B cells and Monocytes as Antigen Presenting cells for Cancer Immunotherapy

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Antibody NK cell T cell


Anti-cancer immunotherapy

Passive
- Monoclonal Ab
- Adoptive T cell Therapy
- Adoptive NK cell Therapy

Active
- Protein based Immunotherapy
- DNA based Immunotherapy
- Cell based Immunotherapy

Checkpoint Blockade

College of Pharmacy, Seoul National University
Anti-tumor immune responses induced by vaccination

- Antigen (Cancer)
  - Protein
  - DNA vector
  - Virus vector
  - Antigen Loaded Dendritic cell

- Production of Ag specific antibody

- Lysis of cancer cell

- Cytotoxic T lymphocyte
  (CTL)

- Effector TH
Using specific antigenic peptide or antigen-loaded antigen presenting cell, we can induce either a strong humoral immunity or a strong cellular immunity.

They are easily administered to outpatients and generally do not cause significant side effects.

As one of the most potent antigen presenting cell, dendritic cells (DCs) has usually been used for this purpose.
Strategy for immunization with autologous peptide-pulsed DCs

One can employ DCs by
(1) Loading with a peptide, protein, or anti-Id Ab
(2) Infecting with a viral vector
(3) Loading with apoptotic bodies from tumor cells
Autologous cellular vaccine.

FDA approved cancer vaccine for prostate cancer.

Dendritic cells (DCs) from patient’s blood is incubated with a fusion protein consisting of two parts, the antigen prostatic acid phosphatase (PAP), which is present in most prostate cancer cells, and an immune signaling factor granulocyte-macrophage colony stimulating factor (GM-CSF) that helps the APCs to mature. Finally, the activated DC is re-infused into the patient to cause an immune response against cancer cells carrying the PAP antigen.
Limitations of DC based therapeutic vaccine

- The rarity of DC in blood and lymphoid tissues
- Short life span
- The difficulty of expanding their number ex vivo
- The costly and labor-intensive method used to differentiate DC from monocytes or bone marrow cells
- In vitro- or ex vivo-generation currently requires several days and produces heterogeneous DC
The properties of the various antigen-presenting cells

<table>
<thead>
<tr>
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<th>Dendritic cells</th>
<th>Macrophages</th>
<th>B cells</th>
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<tbody>
<tr>
<td><strong>Antigen uptake</strong></td>
<td>+++++ Macropinocytosis</td>
<td>Phagocytosis ++</td>
<td>Antigen-specific receptor (Ig) +++</td>
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<td>and phagocytosis by</td>
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<td>tissue dendritic cells</td>
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<td>Viral infection</td>
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<td><strong>MHC expression</strong></td>
<td>Low on tissue</td>
<td>Inducible by</td>
<td>Constitutive</td>
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<td>dendritic cells</td>
<td>bacteria and cytokines</td>
<td>Increases on activation</td>
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<td>High on dendritic</td>
<td>– to +++</td>
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<td>cells in lymphoid</td>
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<td><strong>Co-stimulator delivery</strong></td>
<td>Constitutive by</td>
<td>Inducible – to +++</td>
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<td></td>
<td>mature, nonphagocytic</td>
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<td>cells</td>
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<td><strong>Antigen presented</strong></td>
<td>Peptides</td>
<td>Particulate antigens</td>
<td>Soluble antigens</td>
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<td></td>
<td>Viral antigens</td>
<td>Intracellular and extracellular</td>
<td>Toxins</td>
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<td></td>
<td>Allergens</td>
<td>pathogens</td>
<td>Viruses</td>
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<td><strong>Location</strong></td>
<td>Ubiquitous throughout</td>
<td>Lymphoid tissue</td>
<td>Lymphoid tissue</td>
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<td>the body</td>
<td>Connective tissue</td>
<td>Peripheral blood</td>
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<td>Body cavities</td>
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*Figure 8-18 Immunobiology, 6/e. (© Garland Science 2005)*
B cells & monocytes as an alternative of Dendritic cell

- Abundant in both blood and lymphoid tissues

- After parenteral administration, they home to lymphoid organs

- Despite their many advantages, they have been ignored as a vaccinating APC since they are poorly immunogenic

- Previous studies have demonstrated that ‘activated’ B cells prime both CD4 and CD8 T cells

- When activated by appropriate adjuvants, B cells and monocytes can act as immunogenic APC which, like DC, can induce antigen specific immunity
NKT ligand $\alpha$-Galactosylceramide ($\alpha$GalCer) as a new adjuvant
NKT-mediated immune responses
B cells or monocytes which are loaded NKT ligand and expresses adenovirus-transduced tumor-antigen are converted into strong APCs with the help of NKT.

They can induce CD4, CD8, B cell, NK and NKT cell responses which could eliminate immunologically heterogeneous tumor cells.
Dendritic cell based vaccine vs B cell & Monocyte based vaccine

NKT ligand-loaded, Ag-expressing B cell and monocyte-based vaccine

7~10-day process

B cell & Monocyte based vaccine

1-Day process

NKT ligand (aGC) loading

B cells & monocytes
Development of B & Monocyte based vaccine

2006
- B cell
- Activated B cell
- Epitope peptide
- NKT cell

YE Chung et al. Cancer Res

2008
- aGalCer
- Adenoviral antigen transfer

YJ Kim et al. Int J Cancer

2009
- CD8 T cell, CD4 T cell, Ab, NK cell mediated antitumor immunity

HJ Ko et al. J Immunol

2010
- CFm40L adaptor protein
- CD40L

YS Kim et al. Hum. Gene Ther

2014
- Modified adenoviral vector, Ad.k35HM

EK Kim et al. Al Gene Therapy 2014
Monocytes can be converted into immunogenic APCs with the help of activated NKT cells (2009)

(HJ Ko et. al., J Immunol. 2009)
HER2/neu – Tumor antigen

- Human EGF receptor family, Proto-oncogene
- Almost no expression in normal cells
- Tumor antigen

30% of breast cancer
21% of lung cancer
10–30% of ovarian cancer
45–52% of pancreatic cancer
20–38% of stomach cancer

- HER2/neu-specific Abs and T cells are detected in breast and ovarian cancer patients
- Herceptin®: a humanized anti-HER2/neu mAb used for treatment of HER2/neu expressing tumors
Enhanced antitumor immunotherapeutic effect of B cell based vaccine transduced with modified adenoviral vector containing type 35 fiber structures (2014)

*In vivo NKT activation & Ag presentation*

(EK KIM et. al., *Gene therapy*, 2014)
Enhanced antitumor immunotherapeutic effect of B cell based vaccine transduced with modified adenoviral vector containing type 35 fiber structures (2014)

In vivo NKT activation

Cytotoxic T cell response

Activation of transferred B cells

(EK KIM et. al., Gene therapy, 2014)
Enhanced antitumor immunotherapeutic effect of B cell based vaccine transduced with modified adenoviral vector containing type 35 fiber structures (2014)

Humoral immune response (Ab production & ADCC)

(EK KIM et. al., Gene therapy, 2014)
Enhanced antitumor immunotherapeutic effect of B cell based vaccine transduced with modified adenoviral vector containing type 35 fiber structures (2014)

a  Therapeutic tumor model

D0  D5  D7
2×10⁶ CT26-Her2 s.c.  1.5×10⁶ B cell vaccination (i.v.)  Tumor size measurement (3 times a week)

b  Tumor growth curve

Days after tumor challenge

No treat  B/AdHM  B/AdHM/KBC009  B/AdK35HM  B/AdK35HM/KBC009

C  Immune Response in Humanized mouse

# IFN-γ spots/2×10⁶ cells

B only  B/Ad-k35HM  B/Ad-k35HM/KBC009
Human Papiloma Virus (HPV) tumor antigen: E6/E7 (HPV 16/18)

- HPV: cause of 5% of all cancers globally
- HPV type 16/18: 70% of all cancers caused by HPV
- HPV cancers: cervical cancer, head & neck cancer, anal cancer

The efficacy of the tumor antigen was clinically validated in a DNA vaccine phase I trial.

→ Nature Communications, 2014, Genexine
HPV infection can be prevented by HPV L1, L2 vaccine, and can be treated using therapeutic vaccine that harbors HPV E6/E7 tumor antigens.
Analysis of Antigen delivery efficiency in Human PBMC using Ad-k35 E6E7

( Unpublished Data )
Antitumor effect of B&monocyte-based vaccine

Development of B cells and monocyte based anti-cancer vaccine for HPV 16& 18 positive cervical cancer therapy.(2014~)

- **Day 0**: Tc-1 Injection 1x10^5 cell s.c.
- **Day 7**: Tumor size measurement
- **Day 8**: B&Mo preparation & 100MOI Ad-k35E6E7 transfection
- **Day 9**: 10^6 B&Mo vaccine injection (i.v.)

**Graph:**
- **No Treat**
- **B/Mo/Ad-k35-E6E7**
- **B/Mo/Ad-k35-E6E7/aGC**

Tumor size (mm^3)

Days after tumor challenge

( Unpublished Data )
B cells or monocytes which are loaded NKT ligand and expresses adenovirus-transduced tumor-antigen are converted into strong APCs with the help of NKT.

They can induce CD4, CD8, B cell, NK and NKT cell responses which could eliminate immunologically heterogeneous tumor cells.
• To induce strong immune responses
  - An important factor in the use of cell based vaccines for cancer immunotherapy

• To induce diverse immune responses
  - NKT ligand-loaded and tumor antigen-expressing B cells and monocytes can induce diverse immune responses

• To overcome various barriers
  - Immunosuppressive environment
  - Tumor Heterogeneity
    - Molecular: antigen loss, MHC loss
    - Cellular: Cancer cell vs. Cancer stem cell
  - Expression of negative regulator (Checkpoint)
BVAC-P®
• B cell & monocyte based therapeutic vaccine for the treatment of cervical cancer, head & neck cancer
• HPV16 E6/E7 targeting therapeutic vaccine
• Be on the preclinical stage

BVAC-B®
• B cell & monocyte based therapeutic vaccine for the treatment of gastric cancer, ovarian cancer, pancreatic cancer and breast cancer
• HER-2/neu targeting therapeutic vaccine
• Be on the preclinical stage

BVAC-C®
• B cell & monocyte based therapeutic vaccine for the treatment of cervical cancer, head & neck cancer
• HPV16 E6/E7 targeting therapeutic vaccine
• Be on the preclinical stage

BVAC-P®
• B cell & monocyte based therapeutic vaccine for the treatment of prostate cancer
• Human Prostatic Acid Phosphatase(hPAP) targeting therapeutic vaccine
• Be on the preclinical stage
Product Pipeline: Development plan

- **Product**
  - BVAC-C®
  - BVAC-B®
  - BVAC-P®

**Years**

- 2014~2015
- 2015~2017
- 2018~2020

**Pre-clinical studies**

- Supported by a Government grant

**Phase 1**

- BVAC-C®
- BVAC-B®
- BVAC-P®

**Phase 2 and approval**

- BVAC-C®
- BVAC-B®
- BVAC-P®
Step 1. Sorting PBMC through Leukapheresis

Step 2. T cell depletion by the magnetic separation

Step 3. ① Adenovirus vector transfection
          ② Adjuvant loading
          ③ 12 hour 37°C Incubation

Step 4. Quality Control

Step 5. Freezing (3 treatments + extra)

Step 6. Thawing

Step 7. IV Injection (1x10^8 cells) (6 week interval)
Contributions

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