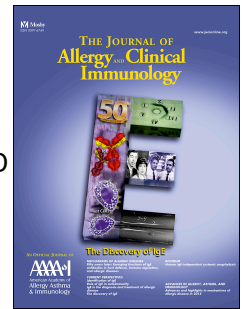


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Impaired Humoral and Cellular Immune Responses to Influenza Vaccination in COPD Patient

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Impaired Humoral and Cellular Immune Responses to Influenza Vaccination in COPD**Patient***Aurélien Parpaleix, PhD^{a*}**Laurent Boyer, MD^{a,b*}**Aurelie Wiedemann, PhD^{c, d}**Christine Lacabaratz, PhD^{c, d}**Laurent Margarit, MS^b**Vincent Enouf, PhD^e**Philippe Le Corvoisier, MD, PhD^f**Anne Lino, MD^b**Ala Covali-Noroc, MD^b**Bruno Housset, MD, PhD^g**Christos Chouaid, MD, PhD^g**Bernard Maitre, MD, PhD^{b,g}**Yves Lévy, MD, PhD^{c, d, h}**Jean-Daniel Lelièvre, MD, PhD^{c, d, h}**Serge Adnot, MD, PhD^{a,b}*

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Short title: Impaired Influenza Vaccine Response in COPD

To the Editor:

Exacerbations are the main sources of hospitalization and mortality in COPD patients. They are related chiefly to lung infections,¹ of which 36% are due to influenza viruses.^{2,3} Exacerbations triggered by viral infections are usually more severe than those related to bacterial infections, with a longer recovery time and higher admission rate.¹ Although influenza vaccination of COPD patients is recommended in many countries, the immune response to the vaccine in this population has not been studied in detail.⁴ Besides humoral response, cellular responses mediated by CD8⁺ and CD4⁺ T cells are now recognized as crucial for the protection against influenza virus during infection, and interferon- γ (IFN γ) production by T cells in response to vaccination may be the most reliable marker for influenza outcomes and live attenuated vaccine-induced protection.⁵⁻⁷ Therefore, we performed a study to explore B and T-cell phenotypes, serum titers of neutralizing anti-hemagglutinin antibodies response and in vitro T-cell recall responses using the whole vaccine in COPD patients and controls without COPD (details are described in this article's methods section in the Online Repository at www.jacionline.org).

We include 15 patients with clinically stable COPD and 15 subjects without COPD (clinical characteristics are given in **Table E1**, and the Methods section in this article's Online Repository at www.jacionline.org). The study consisted of two clinical visits (days 0 and 30). Each participant received a single dose of the 2013 trivalent inactivated influenza vaccine (Vaxigrip[®] 2013) by deep subcutaneous injection on day 0. Blood samples were obtained at baseline and at day30.

Compared to controls, patients with COPD had higher percentages of naive B cells (CD21⁺CD27⁻), and lower percentages of resting memory B cells (CD21⁺CD27⁺) at baseline

(**Table 1**). The activated memory (CD21⁺CD27⁺) and exhausted (CD21⁺CD27⁻) B-cell subsets were not different between the two groups. B cells from COPD patients expressed larger amounts of surface IgD and IgM than those from controls. Marginal zone B cells (CD27⁺, IgD⁺), which are involved in producing plasma cells and antibodies independently from T-cell responses, were not significantly different between the two groups. Switched memory B cell (CD27⁺, IgD⁻) counts were lower in the patients than in the controls. We found no differences in circulating Tfh cells between the patients and controls. The patients and controls showed no differences in the distribution of CD4⁺T cells subsets (**Table 1**). Neither were the groups different for the CD8⁺ T-cell subsets or CD8⁺ T-cell cytotoxicity assessed based on perforin and granzyme B levels.

Regarding humoral response to vaccination, influenza vaccine-specific antibody levels expressed as geometric mean titers (GMT) of hemagglutination-inhibiting antibodies to all three influenza strains were significantly higher 30 days after vaccination than before vaccination in the controls (**Fig 1A** and see **Fig E1** in this article's Online Repository at www.jacionline.org). In contrast, antibody titers to two strains, A/California/7/2009 and A/Texas/50/2012, did not increase significantly between days 0 and 30 in COPD group. The level of A/Texas/07/2009 was higher in COPD patients compared to controls (3.13 +/- 1.95 versus 1.77 +/- 1.18, p=0.022). The percentage of controls and patients who experienced seroconversion was 30.8% vs. 14.3% for A/California/7/2009 (NS), 46.2% vs. 28.6% for A/Texas/50/2012 (NS), and 21.4% vs. 23.1% for B/Massachusetts/02/2012 (NS), respectively.

Next, we used flow cytometry to assess the T-cell cytokine production following *in vitro* whole vaccine stimulation (for 5 days and 6 hours respectively). These tests were

performed before vaccination. We determined the percentage of living CD3⁺ T cells that were CD4⁺ and expressed IFN γ , IL-2, or TNF α (**Fig 1B**). After 5 days of vaccine stimulation, we found a lower percentage of CD4⁺ T cells expressing IFN γ among cells from patients than controls (**Fig 1B**). No difference was observed between smokers and non-smokers regarding IFN-gamma CD4⁺ response in the control group. In contrast, the percentages of CD4⁺ T cells expressing IL-2⁺ or TNF α were not different between the two groups. Moreover, no difference was observed regarding the percentage of CD8⁺ T cells expressing IFN γ , IL2 or TNF α between the two groups in response to the whole vaccine (data not shown). To confirm this result, we performed a Luminex assay to assess cytokine production in culture medium of T cells subjected to whole vaccine stimulation. Of the 29 cytokines assayed, only IFN γ was found in lower levels in media from COPD patients compared to controls (**Fig 1B**). No differences were observed after 6 hours of vaccine stimulation (**data not shown**). To assess whether this response was also altered in response to other virus, we stimulated T cells with cytomegalovirus. The percentages of CD4⁺ T cells producing IFN γ , IL-2, or TNF α were not significantly different between the two groups (see **Fig E2** in this article's Online Repository at www.jacionline.org). We also examined the production of cytokines by CD8⁺ T cells subjected to *in vitro* whole vaccine stimulation but found no difference between patients with COPD and controls (**data not shown**). In addition, we assessed the proliferation of CD4⁺ and CD8⁺ T cells in response to the vaccine, using CFSE dilution. No difference was found between cells from patients and controls, which also exhibited similar telomere length (see **Fig E3, A and B**, in this article's Online Repository at www.jacionline.org).

We looked for associations linking lung characteristics of the participants to vaccine response markers (**Table E2**). IFN γ (pg/mL) production after Vaxigrip stimulation correlated positively with DL_{CO} and negatively with CT emphysema score. Among hemagglutination-inhibiting antibodies to the three influenza strains, only Delta A/California GMT was positively correlated with DL_{CO}.

Our findings show that both the humoral and the cellular responses to influenza vaccination are impaired in patients with COPD, with a blunted antibody response and decreased IFN γ production by CD4⁺ T cells. This is the first evidence of impaired IFN γ production by CD4⁺ T cells in response to influenza vaccination in COPD patients, suggesting an impaired ability of COPD patients to develop cell-mediated immunization against influenza. Therefore, to protect COPD patients from influenza, efforts should focus specifically on developing vaccines that boost both the humoral and the cell-mediated responses.

Whether this decline of immune response in COPD patients is ascribable to a process of immunosenescence as with advancing age remains to be determined.⁸ Immune function in patients with COPD shares several of the characteristics of immunosenescence such as increased susceptibility to infections and altered humoral and cellular responses to vaccination.⁸ However, the increase of naive B-cells and the decrease of memory B-cells and switched B cells in COPD patients, compared to same-age controls without COPD, does not support a defect in B-cell regeneration in COPD. Interestingly, this B cells abnormality resembles more the impairments seen in patients with common variable immunodeficiency, than in the elderly.⁹

FIGURE LEGENDS

Fig 1: A, Geometric mean titers (GMT) of hemagglutination-inhibiting antibodies to A/California/07/2009, A/Texas/07/2009, and B/Massachusetts/2/2012 viral strains in each COPD patients and controls before (D0) and after (D30) vaccination. **B,** Gating strategy, analysis of IFN γ ⁺, IL-2⁺ and TNF α ⁺ CD4⁺ T cells among PBMCs; Luminex assay of IFN γ , IL-2, and TNF α (pg/mL) in supernatants of cultured PBMCs stimulated *in vitro* for 5 days by the whole influenza vaccine (150 ng/mL) in COPD patients and controls. * P <0.05; ** P <0.01. NS: not significant.

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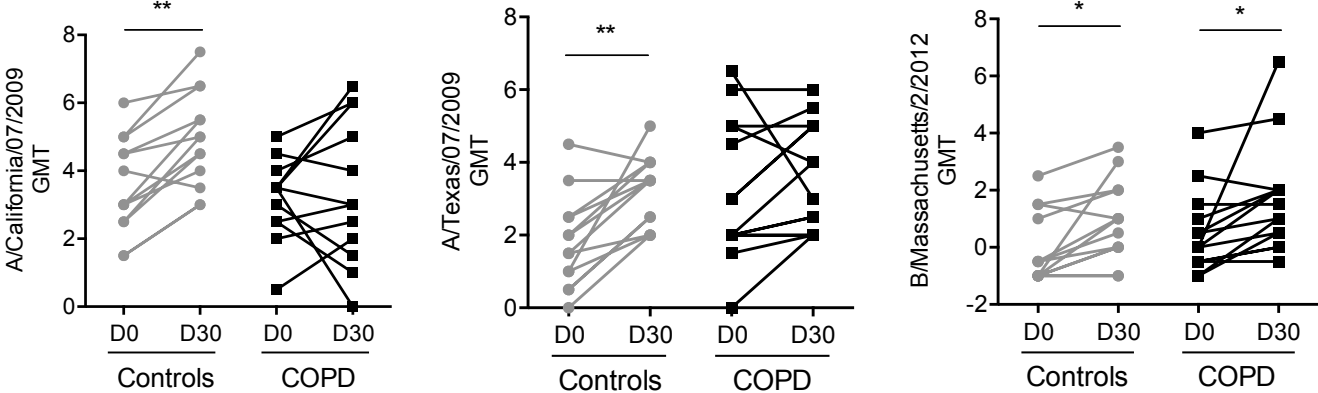
Table 1: B and T cells subsets in participants with COPD and in controls before vaccination.

Parameters	Control	COPD	p value *
<u>B cells</u>			
Naïve B cells, %	52.7 (47.2 - 59.0)	66.9 (65.2 - 79.0)	0.018
Resting memory B cells, %	40.1 (32.4 - 44.5)	28.2 (15.3 - 31.0)	0.036
Activated memory B cells, %	3.41 (2.87 - 5.31)	3.62 (1.66 - 4.23)	0.454
Exhausted B cells, %	2.53 (1.47 - 3.50)	1.95 (1.26 - 2.93)	0.704
Marginal zone B cells, %	8.81 (5.93 - 12.5)	4.44 (3.63 - 8.93)	0.170
Switched B cells, %	36.4 (29.0 - 40.2)	24.4 (16.3 - 29.7)	0.029
CD4 ⁺ CXCR5 ⁺ CCR7 ^{lo} PD1 ^{hi} T cells, %	9.00 (7.54 - 16.1)	10.5 (8.96 - 13.0)	0.282
CD4 ⁺ CXCR5 ⁺ ICOS ⁺ PD1 ^{hi} T cells, %	0.81 (0.23 - 1.38)	0.47 (0.24 - 0.81)	0.560
<u>Circulating lymphocytes</u>	1.79 (1.10 - 3.30)	1.83 (1.20 - 3.80)	0.883
<u>CD4+ T cells</u>			
Naïve CD4 ⁺ T cells, %	40.3 (27.4 - 52.6)	49.7 (37.1 - 58.2)	0.198
Central memory CD4 ⁺ T cells, %	48.7 (33.2 - 58.3)	35.7 (27.4 - 45.3)	0.229
Effective memory CD4 ⁺ T cells, %	9.31 (5.87 - 22.2)	7.79 (4.89 - 18.2)	0.363
Exhausted CD4 ⁺ T cells, %	1.93 (1.39 - 3.01)	2.86 (1.97 - 3.99)	0.184
PD1 ⁺ CD4 ⁺ T cells, %	31.9 (18.4 - 38.3)	25.1 (16.5 - 36.2)	0.467
<u>CD8+ T cells</u>			
Naïve CD8 ⁺ T cells, %	43.7 (12.3 - 58.1)	39.9 (20.6 - 57.0)	0.705
Central memory CD8 ⁺ T cells, %	26.3 (17.1 - 49.8)	27.2 (21.7 - 47)	0.735
Effective memory CD8 ⁺ T cells, %	7.46 (4.88 - 10.7)	7.79 (4.8 - 13.1)	0.588
Exhausted CD8 ⁺ T cells, %	15.7 (6.7 - 27.6)	12.9 (3.7 - 37.8)	0.704
PD1 ⁺ CD8 ⁺ T cells, %	34.1 (28.4 - 46.0)	29.3 (21.0 - 37.4)	0.118
<u>Cytotoxic capacity</u>			
Gz B ⁺ CD8 ⁺ T cells, %	32.1 (13.4 - 39.9)	21.2 (6.1 - 47.2)	0.560
Pfp ⁺ CD8 ⁺ T cells, %	10.9 (4.1 - 26.0)	10.93 (2.1 - 24.6)	0.533

Bold type indicates statistically significant differences defined as p<0.05.

Fig 1

A



B

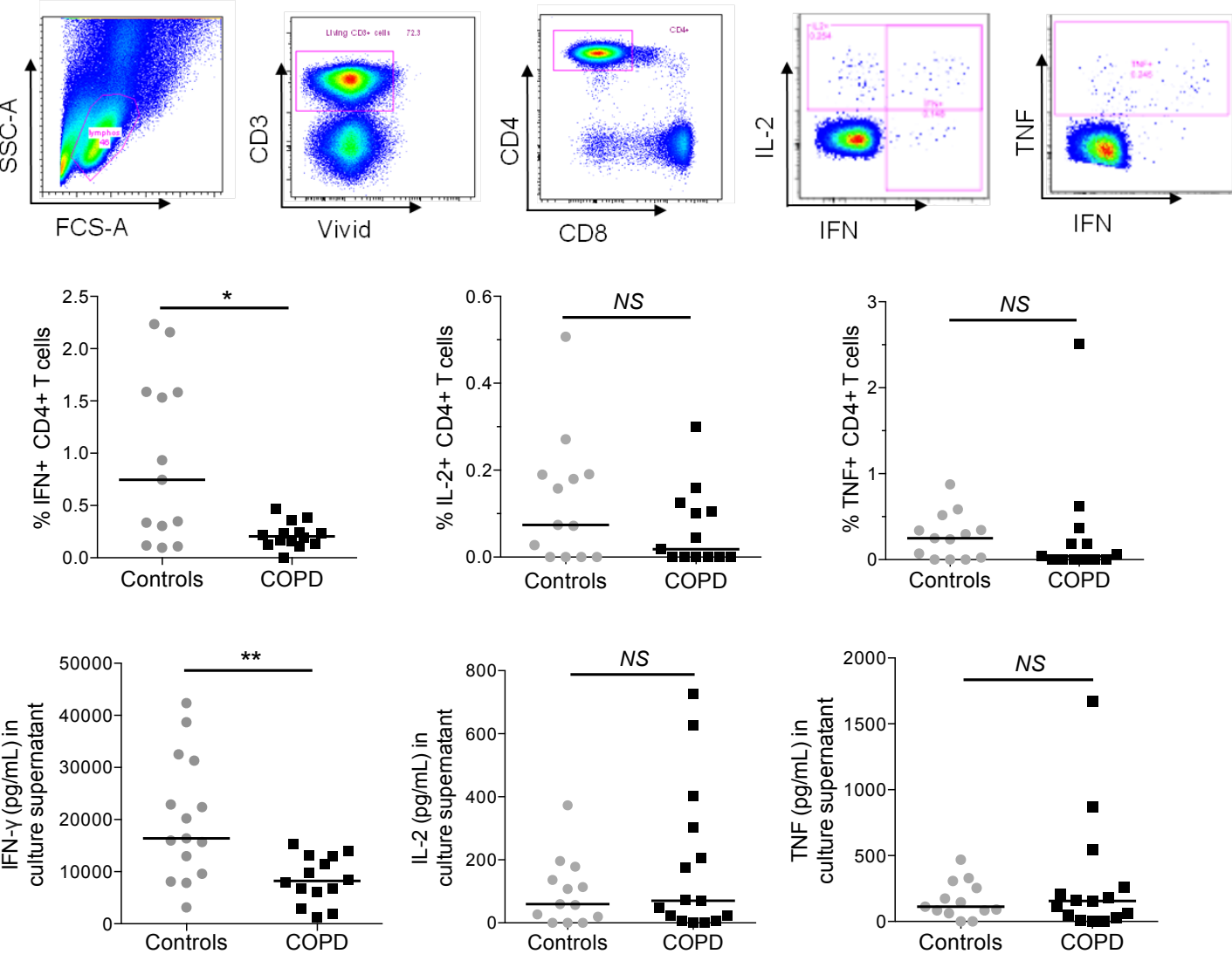


Table E1: Main features in participants with chronic obstructive pulmonary disease and in controls

	COPD n=15	Controls n=15	P value†
Males/Females	10/5	11/4	
Age, years	62.0 ± 1.1	62.4 ± 1.2	0.71
Pack-years	42.3 ± 4.0	22.0 ± 6.1	0.017
Smoker/Non-smoker	15/0	8/7	
BMI, kg/m ²	24.5 ± 2.0	26.4 ± 1.7	0.41
FEV ₁ , % predicted	57 ± 6.2	99.1 ± 4.6	<0.0001
FVC, % predicted	83.6 ± 4.5	100.5 ± 4.3	0.003
FEV ₁ /FVC, % predicted	53.8 ± 4.1	78.6 ± 1.6	<0.0001
DL _{CO} , % predicted	52.1 ± 4.5	86.2 ± 4.2	<0.0001
Exacerbations*	1.5 ± 0.4	0 ± 0	
CRP, mg/L	2.7 ± 1	1.4 ± 0.2	0.45

Definition of abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide; CRP, C-reactive protein

Data are mean ± SEM.

* Number of exacerbations during the 6 months following vaccination.

†P value by nonparametric Mann Whitney test.

Table E2. Correlation coefficient matrix between humoral, cellular vaccine response and lung function parameters.

Parameters	FEV1, % predicted	DLCO, % predicted	Emphysema score AU
Hemagglutination-inhibiting ab			
Delta A/California 07/2009 GMT	0,342	0,489	-0,219
Delta A/Texas/50/2012 GMT	0,112	0,243	-0,300
Delta B/Massachusetts/2/2012 GMT	0,209	0,160	0,155
In vitro IFNγ production by T cells			
IFN γ ⁺ CD4 ⁺ T cells, % (D0)	-0,028	0,267	-0,363
IFN γ (pg/mL) after Vaxigrip stimulation	0,247	0,574	-0,564

Definition of abbreviations: FEV1%, forced expiratory volume in 1 second as the % of the predicted value; DLCO, diffusing capacity of the lung for carbon monoxide; AU, arbitrary units, Bold type indicates statistically, significant differences defined as $p < 0.05$

Fig E1

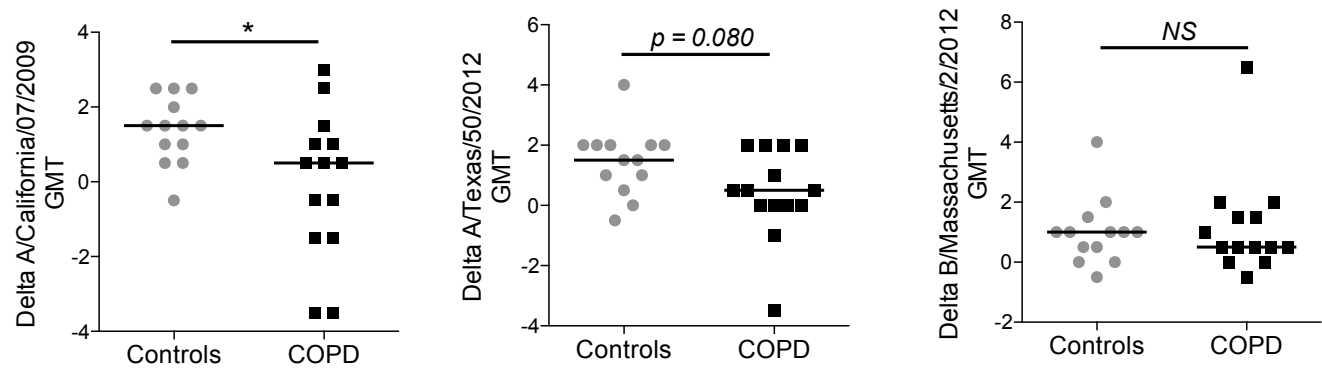
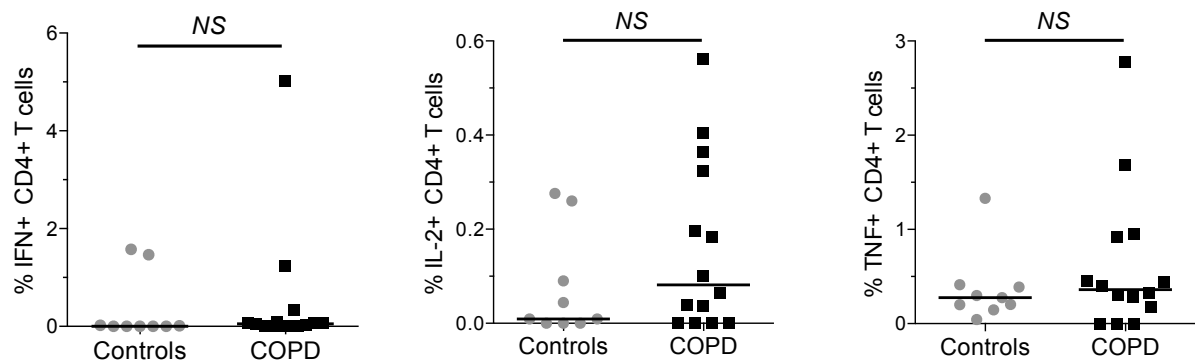


Fig E2

A



B

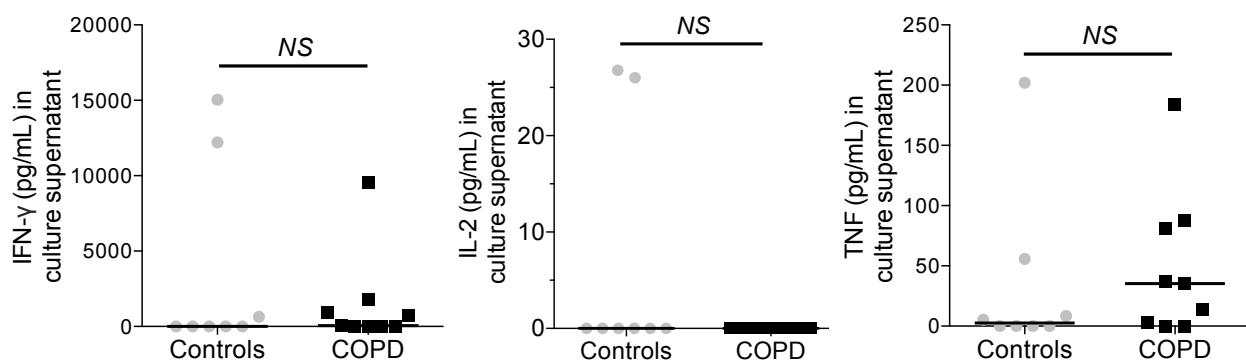
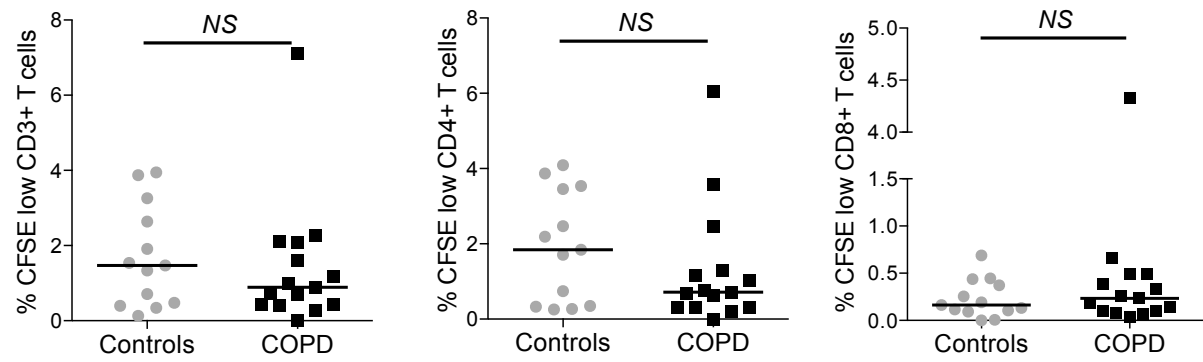


Fig E2

A



B

