The Glycobiology of Inflammatory Diseases and Innate Immunity

4/15/13

HIV infection/AIDS

- 1980-81: CDC reports small clusters of patients with pneumocystis pneumonia, a rare infection, among intravenous drug users and gay men
- Additional clusters of usually opportunistic infections begin to increase in frequency
- Within 3 years, French and American groups propose and identify a lymphotrophic virus as the causative agent (kills T-cells)
- Called HTLV-III (human T-lymphotrophic virus) and LAV (lymphadenopathy associated virus) by the two groups, eventually the virus was named HIV (a compromise name)
- The etiologic progression of HIV reveals the power and importance of innate immunity

Pneumocystis pneumonia, PCP (Pneumocystis carinii)

Extrem lung pathology of PCP (cystic lung pathology)

Dept. Veterans Affairs

department.med.utah.edu/WebPath/TUTORIAL/AIDS
Tracheal epithelium
--a ciliated pseudostratified columnar epithelium

Normal, clear alveolar appearance
P. carinii infection in alveolar spaces

Squashed Ping-Pong ball appearance is characteristic of the pathogen

Another rare disorder, Kaposi’s Sarcoma (skin lesions), caused by an opportunistic human herpes virus, HHV-8

Tumors appear anywhere there is vascularization and associated draining lymphatics
KS is a cancer of the lymphatic endothelium, results in excess vascularization (imparts reddish appearance to tumors).

Cryptococcus neoformans, fungal infection of the nervous system.

HIV virus budding from lymphocyte.

High early viral load seeds lymphatic tissue, subsequent period of "latency" precedes crash in immune function.
HIV infection/AIDS

- Despite decreasing T-lymphocytes, infected individuals are capable of holding-off opportunistic infections for a significant time
- Opportunistic infections increase exponentially once T-cell values drop below threshold (about 200 CD4+ cells/µl of blood)
- What underlies the suppression of opportunistic infections as T-cells drop?
- Innate vs. Adaptive Immunity

Components of Innate Immunity—the first line of defense
- Physical and anatomical barriers
  - Skin
  - Intestinal mucosa
  - Respiratory mucosa (including the ciliated pseudostratified columnar epithelium)
- Secretions from mucosal epithelial cells
  - Tears, mucus, saliva
- Granulocyte and endothelial cell functions
  - Phagocytosis
  - Killing
  - Leukocyte capture
  - Antigen processing and presentation (link to adaptive immunity)
- Molecular mechanism for recognizing and signaling the presence of pathogen
  - Toll like receptors
- Weapons of mass destruction
  - “Redness, heat, pain” = rubor, calor, dolor
  - Granulocyte and leukocyte degranulation
  - Production of Reactive Oxygen Species
  - Alternative and Lectin pathways of complement activation

What innate immunity is not...

- Capable of adapting acute responses to new molecular patterns
- Capable of remembering previous exposure to individual pathogens in order to mount an effective, tailored, targeted response
- Capable of conferring long-lasting protection
- No learning, No memory
Neutrophils among RBCs, circulating first responders

- "Microphages," professional eating machines
- Segmentated nuclei connected by strands
- PMN = neutrophils
- Migrate to infection sites in response to specific cues (cytokines and chemokines)
- Live approximately 6 hours after entering circulation
- Dead neutrophils are major component of pus

Eosinophil, another circulating first responder with different specificity, characteristic granules

- Lobed nuclei
- Eosinophilic characteristic granules
- Not very active phagocytes
- Antigen-presenting cells, activate other granulocytes and other leukocytes in response to specific stimuli
- Efficient killers of parasites

Basophil, another circulating first responder with different specificity, characteristic granules

- Lobed nuclei
- Basophilic granules, frequently obscure the nucleus
- Histamine release from basophils locally increases vascular permeability
- Activation of basophils enhances clotting

Monocytes and lymphocytes, other circulating immune cell types

- Monocytes larger than lymphocytes, clear cytoplasm, frequently nucleus is slightly indented but not lobed, become tissue macrophages
- Lymphocytes have very little cytoplasm, condensed basophilic nucleus
- Cannot tell B-cells from T-cells by histologic stains
Characteristic Granule Content

- Neutrophils: 50-70% of circulating leukocytes; “microphages,” phagocytose bacteria mostly; granules contain lysosomal enzymes, peroxidases, lysozyme
- Eosinophils: 5% of circulating leukocytes; increased during parasitic infection and some inflammatory disorders such as asthma; granules contain peroxidase, lysosomal enzymes, cytotoxic proteins
- Basophils: 1% of circulating leukocytes; associated with allergic hypersensitivity responses, massive degranulation; granules contain histamine and heparin; similar to mast cells
- Lymphocytes: 25-35% of circulating leukocytes; increased during infection; can't tell T from B by histological stains, approximately 2:1:1B
- Monocytes: 3-9% of circulating leukocytes; differentiate into tissue and circulating macrophages; agranular cytoplasm

Circulating Leukocytes

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Capture of lymphocytes at sites of peripheral inflammation requires endothelial P-selectin interaction with PSGL-1 on T-cells.

Lymphocyte extravasation in lymphatic tissues occurs at specialized endothelial domains called high endothelial venules (HEVs), post capillary venules characterized by cuboidal cells.

Lymphocytes express their own selectin, known as L-selectin.

L-selectin binds to a group of ligands known as PNAd proteas which are mucin-type proteins expressed on high endothelial cells.

The shared characteristics of the PNAd proteas is a common carbohydrate structure.
Reciprocal ligand-selectin pairs encode different homing specificities

L-selectin on lymphocytes recognizes specific glycans on PNAd proteins expressed on endothelial cells at the HEV of lymph nodes.

P-selectin on endothelial cells at sites of inflammation recognizes specific glycans on the PSGL-1 protein expressed on lymphocytes.

Note: Cytokines produced by macrophages and by activated granulocytes induce expression of P-selectin on endothelial cells and of the enzymes needed to build the recognized carbohydrate structures in lymphocytes.

Three Possible Models for Functions of CD22-Sialic (Siglec-2) Acid Interactions

Collins, B., 2002

CD22 cis-interactions modulate signaling

Collins, B., 2002
Glycobiology of Lung Inflammatory Disease (LID)

- Asthma is a pulmonary disorder characterized by recurrent wheeze, cough, or shortness of breath, reversible airway obstruction, and airway hyper-responsiveness

- COPD (chronic obstructive pulmonary disease) refers to several diseases, especially emphysema, but also chronic bronchitis and some forms of asthma that evolve into fixed obstruction and remodeling of airways

- Both asthma and COPD involve airway obstruction, but in COPD the obstruction is progressive and largely irreversible. Over time, alveolar damage worsens, air trapping progresses, and the ability of the lung to facilitate gas exchange decreases

- Current epidemiologic estimates suggest that 20 million Americans suffer from asthma and 16 million from COPD. The most recent evaluations indicate that asthma treatment approaches $18 billion annually. Treatment for asthma and COPD currently targets symptoms and not cause.

Asthma

“Chronic inflammatory lung disease characterized by reversible airway obstruction and airway hyper-reactivity”

- Chronic respiratory diseases is the 4th leading cause of death
- 1 in 12 people (about 25 million) in 2009
- ~$3,300/person/year for years 2002-2007

- Increased mucus production
- Infiltration into the site of inflammation
- Eosinophil (asthma)
Psuedostratified columnar epithelium...

...with a ciliated apical surface (ciliated pseudostratified columnar epithelium)
Epithelial morphology along the airway

Bronchi with residual cartilage

Bronchiole with smooth muscle (asthma constriction)

Alveolar epithelium
Alveolar cells

Asthma model—increased smooth muscle (actin) and eosinophilic infiltration

Glycobiology of LID

- Asthma:
  - Eosinophilia in larger airways (trachea, bronchi, bronchioles)
  - Mast cell activation
  - Alveolar infiltration and remodeling in severe asthma
  - Neutrophilia in severe asthma (due to corticosteroid treatment?)

- COPD
  - Neutrophilia in small airways (bronchioles, alveolae)
  - No Mast cell component
  - Remodeling apparent, along with fibrosis

- No curative therapies, only symptom amelioration (corticosteroids for inflammation, β2-agonists for smooth muscle relaxation)
- Animal models available (ova-induced, smoke-induced, interleukin expression)
- Human tissues and primary cell types available
Siglec-8/SAF-2

- Identified and published in 2000 (Bochner) as a cDNA enriched in activated lung eosinophils
- Monoclonal antibody generated against bacterially expressed protein, 2E2
- Antibody treatment of eosinophils demonstrates interesting property....

Siglec-8 cross-linking induces lung eosinophil apoptosis

Siglec-8/F binding specificity

Siglec-9/E binding specificity

Model for possible siglec-mediated cell signaling events
Culture conditions matter

What is the endogenous Siglec-F counter receptor?

1. mTEC (mouse tracheal epithelial cells) lysate
2. mTEC culture medium
3. OVA-sensitized/challenged mouse lung tissue lysate
4. OVA-sensitized/challenged mouse BAL (bronchoalveolar lavage) fluid
Immunoprecipitation of Siglec-F-binding proteins and proteomic analysis

Reduction & alkylation
Trypsin digestion
LC-MS/MS (LTQ Orbitrap)

Further evidence that Muc5b is a Siglec-F ligand

Anti-Muc5b ab stains with a similar pattern to Siglec-F-Fc

Siglec-F i.p. pulls down Muc5b

MS identified Muc5b peptides from 500kDa bands

Muc5b is a strong candidate for being a Siglec-F-binding protein

Mucin-5b (MUC5B)
- MUC5B is one of the gel-forming respiratory mucins and main component of mucus (saliva, nasal mucus, airway mucus)
- The largest secreted protein (human: 5762aa, mouse: 4782aa)
- Mucin-type O-glycosylation
Analysis of O-linked glycans from mMuc5b protein

Muc5b protein purification from mTEC culture media
1. mTEC culture media
2. D-sephrose anion-exchange column chromatography
3. Sepharose CL-4B gel filtration column chromatography
4. Concentration with 100k cut-off filter membrane

O-glycans are responsible for Siglec-F binding

Characterization of O-linked glycans from mMuc5b

Sulfated glycan
- Core 2
- Core 3

Non-sulfated glycan
- Core 1
- Core 2

Determination of the position of the sulfate group in S,NeuAc,Hex,HexNAc,GalNAc-ol

Positive ion mode
MS of m/z 1432

Negative ion mode
MS of m/z 1386
Muc5b dampens eosinophilic airway inflammation by inducing apoptosis through the interaction with Siglec-F.

Trans-engagement triggers Siglec-induced Apoptosis

6'-sulf-o-Lex?

Removal of sulfate from Muc5b

Control

kD

250

150

50

10

Siglec-F-Fc