Immunological Adjuvants
The Indispensable Third Component

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Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
  - T-cells: change MHC anchor residues
  - B-cells: increase antigen stability – GD2 and GD3 lactones
  - xenogenize antigen – N- propionylated polysialic acid

- Modify nearby components
- Immunological adjuvants
- Immune modulators
Reactivity Against SK MEL 28 Cells of Sera from Melanoma Patients Vaccinated with GD3L-KLH+QS-21
IgM ELISA Titers Against Polysialic acid in SCLC Patients After Vaccination
Reactivity Against H345 SCLC with Sera from SCLC Patients Vaccinated against Poly Sialic Acid (PolySA)

NP-POLY SA-KLH+QS-21

Pretreatment sera
Post treatment sera

Poly SA-KLH+QS-21

% Positive Cells (FACS)

0
10
20
30
40
50
60
70
80
90
100

Patients

1 2 3 4 5 6 1 2 3 4 5
Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
- Modify nearby components
- Immunological adjuvants
- Immune modulators
Tumor Antigens are Poor Immunogens

1. Because they are autoantigens and
2. Because they are surrounded by autoantigens

SOLUTION: XENOGENIZATION
Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
- Modify nearby components
  - Xenogenize: T-cell help (conjugate, viral or bacterial vectors)
  - Processing: leader sequence, truncation, glycosylation
- Immunological adjuvants
- Immune modulators
Tumor protection by genetic immunization with *hgp75*

Optimized mGp75 for MHC-I

Tumor free mice

Exo-Opt10

Opt10

Exo + Opt10

Exo-mGp75

mGp75

Time

0 5 10 15 20 25

0 2 4 6 8 10 12 14 16

days

Figure 3
Conjugate GD3 isolysoganglioside vaccines

Polylsine Conjugate

Supercarrier Conjugate

Partial MAP Conjugate

Bovine Serum Albumen

Dominant Malarial T-cell isotope

Full MAP conjugate

Proteosome

High density conjugate with ABH photoactivated cross linker

*= Isolysos GD3

Glucose

Galactose

Sialic acids
## Conjugate Vaccine Components for Optimal Antibody Induction

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Carrier (KLH)</th>
<th>Adjuvant (QS-21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen must mimic expression on tumor cell</td>
<td>Cytokine release is proportional to carrier immunogenicity</td>
<td>Activation of APCs, B-cells and T-cells optimal</td>
</tr>
<tr>
<td>High antigen/carrier ratio needed</td>
<td>Cytokine release sequence may be key</td>
<td>Depot effect</td>
</tr>
</tbody>
</table>
Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
- Modify nearby components
- Immunological adjuvants
  - Activate APCs, B and T-cells
  - Depot effect
  - Access to endogenous pathway
- Immune modulators
## Requirement for Immunological Adjuvants

<table>
<thead>
<tr>
<th>Role</th>
<th>Vaccine Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definite:</strong></td>
<td>Carbohydrates, Peptides, Proteins, Conjugates</td>
</tr>
<tr>
<td></td>
<td>Tumor Cells (BCG)</td>
</tr>
<tr>
<td></td>
<td>DNA (cytokines, electroporation)</td>
</tr>
<tr>
<td><strong>None:</strong></td>
<td>Live Vectors, DCs</td>
</tr>
</tbody>
</table>
## Toll Like Receptors activate NF-κB, Linking Innate and Adaptive Immunity

<table>
<thead>
<tr>
<th>TLR</th>
<th>Pathogen Associated Molecular Patterns</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR 2</td>
<td>Bacterial lipoproteins, viral porins, hemagglutinin</td>
<td>Yeast, bacteria, viruses</td>
</tr>
<tr>
<td>TLR 3</td>
<td>dsRNA</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR 4</td>
<td>LPS</td>
<td>Bacteria</td>
</tr>
<tr>
<td>TLR 5</td>
<td>Bacterial flagellin</td>
<td>Bacteria</td>
</tr>
<tr>
<td>TLR 6</td>
<td>LPS</td>
<td>Bacteria</td>
</tr>
<tr>
<td>TLR 7</td>
<td>U-rich ssRNA, Imiquimod</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR 8</td>
<td>U-rich ssRNA, Imiquimod</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR 9</td>
<td>CpG DNA</td>
<td>Viruses, bacteria</td>
</tr>
<tr>
<td>TLR 11</td>
<td>?</td>
<td>Uropathic bacteria</td>
</tr>
</tbody>
</table>
## Immunological Adjuvants

<table>
<thead>
<tr>
<th>Adjuvants</th>
<th>Cytokine Induction</th>
<th>Depot Effect</th>
<th>Antibody Induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum Salts, Emulsions (MF59, Montanide), Liposomes, particles</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins (QS-21, GPI-0100)</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lipid A (MPLA, Detox) (TLR4)</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>CpG ODN (TLR9)</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Block Copolymers (Titermax)</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Cytokines (GM-CSF, IL2, IL12, IL18)</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Imiquimod (Aldara) (TLR7,8)</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
IFN-$\gamma$ Secretion Induced by 96 hr *in vitro* Stimulation with KLH After Vaccination of Mice with MUC1-KLH and GD3-KLH Plus Various Adjuvants

<table>
<thead>
<tr>
<th>Antigen</th>
<th>InVitro stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI-0100</td>
<td>14,560</td>
</tr>
<tr>
<td>CpG ODN</td>
<td>8902</td>
</tr>
<tr>
<td>QS-21</td>
<td>6573</td>
</tr>
<tr>
<td>MPL-SE</td>
<td>5233</td>
</tr>
<tr>
<td>MoGM-CSF</td>
<td>1164</td>
</tr>
<tr>
<td>TiterMax-G</td>
<td>694</td>
</tr>
<tr>
<td>Saline</td>
<td>180</td>
</tr>
</tbody>
</table>
# Median Antibody Titer after Immunization of Mice with MUC1-KLH and GD3-KLH Plus Adjuvants

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>MUC 1 IgG</th>
<th>MUC 1 IgM</th>
<th>GD3 IgG</th>
<th>GD3 IgM</th>
<th>Adjuvant</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI-0100</td>
<td>3277000</td>
<td>12800</td>
<td>450</td>
<td>12150</td>
<td>GPI-0100</td>
</tr>
<tr>
<td>QS-21</td>
<td>819200</td>
<td>3200</td>
<td>150</td>
<td>4050</td>
<td>QS-21</td>
</tr>
<tr>
<td>Detox-PC</td>
<td>819200</td>
<td>3200</td>
<td>0</td>
<td>4050</td>
<td>TiterMax-G</td>
</tr>
<tr>
<td>MPL-SE</td>
<td>819200</td>
<td>3200</td>
<td>0</td>
<td>1350</td>
<td>MoGM-CSF</td>
</tr>
<tr>
<td>MoGM-CSF</td>
<td>819200</td>
<td>800</td>
<td>0</td>
<td>1350</td>
<td>Detox-PC</td>
</tr>
<tr>
<td>TiterMax-G</td>
<td>204800</td>
<td>3200</td>
<td>0</td>
<td>1350</td>
<td>CpG ODN</td>
</tr>
<tr>
<td>CpG ODN</td>
<td>204800</td>
<td>800</td>
<td>0</td>
<td>930</td>
<td>PG-026</td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>Saline</td>
</tr>
</tbody>
</table>
## Median Antibody Titer after Immunization of Patients with GM2-KLH plus Different Adjuvants

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Adjuvant</th>
<th>Dose</th>
<th>Median Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM2-KLH</td>
<td>none</td>
<td>—</td>
<td>1/80</td>
</tr>
<tr>
<td>GM2-KLH</td>
<td>Detox</td>
<td>250µg CWS/25 µg MPL</td>
<td>1/160</td>
</tr>
<tr>
<td>GM2-KLH</td>
<td>BCG</td>
<td>10⁷ viable units</td>
<td>1/320</td>
</tr>
<tr>
<td>GM2-KLH</td>
<td>QS-21</td>
<td>10µg</td>
<td>1/320</td>
</tr>
<tr>
<td>QS-21</td>
<td></td>
<td>50µg</td>
<td>1/320</td>
</tr>
<tr>
<td>QS-21</td>
<td></td>
<td>100µg</td>
<td>1/1280</td>
</tr>
<tr>
<td>QS-21</td>
<td></td>
<td>200µg</td>
<td>1/1280</td>
</tr>
</tbody>
</table>
ELISPOT Reactivity in Melanoma Patients Immunized With Tyrosinase 368-376 (370B) Peptide plus IFA (Montanide ISA-51), QS-21 or GM-CSF

Adjuvant Active Components of Quillaja Saponins

Triterpene Nucleus
Lipophilic Moiety
Saccharide Groups

R3 = Apiose or Xylose
R2 = H
R1 = H

QS-17
QS-18
QS-21

primary site of hydrolysis
(OH⁻, H₂O)

Glucuronic acid
Xylose
Galactose
Fucose
Glucose
Rhamnose
Arabinose

OH⁻, EtOH, heat

QS-17
QS-18
QS-21
Q. Saponins & Analogs Proposed Mechanism for Adjuvant Effect

- Saponin sugar moieties target the delivery of the saponin-Ag complex to APCs by binding to their cell surface lectins
- Saponin aldehyde forms an imine group with certain T-cell surface -NH2 groups & provides a costimulatory signal, replacing the B7-1 ligand and biasing the immune system toward a Th1 response
Q. Saponins & Analogs Proposed Mechanism for Adjuvant Effect

• Q. saponins and their derivatives having a hydrophobic chain allow the processing of exogenous proteins by the endogenous pathway

• Combination of the costimulatory signal and endogenous process results in the production of Ag specific CTLs
Impact of Immunological Adjuvant on Immunogenicity

MICE
- IFN$_\gamma$ release: KLH 180 $\rightarrow$ 14,560 pg/ml
- Antibody titers: GD3: 0 $\rightarrow$ 1/12,150
  MUC1: 1/100 $\rightarrow$ 1/3X10$^6$
  KLH: 1/160 $\rightarrow$ 1/7290

HUMANS
- Antibody titers: GM2: 1/80 $\rightarrow$ 1/1280
Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
- Modify nearby components
- Immunological adjuvants

- Immune modulators
  Decrease T-regulator cells (Cytoxan, ↑CTLA4)

Increase effector mechanisms
Synergistic Effect of IV 3F8 mAb and PO β-glucan (BG) on Growth of 3 Human Neuroblastoma Cell Lines in Athymic Mice

Cheung, N.K. et al, Clin Ca Res in press
Percent survival

Survival in days post challenge

- PBS
- 3F8
- 3F8 + Bglucan
- PBS + Bglucan

Amputation day 20
3F8 Days 21, 35
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