**Online Data Supplement**

# Dendritic Cell Depletion and Repopulation in the Lung after Irradiation and Bone Marrow Transplantation in Mice

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## Reagents

Phycoerythrin (PE)-labelled anti-mouse CD45.1 monoclonal antibody (clone A20, isotype $IgG_{2a}$), fluorescein isothiocyanate (FITC)-, allophycocyanin (APC)- and PerCPCy5.5-labeled anti-mouse CD45.2 monoclonal antibody (clone 104, isotype $IgG_{2a}$), PECy7-labelled anti-mouse CD11b monoclonal antibody (clone M1/70, isotype $IgG_{2a}$), PE-labelled anti-mouse MHC II monoclonal antibody (clone M5/114, isotype $IgG_{2a}$), biotinylated MHC II (Clone 2G9), APC-labelled streptavidin, APC- and PE-labelled anti-mouse CD103 monoclonal antibody (clone 2E7 and M290, isotype $IgG_{2a}$) were purchased from BD Biosciences (Heidelberg, Germany). APC-labelled anti-mouse CD11c and APC-labelled anti-mouse F4/80 monoclonal antibody was purchased from Caltag Laboratories (Hamburg, Germany) and Serotec (Düsseldorf, Germany), respectively. For determination of apoptosis and necrosis, PE-labelled Annexin V and 7-Aminoactinomycin D (7-AAD) were purchased from BD Biosciences (Heidelberg, Germany).

## Generation of chimeric mice

Briefly, bone marrow cells were isolated from tibias and femurs of CD45.2 alloantigen-expressing donor mice under sterile conditions. Single cell suspensions prepared from bone marrow isolates were filtered through cell strainers to remove aggregates and were then washed in Leibovitz L15 medium (Gibco, Invitrogen, Karlsruhe, Germany). Recipient CD45.1 alloantigen-expressing mice were irradiated with a single dose of 8 Gy at a dose rate of 2.5 Gy/min delivered by a linear accelerator (Siemens MD 2, Hannover, Germany) operating in a 6-MV high-energy photon delivery mode. Within 24 h after irradiation, single cell suspensions ($1 \times 10^7$ cells per mouse) of bone marrow cells from CD45.2 donor mice were intravenously injected into CD45.1 alloantigen-expressing recipients via their lateral tail veins. The resulting chimeric mice are characterized by a $CD45.2^{pos}$ hematopoietic system in a $CD45.1^{pos}$ background. During both reconstitution and experimentation, chimeric mice were housed under specific pathogen-free conditions, using autoclaved food and water and individually ventilated cages.

## Bronchoalveolar lavage and quantification of macrophages and lung DC subsets

Bronchoalveolar lavage (BAL) was performed by repeated intratracheal instillation of 300 µl aliquots of cold PBS supplemented with EDTA (Versen; Biochrom, Berlin, Germany) into the lungs and careful aspiration until a BAL volume of 1.5 ml was collected. Subsequently, BAL was continued until an additional volume of 4.5 ml was collected. Whole lung washes were centrifuged (1400 rpm, 4°C, 9 min) and the cell pellets were pooled, re-suspended in RPMI/10 % FCS and total numbers of BAL fluid leukocytes were determined. Quantification of alveolar macrophages (including resident and newly recruited cells) and determination of their turnover characteristics in BAL fluids of chimeric mice was done

based on their F4/80$^{pos}$ cell surface expression in conjunction with their FACS-based immunophenotypic differences in CD45 alloantigen expression profiles, with resident recipient-type alveolar and lung macrophages expressing CD45.1 but not CD45.2, and newly recruited alveolar and lung macrophages expressing the CD45.2 but not CD45.1 alloantigen. In addition to these markers, resident alveolar and lung macrophages are CD11c$^{pos}$/CD11b$^{neg\text{-}low}$, whereas newly recruited "exudate" alveolar and lung macrophages are CD11c$^{pos}$/CD11b$^{pos}$, corresponding to recent reports (E1, E2).

To determine the turnover of CD11b$^{pos}$ DC and CD103$^{pos}$ DC subsets in lung parenchymal tissue of chimeric mice, lungs were subjected to BAL to remove alveolar macrophages, as described above. Subsequently, lungs were perfused via the right ventricle with Hank's balanced salt solution (HBSS, PAA, Cölbe, Germany) until visually free of blood. Individual lung lobes were removed and dissected into small pieces, while avoiding any contamination with lymph nodes or conducting airways. Lung tissue was then digested in RPMI supplemented with collagenase A (5mg/ml) and DNAse I (1 mg/ml) for 1.5 hours at 37°C. The digested tissue was aspirated with a 1 ml pipette and filtered through 100 and 40 µm cell strainers to obtain single cell suspensions. CD11c$^{pos}$ cells (including DC subsets and macrophages) were further purified from lung parenchymal tissue digests by re-suspending them in MACS buffer (PBS/2 mM EDTA/0.5 % BSA), followed by incubation with magnetic bead-conjugated anti-CD11c antibodies (10 µl/$10^7$ cells) for 15 min on ice. Subsequently, cell suspensions were passed through MS columns, and were then eluted with MACS buffer. Highly enriched (85-90 %) CD11c$^{pos}$ lung mononuclear phagocyte subsets were then analyzed according to their FACS-based immunophenotypic differences in CD11c, CD11b, and MHC class II cell surface expression (E1), as well as their CD103 and CD45 alloantigen expression profiles.

**Analysis of apoptosis and necrosis**

Cells from BAL fluid and lung tissue digests were collected as described above and then stained with APC-labelled anti-F4/80 Ab and FITC-labelled anti-CD45.1 Ab followed by incubation with PE-labelled Annexin V for determination of apoptosis and 7-AAD for determination of necrosis for 15 min at room temperature. Subsequently, cells were gated and analyzed according to their FSC/SCC properties and FSC/F4/80 characteristics as well as their CD45.2 versus CD45.1 alloantigen cell surface expression profiles, using a BD FACSCanto flow cytometer equipped with an argon ion laser and a helium-neon laser.

**Infection experiments with *S. pneumoniae***

For infection experiments, we used the capsular group 19 *S. pneumoniae* strain EF3030, which is characterized by a relatively low virulence in mice (13-15). The bacteria were grown in Todd-Hewitt broth (Oxoid, Wesel, Germany) supplemented with 20 % FCS to mid-log phase and aliquots were snap frozen in liquid nitrogen and stored at -80°C until use. Pneumococci were quantified by plating serial dilutions on sheep blood agar plates (BD Biosciences, Heidelberg, Germany), followed by incubation of the plates at 37°C/5 % $CO_2$ for 18 hours and subsequent determination of colony-forming units (CFU).

Low-dose infection of chimeric CD45.1 mice with *S. pneumoniae* was done using freshly thawed aliquots adjusted to ~1,5 x $10^6$ CFU/mouse in 50 µl THB. Intratracheal instillation of *S. pneumoniae* into the lungs of mice was

performed by orotracheal intubation of mice with a 29-gauge catheter (Abbocath) under visual control with transillumination of the neck region, followed by slow instillation of *S. pneumoniae* into the lungs of mice. For determination of the turnover kinetics of lung DC subsets and alveolar and lung macrophages, chimeric CD45.1 mice were infected with *S. pneumoniae* on day 10 and 35 post irradiation/BMT, respectively.

Bacterial loads within the lungs of *S. pneumoniae*-infected chimeric mice were determined from whole lung washes. Briefly, mice were euthanized with an overdose of isoflurane and BAL was performed as described above. Colony-forming units (CFU) in the respective 1.5 ml and 4.5 ml BAL fluid aliquots collected from *S. pneumoniae*-infected chimeric mice were quantified by plating serial dilutions on sheep blood agar plates (BD, Biosciences, Heidelberg, Germany), followed by incubation of the plates at 37°C/5 % $CO_2$ for 18 hours.

**References**

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E2. Winter C, Herbold W, Maus R, Länger F, Briles DE, Paton JC, Welte T, and Maus UA. Important role for CC chemokine ligand 2-dependent lung mononuclear phagocyte recruitment to inhibit sepsis in mice infected with *S. pneumoniae*. J Immunol 2009;182:4931-4937.