

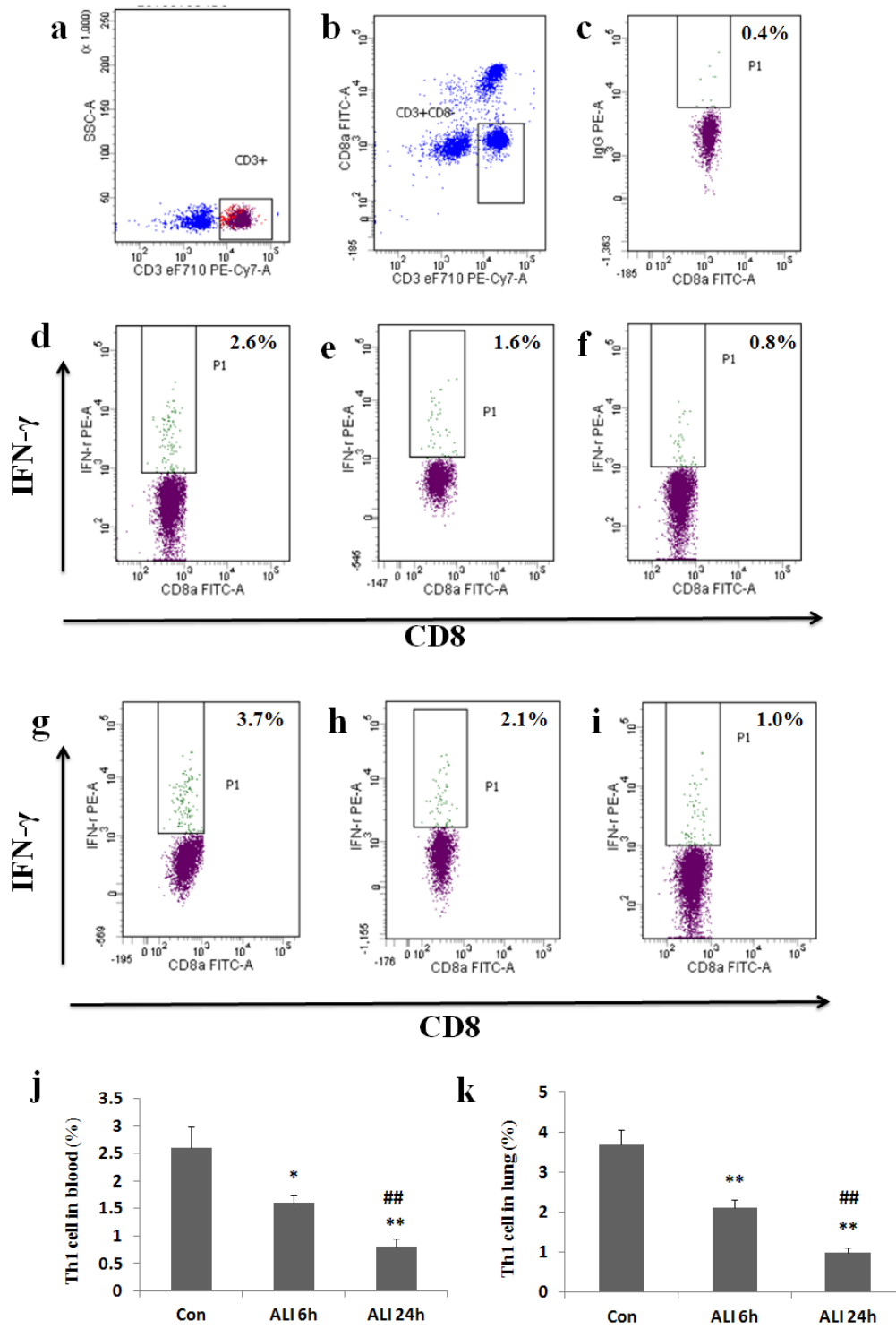
Imbalance of Th17/Tregs in rats with smoke inhalation-induced acute lung injury

Fan Zhang, Mian-yang Li, Ya-ting Lan, Cheng-bin Wang *

Department of Clinical Laboratory Medicine, Chinese People's Liberation Army General Hospital

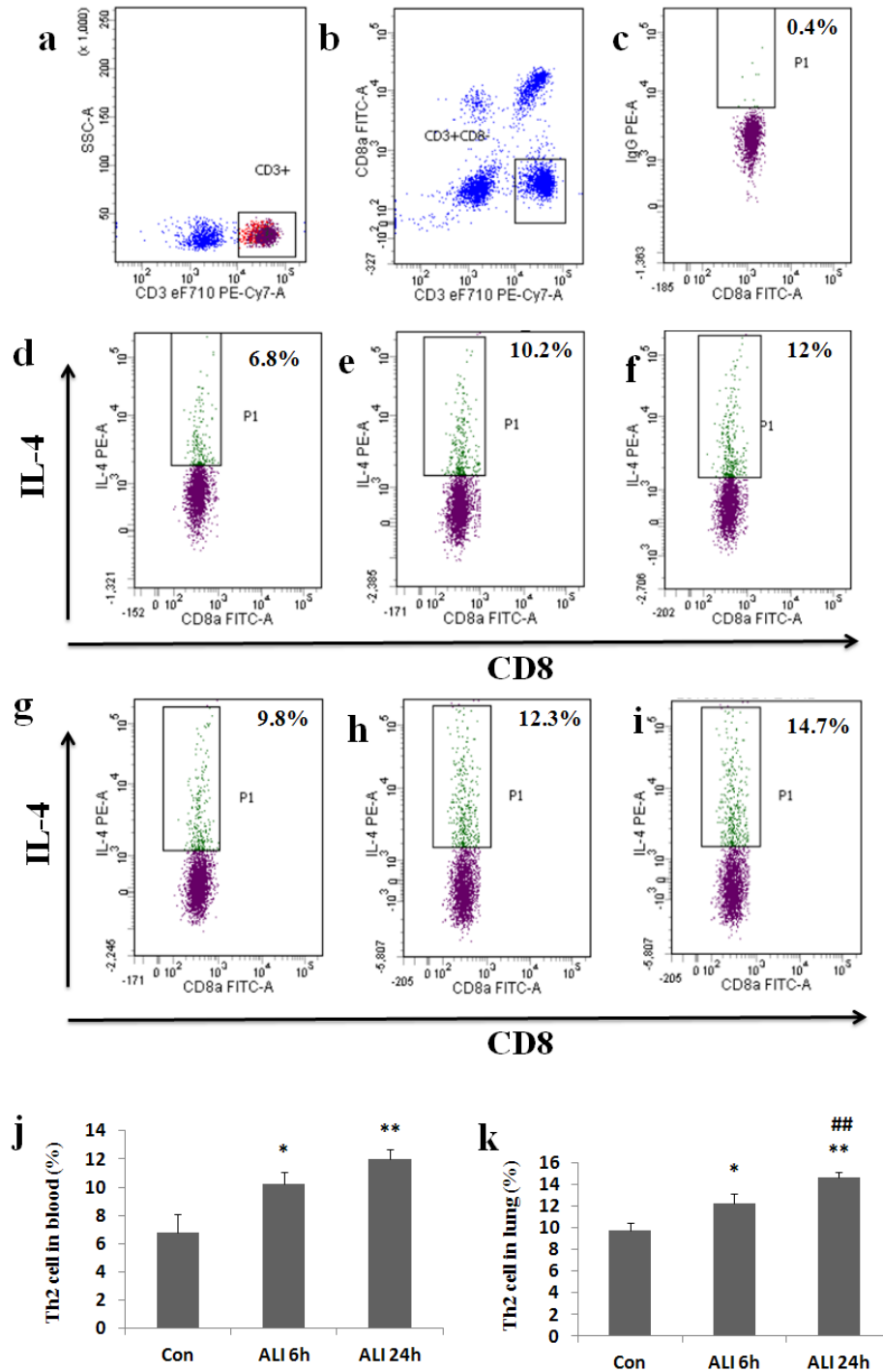
& Postgraduate Medical School, Beijing 100853, People's Republic of China

* Correspondence should be addressed to C.-B.W. (email: wangcb301@126.com)



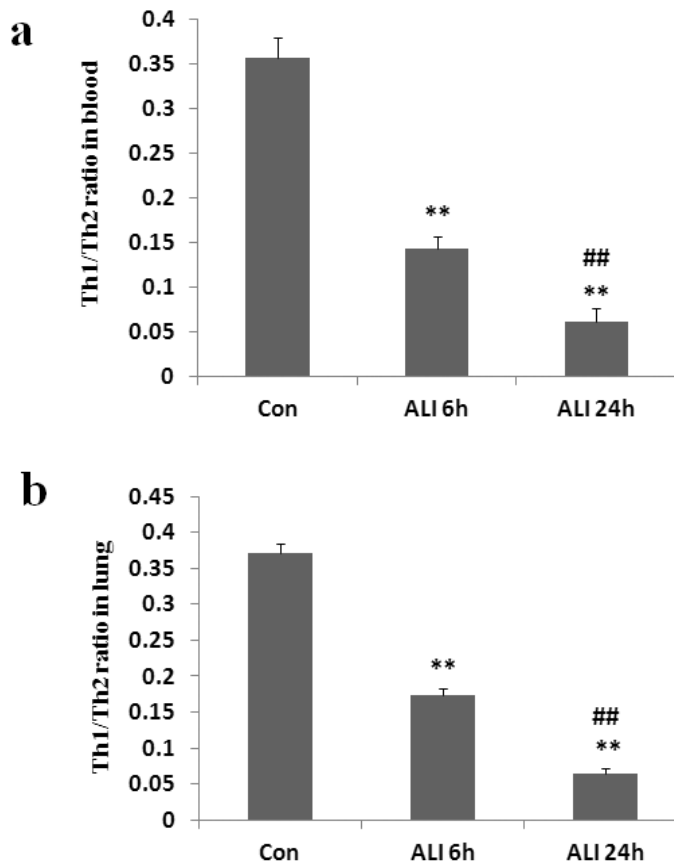
Supplementary Fig. S1. Th1 prevalence decreased in rats with smoke inhalation. Lymphocytes were stimulated with PMA, ionomycin and BFA for 5 h, and then stained with labeled antibodies as described in Materials and methods. **(a)** T lymphocytes were gated by flow cytometry; **(b)** CD4⁺ T subsets were gated by flow cytometry; **(c)** Isotype control; **(d)** IFN- γ expression in CD4⁺

T cells were gated by flow cytometry in Con group in peripheral blood; (e) IFN- γ expression in CD4⁺ T cells were gated by flow cytometry in ALI 6h group in peripheral blood; (f) IFN- γ expression in CD4⁺ T cells were gated by flow cytometry in ALI 24h group in peripheral blood; (g) IFN- γ expression in CD4⁺ T cells were gated by flow cytometry in Con group in lung; (h) IFN- γ expression in CD4⁺ T cells were gated by flow cytometry in ALI 6h group in lung; (i) IFN- γ expression in CD4⁺ T cells were gated by flow cytometry in ALI 24h group in lung; (j) The circulating Th1 cell frequency was presented as mean \pm S.E.M; (k) The Th1 cell frequency in lung was presented as mean \pm S.E.M. Con (normal control group, ambient air inhalation, n=10), ALI 6h (ALI group, smoke inhalation for 6h, n=10), ALI 24h (ALI group, smoke inhalation for 24h, n=10). *P<0.05 and **P<0.01 versus normal control group; #P<0.05 and ##P<0.01 versus ALI 6h group.



Supplementary Fig. S2. Th2 prevalence increased in rats with smoke inhalation. Lymphocytes were stimulated with PMA, ionomycin and BFA for 5 h, and then stained with labeled antibodies as described in Materials and methods. (a) T lymphocytes were gated by flow cytometry; (b) CD4⁺ T subsets were gated by flow cytometry; (c) Isotype control; (d) IL-4 expression in CD4⁺ T

cells were gated by flow cytometry in Con group in peripheral blood; (e) IL-4 expression in CD4⁺ T cells were gated by flow cytometry in ALI 6h group in peripheral blood; (f) IL-4 expression in CD4⁺ T cells were gated by flow cytometry in ALI 24h group in peripheral blood; (g) IL-4 expression in CD4⁺ T cells were gated by flow cytometry in Con group in lung; (h) IL-4 expression in CD4⁺ T cells were gated by flow cytometry in ALI 6h group in lung; (i) IL-4 expression in CD4⁺ T cells were gated by flow cytometry in ALI 24h group in lung; (j) The circulating Th2 cell frequency was presented as mean \pm S.E.M; (k) The Th2 cell frequency in lung was presented as mean \pm S.E.M. Con (normal control group, ambient air inhalation, n=10), ALI 6h (ALI group, smoke inhalation for 6h, n=10), ALI 24h (ALI group, smoke inhalation for 24h, n=10). *P<0.05 and **P<0.01 versus normal control group; #P<0.05 and ##P<0.01 versus ALI 6h group.



Supplementary Fig. S3. The ratio of Th1/Th2. There was a significant decrease in the ratio of Th1/Th2 in peripheral blood and lung from rats with smoke inhalation. **(a)** The ratio of Th1/Th2 in peripheral blood; **(b)** The ratio of Th1/Th2 in lung. Con (normal control group, ambient air inhalation, n=10), ALI 6h (ALI group, smoke inhalation for 6h, n=10), ALI 24h (ALI group, smoke inhalation for 24h, n=10); data are presented as mean \pm S.E.M. *P<0.05 and **P<0.01 versus normal control group; #P<0.05 and ##P<0.01 versus ALI 6h group.

Supplementary Table S1. Th1- and Th2-related cytokines in serum.

Cytokines	Con	ALI 6h	ALI 24h
IFN- γ (pg/ml)	30.0 \pm 2.6	21.0 \pm 4.6*	9.2 \pm 1.4**#
IL-4 (pg/ml)	6.5 \pm 1.6	19.6 \pm 2.5**	67.7 \pm 9.6**##

Con (normal control group, ambient air inhalation, n=10), ALI 6h (ALI group, smoke inhalation for 6h, n=10), ALI 24h (ALI group, smoke inhalation for 24h, n=10); data are presented as mean \pm S.E.M. *P<0.05 and **P<0.01 versus normal control group; #P<0.05 and ##P<0.01 versus ALI 6h group.

[Supplementary Table S2](#).Th1- and Th2-related cytokines in BALF.

Cytokines	Con	ALI 6h	ALI 24h
IFN- γ (pg/ml)	35.0 \pm 4.6	19.3 \pm 3.1**	10.3 \pm 1.6**#
IL-4 (pg/ml)	6.5 \pm 1.6	19.6 \pm 2.5**	67.7 \pm 9.6**##

Con (normal control group, ambient air inhalation, n=10), ALI 6h (ALI group, smoke inhalation for 6h, n=10), ALI 24h (ALI group, smoke inhalation for 24h, n=10); data are presented as mean \pm S.E.M. *P<0.05 and **P<0.01 versus normal control group; #P<0.05 and ##P<0.01 versus ALI 6h group.