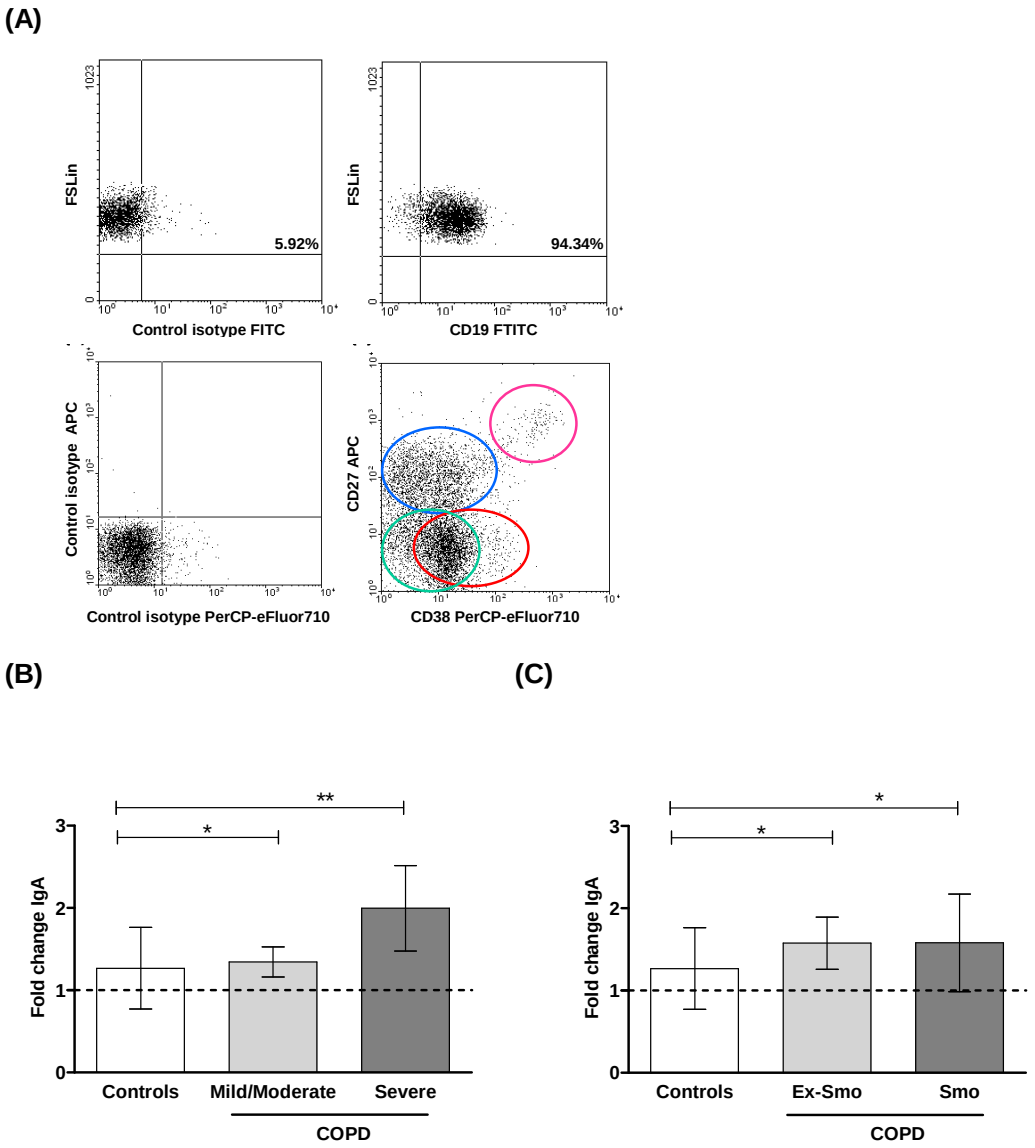
Table E1. Clinical characteristics of patients from whom AEC were derived.

AEC N°	Age (yrs)	Gender M/F	Lung disease	FEV1 (% pred)	FEV1/VC (%)	DLco (% pred)	Smoking (PY, active/former)	Co-morbidity
20	61	M	COPD III	38	61	48	75, active	
25	55	F	No	99	77	82	35, active	
36	70	M	COPD II	56	57	70	58, ex	K. colon
55	66	M	COPD III	44	40	50	120,ex	
57	56	M	COPD III	53	47	45	40, ex	
61	72	F	COPD I	80	67	60	45, ex	K. ovarian
64	66	M	No	106	78	81	0	K. colon
65	71	M	No	103	78	Nd	10,ex	
67	78	M	COPDII +fibrosis	79	76	33	40, active	
70	55	M	COPD II	74	63	64	40, ex	
72	75	F	No	97	70	Nd	0	
74	69	F	No	56	70	79	18,ex	Obesity
77	44	F	No	75	73	46	20, active	Vasculitis
78	74	F	No	109	79	112	0	K. colon
79	62	M	COPD II	58	56	76	30, ex	
80	62	F	No	85	80	Nd	50, active	
82	36	F	No	96	7978	84	17, active	
83	60	F	COPD IV	10	25	Nd	35, ex	Lung transplant

85	43	M	COPD II	74	66	51	13, active	Hepatitis C
86	55	F	COPD I	84	65	75	38, active	
87	50	F	No	99	78	81	0	
88	60	M	No	90	70	81	20, ex	
89	82	M	No	97	71	92	0	
93	61	F	COPD IV	26	31	Nd	50, ex	Lung transplant
94	85	M	No	76	81	92	16, ex	Cardiac failur
96	84	M	No	142	75	68	20, ex	K. skin
97	77	M	COPD I	101	61	73	40, active	
98	61	F	COPD II	67	57	58	35, active	

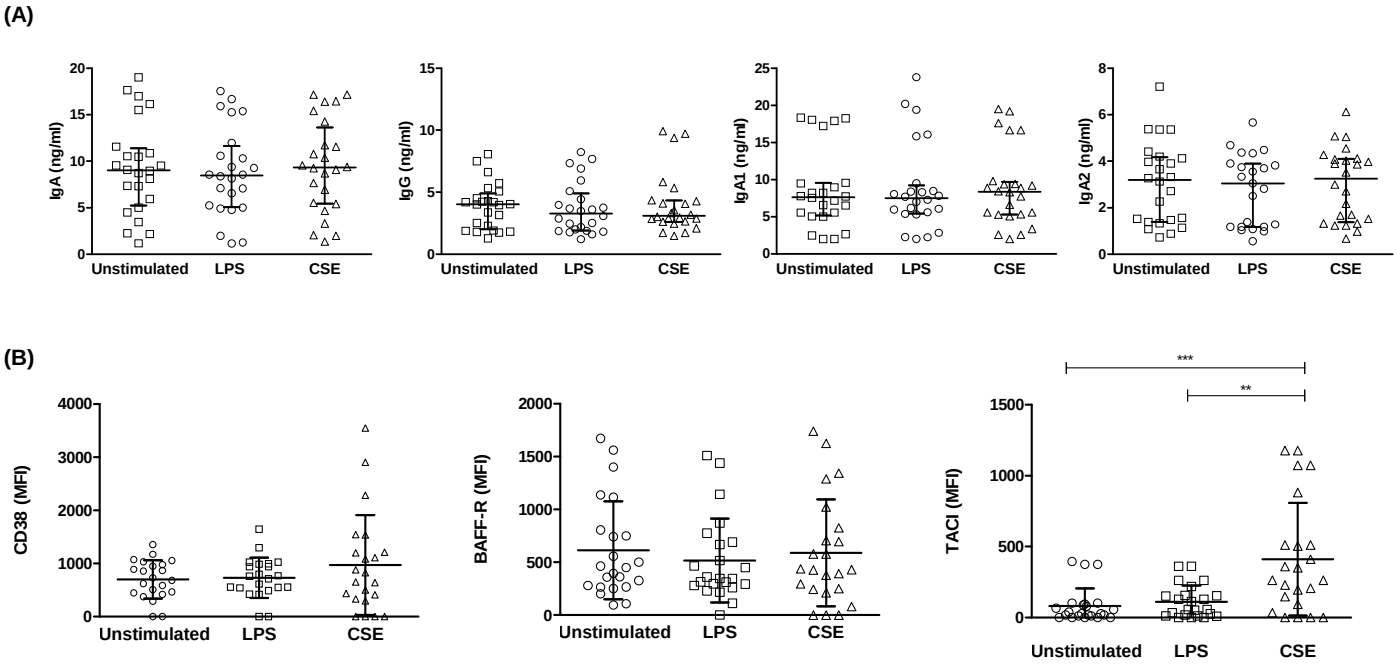
All patients had a confirmed diagnosis of primary lung tumor (NSCLC), except five patients: HBEC64 (colon metastasis), HBEC94 (pleural fibroma), HBEC96 (spinocell skin metastasis) and HBEC 83 / HBEC 93 (lung transplantation for end-stage COPD). Nd: not determined. K: carcinoma.

Figure S1



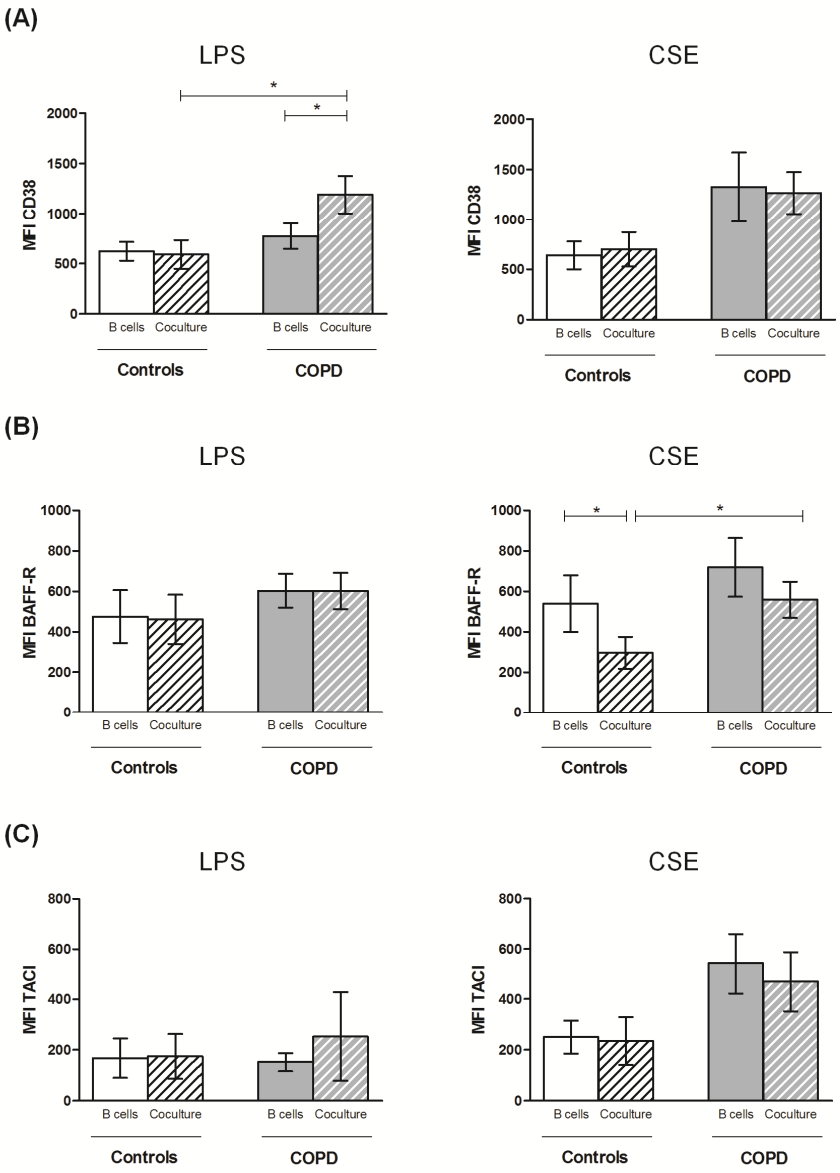
(A) Representative dot plots of B-cell subsets after CD19+ MACS purification from PBMC. Validation of purification of B cells by CD19 staining and phenotype of isolated B cells according to CD27 and CD38 expression (Pink: plasmablasts, blue: memory B cells, green: mature naïve cells and red: immature cells). **(B)** and **(C)** Effect of AEC/B cell co-culture on IgA production. Controls consist of never smokers (non-Smo) and current or former smokers (Smo). Smoker patients with COPD were classified **(B)** according to GOLD into two groups, namely mild/moderate (GOLD stages I and II) and severe (GOLD III and IV) or **(C)** according to the smoking status into two groups, namely former (Ex-Smo) and current (Smo) smokers. Fold change of Ig production in B cells co-cultured with AEC corrected for those obtained in B cells cultured alone. Data represent mean ± SD of 14 experiments performed with control AEC versus 14 experiments with COPD AEC. * *P<0.05*, ** *P<0.01*

Figure S2



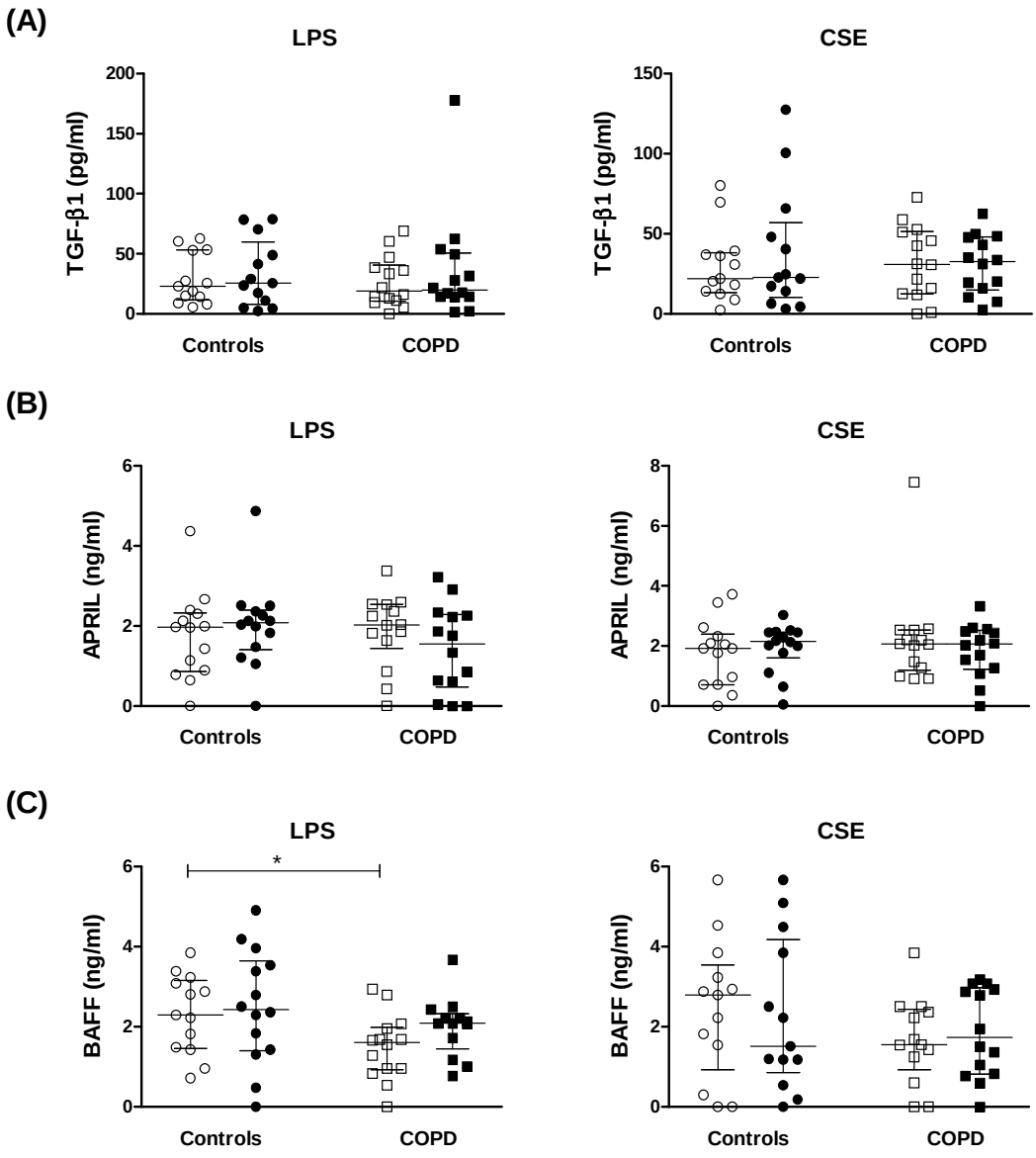
Effect of LPS and CSE on **(A)** Ig production and **(B)** IgA-related markers. **(A)** Ig production levels were assessed by ELISA after 13 days of culture of either resting or stimulated (LPS or CSE) B cells. Data represent mean (ng/ml) ± SD. **(B)** Expression levels of CD38, BAFF-R and TACI on resting or stimulated (LPS or CSE) B cells. Data are presented in terms of Mean Fluoresence Intensity (MFI) minus MFI of the appropriate isotype control. Data represent mean ± SD. ** *P<0.01*, *** *P<0.001*.

FigureS3



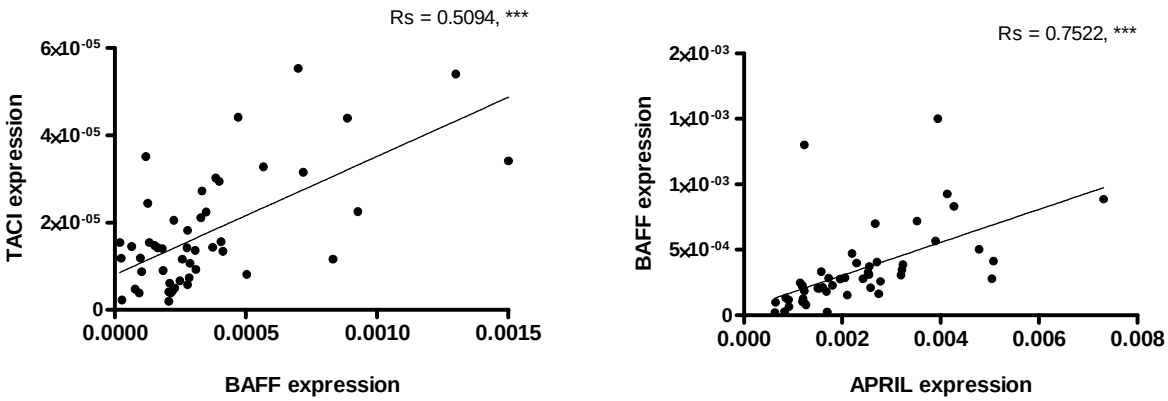
Effect of LPS or CSE on AEC/B cell co-culture IgA-related markers. Expression levels of the differentiation marker CD38 (A), the receptors BAFF-R (B) and TACI (C) (which bind respectively BAFF only or both BAFF and APRIL) on B cells cultured alone (empty bars) or co-cultured with AEC (hatched bars). Data are presented as Mean Fluoresence Intensity (MFI) minus MFI of the control isotype. Data represent mean ± SD of 13 experiments performed with AEC from control patients versus 14 experiments with AEC from COPD patients. * *P<0.05*

Figure S4



Effect of LPS or Cigarette Smoke Extract on AEC/B cell co-culture IgA-inducing factors. Production of TGF-β1 **(A)**, APRIL **(B)** and BAFF **(C)** by AEC cultured alone or in co-culture with B cells. Open symbols represent AEC cultured alone and filled symbols represent AEC in co-culture with B cells (circles for control group and squares for COPD group). Data represent median with interquartile range of 9 to 14 experiments performed with AEC from control patients versus 10 to 14 experiments with AEC from COPD patients. * *P<0.05*

Figure S5



Correlation of TACI/BAFF and BAFF/APRIL expression levels in lung tissue of the same patients. *** *P<0.001*

Methods - Additional details for material

Patients. Fifty five patients, including twenty three control patients (9 never-smokers, 9 ex-smokers and 5 current smokers) and thirty two COPD patients (10 mild, 16 moderate and 6 severe COPD) undergoing lung resection surgery for a solitary tumor were included in the study (Table 1). Lung tissue was sampled away from the tumor site to obtain tissue for immunohistochemistry, RT-qPCR and a large sample for primary airway epithelial cell (AEC) culture. Additional lung tissues were obtained from explanted lungs of two patients with very severe (GOLD stage IV) COPD.

Lung tissue samples. After surgical removal, lung tissue was sampled away from the tumor site, to obtain 2 to 5 proximal and distal samples for immunohistochemistry (IHC; immediately immersed in % formaldehyde in PBS at pH 7.4), 2 samples for RT-qPCR (immediately immersed in RNAlater) and one large sample for primary epithelial cell culture (put in culture medium, see below). Expression analyses (IHC, RT-qPCR) were performed for all patients, and primary cultures were derived from 28 patients (Table E1).

Epithelial cell cultures. Briefly, 12-well polyester membrane inserts (0.4-µm pore size; Corning) were coated with 0.2 mg/ml collagen IV (Sigma Aldrich) and then AEC were seeded at a density of 50,000 cells/well. AEC were cultured in submerged condition until confluence (approximately 10 days), cell differentiation was then allowed by removing medium in the apical compartment, as referred to air-liquid interface (ALI) condition, for 15 to 21 days.

AEC were cultured in BEBM:DMEM (1:1) medium supplemented with penicillin (100U/ml), streptomycin (100µg/ml), bovine pituitary extract (52µg/ml), insulin

(5µg/ml), hydrocortisone (0.5g/ml), transferrin (10µg/ml), epinephrine (0.5µg/ml), Epidermal Growth Factor (0.5ng/ml), triiodothyronine (3.25ng/ml), BSA (1.5µg/ml) and retinoic acid (30ng/ml) (Lonza, Verviers, Belgium).

ELISA. Ig synthesis was assessed in purified CD19+ B cells following co-culture for 13 days with ALI-AEC. Indirect ELISA was performed by coating 96-well plates overnight at 4°C with goat anti-hIgA (ACP17 polyAb made in our laboratory) or mouse anti-hIgG mAb (I5885, Sigma, St Louis, MO, USA) diluted in carbonate-bicarbonate buffer at 1 µg/ml and 5µg/ml respectively for total IgA, IgA1 and IgA2 or IgG production. Plates were then saturated for 1h with PBS containing 1% serum albumin (BSA). After three washes with 0.05% Tween20/PBS, supernatants were added to each well and incubated for 2h at room temperature. Standards consisted of IgA, IgA1 and 2 purified from myeloma sera and IgG was purified from the serum of a healthy donor. For IgG and IgA assays, Horseradish peroxidase-conjugated goat anti-human IgA (A0295, Sigma, St Louis, MO, USA, dilution 1/5000) and rabbit anti-human IgG (6145-05, Southern Biotech, AL, USA, dilution 1/8000) were added after plate washing and incubated for 1h. For IgA1 and IgA2 detection, mouse mAbs were used (B3506 or A9604, respectively) for 1h at 1µg/ml, and sheep anti-mouse IgG-horseradish conjugate (A6782, dilution 1/4000; Sigma, St Louis, MO, USA). One hundred micro-liters of ultra-TMB (TetraMethylBenzidine) including hydrogen peroxide was added for revelation (Thermo fisher, Meridian, Rockford, USA). After stopping the reaction with 100µl/well of 1.8M H_2SO_4, absorbance was measured at 450 nm using a microplate spectrophotometer (Bio-Rad, Model 3550, Hercules, CA, USA).

IL-6 bioassay. IL-6 activity in the culture supernatants was measured using an IL-6-dependent hybridoma clone (7TD1), as previously described{Van Snick, 1986 #142}. Briefly, various dilutions (the first dilution was 1/2) of the culture supernatants were added to the 7TD1 cells and cultured for 4 days. Cell proliferation was estimated by colorimetric determination; rmIL-6 was used as an internal standard. One unit per milliliter of IL-6 was defined by the concentration corresponding to the half-maximal growth of 7TD1 cells.

Flow cytometry. *Reagents.* The following anti-human mAbs were used for flow cytometry: CD19-FITC (Clone HIB19), CD20-FITC (Clone L27), CD27-APC (Clone M-T271), CD38-FITC (Clone HIT2) and isotype-matched control IgG Abs (BD Pharmingen, San Diego, CA, USA); CD27-APCCy7 (Clone O323), BAFF-R-APCCy7 (Clone 11C1), TACI-PE (Clone 1A1) and isotype-matched control IgG-APCCy7 (BioLegend, San Diego, CA, USA).

For cell viability and proliferation Propidium Iodide staining solution (BD Pharmingen, San Diego, CA, USA) and CellTrace CFSE Cell Proliferation Kit (2.5mM; Invitrogen, Gent, Belgium) were used respectively.

Staining. 1-2 x 10^5 cells per sample were saturated with 10µl of FcR Blocking (Miltenyi Biotec, Bergisch Gladbach, Germany) for 15 minutes at 4°C. Cells were then incubated with fluorochrome-labeled Abs for 40 minutes at 4°C and fixed with 1.25% paraformaldehyde, except for cell viability (immediately analyzed, in unfixed non-permeabilized B cells). Flow cytometry was performed using FACSCanto II flow cytometer equipped with DIVA software (BD Biosciences, San Diego, CA, USA).

RNA extraction and RT-qPCR. Total RNA was isolated from lung tissue using the RNeasy® Plus Mini kit (Qiagen, Hilden, Germany). Total RNA (500 ng per sample) was reverse-transcribed with RevertAidTM Reverse transcriptase kit (Fermentas, St. Leon-Rot, Germany) with 0.3 µg of random hexamer (Invitrogen, Gent, Belgium) and 1mM of each dNTP (Invitrogen, Gent, Belgium) following to the manufacturer's protocol in a thermocycler (Applied Biosystems, Carlsbad, CA, USA).

The expression levels of TACI, BAFF and APRIL in lung tissue were quantified by real time quantitative PCR using the iCycler IQ5 PCR (Bio-Rad, Hercules, CA, USA). The reaction mix contained 5µl of 10-fold diluted cDNA, 400nM of each primer (TACI, Forward-GCC TTC TGC AGG TCA CTC AG, Reverse-GCT TAG GGT GCT GTC CAC A; BAFF, Forward-GTC TGG TGA CTT TGT TCG ATG TA , Reverse-TTG CAA TGC CAG CTG AAT AG; APRIL, Forward-CTG TAT AGC CAG GTC CTG TTT CA, Reverse-TCG GAA TAG AGT CTC CTG CCT; RPS18, Forward-TGT GGG CCG AAG ATA TGC T, Reverse-TGA TCA CAC GTT CCA CCT CAT) and 2x iQTM SYBR®Green Supermix (Bio-Rad, Hercules, CA, USA) in a final volume of 20µl. The cycling conditions were 95°C for 3 min followed by 40 cycles of 95°C for 15s and 60°C for 30s. To control the specificity of the amplification products, a melting curve analysis was performed. Samples were run in duplicate and the copy number was calculated from the standard curve. Data analysis was performed using Bio-Rad iQ5 Software (Bio-Rad, Hercules, CA, USA). Expression levels of TACI, BAFF and APRIL were reported to those of RPS18 housekeeping gene.

For IgA1 and 2 expression levels, quantitative real-time PCR was performed by using an ABI PRISM 7900 Sequence Detection System thermal cycler according to manufacturers

instructions (Applied Biosystems, Life Technologies Corporation, CA, USA) using the equivalent of 1.25ng reverse transcribed RNA per reaction. Gene specific primer/probe sets were designed using the on-line Universal ProbeLibrary Assay Design Centre (Roche Applied Science, Burgess Hill, UK). Universal ProbeLibrary probes were purchased from Roche Applied Science and corresponding forward and reverse primers synthesized by Sigma (Sigma-Aldrich Company Ltd, Dorset, UK). The primer/probes sets were as follows: IgA1, Forward-CAT GCC ACG TGA AGC ACT AC, Reverse-GGT AGG TGG AGT TGA GGG AAC T, Probe-UPL Probe 20; IgA2, Forward-CGC CAA CAT CAC AAA ATC C, Reverse-CGT CAC CAG CTC GTT CAG, Probe-UPL Probe-17. SDS software was used to determine the absolute quantification of the target cDNA. Standard curves were created using plasmids containing cDNA for IgA1, I.M.A.G.E cDNA clone 4701069 and IgA2, I.M.A.G.E cDNA clone 4765168 (Source Bioscience UK Limited, Nottingham, UK). Expression levels of IgA1 and 2 were reported to those of RPS18 housekeeping gene.

Immunohistochemistry (IHC). Serial lung sections of 5 µm thickness were cut from paraffin blocks, spread on polylysine-coated glass slides, and dried at 37°C for at least 24 h. The slides were then processed for IHC, each step of the procedure being followed by washing with Tris-buffered saline (pH 7.4). After disembedding and rehydration of the specimen, endogenous peroxidases were inhibited by incubation in 0.03% (vol/vol) H_2O_2 in water for 30 min, and the slides were treated with 1% (wt/vol) BSA in Tris-buffered saline for 30 min. For IgA1 and IgA2 staining, primary Abs were used as for ELISA (respectively at 6µg/ml and 7.5µg/ml) followed by biotinylated anti-mouse IgG (B8520, dilution 1/3000; Sigma, St Louis, MO, USA). Color was developed by incubation with

0.6 mg/ml diaminobenzidine (SigmaFast DAB, D4293; Sigma, St Louis, MO, USA) in 0.03% H_2O_2 for 10 min. After the reaction was stopped by washing in water, slides were counterstained with Mayer's hemalum.