anti-SiglecH (clone 440c). All the mAbs coupled with different fluorochromes were purchased from BD Pharmingen, eBioscience or Biolegend, and the control isotypes were purchased from the corresponding providers.

Staining was performed in the presence of anti-CD32/CD16 (clone 2.4G2, eBioscience) to block non-specific labeling and with DAPI (Thermo Fisher Technologies) or Live Dead Blue (Life Technologies) to characterize live cells. In the experiments where cells were fixed prior to acquisition, the BD Cytofix kit (BD Biosciences) was used, and cell samples were treated according to the protocol provided by the manufacturer. The stained cells were acquired on an LSR Fortessa (BD Biosciences) flow cytometer and were analyzed using FlowJo software (Tree Star).

Transcriptomic analysis of pDCs

Following purification by FACS Aria III, splenic pDCs were either immediately lysed after purification or following *in vitro* culture. pDCs purified from TC-1 and B16-OVA tumors were immediately lysed after FACS sorting. RNAs were extracted using the NucleoSpin® RNA XS kit (Macherey-Nagel) and were quantified using the Qubit fluorometer (Life Technologies). Transcriptomic analysis was performed using the nCounter® PanCancer Immune Profiling Panel (NanoString Technologies) which allowed the study of approximately 800 genes following the manufacturer's procedure. The samples were processed with the fully automated nCounter Prep Station (NanoString Technologies) and were imaged on the nCounter Digital Analyzer (NanoString Technologies). All the samples were normalized with the expression level of housekeeping genes. The normalized data were analyzed with Qlucore Omics Explorer (Lund, Sweden) to perform Principal Component Analysis (PCA) and Heat Map clustering. Pathway analysis and gene ontology enrichment were performed using Ingenuity® Pathways Analysis software (IPA; Qiagen Bioinformatics).

SUPPLEMENTARY REFERENCE

1. Lin KY, Guarnieri FG, Staveley-O'Carroll KF, Levitsky HI, August JT, Pardoll DM, et al. Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. Cancer research 1996; 56:21-6.