Immunological Barriers in Molecular Therapy
Targeted to Skin

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In vivo RRV-directed gene transfer to skin

(Ghazizadeh, et al 1999, Gene therapy 6:1267-1275)

**Transgene expression**
- 2 weeks
- 3 weeks
- 40 weeks

**Immune responses may limit the effectiveness of gene therapy**
1. Transduce stem cells
2. Persistent transgene expression
Delivery of macromolecules often alters the state of target cell

Activation
Metabolic stress
Protein overexpression

Danger signal

Immune response

Cell destruction
Two Arms of Immune System:

**Innate Immunity**
- LPS, endotoxin, dsRNA, ssRNA, CpG DNA, etc
- Pattern Recog. Receptors (TLR)
- No memory
- hours

**Adaptive Immunity**
- antigen receptor
- memory
- days

**Inflammatory Cytokines**
(danger signal)

**Naïve Tcells**

**CD4**

**Th2**

**Th1**

**Activated Tcells**

**B**

**CTL**
Immune responses in molecular therapy are influenced by:

1. Nature of the molecule being transferred
2. Target tissue and tissue compartments
3. Delivery methods
Immune responses must be considered in developing molecular therapy protocols:

- What type of immune responses are generated?
- How can we control/eliminate unwanted responses?

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>C57BL6</th>
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</thead>
<tbody>
<tr>
<td>Serum IgG</td>
<td>+</td>
</tr>
<tr>
<td>CD8+ cells</td>
<td>+</td>
</tr>
<tr>
<td>CD4+ cells</td>
<td>+</td>
</tr>
<tr>
<td>Transgene expression</td>
<td>lost</td>
</tr>
</tbody>
</table>

1. Loss of transgene is mediated by transgene-specific responses

2. Either CD4 or CD8 T cells could mediate clearance of transduced cells

3. Simply blocking one key player may not be sufficient
Immunity

Cross-presentation

Direct presentation

In vivo gene transfer

Ex vivo gene transfer

Mature DC

Lymph node

B cell

Effector T cells

Th0

Ignorance

Ag

DC

KC

Th0

Immunity
Host responses following ex vivo gene transfer to KC
(Lu and Ghazizadeh, 2005, Exp. Dermatol., 14:727)

- Isolate epidermal cells from mice
- Deplete APCs
- Transduce in culture
- Graft cells to syngeneic host
Loss of transgenic cells by transgene-specific immunity
<table>
<thead>
<tr>
<th>Characterization of transgene-specific immune responses:</th>
<th>In vivo</th>
<th>Ex vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue infiltrate:</td>
<td>Lymphocyte</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>Serum IgG isotype:</td>
<td>IgG2α</td>
<td>IgG1</td>
</tr>
<tr>
<td>Cytokine profile:</td>
<td>IFN-γ</td>
<td>IL-4</td>
</tr>
<tr>
<td>In vitro CTL activity:</td>
<td>Yes</td>
<td>NO</td>
</tr>
<tr>
<td>CTL memory:</td>
<td>Yes</td>
<td>NO</td>
</tr>
</tbody>
</table>

Immune responses are distinct between in vivo and ex vivo approaches to gene transfer.
Targeting transgene to suprabasal compartment of epidermis

**In vivo**

- LTR-GFP (n=6)
- INV-GFP (n=6)

**Ex vivo**

- LTR-GFP (n=12)
- INV-GFP (n=12)
Suppression of antigen-specific tissue responses when transgene targeted to suprabasal KC

A potential strategy to control unwanted responses
Conclusion:

- Immune responses are a challenge, not an impassible barrier

Need to develop strategies to suppress unwanted responses

  ↓

  type of immune response

  ↓

  therapeutic approach

  ↓

  Disease
Dominant disorders: Expression of a mutant allele
- Selective suppression by siRNA, antisense, oligos,
- induction of innate immunity (TLR)
- low efficiency of therapy

Focus: controlling innate immunity
- Alter molecules to make them less immunogenic
- Corticosteroids
- Anti inflammatory/cytokines
- New anti-innate immunity therapeutics
Recessive disorders: Loss or reduced gene expression
- introduction of normal cDNA/protein
- induction of innate immunity
- therapeutic protein = neoantigen
- induction of adaptive immunity/loss of transgenic cells

Focus: Modulate adaptive immune responses
- Control tissue inflammation/injury
- Dissociate innate and adaptive immunity at DC
- Immune suppression by blocking co-stimulation
- Induction of regulatory cells/tolerance
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