

Strain Differences Influence Murine Pulmonary Responses to
Stachybotrys chartarum

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MATERIALS AND METHODS

Intratracheal Instillation

The mice were weighed and anesthetized with up to 5% vaporized Halothane (Halocarbons Lab, Inc., North Augusta, SC). They were then placed on a slanted board where they were supported by an elastic band under the upper incisors. A suspension of *S. chartarum* spores was instilled intratracheally at 0.25 ml/100 gm body weight. The spore suspension was delivered to the lungs through the trachea with a ball-tip 22-gauge needle inserted between the vocal cords. A microscope lamp shining on the neck provided transillumination of the larynx. Each animal remained in the slanted position for approximately 1 minute, and then was placed on its back in the cage where it regained consciousness within a few minutes.

Bronchoalveolar Lavage (BAL)

The trachea was carefully exposed and cannulated. The lung was lavaged *in situ* with 12 washes of sterile 0.9% saline using one ml of saline for the first lavage, then 0.75 ml for subsequent washes. Washes 1 and 2 were used for biochemical assays. Cells were separated from the supernatant in all washes (350g at 4°C for 10 minutes). The supernatant fraction of washes 1-2 was clarified by sedimentation at 14,500g for 30 minutes and used to assay various enzymes and albumin determination.

Analysis of Bronchoalveolar Lavage

The total leukocyte cell count in the lavage fluid was estimated in a hemocytometer chamber at 250X magnification. Leukocytes were identified by size and granularity. The

leukocytes were deposited onto a microscope slide by cytopspin (72g for 5 min), fixed, stained, and mounted in Permount. Macrophages, PMNs, eosinophils and lymphocytes in the BAL cell pellet were differentiated by light microscopy at 400X. The supernatant was analyzed for lactic dehydrogenase, myeloperoxidase and albumin by spectrophotometry. Hemoglobin analysis was performed on the combined cell pellet from all 12 lavages. Biochemical analyses were performed according to Beck (48).

Table E1 – *p<0.05 **p<0.01 ***p<0.005 Chart showing mean ± SEM (p-value) of all factors measured in a MAP analysis of bronchoalveolar lavage fluid after instillation of 10 million spores *S. chartarum*/ml (2.5 ml/kg body weight). Percent of baseline is calculated by dividing the mean value after instillation with spores by the mean value after instillation with saline for each mouse strain. † KC percent of baseline calculated based on the least detectable dose of 0.035 ng/ml; SGOT percent of baseline calculated based on the least detectable dose of 0.37 µg/ml.

Table E1. MAP Analysis of Cytokines and Chemokines in BAL Fluid (Online Supplement Only)

	C57BL/6J		BALB/c		Percent of Baseline	
	Saline	Spores	Saline	Spores	C57BL/6J	BALB/c
CYTOKINES						
Interferon- γ (IFN- γ) (pg/ml)	18.38 \pm 0.634	16.16 \pm 2.867	17.03 \pm 0.853	16.8 \pm 3.09	88%	99%
Interleukin -1 α (IL-1 α) (pg/ml)	12.18 \pm 0.471	12.68 \pm 1.323	11.72 \pm 0.966	28.26 \pm 8.445*	104%	241%*
Interleukin-1 β (IL-1 β) (ng/ml)	0.06 \pm 0.005	0.05 \pm 0.006	0.04 \pm 0.001	0.07 \pm 0.014***	83%	175%***
Interleukin-2 (IL-2) (pg/ml)	22.44 \pm 1.541	19.94 \pm 2.591	19.8 \pm 1.53	17.9 \pm 3.548	89%	90%
Interleukin-3 (IL-3) (pg/ml)	4.62 \pm 0.205	4.31 \pm 0.784	4.32 \pm 0.462	7.26 \pm 1.639*	93%	176%*
Interleukin-4 (IL-4) (pg/ml)	30.46 \pm 1.314	28.76 \pm 2.768	29.58 \pm 0.894	29.52 \pm 5.419	106%	100%
Interleukin-5 (IL-5) (ng/ml)	0.02 \pm 0.001	0.02 \pm 0.004	0.02 \pm 0.002	0.03 \pm 0.005	100%	150%
Interleukin-6 (IL-6) (pg/ml)	5.6 \pm 0.708	4.83 \pm 0.515	5.26 \pm 0.332	28.68 \pm 10.657*	116%	545%*
Interleukin-7 (IL-7) (ng/ml)	0.08 \pm 0.002	0.07 \pm 0.014	0.07 \pm 0.003	0.07 \pm 0.013	87.5%	100%
Interleukin-10 (IL-10) (pg/ml)	144.4 \pm 2.561	132.34 \pm 28.96	155.5 \pm 8.18	196.6 \pm 39.781	92%	126%
Interleukin-11 (IL-11) (pg/ml)	28.6 \pm 0.694	24.58 \pm 4.928	25.43 \pm 1.102	33.44 \pm 6.436	86%	131%
Interleukin-12/p70 (IL-12/p70) (ng/ml)	0.25 \pm 0.01	0.26 \pm 0.034	0.22 \pm 0.013	0.22 \pm 0.043	104%	100%
Interleukin-17 (IL-17) (ng/ml)	0.03 \pm 0.001	0.02 \pm 0.003	0.02 \pm 0.002	0.02 \pm 0.005	66%	100%
Interleukin-18 (IL-18) (ng/ml)	0.11 \pm 0.007	0.10 \pm 0.007	0.10 \pm 0.001	0.19 \pm 0.051*	91%	190%*
Interferon- γ Inducible Protein-10 (IP-10) (pg/ml)	16.02 \pm 0.646	14.92 \pm 1.406	17.05 \pm 1.508	23.2 \pm 5.2	93%	136%
Leukemia Inhibitory Factor (LIF) (pg/ml)	17.1 \pm 0.898	14.23 \pm 2.257	15.53 \pm 0.993	33.94 \pm 8.585*	83%	219%*
Macrophage Colony Stimulating Factor (M-CSF) (ng/ml)	0.05 \pm 0.004	0.06 \pm 0.011	0.06 \pm 0.004	0.15 \pm 0.047*	120%	250%*

	C57BL/6J		BALB/c		Percent of Baseline	
	Saline	Spores	Saline	Spores	C57BL/6J	BALB/c
Tumor Necrosis Factor- α (TNF α) (ng/ml)	0.1 \pm 0.004	0.09 \pm 0.013	0.09 \pm 0.006	0.7 \pm 0.263**	90%	778%**
CHEMOKINES						
Eotaxin (pg/ml)	12.18 \pm 0.529	11.93 \pm 0.695	11.5 \pm 0.704	13.92 \pm 2.848	98%	121%
Granulocyte Chemotactic Protein-2/LPS-induced CXC Chemokine (GCP-2 / LIX) (ng/ml)	0.05 \pm 0.015	0.07 \pm 0.037	0.07 \pm 0.012	0.12 \pm 0.035	140%	171%
Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) (pg/ml)	7.11 \pm 0.446	6.97 \pm 1.032	6.77 \pm 0.479	7.89 \pm 2.411	98%	117%
Mouse Homolog of Human Growth Related Protein (KC/GRO α) (ng/ml)	0 \pm 0	0 \pm 0	0 \pm 0	0.37 \pm 0.123*	N/A	1057%* [†]
Lymphotactin (pg/ml)	38.2 \pm 1.117	33.7 \pm 6.246	33.0 \pm 2.209	31.36 \pm 6.005	88%	95%
Monocyte Chemotactic Protein-1 (MCP-1 / JE) (pg/ml)	11.64 \pm 0.53	10.29 \pm 1.496	10.61 \pm 0.484	63.03 \pm 25.713*	88%	594%*
Monocyte Chemotactic Protein-3 (MCP-3) (pg/ml)	14.14 \pm 0.312	12.69 \pm 1.627	12.98 \pm 0.309	37.22 \pm 12.642*	90%	287%*
Monocyte Chemotactic Protein-5 (MCP-5) (pg/ml)	17.98 \pm 1.117	14.59 \pm 2.467	16.23 \pm 1.183	17.18 \pm 3.153	81%	106%
Macrophage Derived Chemokine (MDC) (pg/ml)	22.8 \pm 2.119	20.96 \pm 0.79	20.25 \pm 0.846	48.12 \pm 17.119	92%	238%
Macrophage Inflammatory Protein -1 α (MIP-1 α) (ng/ml)	0.03 \pm 0.003	0.03 \pm 0.004	0.03 \pm 0.001	0.09 \pm 0.03*	100%	300%*
Macrophage Inflammatory Protein -1 β (MIP-1 β) (pg/ml)	94.5 \pm 3.182	75.52 \pm 12.536	83.05 \pm 3.988	399.52 \pm 140.48*	80%	481%*
Macrophage Inflammatory Protein -1 γ (MIP-1 γ) (ng/ml)	0.1 \pm 0.037	0.09 \pm 0.036	0.11 \pm 0.032	0.94 \pm 0.383*	90%	855%*
Macrophage Inflammatory Protein -2 (MIP-2) (pg/ml)	11.5 \pm 1.301	10.84 \pm 1.661	11.77 \pm 1.168	134.14 \pm 58.812*	94%	1139%*
Macrophage Inflammatory Protein -3 β (MIP-3 β) (ng/ml)	0.08 \pm 0.003	0.08 \pm 0.012	0.08 \pm 0.002	0.1 \pm 0.021	100%	125%
Regulated Upon Activation, Normal T-cell Expressed and Secreted (RANTES) (pg/ml)	2.09 \pm 0.383	1.89 \pm 0.376	2.07 \pm 0.453	8.45 \pm 3.474*	90%	408%*
OTHER PROTEINS						

	C57BL/6J		BALB/c		Percent of Baseline	
	Saline	Spores	Saline	Spores	C57BL/6J	BALB/c
Apolipoprotein A1 (µg/ml)	0.04 ± 0.009	0.37 ± 0.394	0.1 ± 0.068	1.96 ± 0.977	925%	1960%
C Reactive Protein (µg/ml)	0.01 ± 0.001	0.01 ± 0.006	0.01 ± 0.001	0.02 ± 0.008	100%	200%
Endothelin-1 (pg/ml)	8.66 ± 0.096	7.74 ± 1.052	8.07 ± 0.163	7.08 ± 1.385	89%	88%
Epidermal Growth Factor (EGF) (pg/ml)	7.33 ± 0.394	6.99 ± 0.888	5.9 ± 0.414	5.4 ± 1.204	95%	91%
Factor VII (ng/ml)	0.19 ± 0.012	0.16 ± 0.024	0.18 ± 0.016	0.26 ± 0.055	84%	144%
Fibrinogen (µg/ml)	0.09 ± 0.011	0.42 ± 0.375	0.26 ± 0.06	1.66 ± 0.85	466%	638%
Fibroblast Growth Factor-9 (FGF-9) (ng/ml)	0.23 ± 0.007	0.22 ± 0.041	0.24 ± 0.014	0.27 ± 0.053	96%	112%
Fibroblast Growth Factor-basic (FGF-basic) (ng/ml)	0.48 ± 0.008	0.45 ± 0.09	0.41 ± 0.017	0.38 ± 0.077	94%	93%
Growth Hormone (ng/ml)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	N/A	N/A
GST (ng/ml)	0.61 ± 0.048	0.58 ± 0.087	0.54 ± 0.022	0.48 ± 0.104	95%	89%
Haptoglobin (µg/ml)	0.34 ± 0.019	0.38 ± 0.067	0.34 ± 0.029	0.45 ± 0.096	112%	132%
Immunoglobulin A (IgA) (µg/ml)	0.02 ± 0.002	0.05 ± 0.029	0.04 ± 0.005	0.24 ± 0.116	250%	600%
Insulin (µIU/ml)	1.32 ± 0.055	1.21 ± 0.16	1.16 ± 0.037	1.5 ± 0.29	92%	129%
Leptin (ng/ml)	0.04 ± 0.001	0.03 ± 0.004	0.03 ± 0.002	0.03 ± 0.005	75%	100%
Myoglobin (ng/ml)	0.57 ± 0.06	6.13 ± 4.444	3.06 ± 0.737	1.98 ± 0.755	1075%	65%
Oncostatin-M (OSM) (ng/ml)	0.08 ± 0.002	0.07 ± 0.011	0.07 ± 0.003	0.07 ± 0.013	88%	100%
Serum Glutamic Oxaloacetic Transaminase (SGOT) (µg/ml)	0 ± 0	0.21 ± 0	0 ± 0	0.41 ± 0.26	N/A	111% [†]
Stem Cell Factor (SCF) (pg/ml)	40.24 ± 2.782	31.82 ± 4.656	34.9 ± 2.86	35.52 ± 6.761	79%	102%
Thrombopoietin (TPO) (ng/ml)	0.58 ± 0.062	0.63 ± 0.123	0.54 ± 0.048	0.93 ± 0.235	109%	172%
Tissue Inhibitors of Metalloproteinases-1 (TIMP-1) (ng/ml)	0.13 ± 0.013	0.12 ± 0.007	0.2 ± 0.02	0.99 ± 0.446*	93%	495%*

	C57BL/6J		BALB/c		Percent of Baseline	
	Saline	Spores	Saline	Spores	C57BL/6J	BALB/c
Tissue Factor (ng/ml)	0.10 ± 0.008	0.12 ± 0.031	0.12 ± 0.017	0.27 ± 0.07*	120%	225%*
Vascular Cell Adhesion Molecule-1 (VCAM-1) (ng/ml)	1.43 ± 0.167	3.36 ± 2.31	1.7 ± 0.167	6.73 ± 2.878	235%	396%
Vascular Endothelial Growth Factor (VEGF) (pg/ml)	84.8 ± 5.644	84.6 ± 14.496	104.98 ± 5.951	147.6 ± 38.368	100%	140%
von Willebrand Factor (ng/ml)	2.94 ± 0.25	2.67 ± 0.478	3.01 ± 0.21	2.92 ± 0.624	91%	97%