Recruitment of activated microglia cells in the spinal cord of mice by ALS IgG

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Mice were injected i.p. with IgG samples of different patients to test whether IgG from amyotrophic lateral sclerosis (ALS) can initiate an immune/inflammatory reaction targeting motor neurons. All IgG samples of five ALS patients and none of the disease controls recruited activated microglia cells in the ventral horn of the spinal cord. CD3 lymphocytes were not accumulated in the same tissue. Similar reaction was evoked by injection of IgG from guinea pigs with experimental autoimmune gray matter disease (EAGMD) induced by immunization with the homogenate of the ventral horn of bovine spinal cord. The results indicate that ALS IgG and anti-motoneuron IgG induce microglia reaction targeting motor neurons without initiating T cell response in the recipient mice. NeuroReport 12:2449–2452 © 2001 Lippincott Williams & Wilkins.

Key words: ALS; IgG; Microglia; Motor neuron

INTRODUCTION

ALS is a devastating human disease with the degeneration of upper and lower motor neurons. It eventually leads to respiratory failure and death due to the atrophy of striated muscles. Autoimmunity has been implicated in the pathomechanism of the degeneration of motor neurons [1]. IgG from immune-mediated models of motor neuron diseases and also from ALS patients transferred to mice is taken up in the spinal motor neurons of the recipients. It increases the frequency of miniature end-plate potentials reflecting enhanced acetylcholine release from the motor axon terminals [2]. Anti-motoneuron IgG produced in goat by immunization of the animal with ventral horn homogenate of bovine spinal cord transferred to mice induces increased density of synaptic vesicles in the axon terminals of the neuromuscular junctions and in synaptic boutons terminating on spinal motor neurons [3]. The IgG also increases intracellular calcium mainly in the abnormal vacuolations of the rough endoplasmic reticulum and in dilated cisterns of the fragmented Golgi system. Similar ultrastructural early signs of degeneration can be evoked in spinal motor neurons of mice injected i.p. with IgG from ALS patients [4,5]. Passively transferred immunoglobulins from ALS patients also raise the level of glutamate in the cerebrospinal fluid of rats [6]. Microglia/macrophage and lymphocytic infiltrates in the spinal cord were reported in one of the developed experimental immune-mediated motor neuron diseases (EAGMD) [7] and also in ALS [8]. The aim of this study was to examine whether microglia accumulation and activation, lymphocyte infiltrates in the spinal cords of recipient mice can be induced by passively transferred IgG from ALS patients. Anti-motoneuron IgG from guinea pigs with EAGMD served as a possible positive control in the experimental paradigm.

MATERIALS AND METHODS

Four groups of male, 6 week-old Balb/c mice (Harlan Sprague Dawley, Inc. Indianapolis IN) were injected i.p. with 20 mg IgG prepared from various sera. An Avid/ALTM column (Bioprobe International, Inc., Tustin CA) was used for the isolation of IgG according to the manufacturer’s instructions. Each IgG sample was administered to three mice in two consecutive days. Group 1 (ALS) received IgG from five patients with ALS (n = 15). Group 2 (disease control group; DC) was injected with IgG obtained from five patients with either multiple sclerosis, Guillain-Barré syndrome, Parkinson disease, Alzheimer’s disease or ischemic stroke (n = 15). The mice in group 3 (GP IgG) received IgG from three guinea pigs treated with complete and incomplete Freund’s adjuvant (n = 9) or were not injected with IgG (n = 3). Finally, group 4 (anti-MN) was treated with IgG prepared from three guinea pigs with EAGMD [7] (guinea pigs immunized with the homogenate of the ventral horn of bovine spinal cord; n = 9).

Twenty-four hours after the second inoculation the mice were perfused with 2% paraformaldehyde. The spinal cords were removed and 20 μm frozen sections of the lumbar segments were immunostained with 1:50 dilution of rat monoclonal antibody to mouse CD11b (Ly-40) (Serotec Ltd, Oxford Implant, UK) specific for CR(complement receptor)3 binding sites which also labels activated microglia. Other sections were immunostained with 1:50 dilution of monoclonal rat antibody to CD3 lymphocyte molecular complex (Pharmingen, San Diego, CA). Sections...
of mouse tonsils served as positive control. The bound monoclonal antibodies were detected with Vectastain Elite ABC kit for rat IgG according to the manufacturer’s instruction (Vector Laboratories, Inc., Burlingame CA) and visualized with Sigma Fast 3,3’ diamino benzidine tetrahydrochloride solution with metal enhancer (Sigma Chemical Co., St. Louis, MO). The labeled cells were counted in every fifth section of the lumbar cord (altogether in 10 sections) of each animal. The mean number of labeled cells in three mice was determined for each IgG preparation. The non-parametric Kruskal-Wallis one-way analysis on ranks was used to compare the numbers of the immunopositive microglia cells in the ventral horns of the spinal cords of mice in the four groups. The Mann-Whitney test served for pairwise post hoc comparison between the group of mice treated with ALS IgG and the group injected with IgG from other neurological diseases. In all tests, an α level of $p < 0.05$ was taken as an indication of statistical significance.

Other sections for light microscopic evaluation were stained with cresyl violet.

RESULTS
Regarding the numbers of CD11b-immunopositive microglia cells the Kruskal-Wallis analysis of variance indicated statistically significant variations among the four groups of mice treated with various IgG samples. In sections of the ventral horns of the lumbar segments of spinal cords of mice in the four groups of mice treated with various IgG samples. In sections of the ventral horns of the lumbar segments of spinal cords of mice injected with IgG from ALS patients and the group of mice treated with IgG from patients with various neurological diseases. The mean number of labeled cells in three mice was determined for each IgG preparation. The Mann-Whitney test served for pairwise post hoc comparison between the group of mice treated with ALS IgG and the group injected with IgG from other neurological diseases. In all tests, an α level of $p < 0.05$ was taken as an indication of statistical significance.

In the sections of the ventral horns of the lumbar cord of the spinal cord of mice injected with IgG from ALS patients high numbers of CD11b-immunopositive microglia cells were observed (Fig. 1, Fig. 4). The mean number of immunopositive cells varied from 47 to 88 in these mice (mean ± s.e. 66 ± 9). Some of the activated microglia cells surrounded spinal motor neurons even if the neurons showed no signs of destruction in light microscopic sections stained with cresyl violet. Fewer immunolabeled cells (9 ± 4) were also noted in the anterior columns in proximity to the ventral horns (Fig. 1). Labeled cells were only scarcely detected in the posterior horns or in the posterior and lateral columns of the spinal cords (1–3/section). The effect of IgG samples of different ALS patients on the numbers of activated microglia cells was uniform and did not differ statistically from each other. In contrast to the response to ALS IgG the mean number of CD11b immunopositive microglia cells was low (8 ± 0.3, range: from 8 to 9) in the ventral horns of mice injected with IgG from patients with the five other neurological diseases (Fig. 2, Fig. 4). The difference in the counts of the labeled cells was significant (Mann-Whitney test) between the group of mice treated with ALS IgG and the group injected with IgG from other neurological diseases ($p < 0.05$). IgG from guinea pigs immunized with the homogenate of the ventral horn of bovine spinal cord injected i.p. in mice also induced the appearance of a high number of activated microglia cells in the ventral horn of the spinal cord. The mean number of labeled cells was 86 ± 2 (ranged 84–90) in those mice. The response to the anti-motoneuron IgG was comparable to the results obtained following the injection of mice with ALS IgG, but the effect was even more uniform (Fig. 4). Some of the activated microglia cells concentrated in the vicinity of spinal motor neurons and only a few (12 ± 7) immunolabeled cells were observed in the anterior columns (Fig. 3). Labeled cells were as barely detected in other locations in the lumbar spinal cords as in ALS IgG treated mice. The mean number of the labeled microglia cells was 5 ± 0.8 (ranged 3–6) in the ventral horns of the spinal cord in the group of mice injected with IgG from control guinea pigs or in untreated animals (Fig. 4). CD3-positive lymphocytes were not detected in the lumbar segments of the spinal cords of mice injected either with ALS IgG, or anti-motoneuron guinea pig IgG. However, they could readily be seen in mouse tonsil tissue treated in the same way.

DISCUSSION
Microglia comprise 5% of the CNS parenchyma [9]. Resting microglia cells are only moderately CD11b immunoreactive; i.e. they bear few type 3 complement receptors [10].
CNS trauma [10], peripheral nerve injury [11], epidural application of kainic acid [12] and CNS inflammatory processes [13] induce a rapid microglial up-regulation of CD11b which indicates the activation of the cells [14]. The activation of microglia is a common feature also in neurodegenerative diseases particularly in ALS [8]. Our previous experiments demonstrated that IgG from ALS patients directed to calcium channels initiates a raise in intracellular calcium in motor neurons which is followed by ultrastructural alterations indicative of early signs of degeneration [4]. Similar changes were noted after i.p. injection of ALS or anti-motoneuron IgG can represent additional damage to the motor neurons. Activated microglia can enhance neuronal injury by the formation of reactive oxygen intermediates and by the production of cytokines [15-17]. In the light of the consecutive microglia activation the ultrastructural alterations observed in motor neurons following i.p. injection of anti-motoneuron or ALS IgG may also be attributed to the harmful effect of activated microglia. Further experiments with inoculation of anti-motoneuron IgG and simultaneous administration of compounds inhibiting microglia activation and recruitment can elucidate the potential role of microglia involvement in the harmful process. However, microglia can also have neuroprotective function [18,19]. It may facilitate the repair of the damage caused by IgG. The signals which trigger microglia response have not been well delineated. IL (interleukine)-1- and TNF (tumor necrosis factor)-α can up-regulate the expression of CD11b (Mac-1, CR3) in cultured mouse microglia cells [20]. IFN-γ does not seem to be involved in signaling for microglia by motor neurons following nerve transection [21]. Intracerebroventricular injection of C10, a novel chemokine expressed in the CNS in experimental inflammatory demyelinating diseases promotes recruitment of macrophages to the CNS, but its effect is rather diffuse than localized to the ventral horns of the spinal cord [13]. The release of C10 by motor neurons is unlikely. The localized microglia activation and infiltration of the ventral horns of the spinal cords of mice injected with ALS and anti-motoneuron IgG suggest the action of a soluble molecule released by motor neurons in response to the primary influence of IgG. The IgG mediated increase in glutamate release from synaptic boutons residing on spinal motor neurons [6] might also play a role in this process. Furthermore, raised intracellular calcium in motor neurons can also activate signaling [3,4].

Infiltration of the ventral horns of the spinal cord of mice by CD3 positive lymphocytes did not occur following i.p. injection of IgG from ALS patients or from guinea pigs with EAGMD. It suggests that a full immune response which is observed in EAGMD [7] and in ALS [8] can not be initiated by passively transferred IgG. It may explain why motor neurons do not die in simple IgG passive transfer experiments [3-5]. However, mice receiving IgG from guinea pigs with EAGMD die due to severe weakness of the striated muscles and consequent respiratory failure. Nevertheless, motor neurons display only the early signs of degeneration even in premorbid animals [3]. The severe

**Fig. 3.** Activated microglia cells are immunostained darkly (indicated by solid arrow) in the ventral horn of the spinal cord of a mouse inoculated i.p. with IgG from a guinea pig with EAGMD. Some of the cells accumulated in the vicinity of spinal motor neurons (empty arrow). MN, motor neuron. Peroxidase reaction. Bar = 140 μm.

**Fig. 4.** The mean numbers of activated microglia cells in the ventral horn sections of the lumbar spinal cords in mice. The symbols mark values obtained in animals inoculated with the same IgG sample. ALS: mice injected i.p. with IgG from ALS patients (filled circles). DC: mice injected with IgG from disease controls (filled circle, MS; empty circle, Guillain-Barré syndrome; empty triangle, Parkinson disease; filled triangle, Alzheimer’s disease; filled rectangle, ischemic stroke). GP IgG: mice inoculated with IgG from guinea pigs immunized with only Freund’s adjuvants (empty triangles) and untreated mice (filled rectangle). Anti-MN: anti-motoneuron IgG from guinea pigs immunized with the homogenate of the ventral horn of bovine spinal cords (filled circles). The error bars represent SE of means. The administration of ALS IgG and anti-motoneuron IgG increased the numbers of activated microglia cells in sections of the ventral horn of the spinal cord of injected mice.
weakness in striated muscles can be attributed to the dysfunction of the motor axon terminals resulting from a deficiency in intraterminal calcium and decreased acetylcholine release [3].

CONCLUSION

IgG from the sera of ALS patients and IgG from guinea pigs with EAGMD injected i.p. in mice do not only induce ultrastructural alterations of spinal motor neurons, but also recruitment of activated microglia cells in the ventral horns of the spinal cord. Activated microglia can enhance neuronal injury initiated by IgG. However, a full immune response targeting motor neurons observed in EAGMD or in ALS can not be triggered by passively transferred antimotoneuron IgG or by ALS IgG in the recipient mice. The absence of CD3 lymphocytes in the ventral horn of the spinal cords of the injected mice may explain the survival of motor neurons in IgG passive transfer experiments.

REFERENCES


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