



Published in final edited form as:

Epilepsia. 2021 March ; 62(3): 671–682. doi:10.1111/epi.16838.

Seizures and memory impairment induced by patient-derived anti-NMDA receptor antibodies in mice are attenuated by anakinra, an interleukin-1 receptor antagonist

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Summary

Objective: Neuroinflammation associated with anti-N-methyl-D-aspartate receptor encephalitis may facilitate seizures. We previously showed that intraventricular administration of CSF from patients with anti-NMDAR encephalitis to mice precipitates seizures, thereby confirming that antibodies are directly pathogenic. To determine if interleukin (IL)-1-mediated inflammation exacerbates autoimmune seizures, we asked if blocking the effects of IL-1 by anakinra, a selective IL-1 receptor antagonist, blunts antibody-induced seizures.

Methods: We infused C57BL/6 mice intraventricularly with purified serum IgG from patients with anti-NMDAR encephalitis or monoclonal anti-NMDAR IgG; subdural EEG was continuously recorded. After a 6-day interval, mice received anakinra (25 mg/kg s.c., twice daily) or vehicle for five days. Following a 4-day washout period, we performed behavioral tests to assess motor function, anxiety, and memory, followed by hippocampus tissue analysis to assess astrocytic (GFAP) and microglial (Iba-1) activation.

Results: Of 31 mice infused with purified patient NMDAR-IgG (n = 17) or monoclonal NMDAR-IgG (n = 14), 81% developed seizures. Median baseline daily seizure counts during exposure to antibodies were 3.9; most seizures were electrographic. Median duration of seizures

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Disclosures

The authors have declared that no conflict of interest exists.

during the baseline was 82.5 sec. Anakinra administration attenuated daily seizure frequency by 60% ($p = 0.02$). Anakinra reduced seizure duration; however, the effect was delayed and become apparent only after the cessation of treatment ($p = 0.04$). Anakinra improved novel object recognition in mice with antibody-induced seizures ($p = 0.03$) but did not alter other behaviors. Anakinra reduced the expression of GFAP and Iba-1 in the hippocampus of mice with seizures, indicating decreased astrocytic and microglial activation.

Significance: Our evidence supports a role for IL-1 in the pathogenesis of seizures in anti-NMDAR encephalitis. These data are consistent with therapeutic effects of anakinra in other severe autoimmune and inflammatory seizure syndromes. Targeting inflammation via blocking IL-1 receptor mediated-signaling may be promising for developing novel treatments for refractory autoimmune seizures.

Keywords

neuroinflammation; IL-1; cytokines; anti-NMDA receptor encephalitis; autoimmune seizures; autoantibodies

1. Introduction

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune disease that manifests with acute confusion, memory loss, and severe seizures in previously healthy people.¹ Status epilepticus is present in 45% of patients treated in intensive care settings, and seizures that do not respond to treatment develop in two-thirds of these patients.² Seizures subside in some patients after immunotherapies aimed to remove antibodies, thereby suggesting that antibody proteins were linked to clinical symptoms.³ We recently established the antibodies' pathogenic role in seizures, in that intraventricular administration of cerebrospinal fluid (CSF) from affected patients, or purified patient anti-NMDAR antibodies, or commercial antibodies directed to the N-terminal domain of GluN1, into mice was shown to precipitate seizures, thereby confirming that antibodies are directly pathogenic for seizures.⁴

Along with modulation of NMDAR channels possibly resulting in increased overall excitability of the seizure network in autoimmune encephalitis, the innate immune system in patients with this condition has been increasingly considered as a potential target for new anticonvulsive therapies.⁵⁻⁷ Seizures or brain injury can trigger neurogenic inflammation.⁸ In particular, both brief and prolonged seizures in mice rapidly induce cyclooxygenase-2 (Cox-2) in forebrain glutamatergic neurons.⁹⁻¹¹ The resulting release of prostaglandins from these neurons exacerbates ongoing inflammatory processes and may further perpetuate seizures.^{12, 13} Proinflammatory cytokines (e.g., interleukin (IL-6, IL-17A and IL-2) are persistently elevated in the CSF and serum of anti-NMDAR encephalitis patients.¹⁴ Furthermore, the levels of C-X-C motif chemokine 13 (CXCL-13) are increased in the CSF of patients with anti-NMDAR encephalitis and are positively correlated with intrathecal anti-NMDAR antibody titer, suboptimal response to treatment and higher rates of disease relapse.¹⁵ Corticosteroids, broad spectrum anti-inflammatory agents, are the first line of therapy for anti-NMDAR encephalitis; however, only half of patients show improvement leaving the remaining patients to be approached with other limited treatment modalities.³

Identifying the role of specific inflammatory pathways in the development of autoimmune epilepsy would uncover new therapeutic targets for seizure attenuation.⁸

One potential target for novel anticonvulsant therapies is IL-1 receptor-mediated signaling^{8, 11}. In inflammatory states following the recruitment of the Toll-like receptor (TLR) system, microglial cells release IL-1 β , a potent proconvulsant.⁵ Similarly, the sustained seizures in status epilepticus cause neuroinflammation partly via the IL-1 β system, thereby promoting epileptogenesis.^{8, 16-18} Anakinra (Kineret[®], Swedish Orphan Biovitrum, Sobi), a recombinant and modified version of the human IL-1 receptor antagonist protein currently approved for the treatment of rheumatoid arthritis, has been regarded as a promising therapy for inflammatory epilepsies.¹⁹ It has been used effectively in a few patients with severe autoimmune seizures, both in the acute and chronic phases.²⁰⁻²³ Anakinra is a hydrophilic protein that penetrates the blood-brain barrier and has a relatively short half-life, which decreases the possibility of side effects when used in affected patients.^{24, 25} To establish the role of IL-1 proteins fundamentally involved in epileptogenesis in autoimmune seizures, we asked if blocking the effects of IL-1 by anakinra, a selective antagonist of IL-1 receptor²⁶, would blunt seizures induced by anti-NMDAR antibodies.

2. Materials and Methods

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Nebraska Medical Center (UNMC). The principles outlined in the ARRIVE guidelines²⁷ and the Basel declaration, including the 3R concept, were followed during experimental planning.

2.1 Animal care

Male C57BL/6N mice (8-10 weeks, 25-30 g from Charles River, Roanoke, IL) were housed in groups of five and maintained on a 12 h light cycle (light on/off at 7 a.m. / 7 p.m.) with *ad libitum* access to food and water. Following the implantation of the guide cannula targeting the lateral ventricle and the EEG mount, mice were housed individually in the EEG recording chambers until the completion of EEG monitoring. All functional measures were acquired in a blinded manner.

2.2 Drugs and experimental antibodies

Human serum IgG fraction containing anti-NMDAR antibodies (provided by A.Z and S.J. P. at Mayo Clinic) were purified from the pooled serum of 12 patients with anti-NMDAR encephalitis (approved by the Mayo Clinic IRB) and the activity against NMDAR-IgG was confirmed using indirect immunofluorescence on human embryonic kidney (HEK) 293 cells transfected with a plasmid encoding the GluN1 subunit of the NMDAR (Euroimmun Lübeck, Germany) and by tissue-indirect immunofluorescence, confirming NMDAR-IgG-specific pattern of staining as previously described.⁴ The serum tested negative for other neural autoantibodies, including anti-neuronal nuclear antibody types 1 and 2 (anti-Hu and anti-Ri, respectively), anti-neuronal antibody type 3, anti-glial neuronal antibody type

1 (anti-SOX1), PCA2 (anti-MAP1B), Purkinje cell cytoplasmic antibody (PCA) types 1 and 2 (anti-Yo and anti-MAP1B, respectively), PCA-Tr (anti-DNER), amphiphysin, collapsing response mediator protein 5, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, gamma amino butyric acid B receptor, leucine glioma inactivated 1 and Caspr 2 protein antibodies, antibodies to metabotropic glutamate receptors 1, 2, dipeptidyl aminopeptidase-like protein 6, and glial fibrillary acidic protein. The IgG protein was dissolved in phosphate-buffered saline (PBS) (0.02 $\mu\text{g}/\mu\text{l}$).

A human monoclonal GluN1 antibody (5F5) specific for GluN1 was derived from memory B cells of a patient with anti-NMDAR encephalitis and seizures.²⁸ Specificity for NMDAR-IgG was confirmed using indirect immunofluorescence on HEK 293 cells.²⁸ The 5F5 antibody binds in the extracellular amino terminal domain of the GluN1 and requires the N368 site.²⁸ Proteins were dissolved in PBS (0.02 $\mu\text{g}/\mu\text{l}$).

Anakinra was a gift from Sobi (Stockholm, Sweden). The drug was dissolved in normal saline and administered subcutaneously (25 mg/kg) twice daily.

2.3 Stereotactic surgery, osmotic minipump insertion, and administration of anakinra

Mice were anesthetized with isoflurane and implanted with a unilateral injector guide cannula into the lateral ventricle, the 2 EEG/1 EMG head mount (Pinnacle Technology Inc., Lawrence, KS), and two cortical screw EEG electrodes to derive signals from the parietal cortex overlying the hippocampus and ipsilateral frontal cortex as previously described.⁴ Seven days later (day 0) mice were re-anesthetized with isoflurane, implanted with subcutaneous micro-osmotic infusion pumps (Alzet, Cupertino, CA) containing purified serum IgG or monoclonal IgG (Fig. 1); head mounts were connected to the EEG acquisition system. IgG solution was continuously perfused at a flow rate of 0.25 $\mu\text{l}/\text{h}$. Following a 6-day assessment of baseline seizure counts, mice were injected with anakinra or vehicle twice daily for five days (Fig. 1). The EEG was continued for an additional four days. Upon completion of the experiments, the contents of the pumps were examined for residual IgG solution, and the residual volumes were negligible. The mean amount of protein (\pm SEM) delivered intracerebroventricular (i.c.v.) to mice was 5.7 ± 0.09 ng/kg/day.

2.4 EEG acquisition and analysis of seizure patterns

Continuous video EEG acquisition (Pinnacle Technology, Inc.) was started on day 0, immediately following the implantation of the infusion pumps (Fig. 1). EEG analysis was carried out retrospectively as previously described (Sirenia Seizure Pro 1.7.6, Pinnacle Technology, Inc.) and verified visually without knowledge of the treatment status.⁴ Briefly, seizures were defined as rhythmic activity for 5 sec or longer that exceeds the baseline amplitude by at least 3-fold. A modified Racine scale was applied to characterize the following behavioral signs: nonconvulsive (0), freezing (1), facial tremor (2), head shaking (3), isolated generalized jerks (4), sustained clonic activity (5), or unclear behavior (6).⁴ All EEGs were analyzed by two investigators with one of them being unaware of the treatment status.

2.5 Open field

An open field test was performed using a video tracking system (Noldus, Leesburg, VA) as previously described.⁴ Briefly, mice were habituated in the testing room for 30 min prior to being placed into the custom-made acrylic chamber (41 L x 41 W x 35 H cm) and were allowed to move freely for 20 min. The motor behavior was measured as the total distance travelled during the trial. Anxiety-related behavior was measured based on the percent time animals spent in the center of the arena (25% of the total area) during a 20-min trial.

2.6 Novel object

A novel object recognition test was performed as previously described⁴. The exploration behavior was defined as orientation of the nose to the object at a distance of 2 cm. The time spent with familiar object (FO) and novel object (NO) was measured and compared for each treatment group. Mice that explored both objects for a total time of < 4.5 sec were excluded. Additionally, to account for normalization on total exploration time, a novel object index (NOI) was calculated as time spent exploring the novel object divided by the sum of times spent exploring both novel and familiar objects.

2.7 Histological evaluations

Upon completion of experiments, animals were deeply anesthetized with isoflurane and transcardially perfused with PBS followed by 4% paraformaldehyde in PBS. Frozen coronal sections (50 μm ; at least 4 sections per mice) were processed for immunohistochemistry for GFAP (astrocytes) and Iba-1 (microglia) as previously described.^{4, 29} Briefly, sections were incubated at 4 °C overnight with polyclonal rabbit anti-GFAP antibodies (1:500, N 1506, Dako, Carpinteria, CA) or anti-Iba-1 antibodies (1:500, 019-19741, Fujifilm Wako Chemicals, Richmond VA). Following extensive washing, goat anti-rabbit biotinylated secondary antibodies (1:300, Vector, Burlingame, CA) were used for signal detection.

2.8 Imaging acquisition and processing

Slide specimens of the CA1 region of hippocampus from both hemispheres between bregma -1.55 and -2.03 were scanned with a Nuance Multi-Spectral Imaging System (Cambridge Research Instruments, Woburn, MA) fitted to a Nikon ECLIPSE 55i microscope (Nikon, Tokoyo, Japan) with a 20X objective (1392 X 1040 pixels, 0.498 $\mu\text{m}^2/\text{pixel}$). The absorbance spectrum for each pixel was scanned from 530 nm to 620 nm at 10 nm increments; the absorbance profile of immunostaining for GFAP or Iba-1 was input to the system; and the quantitative greyscale image corresponding to the GFAP or Iba-1 profile was extracted from the scanned images. Greyscale images were transferred to the Nuance environment for quantification of the intensities (grey scale units, gru) and areas (μm^2) as previously described.³⁰ The GFAP or Iba-1 abundances per the positivity event were computed as the product of mean pixel intensity and area (gsu \bullet μm^2). The immunopositivity in the hippocampal CA1 region was taken as the sum of abundances of all events in the CA1 region scanned. The same area of the CA1 region was analyzed for the specimens from anakinra-treated and control mice; therefore, abundances were normalized to the fixed scanned area. Duplicate slides from each mouse were averaged. The analyses were performed by the investigators who were unaware of the treatment assignment.

2.9 Statistical analysis

Seizure counts and seizure duration were compared between two treatment groups using two-way analysis of variance (ANOVA) followed by Sidak's multiple comparison tests to assess the change from the corresponding baseline in each treatment group (GraphPad Prism 8.4, San Diego, CA). The time course of seizure counts was assessed using repeated measures ANOVA. The times spent with familiar and novel objects in the novel object assay were compared for each treatment group using paired t-tests while NOIs were compared using one-sample t-tests with the assumption that random presence of the animal at the objects would lead to a NOI index of 50%. The distance travelled and percent time spent in the middle in the open field assay were compared between the treatment groups using Student's t-tests. The expressions of GFAP- and Iba-1-immunoreactivity in hippocampus were compared using Student's t-tests.

3. Results

3.1. Anakinra attenuates the frequency and duration of antibody-induced seizures

Thirty-one mice were infused with purified patient anti-NMDAR antibodies ($n = 17$) or the 5F5 anti-NMDAR monoclonal antibodies ($n = 14$). Consistent with our previous report⁴, 25 mice (81%) developed seizures following the exposure to antibodies. Six mice that did not develop seizures and five mice with median daily seizure counts of < 0.5 were excluded; the remaining 20 mice were randomized to receive anakinra ($n = 12$) or vehicle ($n = 8$; Fig. 1). The median baseline seizure counts were not significantly different in mice infused with purified patient antibodies ($n=10$) or monoclonal antibodies ($n=10$; $p=0.86$, unpaired t-test) and therefore these groups were combined.. The median daily seizure count during the baseline period in all mice combined was 3.9 (25-75% Interquartile range, IQR 3.7-4.1). At two weeks from the initiation of antibody infusion (9 days after initiation of anakinra treatment), anakinra reduced the number of seizures by 60% compared to the corresponding baseline counts in anakinra-treated mice ($p = 0.02$; $F_{2, 36}=7.40$, two-way ANOVA, Fig. 2A). Specifically, in post-hoc comparisons, seizure frequency in anakinra-treated mice decreased during the treatment period and remained low upon cessation of treatment ($p = 0.02$ and $p = 0.002$ vs. baseline, respectively; Sidak's multiple comparison tests, Fig. 2A). When examining the temporal distribution of seizures, the daily counts decreased starting at 24-48 h following the initiation of anakinra ($p = 0.02$, $F_{14, 238} = 2.0$, repeated measures ANOVA; Fig. 2C). There was no change in the seizure counts during the treatment or after washout in the vehicle-treated groups ($p = 0.88$ and $p = 0.19$ vs. baseline, respectively; Sidak's multiple comparison tests, Fig. 2A). Of note, seizure responses to anakinra were not different in the groups of mice infused with patient-derived antibodies or monoclonal antibodies ($p=0.65$, unpaired t-test). Of 1096 seizures recorded during the study in both treatment groups, the majority (97.4%) were electrographic only (Racine's score 0; Fig. 2D) while the remaining were accompanied by behavioral arrest (score 1, 0.8%) or their behavioral pattern was unclear (score 6, 1.7%).

The median seizure duration during baseline period in all mice was 82.5 sec (IQR, 76.5-88.7). Anakinra shortened the duration of epileptic activity by 25.2 % compared to the corresponding control ($p = 0.04$, $F_{2, 36} = 3.60$, two-way ANOVA; Fig. 2B). However,

reduction of the seizure duration was not apparent during the treatment period but instead was delayed until after the cessation of treatment ($p = 0.96$ and $p = 0.004$ vs. baseline, respectively; Sidak's multiple comparison tests; Fig. 2B). The median duration of seizures remained unchanged in mice from the vehicle-treated group ($p = 0.99$ and $p = 0.97$ vs. baseline, respectively; Sidak's multiple comparison tests).

3. 2. Anakinra improves memory in mice with seizures induced by antibodies

Since there were no differences in the vehicle-treated mice infused with patient and monoclonal antibodies with respect to times spent exploring the familiar or novel objects ($p=0.31$ and $p=0.88$, respectively), these two groups were pooled together. The time spent with FO and NO in the vehicle treated group were (mean \pm SEM) 8.8 ± 3.1 and 13.6 ± 3.4 sec, respectively (Fig. 3A). There was no significant difference between these latencies ($p = 0.29$, paired t- tests). There were no differences in the anakinra-treated mice infused with patient and monoclonal antibodies with respect to times spent exploring the familiar or novel objects ($p = 0.47$ and $p= 0. 50$, respectively) and the two groups of animals were combined. The times for FO and NO in the anakinra-groups were 6.7 ± 1.5 and 16.5 ± 3.9 sec, respectively. Anakinra induced a significant improvement in the recognition of NO by allowing the prolongation of the time spent with NO by 9.8 sec, compared to that spent with FO ($p = 0.03$, paired t test; Fig. 3A). Sham mice and vehicle-treated mice that had no seizures or developed very low seizure counts (i.e., median daily seizure counts of < 0.5) in response to the antibody infusion did not demonstrate memory impairment in the novel object paradigm (Supplemental data).

The NOIs in the vehicle- and anakinra-treated groups were 0.60 ± 0.27 and 0.69 ± 0.18 , respectively (mean \pm SEM). The direct comparison of the NOIs between the two groups failed to reach statistical significance ($p= 0.45$, unpaired t test). However, the NOI is expected to be higher than 50% when novelty is recognized and NOI was in fact significantly different from chance in the anakinra-treated mice ($p=0.01$, one sample t-test) but not in the vehicle-treated mice ($p = 0.36$, one sample t-test).

When exposed to the open field, the total distance traversed by mice (mean \pm SEM) in the vehicle- and anakinra- treated groups was 77.9 ± 3.2 and 75.5 ± 5.0 m, respectively (Fig. 3B). Furthermore, the proportion of time spent in the inner zone of the arena in the same groups was 17.1 ± 1.6 and 20 ± 4.7 percent, respectively (Fig. 3C). Administration of anakinra did not affect the locomotor activity and emotion-related behavior in mice ($p = 0.94$ and $p = 0.33$, respectively, t-tests).

3.3. Anakinra reduces the expression of astrocytic and microglial markers of inflammation in the hippocampus

Sections of CA1 region of the hippocampus stained for GFAP immunoreactivity demonstrated a characteristic pattern of immunostaining and the glial cells had a typical morphological appearance (Fig. 4A-D). The expression of GFAP in the CA1 region of hippocampus (i.e., GFAP abundance) was computed as the sum of abundances of all positivity events in the region while the GFAP abundance per event was determined as the product of mean pixel intensity and area (gsu \bullet μm^2). The expression of GFAP was

(mean \pm SEM) 44.9 ± 1.9 and 36.8 ± 2.1 gsu $\bullet \mu\text{m}^2 \times (10^3)$ in the vehicle- and anakinra treated mice, respectively (Fig. 4E). Anakinra significantly reduced the expression of GFAP in CA1 region of mice with antibody-induced seizures ($p = 0.02$, Student's t-test).

To assess the extent of Iba-1 expression in the hippocampal CA1 sections (i.e., Iba-1 abundance), we measured the sum of abundances of all events in the region; the Iba-1 abundance per event was determined as the product of mean pixel intensity and area (gsu $\bullet \mu\text{m}^2$; Fig. 5A-D). The expression of Iba-1 immunoreactivity in the CA1 region of hippocampus was 16.3 ± 2.5 and 8.4 ± 0.7 gsu $\bullet \mu\text{m}^2 (\times 10^3)$ in the vehicle- and anakinra-treated groups, respectively. Anakinra reduced the area occupied by microglia in mice with autoimmune seizures (Fig. 5E, $p = 0.02$, Student's t-test).

4. Discussion

In the present study, we examined the role of IL-1 receptor-mediated inflammation in the persistence of autoimmune seizures using the mouse model of anti-NMDAR encephalitis developed in our laboratory.⁴ We showed that repeated systemic administration of anakinra, a potent and selective IL-1 receptor antagonist, significantly attenuated the frequency and duration of seizures induced by anti-NMDAR antibodies and improved memory of mice with seizures without affecting other behaviors. Furthermore, anakinra reduced the hippocampal expression of markers of activated microglia and astrogliosis in mice with autoimmune seizures. These findings suggest that targeting IL-1 signaling may be useful for treatment of seizures associated with anti-NMDAR encephalitis.

Neurogenic inflammation and proinflammatory cytokines, including IL-1 β , are increasingly recognized as contributing to the pathogenesis of seizures.^{8, 11, 31} IL-1 β synthesis in the brain takes place in astrocytes, microglia, and a subset of neurons.^{32, 33} The baseline levels of IL-1 β are low but increase robustly in neurons and glia following the induction of status epilepticus by electrical stimulation of hippocampus or during chemically induced seizures in rodents.³³⁻³⁶ IL-1 β levels were elevated in serum of patients with autoimmune epilepsy presenting with new-onset refractory status epilepticus (NORSE) and in the CSF and serum of patients with anti-NMDAR encephalitis; notably the decline of cytokine concentration in response to anti-inflammatory therapies correlated with clinical improvement.^{6, 37-39} These data suggest that autoimmune encephalitis and other acute devastating epileptic encephalopathies are accompanied by sustained disruption of cytokine-mediated signaling; and thus, targeted attenuation of IL-1 β signaling may provide new therapeutic opportunities for seizures. We did not measure cytokine expression in mice with antibody-induced seizures in this study; however, our preliminary results from related experiments showed that mice with seizures induced by monoclonal anti-NMDAR antibodies showed signs of hippocampal inflammation with increased levels of inflammatory markers, including IL-1 β (unpublished).

Pharmacological blockade of IL-1 receptors in rodent models of limbic epilepsy attenuates chemically- and electrically-induced status epilepticus and reduces inflammation, further supporting the premise that IL-1 receptor-mediated signaling contributes to the onset and persistence of seizures.^{12, 34} Anakinra, a recombinant IL-1 receptor antagonist, has a plasma

half-life of 4-6 hours after subcutaneous administration in humans and blocks the activity of IL-1 β and IL-1 α , the product of a related gene.^{25, 40} In case reports, administration of anakinra to patients with recurrent seizures in FIRES and epilepsy associated with autoinflammatory conditions attenuated epileptic activity and improved encephalopathy; this occurred in parallel to the reduction of serum IL-1 β levels.²⁰⁻²² Furthermore, anakinra was beneficial in combatting relapsing seizures in the late stages of prolonged febrile infection-related encephalopathy syndrome (FIRES), suggesting that targeting IL-1 receptor may also be effective in chronic seizures and may interfere with epileptogenesis.²³ We showed that anakinra reduced seizure burden in mice with antibody-induced seizures by attenuating both frequency and duration of seizures. Taken collectively with previous reports in patients, these data suggest that anti-inflammatory therapy may be an effective approach to reduce autoimmune seizures. This anticonvulsant activity could be due to a direct effect of anakinra on the IL-1 receptor and interruption of the downstream effects triggered by IL-1 β , including blood-brain barrier breakdown, which occurs during seizures.^{11, 12, 41} Interestingly, a transient interruption of IL-1 function in our studies had a long-lasting effect on seizure activity as evidenced by decreased seizure duration following the removal of treatment. The latter suggests the intriguing possibility that anakinra not only reduces acute neuroinflammation but may also have a disease-modifying effect on antibody-induced seizures.

A daily dose of 50 mg/kg anakinra administered to mice in our study corresponds to a daily equivalent of 285 mg in an adult human⁴², which is higher than that recommended for the treatment of systemic rheumatological conditions²⁶. However, we anticipate that anakinra will be administered for a much shorter period of time to patients with autoimmune encephalitis and NORSE compared to those with other chronic autoinflammatory conditions²⁶. Furthermore, previous studies in healthy volunteers treated with 700-800 mg of anakinra did not find any clinically significant differences between the drug and placebo-treated groups in physical examination, complete blood counts, mononuclear cell phenotypes, blood chemistry profiles or serum cortisol levels⁴³. In our study, mice were continuously monitored with video; they showed no overt behavioral signs of toxicity.

The mechanism of seizure reduction achieved by IL-1 receptor blockade is not completely understood. Exogenous application of IL-1 β into the hippocampus increases cytokine production in glial cells and prolongs kainate-induced seizures likely by enhancing glutamatergic neurotransmission.³⁵ The potentiation of NMDA responses occurs via increased Ca²⁺ entry through receptor-associated ion channels, which remains sustained following a transient exposure to IL-1 β .⁴⁴ On the other hand, the release of IL-1 β can cause a reduction of inhibitory tone via attenuation of GABA-evoked currents as observed in tissue from patients with refractory temporal epilepsy.⁴⁵ Anakinra might attenuate epileptic activity by restoring the balance between the inhibitory and excitatory circuits in hippocampus. Since the drug was administered systemically and there is evidence of peripheral inflammatory response in autoimmune encephalitis^{6, 29}, it is possible that in addition to blocking the central effects of the IL-1-mediated signaling, anakinra also attenuated peripheral proinflammatory factors that may have contributed to promoting seizure activity.

Patients with anti-NMDAR encephalitis demonstrate memory impairment that correlates with the loss of functional connectivity in the hippocampus.^{1, 46} In animal studies, mice infused with anti-NMDA-IgG-positive CSF or immunized with NMDA-like holoreceptors develop progressive memory deficits and a corresponding increase of tissue-bound antibodies in the hippocampus.^{47, 48} Consistent with these previous reports from preclinical studies and observations in patients, mice with autoimmune seizures in our study showed impaired hippocampal-dependent memory.^{1, 47, 48} The restoration of memory with anakinra opens an intriguing possibility that the blockade of IL-1 receptor-mediated signaling could repair functional connectivity in the hippocampus and improve memory in patients. In a case series of children with FIRES, anakinra promoted resolution of seizures and cognitive improvement; however, the role of improved seizure control in the neurological recovery could not be teased apart.⁴⁹ We cannot determine from our data if the improved memory is a consequence of the reduced seizure burden or an anti-inflammatory effect of anakinra itself. The effects of persistent ictal and interictal activity are well documented in patients with epilepsy along with the improvement in cognition upon seizure control achieved with surgery or conventional anti-seizure medications.^{50, 51} IL-1 blockade with anakinra was also effective to reduce cognitive impairment in other acute seizure models and in a mouse model of traumatic brain injury.^{52, 53} Of note, in our previous study, we found insignificant memory deficit in mice with seizures induced by infusion of CSF or purified IgG from patients with anti-NMDAR encephalitis.⁴ Our ability to demonstrate memory decline in the present study could be supported by the use of monoclonal anti-NMDAR antibodies that bind to a single epitope and possibly contributes to a more consistent behavioral phenotype as well as technical improvement of the previously used assay to assess memory.^{4, 28, 54}

Histopathology of anti-NMDAR encephalitis in patients demonstrated neuroinflammation, infiltrates of lymphocytes or macrophages and rare neuronal loss.^{55, 56} We previously found morphological changes in glial cells of the CA1 region in the same mouse model, suggestive of inflammation; however, there was no increase in the number of GFAP-positive cells in mice with seizures.⁴ Using a different method of quantification of immunoreactivity in the present study, we showed that anakinra reduced the expression of markers of microgliosis and astrogliosis in the CA1 region of hippocampus. The GFAP and Iba-1 immunoreactivity in hippocampus of mice with experimentally-induced anti-NMDAR encephalitis in another mouse model did not differ from that in control mice after three weeks of florid behavioral presentations of encephalitis but was significantly increased at the fulminant stages of the disease at six weeks.⁴⁸ The authors proposed that the detection of inflammatory changes with these markers in mice with autoimmune encephalitis may depend on the time point at which the tissue is examined.⁴⁸

Targeting IL-1 receptor-mediated signaling has tremendous potential for treating refractory autoimmune seizures and other drug-resistant epilepsies⁵⁷. Thus, the introduction of anakinra in 1993 was followed by the development of canakinumab, a human IL-1 β monoclonal antibody that has a long plasma half-life, and binds selectively with high affinity to IL-1 β .²⁵ Gevokizumab, a novel IgG2 humanized monoclonal antibody, targets a single epitope of IL-1 β and modulates the interaction of the cytokine with the receptor complex.²⁵ While these agents are currently in clinical trials for peripheral autoimmune and inflammatory disorders, their success may catalyze their use for relapsing autoimmune

seizures²⁵, if they are brain-penetrant. Based on the existing literature, our present findings and previously published data from our laboratory⁴, we propose that seizures resulted from exposure to antibodies induce neuroinflammation and facilitate the release of IL-1, which exacerbates both seizures and cognitive deficits.

5. Conclusions

In summary, we demonstrated that the IL-1 receptor antagonist anakinra attenuated seizures induced by antibodies against NMDA receptors and improved memory in mice as tested in the novel object recognition assay. Furthermore, anakinra reduced the expression of the inflammatory astrocytic and microglial markers in the hippocampus of mice with autoimmune seizures. These findings suggest that targeting IL-1 signaling may be a promising novel approach for the treatment of clinical conditions that present with autoimmune seizures and associated encephalopathy.

6. Limitations

In this study, we used a mouse model of anti-NMDAR antibody-induced seizures developed in our laboratory.⁴ While the seizures in mice were frequent, they did not recapitulate the full extent of epileptic activity that occurs in patients with NORSE, FIRES, and other acute devastating seizure encephalopathies. In addition to neuroinflammation and autoantibodies, other currently unidentified insults may perpetuate seizures in these patients and these factors might not be recounted in the present model. Therefore, it remains unclear which population of patients will benefit from blockade of IL-1 receptors. Furthermore, the EEG recordings in mice were limited to two brain areas; thus, the full extent of seizure propagation could not be ascertained. Therefore, the findings may not be generalizable to predict the effects of anakinra in multifocal or generalized seizures, which occur in autoimmune status epilepticus. As acknowledged in other studies on inflammation and seizures^{12, 58} it is difficult to discern the effects of reduced seizures from unrelated anti-inflammatory effects of anakinra on behavior and the expression of astrogliosis and microgliosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Dr. Larisa Poluektova and Mr. Edward Makarov for their expert advice and technical assistance with image processing, and Ms. Robin Taylor for excellent editorial support. We thank Swedish Orphan Biovitrum (Stockholm, Sweden) for providing the supply of anakinra as a gift. SKD holds the Joseph and Ray Gordon Chair for Clinical Oncology and Research at the Lankenau Institute for Medical Research. O.T. received grant support from the American Epilepsy Society Junior Investigator Research Award, and R.D. from NIH award R01NS112308. The work described herein is consistent with the Journal's guidelines for ethical publication.

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Key Points

- Anakinra, an antagonist of interleukin-1 (IL-1) receptor, attenuates seizures in mice induced by neuronal antibodies from patients with autoimmune encephalitis.
- Anakinra also attenuates memory impairment and reduces inflammation in the hippocampus of mice with autoimmune seizures.
- In addition to encouraging clinical data, these findings suggest that IL-1 plays a role in pathogenesis of autoimmune seizures.

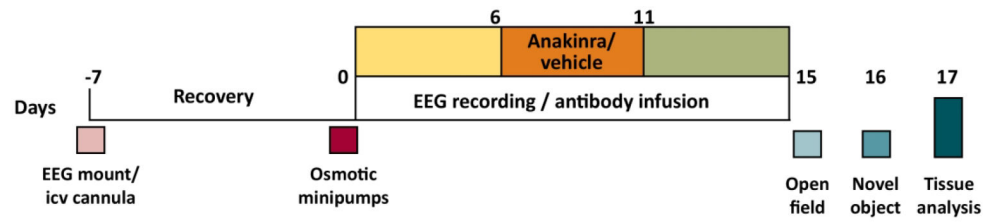


Figure 1.

Experimental protocol to assess the role of anakinra in the persistence of seizures induced by anti-NMDAR antibodies, followed by behavioral and histological analysis.

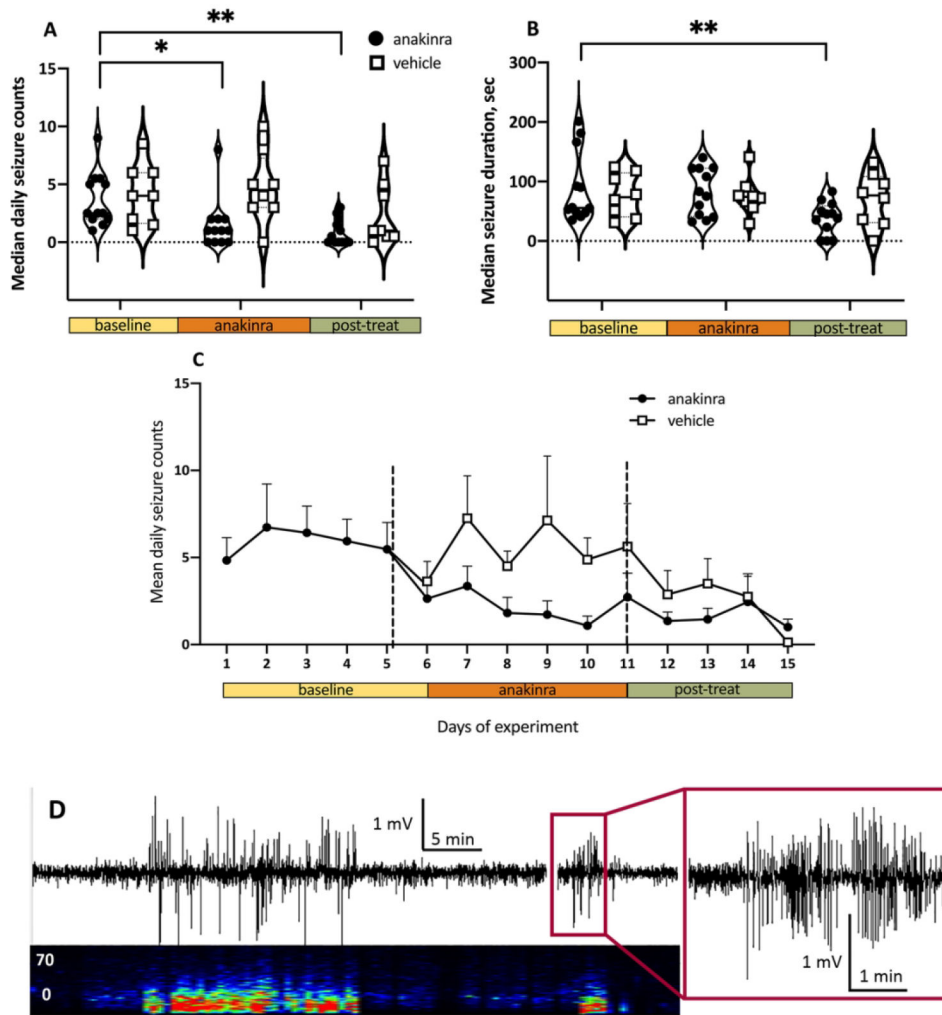


Figure 2.

Administration of anakinra reduced seizures induced by anti-NMDAR antibodies. Data are daily medians and 25-75% interquartile ranges (IQR). Solid and dotted horizontal lines indicate median values and IQRs, respectively *, $p < 0.05$; **, $p < 0.01$, ANOVA with Sidak multiple comparisons tests. (A) Daily seizure counts in anakinra-treated mice ($n = 12$) were reduced during the treatment (orange bar) and post-treatment periods (green bar) compared to the corresponding baseline (yellow bar). The seizure counts were unchanged in the vehicle treated mice ($n = 8$). (B) Delayed effect of anakinra on seizure duration. The duration of seizures was decreased in the washout phase following anakinra. (C) Effects of anakinra on seizure counts were apparent 24-48 h following the initiation of treatment. Each time point represents the mean and SEM at the completion of a 24-h of recording. (D) Representative 60-min EEG recording and the corresponding spectrogram showing clusters of electrographic seizures in the parietal cortex of mice during the continuous intracerebroventricular (i.c.v.) infusion of anti-NMDAR antibodies. The vertical axis of the spectrogram represents frequency from 0 to 70 Hz and the horizontal axis represents time in min. The trace of one seizure is expanded to demonstrate the characteristic pattern of high amplitude sharp rhythmic activity.

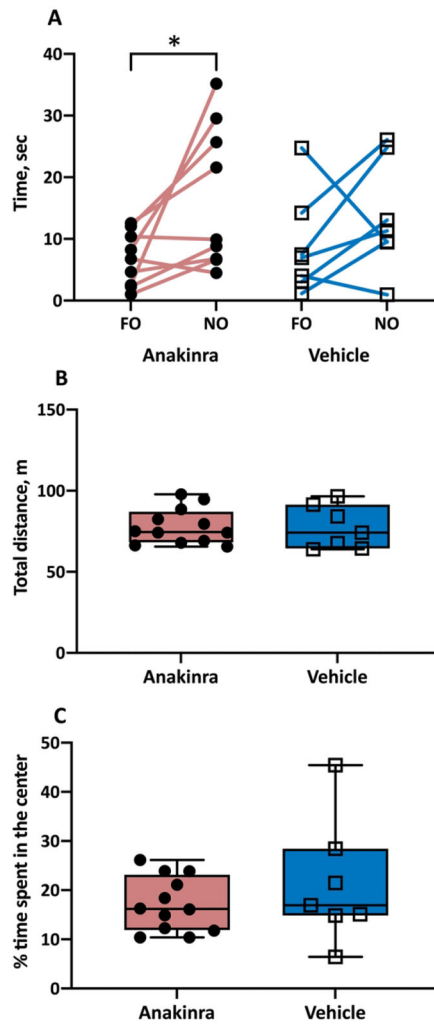


Figure 3.

Behavioral phenotype of mice with autoimmune seizures treated with anakinra. (A) Anakinra rescued an ability to discriminate between familiar object (FO) and novel object (NO) in mice with seizures induced by anti-NMDAR antibodies. $N = 9$ (anakinra-treated), $n = 7$ (vehicle-treated). (B) Anakinra had no effect on locomotor activity in mice with seizures. $N = 12$ (anakinra-treated), $n = 7$ (vehicle-treated). (C) Anakinra did not affect the anxiety scores in mice with seizures. *, $p < 0.05$, paired t-tests. Error bars represent mean \pm SEM.

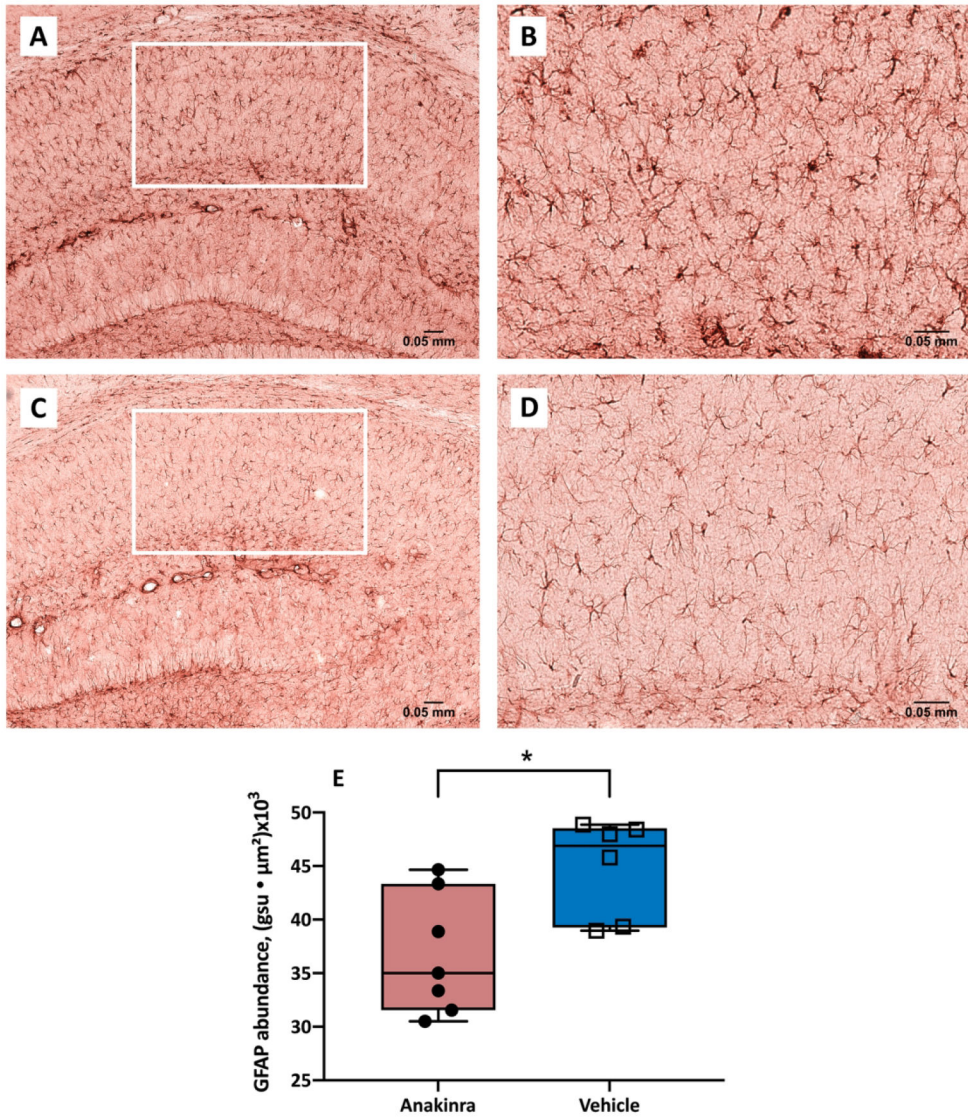


Figure 4. Abundance of GFAP-positive astrocytes in the CA1 region of hippocampus of mice with autoimmune seizures. (A-D) Representative GFAP immunostaining of the CA1 region in vehicle-treated (upper panel) and anakinra-treated (lower panel) mice with seizures induced by continuous infusion of anti-NMDAR antibodies at 10 X (A, C) and 20 X (B, D). (E) Anakinra reduced the expression of GFAP in the CA1 region of hippocampus in mice with seizures. The abundance of GFAP labeling in the CA1 region was determined as the sum of the products of mean pixel intensity (grey scale units, gsu) and area of each event (μ^2) in a fixed scan area. $N = 7$ (anakinra-treated), $n = 6$ (vehicle-treated). * $p < 0.05$, Student's t-test. Error bars represent mean \pm SEM.

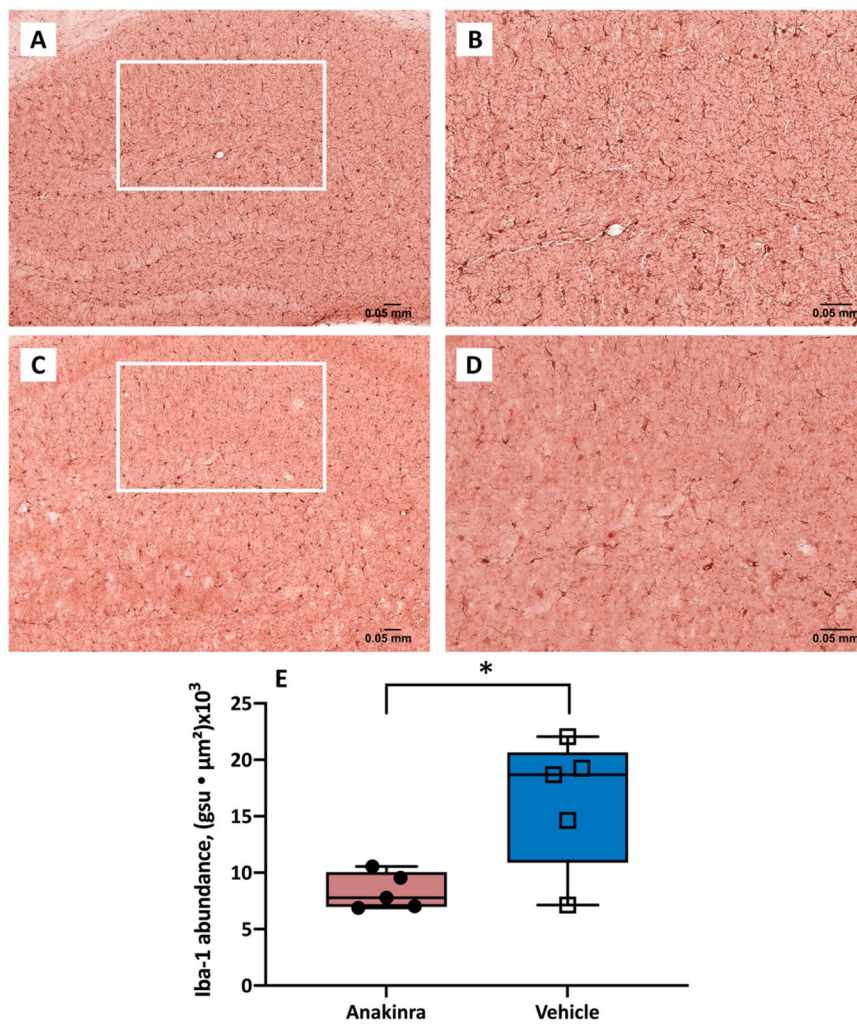


Figure 5. Expression of Iba-1 immunoreactivity in the CA1 region of hippocampus in mice with autoimmune seizures. (A-D) Representative Iba-1 immunostaining images of the CA1 region of vehicle-treated (upper panel) and anakinra-treated (lower panel) mice with seizures induced by anti-NMDAR antibodies at 10 X (A, C) and 20 X (B, D). (E) Anakinra reduced the expression of Iba-1 in the CA1 region of hippocampus in mice with seizures. The abundance of Iba-1 labeling in the CA1 region was determined as the sum of the products of mean pixel intensity (gsu) and area of each event (μ^2) in a fixed scan area. N=5 (anakinra-treated), n=5 (vehicle-treated). * $p < 0.05$, Student's t-test. Error bars represent mean \pm SEM.