FcγR Mediated Regulation of Adaptive Immunity: Implications for Antibody Therapies

Madhav Dhodapkar, MD; Kavita Dhodapkar, MD

Hematology, Immunobiology and Pediatrics
Yale University
New Haven, CT
• mAb mediated enhancement of T cell immunity.

• Effect of activating / inhibitory FcγR balance on DC activation and T cell immunity.

• Induction of adaptive immunity in patients treated with mAb therapy
Mechanisms of Anti-tumor Effects of MoAbs

MoAb

- Direct effect
- Innate effectors
  - ADCC
  - Complement
- Adaptive immunity
  - T cell immunity
Why Harness MoAbs to Elicit Adaptive Immunity

- Scope for improvement…..even with Rituxan.
- May provide a mechanism for durable responses.
- Immunologic memory: booster effect with repeat administration.
- Targeting antigen negative tumor cells (epitope spread)
Targeting tumor antigens to Fcγ receptors of dendritic cells via anti-tumor monoclonal Ab enhances anti-tumor immunity

- The enhanced presentation requires presence of Fcγ receptors on DCs.
Human Fcγ Receptors

FcγRI  CD64  FcγRIIA  CD32  FcγRIIB  CD32  FcγRIIIA  CD16  FcγRIIIB  CD16  FcεRI  FcαRI  CD89
Selective blockade of inhibitory Fcγ receptor leads to DC maturation and induction of IL12p70 in the presence of normal human plasma.

Dhodapkar et al. PNAS 2005
Enhanced Generation of Anti-Tumor Immunity After Blockade of Inhibitory Fcγ receptors on DCs

![Bar chart showing IFN-γ spots per 10^6 cells for different conditions.](chart)

- DC used to detect responses:
  - MAGE-A3
  - NY-ESO-1
  - Survivin

- DC used to expand T cells:
  - DC alone
  - Anti-FcγRIIb antibody

Legend:
- Isotype Control
- DC + Tumor

*Statistically significant differences compared to DC alone.
Balance of Activating / Inhibitory FcγRs
As A Checkpoint for Regulating Ag Presentation
Selective Blockade Of Inhibitory FcγRIIB On Human DCs Leads To A Distinct Gene Expression Profile

- Inflammation associated cytokines / chemokines
- FcR / complement related genes
- Type I IFN response genes
Selective blockade of inhibitory Fc\(\gamma\)Rs on human DCs and monocytes leads to the induction of type I IFN responses genes.

...but no increase in expression of type I interferons themselves !!! (including \(\alpha\), \(\beta\), \(\sigma\), \(\tau\), IFN28A, IFN28B, IFN29).
Blocking the inhibitory Fc receptor on DCs leads to induction of Phospho-STAT1
FcγR mediated induction of P-STAT1 is rapid and not blocked by anti-IFN Abs

Dendritic cells

Monocytes

P-STAT1 induction at 1 Hr

Phospho-STAT1

Fold change in P-STAT1 compared to Isotype treated cells

Similar result with anti-IFNα and anti-IFNγ antibody

IFNα

Anti-RIIB Ab
Suppression of Fc\(\gamma\)R mediated induction of P-STAT1 by blockade of activating Fc\(\gamma\)Rs and Syk inhibition.
Knockdown of STAT1 inhibits FcγR mediated DC maturation

Linking FcγR signaling to IFN pathway

Immune complexes

FcγR

Jak1
Tyk2
Syk
STAT1
STAT2
ISGF3
AAF
ITAM
ITIM
Balance Of Effector Versus Tregs As A Determinant Of Vaccine Efficacy

Tumor Antigen presenting DC

Effector and Memory T cells

Regulatory T cells (Tregs)
FcγR matured DCs induce T effectors with less induction of FoxP3 Tregs.
Functional Diversity of T cell Response

CD4+ → Th1 (T-bet, IFNγ)

CD4+ → Th2 (GATA-3, IL4,5,13)

CD4+ → Tregs (FOXP3)

CD4+ → Th17 (RORγT, IL17A,17F,22,26)
Expression of cytokines/chemokines in response to treatment with 2B6Ab (RIIBDC), isotype control antibody (IsoDC) IgG1 and inflammatory cytokines (CC-DC).

<table>
<thead>
<tr>
<th></th>
<th>IsoDC</th>
<th>RIIBDC</th>
<th>CC-DC</th>
<th>RIIBDCvsIsoDC</th>
<th>RIIBDC vs CC-DC</th>
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<tbody>
<tr>
<td>IL-1a</td>
<td>57.19(29.4)</td>
<td>699.5(184.4)</td>
<td>87.6(80.8)</td>
<td>0.0002</td>
<td>0.0005</td>
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<tr>
<td>IL-1b</td>
<td>11(15.6)</td>
<td>668.9(780)</td>
<td>NE</td>
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<td>IL-2</td>
<td>10.2(4.1)</td>
<td>14.3(4.7)</td>
<td>21.9(14)</td>
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<td>0.170</td>
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<tr>
<td>IL-3</td>
<td>102.2(16.2)</td>
<td>176(56.5)</td>
<td>137.6(35.5)</td>
<td>0.023</td>
<td>0.147</td>
</tr>
<tr>
<td>IL-5</td>
<td>0(0)</td>
<td>0.0</td>
<td>0(0)</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>IL-6</td>
<td>229.6(142.6)</td>
<td>7941.8(4116.4)</td>
<td>NE</td>
<td>0.005</td>
<td>NE</td>
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<tr>
<td>IL-7</td>
<td>0.1(0.2)</td>
<td>27(3.8)</td>
<td>16.4(12.7)</td>
<td>0.000</td>
<td>0.080</td>
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<tr>
<td>IL-8</td>
<td>628.1(408.1)</td>
<td>10000(0)</td>
<td>5727(4992.1)</td>
<td>0.000</td>
<td>0.069</td>
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<td>IL-10</td>
<td>22.5(2.8)</td>
<td>1200.9(987.5)</td>
<td>108.6(111.5)</td>
<td>0.027</td>
<td>0.035</td>
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<td>IL-12p40</td>
<td>40.5(28.4)</td>
<td>4713.3(5019.4)</td>
<td>3191(4483.3)</td>
<td>0.056</td>
<td>0.333</td>
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<td>IL-12p70</td>
<td>15.9(16.6)</td>
<td>148.2(148.5)</td>
<td>29(20.1)</td>
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<td>0.082</td>
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<td>IL-13</td>
<td>8.9(3.4)</td>
<td>28.9(21.1)</td>
<td>39.7(47.2)</td>
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<td>IL-15</td>
<td>3.8(7.7)</td>
<td>0.9(1.8)</td>
<td>0(0)</td>
<td>0.242</td>
<td>0.178</td>
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<td>IFNg</td>
<td>116.5(8.8)</td>
<td>264.2(61.1)</td>
<td>198.3(81.6)</td>
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<td>TNFa</td>
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<td>1059.8(911.3)</td>
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<td>NE</td>
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<td>Eotaxin</td>
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<td>35(11)</td>
<td>43.1(14.5)</td>
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<td>MCP1</td>
<td>1173.1(1074)</td>
<td>2776.34(2655.6)</td>
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<td>Rantes</td>
<td>27.8(16.5)</td>
<td>1531(682)</td>
<td>264.8(356.3)</td>
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<td>0.008</td>
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<td>MIP1α</td>
<td>710.6(351.3)</td>
<td>9746.9(388)</td>
<td>3067.8(2733.7)</td>
<td>&lt; 0.0002</td>
<td>0.001</td>
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<td>IP10</td>
<td>395.3(185.4)</td>
<td>8629.8(2364.7)</td>
<td>637.9(664.9)</td>
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<tr>
<td>IFNa</td>
<td>10.2(9.9)</td>
<td>10.3(12.5)</td>
<td>28.9(15.6)</td>
<td>0.493</td>
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</table>
Does Antibody Therapy Lead To The Induction Of Anti-tumor Adaptive Immunity In Vivo In Humans?
Induction of Adaptive Immunity After mAb Therapy of Cancer

Rituximab

Trastuzumab

Id specific T cell responses

Anti-HER-2/neu Ig\(\lambda\) responses

Conclusions

• Selective engagement of activating FcγRs leads to a distinct form of DC maturation and boosts the generation of anti-tumor immunity by human DCs
  – More anti-tumor effector T cells
  – Fewer FoxP3+ Tregs.
  – ? Increased CD8+ IL17 producing T cells

• Early evidence for induction of adaptive immunity in patients treated with anti-tumor mAbs
  – Enriched in the tumor bed.
  – Both CD4 and CD8+ T cells

• Alteration of activating / inhibitory FcγR balance may impact the ability of DCs to induce adaptive immunity in vivo in mAb treated patients
  – FcγR polymorphisms / Fc engineering

• Antibody mediated activation of tumor specific T cell responses provides an opportunity to further optimize their effects.
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