Gene therapy for leukocyte adhesion deficiency: the road from Irish setters to children

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National Cancer Institute

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Our graduates are outstanding in their field
Synopsis of talk

• Leukocyte adhesion deficiency (LAD)
• Canine leukocyte adhesion deficiency (CLAD)
• Gene therapy for CLAD
• Gene therapy for LAD?
“I am always ready to learn, although I do not always like being taught.”

Winston Churchill
Phenotype of leukocyte adhesion deficiency

- Delayed umbilical cord separation
- Life-threatening bacterial infections
- Severely impaired wound healing
- WBC count very high
- Absence of pus
Leukocyte adhesion deficiency due to mutations in leukocyte integrin CD18
CD18 Mutations in LAD

β₂ subunit/CD18

Signal sequence

“I/A” domain

Cysteine rich domain

MIDAS motif

DXSXSD

N351S

K332-N361del

G284S (2)

G273R

P247ins

K196T

P178L (2)

G169R (2)

L148P

S138P (MIDAS)

D128N

R593G

R586W

G570D

1 22 110 361 449 628 700 723 769
LAD neutrophils have defective adhesion and migration
Defective PMN adhesion in LAD

Normal Donor

LAD Patient

Normal Donor + Anti-CD18 mAb
Neutrophil migration and phagocytosis

QuickTime™ and a decompressor are needed to see this picture.
Two Views of Leukocyte Trafficking

- **Cohnheim (1889)**
  - Inflammatory signal alters endothelium

- **Metchnikoff (1893)**
  - Inflammatory signal alters leukocyte surface
Ilya Metchnikov- Father of the Phagocyte
Treatment of LAD

- Prophylactic antibiotics - Don’t cure
- Granulocyte infusions - Short lived, 6 hrs
- Hematopoietic stem cell transplantation - problems with graft reject and GVHD
- Gene therapy?
What about gene therapy for LAD?

- disease is life-threatening
- only other definitive treatment is BMT
- gene transfer corrects defect in vitro
- <10% CD18+ PMN may reverse phenotype
- Unlike BMT all pts have donor, no GVHD
Variables in hematopoietic stem cell gene therapy

- How to deliver therapeutic gene?
- Is conditioning needed for stem cells to engraft?
- Is immunosuppression required to prevent stem cells from being rejected?
- What model to assess variables?
Potential animal models of LAD

- Murine models of LAD (gene targeting)
- Bovine LAD (BLAD)
- Canine LAD (CLAD)
Bovine LAD (BLAD) in Holsteins

BLAD calves infections, diarrhea, weight loss - US prev 15%

Point mutation in CD18 (Asp to Gly at aa 128)

BLAD calves homozygous

Osborndale Ivanhoe - Godfather of BLAD
Canine granulocytopathy syndrome: neutrophil dysfunction in a dog with recurrent infections

H.W. Renshaw, C. Chatburn, G.M. Bryan, R. C. Bartsch, and W.C. Davis

Journal of the American Veterinary Medical Association
Volume 166 (5), pp 443-7, 1975

Male Irish Setter dog with recurrent bacterial infections, pyrexia and severe neutrophilia. Syndrome resembled granulocytopathies described in man and other animals.
Deficiency of Leukocyte Surface Glycoproteins Mo1, LFA-1, and Leu M5 in a Dog With Recurrent Bacterial Infections: An Animal Model

By Urs Giger, Laurence A. Boxer, Paul J. Simpson, Benedict R. Lucchesi, and Robert F. Todd III

A dog with severe recurrent bacterial infections, impaired pus formation, delayed wound healing, and severe persistent leukocytosis was the result of a mother–son mating. Assessment of leukocyte function revealed profound abnormalities in adherence-dependent activities including impaired granulocyte adhesion to glass/plastic surfaces or nylon wool, decreased granulocyte aggregation and chemotaxis, and diminished lymphocyte blastogenesis, but normal neutrophil oxidative activity, serum immunoglobulin, and complement levels. By immunofluorescence analysis, CD11b and CD18 monoclonal antibodies specific for the 155-kd α polypeptide of Mo1 (gp 155, 94) and the 94 kd β peptide common to Mo1, LFA-1 (gp 170, 94), and Leu M5 (p 150, 94) (surface molecules that promote leukocyte adhesion) failed to bind to unstimulated and A23187 calcium ionophore-stimulated granulocytes or mononuclear cells of the affected dog as compared with strong specific binding to canine control cells. The Mo1 glycoproteins were only barely detectable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of immunoprecipitates from lysates of 125I surface-labeled neutrophils from the affected dog as compared with intense bands seen with canine control cell precipitates. We conclude that this dog has a severe leukocyte surface glycoprotein deficiency syndrome that is similar, if not identical, to that recently recognized in humans. Dogs with deficiency of leukocyte Mo1, LFA-1, and Leu M5 expression may represent a useful animal model to characterize further the molecular basis for an inherited disorder in leukocyte effector function. ©1987 by Grune & Stratton, Inc.
CLAD in Irish setters in Sweden
Swedish CLAD Carrier Pups
CD18 mutation in CLAD

Human CD18 mutations (>50)

Cys36Ser  Asp128Asn
CLAD     BLAD

(Kijas, Bauer, et. al, Genomics, 1999)
Canine leukocyte adhesion deficiency

- Episodes of fever with bacterial infections leading to death by 6 months of age

(Bauer, Peds Res, 2004)
Why is CLAD a good model for gene therapy?

- CD18 molecule is detected by flow cytometry on peripheral blood leukocytes
- Results in canine model translate to humans
- Scale-up for human trial is minimal
Gene transfer of CD18

CLAD cell

CLAD cell after gene transfer

Vector

CD11

CD11/CD18

CD18
Questions for CLAD gene therapy

• What conditioning is required for engraftment?
• Is immunosuppression required to prevent rejection of the CD18+ cells?
• What vectors and transduction conditions?
• Who will do the work?

Laura Tuschong

Dr. Thomas R. Bauer, Jr., PhD
Choices of retroviruses for gene transfer

**Gammmaretrovirus**

**Lentivirus**

**Foamy Virus**
Rationale for foamy virus vector

• Broad host range
• Large packaging capacity >13 kb
• Wild-type virus is non-pathogenic
• Transduction of HSC of mice, dogs, and humans with minimal ex vivo culture
• May integrate less frequently in genes?
Four plasmid foamy vector system

\[ \Delta \Phi \text{Mscv-cCD18 vector} \]

\[ \Delta U3 \text{LTR} \]

\[ \Delta U3 \text{LTR} \]

Helper plasmids

\[ \text{CMV} \]

\[ \text{gag} \]

\[ \text{pA} \]

\[ \text{CMV} \]

\[ \text{env} \]

\[ \text{pA} \]

\[ \text{CMV} \]

\[ \text{pol} \]

\[ \text{pA} \]
Foamy viral vector gene therapy

Re-infusion (Day 0)

CLAD dog

200cGy TBI (Day -1)

BM Harvest CD34 selection

Transduce with $\Delta \Phi M$scv-cCD18 vector supernatants + cGCSF, cSCF, hFlt3L
Foamy vector transduced cell doses infused

<table>
<thead>
<tr>
<th></th>
<th>CD34+/kg</th>
<th>% CD18+</th>
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<tbody>
<tr>
<td>Nugget</td>
<td>2.3 x 10^6</td>
<td>13.7%</td>
</tr>
<tr>
<td>*Jazz</td>
<td>2.2 x 10^6</td>
<td>23.0%</td>
</tr>
<tr>
<td>Ash</td>
<td>1.7 x 10^6</td>
<td>24.6%</td>
</tr>
<tr>
<td>Cactus</td>
<td>3.1 x 10^6</td>
<td>23.2%</td>
</tr>
<tr>
<td>Sequoia</td>
<td>3.4 x 10^6</td>
<td>22.2%</td>
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* Died 7 days post-TBI from intussusception
Kinetics of peripheral blood CD18+ neutrophils

- % CD18+ Neutrophils

- Months post infusion

Graph showing the percentage of CD18+ neutrophils over time post infusion for different groups labeled as Nugget, Ash, Cactus, and Sequoia.
Kinetics of peripheral blood CD18+ lymphocytes

% CD18+ Lymphocytes vs Months Post Infusion

- Nugget
- Ash
- Cactus
- Sequoia
Questions

• Did we reverse the CLAD phenotype?
• Why is the % of CD18+ lymphocytes high?
• Did we have genotoxicity from integrations?
Correction of CLAD phenotype with FV

- UD1
- UD2
- UD3
- UD4
- FD1
- FD2
- FD3
- FD4

Months Post Birth

- Infection
- Infusion
Clinical correction of CLAD phenotype

WBC Count (K/μL)

Months Post Infusion

- Nugget
- Ash
- Cactus
- Sequoia

Normal Range
Questions

• Did we reverse the CLAD phenotype?
• Why is the % of CD18+ lymphocytes so high?
• Did we have genotoxicity from integrations?
CD18+ T-lymphocytes proliferate at low concentrations of mitogen
Enhanced proliferation of CD18+ lymphocytes in response to low levels of SEA mitogen
Neutrophils from foamy vector treated dogs adhere in response to PMA

Blue = Hoechst 33342 - Nucleated cells
Green = CD18+ cells
Preferential migration of CD18+ leukocytes into Pus

CD18 Expression

Peripheral Blood

Pus

Blitzen

Sambuca
Questions

• Did we reverse the CLAD phenotype?
• Why is the % of CD18+ lymphocytes high?
• Did we have genotoxicity from integrations?
First successful gene therapy trial
First gene therapy-related adverse event

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Use of integrating retroviruses in gene therapy

Advantages

• Stable integration of vector into host genome results in long-term expression of therapeutic gene

Disadvantages

• Integration of retrovirus into host genome may have far-reaching mutagenic effects on neighboring genes
Retroviral insertion-induced mutagenesis

Cis-activation of downstream gene by viral 3’LTR promoter

Trans-activation of downstream gene by viral 3’LTR enhancer

Disruption of post-transcriptional elements such as silencers

Fusion of retroviral and nearby gene transcripts, encoding protein with altered function

Assess insertional mutagenesis by LAM-PCR

- Determination of clonality (how many insertions)
  - Integration pattern by LAM-PCR

- Determination of insertion sites (where are insertions)
  - Amplification of sites by LM-PCR or LAM-PCR
  - Cloning and DNA sequencing of insertion sites
**Linear Amplification Mediated PCR (LAM-PCR)**

Proviral DNA (pink) integrated into dog’s genomic DNA (blue)

1. Amplification of LTR plus genomic DNA

2. Synthesis of second DNA strand

3. Restriction digest with 4 base RE

4. Ligation of linker cassette

5. PCR and nested PCR
LAM PCR shows polyclonal insertion sites in PB leukocytes at 24 mo in all 4 dogs

Nugget  Ash
6 12 18 24 6 12 18 24-C H

Cactus  Sequoia
6 12 18 24 6 12 18 24-C H

Mapping sequences to the dog genome
Insertion sites for foamy vector compared to retroviral vector in leukocytes from CLAD dogs

- **Within 15 kb Tx Start Sites**
  - RV: 145 / 387
  - FV: 166 / 466

- **Within Genes**
  - RV: 174 / 387
  - FV: 158 / 466

- **In/Near 30kb Oncogenes**
  - RV: 145 / 387
  - FV: 166 / 466

Percentages:
- **Within 15 kb**: RV 37.5%, FV 43.9%
- **Within Genes**: RV 45.0%, FV 41.6%
- **In/Near 30kb Oncogenes**: RV 37.5%, FV 43.9%

**Notes**:
- RV = gammaretroviral vector
- FV = foamy viral vector

**Statistical Significance**:
- **Within Genes** and **In/Near 30kb Oncogenes** show significant differences (**p < 0.01**).
Foamy vector gene therapy results

- Long-term engraftment of therapeutic levels of CD18+ leukocytes
- No evidence of leukemia/clonal hematopoiesis
- First successful use of foamy vector to correct phenotype in large animal disease

Foamy viral vector gene therapy trial for LAD

- National Cancer Institute, Cincinnati Children's Hospital Medical Center and Univ of Washington

David Russell, MD, PhD
U Washington, with Nugget
Foamy viral vectors with cellular promoters

- MSCV
- LTR
- cCD18

- pgk
- cCD18

- ef1a
- cCD18

- CD11a
- cCD18

- pgk
- cCD18

- OA

- pgk
- cCD18

- OA
SIN lentiviral vectors with cellular promoters
Summary of CLAD gene therapy studies

• Gene therapy clinical trial of LAD-1 using foamy viral vector with MSCV

• Investigate cellular promoters in FV vectors

• Test cellular promoters in SIN LV vectors
The ones who really do the work (except me)
“Professor: one who talks in someone else’s sleep”

W.H. Auden