A pathophysiological role of PDE3 in allergic airway inflammation

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Running title: A Pathophysiological Role of PDE3 in Allergic Airway Inflammation

Supplementary data/figures

Supplementary table 1

Gene		Primer Sequence	Tm (°C)	Position	Product Length (bp)
PDE3A	F	aaagacaagcttgctattccaaa	59	1286 - 1308	74
	R	gtggaagaaactcgtctcaaca	59	1338 - 1359	
PDE3B	F	tgcagtttggtatctgacaacac	60	2594 - 2616	112
	R	aattcgcccatggttaattct	59	2685 - 2705	
PDE4A	F	gccacgcctgcactagat	59	1282 - 1299	89
	R	ccagggtgatccacatcg	60	1353 - 1370	
PDE4B	F	cgctttggagtcaacactga	59	1166 - 1185	104
	R	ttgtgagaatatccagccaca	60	1249 - 1269	
PDE4C	F	ccatgtctcggaactcctct	59	418 - 437	67
	R	gcaaagggcgtcacaatc	60	467 - 484	
PDE4D	F	gacctctctccaaagtctatgtcc	59	542 - 565	72
	R	cacaatcaagtcatctccgtgt	60	592 - 613	

Supplementary table 1. Primers used for investigating PDE3&4 expression in human lung biopsies (forward (F) and reverse (r)).

Supplementary table 2

Gene		Primer Sequence	Tm (°C)	Position	Product Length (bp)
PDE3A	F	cccagaccatgatcttcctg	60	2303 - 2322	95
	R	ccaggtgttcaactgatcca	59	2378 - 2397	
PDE3B	F	agaaagcctgcagggagtta	59	2087 - 2106	94
	R	ggatccagtgacacttcctga	60	2160 - 2180	
PDE4A	F	gcgtctccaaccagttccta	60	1608 - 1627	107
	R	cagcagcttgaatcccaca	60	1696 - 1714	
PDE4B	F	ctttctacgccggcactg	60	1437 - 1454	72
	R	gatggcagctgcaaaaatg	60	1490 - 1508	
PDE4C	F	cacactcacagcgcaggt	59	117 - 134	109
	R	cactggctctcggactcg	60	208 - 225	
PDE4D	F	aaaccaagaaggtgacaagctc	59	1432 - 1453	88
	R	gcacagtgcaccatattctga	59	1499 - 1519	

Supplementary table 2. Primers used for investigating PDE3&4 expression in mouse lung (forward (F) and reverse (r)).

Supplementary Figure 1 PDE3A and PDE3B are expressed in the human airways

Staining with monoclonal antibodies specific for PDE3A protein or PDE3B protein identified PDE3. Staining is seen in the epithelial tissue and lamina propria of human airway mucosal biopsies. A dark violet hematoxylin nucleus-specific staining was performed to characterize the cells. Epithelial cells (a) stained positive for PDE3A. PDE3B protein can be seen**,** pointed out by arrows, in the bronchus mucosa in inflammatory cells (b) and structural cells such as epithelium (c), muscle cells (d) and endothelium cells (e). Messenger RNA transcription levels are shown for PDE3 and PDE4 in human lung mucosal biopsies obtained from healthy controls (n=19) and mild allergic asthma patients (n=28) (C) and Tissue eosinophilic inflammation (number of major basic protein positive cells) is seen in lung mucosal biopsies of asthmatics and healthy controls (D).

Supplementary Figure 2 PDE3A and PDE3B are expressed in the mice airways and immune cells.

Messenger RNA transcription levels are shown for PDE3 and PDE4 of murine lungs obtained from PBS treated control mice (n=3) or from HDM treated asthmatic mice (n=7) (A). Additionally, the gene muc5a has been included in the mouse qPCR assay, as an internal control for asthma severity (A). Shown are PDE3B mRNA expression levels of unstimulated or IgM stimulated B-cells (B) and PBS (unstimulated) and house dust mite stimulated DCs (C). Sorted naïve T-cells and in vitro differentiated Th subsets were analyzed for PDE3 and PDE4 mRNA expression (D). Mann-Whitney U test followed. Error bars show mean and ± SEM, Mann-Whitney U test (*=P<0.05;**=P<0.01)).

Supplementary Figure 3.

Allergic airway inflammation of indicated cells (data represent 2 separate experiments). Kruskal-Wallis test for multiple comparisons was used followed by Mann-Whitney U test. Error bars show mean and ± SEM, (n=3 for all PBS groups, n=7 for WT HDM, n=5 for both PDE3A-/- and PDE3-/- HDM groups (*=P<0.05;**=P<0.01)).

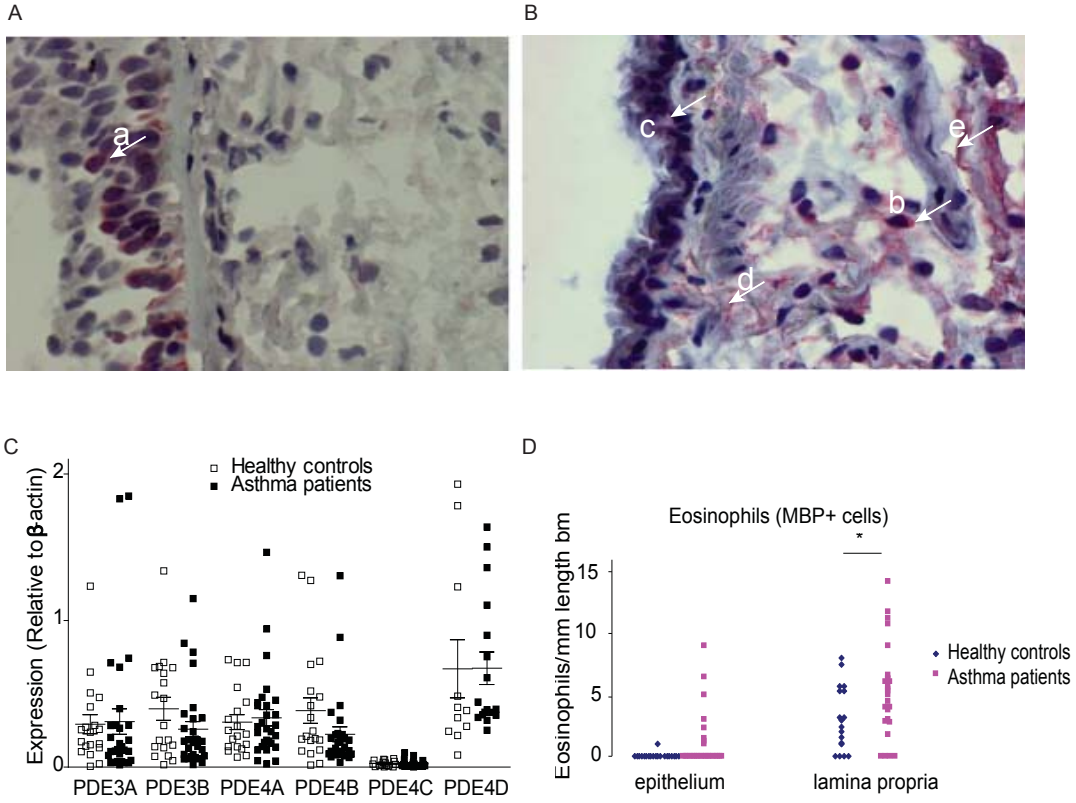
Supplementary Figure 4.

Dose and route optimization of enoximone therapy in mice.

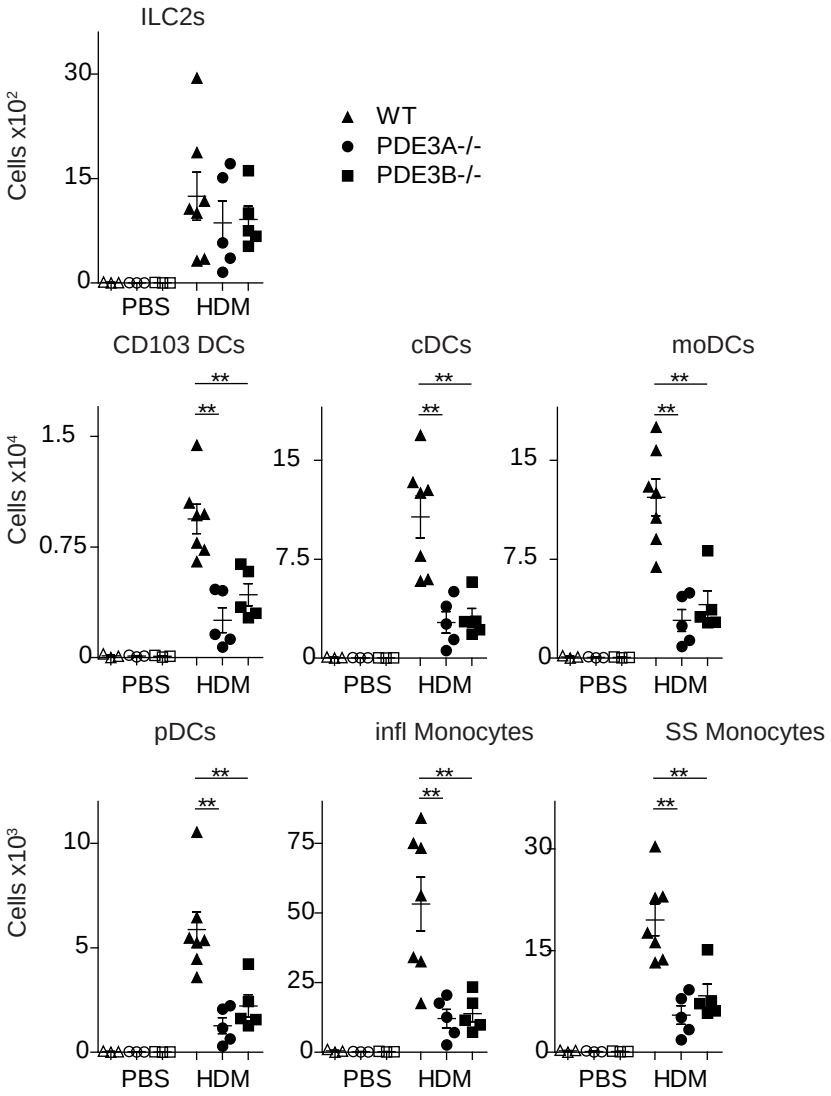
Different treatment modes were tested i.c. route and dosage. Here we tested the oral versus intratracheal route (intratracheal dosing is undertaken simultaneously with HDM treatment) and PBS versus enoximone in a HDM-driven allergic airway inflammation model. For the oral route, Enoximone was precipitated from Perfan®. Since we used clinical grade enoximone, the precipitate was administered with HDM solution and used as a suspension for intratracheal treatment (doses 25 μg and 250 μg) or it was used as a suspension administered in PBS for oral treatment (doses 250 μg or 2.5 mg). Mice underwent enoximone treatment only during challenge. Sensitization was performed with 1 μg HDM and challenge with 2.5 - 5 μg HDM. Experimental HDM asthma study design using intratracheal sensitization (s) and challenge (c) with 10 μg HDM or PBS as a control and enoximone (e) therapy. Mice were treated orally with 250, 25, 2.5 and 0.25 μg enoximone (enox) admixed with PBS or with PBS only as a control during sensitization and challenge. Analyses (a) were performed 1 day after the last challenge (A). Quantification of flow cytometric analyses of the indicated populations of BAL cells (B).

Comparison between oral (systemic) and intratracheal (topical (it)) enoximone therapy during challenge phase either admixed with HDM challenge or admixed with PBS in cases of oral therapy. Quantification of flow cytometric analyses was plotted in indicated populations of BAL cells (C). Ranking score results of albumin leakage judged from the lung tissue sections (D). Kruskal-Wallis test for multiple comparisons was used followed by Mann-Whitney U test. Error bars show mean and ± SEM (n=3 for PBS, n=3-5 for HDM HDM groups; (*=P<0.05;**=P<0.01)).

Supplementary Figure 1

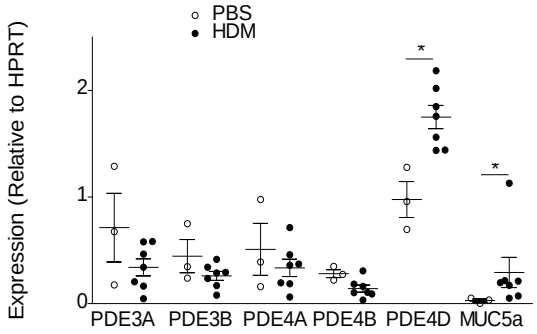


Supplementary Figure 3



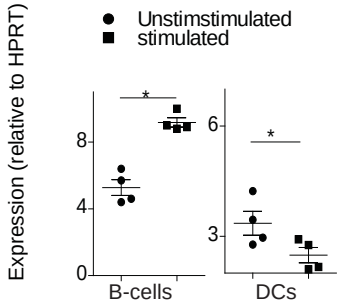
Supplementary Figure 2

A

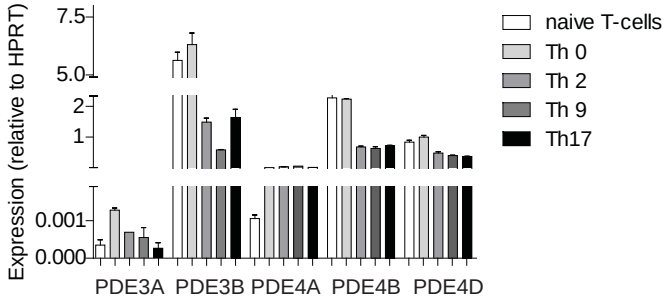


B

C



D



Supplementary Figure 4