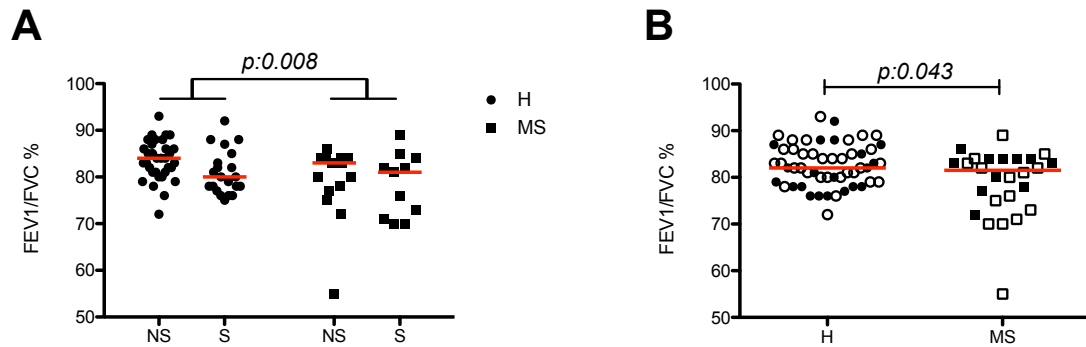


T-cell activation and HLA-regulated response to smoking in the deep airways of patients with multiple sclerosis

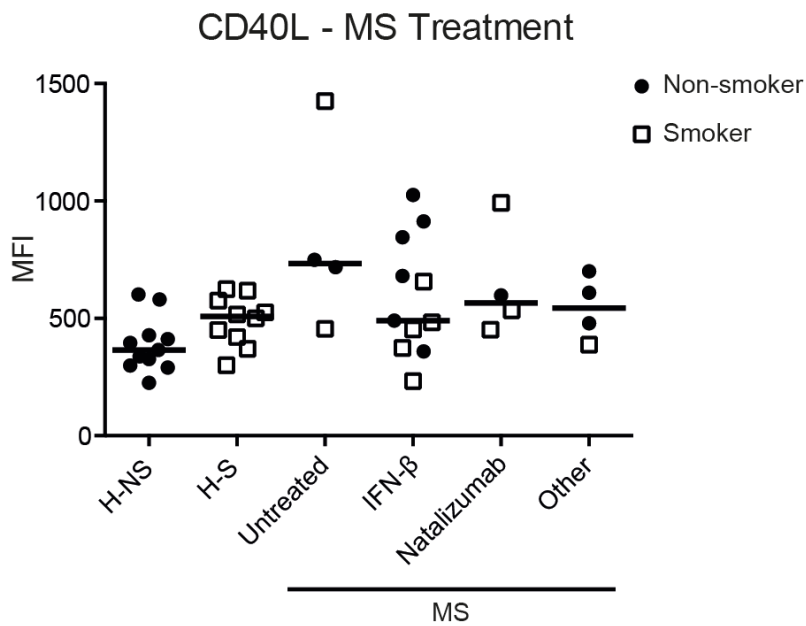
Öckinger et al

Supplementary material



Supplementary Figure 1

FEV1/FVC ratio is reduced in MS-patients, independently of smoke status. A) Regression analysis demonstrates that FEV1/FVC % is associated with diagnosis, but not smoke status (see Supplementary Table 1). B: Analysis of FEV1/FVC in healthy controls and MS-patients, statistics calculated using Mann Whitey. Open symbols indicates non-smokers, filled symbols indicates smokers (B only). Horizontal bars indicates median. H: healthy controls, MS: multiple sclerosis patients, NS: non-smokers, S: smokers.



Supplementary Figure 2

pCD40L is not significantly regulated by current disease modifying treatment (DMT). Median fluorescence intensity of intracellular pCD40L in CD4⁺ T-cells in BALF, measured by flow cytometry. H: healthy volunteers, MS: patients diagnosed with MS. NS: non-smokers, S: smokers. Treatments in MS: Untreated: no current DMT. IFN- β : Interferon- β (Avonex (n=9) or Betaferon (n=2)), Natalizumab: Tysabri, Other: Glatiramer (n=1), Rituximab (n=1), Fingolimod (n=2). Horizontal lines indicate median, filled circles indicates non-smokers, and open boxes indicates smokers. Statistics calculated using Kruskal-Wallis test with Dunn's post-test.

Outcome	Model		Intercept		Diagnosis (MS)		Smoking status (S)		Age (y)	
	Adj. R ²	p	Estim. (SE)	p	Estim. (SE)	p	Estim. (SE)	p	Estim. (SE)	p
Recovery %	0.146	1.65e-03	78.12 (4.44)	< 2e-16	-4.91 (3.33)	0.145	-5.17 (5.56)	0.047	-0.26 (0.15)	0.089
Total cell yield	0.318	3.80e-07	-4.29 (9.04)	0.637	-7.26 (6.79)	0.288	31.55 (5.22)	4.96e-08	0.72 (0.30)	0.019
Cell concentration	0.364	2.77e-08	-43.81 (54.87)	0.427	-32.99 (41.17)	0.426	206.97 (31.65)	6.10e-09	4.98 (1.84)	8.35e-03
Macrophage %	0.218	6.47e-05	95.37 (2.40)	< 2e-16	1.79 (1.97)	0.367	6.08 (1.51)	1.35e-04	-0.23 (0.09)	0.012
Macrophages x 10⁶/L	0.371	2.30e-08	36.38 (47.29)	0.444	22.35 (35.26)	0.528	179.89 (26.55)	2.31e-09	1.49 (1.61)	0.357
Lymphocytes %	0.205	1.19e-04	4.44 (2.21)	0.048	-1.51 (1.81)	0.41	-5.48 (1.39)	1.81e-04	0.19 (0.08)	0.018
Lymphocytes x 10⁶/L	0.197	1.69e-04	-6.16 (3.75)	0.104	-4.02 (2.81)	0.157	-0.47 (2.16)	0.827	0.56 (0.13)	2.69e-05
FVC % predicted		ns								
FEV1 % predicted		ns								
FEV1/FVC	0.126	6.79e-03	88.96 (2.79)	< 2e-16	-11.19 (5.24)	0.0360	-1.69 (1.28)	0.191	-0.21 (0.10)	0.0356
CD4 (%)	0.103	0.029	50.15 (6.93)	1.55e-09	-2.08 (5.06)	0.682	-9.11 (3.85)	0.022	0.37 (0.23)	0.107
CD8 (%)	0.10	0.033	35.61 (6.32)	6.38e-07	-0.007 (4.62)	0.999	9.23 (3.52)	0.011	-0.22 (0.21)	0.284
CD4/CD8		ns								
CD4+ Ki-67+ (%)	0.206	8.10e-03	6.51 (2.77)	0.023	-0.44 (1.71)	0.799	5.65 (1.54)	7.52e-04	0.02 (0.07)	0.793
CD8+ Ki-67+ (%)	0.114	0.050	4.76 (2.76)	0.092	-0.31 (1.71)	0.857	4.36 (1.53)	7.13e-03	0.06 (0.08)	0.395
CD4+ CD40L (MFI)	0.186	0.011	329.72 (93.91)	1.14e-03	119.55 (59.37)	0.049	-31.98 (52.84)	0.548	4.178 (2.73)	0.133

Supplementary Table 1.

Summary of regression analysis on BAL parameters and the major cell populations, including subpopulations and phenotypes of T-cells in BALF, analyzing the influence of smoking status, diagnosis and age independently. Healthy non-smokers was used as reference Gender was not significantly associated with any of the outcomes, and no significant interaction between variables was seen. Non-significant models are not included in the table.. Adj: adjusted, SE: standard error of the mean, MS: multiple sclerosis, S: smoker, ns: not significant

Characteristics	Current study		Previous studies		Extended combined cohort	
	Non-Smokers	Smokers	Non-Smokers	Smokers	Non-Smokers	Smokers
Total number	34	21	63	7	97	28
Gender (M/F)	16/18	6/15	29/34	3/4	46/52	8/19
Age (years)	23.5 (20-57)	25 (19-50)	24 (18-54)	38 (25-56) ^a	24 (18-57)	28 (19-56) [#]
FVC % predicted	105.5 (86-128)	107 (84-127)	111 (85-132)	114 (89-128)	108.5 (85-128)	108 (84-128)
FEV1 % predicted	103.5 (82-122)	103 (80-121)	107 (73-130)	106 (90-119)	106 (73-130)	103 (80-121)
FEV1/FVC %	84 (72-93)	80 (75-92)	82.5 (69-96)	82 (79-86)	83 (69-96)	79.5 (75-92)
Recovery (%)	74 (41-84)	70 (25-77)	68 (31-86)	60 (52-70)	71 (31-86)	67 (25-77) [#]
Total cell yield (x 10⁶)	15.3 (7.7-29.0)	39.8 (15.9-154.0) ^b	13.3 (4.4-31.5)	17.7 (8.8-36.8) ^a	14.1 (4.4-31.5)	30.1 (8.8-154.0) ^{###}
Cell concentration (x 10⁶ /L)	76.4 (14.3-279.5)	257.8 (90.3-987.2) ^b	81.3 (28.7-159.0)	105.6 (50.7-283.1)	83.0 (14.3-279.5)	242.3 (50.7-987.2) ^{###}
Macrophages %	91.7 (61.9-96.6)	96.2 (61.4-99.0) ^b	88.0 (61.0-97.2)	93.6 (83.2-95.8)	89.0 (61.0-97.2)	95.8 (83.2-99.0) ^{###}
Macrophages x 10⁶ /L	70.9 (12.7-261.6)	255.2 (83.1-949.7) ^b	70.9 (25.6-126.4)	92.5 (47.9-245.2)	70.9 (12.7-261.6)	232.1 (47.9-949.7) ^{###}
Lymphocytes %	6.0 (2.4-31.6)	2.2 (0.4-7.8) ^b	9.8 (2.3-37.8)	5.8 (1.6-13.0)	9.2 (2.3-37.8)	2.6 (0.4-13.0) ^{###}
Lymphocytes x 10⁶ /L	5.5 (1.5-50.3)	6.5 (1.0-29.6)	7.3 (0.9-46.0)	5.4 (2.5-16.4)	(1.5-50.3)	6.5 (1.0-29.6)
Neutrophils %	1.2 (0.2-6.4)	0.6 (0.2-2.8)	1.4 (0.0-14.8)	0.6 (0.0-6.8)	1.2 (0.2-6.4)	0.6 (0.2-2.8) [#]
Neutrophils x 10⁶ /L	1.0 (0.1-11.2)	1.5 (0.4-9.2)	1.1 (0.0-16.3)	1.5 (0.0-19.3)	1.0 (0.1-11.2)	1.5 (0.4-9.2)
Eosinophils %	0.0 (0.0-2.3)	0.0 (0.0-1.4)	0.0 (0.0-3.7)	0.2 (0.0-1.2)	0.0 (0.0-3.7)	0.0 (0.0-1.4)
Eosinophils x 10⁶ /L	0.0 (0.0-4.7)	0.0 (0.0-3.9)	0.0 (0.0-2.7)	0.5 (0.0-2.3)	0.0 (0.0-4.7)	0.0 (0.0-3.9)
Basophils %	0.0 (0.0-0.4)	0.0 (0.0-0.2)	0.0 (0.0-2.0)	n.d.	0.0 (0.0-2.0)	0.0 (0.0-0.2)
Basophils x 10⁶ /L	0.0 (0.0-0.4)	0.0 (0.0-0.8)	0.0 (0.0-1.5)	n.d.	0.0 (0.0-1.5)	0.0 (0.0-0.8)
Mast cells/10 fields of vision¹	1 (0-10)	1 (0-6)	0 (0-21)	1 (0-5)	1 (0-10)	1 (0-6)
CD4/CD8 ratio	2.13 (0.71-6.41)	1.13 (0.30-3.81) ^c	2.70 (0.90-11.30)	2.95 (1.30-3.50)	2.30 (0.70-11.30)	1.40 (0.30-3.80) ^{##}

Supplementary Table 2.

Descriptive statistics of healthy controls, recruited for this study, data retrieved from in house database, and the combined extended cohort. Numbers indicate median and range if not otherwise stated.

Statistics calculated using Kruskal-Wallis with Dunn's post test: ^ap < 0.05 compared to non-smokers, from previous studies,

^bp < 0.001 compared to non-smokers, in current study, ^cp < 0.05 compared to non-smokers, in current study

Statistics calculated with Mann-Whitney U test, in the extended combined cohort: # p < 0.05 compared to non-smokers, ## p < 0.01 compared to non-smokers, ### p < 0.001 compared to non-smokers. ¹ calculated in 16x magnification. n.d: not detected