ACRM
AMERICAN CONGRESS OF REHABILITATION MEDICINE

Archives of Physical Medicine and Rehabilitation

journal homepage: www.archives-pmr.org

Archives of Physical Medicine and Rehabilitation 2013;94:369-74

ORIGINAL ARTICLE

Phagocytic and Oxidative Burst Activity of Neutrophils in Patients With Spinal Cord Injury

Selcen Kanyilmaz, MD,[a] Simin Hepguler, MD,[a] Funda C. Atamaz, MD,[a] Nihal M. Gokmen, MD,[b] Omur Ardeniz, MD,[b] Aytul Sin, MD[b]

From the Departments of [a]Physical Medicine and Rehabilitation and [b]Immunology, University of Ege, Izmir, Turkey.

Abstract

Objective: To evaluate phagocytic activity and neutrophil oxidative burst functions in patients with spinal cord injury (SCI) because alterations in neutrophil metabolic activity can be one of the causes of immune mechanism damage contributing to repeated bacterial infections.

Design: A controlled and cross-sectional study.

Setting: Departments of physical medicine and rehabilitation and immunology.

Participants: Patients with SCI (N=34) and 28 healthy controls.

Interventions: Phagocytosis and oxidative burst in whole-blood neutrophils were assessed by flow cytometry. The percentage of phagocytizing cells after in vitro incubation with *Escherichia coli*, phagocytic activity (mean intensity of fluorescence [MIF]) and the percentage of neutrophiloxidative burst, and the MIF value of the production of reactive oxygen intermediates (ROIs) were analyzed. In addition, clinical assessment including the level of injury, American Spinal Injury Association scores, and functional status were carried out.

Main Outcome Measures: Not applicable.

Results: Although the percentage of *E. coli* phagocytizing neutrophils was not different between groups, the MIF value of absorbed *E. coli* was significantly lower in patients with SCI than in controls ($P<.05$). The MIF value of ROI production by neutrophils with both stimulator of phorbol 12-myristate 13-acetate and *E. coli* was significantly higher in patients with SCI ($P<.05$).

Conclusions: In patients with SCI, decreased phagocytic activity of neutrophils may be a result of a regulatory mechanism to minimize the deleterious effects of increased neutrophil burst activity.

Archives of Physical Medicine and Rehabilitation 2013;94:369-74

In spinal cord injury (SCI), infectious complications—mostly pulmonary and urinary tract infections—and their associated morbidity and mortality have been documented.[1-3] Although these infections have been reported in either early or late phases of SCI, they can be an underlying cause of death, especially in the post-acute phase.[1-5] Morbidity is also an important aspect of infections, because they are responsible for a significant number of hospital admissions and total hospital stays in patients with SCI, resulting in a significant social and economic burden. Moreover, it has been shown that infections are associated with worsening of neurologic status after SCI.[6-9]

Considering the serious impact of infectious complications, researchers have focused on immune alterations in patients with SCI. To some point, numerous sociodemographic factors are reported as risk factors for a higher incidence rate of infections in this population,[2,9,10] although it is not sufficient to attribute all to these factors. Therefore, alterations in the immune system after SCI are questioned for susceptibility to infections. It has been shown that decreased levels of $CD3^+$ T lymphocytes, $CD45\ RA^+$ B lymphocytes, major histocompatibility complex class II, and dendritic cells resulted in severe suppression of cellular immune activity and unresponsiveness in some pilot studies of SCI.[11-13] Campagnolo et al[13] reported a decrease in lymphocytic proliferation in a small group of patients with tetraplegia. However, the underlying mechanisms of immune suppression in these patients have not been explained thoroughly.[5]

Neutrophils play the main role in host defense against bacterial and fungal infections. During phagocytosis, migration of white blood cells from the circulation to the site of injury, known as chemotaxis, occurs and numerous responses in neutrophils are

No commercial party having a direct financial interest in the results of the research supporting this article has or will confer a benefit on the authors or on any organization with which the authors are associated.

0003-9993/13/$36 - see front matter © 2013 by the American Congress of Rehabilitation Medicine
http://dx.doi.org/10.1016/j.apmr.2012.09.015

elicited by chemotactic factors, including aggregation, increased adherence, enzyme release, degranulation, and oxidative metabolism.[14] Considering that efficient phagocytosis and the subsequent production of reactive oxygen intermediates (ROIs) play an important role in the intracellular killing of microorganisms by phagocytes, defects in one or both of these functions may lead to a deficiency in phagocytic function.[14,15] The first purpose of this study was therefore to assess neutrophil functions as measured by phagocytosis and oxidative burst in patients with SCI. Second, we aimed to assess whether neutrophil functions were related to clinical status.

Methods

Participants

The sample of the patient population included 34 inpatients in rehabilitation, ranging in age from 18 to 75 years, with traumatic SCI. The International Standards for Neurological and Functional Classification of SCI of the American Spinal Injury Association (ASIA) were used to classify study participants according to their level of neuromuscular function. Twenty-eight healthy age- and sex-matched controls were recruited as a control group. Subjects were excluded if they had known risk factors for immune suppression including active infection; drug exposure (eg, corticosteroid and immunosuppressant); history of diabetes, kidney, thyroid, or liver disease; or any cause that would alter the immune system, such as autoimmune diseases. Patients with SCI secondary to other conditions including neoplasm, neurologic diseases, or vascular diseases rather than trauma were also excluded.

The study was approved by the local institutional review board, and all participants provided written informed consent.

Phagocytosis

The phagocytosis test was carried out using the Phagotest kit.[a] After a sample of 100μL of blood with heparin was mixed with 2×10^7 fluorescein isothiocyanate–labeled *Escherichia coli* cells at 0°C, mixtures of heparinized whole blood and bacteria were incubated for 10 minutes in a 37°C horizontal shaking water bath. Simultaneously, control samples of whole blood and fluorescein isothiocyanate–labeled *E. coli* were incubated at 0°C to reduce the phagocytic potential to a minimum. Then, the fluorescence of the attached bacteria on the cell surface was quenched by adding 100μL of brilliant blue (quenching solution). Erythrocytes were lysed using lysing solution[b] for 20 minutes at room temperature, after 2 washing steps. Finally, 50μL of propidium iodide was added to stain the DNA of the bacteria and the cells.

Oxidative burst

The production of ROIs was determined quantitatively with the Bursttest kit.[a] A sample of heparinized blood was put in a water bath for 15 minutes. Four testing tubes were equally filled with 100μL of blood each and 2×10^7 unlabeled opsonized bacteria *E. coli*, 20μL of substrate solution (negative control), 20μL of peptide *N*-formyl-MetLeuPhe as chemotactic low physiological stimulus (low control), and 20μL of phorbol 12-myristate 13-acetate, a strong nonreceptor activator (high control). All samples were incubated in a 37°C horizontal shaking water bath for 10 minutes. With addition of dihydrorhodamine to the samples, incubation was continued for a further 10 minutes to allow nonfluorescent dihydrorhodamine to convert to fluorescent rhodamine 123 upon the production of ROIs. Lysing solution was added for 20 minutes at room temperature to remove erythrocytes. Samples were washed and centrifuged (5min, 250 revolutions/min, 4°C) and the supernatant was decanted; 200μL of DNA staining solution (centrifuged and incubated for 10min at 0°C in a dark place) was added to discriminate and exclude aggregation artifacts of bacteria and/or cells in cytometric flow analysis.

List of abbreviations:

ASIA American Spinal Injury Association
MIF mean intensity of fluorescence
ROI reactive oxygen intermediate
SCI spinal cord injury

Flow cytometry

Blood samples were taken from all patients and controls between 8:00 and 10:00 AM after a 12-hour overnight fast. Laboratory personnel were blind to case-control status. For analysis, we used FACSort flow cytometer with the Cellquest software program.[b] The device was calibrated and standardized between each analysis using Calibrite beads.[b] The granulocyte populations were gated using their forward- and side-scatter dot plots. During fluorescence-activated cell sorter analysis, free bacteria and aggregates of bacteria were separated from leukocytes on account of their much lower DNA content compared with that of eukaryotic cells. Phagocytosis and oxidative burst were monitored by determining both the proportion of cells fluorescing and the relative fluorescence intensities of the gated granulocytes.

Clinical evaluation

The ASIA Impairment Scale was used for the detailed evaluation of motor and sensory functions of patients besides baseline characteristics (weight, height, body mass index, age, and sex). For functional status of the patient, the FIM motor score was used to assess each patient's motor function.[16]

Statistics

Statistical analyses were performed with the 15.0 Statistical Package for the Social Sciences.[c] All the results were expressed as mean ± SD. A P value below .05 was considered to indicate statistical significance. Because a normal distribution could not be shown in data, the Mann-Whitney U test was used in the comparison of groups. The correlation analyses were performed using Spearman rank correlation coefficients.

Results

Table 1 summarizes participants' characteristics.

Table 2 shows phagocytosis results. While there was no significant difference between patients with SCI and healthy controls in the percentage of neutrophils able to undergo