Supplementary Materials

SUPPLEMENTARY METHODS

Retroviral vectors and virus supernatant

Full-length OVA-sequence was amplified from pLOVASN via PCR using following primer-sequences: 5′-ATAGTCGACCACCATGGGCTCCATCGGTG-3 (fwd) and 5′-ATAGCGGCCGCTTAAGGGGAAACACATCT-3 (rev). Underlined are the introduced restriction-sites for SalI and NotI. After digestion of the pMP71-IRES-GFP vector and the resulting amplicon with SalI and NotI restriction enzymes, both DNA-fragments were ligated using a Rapid DNA Ligation Kit (Roche). The resulting construct, pMP71-OVA-IRES-GFP, was verified by digestion with PstI restriction enzyme as well as DNA sequence analysis. Ecotropic viral vectors were produced using the Platinum-E packaging cell line (1). Viral supernatants were harvested 48 h following transfection, filtrated (0.45-µm pore size) and stored at -80 °C.

Transduction of GL261 cells and murine T cells

GL261 murine glioma cells (National Cancer Institute) were cultured at 37 °C and 5 % CO_2 in RPMI 1640 with 200 mM glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, and 10 % FBS (all from Invitrogen). GL261 cells (5×10^4) were seeded into 24-well plates and after 16 h, 1 ml viral supernatant supplemented with 4 µg/ml protamine sulfate (Sigma-Aldrich) was added prior to centrifugation for 1.5 h (800 g, 32 °C). After 24 h, the supernatant was exchanged with viral supernatant (2 ml) supplemented with protamine sulfate and 48 h later, the cells were analyzed by flow cytometry for expression of GFP and H2kb-SIINFEKL complexes. Transduction efficiency

reached 76% for MP71-OVA-IRES-GFP. To generate a 100 % $GFP^{+}H2kb\text{-}OVA^{+}$ cell population, these cells were bulk-sorted using a FACSARIA II (BD Biosciences).

To transduce murine primary splenocytes, 2×10^6 cells/ml were supplemented with 1 µg/ml anti-CD3 mAb, 0.1 µg/ml anti-CD28 mAb (BD Biosciences) and 10 IU IL-2 (Proleukin Novartis) and cultured overnight. The cell density 1×10^6 per ml were seeded in 24 well plates coated with 12.5 µg/ml RetroNectin (Takara) and supplemented with 4×10^5 beads/ml mouse T-Activator CD3/CD28 (Life Technologies), 10 IU/ml IL-2, 4 µg/ml protamine sulfate and virus-containing supernatant (MOI = 2). Following centrifugation for 1.5 h (800 g, 32 °C), the cells were incubated at 37 °C for 48 h. The medium was replenished with 10 ng/ml human recombinant IL-15 (Peprotech).

MRI methods

For *in vivo* MR measurements, mice were anesthetized shortly before and during the MR session using a mixture of isoflurane (Baxter) as inhalation narcosis (0.5 - 1.5%), pressurized air and oxygen. The core body temperature of the mice was maintained at 37 °C. Respiration rate and temperature were monitored using a remote monitoring system (Model 1025, SA Instruments Inc.).

In vivo assessment of glioma growth using ^{1}H MR microscopy. For comparing glioma tumor size in WT and *Erk1*$^{-/-}$ glioma-bearing mice, mice brain were imaged on day 14 after the intracereberal inoculation of GL261 cells (14 dpi). For comparing glioma tumor size after vaccination, mice were imaged 18 dpi after the intracereberal inoculation of GL261 cells. Anesthetized mice were placed below a cryogenically-cooled quadrature radiofrequency (RF) coil (CryoProbe, Bruker Biospin) tailored for mice brain MR (2). T_2-weighted images (RARE,

repetition time (TR) = 3268 ms, echo time (TE) = 60 ms, RARE factor = 12, 8 averages) were acquired with the same slice geometry (FOV = (18×18) mm^2, matrix size = 350×350, slice thickness = 270 µm, in-plane spatial resolution = 51 µm, 21 coronal slices covering a brain region of 5.67 mm starting at the frontal end of the cerebral cortex (approx. Bregma 3.56 to -2.11 mm) (3). Tumor regions were manually segmented and the volume calculated in Fiji by adding up the voxel volumes.

Imaging inflammatory cell infiltration using in vivo $^{1}H/^{19}F$ MRI. For studying infiltration of immune cells from the peripheral circulation into glioma tissue, glioma-bearing mice were injected with rhodamine-tagged ^{19}F nanoparticles containing 80 µmol perfluoro-15-crown-5-ether (PFCE) 18 dpi via tail vein. After 18 h of injection, $^{1}H/^{19}F$ MRI was performed using an in-house built dual-tunable $^{19}F/^{1}H$ head coil (4) and a protocol consisting of a 3D RARE sequence with an isotropic resolution of 250 $µm^3$ for ^{19}F (TR/TE = 800/5.9 ms, 128 averages) and of 125 $µm^3$ for ^{1}H (TR/TE = 1500/47 ms) MRI. ^{19}F signal intensity was calculated in Fiji.

Visualizing in vivo BMDC migration using $^{1}H/^{19}F$ MRI. BMDC were incubated with ^{19}F nanoparticles (containing 20 µmol PFCE) and 1 µg/ml full-length chicken EndoGrade ovalbumin (endotoxin conc.<1 EU/mg; Hyglos). BMDC were further matured by 0.5 µg/ml lipopolysaccharide (LPS). Following overnight incubation, unbound ^{19}F nanoparticles were washed thoroughly from the culture with warm PBS. BMDC were then harvested and applied intradermally into hind limbs (one limb received $5×10^6$ WT BMDC, the other received $5×10^6$ *Erk1*$^{-/-}$ BMDC) of WT mice and imaged 4 h after injection. Using a $^{1}H/^{19}F$ dual-tunable volume birdcage resonator (Rapid Biomed), gradient echo images were acquired using a 3D-FLASH sequence for ^{19}F (TR/TE = 8/3 ms; 80 averages, flip angle = 10°) and for ^{1}H (TR/TE = 11/4 ms; 8 averages, flip angle = 15°).

In vitro ^{19}F MR Spectroscopy (MRS). Following harvesting, BMDC (1×10^6) labeled with ^{19}F nanoparticles were fixed in 2 % paraformaldehyde (PFA) and transferred to NMR tubes (external diameter: 4.947 ± 0.019 mm; Wall thickness: 0.043 ± 0.02 mm; VWR International GmbH) and the uptake of ^{19}F nanoparticles monitored by ^{19}F spectroscopy. Different BMDC numbers were employed to achieve a ^{19}F signal to cell calibration. We employed an in-house built ^{19}F-tuned loop RF coil (5) for signal transmission and reception and a 90° block pulse with 10 kHz bandwidth for ^{19}F signal excitation. The amount of PFCE in each sample was calculated from the amplitude of the extrapolated monoexponential decay, at t=0 of the free induction decay (FID) ^{19}F MR signal, which is proportional to the ^{19}F concentration. A standard consisting of 500 mM PFCE was used as quantitative reference in all of these experiments. The same MRS method was employed to measure the ^{19}F signal in popliteal LNs extracted from mice following intradermal application of ^{19}F-labeled BMDC. The number of ^{19}F-labeled BMDC in each LN was quantified using the BMDC calibration curves. NMR data processing and analysis were performed in Matlab (R2010a, The MathWorks, Inc.).

References

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