Cigarette smoke-induced reduction of C1q promotes emphysema

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Supplementary Figures 1-8
The heatmap of differentially regulated complement pathway genes. CD1a⁺ APCs isolated from control and emphysema patients were used to generate the heatmap from GSE26296 microarray dataset (N=6; 3 emphysema and 3 healthy controls). Briefly, lung CD1a⁺ APCs were isolated and RNA samples were collected for the analysis. Genes that fall into the complement cascade (Reactome Id: R-HSA-166658.2) are shown in the complied heatmap.
Reduced C1q in emphysema and correlation with lung function (A) The linear regression was used to determine the correlation between plasma C1q concentration and airway obstruction as measured by forced expiratory volume in 1 sec (FEV1) % (N=108), (B) ratio of FEV1 over forced vital capacity (FVC) (N=108), (C) % emphysema as measured by low attenuation area (%LAA) determined by chest CT quantification (N=48). (D) Concentration of plasma C1q in patients categorized by the GOLD stages (N=8 in GOLD I; N=23 in GOLD II; N=22 in GOLD III&IV). P values were determined by the Mann–Whitney nonparametric test, *P<0.05.
Supplementary Figure 3

**The effect of C1q in CD4+ T cell differentiation in vitro**

(A) Murine splenic CD4+ T cells were differentiated under Treg condition (TGFβ, anti-IL-4, anti-IFN-γ) for three days. Mean fluorescent intensity of PD1 was measured in CD25+Foxp3+ cells (N=3 or 4). Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined by one-way ANOVA test with a Bonferroni’s multiple comparisons.

(B) Naïve human CD4+ T cells were isolated from PBMC and cultured under Treg condition for 10 days. Population of Treg in the culture was identified by flow cytometry staining of CD25 and Foxp3 (N=4 in each group). Results are represented as mean±s.e.m, representative of three independent experiments. P values were determined by the Mann–Whitney nonparametric test, *P<0.05, **P<0.01.
**Supplementary Figure 4**

**Intranasal C1q does not cause lung inflammation**
8-week-old WT naïve mice were treated with intranasal C1q (20ug, twice per week) for three weeks. Expression of *Mmp9* (A) and *Mmp12* (A) in BAL cells was measured by quantitative reverse transcription PCR (qPCR) (normalized to 18S); N=4 in each group. Box: median and interquartile range; whiskers: min to max range, representative of two independent experiments. (C) Cumulative flow cytometry analysis showed the population of IL17+ CD4+ T cells in the lungs. N=4 in each group. Results are represented as mean±s.e.m, representative of two independent experiments. (D) Cumulative flow cytometry analysis showed the population of CD11b+ CD11c+ dendritic cells in the lungs. N=4 in each group. Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined the student t-test.
Increased T cell activation in peripheral lymphoid organs in 5-month-old C1qa−/− mice

Total cells from spleen (A), mesenteric lymph node (B) and peripheral lymph nodes (C) were isolated from 5-month-old WT or C1qa−/− mice (N=4 in each group). Representative of flow cytometry analysis and summarized population of CD4⁺CD62L⁻ and CD8⁺CD62L⁻ cells were shown. Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined the student t-test, *P<0.05.
**Human serum albumin (HSA) has no effect on murine emphysema**

Wild type (WT) mice were exposed to cigarette smoke or air for 6 month. Intranasal HSA was given to smoke-exposed mice (20ug, twice per week) for a total of 6 weeks prior to the sacrifice at 6 months. **(A)** Bronchoalveolar Lavage (BAL) fluid analyses showing macrophages (Mac), neutrophils (Neu), eosinophil (Eos) and lymphocytes (Lymph). N=5 in Air group; N=4 in Smoke and Smoke+HSA. Box: median and interquartile range; whiskers: min to max range, representative of two independent experiments. **(B-C)** Cumulative intracellular cytokine staining of Th17 cells and CD11b⁺CD11c⁺ dendritic cells. N=5 in Air group; N=4 in Smoke and Smoke+HSA. Results are represented as mean±s.e.m, representative of two independent experiments. **(D-F)** Expression of Mmp12, Il6 and Il1β mRNA in BAL cells were measured by quantitative reverse transcription PCR (qPCR) normalized to 18S N=5 in Air group; N=4 in Smoke and Smoke+HSA. Results are represented as mean±s.e.m, representative of two independent experiments. P values were determined by a one-way ANOVA test with a Bonferroni’s multiple comparisons.
RNA-Seq and pathway analysis of C1q treated CD4+ T cells. Human CD4+ T cells were isolated from PBMC and cultured with vehicle (no treatment; NT), C1q, TGFβ with or without C1q for 48 hours. Total RNA was isolated from the cells and subjected to RNA-Seq using standard protocols. Unsupervised Heatmap clustering of 125 genes differentially expressed transcripts in C1q + TGF-β (with p<0.01 and 1.4-fold change) compared to NT, C1q, TGF-β groups. Row Z-Score shown in the upper left area.
Impact of C1q on T cell proliferation and apoptosis

(A) Murine splenic CD4+ T cells were labeled with CFSE and differentiated under Th1 condition (IL-2, IL-12 (p70), anti-IL-4) for three days with or without 20ug/ml C1q. Proliferation was quantified using CFSE dilution (N=3 in Naïve; N=4 in Th1 and Th1+C1q). Results are represented as mean±s.e.m, representative of three independent experiments.

(B) Murine splenic CD4+ T cells were differentiated under Treg condition (TGFβ, anti-IL-4, anti-IFNg) for three days. Expression of caspase3 in CD4+ T cells was measured by flow cytometry (N=5); results are represented as mean±s.e.m, representative of three independent experiments. P values were determined by a one-way ANOVA test with a Bonferroni’s multiple comparisons.