

Data Supplement

Detrimental role of the airway mucin Muc5ac during ventilator induced lung injury

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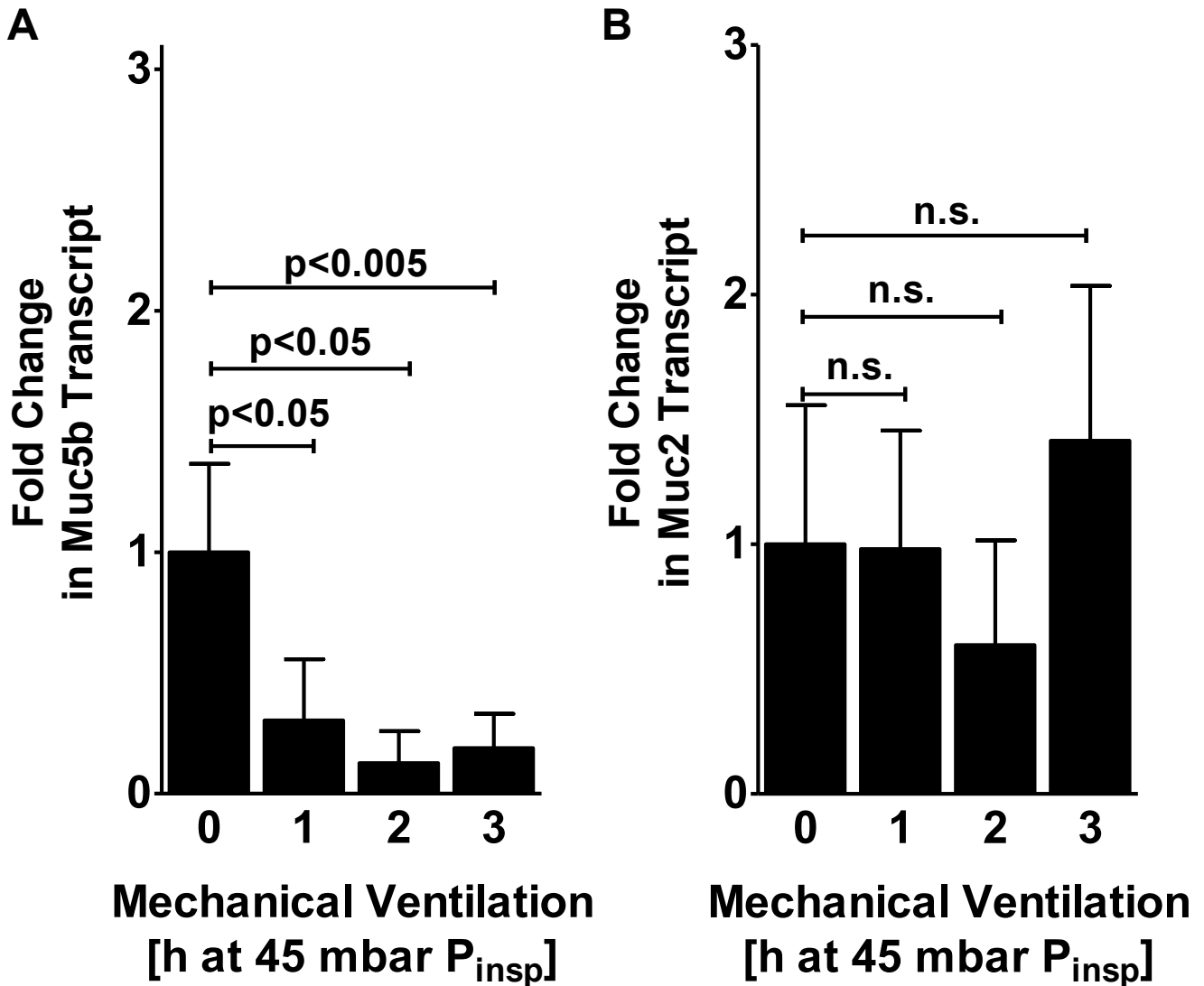
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Supplemental Figure 1. *Muc5b* and *Muc2* transcript levels during acute lung injury *in vivo*. C57BL/6 mice were mechanically ventilated (inspiratory pressure 45 mbar, 100% oxygen). After indicated time periods, lungs were harvested; total RNA isolated and (A) *Muc5b* and (B) *Muc2* mRNA levels were determined by real-time RT-PCR. Data are calculated relative to the internal housekeeping gene (β -actin) and are expressed as mean fold change compared with control (0 h ventilation) \pm SD. (n=4).

Summary of patient sample

Group	Age (years)	Sex	Scores (on day 0)		P _a O ₂ /F _i O ₂	ALI etiology
			APACHE II	SOFA		
ALI	61.2	male	26	8	< 300	Sepsis
	48.1	male	28	7	< 300	Pneumonia
	43	female	5	3	< 300	Pneumonia
	42.1	male	15	9	< 300	Aspiration
	62.2	male	25	4	< 300	Aspiration
Control	39.0	female	0	0	n/a	n/a
	42.0	male	0	0	n/a	n/a
	45.0	male	0	0	n/a	n/a
	54.0	male	0	0	n/a	n/a
	44.0	male	0	0	n/a	n/a

* Acute Physiology and Chronic Health Evaluation II

Sequential Organ Failure Assessment score

Supplemental Table 1. *Patient characteristics of BAL samples.* From patients with acute lung injury (ALI), bronchoalveolar fluid (BAL) was collected and the *Acute Physiology and Chronic Health Evaluation II* (APACHE II) and the *Sequential Organ Failure Assessment* (SOFA) were assessed. Demographic data and samples were made available through clinical trial NCT00201409. Samples from subjects from unconnected study without acute lung injury served as controls. Demographic data and samples of the study subjects were made available through clinical trial NCT00201409

Supplemental Table 2

<u>Alveolar congestions</u>		<u>Hemorrhage</u>		<u>Neutrophil aggregation</u>		<u>Hyaline membrane</u>	
1	Mild	1	Mild	1	Mild	1	Mild
2	Moderate	2	Moderate	2	Moderate	2	Moderate
3	Severe	3	Severe	3	Severe	3	Severe
4	Maximal	4	Maximal	4	Maximal	4	Maximal

Supplemental Table 2. *Histological scoring system for Ventilator induced lung injury .* For each subject (alveolar congestion, hemorrhage, neutrophil aggregation and hyaline membranes), a five-point scale is applied: 0 = minimal (little) damage, 1+ = mild damage, 2+ = moderate damage, 3+ = severe damage, and 4+ = maximal damage.

Supplemental Table 3

Inflammatory Cytokines & Receptors PCR array analysis in *Muc5ac*^{+/+} and *Muc5ac*^{-/-}

Reference sequence	Gene	Description	Fold difference	
			<i>Muc5ac</i> ^{+/+}	<i>Muc5ac</i> ^{-/-}
		ATP-binding cassette, sub-family F (GCN20), member 1		
NM_013854	Abcf1		1.1867	-1.083
NM_009744	Bcl6	B-cell leukemia/lymphoma 6	1.0224	-1.1933
NM_007551	Cxcr5	Chemokine (C-X-C motif) receptor 5	-1.5999	-1.4142
NM_009778	C3	Complement component 3	-1.3784	1.3149
NM_009807	Casp1	Caspase 1	-1.1313	1.1368
NM_011329	Ccl1	Chemokine (C-C motif) ligand 1	1.9212	-1.459
NM_011330	Ccl11	Chemokine (C-C motif) ligand 11	3.121	2.4116
NM_011331	Ccl12	Chemokine (C-C motif) ligand 12	3.0251	4.0558
NM_011332	Ccl17	Chemokine (C-C motif) ligand 17	3.9917	2.4453
NM_011888	Ccl19	Chemokine (C-C motif) ligand 19	1.8622	2.362
NM_011333	Ccl2	Chemokine (C-C motif) ligand 2	9.461	10.7406
NM_016960	Ccl20	Chemokine (C-C motif) ligand 20	6.1988	1.2658
NM_009137	Ccl22	Chemokine (C-C motif) ligand 22	1.7435	-1.1329
NM_019577	Ccl24	Chemokine (C-C motif) ligand 24	-1.4025	1.3287
NM_009138	Ccl25	Chemokine (C-C motif) ligand 25	-1.0091	-1.3059
NM_011337	Ccl3	Chemokine (C-C motif) ligand 3	6.1347	5.6962
NM_013652	Ccl4	Chemokine (C-C motif) ligand 4	6.0503	5.5213
NM_013653	Ccl5	Chemokine (C-C motif) ligand 5	-1.3641	-1.7839
NM_009139	Ccl6	Chemokine (C-C motif) ligand 6	1.0845	1.7532
NM_013654	Ccl7	Chemokine (C-C motif) ligand 7	14.0939	9.9177
NM_021443	Ccl8	Chemokine (C-C motif) ligand 8	-1.9903	-1.1134
NM_011338	Ccl9	Chemokine (C-C motif) ligand 9	1.7925	3.4224
NM_009912	Ccr1	Chemokine (C-C motif) receptor 1	4.9657	8.8458
NM_009915	Ccr2	Chemokine (C-C motif) receptor 2	-1.5562	4.3772
NM_009914	Ccr3	Chemokine (C-C motif) receptor 3	1.3213	3.4462
NM_009916	Ccr4	Chemokine (C-C motif) receptor 4	1.0996	-3.042
NM_009917	Ccr5	Chemokine (C-C motif) receptor 5	-1.1471	4.5002
NM_009835	Ccr6	Chemokine (C-C motif) receptor 6	-2.8442	1.4845
NM_007719	Ccr7	Chemokine (C-C motif) receptor 7	-1.498	-2.2658
NM_007720	Ccr8	Chemokine (C-C motif) receptor 8	-1.0231	1.1096
NM_009913	Ccr9	Chemokine (C-C motif) receptor 9	-2.0463	-2.6574
NM_007768	Crp	C-reactive protein, pentraxin-related	3.1976	1.2527
NM_009142	Cx3cl1	Chemokine (C-X3-C motif) ligand 1	-1.1274	-1.7532
NM_008176	Cxcl1	Chemokine (C-X-C motif) ligand 1	15.4764	10.8528
NM_021274	Cxcl10	Chemokine (C-X-C motif) ligand 10	3.5975	3.3636
NM_019494	Cxcl11	Chemokine (C-X-C motif) ligand 11	-2.5901	1.3996
NM_021704	Cxcl12	Chemokine (C-X-C motif) ligand 12	-2.3587	-2.3376
NM_018866	Cxcl13	Chemokine (C-X-C motif) ligand 13	-1.2728	3.2266
NM_011339	Cxcl15	Chemokine (C-X-C motif) ligand 15	-2.4504	-1.5583
NM_019932	Pf4	Platelet factor 4	-1.0703	1.1567
NM_009141	Cxcl5	Chemokine (C-X-C motif) ligand 5	4.8804	16.7955
NM_008599	Cxcl9	Chemokine (C-X-C motif) ligand 9	-2.8442	3.4224
NM_009910	Cxcr3	Chemokine (C-X-C motif) receptor 3	-1.8	-1.4489
NM_007721	Ccr10	Chemokine (C-C motif) receptor 10	-2.7568	-1.3287
NM_008337	Iifng	Interferon gamma	1.0585	-1.4241
NM_010548	Il10	Interleukin 10	5.6256	1.9185
NM_008348	Il10ra	Interleukin 10 receptor, alpha	-1.1712	1.21
NM_008349	Il10rb	Interleukin 10 receptor, beta	-1.3315	-1.7351

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NM_008350	Il11	Interleukin 11	6.6208	1.5583
NM_008355	Il13	Interleukin 13	2.4829	-1.459
NM_133990	Il13ra1	Interleukin 13 receptor, alpha 1	1.431	1.4241
NM_008357	Il15	Interleukin 15	-1.0446	-1.9319
NM_010551	Il16	Interleukin 16	-1.3928	-1.4241
NM_019508	Il17b	Interleukin 17B	-1.35	-4.1989
NM_008360	Il18	Interleukin 18	-1.4876	-1.3472
NM_010554	Il1a	Interleukin 1 alpha	2.1992	1.4489
NM_008361	Il1b	Interleukin 1 beta	6.7599	3.5064
NM_019450	Il1f6	Interleukin 1 family, member 6	-1.1119	20.6061
NM_027163	Il1f8	Interleukin 1 family, member 8	-1.0161	5.9381
NM_008362	Il1r1	Interleukin 1 receptor, type I	1.4064	1.4845
NM_010555	Il1r2	Interleukin 1 receptor, type II	18.4047	18.6357
NM_021380	Il20	Interleukin 20	3.0994	-1.459
NM_008368	Il2rb	Interleukin 2 receptor, beta chain	-1.7752	-1.4743
NM_013563	Il2rg	Interleukin 2 receptor, gamma chain	1.1543	1.1329
NM_010556	Il3	Interleukin 3	1.5021	-1.9119
NM_021283	Il4	Interleukin 4	1.0439	1.3996
NM_008370	Il5ra	Interleukin 5 receptor, alpha	-2.7473	-1.5422
NM_010559	Il6ra	Interleukin 6 receptor, alpha	1.0882	-1.279
NM_010560	Il6st	Interleukin 6 signal transducer	1.1583	-1.0943
NM_009909	Cxcr2	Chemokine (C-X-C motif) receptor 2	6.4621	4.362
NM_008401	Itgam	Integrin alpha M	2.415	4.0139
NM_008404	Itgb2	Integrin beta 2	1.2897	1.2746
NM_010735	Lta	Lymphotoxin A	-2.148	-1.1607
NM_008518	Ltb	Lymphotoxin B	-1.7876	-2.5403
NM_010798	Mif	Macrophage migration inhibitory factor	-1.2861	-1.1487
NM_007926	Aimp1	Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1	-1.6279	-1.3519
NM_009263	Spp1	Secreted phosphoprotein 1	1.6552	-2.9794
NM_011577	Tgfb1	Transforming growth factor, beta 1	1.5659	1.1096
NM_013693	Tnf	Tumor necrosis factor	2.7076	2.6027
NM_011609	Tnfrsf1a	Tumor necrosis factor receptor superfamily, member 1a	1.431	1.0718
NM_011610	Tnfrsf1b	Tumor necrosis factor receptor superfamily, member 1b	1.6609	1.7839
NM_011616	Cd40lg	CD40 ligand	-1.1352	-2.4967
NM_023764	Tollip	Toll interacting protein	1.0512	-1.2702
NM_011798	Xcr1	Chemokine (C motif) receptor 1	-2.7953	-2.2579
NM_010368	Gusb	Glucuronidase, beta	-1.763	-1.3947
NM_013556	Hprt	Hypoxanthine guanine phosphoribosyl transferase	1.0658	-1.2184
NM_008302	Hsp90ab1	Heat shock protein 90 alpha (cytosolic), class B member 1	1.1909	-1.0943
NM_008084	Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	-1.0852	1.1647
NM_007393	Actb	Actin, beta	1.0073	1.0966

Supplemental Table 3. *PCR Array for cytokine and cytokine receptor regulation.* *Muc5ac*^{-/-} mice or corresponding littermate controls matched in age, sex and weight (*Muc5a*^{+/+}) were mechanically ventilated (inspiratory pressure 45 mbar, 100% oxygen). Total RNA was isolated and transcriptional changes were calculated relative to mRNA levels in control animals of the same genotype using an online analysis tool provided by the manufacturer of the PCR-Array platform (<http://pcrdataanalysis.sabiosciences.com>).

Gene	Description	Fold difference	
		<i>Muc5ac</i> ^{+/+}	<i>Muc5ac</i> ^{-/-}
Ccl20	Chemokine (C-C motif) ligand 20	6.1988	1.2658
Ccl7	Chemokine (C-C motif) ligand 7	14.0939	9.9177
Cxcl1	Chemokine (C-X-C motif) ligand 1	15.4764	10.8528
Il11	Interleukin 11	6.6208	1.5583
Crp	C-reactive protein, pentraxin-related	3.1976	1.2527
Il1b	Interleukin 1 beta	6.7599	3.5064

Supplemental Table 4. Selection of mediators differential regulated between *Muc5ac*^{+/+} and *Muc5ac*^{-/-} from Supplemental table 1. *Muc5ac*^{-/-} mice or corresponding littermate controls matched in age, sex and weight (*Muc5a*^{+/+}) were mechanically ventilated (inspiratory pressure 45 mbar, 100% oxygen). Total RNA was isolated and transcriptional changes were calculated relative to mRNA levels in control animals of the same genotype using an online analysis tool provided by the manufacturer of the PCR-Array platform (<http://pcrdataanalysis.sabiosciences.com>).