

immune cell types and subsequent activation of the inflammatory cascade. In a reported study, NK cells were depleted by administration of antimouse asialo-GM1 antibody in a murine polytrauma model consisting of femur fracture, hemorrhagic shock, and subsequent sepsis. Mortality and immune parameters such as cytokine expression in lung and liver, lymphocyte phenotyping, lymphocyte apoptosis, and organ pathology were determined 96 hours after sepsis induction. NK cell depletion resulted in 50% mortality reduction. Furthermore, reductions in the inflammatory response were observed, represented by IL-6 expression in liver, and a decrease in infiltrating neutrophils in the liver and lung. In addition, lymphocyte apoptosis in spleen was decreased by depletion of NK cells. Taken together, these data demonstrate that NK cells contribute to the pathogenetic pathways in a murine polytrauma model (43). T cell and B cell lymphocytes have not been studied as to their roles in preventing or reducing secondary immunologic injury in surgical and trauma patients following hemorrhagic shock.

**[0012]** Adhesions are fibrous tissues that form between intra-abdominal organs following abdominal and pelvic surgery (56). They are common, occurring following 68 to 100% of all such operations. They result from the peritoneal response to mechanical injury, intra-abdominal ischemia, and the presence of foreign material (e.g. synthetic mesh used for ventral hernia repair) within the abdomen (57). The clinical implications of this wound healing process are profound; and common sequelae including intestinal obstruction, chronic abdominal pain, infertility, pelvic pain, and increased complications during subsequent surgical procedures. Indeed, adhesions are the most common cause of intestinal obstruction in the Western world, accounting for 1% of all hospital admissions and resulting in morbidity costing an estimated \$1-3 billion annually in the United States alone (58-60).

**[0013]** Patients that have had prior bowel obstruction requiring surgical correction are at increased risk for subsequent adhesive obstructions (60, 61). Specifically, the recurrence rate for patients requiring a single exploration for adhesive small bowel obstruction is 18-50% within 10 years. The relative risk of the recurrent obstruction increases with increased number of prior episodes, reaching 81% for patients with four or more admissions for adhesive obstruction. Various types of adhesions (e.g. matted and pelvic) can greatly increase the risk of recurrence and 58% of recurrences occur within the first 5 years following therapy (61).

**[0014]** There is currently no effective preventative therapy for adhesions. Surgical adhesiolysis is therapeutic but results in recurrent adhesive disease and markedly increases the risk of subsequent adhesion related complications. Recent small animal models have demonstrated that T lymphocytes are required for the formation of the abdominal adhesions and their sequelae (62). Clinically, it has been empirically observed that patients undergoing extensive abdominal procedures for pancreatic transplantation do not form adhesions, particularly following therapeutic T cell depleting antirejection therapy.

**[0015]** Many attempts have been made in limiting adhesion formation, one of which was placing mechanical barriers between the intra-abdominal organs and the damaged peritoneum. This procedure has been demonstrated to have limited efficacy particularly in preventing inter-loop and pelvic adhesions (63, 64). The inability of barrier agents to eliminate surgical adhesions stems from failure to arrest the underlying problem: that of dysregulated fibrogenesis during wound

healing. Specifically they do not alter the activity of T lymphocytes, the key regulator of adhesion formation.

**[0016]** Control of pathological T cell responses has been extensively studied and reduced to routine practice in the field of solid organ transplantation. T cell depletion and T cell specific immunosuppression is routinely used in patients undergoing major abdominal operations such as pancreas and liver transplantation. Specific T cell depleting agents included polyclonal rabbit anti-thymocyte globulin (Thymoglobulin, Genzyme, Cambridge, Mass.) which has antibodies directed against many antigens on lymphocytes. Treatment with Thymoglobulin has been shown to result in a profound reduction in the number of T lymphocytes at the time of transplantation, therefore decreasing the frequency of alloreactive cells at a time of increased immune susceptibility. In both kidney and pancreas transplant recipients this has been achieved without an increase in postoperative infections or impaired wound healing relative to transplant patients without T cell depletion, essential elements for the broader application of the prevention of surgical adhesions (65, 66). Therefore, depletion or sequestration of lymphocytes at the time of operation, combined with modest inhibition of Th1 type T cell activation during the perioperative period, may prevent adhesion formation, and therefore eliminate subsequent complications of recurrent adhesive disease.

**[0017]** Antithymocyte globulin (ATG) is a potent lymphocyte depleting agent that is used clinically in induction and anti-rejection therapy for solid organ transplantation, treatment of graft versus host disease, and selected autoimmune diseases. (12, 13) The immunosuppressive activity of anti-lymphocyte sera was first noted at the turn of the century when Metchnikoff described its anti-inflammatory properties. (14) The primary mechanism of immunosuppression involves massive peripheral and central lymphocyte depletion primarily by complement and Fas/Fas-L mediated apoptosis pathways. [15-17] In addition, ATG results in antibody inhibition of nondepleted T cells and functional alteration of several membrane receptors (TCR/CD3) and coreceptors (CD2, CD4, and CD8). [15, 18] Given the innate role of the lymphocyte in IRI, ATG may effectively modulate the post-traumatic inflammatory response.

**[0018]** While ATG is primarily used clinically to suppress adaptive lymphocyte responses, pre-clinical studies have demonstrated the utility of ATG in abrogating innate immune responses. In a non-human primate model, polyclonal ATG conferred a protective effect on reperfusion injury following limb ischemia. (27) In clinical kidney and liver transplantation, ATG (Thymoglobulin) has been shown to reduce graft dysfunction associated with IRI. (28) Specifically, ATG has been shown to reduce delayed graft function following transplantation, an event that is thought to be related to IRI.

**[0019]** FTY720 (NOVARTIS®) is an immunomodulator currently in phase III clinical trials that sequesters lymphocytes to secondary lymphoid organs and reduces circulatory lymphocytes by targeting receptors for sphingosine 1-phosphate. [49] FTY720 has been shown to ameliorate IRI in various animal models and lymphocyte modulation has been mechanistically implicated. [50] In a rat renal transplant model, FTY720 treatment improved renal function and reduced intragraft neutrophil infiltration despite no change in adhesion molecules expression, highlighting the central role of the lymphocyte in IRI. [51]

**[0020]** The innate role of lymphocytes offers the potential for preventing or treating inflammation, especially those