

COMPOSITIONS AND METHODS USING RECOMBINANT MHC MOLECULES FOR THE TREATMENT OF STROKE

CROSS REFERENCES TO RELATED APPLICATIONS

This is a continuation of U.S. application Ser. No. 13/924, 275, filed Jun. 21, 2013, now U.S. Pat. No. 9,050,279, which is a continuation of U.S. application Ser. No. 12/661,038, filed Mar. 8, 2010, issued as U.S. Pat. No. 8,491,913 on Jul. 23, 2013, which claims the benefit of U.S. Provisional patent application 61/209,428, filed Mar. 7, 2009, both of which are incorporated herein in their entirety by reference.

STATEMENT REGARDING GOVERNMENT SPONSORED RESEARCH

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TECHNICAL FIELD

The present invention relates to the use of recombinant polypeptides comprising major histocompatibility complex (MHC) molecular domains that mediate antigen binding and T-cell receptor (TCR) recognition in the prevention and treatment of brain damage caused by stroke.

BACKGROUND OF THE INVENTION

Stroke is a cerebrovascular event which occurs when the normal blood flow to the brain is disrupted, and the brain receives too little blood. Approximately 15 million people worldwide suffer a stroke each year.

There are two forms of stroke: ischemic stroke, caused by a blood clot that blocks or prevents the flow of blood, and hemorrhagic stroke, caused by bleeding into the brain. Ischemic stroke is responsible for about one third of all deaths in industrialized countries and is the major cause of serious, long-term disability in adults over the age of 45.

Ischemic stroke results from insufficient cerebral circulation of blood caused by obstruction of the inflow of blood. The most common cause is narrowing of the arteries in the neck or head such as through atherosclerosis. Blood clots may form that can block the artery where they are formed (thrombosis), or can dislodge and become trapped in arteries closer to the brain (embolism). Another cause of stroke is blood clots dislodged from the heart. Blood clots can occur as a result of irregular heartbeat (for example, atrial fibrillation), heart attack, or abnormalities of the heart valves. Additional causes of ischemic stroke include the use of street drugs, traumatic injury to the blood vessels of the neck, or disorders of blood clotting.

During acute ischemic stroke, the arterial occlusion results in an infarcted core of brain tissue, where cerebral blood flow is reduced to below 10% to 25%. The infarcted core suffers irreversible damage due to significant cell death. The ischemic penumbra, ischemic but still viable tissue, suffers a delayed and less severe infarct.

Administration of thrombolytic agents, such as tissue plasminogen activator (tPA), which dissolve blood clots and

thus restore blood flow to affected regions, have limited applicability. In particular, administration of tPA is only effective if given within three hours from the time of stroke onset. There is therefore an unmet need for other methods for the prevention and treatment of brain damage due to stroke.

SUMMARY OF EXEMPLARY EMBODIMENTS

Innate and adaptive immunity play an important role for the outcome after focal cerebral ischemia (stroke). After stroke, leukocytes home toward the lesion, and brain parenchymal cells (microglia, astrocytes, endothelia, even neurons) transform to an inflammatory phenotype. Macrophages and microglia produce a host of trophic cytokines when activated, and macrophages or T-cells exposed to certain central nervous system (CNS)-specific antigens *ex vivo* partake in tissue repair and recovery after nerve transection and spinal cord injury. However, these benefits may be at least partially offset not only by bystander toxicity of inflammation but also by scar formation, which in peripheral tissues is key to wound closure, but in the brain is a major impediment of regeneration and plasticity. (Dirnagl, Ulrich MD; Klehmet, Juliane MD; Braun, Johann S. MD; Harms, Hendrik MD; Meisel, Christian MD; Ziemssen, Tjalf MD; Prass, Konstantin MD; Meisel, Andreas MD *Stroke*. 38 (2, Part 2) Supplement 1:770-773, Feb. 2007)

The initiation of an immune response against a specific antigen in mammals is brought about by the presentation of that antigen to T-cells by a major histocompatibility (MHC) complex. MHC complexes are located on the surface of antigen presenting cells (APCs); the 3-dimensional structure of MHCs includes a groove or cleft into which the presented antigen fits. When an appropriate receptor on a T-cell interacts with the MHC/antigen complex on an APC in the presence of necessary co-stimulatory signals, the T-cell is stimulated, triggering various aspects of the well characterized cascade of immune system activation events, including induction of cytotoxic T-cell function, induction of B-cell function and stimulation of cytokine production.

This invention is founded on the discovery that mammalian MHC function, including but not limited to, human MHC function, can be mimicked through the use of recombinant polypeptides that include only those domains of MHC molecules that define the antigen binding cleft. The molecules provided herein may be used in clinical and laboratory applications to detect, quantify and purify antigen-specific T-cells, induce anergy in T-cells, or to induce T suppressor cells, as well as to stimulate T-cells, and to treat conditions mediated by antigen-specific T-cells, including, but not limited to, inflammation, autoimmune and neurodegenerative diseases.

It is shown herein that antigen-specific T-cell binding can be accomplished with a monomeric molecule comprising, in the case of human class II MHC molecules, only the $\alpha 1$ and $\beta 1$ domains in covalent linkage (and in some examples in association with an antigenic determinant). For convenience, such MHC class II polypeptides are hereinafter referred to as " $\beta 1\alpha 1$ ". Equivalent molecules derived from human MHC class I molecules are also provided herein. Such molecules comprise the $\alpha 1$ and $\alpha 2$ domains of class I molecules in covalent linkage and in association with an antigenic determinant. Such MHC class I polypeptides are referred to as " $\alpha 1\alpha 2$ ". These two domain molecules may be readily produced by recombinant expression in prokaryotic or eukaryotic cells, and readily purified in large quantities. Moreover, these molecules may easily be loaded with any