As close as you can get to finding the cause of disease with Agent-Based Modeling

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Koch’s Postulates (for cause and effect due to a pathogen)

1. The virus should be found in all animals with the disease, but not in animals without the disease.
2. The virus must be isolated from a diseased animal and grown in pure culture.
3. The virus should cause disease when it is introduced into an animal.
4. The virus should be re-isolated from the animal in #3, and shown to be identical to the virus in #1.
Basic Immune Simulator 2010

Programmed using Repast J 3.0

Recursive Porous Agent Simulation Toolkit
Created by the Social Science Research Computing Department of the University of Chicago, Argonne National Laboratory.

Now managed by the non-profit volunteer Repast Organization for Architecture and Development (ROAD).

http://repast.sourceforge.net
Basic Immune Simulator-Lung

Model Purpose: The BIS-Lung was created to study the behavior of the immune system in the lung, to gain insight into how injuries of unknown origin could lead to a disease called Idiopathic Pulmonary Fibrosis (IPF).

What is IPF?
Idiopathic Pulmonary Fibrosis

• A fibrotic, restrictive, lung disease of unknown cause
• Major symptom: shortness of breath
• Most patients are in their late 50s/early 60s
• By the time a patient is definitively diagnosed (by exclusion) they only have a few years to live
• There is currently no effective treatment
Normal lung alveoli

- Endothelial cell (continuous type)
- Red Blood Cells
- Fibroblast
- Elastic fibers
- Alveolar space
- Alveolar capillary
- Type II Epithelial Cell
- Type I Epithelial Cell
normal lung

Type II Epithelial cells
Idiopathic Pulmonary Fibrosis
Highest level entities represented in the model:

- Respiratory system
- tissues/spaces
  1. lungs
  2. lymph nodes
  3. blood

: Idiopathic pulmonary fibrosis usually affects lower lobes, also the most distal parts of the lung where the alveoli are.
Entities, state variables and scales included in the BIS-Lung:

i. Three virtually connected *spaces* representing the relevant *tissues*.

ii. Many *agent* types, representing the *cells* of the *tissues* system. The *agents* representing cells of the *immune system* travel between the *spaces*.

iii. The *agents* produce *signals*, representing *cytokines* or other *molecules* excreted by *cells*. These communicate the status of an *agent* to other *agents* that have receptors, i.e. the ability to sense the *signals*.
Figure 1a. BIS-Lung Zone 1 at Initiation of Injury

Figure 1a shows the results of a test run of the BIS-Lung with a sterile injury to the epithelium. White space is air space. In the non-injured alveolus, two type I epithelial agent/cells are visible (yellow), and two type II epithelial agent/cells are visible at the left and right (gray). The injured epithelial agent/cells have red Xs in them. The light pink agents/cells are macrophages, the bright green agent/cells are fibroblasts. The capillary has an endothelial agent/cell (purple) around the lumen, and red blood cells (RBCs; blue) in the lumen. Portal Agents are red. Endothelial agent/cells also have red Xs when they are injured.

Figure 1b. BIS-Lung Zone 1 after Progression of Injury

Figure 1b shows how excess collagen or fibrosis (brown) expands the interstitial space and distorts the alveolar spaces. Bare basement membrane is light green.
Time

• Time is represented as discrete, sequential “ticks”, emulating concurrency.

• At each tick, every agent is allowed to examine its environment and execute any conditional behavior.

• Time is abstractly represented in the model, but the correct sequence of events emerges from the behavioral rules of the agents.
Basic Immune Simulator (BIS_2010)
Cell/Agent Types:

- **Parenchymal Cells**  impart tissue function
- **Dendritic Cells**  tissue surveillance, antigen presentation
- **Macrophages**  killing pathogens, phagocytosis/scavenging
- **Granulocytes**  killing pathogens, lysis
- **Natural Killer Cells**  kill stressed cells
- **CTLs**  \( CD8^+ \) T lymphocytes, cell mediated immunity
- **T Cells**  \( CD4^+ \) T lymphocytes (\( T_{\text{H}1}, T_{\text{H}2}, T_{\text{H}17}, T_{\text{reg}}, T_{\text{FH}} \))
- **B Cells**  lymphocytes, humoral immunity (Antibodies)
- **Portals**  blood vessels, lymphatic ducts
New Basic Immune Simulator-Lung
Agent Types:

New Parenchymal Agents

• Epithelial Type I Cells  *Cover alveolar-air interface*
• Epithelial Type II Cells  *Make surfactant, regeneration*
• **Fibroblasts**  *Heal wounds, make collagen*
• Endothelial Cells  *Line the inside of blood vessels*

Other New Agent Types

• **RBCs**  *Carry O₂/CO₂*
• Platelets  *Coagulation*
Laboratory experiments...

• Some of the agents and signals included in the BIS_2010 and BIS_Lung were not previously studied in human lungs, in the context of IPF.

• The **model was incomplete without this information**. This is how an ABM can direct a modeler to the relevant laboratory questions.

• The experimental data yielded some unexpected and interesting results.
<table>
<thead>
<tr>
<th>Antibody name (antibody)</th>
<th>Description</th>
<th>Source</th>
<th>Dilution</th>
<th>Tissue pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR6</td>
<td>Chemokine receptor 6; ligands are CCL20 (MIP-3α or LARC) and human β-defensin-2. Expressed on memory T cells, B cells and dendritic cells; highly expressed on Th-17s.</td>
<td>Abcam</td>
<td>1:200</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD1a</td>
<td>Leu6, OKT8; found on T cells, normal Langerhans cells, immature dendritic cells, and cortical thymocytes. It functions in dendritic cell presentation of glycolipid antigens and dendritic cell anti-tumor response. In disease states, it labels histiocytes and leukemic thymocytes.</td>
<td>Abcam</td>
<td>1:10</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD3</td>
<td>OKT3; an integral membrane protein complex that is part of the T-cell antigen receptor</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD4</td>
<td>OKT4, Ly1, Leu3; marker of T-helper lymphocytes</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD8</td>
<td>OKT8, Ly2/Ly3, Leu2; marker of cytotoxic T lymphocytes; a coreceptor with cCD3 for antigens displayed by MHC Class I antigen-presenting cells</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD20</td>
<td>L26; a common B-cell surface marker for all but the initial and final stages of B-cell differentiation; used as a target for eliminating B cells with rituximab in lymphomas and autoimmune diseases</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD34</td>
<td>Common surface marker for adult hematopoietic progenitor cells, murine hematopoietic cells, fibrocytes, and endothelial cells</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD45</td>
<td>Leukocyte cell surface glycoprotein, mononuclear cells, and fibrocytes</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD45RO</td>
<td>UCHL1; memory T cells, fibrocytes, monocytes, and macrophages</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD56</td>
<td>N-CAM, NKH-1, Leu-19; marker for natural killer cells</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD68</td>
<td>KP1, macrosidein; glycosylated membrane protein expressed by tissue macrophages, Langerhans cells, monocytes, and fibrocytes</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>CD80</td>
<td>B7-1, BB1; a co-stimulatory molecule found on antigen-presenting cells, including dendritic cells, activated B-cells, macrophages, and epithelial cells</td>
<td>Abcam</td>
<td>1:100</td>
<td>None</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>Labels the most of the cytokeratins of the cytoskeleton in normal epithelia and epithelial carcinomas; some abnormal myofibroblasts, and normal smooth muscle cells</td>
<td>DAKO</td>
<td>1:150</td>
<td>Protease</td>
</tr>
<tr>
<td>AE1/AE3</td>
<td>Forkhead-winged helix transcription factor that defines the regulatory T cells</td>
<td>Abcam</td>
<td>1:100</td>
<td>CC1 30</td>
</tr>
<tr>
<td>IL-17</td>
<td>One member of a highly inflammatory cytokine family (IL-17α-δ) with chemotactic properties that binds with receptors on most cell types</td>
<td>Abcam</td>
<td>1:200</td>
<td>CC1 30</td>
</tr>
<tr>
<td>ROR-α</td>
<td>Retinoic acid-related orphan receptor alpha, (NR1F1, a thyroid hormone-like receptor), a transcription factor expressed in the epithelium of many tissues. Required for lymphocyte development. A receptor for cholesterol and its derivatives</td>
<td>Abcam</td>
<td>1:100</td>
<td>CC1 30</td>
</tr>
<tr>
<td>ROR-β</td>
<td>Retinoic acid-related orphan receptor-β (NR1F2), present in the brain and retina</td>
<td>Abcam</td>
<td>1:200</td>
<td>CC1 30</td>
</tr>
<tr>
<td>ROR-γ</td>
<td>Retinoic acid-related orphan receptor gamma (NR1F3), required for the lymphoid tissue development</td>
<td>Abcam</td>
<td>1:200</td>
<td>CC1 30</td>
</tr>
<tr>
<td>S100</td>
<td>A family of intracellular and secreted calcium-binding proteins</td>
<td>Ventana</td>
<td>Ready to use</td>
<td>CC1 30</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Part of the TNF superfamily</td>
<td>Abcam</td>
<td>1:100</td>
<td>Protease</td>
</tr>
</tbody>
</table>

*Note: CC1 30 indicates tissue culture conditions.*
The distribution of immunomodulatory cells in the lungs of patients with idiopathic pulmonary fibrosis

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Funded by R21HL093675 (VAF) from the National Heart, Lung, and Blood Institute of the National Institutes of Health.
H&E stain plus antibodies with different labels.

Fluorescent image of same slide

**CD3:**
T-cells

**IL-17:**
Cytokine (signal) produced by a subset of T-cells (?)

**CD68:**
Macrophages

**IL-17:**
Cytokine (signal) produced by a subset of macrophages
Cyto. AE1/3: Cytoskeletal proteins of epithelia

CCR6 (left): Cytokine receptor highly expressed by Th-17s

Cyto. AE1/3: Cytoskeletal proteins of epithelia

IL-17: Cytokine (signal) produced by Th-17s

a. H&E stain plus antibodies
More questions...

Why are the distinctively abnormal type-II epithelial cells (in the IPF cases) expressing molecules that we don’t normally associate with type-II epithelial cells?

In particular, why are they expressing IL-17, a T-lymphocyte cytokine?
What do we know about IL-17?

• IL-17 (originally called CTLA-8) was cloned from an activated T-lymphocyte cell line, (Rouvier, et al., 1993) and was noted to have homology to a Herpesvirus saimiri gene. (BTW, H. saimiri is a monkey virus.)

• Yao, et al. (1995) also studied this new cytokine in Herpesvirus saimiri, and named it IL-17.

• ~10 years ago, IL-17 was characterized as the cytokine for fighting bacterial, yeast, and parasitic infections. It has also been found to play a role in every autoimmune disease that has been probed for its presence to date.

• Almost all cells have receptors for the various forms of IL-17.

So what happened next?
Saimiri sciureus
Luckily, I have a collaborator that is willing to test my wild ideas.

Thank you, Dr. Gerard J. Nuovo
IPF Lung, abnormal epithelial type II cells

**Blue**: Herpesvirus saimiri

**Red**: IL-17

**Yellow**: Colocalization
Idiopathic pulmonary fibrosis is strongly associated with productive infection by herpesvirus saimiri

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Squirrel monkeys

Cotton Top Tamarins

Common Marmosets

Owl or Night monkeys
Conclusions:

• Agent-based modeling directs the thought process for solving a problem. It is done incrementally, with the potential for insight at any stage. (North and Macal, 2007)

• Creation of a model requires going back to the laboratory at times to fill in the blanks in the knowledge base for the model.

• Agent-based modeling can lead an investigator to the questions that need to be asked, and in this way, help find the answer to the problem.