Altered T Cell Cytokine Production Following Mechanical Trauma*†

KATHLEEN L. MEERT, M.D.,
JOHN P. OFENSTEIN, Ph.D.,
and ASHOK P. SARNAIK, M.D.

Department of Pediatrics,
Children's Hospital of Michigan,
Wayne State University School of Medicine,
Detroit, MI 48201

ABSTRACT

Background: Cell mediated immunity is suppressed following traumatic injury. The objective is to determine whether there is a shift from T helper type 1 (TH1) to TH2 cell cytokine production following mechanical trauma in a rat model.

Methods: Male Sprague-Dawley rats were anesthetized and subjected to bilateral femur fractures or sham injury. Spleens were removed 3 days later. T cell proliferation and cytokine production were stimulated by culturing spleen cells with the T cell mitogen concanavalin A (con A). Interleukin-2 (IL-2), interferon-γ (IFN-γ), IL-10 and IL-4 concentrations were measured in spleen cell supernatants using enzyme linked immunosorbent assays.

Results: Con A-induced spleen cell proliferation was decreased in traumatized rats compared to controls (p < 0.05). Spleen cell supernatant concentrations of the TH1 cytokines IL-2 and IFN-γ were decreased in the trauma group (p < 0.05). Supernatant concentrations of the TH2 cytokine IL-10 were also decreased in traumatized rats (p < 0.01). The IL-4 concentrations were below the detection limit (<15 pg/mL) in all cell supernatants.

Conclusions: Mechanical tissue injury leads to generalized suppression of T helper cell cytokine production rather than a shift from TH1 to TH2 cell activity. Post-trauma cellular immunosuppression is not mediated via excess IL-10 production by TH2 cells.

Introduction

Cell mediated immunity is suppressed following trauma, hemorrhage and burns.1,2,3,4 Several factors may contribute to the development of cellular immunosuppression including the type of injury, species and genetic background of the organism, nutritional status, clinical interventions such as blood transfusions, anesthetics and operative procedures, as well as the presence of secondary infection. The mechanism of post-trauma immunosuppression has been an area of intense investigation.1,2,3,4,5,6,7,8,9,10,11 Understanding the mechanism may allow for the development of effective immunomodulation, potentially improving outcome for the traumatized patient.
The T cells are involved in all aspects of cell mediated immunity and exert their effects by direct cell to cell contact or via production of soluble mediators. The T helper type 1 (TH1) cells up-regulate cellular immunity through the production of interleukin-2 (IL-2) and interferon-γ (IFN-γ).12 Conversely, TH2 cells down-regulate cellular immunity via production of the immunosuppressive cytokine IL-10 but up-regulate humoral immunity via IL-4. Recent studies in mice have suggested that hemorrhage, with and without associated tissue trauma, leads to a change in the relative proportions of T helper cell subpopulations within the spleen. Increased production of IL-10 following hemorrhage has been associated with depressed T cell proliferation. Whether or not mechanical and/or thermal tissue injury leads to alterations in T helper cell subpopulations remains controversial. Decreased spleen cell proliferation and IL-2 production developing 3 days after closed bilateral femur fractures in rats has been previously demonstrated. Our objective is to determine whether mechanical trauma in the rat model leads to a shift within the T helper cell population from TH1 and TH2 cells, identified by their respective cytokine profiles.

Materials and Methods

ANIMALS

Male Sprague-Dawley rats* weighing 250 to 310 g were used in all experiments. All procedures were carried out in accordance with the guidelines set forth by the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. The study was approved by the Animal Investigation Committee of Wayne State University.

FRACTURE MODEL

Fifteen rats were divided into two groups: control (N = 7) and trauma (N = 8). Anesthesia was induced in all rats by intraperitoneal injection of xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (87 mg/kg). The trauma group received bilateral femur fractures by releasing the arm of a common spring-loaded rat trap onto each femur. This produced uniform closed fractures and soft tissue damage. All rats were returned to individual cages and provided free access to food and water. Food was placed on cage floors and water provided through long straws to allow accessibility without standing. The handling of control rats was identical to that of the trauma group, except for subjection to femur fractures. Three days following femur fracture or sham injury, rats were again anesthetized. Spleens were aseptically removed from animals and processed.

PREPARATION OF SPLEEN CELLS

Spleens were individually placed into sterile tubes containing tissue culture medium† supplemented with 10 percent fetal calf serum, 2 mM L-glutamine, 5 x 10⁻⁵ M 2-mercaptoethanol, 25 mM Hepes buffer, and 50 μg/mL gentamicin. Cell suspensions were made by passing each spleen through a wire mesh and removing debris by gravity sedimentation. Red blood cells were lysed using buffered ammonium chloride. The remaining cells were washed twice with tissue culture medium by repeated centrifugation at 24°C for 5 minutes at 300 x g. Viability was tested using trypan blue exclusion. All cell preparations had greater than 95 percent viability.

SPLICEEN CELL PROLIFERATION

Spleen cells were cultured at a concentration of 5 x 10⁵ cells/mL in 96 well microtiter plates with and without concanavalin A (con A, 1 μg/mL).‡ The cells were incubated at 37°C

* Charles River Laboratories, Portage, MI.
† RPMI 1640, JRH Biosciences, Lenexa, KS.
‡ Sigma Chemical Co., St. Louis, MO.
in a 5 percent CO₂ humidified environment for 72 hours. Cells were pulsed with 1 μCi of tritiated thymidine during the last 4 hours of culture. Each determination was performed in triplicate. Cultures were harvested and counted in a liquid scintillation counter. Results are expressed as counts per minute (cpm) tritiated thymidine uptake.

Cytokine Production

Spleen cells were cultured at a concentration of 1 x 10⁶ cells/mL in 24 well plates with conc A (1 μg/mL). Cells were incubated for 48 hours at 37°C in a 5 percent CO₂ humidified environment. Cell supernatants were collected, centrifuged to remove cells and stored at -70°C until assayed.

Cytokine Assays

Spleen cell supernatants were analyzed for IL-2, IFN-γ, IL-10 and IL-4 using commercially available enzyme linked immunosorbent assays specific for the rat species. All samples were analyzed in duplicate and standard curves were generated with each assay.

Statistical Analysis

Data are expressed as the median and range. Trauma and control groups were compared using Mann-Whitney U tests.

Results

All rats survived the anesthesia and femur fracture injury. The baseline weights of trauma and control rats were similar (276 g, 262 to 303 g vs 280 g, 258 to 310 g, p = 0.8). By 3 days post-injury, the body weights of traumatized

Figure 1. Concentration of IL-2 in supernatants of spleen cells obtained from rats 3 days after bilateral femur fracture or sham injury. The top of each box represents the 75th percentile and the bottom of each box represents the 25th percentile. The line in the middle of the box indicates the median. The bars display the 90th and 10th percentiles. The circles represent data points outside the 90th to 10th percentiles. *p < 0.05 compared to control values.

§ Cytoscreen™ Rat IL-2, IFN-γ, IL-10 and IL-4 Immunoassay Kits, BioSource International, Camarillo, CA.

* SPSS for Windows version 6.0, SPSS, Chicago, IL.
rats decreased from baseline by 2.2 percent (-4.3-+2.3 percent), whereas the body weights of controls increased by 4 percent (+2.9-+11.7 percent, p < 0.01).

The number of spleen cells obtained per rat, after lysis of red blood cells, was similar in trauma and control groups (1.1 x 10^8 cells/spleen, 0.5–3.5 x 10^8 cells/spleen vs 1.6 x 10^8 cells/spleen, 0.9–2.2 x 10^8 cells/spleen, p = 0.4). After resuspending the cells to a concentration of 5 x 10^5 cells/mL, con A-induced spleen cell proliferation was significantly decreased in the traumatized rats (33 x 10^3 cpm, 23–123 x 10^3 cpm vs 75 x 10^3 cpm, 43–123 x 12^3 cpm, p < 0.05).

Spleen cells were resuspended to 1 x 10^6 cells/mL prior to stimulation with con A for cytokine production. The concentrations of the T_{H1} cytokines, IL-2 and IFN-γ, were significantly decreased in the spleen cell supernatants of traumatized rats compared to controls (figures 1 and 2). The concentration of the T_{H2} cytokine, IL-10, was also decreased in supernatants from the trauma group (figure 3). IL-4 concentration was below the detection limit (<15 pg/mL) in spleen cell supernatants of all rats.

**Discussion**

The T lymphocytes are major regulatory and effector cells of the immune system.13 Lymphocytes which express T cell receptors can be divided into two subsets based upon the coreceptor molecules they express. The CD4+ T cells provide helper functions for B cells, whereas CD8+ T cells develop into cytotoxic lymphocytes which destroy target cells by direct contact. Helper T cells can be differentiated into T_{H1} and T_{H2} type cells based on the pattern of cytokines they produce.5,13 The T_{H1} cells mediate cellular immunity via the production IL-2 and IFN-γ. The T_{H1} cells induce delayed type hypersensitivity, activate macrophages and stimulate production of opsonizing IgG2a antibody. The T_{H2} cells produce IL-4 and IL-10, and favor the development of humoral immunity. The T_{H2} cells stimulate IgE production, eosinophilia and

![Figure 2. Concentration of IFN-γ in supernatants of spleen cells obtained from rats 3 days after bilateral femur fracture or sham injury. The top of each box represents the 75th percentile and the bottom of each box represents the 25th percentile. The line in the middle of the box indicates the median. The bars display the 90th and 10th percentiles. The circles represent data points outside the 90th to 10th percentiles. *p < 0.05 compared to control values.](image-url)
ALTERED T CELL CYTOKINE PRODUCTION

Figure 3. Concentration of IL-10 in supernatants of spleen cells obtained from rats 3 days after bilateral femur fracture or sham injury. The top of each box represents the 75th percentile and the bottom of each box represents the 25th percentile. The line in the middle of the box indicates the median. The bars display the 90th and 10th percentiles. The circles represent data points outside the 90th to 10th percentiles. \( *p < 0.01 \) compared to control values.

Many heterogeneous cell types reside within the spleen. Spleen cell suspensions as prepared in this protocol are primarily comprised of T cells, B cells, and macrophages. When spleen cells are cultured in the presence of the T cell mitogen con A, T cell proliferation and cytokine production are stimulated. The magnitude of the proliferative response to con A depends on the number of T cells present as well as the cytokines produced. In previous studies using the rat model, the effect of traumatic injury on the spleens' proportions of CD4+ and CD8+ T cells, B cells, and macrophages was investigated. No changes in the proportions of the various spleen cell phenotypes were identified using monoclonal antibody or non-specific esterase staining. Because each spleen was resuspended to the same cell concentration, decreased spleen cell proliferation following trauma could not be attributed to a decrease in the absolute number of T lymphocytes. In the present study, the changes in \( T_H1 \) and \( T_H2 \) cell cytokine production which occur as a result of mechanical tissue injury were evaluated. The results demonstrated that decreased spleen cell proliferation is associated with generalized suppression of T helper cell cytokine production rather than a shift from \( T_H1 \) to \( T_H2 \) cell activity. These findings are important since therapies directed at enhancing \( T_H1 \) cell development in order to restore immunologic homeostasis after traumatic injury have been proposed.

The IL-10 is produced by macrophages, \( T_H2 \) cells, B cells, and keratinocytes. The IL-10 suppresses T cell proliferation by inhibiting the co-stimulatory functions of macrophages and IL-2 production by \( T_H1 \) cells. Previous studies have suggested that IL-10 is responsible for the immunosuppression occurring after hemorrhage and trauma. Although circulating concentrations of IL-10 are elevated in trauma patients, the cellular
source of plasma IL-10 remains controversial. Plasma IL-10 concentration peaks within hours of injury whereas maximal suppression of cellular immunity takes several days to develop. If elevated circulating levels of IL-10 were due to a predominance of TH2 cells, plasma IL-10 and cellular immunosuppression should peak concurrently. The results demonstrate that defects in cellular immunity owing to direct tissue injury are not the result of excess IL-10 production by TH2 cells. Our findings are in agreement with those of Miller-Graziano et al. In their studies, trauma patients with depressed peripheral blood mononuclear cell or isolated T cell proliferation also had decreased IL-10 released into the corresponding cell supernatants. Decreased IL-10 production by T cells was associated with an increase in post-trauma complications and mortality.

It is concluded that mechanical tissue injury leads to decreased production of the TH1 cytokines IL-2 and IFN-γ, and the TH2 cytokine IL-10. Post-trauma cellular immunosuppression is not mediated via excess IL-10 production by TH2 cells.

References