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A Complementary Peptide Vaccine That Induces T Cell Anergy and Prevents Experimental Allergic Neuritis in Lewis Rats1

Shigeru Araga,2 Masahiro Kishimoto, Satoko Doi, and Kenji Nakashima

We have developed and described a new method of altering T cell-mediated autoimmune diseases by immunization with the complementary peptide against T cell epitopes. The complementary peptide (denoted NAE 07-06) to the bovine P2 protein, residues 60–70 (denoted EAN 60–70), was tested in the Lewis rat model of experimental allergic neuritis (EAN). Immunization with NAE 07-06 induced polyclonal and monoclonal Abs that inhibited the proliferation of the P2-specific T cell line, stimulated with EAN 60–70, and recognized V\(\beta\), but not V\(\alpha\), of TCRs. Proliferation of T cells treated with anti-NAE 07-06 Abs could be partially restored by treatment with rIL-2, in accordance with an anergy model. A homologous sequence was found between NAE 07-06 and the VDJ junction of the TCR \(\beta\)-chain from an EAN 60–70-specific T cell line. Rats preimmunized with NAE 07-06 in vivo before EAN induction showed less disease severity clinically and histologically. These data suggest a new therapeutic approach for T cell-mediated autoimmune disorders through the induction of anti-TCR Abs with complementary peptide Ags. The Journal of Immunology, 1999, 163: 476–482.

N eurological diseases include many disorders caused by autoimmune T and B cells. Guillain-Barré syndrome (GBS)3 is the popular disorder of the peripheral nervous system the central nervous system counterpart of which is multiple sclerosis (MS). Some cases of GBS show a long term bed-ridden state requiring respiratory assistance and long lasting severe paraparesis. Immnomodulatory therapies such as corticosteroid or plasmapheresis are effective for GBS. However, such immunosuppressive treatments are nonspecific and have severe side effects such as allowing opportunistic infection. In theory, anti-Id Abs reactive with disease-causing Id Ab or clonotypic T cells represent ideal therapeutic agents. However, it is difficult to know the appropriate Id Ab or clonotypic T cell to use for the induction of anti-Id Ab. We have previously reported a technique that overcomes this problem by actively inducing anti-Id Ab with a peptide immunogen rather Id Ab (1, 2). Our approach is based on the observation that peptides specified by complementary nucleotide sequences can specifically bind to each other, apparently as a result of their having complementary shapes (3–5). These interacting peptides, with presumed complementary shapes, can in turn induce the formation of interacting pairs of polyclonal or monoclonal Id and anti-Id Abs the combining sites of which are complementary (6, 7).

Experimental allergic neuritis (EAN) provides an opportunity to test this new approach for the treatment of T cell-mediated autoimmune disorders. EAN is an animal model of GBS and is caused by immunization with bovine P2 protein (8). Histological examination shows infiltration of lymphocytes and demyelination in the peripheral nerve, similar to that seen in GBS. Passive transfer with P2 protein-sensitized T cells, but not with Abs against the myelin components including P2 protein, demonstrate the cell-mediated origin of EAN (9–11). As demonstrated in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, Th1 lymphocytes against the P2 protein play a major role in development of EAN (12). Bovine P2 protein, residues 53–78, can induce the EAN clinically and histologically in Lewis rats (13, 14). Furthermore, a recent study showed that the minimal epitope was located in the bovine P2 protein, residues 60–70 (15, 16). In this article, we demonstrate that a complementary peptide for this T cell epitope induced polyclonal and mAbs against TCR on P2-reactive T cells. The mAbs specifically recognized TCR \(\beta\) and induced T cell anergy in the EAN model. Furthermore, we have demonstrated in vivo protection against EAN using the complementary peptide as a vaccine.

Materials and Methods

Bovine peripheral myelin purification

Bovine peripheral myelin was prepared from bovine dorsal roots according to the method of Cammer (17) and stored at −80°C until used.

Complementary peptide design for bovine P2 protein, residues 60–70

Bovine P2 protein was reported to induce EAN in Lewis rats (8). However, the nucleotide sequence for bovine P2 protein has not yet been reported. In contrast, nucleotide sequences for the P2 protein in rabbits and mice are available (8, 18). Furthermore, the amino acid sequence of P2 protein, residues 60–70 (denoted EAN 60–70) are conserved in bovine, rabbit, and mouse. Using the rabbit nucleotide sequence, we designed a complementary peptide (denoted NAE 07-06) for EAN 60–70 peptide. Namely, the amino acid sequence of NAE 07-06 peptide, was derived by 5’ to 3’ assignment of amino acids to the nucleotide sequence complementary to the mRNA of rabbit P2 protein encoding EAN 60–70 peptide (Fig. 1).
As expected, the complementary peptide NAE 07-06 has an inverted hydropathic profile compared with the target peptide, EAN 60–70 (Fig. 2). The amidated form of the peptide, EAN 60–70, and NAE 07–06 were commercially synthesized with F-moc chemistry and were purified by reverse phase HPLC (Kurabo Biomedical, Tokyo, Japan). An amidated control peptide denoted PBM 9-1 was similarly synthesized and purified. PBM 9-1 is a complementary peptide for the first 9 residues of human myelin basic protein and has the sequence NH₂-Arg-Ser-Leu-Leu-Ser-Gly-Gly-Leu-Pro-NH₂ (7).

**FIGURE 1.** Bovine and rabbit peripheral nerve myelin P2 protein, residues 60–70 (denoted EAN 60–70), and their complementary peptide (NAE 07-06).

**FIGURE 2.** Hydrophatic profile of bovine P2 (EAN 60–70) and its complementary peptide (NAE 07-06). Hydrophatic score is based on Kyte and Doolittle values. Scale: most hydrophobic, +4.5, to least hydrophobic, −4.5.

CTCR8 (IgG2b, κ), against the complementary peptide for *Torpedo acetylcholine* receptor, residues, 100–116, was used for control mAb (21). mAbs were purified by ammonium sulfate precipitation, followed by passing over a protein G column (HiTrap affinity column, Pharmacia Biotech, Uppsala, Sweden). The purity and reactivity were checked by ELISA and electrophoresis. mAb CTCR8 was used as a control mAb and has been described (21).

**Bovine peripheral myelin-reactive T cells**

Female Lewis rats (8 wk old) were immunized into the rear footpads with 2 mg of purified bovine peripheral myelin protein emulsified with an equal volume of CFA. Eight days after immunization, rats were sacrificed under ether anesthesia. The inguinal and popliteal lymph nodes were removed aseptically. The lymphocytes were collected by passing through a stainless mesh. The tissue debris and dead cells were removed by Ficol-Hypaque centrifugation and resuspended in complete medium (RPMI 1640, 10% FCS, 5 × 10⁻³ M 2-ME, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.25 μM Fungizone, 10 mM HEPES (pH 7.0), 10 mM nonessential amino acids, and 20 mM L-glutamine, and 10 mM sodium pyruvate). Macrophages were depleted by a plastic plate adherence method.

**Irradiated spleen cell preparation**

Normal Lewis rats (8 wk old) were killed, and spleens were removed aseptically. The splenocytes were collected by Ficol-Hypaque centrifugation and suspended in the above medium. The splenocytes were irradiated with 3000 rad. Then, irradiated splenocytes were incubated in complete medium with 100 μg/ml EAN 60–70 peptide for 1 h in a humidified 5% CO₂ incubator. These irradiated cells were used for educated APC.

**EAN 60–70-specific T cell line**

Lewis rats were immunized s.c. with 2 mg purified bovine peripheral myelin, emulsified with an equal volume of CFA. Eight days later, their draining popliteal and inguinal lymph nodes were removed, and a single-cell suspension was made. The primed lymph node cells were stimulated in the presence of EAN 60–70 peptide at a concentration of 100 μg/ml and irradiated APC for 3 days, followed by a 7-day incubation with irradiated APC alone. After three passages through the above procedure, T cell lines were expanded by EAN 60–70 peptide and irradiated APC in the presence of 10% IL-2 without Con A (Genome Therapeutics, Waltham, MA).

For control cell lines, Lewis rats were immunized s.c. with 50 μg OVA, emulsified with an equal volume of CFA. This was followed by an ordinary preparation procedure for T cell line as mentioned above.

**Inhibition of bovine peripheral myelin-reactive lymphocyte proliferation by anti-NAE 07-06 Abs**

The sensitized T cells (2 × 10⁵/well) and educated APC (5 × 10⁵/well) were coincubated with the indicated concentrations of either anti-NAE
Materials and Methods

To test the inhibition of proliferation by mAb NAE3, before coincubation of educated APC (5 \times 10^5/well) and sensitized lymphocytes (2 \times 10^4/well), educated APC or sensitized lymphocytes were preincubated with mAb NAE3 or control mAbs (CTCR8) at a concentration of 1 \mu g/well for 1 h at room temperature and then were washed with complete medium twice, followed by the proliferation assay. OVA-specific T cell lines were used as control cell lines.

Measurement of IL-2 production

P2 residues, 60–70-specific T cells (2 \times 10^4), and APC (2 \times 10^4) were cocultured with 10 \mu g/ml EAN 60–70 peptide in the presence or absence of mAb NAE3 (1 \mu g/ml) for 3 days of culture. The supernatants were stored at −20°C until used. The amount of IL-2 in the supernatant was determined by bioassay using CTLL-2 cells (22). The activity of the IL-2 was determined by the proliferation of the CTLL-2 line as described above.

Restoration of mAb NAE3-induced T cell anergy by coculture with exogenous rIL-2

EAN 60–70-specific T cells (2 \times 10^4) and irradiated APC (2 \times 10^4) were cocultured with indicated amount of EAN 60–70 peptide in the presence or absence of mAb NAE3 (1 \mu g/ml) for 3 days of culture. Then, NAE3-treated EAN60–70-specific T cells were restimulated with fresh irradiated APC and EAN 60–70 peptide in the presence or absence of rIL-2 (50 U/ml) for 5 days. To demonstrate the Ag specificity of the restoration, PB9-1 peptide was used for restimulation. Finally, ordinary proliferation assays were done.

Flow cytometric analyses of T cell line

T cell line reactive to EAN 60–70 peptide was analyzed with the use of FACScan (Becton Dickinson, Mountain View, CA) with propidium iodide and FITC-labeled anti-CD3 (W3/13), anti-CD4 (W3/25), or anti-CD8 (OX-8) (Serotec, Oxford, U.K.). EAN 60–70-specific T cell clones also were analyzed by biotin-labeled NAE3, followed by FITC-labeled avidin (Becton Dickinson) or Abs to NAE 07-06, followed by biotin-anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) and FITC-labeled avidin (Becton Dickinson). All T cell lines were phenotyped to be CD3^+ , CD4^+ and CD8^+ (data not shown).

Immunoblotting with mAb NAE3

EAN 60–70-specific T cells or OVA-specific T cells were lysed in 10 mM Tris-HCl buffer, pH 7.4, containing 1% (w/v) Nonidet P-40 (Sigma), 150 mM NaCl, 1 mM EDTA, supplemented with the protease inhibitors 2 

\mu g/ml leupeptin, 2 \mu g/ml aprotinin, 4 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, hydrochloride (Boehringer Mannheim, Indianapolis, IN), and 20 \mu M iodoacetamide. The lysate was then treated with streptavidin agarose (Life Technologies) to preclear lysate. The precleared lysate was reduced with 100 mM DTT. We used this preparation as total soluble membrane proteins. Furthermore, part of the lysate was dialyzed against 10 mM Tris-HCl buffer, pH 7.4, containing 1% (w/v) Nonider P-40, 150 mM NaCl, 1 mM EDTA to remove excess DTT. The lysate was immunoprecipitated with mAb R73 (IgG1, Serotec) using agarose anti-mouse Ig (Sigma) to remove the TCR-Vβ molecule. We used this preparation as R73-precleared lysate. These total soluble membrane samples and R73-precleared lysate were analyzed by 10% acrylamide gel and then electrotransferred to polyvinylidene difluoride membrane (Bio-Rad, Richmond, CA). After blocking with 10% skin milk in PBS, membranes were incubated with either biotin-labeled mAb R73 or biotin-labeled mAb NAE3 at the concentration of 10 \mu g/ml in TPBS (PBS, containing 0.1% Tween) using a 10-well-slot apparatus (CosmoBio, Tokyo, Japan). After being washed in TPBS, membranes were incubated with alkaline phosphatase-labeled streptavidin. Finally, bands were detected with CDP-Star Western blot chemiluminescence reagent (DuPont NEN) on exposure to x-ray films.

The blot of soluble membrane proteins of OVA-specific T cells was checked by reactivity against either mAb R73 or mAb NAE3. This was then followed by an ordinary Western blotting technique as mentioned above.

TCR Vβ chain analysis

TCR Vβ chain usage was analyzed by the RT-PCR method. RNA was prepared from cloned T cell lines with TRIZOL LS reagent (Life Technologies). cDNA was synthesized from total RNA using SuperScript II

\newpage

FIGURE 3. A. Immunoblots of membrane protein from an NAE 60–70 T cell line. Membrane were blotted as described in Materials and Methods and stained with mAbs R73 (lane a) or NAE3 (lane b). Lane c is an R73 precleared lysate developed with NAE3. B. Immunoblots of membrane protein from an OVA-specific T cell line. Membranes were blotted as described in Materials and Methods and stained with mAbs R73 (lane a) or NAE3 (lane b).

FIGURE 4. Inhibition of bovine peripheral myelin-sensitized lymphocyte proliferation by Ab to NAE 07-06. Purified bovine peripheral myelin-sensitized lymphocytes were incubated with irradiated macrophages, pulsed with EAN 60–70 peptide and the indicated dilutions of rat antisera to NAE 07-06 or PBM 9-1. Proliferation assays were done as described in Materials and Methods. Each point represents the mean ± SEM; 100% of [^3H]thymidine uptake represents 85,740 cpm.

FIGURE 5. Inhibition of bovine peripheral myelin-sensitized lymphocyte proliferation by pretreatment of APC or EAN 60–70-sensitized lymphocytes with NAE3. EAN 60–70-sensitized lymphocytes, depleted of macrophages (sen-T), were coincubated with EAN 60–70 or EAN 60–70 educated (ed) irradiated APC. In each assay, sensitized T cells or educated APC were preincubated or coincubated with 1 mg/ml of either mAb NAE3 or CTCR8, followed by the proliferation assay described in Materials and Methods. Sensitized T cells and APC (nonloaded with EAN 60–70) gave a background incorporation of 1015 cpm. No inhibition was induced on proliferation of OVA-sensitized lymphocytes (sen-OVA), pretreated with mAb NAE3. Each bar represents mean ± SEM; 100% of [^3H]thymidine uptake represents 67,960 cpm.
RNase H\(^{-}\) reverse transcriptase (Life Technologies) and a random hexamer. The cDNA was then amplified using an antisense C\(\beta\) and specific primers for V\(\beta\)s (V\(\beta\)1, V\(\beta\)2, V\(\beta\)3.3, V\(\beta\)4, V\(\beta\)5.1, V\(\beta\)6, V\(\beta\)7, V\(\beta\)8.1, V\(\beta\)8.2, V\(\beta\)8.3, V\(\beta\)8.5, V\(\beta\)9, V\(\beta\)10, V\(\beta\)12, V\(\beta\)13, V\(\beta\)14, V\(\beta\)15, V\(\beta\)16, V\(\beta\)17, V\(\beta\)18, V\(\beta\)19, V\(\beta\)20) (23) in a typical PCR reaction for a total of 40 cycles consisting of 94°C/1 min, 54°C/1 min. ending with a 10-min extension at 72°C. PCR products were size-selected using a 2% agarose gel. PCR products were directly ligated into pGEM-T vector (Promega, Madison, WI). V-D-J genes were identified by comparisons to previously published sequences in the EMBL-GDB (European Bioinformatics Institute) and LASL-GDB (GenBank, National for Biotechnology Information) with the use of genetic Mac/CD software (Software Development, Tokyo, Japan). Rearranged V-D-J sequences of each T cell clone were determined by sequencing at least 10 isolates.

**Statistical analysis**

Statistical analysis was conducted with the two-tailed Student \(t\) test and the two-tailed Mann-Whitney test. A significant difference was considered to be \(p < 0.01\).

**Results**

Abs and mAb to NAE 07-06 peptide recognize a V\(\beta\) of EAN 60–70-specific T cell line

In EAN, Th1 cells are reported to be effector T cells. This T cell epitope is located in the bovine P2 protein, residues 53–78 (14). Recently, the minimal epitope was reported to be located in the bovine P2 protein, residues 60–70 (15). Therefore, rats were immunized with purified bovine peripheral myelin, and the sensitized T cells were pulsed with APC, educated with an appropriate concentration of EAN 60–70 peptide. Flow cytometric analysis showed that these cells were CD4\(^{+}\)CD8\(^{-}\) lymphocytes (data not shown).

To test whether or not mAb NAE3 recognize TCR, EAN 60–70-sensitized T cells were lysed, followed by electrophoresis and Western blotting to polyvinylidene difluoride membrane. Fig. 3 shows that NAE3 immunostained cell surface proteins from this T cell line which reacted with one recognized as well by mAb against rat TCR \(\alpha\beta\) (mAb R73). Precleared lysate with mAb R73 shows no visible bands around 38 kDa by mAb NAE3. The specificity of TCR molecules was demonstrated by the ability of mAb

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**Table I. Sequence homology between NAE 07-06 and the VDJ junctional regions from EAN 60–70-specific clones**

<table>
<thead>
<tr>
<th>Peptide or Clones</th>
<th>Abs to NAE 07-06 Reactivity (a)</th>
<th>NAE3 Reactivity (b)</th>
<th>Amino Acid Sequence</th>
<th>Homology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAE 07-06</td>
<td>+</td>
<td>+</td>
<td>S L D G E L K --</td>
<td></td>
</tr>
<tr>
<td>A6 clone</td>
<td>+</td>
<td>+</td>
<td>S L D G G A R --</td>
<td>64</td>
</tr>
<tr>
<td>H10 clone</td>
<td>+</td>
<td>+</td>
<td>--</td>
<td>54</td>
</tr>
<tr>
<td>B5 clone</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>36</td>
</tr>
<tr>
<td>1H7 clone</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>36</td>
</tr>
<tr>
<td>2A6 clone</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>27</td>
</tr>
</tbody>
</table>

\(\alpha\beta\) T cell clones were stained by Abs against NAE 07-06, followed by biotin-anti-rat IgG and FITC-avidin (a), or were stained by biotin-labeled NAE3, followed by FITC-avidin (b). T cell clones were analyzed using FACScan flow cytometric analysis (Becton Dickinson). VDJ regions of the indicated clone were aligned from N/DJ\(\beta\) into I\(\beta\). The sequence was then compared with that of NAE 07-06. Significant homology was found with the VDJ junction from A6 and H10 clones.

○, identical amino acid; ○, conservative substitution; △, amino acid with a similar hydropathy.
R73 but not mAb NAE3 to immunostain a TCR from a rat OVA-specific T cell lines (Fig. 3B). Recently, mAb R73 has been reported to recognize a constant region of rat TCR β-chain (24). Thus, these results suggest that NAE3 recognizes a TCR-β molecule from EAN 60–70–specific T cells.

We next examined whether or not Abs against the NAE 07-06 inhibited the proliferation of EAN 60–70–specific T cells. Fig. 4 shows that rat antiserum to NAE 07-06 caused a dose-dependent inhibition of the proliferation of bovine peripheral myelin-reactive lymphocytes. The specificity of the inhibition was demonstrated by the inability of antiserum to control peptide PBM 9-1 with an inverted hydrophobic profile relative to that of myelin basic protein residues 1–9, to block the bovine peripheral myelin-reactive lymphocytes. Fig. 5 similarly shows that mAb NAE3 block the proliferation of EAN 60–70–sensitized lymphocytes by pretreatment of EAN 60–70–sensitized T cells with mAb NAE3, not by pretreatment of EAN 60–70–educated APC with mAb NAE3. The control mAb CTCR8 showed no inhibition. No inhibitory effect was demonstrated on the proliferation of OVA-specific T cell lines using mAb NAE3. Not unexpectedly, the inhibition of proliferation of T cell lines by mAb NAE3 was weaker than that by polyclonal Abs against NAE 07-06. This is probably due to the recognition by mAb NAE3 of only a subpopulation of the total T cell lines reactive to EAN 60–70–peptide. In fact, the ability of inhibition by mAb NAE3 was demonstrated in only two of five clones we established from the aforementioned T cell lines, whereas Abs to NAE 07-06 induced an inhibitory effect on all clones (Fig. 6A). These two clones (denoted A6 and H10) were also stained by mAb NAE3 using FACScan analysis (Fig. 6B, Table I). All clones were further studied for analysis of Vβ usage in the TCR. Taken together with Western blot analysis, mAb NAE3 specifically recognized a TCR β molecule of EAN 60–70–sensitized T cells and blocked the proliferation of these cells. IL-2 production was also inhibited by treatment with mAb NAE3 (Fig. 7). The proliferation of these T cells was partially restored by following coculture with EAN 60–70–peptide and rIL-2, not with PBM 9-1 and rIL-2 in the culture (Table II). These results suggest that Abs to NAE 07-06 or mAb NAE3 induced T cell anergy.

To ascertain whether there was restricted Vβ region usage in the TCR that was recognized by mAb NAE3, two clones (H10 and A6 clones) were analyzed. These clones were found to utilize Vβ 8 family, which have a unique VDJ region (PLNTEV and SLDG-GAR). A homology check showed that these VDJ region sequences are similar to that of NAE 07-06 (Table II). Taken together with the results of Western blotting, these results suggest that immunization with NAE 07-06 can induce polyclonal and monoclonal Abs that block the proliferation of EAN 60–70–specific T cells, in accordance with T cell anergy through recognition of their TCR-β.

**Prevention of EAN**

Because we found that Ab to NAE 07-06 recognized Vβ of EAN 60–70–sensitized T cells and blocked their proliferation in vitro. We tested whether preimmunization with NAE 07-06 could induce polyclonal Abs to this peptide and block the development of EAN. Preimmunization with NAE 07-06 lessens the severity of disease when compared with animals immunized with control peptide, PBM 9-1 (Table III). This effect was demonstrated in animals in which disease was induced by EAN 60–70–peptide (Fig. 8). The same effect was also demonstrated in animals in which disease was induced by purified bovine peripheral myelin (data not shown). These results support the notion that the T cell epitope in EAN is located in bovine P2 protein, residues 60–70. Histological examinations showed that no visible cell infiltration was detected in sciatic nerves of rats (EAN induced by EAN 60–70) preimmunized with NAE 07-06. The control peptide, PBM 9-1, did not have this effect (Fig. 9). These results suggest that clonal expansion of EAN-specific T cells are completely blocked by polyclonal Abs to NAE 07-06. Furthermore, these polyclonal Abs did not influence the total subset of T cells in the peripheral lymphocytes checked by flow cytometry using W3/13, W3/25, and OX8 mAbs.

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### Table II. Restoration of NAE3-induced T cell anergy by stimulation with Ag and IL-2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pretreatment with NAE3</th>
<th>Stimuli</th>
<th>IL-2 (U/ml)</th>
<th>Proliferation (cpm) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>+</td>
<td>EAN 60-70</td>
<td>0</td>
<td>13282.3 ± 1062.5</td>
</tr>
<tr>
<td>b</td>
<td>+</td>
<td>EAN 60-70</td>
<td>50</td>
<td>42946.2 ± 8766.3</td>
</tr>
<tr>
<td>c</td>
<td>+</td>
<td>PBM 9-1</td>
<td>50</td>
<td>17709.8 ± 2833.5</td>
</tr>
<tr>
<td>d</td>
<td>−</td>
<td>EAN 60-70</td>
<td>50</td>
<td>88549.0 ± 11954.1</td>
</tr>
</tbody>
</table>

* After anergy induction with EAN3, EAN 60-70–specific lymphocytes were cocultured with EAN 60-70–educated irradiated APC in the absence (a) or presence (b) of IL-2 or with PBM 9-1–educated irradiated APC in the presence of IL-2 (c). EAN 60-70–specific lymphocytes without pretreatment with NAE3 were used as control (d).

### Table III. Histological scores*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2 ± 0.45</td>
</tr>
<tr>
<td>B</td>
<td>2.2 ± 0.45</td>
</tr>
</tbody>
</table>

* Rats were sacrificed 3 wk after inoculation with bovine P2 protein, residues 60-70. Group A was preimmunized with NAE 07-06 peptide. Group B was sham-treated with PBM 9-1 peptide (control group). Each group consists of six rats.
(Serotec) (data not shown). Namely, selective T cells (EAN 60–70 specific T cells) were blocked.

Discussion

The two-signal model for complete T cell activation has been previously described and reviewed (25–27). In short, T cell anergy was described as the results of a lack of costimulation during initial TCR engagement with Ag (28). Namely, T cells do not proliferate well in the absence of a costimulatory signal during an Ag presentation (29). Recent investigations demonstrated that anergy of regulatory T cells may play an important role in susceptibility to autoimmune disease (30). In T cell-mediated disorders such as MS, Th2 cells have regulatory function against the effector T cells (Th1). Thus, anergy of Th2 cells consequently activates and elicits the disease (30). Similarly, induction of T cell anergy of effector cells could also be one of the ultimate therapeutic methods for T cell-mediated disease. The procedures presented here demonstrate T cell anergy induction using a complementary peptide as an immunogen.

The immune network theory has classically been characterized as physiological autoimmunity. The formation of anti-Id Abs could regulate the formation of Id Abs (disease-causing Abs) in experimental autoimmune myasthenia gravis (EAMG) (1). The essence of the network theory is also applied to the T-B cell interaction. Namely, the formation of anticlonotypic Abs could recognize the paratope of TCR and could regulate the Th2 function to protect against the development of EAMG (21). Thus, anticlonotypic Abs induced by a complementary peptide could also protect against the development of EAN, which is caused by a Th1 response. The anti-Id Abs that are complementary to the Id of the binding site for Ag could mimic the structure of the Ag (mirror image). Thus, these anti-Id Abs could enhance or suppress the idiotypic response to that Ag. Zhou and Whitaker (31–33) have also had success with certain aspects of this procedure in an EAE model. In our experiment, monoclonal and polyclonal Abs against the complementary peptide were anti-Id Abs and blocked the proliferation of Ag-specific T cell lines. Although Abs to NAE 07-06 were not detected in the natural course of EAN using a ELISA (data not shown), it is possible that Abs to NAE 07-06 may be induced during the course of EAN.

As mentioned above, vaccination with certain CDRs of TCR effector cells is also reported to alter the regulatory T cells and to protect against the EAE (34, 35). These regulatory T cells recognize the CDR of effector T cells and suppress the effector cell
function (36). In our experiments, the complementary peptide shared homology with the VDJ junction of TCR-β. Thus, it is possible that immunization with a complementary peptide could also induce the regulatory T cells and play a role in regulation of the disease. As demonstrated in a previous report, Th2 responses were not completely ablated in EAMG with this technique because of the existence of other T cell epitopes on the acetylcholine receptor. However, in the present experiments, Th1 responses were completely depleted and were nonexistent in the peripheral nerve in EAN. Because immunization with a complementary peptide for EAN 60–70 peptide can block the EAN induced by whole bovine myelin or bovine P2 protein 60–70, our experimental results support the idea that bovine P2 protein 60–70 is the major epitope of the Lewis rat model of EAN. Furthermore, no occurrences of disease were observed for 3 mo in our experiment.

Our goal is to achieve an ideal therapeutic procedure to correct aberrant immune responses in autoimmune diseases. The procedures presented here and elsewhere (1, 2, 21) may have wide utility of selective immunological suppression for T and/or B cells. As mentioned in a previous publication (1), the procedure requires no knowledge of the paratope sequences of B or T cells. The role of the precise pattern of amino acid hydrophathy in protein and peptide shape or structure allows one to construct peptides, which presumably assume shapes or structures complementary to disease-associated epitopes. Thus, vaccination with a complementary peptide induces the production of antidiotypic and anticonalotypic Abs the combining sites of which are complementary to and therefore reactive with Ag receptors on disease epitope-specific T and B cells. Also, Ab-induced T cell anergy may provide more precise information for understanding the mechanism of T cell regulation and T-B cell interaction.

Acknowledgments

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References


