Cow Ultralong CDR3 Antibodies in Immunology: Targeting HIV and Exhausted T-cells

Antibody Engineering
San Diego
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Molecular Medicine
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Cluster of Differentiation “CD” markers define cells and can be drug or diagnostic targets

CD markers range from CD1 to CD371

Some CDs cover a group of closely related proteins or carbohydrates (e.g., CD1a, CD1b, CD1c, and CD1d)

- It is estimated that only 33% of surface markers have been discovered/characterized for leukocytes!!

- “…a large number of these Abs remain poorly validated. One common observation is that mAbs often recognize recombinant proteins, typically the immunogen, but are unable to react with the endogenous or native protein on live primary cells.”

CD Molecules are defined by antibodies, originally discovered through cell immunization.

OKT3/Muromonab (the first FDA approved mAb) defined CD3

Kung P, Goldstein G, Reinherz EL, Schlossman SF
FDA Approved antibodies originally discovered through immunization with cells

- **Muromonab/OKT3 (CD3); T-cells**
  Kung P, Goldstein G, Reinherz EL, Schlossman SF
  Monoclonal antibodies defining distinctive human T cell surface antigens.

- **Rituximab/C2B8 (CD20); lymphoblastoid cell line SB**
  Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20.

- **Trastuzumab/4D5 (Her2); NIH 3T3/Her2**
  Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A
  p185HER2 Monoclonal Antibody Has Antiproliferative Effects In Vitro and Sensitizes Human Breast Tumor Cells to Tumor Necrosis Factor
CD antigens can be complex…

CD3

CD20
Some proteins can not be easily purified

Multispanning membrane proteins:
GPCRs, ion channels, membrane complexes
Targets on cells can be different than purified protein.

Many useful epitopes may be missed in antibody discovery efforts using purified protein.
66% of leukocyte surface determinants are uncharacterized

Why??

– Tolerance
– Researchers have new technologies, and other more interesting things to do (who wants to keep immunizing mice with cells?)
– Mouse antibodies may have biases towards certain epitopes
Different paratoposes have evolved in different organisms.
Cows have long CDR H3s, with an ultralong subset (40-70 amino acids)
Cow ultralong CDR H3s have protruding ‘stalk’ and ‘knob’ minidomains

Wang et.al. (2013) *Cell* 153: 1379-1393
Five structures reveal conserved and diverse features of ultralong CDR H3s

Stanfield, et.al. (2016) Science Immunology: 1(1)
A conserved 3-strand core forms a scaffold for two highly diverse loops and disulfide connectivity

“CDR-like” loops?
Diversity in:
• Sequence content
• Length
• Shape/disulfide connectivity

Stanfield, et.al. (2016) Science Immunology: 1(1)
Loops within ultralong CDR H3s may be CDRs themselves

CDR = Complementarity Determining Region

CDR Loops?
Cow antibodies have remarkable structural diversity

Cows use only one V-D-J recombination event to produce ultralong CDR H3 antibodies.

Combinatorial diversity through V(D)J recombination is severely limited!

Wang et.al. (2013) Cell 153: 1379-1393
The D region IGHD8-2 is primed for mutation to cysteine

**Bovine D<sub>H</sub>2**

<table>
<thead>
<tr>
<th>GT</th>
<th>AGT</th>
<th>TGT</th>
<th>CCT</th>
<th>GAT</th>
<th>GGT</th>
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</table>

Red: amino acids that can be mutated to cysteine with a single nucleotide change

Boxes: Somatic hypermutation “hotspots”
Ultralong CDR H3 sequences are enormously diverse

<table>
<thead>
<tr>
<th>V_H</th>
<th>N(?)</th>
<th>D_H</th>
<th>J_H</th>
<th>(L)</th>
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<td>D_H2 Germ</td>
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<td>J_H1 Germ</td>
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<td>BLV5B8</td>
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<td>(12 cys) CSPVHQ</td>
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<td>HVDWT</td>
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</tbody>
</table>
Other creatures have IGHV1-7 genes

- **Bos frontalis**
  - Gayal

- **Bos indicus**
  - Zebu

- **Bos grunniens**
  - Yak

- **Bison bonasus**
  - European bison

- **Bison bison**
  - American bison

- **Bos primigenius**
  - Auroch (extinct, Lascaux cave painting, France)
Can cow antibodies bind unusual or challenging targets?
Broadly neutralizing anti-HIV antibodies have long CDR H3s
The surface of HIV has evolved to minimize induction of and recognition by broadly neutralizing antibodies.

Few Env spikes irregularly spaced

Env spike = (gp120)₃(gp41)₃

Spike shows great sequence variability, is metastable and is glycan coated.
Can cows develop broadly neutralizing antibodies against HIV?

HIV gp120 (BG505) → Neutralizing Antibodies?
Ultralong CDR H3 sequences are selected in BG505 SOSIP immunized cows
Serum from immunized cattle are broadly neutralizing against a 12-strain HIV panel

Broadly neutralizing titers develop rapidly

Breadth is maintained against 117 diverse HIV-1 strains

Monoclonal antibodies broadly neutralize HIV by binding the CD4 binding site on gp120

<table>
<thead>
<tr>
<th>V</th>
<th>N</th>
<th>D</th>
<th>J</th>
<th>L</th>
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<tr>
<td>NC-Cow1</td>
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<td>NC-Cow8</td>
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</table>

**NC-Cow1**

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<tr>
<th>Clade</th>
<th>n</th>
<th>% Breadth</th>
<th>Median IC50</th>
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<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>100%</td>
<td>0.021</td>
</tr>
<tr>
<td>B</td>
<td>23</td>
<td>83%</td>
<td>0.151</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>67%</td>
<td>0.021</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>50%</td>
<td>0.007</td>
</tr>
<tr>
<td>G</td>
<td>7</td>
<td>57%</td>
<td>0.019</td>
</tr>
<tr>
<td>AC</td>
<td>5</td>
<td>40%</td>
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<tr>
<td>AE</td>
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<td>75%</td>
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<tr>
<td>AG</td>
<td>7</td>
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<td>BC</td>
<td>10</td>
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<tr>
<td>ACD</td>
<td>2</td>
<td>100%</td>
<td>0.057</td>
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</table>

**Legend**

- **VRC01-class**: VRC01, 12A12, 3BNC17, b12, CH103, PG121, PG128, PGDM1400, PG9, PG7151, 35022
- **CD4bs**: self, V3-glycan, V2-apex, Interface

**Graphical Representation**
NC-Cow-1 binds the CD4 binding site

Anti-gp120 antibodies have high affinity

<table>
<thead>
<tr>
<th>IgG</th>
<th>$K_D$ (M)</th>
<th>$k_a$ (1/Ms)</th>
<th>$k_d$ (1/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC-Cow-1</td>
<td>$4.198 \times 10^{-12}$</td>
<td>$5.592 \times 10^5$</td>
<td>$2.347 \times 10^{-6}$</td>
</tr>
<tr>
<td>P1F1</td>
<td>$4.640 \times 10^{-11}$</td>
<td>$6.508 \times 10^6$</td>
<td>$3.020 \times 10^{-4}$</td>
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<td>P3H4</td>
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<td>$1.315 \times 10^6$</td>
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<td>P4F8</td>
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<tr>
<td>P4G8</td>
<td>$1.345 \times 10^{-11}$</td>
<td>$1.829 \times 10^6$</td>
<td>$2.460 \times 10^{-5}$</td>
</tr>
</tbody>
</table>
LETTER

Rapid elicitation of broadly neutralizing antibodies to HIV by immunization in cows

Devin Sok¹,²,³,⁴, Khoa M. Le¹,²,³,⁴, Melissa L. Vudnais⁵, Karen L. Saye-Francisco¹,²,³, Joseph G. Jardine¹,²,³, Jonathan L. Torres⁶, Zachary T. Berndsen⁶, Leopold Kong⁶, Robyn Stanfield⁶, Jennifer Ruiz¹,²,³,⁴, Alejandra Ramos¹,²,³,⁴, Chi-Hui Liang¹,²,³, Patricia L. Chen⁷, Michael F. Criscitiello⁷, Waithaka Mwangi⁸, Ian A. Wilson²,³,⁶, Andrew B. Ward²,³,⁶, Vaughn V. Smider⁵ & Dennis R. Burton¹,²,³,⁹

Can we use cow antibodies to identify new surface determinants or epitopes on leukocytes?

- **Exhausted T-cells**
  - Chronic infection
  - Cancer
  - Therapeutic MOA (PD-1, checkpoint inhibitors)
Exhausted T-cells form through persistent antigen stimulation

Naïve T-cell → Antigen stimulation (acute phase) → Effector T-cell → Memory T-cell

Antigen clearance

Effector T-cell → Antigen persistence (chronic infection, tumors) → Exhausted T-cell

- PD-1
- LAG3
- TIM3

Checkpoint molecules impact the function of exhausted T-cells

Immunization of a cow with exhausted T-cells

Exhausted T-cells (LCMV clone 13)

Unique mAbs? Antigens? Epitopes?
Immune serum binds known markers of exhausted T-cells
Western blot with serum detects unique bands in exhausted T-cells
Serum binds exhausted T-cells by flow cytometry

![Graph showing flow cytometry results for pre-bleed and 3rd bleed with Anti-cow IgG-FITC and FITC MFI measurements.](image)
Screening monoclonal bovine antibodies for binding exhausted T-cells
Two clones have identical CDR H3s with an unpaired Cys, and a non-canonical Cys in CDR H2

Heavy Chain

Light Chain

121

QVQLRESGPSLVKPSQTLSLTCTVSGFSLRSYAVTWVRQAPGKAELCLGRRTGTSEEYNPALKSRLSTKDNSKSQVSLSVSSVTPEDTATYYCAKHAYNGCAGDAIGHLDWQGGLLVTVSS

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V2

V30

18
Ultralong CDR H3 sequences are found in hits

41

QVQLRESGPSLVKPSQLLTCTASGFSLSDKAVGWVRQAPGKALEWLGSIDTGGSTGYNPGPLSRSLSTKDNSKSQVSLSSVTTEDSATYYCTTVHQETKESC\PAGIYITTRLRC\DDYND\CDYDARGG\CSSR\DVI\TYFY\V\DAWGQ\L\LV\V\SS

50

127

QVQLRESGPSLVKPSQTL\LTCTVSGFS\SSYAVNWVRQAPGKALEW\GAIGSGGGTNPPL\SK\RS\LSTKD\NS\KSQV\S\LS\MN\SV\TPEDTATYY\CTTVHQETTK\QR\CPDDHI\YW\GCD\SSCCD\SD\SC\RCTKPSGSGWYGAPN\R\TY\IANL\HIDS\WGRG\LL\LV\V\SS

61
Conclusions

- Cows have an unusual ultralong CDR H3 comprising “stalk” and “knob” features
- A rich diversity of sequence and cysteine content is found in ultralong CDR H3s
  - Unique disulfide bond patterns are formed within the knob
  - A germline DH region is predisposed to mutate to cysteine
  - A conserved β-sheet and two diverse loops comprise the knob (new CDRs?)
- Cows produce a broadly neutralizing response against HIV, unlike other organisms
  - Breadth is rapid and mediated by ultralong CDR H3 antibodies
  - The monoclonal antibody NC-Cow-1 neutralizes 72% of HIV strains
  - NC-Cow-1 binds the CD4 binding site on gp120 with high affinity
- The novel structural diversity of cow antibodies may allow identification of antibodies to unique epitopes and antigens to potentially expand the known cell surface determinants (CDs)
  - Cows make a robust immune response against exhausted T-cells
  - Serum reacts to LAG3, TIM3, PD1
  - Screen, screen, screen: New monoclonal antibodies with unusual CDR H3s will be characterized for known antigen binding, cellular specificity, and function.
Funding: NIH, DoD, American Cancer Society, USDA

References:
Stanfield, et al. (2016) Science Immunology: 1(1)
Collaborators

**Scripps**
- **Dennis Burton**
- **Devin Sok**
- Khoa Le
- Karen Saye-Francisco
- Joseph Jardine
- Jennifer Ruiz
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- Chi-Hui Lang

**Texas A&M**
- **Andrew Ward**
  - Jonathan Torres
- **Ali Torkamani**
- **John Teijaro**

- **Ian Wilson**
- **Robyn Stanfield**
- Damien Eckiert
- Wenli Yu
- Leopold Kong
- Michael Criscitiello
- Waithaka Mwangi
- **Thad Deiss**
- Patricia Chen
- Terje Raudsepp
Thank You!

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