A Modular Cell-Type Focused Inflammatory Process Network Model for Non-diseased Pulmonary Tissue

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Introduction and Objective

Exposure to environmental stressors such as cigarette smoke (CS) elicits a variety of biological responses in humans, including the induction of an inflammatory response. This response is especially pronounced in the lung, where pulmonary cells sit at the interface between the body's internal and external environments. Since prolonged exposure to CS has been linked to the development and progression of inflammation-related pulmonary diseases, including COPD, a thorough mechanistic understanding of the initial inflammatory pathways modulated by CS is central to understanding disease pathogenesis.
Thus, the objective was, by combining a survey of relevant published literature with the computational analysis of multiple transcriptomic data sets, to construct a fully referenced and computable network model (the Inflammatory Process Network [IPN]) of the main pulmonary inflammatory processes.

Materials and Methods

The Inflammatory Process Network (IPN) was constructed using "Biological Expression Language" (BEL), Selventa™'s computable framework for biological pathway representation (ref. 1). The nodes of this network correspond to biological entities encoded in the BEL language. The edges of this network describe the causal connections between two nodes, supported by explicit literature evidence.

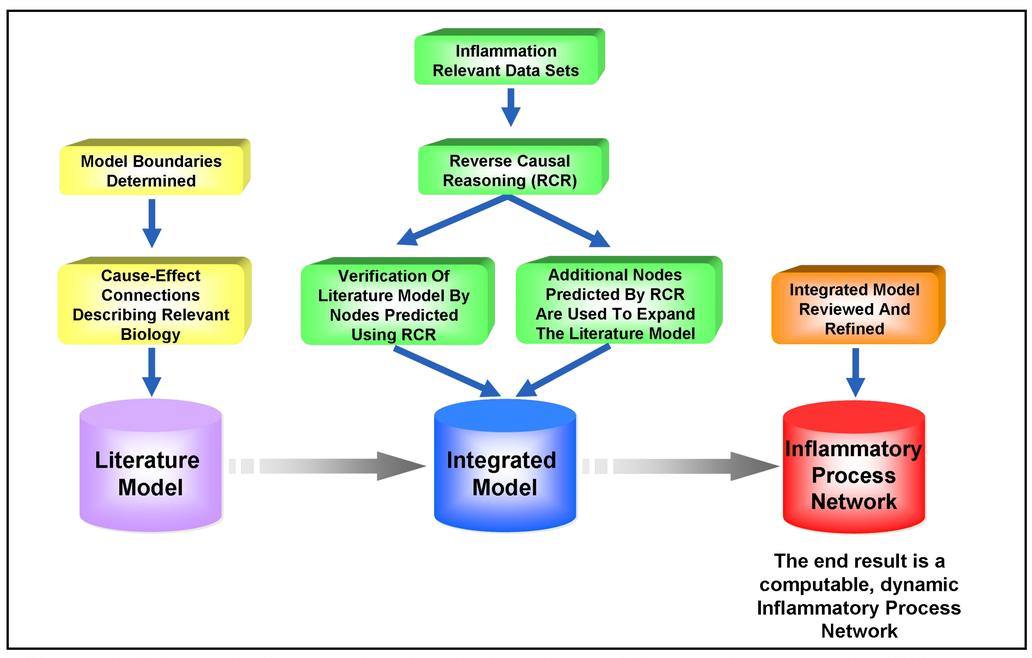

Figure 1: Schematic diagram showing the iterative workflow used to create the IPN.

Figure 1 represents the iterative process of the IPN construction.
A) Literature Model (in purple): It was built from causal connections from the Selventa™ Knowledgebase (1.5 million biological concepts and entities; 7.5 million edges) and from additionally curated literature. The boundaries were chosen with a focus on the inflammatory signaling in a specific set of cell types found in non-diseased pulmonary tissue or normal lung cells predominantly from human origin. A further focus was on initiators of, and responses to inflammation, in order to link different cell-type specific sub-networks. Finally, areas of biology known to be modulated by CS were included.
B) Integrated model (in blue): The content of the Literature Model was evaluated by performing Reverse Causal Reasoning (RCR) on three publicly available relevant data sets (ref. 2-4). Then, the Literature Model was augmented with additional relevant RCR-derived nodes creating the Integrated Model.
C) Final IPN (in red): It resulted from a comprehensive review of the Integrated Model, and it was validated by a set of 4 independent data sets (ref. 5-8).

Results

Network structure and content

A) Literature Model

The IPN was constructed using causal relationships extracted from over 800 unique references, with over 75% of the edges containing literature support from human contexts. It was built in a modular fashion, consisting of 23 sub-models that describe biological signaling in 8 distinct cell types and can be linked via inputs and outputs (e.g., macrophage chemotaxis via epithelial cell IL-8 release) from different sub-models (**Figure 2**).

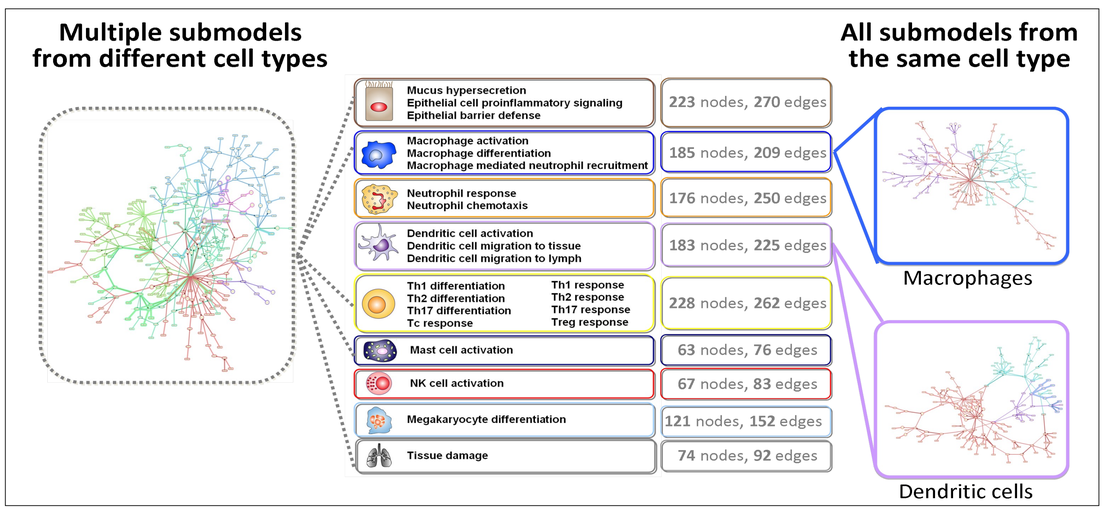

Figure 2: 23 IPN sub-models can be flexibly applied to analyze different experimental systems.

B) Integrated Model

Reverse Causal Reasoning (RCR), which identifies upstream controllers ("hypotheses") that can explain significant mRNA expression changes, was performed on sets of stringently selected transcriptomic data (**Figure 3 A**; ref. 1-3). Selection criteria included species (human, mouse), non-disease context, defined stimuli, the presence of measured inflammation endpoints, and the availability of raw data with at least three biological replicates for each sample group. The sub-models were evaluated using RCR by mapping of known inflammatory pathways onto the literature-derived models. Thereby, over 50 additional nodes were identified, eventually resulting in the integrated network model.

C) Final Model

After another round of review the final IPN contained over 1,200 unique nodes (= biological entities such as mRNA, protein, enzymatic activity, or cellular processes) and 1,500 unique edges, which link the nodes (**Figure 2**) and can be interrogated interactively with respect to the underlying literature information.

A

Data Set	Tissue	Stimulus
Smith et al. (Mouse)	Whole lung	LPS
Abbas et al. (Human)	T-cells	IL4 (Th2 differentiation) IFNG (Th1 differentiation)
	Dendritic cells	LPS
	Macrophage	Cell culture induced differentiation
	NK cell	IL15
Coldren et al. (Human)	Lung Neutrophil	Endotoxin

B

Data Set	Tissue	Stimulus
Spira et al. (Human)	Bronchial epithelial cell	Cigarette smoke
Mathis et al. (Human)	Bronchial epithelial cell	Cigarette smoke
Shaykhiev et al. (Human)	Alveolar Macrophage	Cigarette smoke
Gebel et al. (Mouse)	Whole lung	Cigarette smoke

Figure 3: Transcriptomic data sets for building, evaluating **(A)** and independently validating **(B)** the IPN.

Network validation

Four independent data sets, chosen by the same criteria as for network building, were used to validate the network model (**Figure 3B**; ref. 5-8). For the example of mucus hypersecretion **Figure 4A** shows the work-flow of the RCR analysis in normal human bronchial organotypical tissue (MatTek) directly exposed to cigarette smoke (ref. 6). This analysis identified two well-described pathways leading to MUC5AC expression (**Figure 4B**) (ref. 9,10), thereby validating this sub-model.

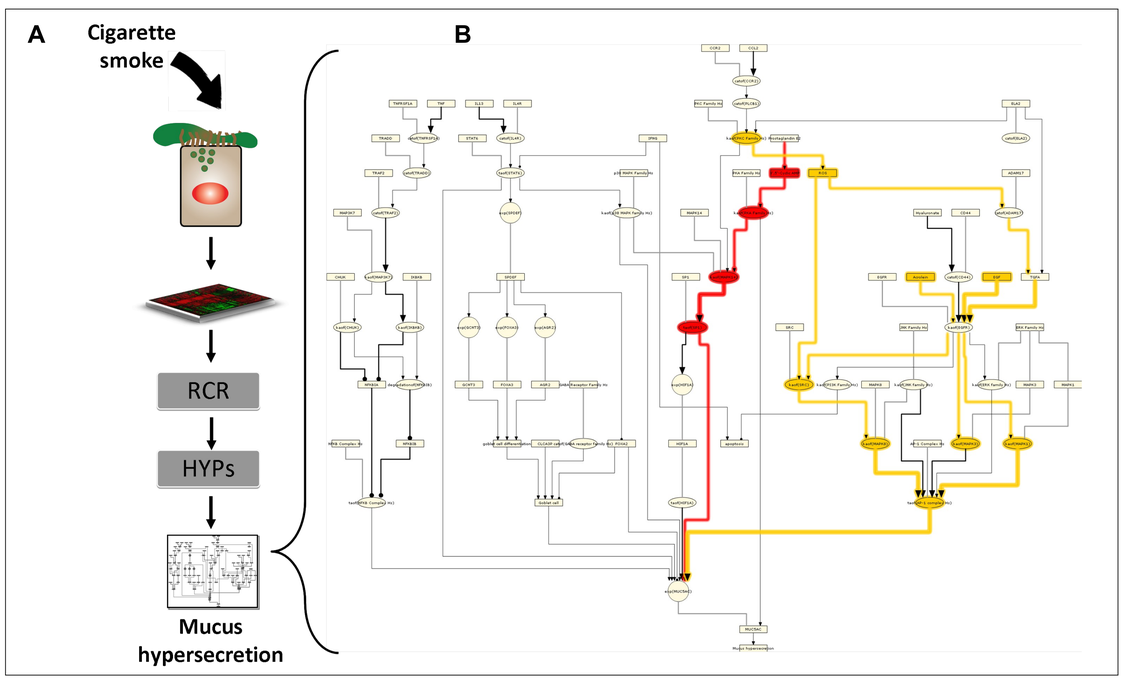

Figure 4: RCR-analysis of mucus hypersecretion in normal human organotypical bronchial tissue (MatTek) directly exposed to CS. **A)** Overview of work-flow. **B)** Pathways leading to MUC5AC expression: ROS / AP-1 (yellow); SP1 (red). Nodes filled in are predicted as HYPs in a direction consistent with increased pathway activation.

Summary and Conclusion

The IPN model was constructed with a modular architecture that captures different processes occurring in key resident pulmonary cells as well as immune cells recruited from the systemic circulation following exposure to cigarette smoke (CS). Its 23 cell-type specific subsets were initially built based on literature, then augmented and evaluated by transcriptomic data and finally validated by an independent set of such data.

The 23 IPN lung-focused sub-models can be flexibly applied to analyze different experimental systems *in vitro* and *in vivo*. The lung focused IPN described here represents the most comprehensive and fully referenced mechanistic representation of the signaling pathways that regulate pulmonary inflammatory processes in existence.

As with our previously built network models ("cell proliferation" (ref. 11) and "cell stress"), the IPN will be freely available and, thus, will provide a novel resource to the scientific community with broad applicability to aid in the investigation of inflammatory signaling mechanisms affected by CS.

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