

Kainic acid-induced excitotoxic hippocampal neurodegeneration in C57BL/6 mice: B cell and T cell subsets may contribute differently to the pathogenesis

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Abstract

The roles of T cells and B cells in kainic acid (KA)-induced hippocampal lesions were studied in C57BL/6 mice lacking specific T cell populations (CD4, CD8, and CD4/CD8 cells) and B cells [Igh-6(–/–)]. At 48 mg/kg of KA administered intranasally, KA-induced convulsions were seen in all groups. However, CD4/CD8(–/–) mice exhibited the mildest seizures; the responses of CD8(–/–), Igh-6(–/–) and wild-type mice were intermediate, whereas CD4(–/–) mice displayed much more severe clinical signs and 100% early mortality, indicating that a deficiency of CD4 T cells obviously increased susceptibility to KA-induced brain damage. Histopathological analysis of the mice that survived 7 days after KA administration revealed that CD4/CD8(–/–) mice had the fewest pathologic changes but Igh-6(–/–) mice showed more severe lesions in area CA3 of the hippocampus than CD8(–/–) and wild-type mice. Reactive astrogliosis were prominent in all KA-treated mice. Locomotor activity as assessed by open-field test increased after KA administration in Igh-6(–/–) and wild-type mice only. These results denote the influence of the adaptive immune response on KA-induced hippocampal neurodegeneration and suggest that B cell and T cell subsets may contribute differently to the pathogenesis. © 2004 Elsevier Inc. All rights reserved.

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1. Introduction

Kainic acid (KA) is commonly used to elicit a selective neurodegeneration in rodents that mimics such human neurological disorders as Huntington's chorea and Alzheimer's disease (AD). In adult rats, systemic administration of KA causes recurrent seizures and neuronal degeneration in selected brain regions (Pollard et al., 1994). KA administration also causes the behavioral changes in mice, which are associated with neuronal pyknosis and cell loss in the pyramidal CA3 cell layer in hippocampus as well as astrogliosis (Royle et al., 1999). Ligation of KA to its receptors leads to an influx

of calcium into neurons, which is believed to play a critical role in seizure genesis (McNamara, 1992; Meyer, 1989; Speckmann et al., 1989). Calcium entry can also result in neuronal death through a series of reactions. During this process, neurons may communicate with structures in the surrounding environment, such as microglia and astrocytes.

Additionally, the immune system may be involved in this communication. The damage can promote up-regulation of endothelial adhesion molecules, MHC I molecules and nuclear factor- κ B expression as well as enhancement of proinflammatory cytokine production in the lesion areas (Akiyama et al., 1994; Corriveau et al., 1998; Eriksson et al., 1999; Matsuoka et al., 1999; Lerner-Natoli et al., 2000). The humoral immune response can also increase in the brain and periphery

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(Akiyama et al., 1994; Pan and Long, 1993). It has been demonstrated that CD40–CD40 ligand interaction results in microglial activation after β -amyloid stimulation, suggesting that the adaptive immune response is an early event in AD pathogenesis (Tan et al., 1999). Experimental and clinical studies indicate impairments in both humoral and cellular immunity in an animal model of AD as well as in AD patients (Popovic et al., 1998). Furthermore, the effects of KA-induced lesions in the lateral septal area influenced cell-mediated immune function (Wetmore et al., 1994). These and other findings suggest that KA-induced seizures and neurodegeneration may be associated with altered immune cell functions (Calvo et al., 1996; de Lecea et al., 1994; Kaur and Ling, 1992; Nance et al., 1987; Smith et al., 2000).

Techniques for producing transgenic and knockout animals have provided a powerful experimental tool for studying the functions of specific genes although there are still some limitations with this technique.

To investigate whether T cells and/or B cells participate in KA-induced seizures, and characterize subtypes involved in the related neurodegeneration, we established the model of KA-induced neurodegeneration in the C57BL/6 mice deficient in specific T cells [CD4(–/–), CD8(–/–), and CD4/CD8(–/–)] (Bachmann et al., 1995; Fung-Leung et al., 1991; Penninger et al., 1995; Rahemtulla et al., 1991; Rahemtulla et al., 1994; Schilham et al., 1993; Tada et al., 1996) and B cells [Igh-6(–/–)] (Kitamura et al., 1991; Beutner et al., 1994; Brundler et al., 1996). In Igh-6(–/–) mice, the gene that produces the heavy chain of IgM in precursor B cells is disrupted; consequently, these mice have no mature B cells.

In a previous study, we successfully established this model of neurodegeneration after administering KA by the intranasal route in C57BL/6 mice, which is the background for most available knockout mice and until now it has been reputedly resistant to the KA-induced brain damage by administering subcutaneously or intraperitoneally (Schauwecker and Steward, 1997). The neuropathological change in C57BL/6 mice was only found in the hippocampus (Chen et al., 2002). However, the exact mechanisms of KA-induced neurodegeneration are not clear. In the present study, we used the immunodeficient mice to elucidate the mechanisms underlying the hypothesis that immune cells, such as subsets of T cells and B cells are involved in KA-induced neurodegeneration and these cells may contribute differently to the pathogenesis.

2. Materials and methods

2.1. Animals

Male C57BL/6 (wild-type) ($n = 25$) and corresponding knockout mice CD4(–/–) ($n = 17$), CD8(–/–)

($n = 19$), CD4/CD8(–/–) ($n = 15$) and Igh-6(–/–) ($n = 14$) of C57BL/6 background were 5–6 weeks old when used in the three separate experiments. All immunodeficient mice were obtained from the Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden. The immunological characteristics of all immunodeficient mice used in the present study have been confirmed by flow cytometry analysis and western blot.

Seven-day-old littermates (6–8 pups) from the wild-type and above immunodeficient groups were used for cerebellar granule cell (CGC) culture. All mice were housed on a 12-h light–dark schedule. Water and food were available ad libitum.

2.2. KA administration

Mice were partially anesthetized with Isofluen (Abbott Laboratories, Kent, England) and held on their backs by hand. KA (Sigma–Aldrich, Stockholm, Sweden) dissolved in water (10 mg/1.3 ml) was slowly and gently dropped by micropipette into the noses of 46 mice at the dose of 48 mg per kilogram body weight. A total of 40 μ l of the KA solution (20 μ l for each naris with a 2 min break between insertion into the first and second nostril) was applied over a 10 min period. For volumes of more than 40 μ l KA solution, the first 40 μ l were administered over a 10 min period, with a break of 10 min, after which the next 40 μ l was administered. Three age- and body weight-matched control C57BL/6 mice received the same amount of water intranasally. Mice were monitored continuously for 5 h to register the onset and extent of seizure activity. Seizures were rated as follows: 0, normal; 1, immobilization; 2, rearing and falling; 3, seizure for less than 1 h; 4, seizure for 1–3 h; 5, seizure for more than 3 h; and 6, death.

2.3. Histopathological analysis

Mice were anesthetized with sodium pentobarbital and transcardially perfused with phosphate-buffered saline (PBS) 7 days after the administration of KA. The brains were obtained, fixed in 4% buffered formaldehyde, and embedded in paraffin. Coronal sections (7 μ m slices) from –1.15, –1.94, and –2.80 mm, respectively, relative to the bregma were prepared according to the information in Franklin's brain atlas (Franklin and Paxinos, 1997). Preliminary experiments by continuous sectioning showed that sections at these three levels could represent the overall changes in the hippocampus. The sections were stained by Nissl's method to evaluate the morphology of neurons. Using a blinded protocol, the counting was duplicated by two different examiners. For assessments of the severity and extent of neurodegeneration in the hippocampus by Nissl's staining, the sections were scored using a semiquantitative grading

system: 0, normal; 1, slight shrinkage of neurons (1–4% pycnotic neurons in area CA3); 2, moderate shrinkage of neurons (5–15% pycnotic neurons in area CA3); 3, severe shrinkage of neurons (more than 15% pycnotic neurons in area CA3); 4, slight loss of neurons (5–10% neuron loss in area CA3); 5, moderate loss of neurons (11–40% neuron loss in area CA3); 6, severe loss of neurons (more than 40% neuron loss in area CA3); mean values of pathological changes in the two sides of hippocampus were presented.

2.4. Cell culture and neurotoxicity assay

Cerebellar granule cells (CGCs) were prepared from 7-day-old mouse pups in the different groups and were plated in 1 ml Eagle's basal medium with 25 mM KCl, 2 mM glutamine, 100 µg/ml gentamycin, and 10% fetal bovine serum on poly-L-lysine-coated coverslips in 24-well plates at 10^6 cells/well. Cells were maintained at 37 °C in 95% O₂/5% CO₂ atmosphere and 90% humidity. The starting day of cell culture was considered as day in vitro (DIV) 0. After 24 h, cytosine arabinoside (C-Ara) was added at a final concentration of 10 µM to inhibit the proliferation of non-neuronal cells. On DIV 8, the conditioned medium was removed and filtered. Because preliminary experiments indicated that 120 µM KA was the optimal concentration for these experiments, we added 120 µM KA in complete Locke's solution (154 mM NaCl, 5.6 mM KCl, 3.6 mM NaHCO₃, 2.3 mM CaCl₂, 5.6 mM glucose, 5 mM Hepes, and 1.2 mM MgCl₂, pH 7.4) to each well (0.5 ml/well) and maintained this mixture at 37 °C in 95% O₂/5% CO₂ atmosphere and 90% humidity for 1 h. After 24 h, neuron survival was quantified by a colorimetric method utilizing the metabolic dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Culture medium was removed and replaced by 0.2 ml 5 mg/ml MTT in complete Locke's solution. After incubation at 37 °C for 30 min, the MTT solution was removed and the reaction product dissolved in 300 µl 20% SDS in 40% dimethylformamide. Absorbance was read at 570 and 630 nm, respectively, and the results were expressed as percentage of viability relative to the control culture (= 100%).

2.5. Immunohistochemistry of brain sections

Paraffin sections prepared as described for histopathological analysis were dewaxed and immersed in citric buffer (pH 6.0) and heated by microwave at 94 °C for 9 min. After washes with water and Tris buffer, the sections were blocked by "protein block" (DAKO A/S, Copenhagen, Denmark) at room temperature for 30 min. Subsequently they were exposed to rabbit antibodies to glial fibrillary acidic protein (GFAP) (1:1600; DAKO), goat antibodies to mouse cyclooxygenase 2

(COX-2) (1:600; RDI, Flanders NJ, USA), rabbit antibodies to rat (cross-reacting with mouse) tumor necrosis factor- α (TNF- α) (1:100, Central Laboratory Animal Institute (CLAI), Utrecht, Netherlands) and to rat (cross-reacting with mouse) interferon- γ (IFN- γ) (1:200)(CLAI), respectively. Sections were stained by using the avidin-biotin technique (Vectastain Elite Kit; Vector Labs, Burlingame, CA, USA). Omission of primary antibodies served as the negative controls. Peroxidase-substrate solution DAB (Sigma-Aldrich, Stockholm, Sweden) was added until the desired intensity of color (yellow) developed. The staining of COX-2 was scored using a semi quantitative grading system: 0, no staining; 1, positive staining in 1–4% area of CA3; 2, positive staining in 5–15% area of CA3; and 3, positive staining in more than 15% area of CA3. Data were presented as mean value \pm SEM.

2.6. Behavioral tests

2.6.1. Hole-board test

Exploratory behavior of mice was examined in the afternoon 1 day before and 7 days after administration of KA. The board (Ugo Basle, 6652, Comerio VA, Italy) used for the hole-board test was made of acrylic plastic (Perspex) panels (40 \times 40 cm, 3.5-cm thick) with 16 holes, each 3 cm in diameter, equally spaced in the floor. Head dipping of mice was measured by infrared cells placed under the holes. For hole-board testing, a mouse was gently placed in the center of the floor, and its dipping behavior was observed under white light every 3 min, for a period of 9 min. The number of dips was counted in a double-blind manner.

2.6.2. Open-field test

Open-field activity was measured in the afternoon 2 days before and 6 days after administration of KA. The apparatus consisted of a transparent acrylic plastic (Plexiglas) box (35 \times 35 \times 17 cm) in which the floor was subdivided into 64 equal squares (4.38 \times 4.38 cm). The box was placed under white light, and a mouse was gently placed in the center of the box. Locomotor activity and rearing were recorded every 3 min for a period of 9 min. Locomotor activity was evaluated in a double-blind manner by counting the number of times the mouse crossed the floor squares with both hind paws. Rearing was evaluated by counting the number of times the mouse was vertical with the forepaws raised or placed on the walls. The enclosure was washed with water and 70% ethanol after each test.

2.7. Statistics

Differences between more than two groups were tested by using one-factor analysis of variance (ANOVA). Differences between two groups were tested by using

Student's *t* test. All significance tests were two-sided. The level of significance was set at $p < .05$.

2.8. Ethics

KA-induced neurodegeneration model in mice was approved by the South Stockholm Research Animal Ethics Committee, Huddinge County Court, Stockholm, Sweden.

3. Results

3.1. The roles of T and B cells differ in KA-induced clinical seizures

All wild-type and knockout mice without KA treatment or treated with water did not show any clinical signs. After intranasal administration of KA, all mice treated with KA, except two without seizure in the CD4/CD8(−/−) group, exhibited some level of seizures. The average clinical scores of the individual groups are presented in Fig. 1. Within 15 min after KA administration, the mice were catatonic and staring. This behavior was followed by twitching and frequent rearing and falling. Thirty to forty min after KA administration, the mice exhibited continuous seizures and during that period some mice died. In the CD4(−/−) group, all the 8 mice displayed severe seizures and died less than 3 h after onset. Clinical signs of the CD4/CD8(−/−) group were significantly lower and of the CD4(−/−) group significantly higher than in the wild-type group (both $p < .01$) (Fig. 1). However, the responses of CD8(−/−) and Igh-6(−/−) mice did not differ notably from those of the wild-type group (Fig. 1).

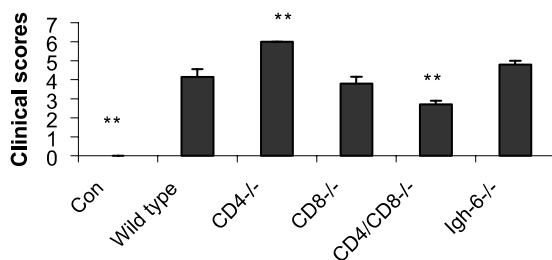


Fig. 1. Clinical changes after KA treatment. Clinical scores of mice from the following groups: control group including wild-type or each knockout mice with water treatment ($n = 10$), C57BL/6 wild-type ($n = 8$), CD4(−/−) ($n = 8$), CD8(−/−) ($n = 7$), CD4/CD8(−/−) ($n = 7$), and Igh-6(−/−) mice ($n = 8$) with KA treatment. Mice were treated with 48 mg KA per kilogram body weight. Seizures were rated as follows: 0, normal; 1, immobilization; 2, rearing and falling; 3, seizure for less than 1 h; 4, seizure for 1–3 h; 5, seizure for more than 3 h; and 6, death. Scores appear as average values for each group. Mean values and SEM are indicated. p values refer to comparisons between KA-treated immunodeficient (or water-treated control mice) and KA-treated wild-type mice. ** $p < .01$.

3.2. CD4/CD8(−/−) mice show less neurodegeneration, but Igh-6(−/−) mice exhibit severe neuropathological changes

KA administration led to a selective hippocampal neurodegeneration in region CA3. The average scores of pathological changes at anterior (−1.15 mm to bregma), middle (−1.94 mm), and posterior (−2.80 mm) hippocampus in mice 7 days after KA treatment are presented in Fig. 2. CD4/CD8(−/−) mice had the mildest hippocampal changes and few pycnotic neurons detectable by the Nissl's staining, with most scores from 0.5 to 2 (Figs. 2 and 3A). However, in the Igh-6(−/−) group, histopathological changes were marked and most sections exhibited more severe neuronal degeneration (Figs. 2 and 3B) in comparison with wild-type mice (Figs. 2 and 3C). All CD4(−/−) mice died less than 3 h after KA administration, and pycnotic neurons were found in area CA3 of the brains collected as soon as the mice died (data not shown). The control wild-type and immunodeficient mice treated with water did not show any pathological change in the brain (Figs. 2 and 3D).

3.3. Lack of T cell subsets or B cells does not influence the viability of CGC neurons treated with KA

The viability of CGCs after in vitro administration of KA was tested by the MTT assay. The pilot experiments demonstrated that the purity of the CGCs was more than 95% as determined by staining the neurons for MAP2 and non-neuronal cells for GFAP. The data on the rates of survival of CGCs from wild-type mice in the different concentrations of KA are presented in the Fig. 4A, which indicated that 120 μM KA had an optimal effect on viability of CGCs among the tested concentrations, since the rate of survival of CGCs was 65% at 120 μM KA in vitro (Fig. 4A). However, no significant difference was found between any two KA-treated groups, indicating that these neurons from T cell- and B cell-deficient mice had similar susceptibilities to KA treatment at 120 μM concentration in vitro (Fig. 4B).

3.4. Lack of T cell subsets or B cells increases GFAP expression

GFAP-positive immunostaining is an indicator of activated astrocytes. Very few activated astrocytes were found in the hippocampal neurons of control wild-type C57BL/6 mice or each knockout type treated with water (Figs. 5a and A). The wild-type mice with pathological scores of 1–3 showed only a few activated astrocytes (Figs. 5b and B), whereas those with a score of 4 exhibited more astrocytes, and they clustered on both sides of the lesions. For CD8(−/−) (not shown)

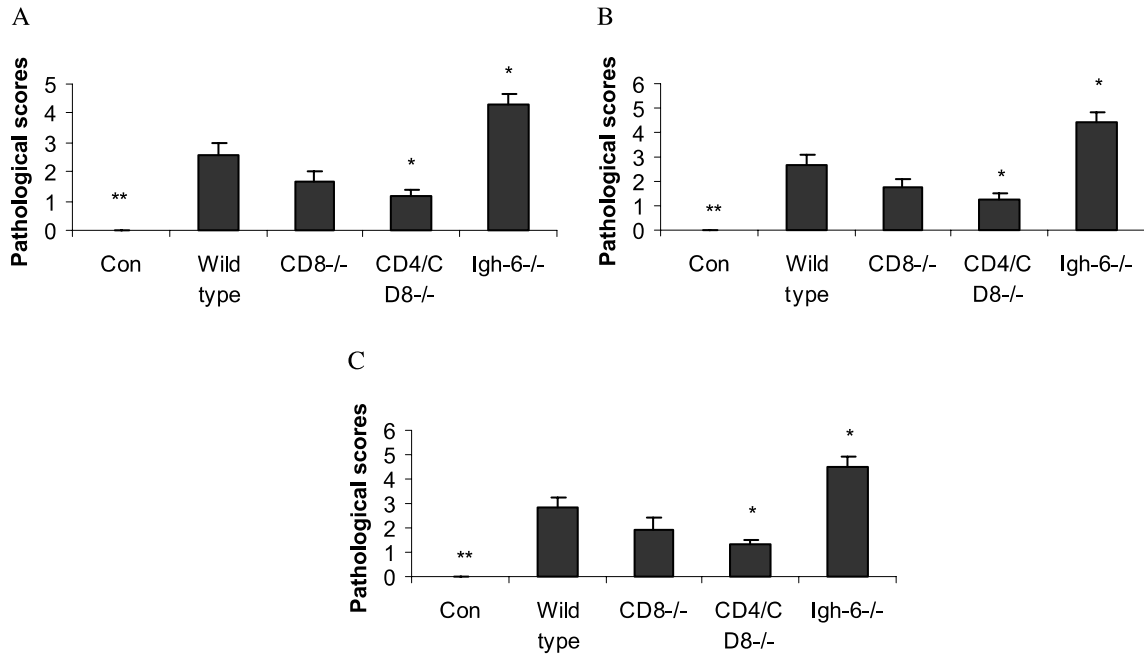


Fig. 2. KA induced pathological changes in hippocampus. After KA administration, a selective hippocampal neurodegeneration in region CA3 was observed. The average scores of histopathological changes at anterior (–1.15 mm to bregma, A), middle (–1.94 mm to bregma, B), and posterior (–2.80 mm to bregma, C) hippocampus in mice 7 days after KA treatment are presented. Wild-type or each knockout mice with water treatment ($n = 9$), C57BL/6 wild-type ($n = 6$), CD8(–/–) ($n = 8$), CD4/CD8(–/–) ($n = 7$), and Igh-6(–/–) mice ($n = 5$) after administration of 48 mg KA per kilogram body weight were judged for their pathological changes in the hippocampus (region CA3). All the CD4(–/–) mice died less than 3 h after KA administration and are not included in the pathological analysis. Changes were scored as described in the materials and methods. The mean values and SEM of pathological changes are indicated. p values refer to comparisons between KA-treated immunodeficient (or water-treated control mice) and KA-treated wild-type mice. * $p < .05$ and ** $p < .01$.

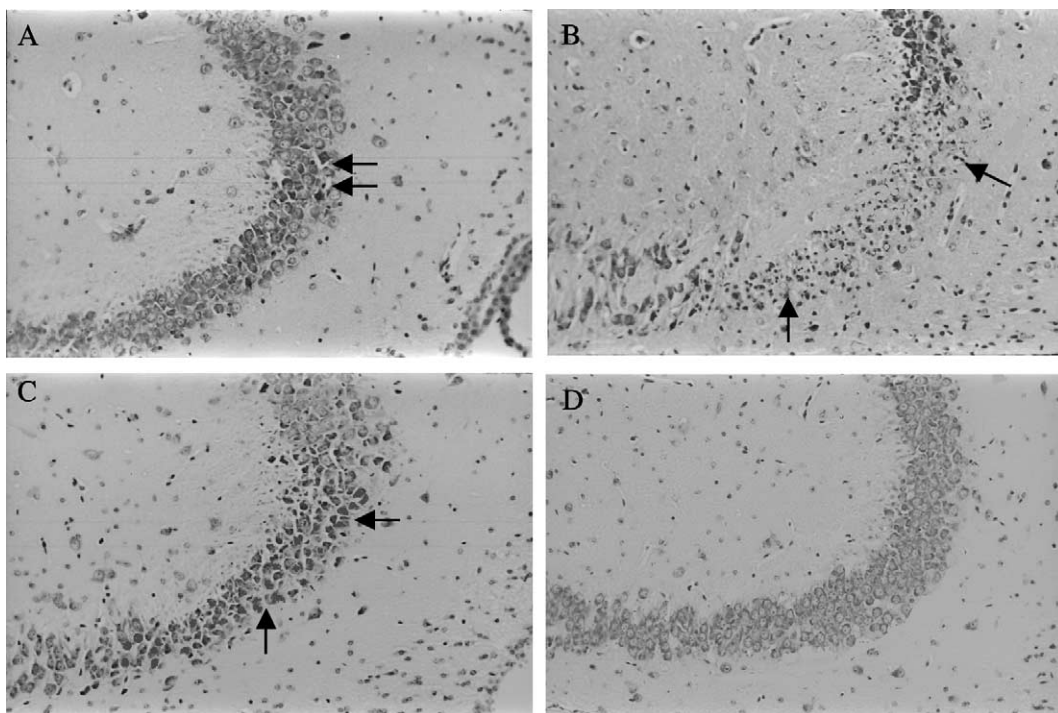


Fig. 3. KA induced neurodegeneration in region CA3 of hippocampus stained by Nissl's method. All sections were from mice 7 days after KA or water administration. (A) KA-treated CD4/CD8(–/–) mice with pathological score 1; (B) KA-treated Igh-6(–/–) mice with pathological score 5; (C) KA-treated C57BL/6 wild-type mice with pathological score 3; and (D) C57BL/6 control mice treated with water (also representing each knockout mice treated with water). Arrows indicate the pathologically changed neurons (200 \times).

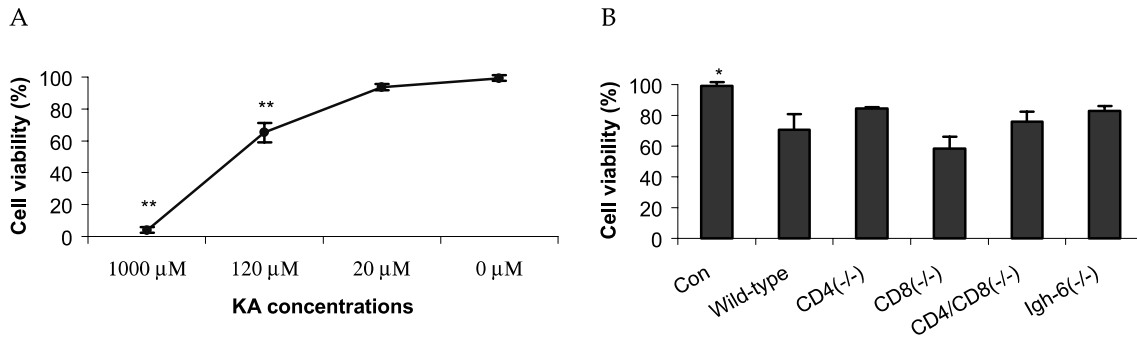


Fig. 4. Neurotoxicity assay of cerebellar granule cells (CGCs) in vitro. (A) The rates of survival of CGCs from wild-type mice in the different concentrations of KA. (B) CGCs from different immunodeficient and wild type mice at day 8 after cell culture were treated with 120 μ M KA for 1 h. Control samples (Con) were treated with buffer only. Neuronal survival was quantified 24 h later. Results are expressed as relative to buffer treated cultures (= 100%). Values are means \pm SEM of three experiments in A and four experiments in B. ** $p < .01$, significant difference versus the samples that received 0 μ M KA in A and * $p < .05$, significant difference between control sample and each of the other KA-treated samples in B. No significant differences were found between any two KA-treated groups in B.

and CD4/CD8(-/-) mice with scores ranging from 1 to 3, a few activated astrocytes were localized along hippocampal neurons (Figs. 5c and C). However, the intensity of these activated astrocytes was greater than those in KA-treated wild-type mice with the same neuropathological scores. Severe tissue damage accompanied by obviously activated astrocytes was found in the CA1, CA2, and CA3 hippocampal regions of Igh-6(-/-) mice, and the immunostaining reactivity was particularly strong around areas of debris (Figs. 5d and D; the asteroid indicates the areas of debris in Fig. 5d).

3.5. T cell subsets or B cell may affect COX-2, but not TNF- α and IFN- γ expression

In the wild-type and each knockout mice treated with water, there was almost no immunostaining for COX-2, TNF- α or IFN- γ (Fig. 6A). The proportions of cells expressing COX-2 approximately paralleled the histopathological manifestations in area CA3 of the hippocampus. Damaged neurons as well as neuronal debris were COX-2 positive (Figs. 6B–D). No apparent difference in the expression of COX-2, TNF- α , and IFN- γ was found in wild-type mice compared to each set of immunodeficient mice with the same level of pathological changes (data not shown). But, in agreement with the histopathological changes, the expression of COX-2 was generally lower in CD4/CD8(-/-) mice (1.0 ± 0.32 , $n = 5$, $p < .05$), higher in Igh-6(-/-) mice (2.9 ± 0.10 , $n = 5$, $p < .05$) and not significantly different in CD8(-/-) mice (1.5 ± 0.22 , $n = 5$, $p > .05$) compared to the wild-type mice (2.0 ± 0.16 , $n = 5$).

TNF- α and IFN- γ expression was also detected in the degenerative neurons and glial cells. However, the most abundant expression of TNF- α and IFN- γ appeared in sections with a pathological score of 3 (Figs. 6E and F), but the debris itself showed no immunostaining.

3.6. KA administration alters behavioral performance only in B-cell depleted and wild-type mice

As judged from the hole-board test of exploratory behavior, KA did not affect the total number of head dips in any group of mice (data not shown). However, the open-field test revealed a significantly greater number of crossings after administration of KA in Igh-6(-/-) and wild-type mice (Figs. 7A and B). CD8(-/-) and CD4/CD8(-/-) mice had no significant increase of locomotor activity after KA administration, except after 6–9 min in CD8(-/-) mice (Figs. 7C and D). For all mice tested except the wild-type mice, no significant increase in rearing activity was found (data not shown).

4. Discussion

In this study, we used a model of KA-induced hippocampal lesion to expand our understanding of excitotoxic neurodegeneration, a pathogenic process in which the immune system has been implicated (Akiyama et al., 1994; Lerner-Natoli et al., 2000; Smith et al., 2000). We found, first, that lymphocytes contribute to KA-induced hippocampal neurodegeneration and, second, that CD4+ T cells and B cells may act effectively to halt and even prevent the neurodegenerative process.

It has been a controversy regarding the role of lymphocytes in neurodegeneration. Our data show that after KA treatment, mice lacking CD4 T cells developed a fatal seizure associated with neurodegeneration of area CA3, and the B cell deficiency also led to severe pathological changes. In contrast, mice lacking both CD4 and CD8 T cells developed only slight seizures and mild pathological changes, which are speculated that CD8 T cells may play a neuroexcitotoxic function.

The behavioral analysis also provided evidence consistent with a protective role for CD4 T cells and B cells

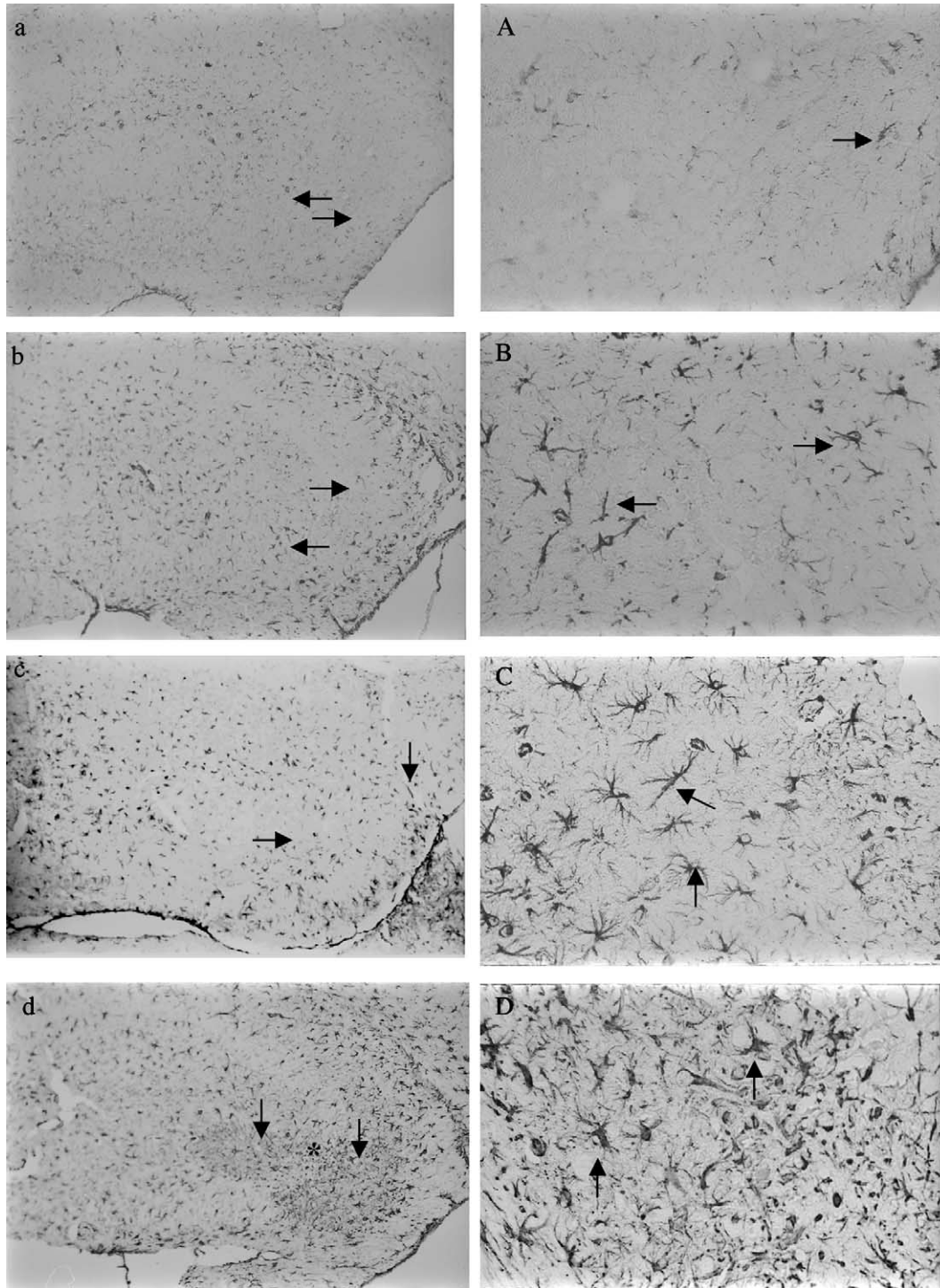


Fig. 5. Immunohistochemical staining for GFAP in the hippocampus (region CA3). (a and A) Control mice treated with water without histopathological changes; (b and B) KA-treated wild-type mice with pathological score of 3; (c and C) KA-treated CD4/CD8(-/-) mice with pathological score of 1; and (d and D) KA-treated Igh-6(-/-) mice with pathological score of 5. Arrows indicate positive GFAP staining around area CA3 and asteroid indicates the debris area (a, b, c, and d, 100 \times ; A, B, C, and D, 400 \times).

and a speculated disease-promoting role for CD8 T cells. The number of open-field crossings was significantly greater after administration of KA for the wild-type and Igh-6(-/-) groups, but not the CD4/CD8(-/-) or CD8(-/-) group. The total number of line crossings in

the open-field test provides a measure of horizontally directed locomotor activity (O'keefe and Nadel, 1978). An increase in the aggregated horizontal locomotor activity presumably reflects a lower level of anxiety (van der Staay et al., 1990); i.e., a higher number of line

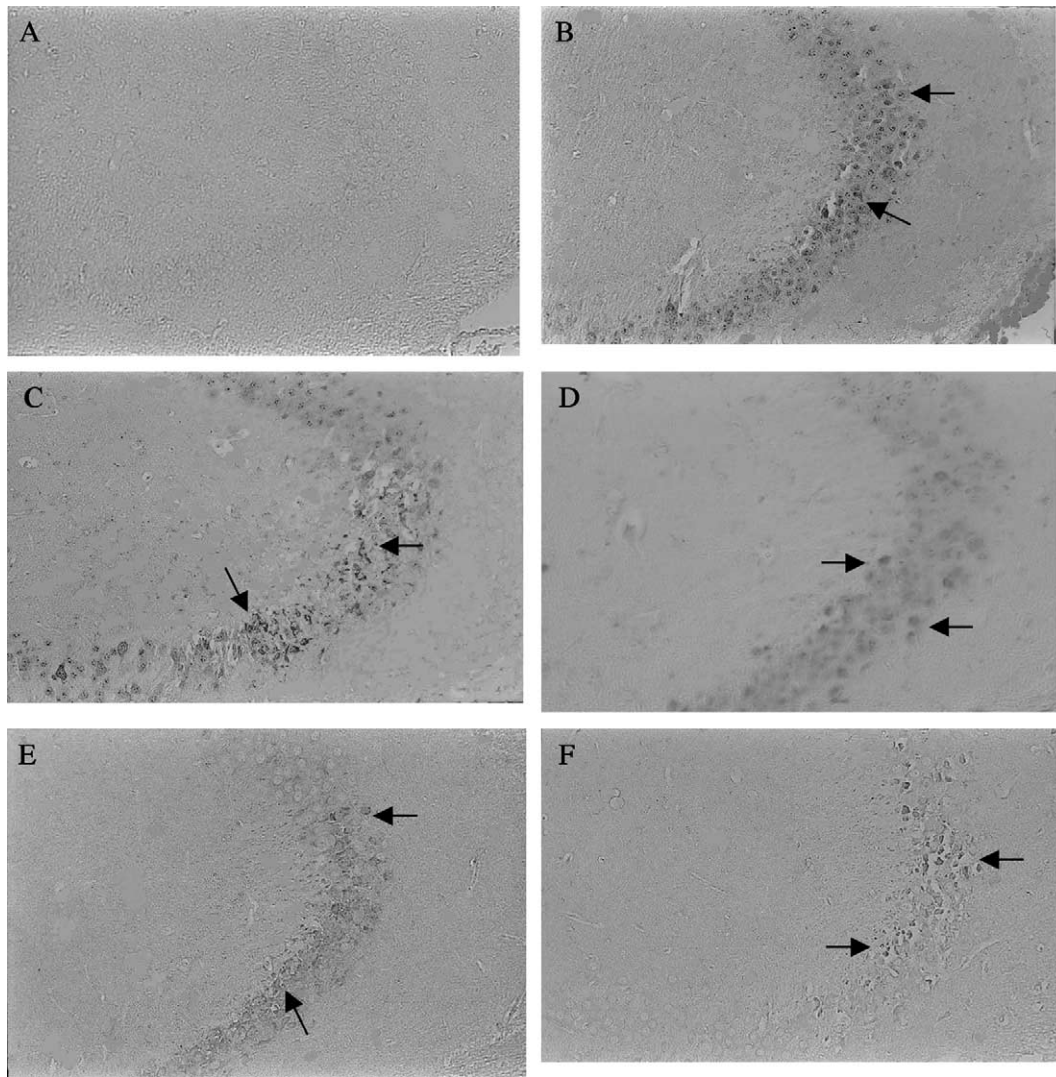


Fig. 6. The expression of COX-2, TNF- α and IFN- γ by immunohistochemical staining. (A) water-treated wild-type mice (also representing each water-treated knockout mice); (B) KA-treated wild-type mice with pathological score 3 for COX-2; (C) KA-treated Igh-6(-/-) mice with pathological score 5 for COX-2; (D) KA-treated CD4/CD8(-/-) mice with pathological score 1 for COX-2; (E) KA-treated wild-type mice with pathological score 3 for TNF- α ; and (F) KA-treated wild-type mice with pathological score 3 for IFN- γ . Arrows indicate positive staining (200 \times).

crossings indicates a lack of fear. Rats with hippocampal lesions have a decreased level of anxiety (O'keefe and Nadel, 1978). Hence, the hippocampal functions necessary for the normal open-field behavior in wild-type and Igh-6(-/-) mice were adversely affected by the KA administration.

CD4 T cells are the source of a wide range of cytokines, including IFN- γ and TNF- α . After injury of the brain, microglia are activated and have been proved helpful for the healing process (David et al., 1990; Lazarov-Spiegler et al., 1996; Schwartz and Moalem, 2001). IFN- γ produced from CD4 T cells can strongly activate microglia and could partially account for CD4 T cells' beneficial effects. Other positive effects of CD4 T cells could stem from the expression of nerve growth factor (NGF), brain-derived neurotrophic factor

(BDNF) and neurotrophins NT-3 and NT-4/5 that could enforce brain recovery from neurodegenerative disease (Kerschensteiner et al., 1999). However, the exact mechanism underlying the protective role of CD4 T cells still remains unclear. CD8 T cells are cytotoxic lymphocytes and can kill target cells when present simultaneously with MHC class I molecules and co-stimulatory signals. Although specific assays for MHC expression in neurons *in vivo* have generally been negative (Lampson, 1995), some studies have suggested that neuronal MHC gene expression may be inducible following KA-induced seizures (Corriveau et al., 1998). Seven days after KA treatment, CD4 and CD8 T cells may have infiltrated through BBB and exerted some influence on neuronal survival in the brain. However, why the lack of different T cell subsets could affect acute

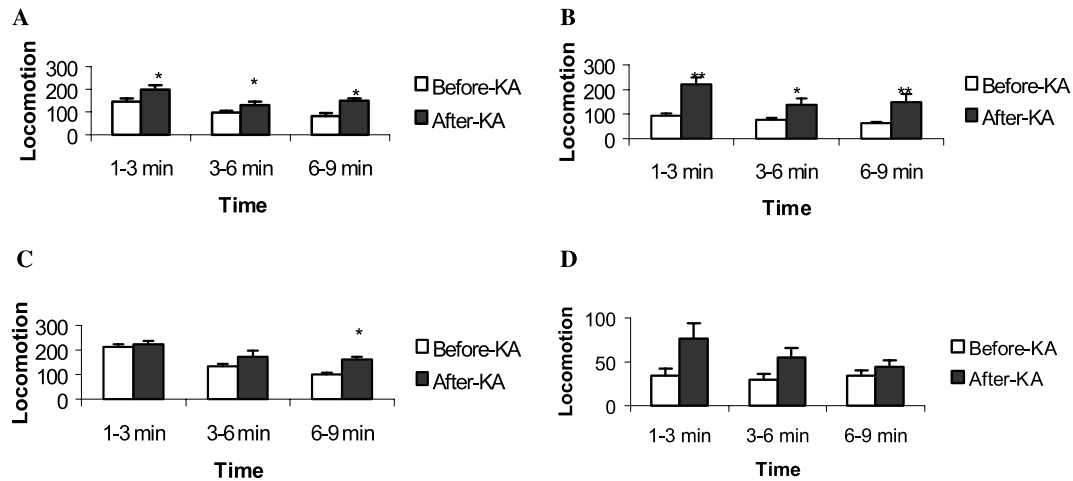


Fig. 7. Open-field test. Behavioral performance was defined as locomotor activity in the open-field test before and after administration of KA. Results were determined every 3 min for a period of 9 min by counting the number of times each mouse crossed the floor squares with both hind paws in (A) Igh-6(-/-) mice; (B) wild-type mice; (C) CD8(-/-); and (D) CD4/CD8(-/-) mice. Mean values and SEM are indicated. Locomotor activity was significantly increased after administration of KA in wild-type and Igh-6(-/-) mice. (** $p < .01$).

seizure activity is still a mystery and further studies are needed to address this issue.

Astrocytes are an important cell population in the CNS that can respond to brain injury by secreting soluble mediators, such as IL-6, TNF- α , NGF, and prostaglandins (Yoshida and Toya, 1997; Aschner, 1998; Minghetti and Levi, 1998). Astroglia is known to possess neuroprotective as well as neurotoxic properties (Chen and Swanson, 2003) and the collective effect of astroglia may differ in specific cases. In the present study, mice lacking both CD4 and CD8 or only CD8 T cells had enhanced GFAP expression in the hippocampus, which indicated that the activated astrocytes could play, as a net effect, protective roles in such an excitotoxic situation. Quite possibly, astrocyte activation balances the effect of CD4 T cells and confines their function within a beneficial limit.

An enhanced humoral immunity was found to accompany lesions of the hippocampus in rats (Nance et al., 1987; Pan and Long, 1993). The antibodies secreted by B cells could facilitate the removal of myelin by microglia/macrophage or T cells and, thereby, heal the injured area. Additionally, B cell activation could also shift T cell differentiation toward the Th2/3 phenotype (Lenschow et al., 1996) and avoid Th1-induced brain damage due to over-activation of inflammatory molecules. In the present study, B cell knockout mice displayed severe pathological signs after KA insult. These findings indicate the involvement of B cells in the neurodegenerative processes. However, lack of B cells led to severe neuropathological signs without significant change of seizure activity. The reason for the results may be owing to B cells exerting their role at the late phase of KA-induced neurodegeneration. Therefore, it could be

assumed that B cells and B cell-mediated humoral immunity might reduce KA-induced brain inflammation to a certain extent, and thus deficiency of B cells led to severe neuropathological changes.

The absence of T and B cells as well as their products may break down the homeostasis in the brain and influence the brain cells in response to KA exposure. The increased permeability of blood-brain barrier (BBB) induced by KA administration may allow influx of peripheral lymphocytes into the brain parenchyma, which may initiate the adaptive immune response in the brain. However, the mechanisms by which T and B cells participate in the pathogenesis of KA-induced neurodegeneration in mice are not fully understood and further studies are needed to address this issue.

The present studies demonstrate a protective capability of CD4 T cells and B cells in the face of KA-induced excitotoxic damage in the brain of C57BL/6 mice. These findings may provide new insight into the contributions of defined B and T cell populations to the pathogenesis of the CNS diseases.

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