Cutting Edge: DNA Immunization with Minigenes of Carbohydrate Mimotopes Induce Functional Anti-Carbohydrate Antibody Response

Thomas Kieber-Emmons, Behjatolah Monzavi-Karbassi, Bin Wang, Ping Luo and David B. Weiner

*J Immunol* 2000; 165:623-627; doi: 10.4049/jimmunol.165.2.623
http://www.jimmunol.org/content/165/2/623

**References**
This article cites 21 articles, 11 of which you can access for free at:
http://www.jimmunol.org/content/165/2/623.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Cutting Edge: DNA Immunization with Minigenes of Carbohydrate Mimotopes Induce Functional Anti-Carbohydrate Antibody Response

Thomas Kieber-Emmons, Behjatolah Monzavi-Karbassi, Bin Wang, Ping Luo, and David B. Weiner

To date, the generation of anti-carbohydrate Th1 immune responses, which would be useful for both tumor immunotherapy as well as in pathogen vaccine strategies, has been elusive. To augment Th1 immune responses to carbohydrate Ags, we describe results of DNA vaccination studies in mice using plasmids encoding designed peptide mimotopes (minigenes) of the neolactoseries Ag Lewis Y (LeY). In contrast to LeY immunization, immunization with mimotope-encoded plasmids induced LeY cross-reactive IgG2a Abs. Minigene immunization primed for a LeY-specific response that is rapidly activated upon encounter with nominal Ag upon subsequent boost. The resulting IgG2a response mediated complement-dependent cytotoxicity of a LeY-expressing human tumor cell line in the presence of human complement. These studies establish that peptide mimotopes of carbohydrate Ags encoded as DNA plasmids are novel immunogens providing a means to manipulate carbohydrate cross-reactive Th1 responses. The Journal of Immunology, 2000, 165: 623–627.

Targeting carbohydrate Ags associated with either pathogens or expressed on tumor cells is a challenge in the design of effective vaccines. Carbohydrates are traditionally viewed as T cell-independent Ags with a number of unique and important immunological properties that are not encountered when inducing an immune response to protein (1). These properties have precluded the use of pure carbohydrate or polysaccharide (PS) vaccines in those patients most at risk. Conjugate vaccine technology has overcome some of the limitations of carbohydrates as vaccine Ags because of the T-dependent help conferred by the protein (2). Nevertheless, PS conjugates induce responses that are often deficient in many respects, including the lack of induction of the Th1-associated murine IgG2a isotype (3), while inducing predominately IgG1 and IgG3 isotypes.

An alternative approach to develop T-dependent responses to carbohydrate Ags is the use of peptide or polypeptide surrogates of carbohydrates. Carbohydrate-mimicking peptides could significantly improve vaccines against infectious pathogens or tumor cells (4). Peptides that mimic carbohydrate structure have significant advantages as vaccines compared with carbohydrate-protein conjugates. Most notably, peptides can be engineered to induce Th1 responses by their incorporation into DNA plasmids for vaccination. The induction of a Th1-like response is the predominant response to DNA vaccines (5, 6). It is now accepted that Th1-dominant immunity, which is regulated by IL-12 and IFN-γ, plays a pivotal role in the eradication of tumors in vivo and in mediating pathogen clearance. Therefore, immunization with peptide mimotope-encoding minigenes can provide a cellular-associated response to carbohydrate Ags not achievable by PS conjugates alone. Redirection of the immune response to a Th1-like profile may augment vaccine responsiveness to these otherwise challenging Ags. Here, we report for the first time that peptide mimotopes constructed into DNA plasmids can prime for the induction of a carbohydrate Th1-associated IgG2a cross-reactive immune response. The feasibility of immunization with peptide-mimotope DNA plasmids (minigene) was investigated in mice by studying whether minigene vaccination can prime for the induction of a carbohydrate Th1-associated IgG2a Ab cross-reactive with the human tumor-associated Histo-Blood group-related neolactoseries Ag Lewis Y (LeY). A Th1 response with the expression of the IgG2a isotype is a desirable response as IgG2a Abs are opsonizing and complement fixing.

Materials and Methods

Construction of the expression vectors

Oligonucleotides were synthesized and inserted into pcDNA3 or pcDNA1 vectors. In the design of pcDNAaggi and pcDNAaari, we included a leader

3 Abbreviations used in this paper: PS, polysaccharide; LeY, Lewis Y; CDC, complement-dependent cytotoxicity.
Table I. Plasmids used in this study

<table>
<thead>
<tr>
<th>Plasmid Designation</th>
<th>Vector</th>
<th>Leader Sequence</th>
<th>T Cell Sequence*</th>
<th>Peptide Sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pcDNAari</td>
<td>pCDNA3</td>
<td>ARIYYRYDGFAY(60)</td>
<td>KQINMNGQVKAMYA(36)</td>
<td>-</td>
</tr>
<tr>
<td>pcDNAggi</td>
<td>pCDNA3</td>
<td>ARIYYRYDGFAY(60)</td>
<td>KQINMNGQVKAMYA(34.5)</td>
<td>GGIYKRYDIYKRYTV(2000)</td>
</tr>
<tr>
<td>pVHSOL</td>
<td>pcDNA1</td>
<td>-</td>
<td>-</td>
<td>ARIYYRYDFAFY(60)</td>
</tr>
</tbody>
</table>

*Values in parenthesis are estimated half time of dissociation of a molecule containing this sequence using a subsequence of 9 or 10 for scoring (13).

(7) and Th epitope (8) in the beginning of the peptide sequence. The plasmid pcDNAggi was generated by inserting the leader sequence oligonucleotide AGCTTCCACATGAGGTACATGATTTTAGGCTTGCTCG CCCCCTGCCAGCTTGACGC between the restriction sites HindIII and NotI in the polylinker region. The T1 and peptide mimotope-encoded oligonucleotide GCCCGCGAAGCAGCATCTAACATGCGAGGA GGTGGGCAAGCCCAATGACCGCGCGACATCTAACCCTGAC GCCTTGCGCCATTA was cloned into pcDNAari plasmid. All inserts were sequenced after construction. Plasmids were grown in Escherichia coli DH5α strain. DNA was purified using a maxi prep kit (Qiagen, Valencia, CA). Plasmid pVHSOL was generated in pCDNA1 (Invitrogen, San Diego, CA) in the same way as pcDNAari but without the leader and the Th epitope-encoding sequences. The gene expression of both vectors is virtually the same.

The cloning of IL-4 and IL-12 under the control of the CMV promoter. Expression is virtually the same.

cell line MCF7 (American Type Culture Collection) and human complement (Sigma) as previously described (10). The anti-LeY mAb BR55-2 was used as a positive control and for IgG standardization (10).

**Statistical analysis**

Data were expressed as arithmetic mean ± SD and analyzed by the Statview 4.1 program (Abacus Concepts, Berkeley, CA). Data were analyzed for normal distribution, and the statistical significance of the difference between groups was determined by the two-tailed Student’s t test.

### Results

**Choice of peptides as DNA immunogens**

Plasmids pcDNAari and pcDNAggi (Table I) were designed to express chimeric peptides that include a secretory leader sequence, a T cell epitope from HIV-1 gp120 (referred to as the T1 peptide; Ref. 8), and respective carbohydrate mimotope-encoding sequences that mimic the meningococcal group C capsular PS of Neisseria meningitidis (11), Lewis Ags expressed on tumor cells (10) and mannosyl, lactosides, and sialyl residues on HIV-1 gp120 (12). It was rationalized that the leader sequence would allow exogenous expression with the inclusion of the T cell epitope to augment T cell help. In plasmid pVHSOL, we cloned only the peptide mimic sequence of pcDNAari without the leader and T1 sequences.

The T1 epitope is suggested to bind to both MHC class II and class I. MHC class I binding sequences can also be subsets of MHC class II binding peptides. Representative HLA binding predictions identify an H2-K (BALB/c) class I binding motif centered on two 9-mer sequences of the T1 peptide (Table I) (13). Likewise, the mimotope sequences encoded in pcDNAari and pcDNAggi contain 10-mer sequences predicted as class I binding peptides (Table I).

**DNA vaccination results in LeY cross-reactive immune response**

Separate groups of animals were immunized with the respective constructs and 3 wk after the immunization, serum reactivity with LeY was quantified by ELISA. Results with 10-fold diluted serum showed a statistically significant increase in the reactivity of IgG Ab after immunization with these constructs. No IgM was detected.

Mice were again immunized at week 3, and the titration of anti-LeY immune response at week 5 was determined (Table II). The LeY cross-reactivity was persistent to a 1:256 dilution for pcDNAggi-immunized animals, which is statistically significant (p < 0.05) compared with serum from control vector-immunized mice. Immunization with pcDNAggi induced a consistently growing LeY cross-reactive response that appears superior to the other two plasmids. The mimotope in pcDNAggi reflects a repetitive Ag, which is typically more immunogenic (14) or the peptide represents a better mimic of the LeY epitope. Isotyping and quantitation of serum Abs of pcDNAggi-immunized mice for subsequent weeks indicate a LeY cross-reactive IgG2a-dominant response compared with IgG1 and IgG2b, which is indicative of a Th1 immune response (Fig. 1). At week 7, IgG2a showed a 10-fold increase compared with preimmune serum with a concentration of...
0.12 μg/ml. However, IgG production dropped significantly after week 7. These results are in agreement with other reports, where a predominant IgG2a response is generated following i.m. DNA immunization. Again, no IgM was detected.

DNA vaccination primes for carbohydrate-inducible IgG Ab response

DNA priming and protein boosting can enhance the production and functionality of induced Ab. Here, we tested this boost strategy with carbohydrate Ag. Minigene-primed mice and a vector-injected control group were boosted with 40 μg of polyvalent synthetic LeY with QS-21 on week 8 and bled on week 9 and 11. Minigene immunization induced the formation of LeY cross-reactive memory cells that were rapidly and specifically stimulated upon encounter with LeY (Table II). Enhancement of IgG was persistent with a statistically significant difference compared with vector alone-primed/LeY-boosted mice. Anti-LeY reactivity of serum from pcDNAagg-prime/LeY-boosted mice increased more than three times in titer from 1 to 256 (before boost) to 1 to 800 titer (after boost) (Table II). As expected, boosting with LeY led to an enhanced anti-LeY IgM response (data not shown). IgM levels increased a week after immunization and dropped 3 wk after the immunization.

We also determined the isotype components at week 11 (Fig. 2). IgG2a remained the predominant component of the immune response, with a 4-fold higher titer for pcDNAagg compared with the two other minigenes (Table II), suggesting that the WRYDI-con-
Several relevant peptides including peptide 106 were used to stimulate T cells. We did not observe any significant differences between immunized groups in T cell proliferation (data not shown). To further determine whether augmentation of Th activity would affect the magnitude of the immune response, we coimmunized additional groups of mice with the plasmids in Table I along with plasmids encoding IL-12 and IL-4. Coimmunization with either cytokine-encoding plasmid did not influence any further the Ab response to LeY relative to that previously shown with peptide-encoded plasmid alone. However, we observed a significantly higher T cell proliferation of splenocytes derived from mice immunized with the pcDNAggi plasmid with coimmunization with IL-12 (data not shown). Detected IFN-γ released (not shown) in supernatant collected from in vitro peptide-activated splenocytes again supported Th1-type activation consistent with the observed IgG2a production. Expectedly, the Th1 response leads to a high cytotoxic T lymphocyte activity against peptide-pulsed class I* class II+ mastocytoma P815 target cells in coimmunized animals (Fig. 4). As expected, the removal of CD8+ T cells resulted in the suppression of specific lysis enhancement observed with coimmunization of IL-12 gene (Fig. 4). Parental P815 cells were not lysed. We did not detect any enhancement in T cell proliferation, CTL, or Ab response upon coimmunization with IL-4, exactly as previously reported (20).

We observe that DNA immunizations with plasmids encoding peptide mimotopes of carbohydrate Ags induce carbohydrate cross-reactive humoral responses, albeit of low titer. Packaging of these epitopes into more effective T cell stimulators or presenting them as multivalent Ags (21) along with adjuvants that further potentiate Th1 response should improve the titers. IgG2a and IgG3 isotypes have been reported to be particularly effective in conferring protection against encapsulated organisms, and Th1 responses are associated with more efficacious tumor vaccination. It is clearly a unique property of this approach to induce a humoral carbohydrate cross-reactive, a Th, and a CTL response in one simple inoculation. It should be possible to further engineer peptides that mimic glycopeptides that are processed on native Ags to further augment T cell responses. It is also likely that engineering mimotopes into hybrid plasmids, which replace the model Th epitope with Th or CTL epitopes from a pathogen or tumor target itself, would be expected to build in a memory response that is relevant in the context of challenge.

Acknowledgments

We thank Kaitly Y. Lin, Shahram Shamloo, Matthew Kieber-Emmons, and Habib Rahbar for technical assistance in DNA purification and ELISAs. We thank Charlotte Read Kensil of Aquila Biopharmaceuticals for the QS-21.

References


FIGURE 3. CDC assay with human MCF7 cells. A, This experiment was performed using serum from immunized mice before and 2 wk after carbohydrate boost. B, Titration of CDC-mediated killing of MCF7 cells using anti-LeY (BR55-2) Ab. ***p < 0.001 and **p < 0.01 compared with vector immunized animals using Student’s t test. *, p < 0.025 compared with pcDNAggi-immunized animals before boost using Student’s t test.

FIGURE 4. IL-12 coimmunization with pcDNAggi induces a significant CD8+–dependent CTL activity. Splenocytes were stimulated for 5 days. Cytotoxicity was measured on peptide-pulsed P815 cells as targets. ■pcDNAggi + IL-12; ▲pcDNAggi + IL-12 (CD8+ cells depleted); ●pcDNAggi.


