Targeting IL-5 pathway against airway hyperresponsiveness: a challenge between benralizumab and mepolizumab

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Online data supplement

e-Table 1. Main characteristics of donors and normal ranges in agreement with GINA recommendations (GINA, 2020).

Variables	Values	Normal range
Gender (male/female)	8/8	/
Age (years)	50.0±3.0	/
Height (cm)	164.8±2.0	/
Weight (Kg)	68.3±3.0	/
Smoking status:		
current	4	/
former	5	/
never	7	/
IgE	55.8±5.7	<100
Pack years	24.4±5.6	/
FEV_1 (L)	2.71±0.07	/
FEV_1 (% predicted)	93.1±2.4	>80
FEV_1 reversibility (%)	4.8±1.3	<12%
FVC (L)	3.34±0.09	/
FEV_1/FVC	0.81±0.01	>0.7

FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GINA: Global Initiative for Asthma; IgE: immunoglobulin E; IU: international units.

e-Table 2. Dataset of sectional tissues used in this study.

Treatment	Concentraton	Contractile stimuli					Total Stimuli	n bronchial ring each stimulus	Total bornchial rings
		CRC to histamine	Plateau to histamine ($EC_{50-70-90}$)	FRC to EFS	Specific EFS ($EF_{50-70-90}$)	QS			
C-	/	1	1	1	1	1	5	5	25
C+	/	1	1	1	1	1	5	5	25
Benralizumab	0.1 µg/ml	0	1	0	0	0	1	5	5
Benralizumab	0.3 µg/ml	0	1	0	0	0	1	5	5
Benralizumab	1 µg/ml	1	1	1	1	1	5	5	25
Benralizumab	3 µg/ml	1	1	1	1	1	5	5	25
Benralizumab	10 µg/ml	1	1	1	1	1	5	5	25
Benralizumab	30 µg/ml	1	1	1	1	1	5	5	25
Benralizumab	100 µg/ml	1	1	1	1	1	5	5	25
Mepolizumab	0.1 µg/ml	0	1	0	0	0	1	5	5
Mepolizumab	0.3 µg/ml	0	1	0	0	0	1	5	5
Mepolizumab	1 µg/ml	1	1	1	1	1	5	5	25
Mepolizumab	3 µg/ml	1	1	1	1	1	5	5	25
Mepolizumab	10 µg/ml	1	1	1	1	1	5	5	25
Mepolizumab	30 µg/ml	1	1	1	1	1	5	5	25
Mepolizumab	100 µg/ml	1	1	1	1	1	5	5	25
								Time controls	46
								Total	366

Results

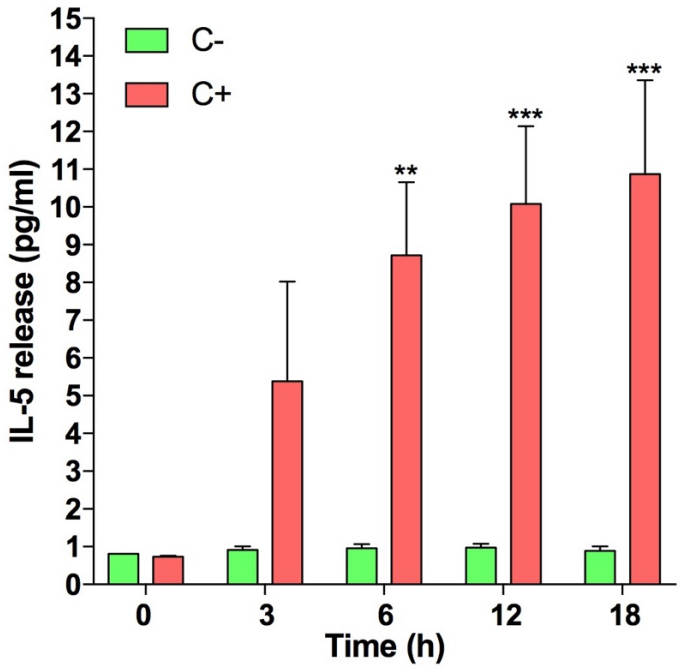
Baseline characteristics of isolated airways

Independently by the smoking habit of donors, there were no significant differences (P>0.05) between the wet weight of the human isolated bronchial rings used in the different treatments arms (C- 87.50±7.01 mg; C+ 92.50±7.91 mg; benralizumab 90.50±7.63 mg; mepolizumab 86.75±7.92 mg).

The E_{max} in C- specimens stimulated by histamine, EFS, and QS was 1857.00±171.60 mg/100 mg bronchial tissue, 458.90±24.28 mg/100 mg bronchial tissue, and 291.52±90.27 mg/100 mg bronchial tissue, respectively. Passive sensitization induced AHR in C+ airways, leading to significant overall increase in ASM contractile response to histamine (+227.85±24.23 % vs. C-, P<0.001), EFS (+117.45±12.63 % vs. C-, P<0.001), and QS (+113.58±17.39 % vs. C-, P<0.05).

In C+ airways the EC_{50}, EC_{70}, and EC_{90} to histamine were 0.8 μM, 2.2 μM, and 28.0 μM, respectively; the EF_{50}, EF_{70}, and EF_{90} to EFS were 14.1 Hz, 20.9 Hz, and 32.8 Hz, respectively.

The levels of IL-5 detectable in the supernatant increased after 3 h incubation of bronchial rings with sensitizing serum. The concentrations of IL-5 released by C+ bronchi were significantly greater (P<0.01) than those release by C- airways between 6 h of incubation with sensitizing and non-sensitizing sera, respectively (e-Figure 1). After 18 h of incubation, the maximal concentration of IL-5 in the supernatant was 12.23±2.80 fold greater in C+ than in C- tissues (P<0.001). The quantification of IL-5 released by bronchial tissue and the data reported in e-Figure 1 have been calculated by detracting the background amount of IL-5 provided by the sensitizing serum.



e-Figure 1. Levels of IL-5 detectable in the supernatant of C- and C+ during the sensitizing procedure. ** P<0.01 and *** P<0.001 vs. C- (statistical analysis assessed by two-way ANOVA); bars represent the mean±SEM of n=5 bronchial tissue from different subjects. C+: positive control, isolated bronchi incubated with sensitizing serum; C-: negative control, isolated bronchi incubated with non-sensitizing; IL-5: interleukin 5.

e-Table 3. Effect of overnight incubation with different concentrations of benralizumab and mepolizumab on the FRCs to EFS in passively sensitized bronchi.

	C-	C+	Benralizumab					Mepolizumab				
			1 µg/ml	3 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml	1 µg/ml	3 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml
EFS E_{max} (mg/100mg bronchial tissue)	197.60±29.86	458.90±24.28 ###	452.00±43.83	315.10±26.76 **	255.00±33.32 ***	211.50±15.48 ***	182.30±43.64 ***	402.90±71.97	404.40±49.58	294.19±62.61 *	196.10±34.03 ***	191.90±47.28 ***
EFS pEF_{50}	1.21±0.11	1.15±0.03	1.09±0.07	1.00±0.12	0.93±0.18	1.12±0.05	1.19±0.17	1.08±0.13	1.14±0.10	1.09±0.18	1.27±0.11	1.20±0.19

P<0.001 vs. C- (statistical analysis assessed by Student's t-test); *P<0.05, * P<0.05, ** P<0.01, and *** P<0.001 vs. C+ (statistical analysis assessed by one-way ANOVA); data represent the mean±SEM of n=5 bronchial tissue from different subjects. C+: positive control, passively sensitized bronchi; C-: negative control, non-sensitized bronchi; EFS: electrical field stimulation; FRC: frequency-response curve; EF_{50}: frequency inducing 50% E_{max}; E_{max}: maximal effect; pEF_{50}: negative logarithm of EF_{50}.

e-Table 4. Efficacy and potency of benralizumab and mepolizumab after overnight incubation on the AHR to different EFS frequencies (EF_{50-90}) in passively sensitized bronchi. The pharmacological analysis was performed by assessing E_{max} as the difference in airway contractility between passively sensitized and non-sensitized bronchi.

	Benralizumab			**Mepolizumab**		
Contractile tone to EFS at:	EF_{50}	EF_{70}	EF_{90}	EF_{50}	EF_{70}	EF_{90}
Benralizumab or mepolizumab E_{max} (mg/100mg bronchial tissue)	-120.69±27.15	-105.13±25.01	-105.44±26.53	-110.34±20.33	-107.87±20.68	-108.51±21.52
Benralizumab or mepolizumab pIC_{50}	6.84±0.17	7.00±0.18	7.63±0.16 *	7.06±0.18	7.13±0.17	7.18±0.10

* P<0.05 vs. mepolizumab (statistical analysis assessed by Student's t-test); Data represent the mean±SEM of n=5 bronchial tissue from different subjects. AHR: airway hyperresponsiveness; C+: positive control, passively sensitized bronchi; C-: negative control, non-sensitized bronchi; EF_n: frequency inducing n% E_{max}; E_{max}: maximal effect; EFS: electrical field stimulation; IC_{50}: concentration inducing 50% inhibition AHR to histamine in passively sensitized bronchi; pIC_{50}: $-\log IC_{50}$.

References

GINA (2020). Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Available at: https://ginasthma.org/wp-content/uploads/2020/04/GINA-2020-full-report_-final-_wms.pdf. Last accessed August 21, 2020.