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Amelioration of Experimental Autoimmune Uveoretinitis by Pretreatment with a Pathogenic Peptide in Liposome and Anti-CD40 Ligand Monoclonal Antibody

Kenichi Namba,*† Kazumasa Ogasawara,*‡ Nobuyoshi Kitaichi,*† Taiki Morohashi,* Yoichi Sasamato,* Satoshi Kotake,* Hidehiko Matsuda,* Kazuya Iwabuchi,* Chikako Iwabuchi,* Shigeaki Ohno,* and Kazunori Onoe³

We have defined a peptide K2 (ADKDVVVLTSSRTGGV) that corresponds to residues 201–216 of bovine interphotoreceptor retinoid-binding protein that induces experimental autoimmune uveoretinitis (EAU) in H-2A<sup>k</sup>-carrying mice (H-2A<sup>k</sup> mice). In this study, we attempted to ameliorate EAU in the H-2A<sup>k</sup> mice without nonspecific suppression of T cell responses. Preceding s.c. administration of liposomes including K2 (liposomal K2) specifically inhibited subsequent generation of T cell response to K2. The same result was obtained with a combination of OVA<sub>323–339</sub> peptide and the OVA-specific TCR-transgenic T cells. It was suggested that the inhibition was mainly attributed to peripheral anergy induction of T cells specific for the peptide Ag, although specific cell death might also be involved in the inhibition. Pretreatment with liposomal K2 also considerably abolished IFN-γ production but not IL-4 production. The specific inhibitory effect of the pretreatment with liposomal peptide was augmented by a simultaneous administration of anti-CD40 ligand (anti-CD40L) mAb. Moreover, it was shown that the pretreatment with liposomal K2 reduced both the incidence and severity of the subsequent K2-induced EAU, and the simultaneous administration of anti-CD40L mAb augmented this preventive effect by liposomal K2. Our findings demonstrate that the s.c. administration of liposomal pathogenic peptide and anti-CD40L mAb can be applied to preventing autoimmune diseases without detrimental nonspecific suppression of T cell responses. *The Journal of Immunology, 2000, 165: 2962–2969.

Experimental autoimmune uveoretinitis (EAU) is an organ-specific, T cell-mediated autoimmune disease that can be induced in several animal models by immunization with retinal Ag, e.g., interphotoreceptor retinoid-binding protein (IRBP) emulsified in CFA, or by the adoptive transfer of retinal Ag-specific T lymphocytes into naive syngeneic recipients (1–3). Histopathology in the animal model resembles several human endogenous uveoretinitis (e.g., Behçet’s disease, sarcoidosis, Vogt-Koyanagi-Harada syndrome, birdshot retinochoroidopathy, and sympathetic ophthalmitis) and is characterized by posterior retinal and choroidal lesions, photoreceptor damage, vasculitis, vitritis, granuloma formation, and varying degrees of anterior chamber infiltration (4–6). However, cumbersome IRBP preparation from bovine eyes and complex antigenicity of the IRBP had been an obstacle to precise immunological analyses of EAU. To circumvent this obstacle, referring to the H-2A<sup>k</sup>-binding motif, we have defined a peptide, K2, in bovine IRBP that induces EAU in H-2A<sup>k</sup> mice (7).

A desirable therapy of autoimmune diseases involving autoreactive CD4<sup>+</sup> T cells would be selective inactivation, elimination, or functional deviation of the disease-causing T cells. Various approaches have been used to regulate Ag-specific responses in experimental allergic encephalomyelitis (EAE), which is another type of organ-specific autoimmune animal model. It was reported that administration of a low number of encephalitogenic T cells (8), immunization with peptide derived from TCR of encephalitogenic clones (9), or administration of mAb specific for TCR of encephalitogenic clones (10) prevented development of EAE. It was also reported that an i.p. injection or repeated s.c. injections of an encephalitogenic peptide in IFA ameliorated EAE by inducing anergy of encephalitogenic T cells (11, 12). In contrast, Forsthuber et al. (13) demonstrated that the administration of encephalitogenic peptides in IFA induced differentiation of potentially encephalitogenic T cells into nonencephalitogenic, possibly protective cells of Th2 phenotype. The precise mechanism underlying the induction of T cell tolerance by administration of the peptide in IFA is still unclear.

In this study, we attempted to prevent K2-induced EAU by the preceding administration of liposomal K2. We used liposome (phosphatidylidyolpholine/phosphatidylserine = 7:3) instead of IFA because we observed that the liposome promoted effective incorporation of peptide Ag into dendritic cells (DC), a professional APC (14, 15). We report herein that the liposomal K2 induces T cell tolerance more efficiently than K2 in IFA and inhibits EAU induction.

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Furthermore, we show that simultaneous administration of liposomal K2 and anti-CD40 ligand (CD40L) mAb inhibits considerably the development of EAU as compared with the administration of liposomal K2 alone.

### Materials and Methods

#### Animals

B10.BR female mice (H-2A b mice) and BALB/c mice (H-2A b mice) were obtained from Shizukura Laboratory Animal Corporation (Hamamatsu, Japan). OVA-specific TCR-transgenic mice (DO11.10 TCR mice) (16) were obtained from Dr. Dennis Loh (Washington University, St. Louis, MO) and were maintained in a specific pathogen-free condition of our animal facility at Hokkaido University.

#### Peptides and adjuvants

Peptide K2 (ADKDVVVLTSSRTGGV) that corresponds to bovine IRBP201-216, and OVA323-339 peptide (IQAVHAHAAHAEINEAGR) were synthesized using automatic peptide synthesizer PSSM-8 (Shimadzu, Kyoto, Japan) and purified by reverse-phase HPLC (Waters Japan, Tokyo, Japan) as previously described (7). CFA, IFA, and *Mycobacterium tuberculosis* H37Ra were purchased from Difco (Detroit, MI). Bordetella pertussis inactive bacterial suspension was purchased from Wako Pure Chemical Industries (Tokyo, Japan).

#### Liposome and pretreatment

Multilamellar liposome was prepared as previously described (17). Because preliminary experiments showed that 50- to 200-nmol K2 peptides exerted almost the same levels of influence, 100-nmol peptides were used throughout the study. The peptide (100 nmol) was mixed with 1.25 μmol of phosphatidylserine (Avanti Polar Lipids, Alabaster, AL) and 2.75 μmol of phosphatidycholine (Avanti Polar Lipids) in chloroform. In a glass tube, the mixture was blown by N2 gas, evaporated at 40°C, and aspirated for 3 h. The peptide incorporated in multilamellar liposome (liposomal peptide) that adhered to the inside of the glass tube was dissolved in 300 μl PBS by vortexing and sonication with or without anti-CD40L mAb (50 μg; PharMingen, San Diego, CA). The dose (50 μg) of anti-CD40L mAb was determined by preliminary experiments in which 50–500 μg/mouse mAb showed the same level of inhibition. The liposomal peptide with or without anti-CD40L mAb, liposome alone, or PBS (as a negative control) was s.c. injected into the backs of mice (three mice per group) (pretreatment) 10 days before immunization as described below. One group of mice was treated with peptide (100 nmol) in IFA.

#### Immunization

To analyze T cell proliferative response, B10.BR mice were immunized in the footpads and the tail base with peptide (50 nmol) in emulsion with CFA. A representative finding, EAU clinical scores were graded 0–4 as described by Thurau et al. (20) (Table I).

At day 37 after immunization, eyes were enucleated and fixed for 1 h in 4% phosphate-buffered glutaraldehyde and transferred into 10% phosphate-buffered formaldehyde until processing. Fixed tissues were processed and the histological severity was graded on a scale of 0–4 as reported previously (7).

#### Statistical analysis

The data of mean thymidine uptake (cpm) or mean EAU clinical score were analyzed with Student’s t test.

### Results

#### Pretreatment with liposomal K2 peptide decreased K2-specific T cell responses

B10.BR (H-2A b) mice were s.c. administered PBS, liposome, K2 emulsified with IFA, or liposomal K2, and, 10 days later, immunized in the footpads with K2 emulsified with CFA. A representative result from three separate experiments is shown in Fig. 1. In the PBS- and liposome-pretreated mice, K2-primed T cells reached almost the same levels of response against K2 (Fig. 1, a and b). As reported earlier by Gaur et al. (11), the pretreatment with K2 in IFA considerably decreased the K2-specific T cell responses (Fig. 1c). It should be noted that the pretreatment with liposomal K2 reduced T cell responses more profoundly than that with K2 in IFA (Fig. 1d). Almost the same levels of PPD-specific T cell responses were detected in these mice pretreated with PBS, liposome alone, K2 in IFA, or liposomal K2 (5689, 5917, 7567, or 6619 Δcpm).

### Table 1. Clinical score of EAU in the mouse

| Grade | Criteria
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<tr>
<td>0</td>
<td>No change</td>
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<tr>
<td>1</td>
<td>Mild vasculitis; &lt;5 focal lesions; ( \geq 1 ) linear lesion</td>
</tr>
<tr>
<td>2</td>
<td>Multiple (&gt;5) choroidal lesions and/or infiltrations; severe vasculitis (large size, thick wall, infiltrations); &lt;5 linear lesions</td>
</tr>
<tr>
<td>3</td>
<td>Pattern of linear lesions; large confluent lesions; subretinal neovascularization; retinal hemorrhages; papillodema</td>
</tr>
<tr>
<td>4</td>
<td>Large retinal detachment; retinal atrophy</td>
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* According to Thurau et al. (20).
pretreatment with liposomal K2 (23), the T cells hardly responded.

mice were immunized with K2 emulsified in CFA 3 days after the

termed RR, were shown in Fig. 1 values of T cell responses to K2 by those to PPD. These ratios, termed RR, were shown in Fig. 1e (see Materials and Methods). It seems that liposomal K2 diminishes the K2-specific T cell responses more profoundly than K2 in IFA.

Time course of the inhibitory effect of pretreatment with liposomal K2 on K2-specific T cell responses

We then examined the time course of the inhibitory effect of the liposomal K2 pretreatment on the K2-specific T cell responses. Fig. 2 shows a representative result obtained with two separate experiments. This figure illustrates T cell-proliferative responses in mice pretreated with liposomal K2 at different times. When H-2Aa mice were immunized with K2 emulsified in CFA 3 days after the pretreatment with liposomal K2 (−3), the T cells hardly responded to K2. The Ag-specific inhibitory effect of the liposomal K2 continued for 2–3 wk, although the inhibitory effect of the liposomal K2 was reduced compared with that seen in the 3-day group (Fig.

To elucidate the mechanism underlying the specific T cell inhibition

To elucidate the mechanism underlying the specific inhibition of T cell responses by the pretreatment with liposomal K2, various Ags and TCR-transgenic T cells in this experiment, a combination of a peptide, OVA(323–339), which can be recognized by T cells bearing the transgenic DO11.10 TCR in the context of H-2Aa (16), and BALB/c (H-2Aβ) mice transferred with the DO11.10 T cells, was used. It has been demonstrated that BALB/c mice-transferred DO11.10 T cells (DO11.10→BALB/c) are useful for analyzing the mechanism underlying T cell tolerance in vivo (21–23). In these mice, the T cells expressing DO11.10 TCR show normal lymphocyte functions and are readily detected with a clonotypic mAb, KJ1-26.

(Do11.10→BALB/c) mice were treated with liposomal OVA(323–339), liposome alone, or OVA(323–339) emulsified with IFA three times. Three days after the last pretreatment, these mice were immunized with OVA(323–339) in CFA, and 3 days later, the OVA(323–339) specific T cell responses were analyzed. At this stage, normal BALB/c mice that had not received DO11.10 T cells generated no T cell responses to OVA(323–339) (data not shown). Fig. 3a shows results of two representative mice in each group. Pretreatment with liposomal OVA(323–339) considerably inhibited the proliferative responses of the lymph node cells. The inhibition was again more profound than that seen in mice pretreated with peptide in IFA (Fig. 3a). When amounts of IL-2 in the CS were quantitated, almost complete inhibition was observed in (DO11.10→BALB/c) mice pretreated with liposomal peptide, and moderate inhibitions were seen in mice treated with peptide in IFA (Fig. 3b). Similar results were obtained with IFN-γ production (data not shown).

Then, to evaluate the responsiveness per T cell, (Do11.10→BALB/c) mice that had been pretreated with liposomal OVA(323–339) or liposome alone were immunized with OVA(323–339) in CFA, and...
both the proportion of KJ1-26^CD4^+ cells and the proliferative responses were quantitated. The proportion of KJ1-26^CD4^+ T cells recovered from the lymph nodes of liposomal peptide-pretreated mice was lower than that in liposome alone-pretreated mice (data not shown). Then Δcpm values were adjusted to those per 5000 KJ1-26^CD4^+ T cells. Fig. 3 shows a representative result from two separate experiments. DO11.10 T cells from liposomal OVA 323–339-pretreated (DO11.10 BALB/c) mice (P1 and P2) showed significantly low responsiveness as compared with that in the liposome alone-pretreated (DO11.10 BALB/c) mice (C). Almost the same result was obtained with the other experiment (data not shown). These findings suggest that the s.c. administration of liposomal OVA323–339 induces anergy and/or perhaps the depletion of KJI-26^CD4^+ T cells.

Additional effect of anti-CD40L mAb administration on inhibition of specific T cell responses by pretreatment with liposomal K2

B10.BR mice (three mice per group) were pretreated with liposome alone, liposomal K2, or liposomal K2 plus anti-CD40L mAb and, 2 or 3 wk later, were immunized with K2 in CFA. After 10 days, T cell proliferative responses were analyzed. Fig. 4a shows a representative result from two separate experiments. Again, liposomal K2 induced considerable inhibition of T cell responses to K2. The inhibitory effect of the liposomal K2 on the induction of K2-specific T cell responses was slightly but significantly augmented by the addition of anti-CD40L mAb to the liposomal K2.

FIGURE 3. Inhibition of OVA-specific T cell responses by liposomal peptide in mice adoptively transferred OVA-specific TCR-transgenic T cells. BALB/c mice that had been i.v. injected with KJ1-26-positive T cells (5 × 10^6/mouse) from DO11.10 TCR-transgenic mice were s.c. administered either liposomal OVA323–339 (P1, P2; the numbers indicate individual mice), liposome alone (C1, C2), or OVA323–339 in IFA (IFA1, IFA2) 3, 6, and 9 days after the transfer. Three days after the last treatment, these mice were immunized with OVA323–339 in CFA. Three days later, T cell proliferation to OVA323–339 was analyzed (a). Amounts of IL-2 in the CS were analyzed (b). Liposomal peptide-pretreated (P1, P2) or liposome alone-treated (C) mice were immunized with OVA323–339 in CFA, and 3 days later KJ1-26^CD4^+ T cells in the draining lymph nodes were quantitated by FACS analysis, and the proliferative responses against OVA323–339 were examined (c). The mean cpm values at each peptide dose were adjusted to those per 5000 KJ1-26^CD4^+ T cells in the culture. (×, p < 0.05 vs C)

FIGURE 4. Augmented K2-specific inhibition by simultaneous administration of anti-CD40L mAb and liposomal K2. a, B10.BR mice were pretreated with liposomal K2 (P), liposomal K2 and anti-CD40L mAb (PA), or liposome alone (C). Two weeks after the pretreatment, the B10.BR mice were immunized with K2 in CFA, and, 10 days later, T cell proliferation was evaluated (×, p < 0.01 vs P; **, p < 0.05 vs P). b, B10.BR mice were pretreated with liposomal K2 and anti-CD40L mAb. These mice were immunized with K2 in CFA 14 or 21 days later, and, after 10 days, the [3H]thymidine uptake was evaluated. C, Pretreatment with liposome alone; −14 or −21, pretreatment 14 or 21 days before immunization (×, p < 0.01 vs −21; **, p < 0.05 vs −21). c, B10.BR mice were pretreated with liposomal K2 and anti-CD40L mAb (PA), anti-CD40L mAb alone (A), or PBS (C). Three weeks after the pretreatment, the B10.BR mice were immunized with K2 in CFA, and the T cell proliferative response was determined. (×, p < 0.01 vs C)
Levels of IFN-γ and IL-4 seen in the CS of T cells from mice pretreated with liposomal K2 and anti-CD40L mAb were almost the same as those in the CS of T cells treated with liposomal K2 alone. Recent studies demonstrated that the administration of anti-CD40L mAb altered Th1-type responses to the Th2-type responses in vivo (24–26). However, the shift of the cytokine production pattern from Th1 to Th2 was not augmented by simultaneous administration of anti-CD40L mAb in our experimental system. In addition, it was shown that administration of anti-CD40L mAb alone showed no influence on the cytokine production by the K2-primed T cells (Fig. 5, a and b). Almost the same low levels of IL-2 were detected in CS of the lymph node cells, irrespective of administration of liposomal peptide or PBS (data not shown). These findings suggest that the administration of liposomal K2 inhibits Th1 response but not that of Th2, and that the augmentation of the inhibitory effect of liposomal K2 by anti-CD40L mAb results from anergy induction but not from the Th2 shift.

Inhibition of Th1-type cytokine production by pretreatment with liposomal K2

We examined whether inhibition of K2-specific T cell-proliferative responses was associated with the inhibition of cytokine production. K2-primed T cells from B10.BR mice that had been pretreated as described above were cocultured with APC and 30 μM K2 peptide, and amounts of IFN-γ, IL-2, and IL-4 in the CS were determined. Fig. 5a shows that T cells from mice pretreated with liposomal K2 in vivo produced a significantly smaller amount of IFN-γ than those treated with PBS or liposome alone (controls). In contrast, no influence on the level of IL-4 production in the CS was observed in these mice (Fig. 5b). Thus, the reduction of IFN-γ but not of IL-4 appeared to alter the IFN-γ-IL-4 ratio and polarize toward a Th2 shift.

FIGURE 5. Depressed production of IFN-γ but not of IL-4 in CS of T cells from liposomal K2-pretreated mice. B10.BR mice (three mice per group) were pretreated with liposomal K2 plus anti-CD40L mAb, liposomal K2, anti-CD40L mAb, liposome alone, or PBS and then immunized with K2 in CFA. Ten days later, lymph node T cells of the pretreated mice were cultured with APC and K2 (30 μM) for 3 days and amounts of IFN-γ (a) and IL-4 (b) in the CS were quantitated.

Prevention of EAU development by pretreatment with s.c. administration of liposomal K2 and anti-CD40L mAb

It has been demonstrated that EAU is induced by Th1 cells (6, 7). We then examined whether the pretreatment with liposomal K2 and anti-CD40L mAb prevents EAU development in H-2A\(^b\) mice. Fig. 6 shows two separate experiments. In each experiment, five mice (10 eyes) in one group were analyzed. Ten days before EAU induction by immunization with K2 in CFA, H-2A\(^b\) mice were administered s.c. either liposomal K2, anti-CD40L mAb alone, liposomal K2 together with anti-CD40L mAb, or PBS. Thereafter, the clinical score of EAU was sequentially determined. Compared with the PBS pretreatment as a control, liposomal K2 administration significantly decreased EAU clinical score (p < 0.01 at days 27–37) (Fig. 6a). Furthermore, additional treatment with anti-CD40L mAb augmented the inhibitory effect of liposomal K2 on EAU development, both onset and severity (Fig. 6b). Fig. 6c

FIGURE 6. Prevention of EAU development by pretreatment with liposomal K2 or liposomal K2 plus anti-CD40L mAb. a and b, B10.BR mice were pretreated with PBS (C), liposomal K2 (P), or liposomal K2 plus anti-CD40L mAb (PA). Thereafter, the mice were immunized in the footpads and base of the tail with 100 nmol K2 in CFA. Concurrently, B. pertussis inactive bacterial suspension (10\(^{10}\)) in a volume of 100 μl was injected intraperitoneally as an additional adjuvant. Clinical score was determined as described in Materials and Methods. c and d, B10.BR mice were pretreated with PBS (C), liposomal K2 plus anti-CD40L mAb (PA), or anti-CD40L mAb alone (A).
shows the same set of experiments as Fig. 6b. Although the EAU onset in the control group was detected at early stages in this particular experiment, almost complete inhibition of EAU by both anti-CD40L mAb and liposomal K2 was obtained. In contrast, pretreatment with anti-CD40L mAb alone showed no considerable influence on the EAU development as compared with that in PBS-treated mice (p > 0.1 at days 20, 23, 27, and 34) (Fig. 6d). Thus, it was demonstrated that prior administration of liposomal K2 plus anti-CD40L mAb inhibited the subsequent development of K2-induced EAU more profoundly than that of liposomal K2 alone.

Fig. 7 summarises pathological scores of eye tissues from mice used for clinical assessment in the prior two experiments. Eyes were enucleated immediately after the last clinical assessment of EAU and examined histologically. Because the inflammation area of the eyes was limited, the pathological EAU was not always observed in the eye sections of positive control groups, where almost all mice showed clinically detectable EAU. Nevertheless, it seemed clear that no or few histological EAU areas were detected in liposomal K2 and liposomal K2 plus anti-CD40L groups, whereas histological EAU could be observed in substantial proportions of the eyes from PBS control group or liposome alone group (Fig. 7).

Discussion
In this study, we showed that pretreatment of H-2A{k mice with the liposomal K2 considerably inhibited the subsequent K2-specific T cell-proliferative response and IFN-γ production, but not IL-4 production. In contrast, almost the same levels of T cell-proliferative response to PPD were observed among liposomal K2-treated mice and control mice pretreated with PBS or liposome alone. Thus, the inhibition appeared to be induced in Th1 cells specific for the K2. The inhibitory effect of the liposomal K2 on the K2-specific T cell response was greater than that by K2 in IFA. Similar results were obtained in the combination of DO11.10 T cells and OVA323–339.

It was suggested that T cell tolerance by repeated s.c. injections of peptide in IFA was attributed to the T cell anergy specific for the peptide Ag (11, 13). Thus, we attempted to determine whether the T cell tolerance induced by s.c. administration of liposomal peptide was also attributed to the T cell anergy. To address this issue, we used a combination of OVA323–339 peptide and H-2A{k (BALB/c) mice that had been transferred T cells bearing DO11.10 TCR (21–23). However, Hayashi et al. (27) reported that naive DO11.10 T cells were resistant to the anergy induction. Indeed, in this experimental system, a single treatment with liposomal peptide (100 nmol) showed only a slight effect (data not shown). Then, the (DO11.10→BALB/c) mice were s.c. administered liposomal OVA323–339 (50 nmol) or OVA323–339 in IFA three times (12). Three days after the last pretreatment, these mice were immunized with OVA323–339 in CFA. The T cell responses were marked inhibited in liposomal peptide-treated mice as compared with those treated with liposome alone (control mice) and those treated with peptide in IFA. The number of the transferred DO11.10 T cells in the draining lymph nodes of (DO11.10→BALB/c) mice pretreated with liposomal peptide was lower than that in those treated with peptide in IFA or liposome alone. Nevertheless, the responsiveness against OVA323–339 Ag per 5000 DO11.10 T cells in vitro was considerably decreased in the former mice pretreated with liposomal peptide as compared with that in control mice. These findings suggest that the s.c. administration of liposomal peptide induces both peptide-specific T cell, perhaps Th1, anergy, and selective death among the Th1 cells. We consider that rapid uptake and presentation of the peptide Ag in liposomes by APC may result in the engagement of TCR before sufficient expression of the costimulatory molecules such as B7 on the APC and induce anergy and/or death in the T cells (14, 15).

It has been reported that a single i.p. injection or repeated s.c. injections of peptide in IFA not only induces the peptide-specific T cell tolerance but also prevents development of autoimmune diseases in mice (11, 13). Thus, it was anticipated that liposomal peptide could be used for preventing autoimmune diseases. Indeed, the same pretreatment with liposomal K2 ameliorated EAU in the H-2A{k mice that would have been induced by immunization with K2 in CFA 10 days later.

In various autoimmune disease models, including EAE and rheumatic arthritis, i.p. administration of anti-CD40L mAb has been used to successfully prevent development of the diseases (25, 28–31). However, we show herein that s.c. administration of anti-CD40L mAb (50 μg) alone exerts no influence on the development of EAU. Although it has been reported that anti-CD40L mAb functions at various sites and stages of the immune responses (32), a single s.c. administration of anti-CD40L mAb alone might no longer influence the priming of T cells by K2 in CFA administered 10 days later. This finding seems to be compatible with the result that PPD-specific T cell response (a representative Th1-type response) (33–35) was not inhibited by the administration of anti-CD40L mAb at all. H37Ra bacterial Ag including PPD was given 10 days after anti-CD40L mAb administration, and no inhibitory effect was observed in the response to PPD. Similarly, when mice were treated with anti-CD40L mAb alone, and immunized K2 in CFA 3 wk later, inhibition of the generation of the K2-specific T cell response was almost negligible. Perhaps at these stages the effect of anti-CD40L mAb no longer lasted.

Nevertheless, simultaneous s.c. administration of the same dose of anti-CD40L mAb in addition to liposomal K2 augmented the inhibitory effect of the liposomal K2 on T cell-proliferative responses and the liposomal K2-induced prevention of EAU development. Upon CD40 ligation, professional APC such as DC up-regulate costimulatory activity and efficiently activate T cells. Thus, lack of APC activation through CD40 appears to result in insufficient priming of CD4+ T cells (36, 37). Liposomal peptide
Ag and anti-CD40L mAb were mixed exclusively and administered s.c. At this early stage of Ag presentation, anti-CD40L mAb blocked important costimulatory functions of APC, and T cell energy specific for the peptide Ag in liposome might efficiently be induced. Thus, we consider that the simultaneous administration of anti-CD40L mAb and peptide Ag resulted in the synergistic induction of T cell anergy shown in this study.

Although the main mechanism underlying the augmenting effect of anti-CD40L mAb on the inhibition of disease onset and severity of EAU induced by liposomal K2 appears to be anergy induction in Th1 cells and the subsequent Th2 shift, there may be other possibilities. It has been reported that CD40 ligation activates endothelial cells and up-regulates expression of adhesion molecules (38) as well as production of proinflammatory cytokines (39). These alterations seem to promote extravasation of lymphocytes. Thus, it seems possible that anti-CD40L mAb blocks transmigration of T cells across the blood-ocular barrier and eventually inhibits development of EAU. However, this possibility appears to be unlikely, because anti-CD40L mAb may not be present at the stage of EAU development.

It has been postulated that anti-CD40L mAb promotes to shift Th1-type to Th2-type responses. In the absence of a signal through CD40, DC produce no or negligible IL-12 that results in prevention of Th1 priming necessary for developing various autoimmune diseases and allograft rejections (28–31, 40, 41). However, in this study, we demonstrated that pretreatment with anti-CD40L mAb either with liposomal K2 or PBS showed no apparent influence on the productions of IFN-γ and IL-4 by T cells from these mice, which were immunized with K2 in CFA 10 days after pretreatment (Fig. 5). Perhaps production of a Th1 cytokine, IFN-γ, was inhibited sufficiently by liposomal K2 alone, and additive effects of anti-CD40L mAb might be detected only in inhibition of the Th1 cell-proliferative responses but not apparently seen in inhibition of Th1 cytokine production in our experimental system.

In this study, we demonstrated that pretreatment with liposomal peptide and anti-CD40L mAb induced the peptide-specific T cell tolerance. We consider that our procedure would be useful for inducing Ag-specific immune suppression and preventing autoimmune diseases in which pathogenic epitope peptides are identified (42). To apply our method to clinical use, it should be pursued in autoimmune diseases in which pathogenic epitope peptides are identified and inducing Ag-specific immune suppression and preventing autoimmunity. We consider that our procedure would be useful for induction of autoimmune encephalomyelitis by myelin basic protein-derived peptide. J. Exp. Med. 186:507.


