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Preferential Recognition of TCR Hypervariable Regions by Human Anti-Idiotypic T Cells Induced by T Cell Vaccination

Ying C. Q. Zang,* Jian Hong,* Victor M. Rivera,* James Killian,* and Jingwu Z. Zhang‡

T cell responses to myelin basic protein (MBP) are potentially involved in the pathogenesis of multiple sclerosis (MS). Immunization with irradiated MBP-reactive T cells (T cell vaccination) induces anti-idiotypic T cell responses that suppress circulating MBP-reactive T cells. This T cell-T cell interaction is thought to involve the recognition of TCR expressed on target T cells. The study was undertaken to define the idiotypic determinants responsible for triggering CD8⁺ cytotoxic anti-idiotypic T cell responses by T cell vaccination in patients with MS. A panel of 9-mer synthetic TCR peptides corresponding to complementarity-determining region 2 (CDR2) and CDR3 of the immunizing MBP-reactive T cell clones were used to isolate anti-idiotypic T cell lines from immunized MS patients. The resulting TCR-specific T cell lines expressed exclusively the CD8 phenotype and recognized preferentially the CDR3 peptides. CDR3-specific T cell lines were found to lyse specifically autologous immunizing MBP-reactive T cell clones. The findings suggest that CDR3-specific T cells represented anti-idiotypic T cell population induced by T cell vaccination. In contrast, the CDR2 peptides were less immunogenic and contained cryptic determinants as the CDR2-specific T cell lines did not recognize autologous immunizing T cell clones from which the peptide sequence was derived. The study has important implications in our understanding of in vivo idiotypic regulation of autoimmune T cells and the regulatory mechanism underlying T cell vaccination. * The Journal of Immunology, 2000, 164: 4011–4017.

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3 Abbreviations used in this paper: CDR, complementarity-determining region; MBP, myelin basic protein; MS, multiple sclerosis; MBP83–99, the immunodominant 83–99 peptide of MBP.

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that specifically recognized and lysed the immunizing T cell clones but not unrelated autologous T cells (12, 18).

This study was undertaken to define the recognition pattern and functional properties of CD8<sup>+</sup> anti-idiotypic T cells induced by T cell vaccination. A panel of anti-idiotypic T cell lines was isolated from immunized MS patients using overlapping TCR peptides corresponding to CDR2 and CDR3 of the immunizing MBP-reactive T cell clones. Based on the sequence diversity and immunogenic properties of CDR2 and CDR3, the idiotypic determinants recognized by the anti-idiotypic T cells are most probably localized within these regions. The use of TCR peptides over the irradiated T cells as stimulator offered several advantages. First, the TCR peptides were designed as 9-mers to selectively isolate CD8<sup>+</sup> anti-idiotypic T cells, as the majority of the anti-idiotypic T cells induced by T cell vaccination were previously shown to express the CD8 phenotype and exhibit MHC class I restriction (12, 18). Therefore, the resulting anti-idiotypic T cell lines isolated by the 9-mer TCR peptides most likely represent the same T cell population elicited by T cell vaccination. Second, immunization with irradiated MBP-reactive T cell clones also induced the T cell reactivity to other surface molecules present on the immunizing T cells. For the purpose of this study, the use of TCR peptides would selectively activate the anti-idiotypic T cells, sparing unrelated T cells that may obscure anti-idiotypic T cell responses. The study described herein provided important experimental evidence indicating that T cell vaccination induces CD8<sup>+</sup> cytotoxic anti-idiotypic T cell responses that are preferentially directed at CDR3 sequences of the immunizing MBP-reactive T cell clones. CDR2 contains cryptic determinants and is less immunogenic. The findings are important in our understanding of the mechanism underlying in vivo idiotypic regulation that can be boosted by T cell vaccination.

Materials and Methods

Reagents and peptides

Medium used for cell culture was Aim-V serum-free medium (Life Technologies, Grand Island, NY). Recombinant human IL-2 was purchased from Boehringer Mannheim (Indianapolis, IN). The immunodominant peptide (residues 83–99) of MBP was kindly provided by Dr. Stefan Boheme (Neurocine Biosciences, San Diego, CA). A set of overlapping TCR peptides of 9 aa in length was synthesized by Chiron Technologies (San Diego, CA). The purity of the peptides was greater than 95%.

Myelin-reactive T cell clones

A panel of six MBP-reactive T cell clones were generated from three patients with MS using a microculture system combined with split-well technique (12, 15, 16). Briefly, PBMC were plated out at 200,000 cells/well in U-bottom 96-well plates in the presence of MBP<sub>83–99</sub> peptide (10 μg/ml) in Aim-V serum-free medium. Seven days later, the cultures were restimulated with MBF<sub>83–99</sub> peptide and irradiated autologous PBMC as a source of APC. After another 7 days, the cultures were tested for specific response to MBP<sub>83–99</sub> peptide in a proliferation assay. The positively identified T cell lines were cloned using limiting dilution assay in the presence of PHA-protein (PHA-P) at 2 μg/ml. Cultures were fed with fresh medium every 3–4 days. Growth positive wells were tested for specific reactivity to the MBP<sub>83–99</sub> peptide in proliferation assays. The resulting MBF<sub>83–99</sub>-specific T cell clones were further characterized and used for T cell vaccination.

TCR V gene analysis of MBF<sub>83–99</sub>-reactive T cell clones

TCR V gene rearrangements of the immunizing MBP-reactive T cell clones were analyzed using reverse-transcribed PCR. TCR α- and β-chain genes were amplified and directly sequenced as previously described (19, 20). Briefly, total RNA was extracted from 10<sup>6</sup> cells of each MBF<sub>83–99</sub>-reactive T cell clone using RNAeasy mini kit (Qiagen, Santa Clarita, CA). First-strand cDNA reverse transcribed from total RNA was subject to PCR amplification with a set of primers specific for TCR Vα and Vβ gene families whose sequences were published previously (19, 20). The amplified PCR products were separated in a 1% agarose gel by electrophoresis and stained with ethidium bromide. The visualized PCR products were cut and purified subsequently using a QIAquick gel extraction kit (Qiagen) before sequence analysis. The purified PCR products were directly sequenced with the T7 sequencing kit (Pharmacia, Uppsala, Sweden). A total of 1.5 μg of template was sequenced with 2 pmol of the corresponding V gene primer using the method of dideoxy chain termination (21).

Immunization of MS patients with irradiated autologous MBP-reactive T cell clones

Three patients with clinically definite MS confirmed by magnetic resonance imaging were included in this study (22). They were diagnosed as having relapsing-remitting MS for more than 2 years, and mean baseline Expanded Disability Scale Score (EDSS) was 1.67. The patients had not taken any immunomodulatory drugs at least 3 mo before the study. Immunizations with irradiated autologous MBF<sub>83–99</sub>-reactive T cell clones was performed as previously described (12, 17). Briefly, MBF<sub>83–99</sub>-reactive T cell clone was activated and expanded in the presence of PHA 7 days before injection. T cells were then irradiated at 10,000 rads (a 60 Co source) and thoroughly washed with sterile saline. A total of 2 × 10<sup>7</sup> cells of each T cell clone was resuspended in 1 ml of sterile saline and was injected s.c. in the arms. The immunization was repeated every 2 mo for 8 mo to achieve an adequate immune response as defined by the proliferation of PBMC to the immunizing T cell clone(s). The protocol was approved by the Institutional Human Subjects Committee at Baylor College of Medicine. Consent forms were obtained from the patients after explaining the experimental procedures. The patients were evaluated for adverse events and disability score (the EDSS) before and after each immunization. Gadolinium-enhanced MRI scans were performed before and at different time points after immunization. The clinical and radiographic evaluation was part of a separate clinical study (Zang et al., manuscript in preparation).

The generation and characterization of anti-idiotypic T cell lines raised against TCR peptides and the immunizing T cell clones

To generate anti-idiotypic T cell lines recognizing the TCR peptides, PBMC obtained from the immunized patients were plated out at 100,000 cells/well in U-bottomed plates (Costar, Cambridge, MA) in the presence of 10 μg/ml respective peptides (40 wells for each peptide). Seven days later, all cultures were restimulated with the corresponding peptide in the presence of irradiated autologous PBMC as a source of APC. After another week, each culture was examined for specific proliferation to the TCR peptide in proliferation assays. Briefly, each well was split into four aliquots (∼10<sup>4</sup> cells/aliquot) and cultured in duplicate in the presence of 10<sup>5</sup> APC pulsed with the TCR peptide or a control peptide, respectively. Cells were cultured for 72 h and pulsed with [3H]thymidine (Amersham, Arlington Heights, IL) at 1 μCi/well during the last 16 h of the culture. Cultures were then harvested and [3H]thymidine incorporation was measured in a liquid scintillation counter (Wallac, Turku, Finland). A T cell line was considered to be specific for the peptide when the cpm were greater than 1500 and exceeded the reference cpm (in the presence of the control peptide) by at least three times. T cells generated at this stage were considered as short-term T cell lines and were expanded for further characterization.

The protocol for the generation of anti-idiotypic T cell line recognizing the original immunizing MBP-reactive T cell clones was described previously (12, 18). Briefly, fresh PBMC (5 × 10<sup>6</sup> cells/well) from the immunized patients were plated out in the presence of irradiated immunizing T cells as stimulator cells (5 × 10<sup>5</sup> cells/well). Seven days later, cultures were then restimulated with irradiated immunizing T cells and supplemented with human rIL-2. On day 14, the growth-positive wells were examined for their inhibitory effect on the proliferation of the immunizing MBP-reactive T cell clones (15). For this purpose, the wells were split into four aliquots (∼40,000 cells from each well were lightly irradiated (2,000 rads) and used as effector cells. The 40,000 immunizing MBP-reactive T cells were used as responder cells in the presence of MBP and APC in a proliferation assay as described above. The percent inhibition was calculated as [1 − (cpm of MBP-reactive T cell clones in the presence of irradiated regulatory T cells/cpm of MBP-reactive T cell clones in the absence of irradiated regulatory T cells)] × 100%. T cell lines exhibiting more than 50% specific inhibition on MBP-reactive T cell clones but not on autologous PHA-stimulated T cells (control T cells) were selected for further characterization.

As a general rule, the identified anti-idiotypic T cell lines were expanded in two stimulation cycles (7 days for each cycle) with the corresponding TCR peptides or irradiated regulatory T cells under optimal experimental conditions described above. The cell lines were then tested in proliferation assays to confirm the reactivity to the TCR peptides or the immunizing T cell clones before further characterization.
Anti-idiotypic T cell reactivity to TCR peptides and immunizing T cell clones

A total of 20,000 cells of each TCR-specific T cell lines was cultured with irradiated autologous PBMC (100,000 cells/well) as a source of APC in the presence of a corresponding TCR peptide (10 μg/ml), or irradiated immunizing T cells (20,000 cells per well), respectively. Cells were cultured for 72 h, and the cell proliferation was measured in [3H]thymidine incorporation assays as described above.

Cytotoxicity assay

Immunizing T cell clones and control clones were labeled by incubating the cells with 200 μCi of 51Cr for 45 min. Cells were washed subsequently and used as target cells in a chromium release assay. The effector (anti-idiotypic T cells) to target (the immunizing clones and control T cells) ratio was 10. After incubation at 37°C for 4 h, supernatants were harvested and radioactivity was measured in a gamma counter. The maximum and spontaneous chromium release was determined in wells containing detergent or medium alone. The percent specific cytolysis was calculated as [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100%.

Results

The functional and structural characteristics of the immunizing MBP-reactive T cell clones and the induction of anti-idiotypic T cell responses by T cell vaccination in patients with MS

A panel of six MBP-reactive T cell clones was generated from three MS patients with relapsing-remitting MS. They expressed the CD4 phenotype and recognized the 83–99 immunodominant peptide of MBP in the context of the DR2 (DRB1*1501) or DR4 molecules (Table I). As shown in Tables II and III, all the MBP-reactive T cell clones derived from three patients displayed distinct TCR Vα and Vβ genes. These T cell clones were used for T cell vaccination according to the protocol employed in previous clinical trials (see Materials and Methods). Each patient received a total of 4 s.c. inoculations with two autologous MBP-reactive T cell clones (2 × 10⁶ cells/clones) at a 2-mo interval. The T cell responses to the autologous immunizing T cell clones were examined before and after each immunization.

Table I. Characteristics of the immunizing MBP-reactive T cell clones

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clone</th>
<th>MBP&lt;sub&gt;83-99&lt;/sub&gt;</th>
<th>Medium Alone</th>
<th>MHC Restriction</th>
<th>TCR V Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS1</td>
<td>K2B9</td>
<td>18,199 ± 876</td>
<td>230 ± 19</td>
<td>DRB1*1501</td>
<td>AV16</td>
</tr>
<tr>
<td></td>
<td>K1D9</td>
<td>31,827 ± 1827</td>
<td>1,172 ± 192</td>
<td>DRB1*1501</td>
<td>AV3</td>
</tr>
<tr>
<td>MS2</td>
<td>F2D2</td>
<td>4,918 ± 333</td>
<td>399 ± 91</td>
<td>DR4</td>
<td>AV9</td>
</tr>
<tr>
<td></td>
<td>F1F3</td>
<td>4,152 ± 280</td>
<td>923 ± 28</td>
<td>DRB1*1501</td>
<td>AV22</td>
</tr>
<tr>
<td>MS3</td>
<td>A1C3</td>
<td>5,492 ± 213</td>
<td>839 ± 102</td>
<td>DR4</td>
<td>AV17</td>
</tr>
<tr>
<td></td>
<td>A1D4</td>
<td>7,328 ± 128</td>
<td>827 ± 110</td>
<td>DR4</td>
<td>AV22</td>
</tr>
</tbody>
</table>

* MBP-reactive T cell clones recognizing the immunodominant 83-99 peptide (MBP<sub>83-99</sub>) were generated from the blood of three MS patients. The reactivity of the clones to the 83-99 peptide is expressed in cpm ± SEM as compared to background cpm ± SEM (in the absence of the peptide). MHC restriction of the clones was tested in proliferation assays using mouse L cell lines transfected with DR4 and DR2 (DRB1*1501). All T cell clones were examined for TCR V gene usage by PCR analysis with oligonucleotide primers specific for Vα and Vβ families.

As shown in Fig. 1A, the proliferative responses to irradiated immunizing T cell clones were increased progressively with each immunization and exceeded substantially the baseline value (>10-fold) after the fourth immunization in all three patients. The increased response to the immunizing T cells was found to inversely correlate with a decline of the frequency of circulating MBP-reactive T cells in the immunized patients (Fig. 1B). The results have confirmed that the anti-idiotypic T cell responses directed at MBP-reactive T cells can be induced in patients with MS by repeated immunizations with irradiated T cell clones (12, 17, 18).

Isolation and characterization of anti-idiotypic T cell lines raised against the TCR peptides

Based on the extreme sequence diversity in CDR3, and to a lesser degree in CDR2 (8), it was predicted that the idiotypic determinants responsible for triggering anti-idiotypic T cells might reside within these TCR regions. A set of overlapping peptides was synthesized and used to selectively isolate CD8<sup>-</sup> TCR-specific T cell lines (4/21) were found to recognize CDR2 peptides and recognize immunizing T cells. As the majority of the anti-idiotypic T cells induced by T cell vaccination were found to express the CD8 phenotype and recognize immunizing T cells in the context of MHC class I molecules (12, 17, 18), 9-mer peptides were synthesized and used to selectively isolate CD8<sup>-</sup> anti-idiotypic T cells induced by T cell vaccination. As all immunizing T cell clones had 15–16 aa in the CDR2 and less than 8–9 aa in the CDR3, two overlapping CDR2 peptides and one CDR3 peptide were made for each α- or β-chain. TCR-specific T cell lines were generated from PBMC specimens obtained from three patients 1–2 mo after the fourth immunization, at which time substantial T cell responses to the immunizing T cell clones were documented (Fig. 1).

As shown in Fig. 2, the majority of anti-idiotypic T cell lines obtained from immunized patients reacted with the CDR3 peptides (17/21 resulting T cell lines), suggesting the preferential recognition of the CDR3 determinants. In contrast, only four of the TCR-specific T cell lines (4/21) were found to recognize CDR2 pep-

Table II. TCR VJ region sequence of the immunizing MBP<sub>83-99</sub>-reactive T cell clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>AV</th>
<th>V-J</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS1-K2B9</td>
<td>AV16</td>
<td>YYCLVGIDDMRFAGGTLTVP</td>
<td>NQK</td>
</tr>
<tr>
<td>MS1-K1D9</td>
<td>AV3</td>
<td>YFCAFDGATYKIVGFGLKVLVA</td>
<td>NQK</td>
</tr>
<tr>
<td>MS2-F2D2</td>
<td>AV9</td>
<td>YYCAKLKNFNGKLFITGTRTLTI</td>
<td>NQK</td>
</tr>
<tr>
<td>MS2-F1F3</td>
<td>AV22</td>
<td>YFCAALSRSGNYLKTGKTLTLTVNP</td>
<td>NQK</td>
</tr>
<tr>
<td>MS3-A1C3</td>
<td>AV17</td>
<td>YFCAANQDGFGNEKLFTGTRTLTI</td>
<td>NQK</td>
</tr>
<tr>
<td>MS3-A1D4</td>
<td>AV22</td>
<td>YFCAVSAGGTYSGLTFQGAGTLYHP</td>
<td>NQK</td>
</tr>
</tbody>
</table>

* The amino acid sequences of synthetic CDR3 peptides are underlined.

Table III. TCR VDJ region sequence of the immunizing MBP<sub>83-99</sub>-reactive T cell clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>BV</th>
<th>V-D-J</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS1-K2B9</td>
<td>BV17</td>
<td>YLCASSTRQGPOPQYFGGTRLVL</td>
<td>ELDKN</td>
</tr>
<tr>
<td>MS1-K1D9</td>
<td>BV7</td>
<td>YLCASSQDRAVPOTYFGGTRLVL</td>
<td>ELDKN</td>
</tr>
<tr>
<td>MS2-F2D2</td>
<td>BV11</td>
<td>YFCASSAMGETQYFGGTRLVL</td>
<td>ELDKN</td>
</tr>
<tr>
<td>MS2-A1F3</td>
<td>BV9</td>
<td>YFCASSQDFWGGTVNTAEFFGQGTRTV</td>
<td>ELDKN</td>
</tr>
<tr>
<td>MS3-A1C3</td>
<td>BV13.1</td>
<td>YFCASSLGRATQFQYFGGTRLTV</td>
<td>ELDKN</td>
</tr>
<tr>
<td>MS3-A1D4</td>
<td>BV9</td>
<td>YFCASSPTNYTGGTGSQGTV</td>
<td>ELDKN</td>
</tr>
</tbody>
</table>

* The amino acid sequences of synthetic CDR3 peptides are underlined.
tides. The resulting CDR2- and CDR3-specific T cell lines expressed exclusively the CD8 phenotype. However, not all TCR peptides used here were equally immunogenic in the generation of TCR-specific T cell lines. In patient MS1, CDR3-specific T cell lines were isolated from both immunizing T cell clones, while in MS2 and MS3 only the TCR peptides derived from one of the immunizing T cell clones were effective in eliciting anti-idiotypic T cell responses.

Furthermore, attempts were made to isolate anti-idiotypic T cells from pre-immunization specimens using the same TCR peptides and the same experimental settings. A small panel of five TCR-specific T cell lines (three for CDR3 and two for CDR2) were identified from these patients. The number was significantly lower than that obtained from postimmunization specimens in the same patients, suggesting that pre-immunization specimens contained a lower precursor frequency of TCR-specific T cells. These CD8+ T cell lines exhibited poor growth characteristics (stimulation index of 1.5–2.5) and lost in subsequent cell passages. Taken together, the finding suggests that CDR3-specific T cells but not T cells recognizing CDR2 occurred at a higher precursor frequency in immunized patients, which may reflect high immune state induced by T cell vaccination.

FIGURE 1. The T cell responses to the immunizing MBP-reactive T cell clones and changes in the frequency of circulating MBP-reactive T cells in immunized MS patients. A, The proliferative responses of PBMC obtained from three immunized patients are expressed as stimulation indexes defined as cpm of PBMC cultured with irradiated immunizing MBP-reactive T cells (1:1 mix of two clones)/the sum of cpm of PBMC cultured alone and cpm of irradiated T cells cultured alone. B, The precursor frequency of T cells specific for MBP was estimated in the patients before and at the indicated time points after immunization. The arrows represent the time points corresponding to the immunizations.

FIGURE 2. The reactivity pattern of TCR-specific T cell lines generated from immunized MS patients. The obtained TCR-specific T cell lines (Tid lines) were stimulated with corresponding CDR3 or CDR2 peptides, respectively, in the presence of APC. Cell proliferation in response to the peptide was measured in [3H]thymidine uptake assays and expressed as cpm. Control cpm was obtained in the absence of peptides (medium alone). All TCR-specific T cell lines expressed the CD8 phenotype. In all cases, SEM is less than 12%. The amino acid sequences of the additional CDR2 peptides that were tested here are listed as follows: PRFIQ GYKTKVTNE (2B9VA16), LILIRS NEREKHSGRGL (1D9VA3), LQLLL RHISSRESIKG (2D2VA9), LLLK ATKADDKGNK (1D4AV22), LISYY FQNIQVNDQK (2B9VB17), AKKPLE LMFSYSLERV (1D9VB7), LQLQIQ YNGEERA (2D2VB1), HIVSGVEC TTARKGEV (1C3VB13.1), FQISSLKHV NLQKEL (1D4VB9).
Recognition pattern and cytotoxicity of TCR3-specific anti-idiotypic T cell lines

The obtained TCR-specific T cell lines were further characterized for their reactivity to the autologous immunizing T cell clones used for T cell vaccination. Of 17 CDR3-specific T cell lines examined, eight T cell lines recognized the autologous immunizing MBP-reactive T cell clones but not autologous nonspecific T cells. The result of a representative experiment is presented in Fig. 3. In contrast, none of the CDR2-specific T cell lines obtained reacted with the immunizing T cell clones (data not shown). All eight CDR3-specific T cell lines examined exhibited specific cytotoxic activity against the original immunizing T cell clones but not autologous PHA-induced T cells in chromium-release assays (four representative T cell lines shown in Fig. 4). The cytotoxicity could be blocked by an Ab directed at MHC class I (W6/32) but not class II molecules (HB55) (Fig. 4). The results indicate that a significant proportion of anti-idiotypic T cell lines raised against the CDR3 peptides but not the CDR2 peptides may represent the CD8\(^{+}\) anti-idiotypic T cell populations induced by T cell vaccination.

Reactivity of anti-idiotypic T cell lines raised against the immunizing T cells to the CDR3 peptides

As the CDR3-specific T cell lines recognized the original immunizing MBP-reactive T cells, it remained to be tested whether the anti-idiotypic T cell lines raised against original immunizing T cell clones would recognize the TCR peptides. To this end, a small panel of eight anti-idiotypic T cell lines were generated from PBMC of two MS patients (MS1 and MS3) using the original immunizing MBP-reactive T cell clones as stimulator (see Materials and Methods). As shown in Fig. 5, the resulting CD8\(^{+}\) anti-idiotypic T cell lines specifically reacted with the original MBP-reactive T cell clones but not unrelated MBP-reactive T cell clones of autologous origin that were not used for immunization. Of eight T cell lines examined, five lines reacted specifically with the CDR3 peptides derived from CDR3 of the immunizing MBP-reactive T cell clones (Fig. 5). The remaining T cell lines exhibited no reactivity to any of the TCR peptides, suggesting that at least some of the CDR3 peptides contained idiotypic determinant(s) recognized by CD8\(^{+}\) anti-idiotypic T cells induced by T cell vaccination.

FIGURE 3. The reactivity of CDR3-specific T cell lines toward the immunizing MBP-reactive T cell clones. CDR3-specific T cell lines obtained from MS patients immunized with MBP-reactive T cell clones were examined for their reactivity to autologous immunizing T cell clones. Each CDR3-specific T cell line (T\(_{id}\) line) was examined with the corresponding CDR3 peptides and irradiated immunizing T cells, respectively. Autologous nonspecific T cells prepared by PHA stimulation of peripheral blood T cells were used as control T cells and a CDR2 peptide derived from the same immunizing T cell clone was employed as control peptide. The proliferation of CDR3-specific T cell lines was measured in [\(^{3}\)H]thymidine uptake assays and expressed as cpm.

FIGURE 4. Cytotoxicity of CDR3-specific T cell lines toward the immunizing MBP-reactive T cell clones. The representative CDR3-specific T cell lines derived from three MS patients were examined for cytotoxicity toward autologous immunizing clones and nonspecific T cells that were labeled with \(^{51}\)Cr as target cells. mAbs to MHC class I (W6/32) and class II (HB55) products were added at 20 \(\mu\)g/ml in parallel wells to determine the MHC restriction element. The specific cytotoxicity was determined in a standard chromium-release assay at an E:T ratio of 10. Control T cells (nonspecific T cells) were prepared from autologous PBMC by repeated PHA stimulation.
The recognition of the CDR3 peptides by CD8\(^+\) anti-idiotypic T cell lines raised against the immunizing T cell clones. Anti-idiotypic T cell lines raised against the immunizing MBP-reactive T cell clones were tested for their recognition of the CDR3 peptides using CDR2 peptides of the same T cell clones as a control. The CD8\(^+\) cytotoxic anti-idiotypic T cell lines were stimulated with the CDR3 peptides whose sequence corresponded to the CDR3 of the immunizing T cell clones. Irradiated autologous PBMCs were used as a source of APC. The anti-idiotypic T cell lines were tested in parallel wells with irradiated immunizing T cell clones or non-specific T cells (control T cells) prepared from autologous PBMC by repeated PHA stimulation, respectively. The proliferation of the anti-idiotypic T cell lines was measured in a[^3]H]thymidine uptake assay. T cell lines AC1D9 and AC2B9 were raised against clones 1D9 and 2B9 from patient MS1, and T cell lines AC1C3-1 and AC1C3-2 and ACIF3 were raised against clones 1C3 (MS-3) and 1F3 (MS-2), respectively. The CDR3 peptides used in the experiments have the following sequences: ATDAGGTYK for AC1D9, STRGQPQET for AC2B9, LGRAGLTYE for AC1C3-1 and AC1C3-2, and QDRFWGGTV for ACIF3.

**Discussion**

The findings described here indicate that immunization with irradiated MBP-reactive T cell clones induces CD8\(^+\) cytotoxic anti-idiotypic T cell responses preferentially directed at the CDR3 of the immunizing T cell clones. The conclusion is based on a series of experiments described here. First, the CDR3-specific T cell lines represent the anti-idiotypic regulatory T cell populations elicited by T cell vaccination as they are present at a higher precursor frequency in immunized patients and recognize the original immunizing T cells. In contrast, the idiotypic determinants within CDR2 are less immunogenic and are cryptic in nature, as suggested by the failure to recognize the original immunizing T cell clones from which the CDR2 sequences are derived. The CDR2-specific T cells described here responded to the corresponding CDR2 peptides but exhibited no cytotoxic activity toward the immunizing T cells. The findings suggest that anti-idiotypic T cell responses to CDR2 determinants may represent part of the normal T cell repertoire and are not induced by T cell vaccination.

The CDR3-specific T cell lines obtained from immunized patients represent distinct anti-idiotypic T cell populations from those reported in other studies where anti-idiotypic T cells were isolated from nonimmunized healthy individuals (3, 4). They recognize CDR3 peptides in the context of MHC class I molecules, and differ in MHC restriction from anti-idiotypic T cells reported by Jiang et al. (23, 24). The CD8\(^+\) cytotoxic anti-idiotypic T cell responses described here are not readily detectable in nonimmunized patients and healthy individuals, as reported previously (3, 4). It is conceivable that the unique sequence diversity and structural properties within CDR3 may contribute to its immunogenicity and preferred role in eliciting anti-idiotypic T cell responses in T cell vaccination. On the contrary, CDR2-specific T cells generated from these immunized MS patients resemble anti-idiotypic T cells found in nonimmunized individuals as they do not recognize the original MBP-reactive T cell clones or lines (3, 4), which is consistent with our hypothesis that they may recognize cryptic determinants within CDR2 and are not involved in T cell vaccination.

There are several issues emerged from the study. 1) The results of the study do not completely rule out the possibility that the TCR regions other than CDR3 and CDR2, such as CDR1 and the framework regions, may also be involved in the anti-idiotypic T cell responses induced by T cell vaccination. This possibility may account partially for the observation that not all cytotoxic anti-idiotypic T cell lines isolated from the immunized patients recognize the CDR2 and CDR3 peptides. However, they are unlikely to represent dominant idiotypic determinants responsible for the anti-idiotypic T cell responses in T cell vaccination as these regions are less variable and encoded by germline sequences to which the immune system may be tolerant. 2) The study also indicates that not all CDR3 or CDR2 peptides examined are equally effective in activating anti-idiotypic T cell responses in the immunized patients. Some of the CDR3 and CDR2 peptides failed to elicit sufficient anti-idiotypic T cell responses. It is likely that some of the TCR peptides may have poor immunogenic properties for the following reasons. The CDR3 peptides used in these experiments were single 9-mer peptides corresponding to the V\(\alpha\)-J\(\alpha\) or V\(\beta\)-D\(\beta\)-J\(\beta\) junctional regions of a given T cell clone, excluding the flanking residues. As a result, the T cell recognition of some idiotypic determinants may be affected by the lacking of certain flanking residue(s) that may contribute to the T cell recognition. An alternative explanation is that immunization to some of these MBP-reactive T cell clones was not equally sufficient. This possibility is supported by relatively lower anti-idiotypic T cell responses (stimulation index) to the immunizing T cell clones in patients MS2 and MS3. 3) At this time, it is unclear whether there are functional differences in cytotoxic anti-idiotypic T cell response between MS patients and normal individuals. The likelihood of a functional deficit in the idiotypic regulatory network needs to be exploited in future investigations.

There are several existing models that may explain how the idiotypic determinants of target TCR are presented to and recognized by anti-idiotypic T cells. There is experimental evidence indicating that peptides of cell surface molecules are often presented by MHC class I molecules, and that peptide binding motifs for MHC class I molecules have been identified (25, 26). Several recent studies have demonstrated that endogenous TCR peptides can be presented by self-MHC to anti-idiotypic T cells (11, 27). The CD8\(^+\) cytotoxic anti-idiotypic T cells seem to be characterized by the failure to recognize the original intact T cell repertoire and are not induced by T cell vaccination. Alternatively, the route of administration, the amount of T cells administered and potentially altered biological/immunologic properties of the cell surface molecules by irradiation may all account for selective activation of CD8\(^+\) cytotoxic anti-idiotypic T cell responses in T cell vaccination.

The CD8\(^+\) cytotoxic anti-idiotypic T cell responses to MBP-reactive T cells induced by T cell vaccination may favorably alter the clinical course of MS. This is suggested by an inverse correlation of anti-idiotypic T cell responses with depletion of circulating MBP-reactive T cells and with clinical improvement seen in immunized patients with MS (Ref. 17, Zang et al., manuscript in...
preparation). However, the clinical effect of T cell vaccination needs to be proven in a controlled clinical trial. The findings described herein have important implications in advancement of the current T cell vaccination protocol by designing a more practical peptide-based immunization approach. Although CDR2 peptides have been used in clinical trials to suppress MBP-reactive T cells (28), our findings suggest that immunization with short CDR3 peptides may be more effective in depleting circulating MBP-reactive T cells by inducing CD8+ cytotoxic anti-idiotypic T cell responses. On the other hand, the use of CDR3 peptides significantly limits the applicability of the peptide-based immunization approach because CDR3 sequences are extremely diverse and unique for each T cell clone. As we demonstrated recently, common sequence motif(s) does exist within CDR3 of MBP-reactive T cells derived from different MS patients that uniformly recognize the immunodominant 83–99 peptide of MBP in the context of DR2 (DRB1*1501) molecules (29). Further investigations in exploring common CDR3 motifs among the immunodominant MBP-reactive T cells in a selected group of MS patients may provide a solution to overcome these limitations.

References