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RESEARCH REVEALS NEW UNDERSTANDING, WARNING SIGNS, AND POTENTIAL TREATMENTS FOR MULTIPLE SCLEROSIS

SAN DIEGO — Scientists are gaining a new level of understanding of multiple sclerosis (MS) that may lead to new treatments and approaches to controlling the chronic disease, according to new research released today at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

MS is a severe, often crippling, autoimmune disease caused by the body's immune system attacking the nervous system. Today, more than two million people worldwide suffer from MS and other neuroinflammatory diseases. MS usually strikes in early adulthood and manifests with symptoms including vision loss, paralysis, numbness, and fatigue. The disease can be intermittent or progressive and currently has no cure.

Today's new findings show that:

- Scientists are one step closer to understanding how antibodies in the blood stream break past the brain's protective barrier to attack the optic nerves, spinal cord, and brain, causing the symptoms of neuromyelitis optica, a rare disease similar to MS. Understanding how the antibodies bypass the protective blood-brain barrier could provide new approaches to treating the disease (Yukio Takeshita, MD, PhD, abstract 404.09, see attached summary).
- A protein involved in blood clotting mightserve as an early detection method for MS before symptoms occur. Early detection of the disease could lead to more effective early treatments (Katerina Akassoglou, PhD, abstract 404.11, see attached summary).
- Low levels of a cholesterol protein correlate with the severity of a patient's MS in both human patients and mouse models. The finding suggests the protein, known to protect against inflammation, may protect against developing MS, and possibly even aid in the regeneration of damaged neurons. This research opens the door to cholesterol drugs as a possible new avenue for MS treatment (Lidia Gardner, PhD, abstract 404.01, see attached summary).

Other recent findings discussed show that:

- A type of immune system cell has been found to directly target and damage nerve cell axons, a hallmark of MS. This may reveal a target for new therapies (Brian Sauer, PhD, presentation 404.06, see attached speaker summary).
- While no treatments to rebuild cells damaged by MS currently exist, scientists have found that when exosomes — tiny, naturally occurring "nanovesicles" — are produced by dendritic cells and applied to the brain, they can deliver a mixture of proteins and RNAs that promote regeneration of protective myelin sheaths and guard against MS symptoms (Richard Kraig, MD, PhD, presentation 812.02, see attached speaker summary).

"The findings shown today represent real promise for the millions suffering from MS," said press conference moderator Jeffrey Rothstein of Johns Hopkins University and an expert in neurodegenerative diseases. "These studies are breakthroughs in understanding and treating a disease that remains uncured, difficult to diagnose, and for which it is very difficult to prevent progression."

This research was supported by national funding agencies such as the National Institutes of Health, as well as private and philanthropic organizations. Find more information on MS at *BrainFacts.org*.

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Abstract 404.09 Summary

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Brain Cells in a Dish May Solve a Mystery of Inflammatory Diseases

Research tackles how antibodies cross the protective barrier of the brain to produce symptoms of neuromyelitis optica

A new model allows researchers to look into how antibodies shuttle across the blood-brain barrier (BBB), a network of blood vessels that prevents 95 percent of chemicals from reaching (and damaging) the brain from the bloodstream. This research aims to achieve a better understanding of the often debilitating disease neuromyelitis optica (NMO). The results were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Until now, we've been unsure how these antibodies were getting past the central nervous system's security system to mount their attack," said lead author Yukio Takeshita, MD, PhD, of the Cleveland Clinic Foundation Lerner Research Institute. "This research opens the door to understanding in detail how symptoms arise in this particular disorder, and it may give us new targets for treatment."

NMO is a condition resembling multiple sclerosis in which the human immune system attacks the central nervous system. Antibodies in the blood stream somehow penetrate the BBB and cause inflammation in the brain, spinal cord, and optic nerves, resulting in painful and debilitating symptoms.

To examine how this occurs, the researchers created a BBB in a dish using brain and blood vessel (endothelial) cells grown outside the body. The research team then examined the behavior of NMO antibodies in their model and found that the antibodies were able to cross the barrier, possibly using other proteins to help travel. The researchers plan to identify these other proteins to better understand the underlying mechanisms and best treatments for this rare disease.

Research was supported with funds from The Guthy-Jackson Charitable Foundation and the National Institutes for Health.

Scientific Presentation: Monday, Nov. 11, 1–3:30 p.m., Room 24A

404, Construction of In vitro Neuromyelitis optica (NMO) models and effect of NMO-IgG at the Blood-Brain Barrier

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TECHNICAL ABSTRACT: Background: Neuromyelitis optica (NMO), an inflammatory disease of the human central nervous system, is characterized by antibodies to the astrocyte water channel aquaporin 4 (AQP4). It remains uncertain whether NMO-IgG affects endothelial cells at the blood brain barrier (BBB) and unknown how NMO-IgG accesses AQP4 on astrocytes. Current in vitro BBB models for NMO that utilize primary endothelial and astrocyte cell cultures are restricted by limited availability of human tissue and loss of AQP4 expression on human astrocytes in vitro. We established static and flow based dynamic BBB models incorporating (1) a conditionally immortalized human brain microvascular endothelial cell (EC) line and (2) a conditionally immortalized human astrocyte cell line with (A4) or without (A) expression of AQP4. Co-cultures including either A4 or A enhanced leukocyte transmigration across EC.
Aim: We examined effects of NMO-IgG on endothelial cells at 'vascular' side and on astrocytes at 'intrathecal' side of the BBB.
Method: NMO-IgG was pooled from therapeutic plasma exchange and control IgG from a healthy human plasma pool; IgG was purified by protein G affinity. In the first experiment EC/A4 or EC/A were co-cultured on a membrane, activated with TNF-α and IFN-γ, and the EC exposed to NMO-IgG. In the second condition, EC/A4 were co-cultured on a membrane, and EC or A4 exposed to NMO-IgG after activation. We measured the BBB permeability, IgG accumulation, the expression of ICAM-1 (adhesion molecule) and Claudin-5 (tight junction molecule) on EC by qRT-PCR; and leukocyte transmigration across the BBB under flow.
Results: The vascular application of NMO-IgG elevated EC expression of ICAM-1 in EC/A4 and EC/A co-cultures. The vascular application of NMO-IgG increased permeability and IgG transport in EC/A4 and EC/A. Intrathecal application of NMO-IgG decreased EC Claudin-5 expression, increased permeability in EC/A4, and increased leukocyte transmigration across BBB.
Conclusion: These results indicate that 1) that there are NMO-IgG factors which directly modulate EC function in EC-astrocyte co-cultures regardless of AQP4 expression by astrocytes; and 2) intrathecal NMO-IgG signals to astrocytes to decrease EC tight junctions and increase leukocyte migration.

Abstract 404.11 Summary

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A Potential Early Detection Method for Multiple Sclerosis

New indicator could pinpoint MS lesions before symptoms arise

A protein involved in blood clotting may be a new indicator to help detect multiple sclerosis (MS) lesions before symptoms arise. The presence of the clotting protein, thrombin, signals an early stage of the disease when the blood-brain barrier is breached and the brain's immune response is set into motion. The research was presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Our research shows this indicator is a promising approach for detecting MS-like lesions early even before major symptoms appear," said senior author Katerina Akassoglou, PhD, of the Gladstone Institutes and the University of California, San Francisco. "Such sensitive indicators could act as red flags that signal neuroinflammatory changes in the brain not only in MS, but also in other diseases such as Alzheimer's."

MS is a debilitating disorder that can be intermittent or progressive, and causes numbness, fatigue, difficulty walking, paralysis, and loss of vision in 2 million people worldwide. MS arises when the body's immune system attacks its own myelin sheaths, the protective coverings that surround neurons and allow signals to move from one cell to the next.

The researchers found that thrombin, usually a beneficial protein involved in blood clotting, builds up in the central nervous system as MS progresses. Thrombin enters in the brain together with fibrinogen, another clotting protein when the protective barrier between the blood and brain becomes leaky. Thrombin converts the fibrinogen to fibrin which activates brain's immune cells that break down the protective myelin sheath that surrounds neurons in the central nervous system.

Because thrombin levels increase as the disease progresses, the researchers conclude that it could be used as an early detector of the disease. In their studies, the researchers used a mouse model and demonstrated that MS symptoms increased as thrombin levels rose. Early detection of MS could result in more successful treatment of the disease.

Research was supported with funds from the National Multiple Sclerosis Society, the Nancy Davis Foundation for Multiple Sclerosis, and the National Institutes of Health.

Scientific Presentation: Monday, Nov. 11, 1–3:30 p.m., Room 24A

404.11, Early detection of thrombin activity in neuroinflammatory disease

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TECHNICAL ABSTRACT: The development of neuroinflammatory disease has been linked with multiple components of the coagulation system. In addition to proteomics-based association studies, genetic as well as pharmacological interventions at the level of hemostatic factors have been shown to result in significant protection from paralysis in animal models of multiple sclerosis (MS). However, the temporal and spatial regulation of coagulation activity in neuroinflammatory lesions remains largely unknown. We employed a fluorescent probe that detects thrombin protease activity to image the precise activity pattern of this key coagulation protease within the CNS as a function of time in mice challenged with experimental autoimmune encephalomyelitis (EAE). In vivo thrombin activity was detected in the spinal cord of mice before the onset of clinical signs and was increased at the peak of EAE, but not detected in healthy controls. Thrombin activity was prominent in neuroinflammatory lesions and correlated with fibrin deposition, microglial activation, and demyelination. Notably, thrombin activity also correlated with signs of axonal damage and was a strong indicator of clinical severity. Detection of thrombin activity was significantly reduced in mice with genetically reduced or eliminated prothrombin, confirming the specificity of the thrombin-sensitive peptide. Identification of thrombin-activity as a reliable molecular marker of neuroinflammation could be exploited as a novel direction for the development of sensitive molecular probes for preclinical detection of MS lesions.

Abstract 404.01 Summary

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Low Levels of Cholesterol Transport Protein Implicated in Multiple Sclerosis

Human and animal research suggests protein in HDL protects against brain cell damage and inflammation

A protein that is the most abundant component of HDL cholesterol (or "good" cholesterol) may protect the body from inflammation and the effects of multiple sclerosis (MS), according to new human and animal research. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Considering that the levels of this protein can be modified by currently used cholesterol medications, better understanding of this protein could lead to new treatments for multiple sclerosis," said lead author Lidia A. Gardner, PhD, of the Memphis VA Medical Center and the University of Tennessee Health Science Center.

The protein, called apolipoprotein A1 (ApoA1), is known to protect the body from inflammation, a symptom of MS that results from the body's immune system attacking cells of the central nervous system.

The researchers investigated the levels of Apo A1 in both humans and mouse models. Levels of the protein in the blood of human patients with MS were between 25 percent and 75 percent lower than in healthy individuals. Importantly, the protein was lowest in progressive MS, the most severe form of the disease. Mouse models confirmed that mice with low Apo A1 exhibited worse symptoms of the mouse model of MS, experimental autoimmune encephalomyelitis.

The results suggest that ApoA1 plays a protective role against inflammation and the development of MS symptoms. Manipulation of the levels of this protein in MS patients may present a new treatment opportunity.

Research was supported with funds from the National Multiple Sclerosis Society.

Scientific Presentation: Monday, Nov. 11, 1–3:30 p.m., Room 24A

404.01, A potential role for apolipoprotein A1 in multiple sclerosis
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TECHNICAL ABSTRACT: Apolipoproteins play important roles in cholesterol transfer and lipid metabolism in the central nervous system. Among six major classes of Apolipoproteins (A, B, C, D, E and H) only apolipoprotein E (ApoE) has been studied extensively in neurobiology. The importance of Apolipoprotein A1 (ApoA1) has been identified in atherosclerosis, cardiovascular and cognitive diseases, however its function in multiple sclerosis (MS) has not been fully investigated. ApoA1 is the most abundant component of high-density lipoprotein (HDL). HDL-associated ApoA1 may play a role in neuronal regeneration by acting as a constitutive anti-inflammatory factor.

We measured ApoA1 expression in serum and plasma of MS patients and healthy controls. Samples were depleted of albumin and immunoglobulins, subjected to isoelectric focusing (IEF), separated on 1-D SDS-PAGE gels and probed with anti-ApoA1 antibodies. MS patients with progressive disease exhibited decreased ApoA1 expression in their plasma. The data was confirmed with 2-D PAGE analysis. The intensity of each spot from the control gel was quantified and compared to identical spots on gels from the three types of MS compared to age-matched healthy controls. Differential ApoA1 expression between controls and MS patients was evident on 2-D gels (pI 4.9-5.5 and molecular weight between 22873-23454Da). Remarkably, all MS patients had less ApoA1 than controls. ApoA1 was reduced by approximately 25%, 50%, and 75% in relapsing remitting MS, secondary progressive MS and primary progressive MS samples respectively compared to the control. Importantly, ELISA assays confirmed this trend.

Further, we investigated the role of ApoA1 in a mouse model of MS. ApoA1 deficient female mice (C57Bl/6-Tg(ApoA1)1Rub/J) demonstrated higher incidence and severity of the experimental autoimmune encephalomyelitis (EAE) in comparison to the wild type control mice (C57Bl/6J). Demyelination was scored on histological brain and spinal cord sections.

In summary, we show that ApoA1 plays an important role in MS. Our study highlights the protective role of ApoA1 in both acute and chronic inflammation. Further investigation into the mechanisms of ApoA1 formation and disease-associated loss might lead to novel therapies in MS.

Speaker Summary (404.06)

Speaker: Brian Sauer (412) 916-3662
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Axons are MHC Class I-Dependent Targets of Antigen-Specific CD8+ T Cells and are Injured Through a Granzyme B-Dependent Mechanism (404)

Nanosymposium: *Multiple Sclerosis: Molecular and Cellular Mechanisms of Demyelination and Neurodegeneration*
Monday, Nov. 11, 1–3:30 p.m., Room 24A

Multiple sclerosis (MS) is the leading cause of non-traumatic neurological disability for young people in Western countries. It is a complex, heterogeneous disease in which the immune system damages the nervous system. The mechanisms by which specific disease-associated immune cell types recognize and damage nervous system targets are poorly understood. Our research identifies a novel mechanism by which an immune system cell type, CD8+ T cells, may directly damage the nervous system and lead to neurological dysfunction.

CD8+ T cells are an immune cell type normally involved in the control of viral infection. Previous studies from our laboratory and the laboratories of others indicate that in the context of MS, CD8+ T cells may abnormally target and damage axons, the nervous system cables that transmit information between the brain and body. Axon damage is a central cause of irreversible neurological disability in MS patients.

In this study we delineate a specific mechanism by which CD8+ T cells target and directly injure axons. We show that when axons are exposed to interferon gamma, a molecular danger signal present at sites of nervous system injury, they begin to express major histocompatibility class I molecules which are the molecular flags necessary for CD8+ T cell target recognition. We demonstrate that CD8+ T cells directly recognize, bind, and injure axons. Finally, we reveal that CD8+ T cell-mediated axon injury is dependent upon granzyme B, an effector molecule used by CD8+ T cells to initiate target cell injury.

Our study offers a novel mechanism of immune-mediated nervous system injury which may contribute to the neurological deficits that MS patients experience. Our work provides a mechanistic rationale for the continued use of currently available anti-CD8+ T cell therapies, such as natalizumab, in the treatment of MS. Furthermore, our work presents a novel therapeutic target molecule, granzyme B, for which new, rationally-designed inhibitors may prove effective in protecting axons and preventing neurological deficits in MS patients.

Research was supported with funds from a gift from Donald and Frances Herdrich, National Multiple Sclerosis Society, and the National Institutes of Health.

Speaker Summary (812.02)

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IFNγ Stimulated Dendritic Cell Exosomes As A Therapeutic For Remyelination

Wednesday, Nov. 13, 2–3 p.m., Halls B–H

Currently, no multiple sclerosis (MS) treatments promote remyelination. Our research shows that dendritic cells, a type of immune cell present in blood, can be cultured from bone marrow and stimulated to release small particles called exosomes. When administered to the brain, these exosomes significantly increase myelination and improve remyelination following a demyelinating injury, like that caused by MS.

MS is an inflammatory disease involving oligodendrocyte loss, demyelination, and failure to remyelinate damaged brain areas. Oligodendrocytes in the central nervous system produce myelin, the insulation surrounding axons, which is necessary for neuronal signaling. Damage to oligodendrocytes and demyelination — loss of this insulation — can lead to severe neurological disability. Remyelination is a spontaneously occurring repair process mediated by recruitment of oligodendrocyte precursor cells to damaged areas. Their subsequent differentiation into mature oligodendrocytes is capable of replacing lost myelin. Initially, MS patients follow a relapsing-remitting disease course, characterized by periods of partial recovery associated with incomplete remyelination. However, over time this ability to repair declines and patients develop a secondary-progressive, steadily worsening disease course. With over 400,000 people currently suffering from MS in the United States, it is a significant and devastating healthcare burden.

Recent evidence shows that linking the circulatory system of an aged animal to a young one improves the aged partner's recovery from a demyelinating injury. We have previously shown that this effect likely involves production of exosomes that impact oligodendrocyte development and production of myelin. Exosomes are exported by many cell types and have the potential for targeting specific cells to deliver their cargo of proteins, mRNA, and importantly, microRNA. MicroRNAs are small non-coding RNA molecules that are increasingly recognized for their role in regulating gene expression.

Here, we found that interferon gamma (IFNγ)-stimulated, dendritic cell-derived exosomes increase baseline myelination. In addition, they improve recovery from MS modeled by inducing demyelination.

Furthermore, when nasally administered to whole animals, these exosomes stimulate an increase in brain myelin. All this suggests that these nutritive exosomes can be crafted into a novel therapy for MS.

We are interested in better understanding why and how these exosomes improve myelination. Studies to determine the content of IFNγ-stimulated, dendritic cell-derived exosomes are ongoing in our laboratory. To date, we have found high levels of a microRNA species known to facilitate oligodendrocyte precursor differentiation into mature cells that are capable of producing myelin. Additional microRNA species involved in increasing oxidative tolerance are also present at high levels in stimulated exosomes. These microRNAs may be responsible for the reduced oxidative stress and increased antioxidant levels observed in the exosome-treated brain. We believe reduction of oxidative stress also plays a role in remyelination, since antioxidants protect oligodendrocytes and enhance their production of myelin. Additionally, IFNγ-stimulated dendritic cell-derived exosomes are preferentially taken up by oligodendrocytes, suggesting that they directly stimulate these cells.

This proof-of-principal work paves the way for further development of dendritic cell-derived exosomes as a remyelination therapy. Future directions include re-engineering exosomes to contain only desired microRNAs and optimizing targeting methods to direct them to specific cells.

Treatment options for MS are limited and consist of immunosuppressors or agents to prevent immune infiltration of the brain. These therapies potentially have harmful side effects, and do little to promote myelin repair. Instead, we suggest using exosomes, naturally occurring vesicles that exert influence through delivery of mRNAs, microRNAs and proteins. They are a non-toxic, ideal delivery platform that can easily cross the blood brain barrier, and have great potential as an adjunct approach to increasing remyelination post-injury. Thus, our results show great potential for use of these exosomes as a potential therapeutic to promote remyelination in MS.

Research was supported with funds from the National Institutes of Health.