Delayed Anti-Nogo-A Therapy Improves Function After Chronic Stroke in Adult Rats

Shih-Yen Tsai, MD, PhD; Catherine M. Papadopoulos, PhD; Martin E. Schwab, PhD; Gwendolyn L. Kartje, MD, PhD

**Background and Purpose**—We have shown that anti-Nogo-A immunotherapy to neutralize the neurite growth inhibitory protein Nogo-A results in functional improvement and enhanced plasticity after ischemic stroke in the adult rat. The present study investigated whether functional improvement and neuronal plasticity can be induced by this immunotherapy when administered to the chronic stroke-impaired rat.

**Methods**—Adult rats were trained to perform the skilled forelimb reaching test, followed by permanent middle cerebral artery occlusion to impair the preferred forelimb. Nine weeks after stroke, animals showing a profound deficit were randomly distributed to 3 groups: no treatment, control antibody, or anti-Nogo-A antibody (11C7). Animals were tested weekly after stroke surgery and daily after antibody treatment until the end of the study. Biotin dextran amine tracing was injected into the nonlesioned forelimb motor cortex at the end of behavioral testing to determine axonal plasticity.

**Results**—All rats showed similar forelimb impairment before treatment. Animals treated with anti-Nogo-A immunotherapy started to show improvement 3 weeks after treatment. Such improvement became significantly better than stroke-only control and control Ab-treated animals, and persisted to the end of the study. Biotin dextran amine-labeled axonal fiber analysis also showed significant enhanced corticorubral axonal sprouting from the contralesional forelimb motor cortex to the deafferented red nucleus in the anti-Nogo-A immunotherapy rats.

**Conclusions**—These results indicate that improvement of chronic neurological deficits and enhancement of neuronal plasticity can be induced in the adult rat with anti-Nogo-A immunotherapy, and that this therapy may be used to restore function even when administered long after ischemic brain damage has occurred. (Stroke. 2011;42:186-190.)

**Key Words:** neuroplasticity ■ Nogo-A ■ stroke recovery

**Stroke** is the nation’s third leading killer and the most common cause of adult neurological disability. Each year, nearly 800,000 Americans are stricken by ischemic stroke, during which blood flow to the brain is interrupted and neurons are permanently damaged. More than 232,000 Americans die from stroke-related causes each year, and there are nearly 800,000 Americans who survive the acute stroke period and suffer with their disability for many subsequent years. Patients who have had a stroke receive acute treatment when administered immediately, or 24 hours after, or 1 week after ischemic stroke in adults or aged rats. Because many stroke victims survive the acute stroke period and suffer with their disability for many subsequent years, several treatments are being developed to improve functional outcome beyond the acute and into the chronic stroke stage.

The purpose of the present study was to examine the effectiveness of anti-Nogo-A immunotherapy as a treatment to restore lost functions in the chronic stage after ischemic stroke. Our results indicate that anti-Nogo-A immunotherapy is effective as a treatment, even when administered up to 9 weeks after stroke in adult rats.

**Materials and Methods**

Adult male, Long Evans black-hooded rats (mean weight of 300 grams) were first trained and tested on the skilled forelimb reaching task (Figure 1A) as previously described. This task requires fine digit movement and intact motor and sensory neural pathways. Animals that successfully retrieved 15 out of 20 pellets during 3 consecutive trials were selected for further study. In addition, we did not include animals with abnormalities in gait or motor coordination.

Animals were then trained for 30 minutes daily on the skilled forelimb reaching task for 10 days. At the end of training, rats received hemilaminectomy to impair the preferred forelimb. Nine weeks after stroke, animals showing a profound deficit were randomly distributed to 3 groups: no treatment, control antibody, or anti-Nogo-A antibody (11C7). Animals were tested weekly after stroke surgery and daily after antibody treatment until the end of the study. Biotin dextran amine tracing was injected into the nonlesioned forelimb motor cortex at the end of behavioral testing to determine axonal plasticity.
consecutive training sessions received permanent middle cerebral artery occlusion to impair the preferred forelimb as described. After middle cerebral artery occlusion, animals continued to use their impaired forelimb to reach, despite showing deficits in performing the task. To reduce possible effects of rehabilitation through daily testing, animals were tested Monday through Friday for the first week after stroke and once per week until time of treatment, ie, 9 weeks after stroke. Any animal that improved spontaneously to exhibit improvements in the pellet reaching success rate at 3 weeks after treatment (ie, 12 weeks after stroke) and showed a significant difference starting 5 weeks after treatment when compared to the stroke-only group (*P < 0.05; Figure 1B). At the end of the study, animals treated with anti-Nogo-A Ab treatment began to exhibit improvements in the pellet reaching success rate at 3 weeks after treatment (ie, 12 weeks after stroke) and showed a significant difference starting 5 weeks after treatment when compared to stroke-only animals (*P < 0.01) or stroke/control Ab-treated animals (P < 0.05; Figure 1B). At the end of the study, animals treated with the anti-Nogo-A Ab demonstrated an improvement up to 78% of their prestroke performance, which was significantly different from the stroke only animals (47%; P < 0.05) or stroke/control Ab animals (33%; P < 0.01). There was no significant difference in success scores between the 2 control groups at any time point (1-way ANOVA). Furthermore, animals receiving anti-Nogo-A Ab treatment demonstrated a significant increased rate of improvement when compared to the stroke-only group (P < 0.05) and stroke/control Ab group (P < 0.05; Figure 1C), with no difference between the 2 control groups. These results demonstrate that neutralization of Nogo-A with a specific function blocking antibody started at 9 weeks after ischemic

Results

Skilled forelimb reaching, which is a complex motor cortex-dependent behavior, was analyzed in the single pellet retrieval task. Before stroke surgery, animals in all groups showed excellent skilled reaching and no difference in performance. One week after stroke, all animals had significant deficits in obtaining pellets with the stroke-impaired limb, and there was no spontaneous improvement over the subsequent 8 weeks (before treatment; Figure 1B). However, animals that received anti-Nogo-A Ab treatment began to exhibit improvements in the pellet reaching success rate at 3 weeks after treatment (ie, 12 weeks after stroke) and showed a significant difference starting 5 weeks after treatment when compared to stroke-only animals (P < 0.01) or stroke/control Ab-treated animals (P < 0.05; Figure 1B). At the end of the study, animals treated with the anti-Nogo-A Ab demonstrated an improvement up to 78% of their prestroke performance, which was significantly different from the stroke only animals (47%; P < 0.05) or stroke/control Ab animals (33%; P < 0.01). There was no significant difference in success scores between the 2 control groups at any time point. Furthermore, animals receiving anti-Nogo-A Ab treatment demonstrated a significant increased rate of improvement when compared to the stroke-only group (P < 0.05) and stroke/control Ab group (P < 0.05; Figure 1C), with no difference between the 2 control groups. These results demonstrate that neutralization of Nogo-A with a specific function blocking antibody started at 9 weeks after ischemic

Figure 1. Behavioral assessment of animals. A, Sequential snap frames of the skilled forelimb reaching task performed by an adult rat grasping a pellet. B, All groups showed marked deficits in successfully obtaining pellets with the stroke-impaired limb before treatment, with no significant difference between groups. Animals treated with anti-Nogo-A Ab exhibited significant improvement greater than the stroke/control Ab group and the stroke-only group at 5 weeks after treatment that persisted until the end of the study. There was no difference between stroke/control Ab animals (n = 5) and stroke/control Ab treatment (*P < 0.05; Figure 1B). However, animals that received anti-Nogo-A Ab treatment began to exhibit improvements in the pellet reaching success rate at 3 weeks after treatment (ie, 12 weeks after stroke) and showed a significant difference starting 5 weeks after treatment when compared to stroke-only animals (P < 0.01) or stroke/control Ab-treated animals (P < 0.05; Figure 1B). At the end of the study, animals treated with the anti-Nogo-A Ab demonstrated an improvement up to 78% of their prestroke performance, which was significantly different from the stroke only animals (47%; P < 0.05) or stroke/control Ab animals (33%; P < 0.01). There was no significant difference in success scores between the 2 control groups at any time point. Furthermore, animals receiving anti-Nogo-A Ab treatment demonstrated a significant increased rate of improvement when compared to the stroke-only group (P < 0.05) and stroke/control Ab group (P < 0.05; Figure 1C), with no difference between the 2 control groups. These results demonstrate that neutralization of Nogo-A with a specific function blocking antibody started at 9 weeks after ischemic

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infarction can result in remarkable improvement on the skilled forelimb reaching task.

Lesion analysis showed that all stroke lesions included the ipsilateral sensorimotor cortex without involving subcortical structures, such as the striatum or the hippocampus (Figure 2 A–D). There was no statistical difference in stroke size between groups (stroke only, 10.2% ± 3.2 SEM; stroke/control Ab, 12.63% ± 4.32 SEM; stroke/anti-Nogo-A Ab, 16.49% ± 3.83 SEM; 1-way ANOVA), indicating no apparent effect of anti-Nogo-A immunotherapy on lesion size.

Compensatory sprouting and neuroanatomical plasticity were analyzed for corticorubral midline crossing fibers from the contralesional forelimb cortex to the deafferented red nucleus using the anterograde neuroanatomical tracer BDA. Control stroke animals consistently showed few BDA-labeled midline-crossing fibers (253±60 SEM; Figure 3A). More than twice the number of midline crossing projections into the deafferented red nucleus were observed in the animals receiving anti-Nogo-A Ab therapy (527±74; Figure 3B–D; P<0.05; Student t test). This result suggests that anti-Nogo-A immunotherapy administered at 9 weeks after ischemic infarction can induce remarkable compensatory sprouting and fiber growth, indicating the responsiveness of the chronically injured brain to form new neural networks under the proper growth conditions.

Discussion

The present study shows that treatment with anti-Nogo-A immunotherapy started at 9 weeks after ischemic stroke in the adult rat results in significant improvement in a chronic lesion-induced deficit of skilled forelimb reaching. Furthermore, this therapy also enhanced sprouting and midline crossing of corticorubral axons originating from the contralesional sensorimotor cortex to innervate the deafferented red nucleus, which is an important neural structure for motor control.

Studies have shown that anti-Nogo-A immunotherapy improves functional recovery, neuroregeneration, and compensatory fiber growth after central nervous system lesions in adult rats11,13 and primates.14 Our laboratory was the first to show that anti-Nogo-A immunotherapy administered immediately after ischemic stroke in adult rats resulted in improvement in skilled forelimb reaching.4 Further studies using different function blocking anti-Nogo-A antibodies confirmed this result and showed that when anti-Nogo-A immunotherapy was delayed for either 24 hours5 or 1 week6 after stroke, significant improvement of sensorimotor function was still observed. This treatment was also effective in improving functional outcome when applied in a lesion-induced neglect model in the rat.15 Therapy targeting a Nogo-A–related receptor, NgR, also resulted in beneficial effects in rats when administered at 1 week after stroke.16 A recent report further showed that motor rehabilitation facilitated the effect of NEP1–40, which is a NgR competitive antagonist, in functional improvement after ischemic stroke in rats.17 All these findings suggested that blocking Nogo-A activities is an important intervention to restore lost function after central nervous system lesions.
In the present study, although treatment was delayed for 9 weeks after stroke, animals improved significantly in a skilled forelimb reaching task by 5 weeks after the start of Ab treatment and reached a mean of 78% of their baseline performance at the end of the study. This result closely paralleled our earlier reports with acute or 1-week delayed antibody infusions. In our earlier studies, animals receiving immediate treatment showed significant improvement at 6 weeks after the start of anti-Nogo-A Ab infusion and improved to 77% of baseline performance at the end of the study.4 In our other study, animals received treatment 1 week after stroke and showed significant improvement at 5 weeks after the start of anti-Nogo-A Ab treatment, and these animals reached 75% of the baseline level at the end of the study.6 Therefore, even when starting treatment at a much longer time point after stroke, such as in the present study, similar mechanisms may be underlying the improved functional outcome.

We investigated the possibility of neuroanatomical plasticity in the improved animals and found enhanced corticorubral axonal fiber sprouting originating from the contralesional cortex and targeting the deafferented red nucleus. This result is consistent with our previous reports that anti-Nogo-A immunotherapy enhanced corticorubral plasticity when administered immediately,4,6 24 hours after,5 or 1 week after stroke.6 The similarity in the corticorubral crossing index between the present study (527±74) and that of the immediate treatment study (469±81)4 and the similarity of control groups at 263±60 and 232±37, respectively, suggest that anti-Nogo-A immunotherapy administered at 9 weeks after stroke is as effective as immediate therapy in inducing corticorubral axonal plasticity.

The critical window for inducing plasticity and improving functional outcome after central nervous system lesions, particularly ischemic stroke, has not been established. Enhancing neuronal plasticity by modulation of the inhibitory environment has become an important mechanism to improve lost function in the adult brain after injury. Interestingly, blocking Nogo-A function alone (no lesion) induces transient expression of various trophic factors, growth-associated factors, and neuronal cytoskeletal components, and results in transient axonal18–22 and dendritic23 arborization. Also, chondroitinase ABC treatment degraded chondroitin sulfate proteoglycans, reactivated visual cortical plasticity, and, when paired with reverse environmental manipulation, completely restored ocular dominance, visual acuity, and dendritic spine density in adult rats with shifted ocular dominance attributable to early monocular deprivation.24 These studies suggest that modulation of neuronal plasticity can be achieved even in the unlesioned mature brain that has sustained an earlier insult. This further supports our findings that appropriate interventions, even when administered at a chronic stage after stroke, may be sufficient to induce neuronal plasticity in a stable, chronically lesioned brain with a “fixed” neurological deficit and may ultimately improve functional outcome.

**Conclusion**

In conclusion, this study demonstrates that the adult brain is capable of improved functional outcome and enhanced com-

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**Figure 3. Corticorubral plasticity.** Representative photomicrographs show obvious differences of corticorubral midline crossing fibers (arrows) between a stroke/control Ab animal (A) and a stroke/anti-Nogo-A Ab animal (B). Dotted lines indicate the midline. C, Schematic diagram showing corticorubral plasticity after stroke and anti-Nogo-A Ab treatment (adapted from Seymour et al6) D, Stroke/anti-Nogo-A Ab-treated animals exhibit significant increase in the midline crossing corticorubral fibers in comparison with control animals (*P*<0.05; Student *t* test). Bars indicate 50 μm.
pensatory fiber growth and circuit plasticity induced by anti-Nogo-A immunotherapy, even long after ischemic stroke. Our findings are of great clinical importance because anti-Nogo-A immunotherapy may benefit not only patients with spinal cord injury or patients in the early stage of stroke recovery but also patients in later stages who have neurological disability attributable to brain damage from stroke or other causes.

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Disclosure
None.

References
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Abstract

慢性脳卒中の状態が続いた後に抗 Nogo-A 療法を実施すると成熟ラットの機能が改善する

Delayed Anti-Nogo-A Therapy Improves Function After Chronic Stroke in Adult Rats

Shuh-Yen Tsai, MD, PhD; Catherine M. Papadopoulos, PhD; Martin E. Schwab, PhD; Gwendolya L. Kantje, MD, PhD1,2,3

1Neurology and Research Service, Edward Hines Jr VA Hospital, Hines, Ill; 2Brain Research Institute, University of Zurich, and Department of Biology, Swiss Federal Institute of Technology, Zurich, Switzerland; 3Neuroscience Institute, Department of Molecular Pharmacology & Therapeutics, Department of Neurology, Loyola University Chicago, Maywood, Ill

脳卒中後、神経変化は特性があるNogo-Aを中和する抗Nogo-A免疫療法により、新鮮脳卒中後の成熟ラットの機能が改善し、可塑性が強化されることが示された。今回、新鮮脳卒中後、同様の経過を作成したラットに抗Nogo-A免疫療法を実施し、機能的改善および神経細胞の可塑性を観察した。

方法：前肢にskilled reaching test が行えるよう成熟ラットを訓練した後、脳卒中誘発を示す陰極を用い、使用規模のある方の成長に障害を及ぼした。脳卒中作製後9週後に、障害を顕著に見られた側を、対側側面、同側側面、抗Nogo-A抗体 (1/100) 溶液で1週間ずつを治療対象にした。脳卒中療法後は週1回、また体外包装は毎日の顎内で、試験終了まで検査を実施した。行動検査終了時に、トーサであるビオチンキストラフアミンを障害のない側の脳の運動欠損に注射し、検査の可塑性を調べた。

結果：脳卒中療法には、すべてのラットで同程度の障害の改善が観察された。抗Nogo-A免疫療法群では、治療終了3週後から改善がみられるようになった。改善は、非治療群および対側側面に比べて著しく大きくなり、試験終了まで持続した。ビオチンキストラフアミンでの標識突起の細胞数の解析で、抗Nogo-A免疫療法群は、対側側面の運動皮質から全頭路が遮断された側に比べて皮質系続線の重点発芽が有意に増加していた。

結論：本研究の結果から、抗Nogo-A免疫療法を施行した成熟ラットには、神経変化を神経細胞型の改善と神経細胞の可塑性改善がみられる。本治療法を用えば、皮質系続線の発芽は時間が経っても機能が回復する可能性がある。