

**METHOD FOR TREATING INFLAMMATION  
BY LYMPHOCYTE DEPLETION OR  
SEQUESTERING**

**CROSS-REFERENCE TO RELATED  
APPLICATIONS**

**[0001]** This application claims priority to U.S. Provisional application 61/301,383, filed Feb. 4, 2010.

**BACKGROUND**

**[0002]** This invention relates to a method for treating and preventing inflammation. More specifically, the invention is related to a method for treating or preventing ischemia injuries by depleting or sequestering lymphocytes prior to reperfusion.

**[0003]** Inflammation is part of the complex biological response to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by mammal to remove the injurious stimuli and to initiate the healing process. Inflammation is typically caused by an infection or an injury. Without inflammation, wounds and infections would never heal. However, chronic inflammation can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis.

**[0004]** Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli, and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates, and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

**[0005]** Mammalian immune system has evolved to respond to localized injury and infection. Such response is necessary for injury recovery and often has three main focuses: maintaining homeostasis, protection against microorganism invasion, and initiation of tissue repair. However, severe trauma and hemorrhagic shock induce a systemic activation of the immune system, which provokes the simultaneous activation of potent molecular and cellular components of innate immunity. These components include complements, cytokines, chemokines, neutrophils, and monocytes (21). Inappropriate inflammatory response often leads to secondary injuries to the host, which manifests clinically as systemic inflammatory response syndrome, acute respiratory distress syndrome, or even multiple-organ dysfunction syndrome. All of which are common posttraumatic complications found in intensive care units (1). Therefore, it is important to study therapeutic modulation of the immunologic response to injury in order to reduce trauma-associated morbidity and mortality.

**[0006]** Ischemia is a restriction in blood supply, generally due to factors in the blood vessels, with resultant damage or dysfunction of tissue. After an injury, ischemic reperfusion injury refers to tissue damage caused when blood supply returns to the tissue after a period of ischemia. The absence of oxygen and nutrients from blood creates a condition in which the restoration of circulation results in inflammation and oxidative damage through the induction of oxidative stress rather

than restoration of normal function. The damage of reperfusion injury is due in part to the inflammatory response of damaged tissues. White blood cells, carried to the area by the newly returning blood, release a host of inflammatory factors such as interleukins as well as free radicals in response to tissue damage. The restored blood flow reintroduces oxygen within cells that damages cellular proteins, DNA, and the plasma membrane. Damage to the cell's membrane may in turn cause the release of more free radicals. Such reactive species may also act indirectly in redox signaling to turn on apoptosis. Leukocytes may also build up in small capillaries, obstructing them and leading to more ischemia.

**[0007]** Repeated bouts of ischemia and reperfusion injury also are thought to be a factor leading to the formation and failure to heal of chronic wounds such as pressure sores and diabetic foot ulcers (3). Continuous pressure limits blood supply and causes ischemia, and the inflammation occurs during reperfusion. As this process is repeated, it eventually damages tissue enough to cause a wound (3).

**[0008]** The majority of research on clinical therapeutics for ischemia reperfusion injury has been focused on monocyte and neutrophil adhesion blockade. However, despite promising preclinical data, results of phase 2 and 3 trials of neutrophil anti-adhesion therapy in ischemia-reperfusion disorders have been disappointing (2) In two clinical trials testing humanized CD18 monoclonal antibodies in the setting of traumatic injury, mortality and other primary end points were not significantly affected. (3, 4) These failures are likely the result of the redundancy of adhesion pathways, but also suggest that the neutrophil is not central in the innate immune response to ischemia-reperfusion injury (IRI).

**[0009]** Lymphocytes are major components of adaptive immunity and influence immune dysfunction following severe injury (5-7). Lymphocyte activation is classically described in the presence of foreign antigen bound to self-MHC molecules together with antigen-presenting cell costimulation signals. However, there is emerging evidence that lymphocytes are rapidly activated in an alloantigen-independent manner in the setting of ischemia reperfusion injury (IRI)(46). Danger signals released in IRI may activate lymphocytes and lead to innate function prior to classical adaptive function. (22) Furthermore, hypoxia may be sufficient for lymphocyte activation, as CD4+ T cells have been shown to increase adhesion to endothelial monolayers following anoxia modulation. (23, 24) Compelling pre-clinical investigation has established the innate role of lymphocytes in renal (8), gut (9), and liver (10, 11) IRI. In a renal IRI model, genetically engineered mice deficient in both CD4+ and CD8+ lymphocytes had substantially less kidney dysfunction after renal ischemia than did wild-type control mice (8). Interestingly, mice deficient in CD4+ and CD8+ lymphocytes demonstrated less tissue neutrophil infiltration, suggesting that lymphocytes orchestrate cell-mediated innate responses to ischemia. This was associated with a decrease in neutrophil infiltration, which was restored with adoptive transfer of wild type T cells into the athymic (nu/nu) mice.

**[0010]** Therefore, immunomodulation of lymphocytes may offer a novel approach to attenuate detrimental immune responses to inflammation. For example, depletion or sequestering lymphocytes may ameliorate secondary morbidity and mortality associated with severe hemorrhage.

**[0011]** Natural killer (NK) cells are part of the innate immune system. They are thought to play an important role in the development of such syndromes by interplay with other