Sialoside-based pattern recognition in self-nonself discrimination of innate immunity

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Two Types of Immune Recognition

• By clonally distributed receptors
  TCR/Ig: VDJ re-arrangement, hyper mutations (Ig)
  Plus: Diversity, Precision; Minus: Low frequency, slow

• By broadly cross-reactive receptors: pattern recognition
  Plus: high frequency, rapid response
  Minus: Imprecise and therefore it can be pathogenic
Approaching the asymptote? Evolution and revolution in immunology.

*Cold Spring Harb Symp Quant Biol.*


Father of pattern recognition in self-nonself discrimination
Linking innate to adaptive immunity through Pathogen-associated molecular Patterns (PAMPs)

- Pathogen patterns form the basis of self-nonself discrimination of the immune system (Janeway 1989)
- TLR as the PAMP receptor that can induce expression of B7 and inflammatory cytokines (Medzhitov and Janeway, 1997).
DAMPs: Danger-associated molecular pattern

Immune system detects and protects against danger (Matzinger, 1994)

Hsp70 as a signal of cellular injury

HMGB1 as a prototype of Alarmin (DAMP)

DAMP response also depends on TLR
The paradox

If the same pattern recognition receptors can be triggered by both microbes and intracellular components, how can the immune system discriminate infectious nonself from noninfectious self?

A surprising observation in mice with targeted mutation of CD24
What is CD24 to Immunologists

First identified by Springer as the heat-stable antigen (1977).

Small GPI-anchored molecule expressed on multiple lineage of hematopoietic cells.

Widely used as markers for development of T and B lymphocytes.

Implicated in T cell costimulation, traffic and autoimmune diseases
What is CD24 to Cancer Biologists

Over expression observed in more than 70% of all cancer.

In and on cancer cells.

A marker for cancer stem cells (positive or negative).

Expression level is a prognostic marker.

Polymorphism affects cancer response to chemotherapy.
The CD24 headache

Cd24-/-
CD24 confers resistance to Tylenol acetaminophen10 mg/mouse, dissolved in H₂O

CD24⁺/⁺
11/11 survive

CD24⁻/⁻
0/11 survive

Survival (%)
Detoxication reaction of tylenol (paracetamol) in liver

paracetamol

↓

NAPQI (N-acetyl-p-benzoquinone imine)

GSH

Glutathione conjugate

↓

Paracetamol covalently bound cell macromolecules

↓

Mercapturic acid Conjugate (Excreted in urine)

↓

Necrosis

↓

Innate immunity leading to liver damage
**A**

- **IL-6 (pg/ml)**
  - WT: Lower levels
  - CD24^{-/-}: Higher levels
  - AAP: No significant difference

- **MCP-1 (pg/ml)**
  - WT: Lower levels
  - CD24^{-/-}: Higher levels
  - AAP: No significant difference

- **TNF (pg/ml)**
  - WT: Lower levels
  - CD24^{-/-}: Higher levels
  - AAP: No significant difference

**B**

- **ALT (Units/L)**
  - WT: Lower levels
  - CD24^{-/-}: Higher levels
  - AAP: No significant difference

**C**

- **Histological images**
  - WT: Normal liver tissue
  - CD24^{-/-}: Increased inflammation and fibrosis
How is CD24 involved?

Anti-CD24 immunoprecipitation

Elute with EDTA to release CD24 associated molecules

Bulk sequencing of released molecules By Mass Spectrometry
# HMGB-1

## Peptide matches

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>57-64</td>
<td>GKFEDMAK</td>
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<tr>
<td>154-162</td>
<td>YEKDIAAYR</td>
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<tr>
<td>76-85</td>
<td>TYIPPKGETK</td>
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<tr>
<td>30-42</td>
<td>HPDASVNFSEFSK</td>
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<tr>
<td>114-126</td>
<td>GEHPGLSIGDVAK</td>
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<tr>
<td>29-42</td>
<td>KHPDASVNFSEFSK</td>
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<tr>
<td>112-126</td>
<td>IKGEHPGLSIGDVAK</td>
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<tr>
<td>128-145</td>
<td>LGEMWNNTAADDKQPYEK</td>
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<tr>
<td>127-145</td>
<td>KLGMWMWNNTAADDKQPYEK</td>
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</table>
HMGB1 release responsible for enhanced toxicity

Survival (%)

0/8

7/8

PBS anti-HMGB-1

Survival (%)

0

50

100

PBS anti-HMGB-1

ALT (Units/L)

PBS anti-HMGB-1

**

*   *

PBS anti-HMGB-1

WT CD24 -/-

ALT (Units/L)

0

300

600

900

1200

1500

PBS anti-HMGB-1

**

PG/ml

IL-6 MCP-1 TNF-α

*   *

**
CD24: a GPI-anchored glycoprotein

AA sequence:
SETTTGTSSNSSQSTSNSSLAPNPTNATTKV

Crocker et al.
Sandwich ELISA to identify CD24-binding Siglecs

Coated Siglec-Fc fusion proteins or control Fc on plate.

Added lysates of spleen cells. Those from CD24-deficient mice were used as negative control.

The CD24 that bound to Siglecs were detected with biotinylated anti-CD24 mAb followed by HRP-conjugated streptavidin.
Tri-molecular complex: HMGB1-CD24-Siglec10/G

IP

WT
Input  Fc  Siglec-10-Fc  Input  Fc  Siglec-10-Fc

CD24

HMGB-1

hlg-Fc

IB

WT
Anti-Siglec-G  Mouse IgG

CD24-/-
Anti-Siglec-G  Mouse IgG

IB

Siglec-G

CD24

HMGB-1
Siglec G mutation phenocopies CD24-/-
No impact on survival to endotoxin challenge

Survival fractions

Hours after LPS injection

WT (n=10)
Siglecg<sup>-/-</sup> (n=10)
CD24<sup>-/-</sup> (n=8)
PAMPs

A

TLR-Ligand

TLR

MYD88/TRIF

NFkB

Inflammatory cytokines

DAMPs

B

HMGB1/HSP70/90

CD24

TLR

Siglec-G

MYD88/TRIF

NFkB

Inflammatory cytokines

Science 2009
Trends Immunol 2009
Regulation of host response to DAMPs and autoimmune diseases

- Lupus and rheumatoid arthritis are characterized by defective clearance of intracellular components

- Nucleosomes isolated from lupus-prone mice induces inflammatory cytokines via TLR2, TLR9, TLR4

- Genetic manipulations that reduce clearance of these components leads to arthritis and other lupus like symptoms
Implications of host response to DAMPs to therapy of autoimmune diseases

• Inflammatory cytokines such as IL-6, IL-1β and TNFα are major therapeutic targets for autoimmune diseases, such as rheumatoid arthritis, Crohn’s diseases

• Very little headway has been made at targeting the cause of the production of these cytokines.
Human CD24 polymorphism and autoimmune diseases


Replicated by multiple studies in MS, RA and SLE

Not showing up in any GWAS before the region was not evaluated
<table>
<thead>
<tr>
<th>CD22</th>
<th>NEU3</th>
<th>4</th>
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<tr>
<td>CD22</td>
<td>K84E</td>
<td>K371R</td>
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<td>CD33</td>
<td>T30A</td>
<td>F243L</td>
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<td>NEU1</td>
<td>T76P</td>
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<td>NEU3</td>
<td>H366P</td>
<td>T383P</td>
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<tr>
<td>Siglec5</td>
<td>G284C</td>
<td>H399D</td>
</tr>
<tr>
<td>Siglec7</td>
<td>T80A</td>
<td>W290R</td>
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<tr>
<td>Siglec8</td>
<td>R171K</td>
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<tr>
<td>Siglec14</td>
<td>E305K</td>
<td>P307A</td>
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Novel Siglec coding Variants among African American RA patients.
CD24 Fc interact with DAMPs

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**Diagram:**
- CD24 Fc
- C1, C2, C3
- Disulphide bond
- Hinge region

**Table:**
- IP
- HMGB-1 input
- CD24-Fc
- CD24-Fc + HMGB-1
- Fc + HMGB-1
- CD24-Fc + HMGB-1 + EDTA

**Results:**
- IB anti-hIgG anti-HMGB-1
CD24Fc binds to Siglec 10: dependence on sialylation

Chen et al. Nat Biotechnol 2011
CD24 induces recruitment of SHP-1 to Siglec G

CD24−/− spleen cells

HMGB1/HSP70/90 → TLR → MYD88 → SHP1 → NFκB → Inflammatory cytokines

IP | Ctrl serum | Anti-Siglec G
---|-----------|-------------
Stimuli | PBS | PBS | IgG Fc | CD24 Fc
SHP-1
p-Tyr (Siglec G)
Siglec G
CD24 regulates production of inflammatory cytokine by human macrophages
CD24Fc suppression production of multiple inflammatory cytokines
Bovine collagen-induced arthritis: DBA/1 mice

a. Prophylactic CIA

CD24Fc 1mg (n=7)  
PBS (n=9)

Days after the first immunization

Scores

Fisher PLSD P=0.02

b. Therapeutic CIA

CD24Fc 0.2mgx5 (n=5)  
PBS (n=6)

Fisher PLSD P=0.02
Similar results observed on two models of collagen-induced arthritis, therapeutic effect can be observed after onset of diseases.

Does the therapeutic effect rely on its interaction with Siglec G?
Clinical Scores

WT

CD24Fc (n=5)

PBS (n=5)

p<0.0001

P=0.02

Siglecg−/−
Does the therapeutic effect rely on its interaction to Siglec G? Yes but
Clinical Score

Fisher’s PLSD test  p<0.0001

CD24Fc

PBS

Fisher’s PLSD test  p<0.0001

WT

% inhibition=70%

% inhibition=52%

Days after induction
Does the therapeutic effect rely on its interaction to Siglec G? Partially.

Other possibilities: reduction of DAMPs? Other receptors?
Summary II

CD24Fc interacts with DAMPs and Siglecs to suppress inflammatory cytokine production.

CD24Fc show therapeutic effect in multiple models of autoimmune diseases including those for rheumatoid arthritis and multiple sclerosis.
Infection = PAMPs + DAMPs

- TLR-Ligand
  - TLR
  - MYD88
  - NFkB
  - Inflammatory cytokines

- HMGB1/HSP70/90
  - CD24
  - Sglec G
  - TLR
  - MYD88
  - NFkB
  - Inflammatory cytokines
Polybacterial Sepsis model

Cecal ligation and puncture
Microbial disarming of CD24-Siglec G mediated negative regulation

Pathogens

PAMP

TLR

Inflammatory cytokines

Sialidase

CD24

CD24

DAMP

Sialidase

CD24

TLR

Inflammatory cytokines

Desialylation of CD24 in sepsis

Sialidase activity

<table>
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<tr>
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<th>Sham</th>
<th>CLP</th>
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<tbody>
<tr>
<td>WT, shanm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WT, LPS</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Sipteckg -/-</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>CD24 -/- CLP</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

IB:CD24

Total spleen cells

Cell Number (% max)

SNA(α2,6) MAA(α2,3)

CD11c positive cells

MAA sham CLP

SNA sham CLP
Sera from CLP mice desialyate CD24Fc and disrupt its binding to Siglec 10.
Sialidase inhibitors protects mice against sepsis

AC: 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (Neu5AC2en)
GC: 2,3-dehydro-2-deoxy-N-glycolylneuraminic acid (Neu5GC2en)

Nat Biotechnol 2011
S. Pneumoniae sialidases exacerbate sepsis by targeting CD24-Siglec 10 interaction

D39: NanA⁺NanB⁺
mD39: NanA⁻NanB⁻
Central dogma in translational medicine:
Write a transcript before translation

1. CD24-Siglec G/10 interaction represses response to DAMPs, ignores that to PAMPs
   CD24Fc as a biological therapeutic for RA and MS
   FDA approval for first in human studies received

2. Microbes disrupt sialidase-based pattern recognition through desialylation
   Sialidase as potential therapeutic targets for sepsis
Take home messages

1. CD24-Siglec G interaction inhibits inflammatory responses to damage-associated molecular pattern but not those to pathogen-associated molecular patterns.

2. Preserving the sialoside-based pattern recognition may have therapeutic implications for autoimmune diseases and sepsis.
Physiological function of inflammation

Bring immune system into infected tissues to execute its effector function.

Inflammatory cytokines limit infection, directly or indirectly.

Fine-tuned inflammation promotes tissue remodeling (regeneration) following injuries.
Inflammation

- Autoimmune diseases
- Sepsis
- Metabolic syndrome
- GvHD
- AIDS

DAMP
CD24
Siglec
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