Topic I – DNA Vaccines

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DNA vaccines with single-chain Fv fused to fragment C of tetanus toxin induce protective immunity against lymphoma and myeloma.

Outline

• Introduction – Concepts and definitions
  ➢ Traditional vaccination
  ➢ DNA vaccines
  ➢ Definition of terms
  ➢ Tumor models

• Presentation of the paper
  ➢ Methods
  ➢ Results and figures

• Conclusions and discussion
Introduction – Traditional vaccination (1)

Purpose: - Induction of an immune response to achieve protection against an antigen to protect a future challenge or to initiate a reaction against already present antigens

Method: - Injection or oral uptake of antigen (e.g. protein, toxin, attenuated pathogen, etc.) together with adjuvant or carrier
Introduction – Traditional vaccination (2)

Applications:
- Protection against infectious diseases (e.g. Mumps, Influenza, Hepatitis, Flu, etc); increasingly used in cancer therapy

Limitations:
- Protein antigens may be difficult and expensive to produce
- No CTL response induced without cytosolic production of antigens (use of live attenuated viruses)
Introduction – DNA vaccines

Principle: - Injection of DNA, (e.g. in muscle tissue) leads to ectopic expression and presentation of the antigen

Advantages: - DNA vaccines are much easier to produce
- Access to multiple antigen-presentation pathways

Limitations: - Lower magnitude of response than with conventional antigens

→ idea of the paper:
find a strategy to overcome the problem of generally insufficient immune response
Introduction – Definitions

• Idiotype: unique features of the antigen-binding site

• sc-Fv: single-chain variable fragment
Introduction – Tumor models

• A31 cells: mouse B-cell splenic lymphoma expressing surface IgM/κ
• 5T33 cells: murine myeloma, expressing IgG_{2b}/κ in the cytoplasm
• BCL_{1} cells: B-cell lymphoma, expressing surface IgM/λ

all tumors are transplantable, i.e. disease can be transferred to healthy animals by intravenous injection of cells
Presentation of the paper – applied methods

- Preparation of idiotypic proteins
  - A31: IgMκ prepared from supernatant
  - 5T33: Intraperitoneal injection in mice, purification of IgG_{2b} from ascites fluid and preparation of Fabγ fragment by pepsin digestion

- Preparation of DNA vaccine constructs
  - V_H and V_L sequences obtained by PCR with primers mapping in the framework region 1 and in the J_H or C_k segment, respectively
  - Assembly of constructs into expression vectors and purification
Presentation of the paper – applied methods

- Vaccination and tumor challenge
  - Intramuscular injection of DNA vaccines (50μg) on days 0, 21 and 42
  - Subcutaneous injection of idiotypic protein with CFA on days 0, 21 and 28
  - Tumor challenge by intravenous injection of $1 \times 10^4$ A31 or 5T33 cells on day 63
Presentation of the paper – applied methods

• Measurement of antibody responses by sandwich ELISA and FACS
  - ELISA: Idiotypic A31 IgM and 5T33 Fab as capture antigens Detection with HRP-conjugated anti-mouse Fcγ or isotype specific anti-mouse IgG1 or IgG2b antibodies
  - FACS: Determination of antibody binding to A31, 5T33 or BCL1 cells with FITC-conjugated anti-mouse Fcγ antibody
Presentation of the paper – applied methods

• Cytotoxicity assay
  - CTLs taken from spleens of vaccinated mice on day 56
  - Activation either by incubation with FrC peptide-pulsed normal splenocytes for 7 days in the presence of human rIL-2 or with irradiated 5T33 cells
  - $^{51}$Cr-release assay by incubation with $^{51}$Cr-labelled EL-4 target cells (peptide-pulsed) or 5T33 cells (alone or peptide-pulsed)
Presentation of the paper – Results and figures

Fig. 1
Antibody responses against FrC or A31IgM induced by DNA vaccines

- Considerable immune response both to FrC and scFvA31 only upon injection of the fusion construct
- Boost on day 42 did not raise mean antibody levels, but greatly increased number of responding mice
Presentation of the paper – Results and figures

Fig. 2
Reactivity of antibodies induced by DNA fusion vaccines with target tumor cell lines

- Significant reactivity only with the surface-Ig positive A31 cell line
- No binding to 5T33 cells due to lack of surface Ig
Presentation of the paper – Results and figures

Fig. 3
Antibody responses against FrC or 5T33Fab induced by DNA vaccines

• Considerable immune response both to FrC and 5T33Fab in all animals after the first injection on day 0

• Clearly visible boost effect after third injection on day 42
Presentation of the paper – Results and figures

Fig. 4
Antibody responses against 5T33Fab induced by DNA vaccines requires gene fusion

- significant levels of anti-5T33Fab antibodies only upon expression of the p.scFv5T33-FrC fusion construct
- co-expression of single constructs is insufficient and only induces anti-FrC antibodies
Presentation of the paper – Results and figures

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Ab IgG subclass</th>
<th>Ratio IgG1: IgG2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.scFvA31–FrC</td>
<td>A31IgM</td>
<td>18:1</td>
</tr>
<tr>
<td></td>
<td>FrC</td>
<td>0.4:1</td>
</tr>
<tr>
<td>A31IgM/CFA</td>
<td>A31IgM</td>
<td>137:1</td>
</tr>
<tr>
<td>p.scFv5T33–FrC</td>
<td>5T33Fab</td>
<td>28:1</td>
</tr>
<tr>
<td></td>
<td>FrC</td>
<td>22:1</td>
</tr>
<tr>
<td>5T33IgG/CFA</td>
<td>5T33Fab</td>
<td>∞</td>
</tr>
</tbody>
</table>

- Ig subclass profile of antibodies induced by DNA vaccines differs from protein antigen-induced subclass profile

- Dominance of IgG1, but induction of IgG2b antibodies through DNA vaccines indicates activation of a T<sub>H</sub>1-cell response
Presentation of the paper – Results and figures

- Target cells coated with synthetic FrC peptide are efficiently lysed, indicating the induction of a potent CTL response against FrC.

- 5T33 cells alone are not killed, confirming that protective immunity is not mediated by CTLs (no candidate MHCI-binding peptide in scFv5T33 sequence).

Fig. 5
Induction of CTLs against FrC by the DNA fusion vaccine.
Presentation of the paper – Results and figures

Fig. 6
Induction of protective immunity against the A31 lymphoma by the DNA fusion vaccine

- Vaccination of mice with p.scFvA31-FrC DNA vaccine promotes survival upon induction of lymphoma by injection of A31 cells

- Non-vaccinated animals and animals vaccinated with p.scFvA31 or an unrelated DNA vaccine die rapidly
Presentation of the paper – Results and figures

Fig. 7
Induction of protective immunity against the 5T33 myeloma by the DNA fusion vaccine

- Vaccination of mice with p.scFv5T33-FrC DNA vaccine promotes survival upon induction of myeloma by injection of 5T33 cells

- Vaccination with 5T33 IgG protein induces high levels of anti-idiotypic antibody but does not protect against challenge
Presentation of the paper – Results and figures

Fig. 8
Effect of pre-vaccination with tetanus toxoid on induction of anti-Id antibodies by the p.scFv5T33-FrC construct.

• Pre-existing TT antibodies do not inhibit induction of anti-idiotypic antibodies

• Previous vaccination with any antigen delivered in alum even seems to boost the immune response
Conclusions and discussion

- scFv DNA vaccines are easier to generate and manipulate than the respective idiotypic proteins. Important: Idiotypes differ between individual patients. Vaccines must therefore be prepared specifically for each patients!

- when fused to a pathogen sequence such as the C fragment of tetanus toxin, they are able to induce high levels of anti-idiotypic antibody when injected into muscle

- vaccination with fusion constructs provides definite protection against tumor challenge in mice
  - antibody-mediated against A31 lymphoma
  - CD4\(^+\) T-cell mediated against 5T33 myeloma (?)
Conclusions and discussion

• Potential clinical application:
  - Vaccination during remission phase after conventional therapy to mobilize the immune system against residual disease → complete remission without eventual relapse

• Current status of preliminary clinical testing in 10 lymphoma patients:
  • Response to FrC in 8 of 10 patients
  • T cell responses to Id in 5 of 7 patients evaluated → all five patients remain in complete remission
  • No evident toxicity
  • Problem of evaluating significant responses objectively
Conclusions and discussion

Questions remaining to be addressed:

• Mechanism of protection against sId-negative myeloma:
  - helper T-cell-associated effector function?
  - cytokine release?

• Potential induction of tumor-specific CTLs through tumor challenge in vaccinated hosts?
  - tumor cells undergo vaccine-associated apoptosis
    → uptake of tumor cell 'corpses' by host APCs and cross-presentation of additional, Id-independent tumor-specific antigens
    → expansion of a clinically relevant CTL repertoire specific for tumor-associated antigens unrelated to Id and vaccination
References:

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