

Supplementary methods

Antibodies, flow cytometry and immunohistochemistry

Cells isolated from lungs, livers and spleens were pretreated with Fc-Block (anti-CD16/32, eBioscience, San Diego, CA, USA) to reduce nonspecific staining.

Monoclonal antibodies to the following mouse antigens were conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-cyanine7 (PE-Cy7), allophycocyanin (APC), AlexaFluor 488 or biotin: CD3 (145-2C11), NK1.1 (PK-136), CD8 (53-6.7), CD44 (IIM7), and STAT-5 (pY694) were obtained from BD Biosciences (San Diego, CA, USA). Biotinylated antibodies were visualized with streptavidin (Sav)-APC-Cy7 (BD Biosciences). FACSCanto and FACSCalibur cytometers were used for cell acquisition, and data analysis was performed using FACS DiVa (BD Biosciences) and FlowJo 7.2.1 (Tree Star Inc., Ashland, OR, USA).

Immunohistochemistry

For H&E and immunohistochemical stainings harvested organs were soaked in 4% formaldehyde (Panreac) during 24h. Then, they were washed with 70% ethanol and included in paraffin. Immunohistochemical staining was performed using the EnVisionTM+ System (Dako, K4011; Glostrup, Denmark) according to the manufacture's recommendations. Paraffin lung sections (3 µm thick) were cut, dewaxed and hydrated. Antigen retrieval was performed for 30 min at 95 °C in 0.01 M Tris-1 mM EDTA buffer (pH=9) in a Pascal pressure chamber (S2800, Dako). Slides were allowed to cool for 20 min, then endogenous peroxidase was blocked with 3% H₂O₂ in deionized water for 10 min and sections were washed in TBS-0,05% Tween 20 (TBS-T). Sections were incubated overnight at 4°C with anti-CD3 (1:300, clon SP7, Neomarkers, RM9107). After rinsing in TBS-T, the sections were incubated with goat anti-rabbit

labelled polymer for 30 min at room temperature and peroxidase activity was revealed using DAB⁺. Finally, sections were lightly counterstained with Harris hematoxylin, dehydrated, and coverslipped.

Isolation of mononuclear cells from spleen, lung and liver

At the indicated time points, spleens, lungs and livers were surgically harvested from the treated mice. The organs were incubated in collagenase and DNase (Roche) for 15 min at 37°C, mechanically disrupted and passed through a 70 µm nylon mesh filter (BD Falcon, BD Biosciences). Dissociated cells from livers and lungs were centrifuged with Percoll[®] (GE Healthcare, Chalfont St Giles, UK) at 35% (500g, 10 min, 20°C) making a gradient in order to eliminate parenchymal cells. In either case, erythrocytes were lysed with ACK buffer and all the cells were washed before further use. Total cell number was counted with a Z1 Coulter Particle Counter (Beckman Coulter).