SUPPLEMENTARY SECTION

Plasma cell but not CD20-mediated B cell depletion protects from bleomycin-induced lung fibrosis

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Supplementary Methods

Mouse lung and spleen dissociation and B cell phenotype analysis using flow cytometry.

Mouse lung tissue was harvested and dissected into 1-2 mm^3 pieces and incubated in 3 mL digestion medium consisting of 48 µg/mL Liberase™ (Roche), 0.1 mg/mL of DNase I in RPMI (Gibco® Life Technologies™) for 2 h at 37°C with constant shaking. The enzymatic activity within the digestion medium was neutralised with 4 mL RPMI/10% FCS and the lung cells collected by centrifuging at 300 g for 5 min.

Spleen cells were isolated by teasing the spleen with two needles and then washed in PBS, pH7.4. The isolated cells were resuspended in hypotonic cell lysis buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4), incubated at room temperature (RT) for 7 min and then topped up with PBS to lyse the red blood cells. PBMC were isolated on a lymphoprep gradient using Cell SepMate Tubes (Stem Cell Technologies, UK) in accordance with the manufacturer's guidelines. All cells were resuspended and frozen in cyro-preservation medium (RPMI 50% FCS/10% DMSO).

Flow cytometry antibodies were diluted in a solution of 50% PBS and 50% BD Horizon Brilliant Stain™ (BD Biosciences, Franklin Lake, NJ, USA). Mouse PBMCs, lung and spleen cells were stained in 50 µL of the B cell antibody cocktail (Supplementary Table S1) for 30 min on ice, washed twice in 100 µL of PBS/2% FCS and centrifuged at 300 g for 5 min. The cells were then resuspended in 200 µL of PBS/10% FCS and fixed with BD Cytofix/Cytoperm™ kit and stored at 4°C for up to 48 h. Flow cytometry was performed on BD LSRFortessa™ and analysis performed using FlowJo 10.3. B cell subsets were identified by flow cytometry; mature follicular B cells ($CD45.2^+CD19^+CD20^+$), B1 regulatory B cells ($CD45.2^+CD19^+CD20^-CD38^+CD5^+CD43^+$) and

plasma cells ($CD45.2^{+}CD19^{+}CD20^{-}CD138^{+}CD43^{+}CD38^{+}$ $CD27^{+}$) or plasmablasts ($CD45.2^{+}CD19^{+}CD20^{+}CD138^{-}CD43^{+}CD38^{+}$ $CD27^{+}$).

Histochemical and immunohistochemical analysis of fibrosis and immune cell infiltration in tissue. Mouse and human lung sections (3 µm) were dewaxed, and antigen retrieval performed in a pressure cooker by boiling slides in citrate buffer for 10 minutes and cooling. Endogenous peroxidase and non-specific antibody binding blocking steps were performed and then the tissues were incubated with primary antibody or appropriate control mouse or rabbit IgG overnight at 4°C. Tissue sections were washed three times in TRIS Buffered Saline (TBS) and then incubated in the appropriate biotinylated secondary antibody for 45 min followed by streptavidin/HRP for 30 min at RT. Immunolabelling was visualised by incubating tissue sections in 3,3′-Diaminobenzidine solution (DAB) (Sigma-Aldrich®) for up to 5 min. For CD138 staining of mouse lung tissue the Vector® M.O.M™ Immunodetection kit was used according to the manufacturer's instructions. For the detection of CD19 and CD138 by immunofluorescence the sections were incubated in the appropriate AF568- and AF488-conjugated secondary antibodies for 30 min at RT. The nuclei were visualised by incubating the section in 4',6-diamidino-2-phenylindole (DAPI) for 5 min at RT. Representative tissue sections were also stained for hematoxylin and eosin, Masson's trichrome or Martius scarlet blue (MSB) trichrome staining using a Tissue-Tek DRA autostainer (Sakura, Japan). Slides were imaged using either the Aperio ScanScope® XT (Leica Biosystems, Centre for Microscopy, Characterisation and Analysis, UWA) or Nanozoomer HT slide scanner (Hamamatsu, Japan; University College London).