Compromised T-cell immunity in patients with spinal cord injury and its relationship with injury characteristics

Ozgur Kartal[1], Bilge Yilmaz[2], Mustafa Gülec[3], Umüt Guzelkucuk[2], Arif Kenan Tan[2], Osman Sener[1], Uğur Muşabak[4]

[1]Department of Immunology and Allergic Diseases, Gülhane Faculty of Medicine and Gülhane Training and Research Hospital, University of Health Sciences, Ankara, Türkiye
[2]Department of Physical Medicine and Rehabilitation, University of Health Sciences, Ankara Gaziler Physical Medicine and Rehabilitation Training and Research Hospital, Ankara, Türkiye
[3]Department of Immunology and Allergic Diseases, Private Güven Hospital, Ankara, Türkiye
[4]Department of Immunology and Allergic Diseases, Baskent University, Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objectives: The aim of this study was to investigate *in vivo* and *in vitro* cellular immune responses in patients with chronic (spinal cord injury; SCI), determine the effects of autonomic dysfunction on cellular immune response, and determine the effect of completeness of the injury at different levels on cellular immune response.

Patients and methods: Forty-nine patients (42 males, 7 females; mean age: 35.5±13.4 years; range, 18 to 68 years) with chronic (time since injury >6 months) traumatic SCI were included in this cross sectional study between March 2013 and December 2013. Patients were allocated into two groups: Group 1, patients with an injury at T7 or below, and Group 2, patients with an injury at T6 or above. All patients in Group 2 had a history of autonomic dysreflexia and orthostatic hypotension. Intradermal skin tests were applied to the participants to reveal delayed T-cell responses. The percentages of cluster of differentiation (CD)3+ T cells and CD3+ T cells expressing CD69 and CD25 were analyzed by flow cytometry for the detection of activated T cells including all T-cell subsets.

Results: When patients with complete injuries were compared, the CD45+ cell percentage was found to be significantly higher in patients in Group 2. Patients with an incomplete SCI had increased skin response to candida antigens compared to complete SCI patients. Incomplete SCI patients also had higher percentages of lymphocytes and CD3+CD25+ and CD3+CD69+ T cells compared to patients with complete SCI.

Conclusion: T-cell activity is impaired in chronic SCI patients with higher levels of injury, and the completeness of injury and autonomic dysfunction gain prominence as compromising factors in T-cell immunity.

Keywords: Autonomic dysfunction, spinal cord injury, cellular immune response.

Spinal cord injury (SCI) is a type of injury that has multisystem consequences and a wide range of complications related to these consequences. As a result of the changes in the immune system after SCI, infections in respiratory and urinary tracts are one of the leading causes of increased mortality and morbidity rates in patients with SCI.[1]

Although there are many studies in the literature related to immune changes after SCI, most of these studies are about acute changes after the injury, aiming to determine the factors related to inflammation and neural regeneration. What is known about the changes at immune system in chronic periods after SCI is based on only a few human studies. One of

these studies showed that suppression in the immune response occurred in acute phases and recovered to control levels in chronic phases in patients with SCI.[2] During this immune suppression in the acute period, it was found that the number of cluster of differentiation (CD)14^{+} monocytes, CD3^{+} T cells, and CD19^{+} B cells decreased, whereas the number of CD15^{+} granulocytes was not changed. In chronic phases, when the immune suppression recovered, the number and cytotoxic activity of natural killer cells and macrophages were found to be reduced in patients with SCI.[3-5] On the other hand, T-cell functions were reported to be in the normal range.

Another important aspect of the immune system is its close relationship with the autonomic nervous system.[6] Neurotransmitters released by sympathetic and parasympathetic nerve endings bind to their respective receptors on the surface of immune cells and induce immunoregulatory responses. Preganglionic sympathetic axons control lymphoid organ functions by releasing catecholamines.[7] Accordingly, T helper (Th) cell polarization (Th1 or Th2) is partially under the control of β2 adrenergic receptors by epigenetic mechanisms.[8] Human studies pointed out that the level of injury in SCI determined the severity of immune system dysfunction in which the patients with SCI at cervical or thoracic levels had more impaired immune system functions than those of the patients with SCI at lower levels.[9] This might clue in defining the relationship between the autonomic nervous system and the immune system.

The aim of this study was to investigate *in vivo* and *in vitro* T-cell activity in patients with chronic SCI, determine the effects of autonomic dysfunction, and determine the effect of completeness of the injury at different levels.

PATIENTS AND METHODS

The cross sectional study was conducted at the Ankara Gaziler Physical Medicine and Rehabilitation Training and Research Hospital between March 2013 and December 2013. All patients were assessed according to the International Standards for Neurological Classification of SCI by an experienced physiatrist.[10] We did not employ any probability sampling methodology. By using convenience sampling, we chose the patients who came to the clinic in a two-month period. Forty-nine adult traumatic SCI patients (42 males, 7 females; mean age: 35.5±13.4 years; range, 18 to 68 years) who were injured at least six months ago and who completed their acute

rehabilitation care were included in this study. Of these patients, 19 were tetraplegic, and 30 were paraplegic. The exclusion criteria for this study were active infection, nontraumatic SCI, malignancy, and a history of visceral organ pathology.

Autonomic dysfunction occurs in people with spinal lesions at or above the T6 level. The patients were allocated into two subgroups to assess the effect of autonomic dysfunction: Group 1, patients with an injury at T7 or below, and Group 2, patients with an injury at T6 or above. The existence of autonomic dysfunction, such as autonomic dysreflexia and orthostatic hypotension attacks, was confirmed in all patients in Group 2. The reason why these levels were chosen was to determine whether sympathetic derangement had an impact on immune function in patients with SCI. Thus, the patients were also divided according to whether they had a complete or incomplete injury.

Flow cytometric analyses

Flow cytometric analyses were performed to reveal *in vitro* T-cell activity in the cases. The measurements were performed as soon as peripheral blood samples were obtained by venipuncture. Lymphocytes from whole blood were directly stained without being isolated by appropriate fluorochrome-labeled monoclonal antibodies (CD45 FITC [fluorescein isothiocyanate], CD3 FITC, CD69 PE [phycoerythrin], and CD25 PE; Becton Dickinson, San Jose, CA, USA). After the incubation period of 15 min, the stained cells were read in flow cytometry (FACSCanto-II™; Becton Dickinson, San Jose, CA, USA), and then analyzed.

In the statistical analyses, the percentages and the absolute counts of CD3^{+} T cells and CD3^{+} T cells bearing activation markers (CD69 and CD25) on their surfaces (CD3^{+}CD69^{+} and CD3^{+}CD25^{+}) were compared with each other for showing the differences between the study groups with respect to the activity status of T cells. The absolute count of T cells (CD3^{+}) and their subsets (CD3^{+}CD69^{+} and CD3^{+}CD25^{+}) was calculated using the simultaneous results of complete blood count and flow cytometric measurements of each case.

T-cell responses

The CD69 molecule is the earliest activation marker expressed on lymphocytes, such as T cells, B cells, and natural killer cells. Its expression on the cell surfaces reaches peak levels within 24 h of stimulation with mitogens.[11] The other activation marker, CD25 (IL [interleukin]-2 receptor), is expressed two to three days later during this process. The reason why

these markers were chosen was to determine T-cell activity. On the other hand, the fluorochrome-labeled monoclonal antibody to CD45 molecule (leukocyte common antigen) is used for the CD45/side scatter gating procedure in flow cytometric studies to improve phenotypic determination of the leukocyte subsets.[12]

Skin delayed hypersensitivity testing

The presence of positive cutaneous delayed-type hypersensitivity skin tests generally indicates an intact T cell-mediated immunity. The following intradermal skin test antigens/materials and products were used: tuberculin-purified protein derivative intradermal test (PPD-IDT; BB-NCIPD Ltd., Sofia, Bulgaria), Candida albicans allergen intradermal test (C-IDT; ALK-Abello, Port Washington, NY, USA), and tetanus toxoid intradermal test (T-IDT; Serum Institute of India Ltd., Hadapsar, Pune City, India) to evaluate the delayed T-cell response. Intradermal skin tests were performed according to the standard intradermal test procedures. Isotonic saline solution was used as a negative control to exclude false positive reactions (Stallergènes Greer, Antony, France). The C-IDT and T-IDT results were read at 48 h and 72 h by palpating the indurated area. A positive result was defined when the diameter of the induration was ≥5 mm. The PPD-IDT results are given with the measurement of the induration diameter results obtained at 48 h.

Statistical analysis

All analyses were performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to assess the normality assumption, and the equality of variances of numeric variables was tested by the Levene test. Descriptive statistics for numeric variables were presented as the mean ± standard deviation (minimum-maximum) for normally distributed variables, and median with the first and third quartiles for the nonnormally distributed variables. Categorical variables were presented as frequencies and percentages. The differences between two independent groups were evaluated by independent samples t test if the normality assumption was satisfied, otherwise Mann-Whitney U test was used. Yates corrected chi-square test was used to examine differences between categorical variables where the assumption that the expected count less than 5 should not exceed 20% for variables was satisfied; if otherwise, we used Fisher exact test or Pearson chi-square test with Exact Sig (2-sided). P values less than or equal to 0.05 were accepted as statistically significant.

RESULTS

The mean time since injury was 27.9±35.8 (6-168) months. There was no significant difference between the patients in Groups 1 and 2 in terms of demographic characteristics, such as age (p=0.649), sex (p=0.683), and time since injury (p=0.285). There was no significant difference between the patients with complete and incomplete SCI in terms of demographic characteristics, such as age (p=0.653), sex (p=0.407), and time since injury (p=0.835, Table 1).

Patients with incomplete SCIs had a higher skin response to candida (p=0.01, post hoc power=0.72), higher percentages of CD3+CD25+ (p<0.001, post hoc power=0.96), and more CD3+CD69+ T cells compared to patients with complete SCIs (p=0.02, post hoc power=0.73). Accordingly, incomplete SCI patients had higher percentages of lymphocytes compared to the complete SCI patients (p=0.002, post hoc power=0.89, Table 2).

There was no significant difference between Groups 1 and 2 regarding other skin tests and lymphocyte subsets (p=0.422 for C-IDT; p=0.638 for T-IDT; p=0.738 for PPD-IDT; p=0.388 for CD3+ T cell; p=0.299 for CD3+CD25+ T cell; p=0.916 for CD3+CD69+ T cell; p=0.046 for CD45+ cell; p=0.174 for lymphocytes; Table 3).

DISCUSSION

The results of this study revealed that T-cell functions were impaired in SCI patients with higher levels of injury in the chronic period. Completeness created significant differences in both *in vivo* and *in vitro* T-cell functions at all levels, and the difference was pronounced at *in vivo* tests when the patients were compared according to autonomic dysfunction.

In this study, patients were allocated into two groups according to the sympathetic outflow levels to determine the effect of autonomic dysfunction on the immune system. There was no significant difference between the groups in terms of *in vivo* and *in vitro* cellular immune responses. This result was unexpected when the close relationship between the immune system and autonomic system as explained above was considered. However, the findings of Campagnolo et al.[3] pointed out the same result in terms of natural killer activity in patients with chronic SCI.

However, it was difficult to standardize the effect of autonomic dysfunction on the immune system when the injury was incomplete and the circuitry between the segments above and below the level of injury was

TABLE 1
Demographic characteristics of the study group

	T7-↓ (n=21)						T6-↑ (n=28)						p	Complete (n=30)						Incomplete (n=19)						p
	n	%	Mean±SD	Median	Q1-Q3	Min-Max	n	%	Mean±SD	Median	Q1-Q3	Min-Max		n	%	Mean±SD	Median	Q1-Q3	Min-Max	n	%	Mean±SD	Median	Q1-Q3	Min-Max	
Age (year)				36.00	30.0-43.0	18-52				31.0	22.0-48.0	18-68	0.649[d]			34.8±12.9			18-61			36.6±14.5			19-68	0.653[d]
Time since injury (month)				11.0	6.0-23.0	6-85				14.0	7.5-43.0	6-168	0.285[a]				11.0	6.0-29.0	6-168				13.0	6.0-26.0	6-85	0.835[a]
Sex													0.683[b]													0.407[b]
Female	2	9.5					5	17.9						3	10.0					4	21.1					
Male	19	90.5					23	82.1						27	90.0					15	78.9					
AIS													0.785[c]													<0.001[c]*
A	13	61.9					17	60.7						30	100.0					-	-					
B	4	19.0					8	28.6						-	-					12	63.2					
C	2	9.5					2	7.1						-	-					4	21.1					
D	2	9.5					1	3.6						-	-					3	15.8					

SD: Standard deviation; Q1: First quartile; Q3: Third quartile; AIS: Asia Impairment Scale; T7-↓: Injury at or below T7; T6-↑: Injury at T6 or above; a: Mann-Whitney test; b: Fisher exact test; c: Pearson chi-square test with Exact Sig (2-sided); d: Independent Samples t test; * Statistically significant.

TABLE 2
In vivo and *in vitro* test results in patients with SCI

	Group 1 (T7-↓) (n=21)					Group 2 (T6-↑) (n=28)					p	Complete (n=30)					Incomplete (n=19)					p
	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max		n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	
C-IDT											0.09[a]											0.01[a]*
Negative	8	40.0				18	64.3					20	69.0				6	31.6				
Positive	12	60.0				10	35.7					9	31.0				13	68.4				
T-IDT											0.49[a]											0.41[a]
Negative	7	33.3				12	42.9					13	43.3				6	31.6				
Positive	14	66.7				16	57.1					17	56.7				13	68.4				
PPD-IDT											0.54[b]											0.59[b]
Negative	11	52.4				19	67.9					19	63.3				11	57.9				
Induration 3-9	4	19.0				3	10.7					3	10.0				4	21.1				
Induration 10+	6	28.6				6	21.4					8	26.7				4	21.1				
CD3+			71.5±8.1					72.2±7.1			0.74[c]			72.4±8.3					71.1±6.1			0.57[c]
CD3+CD25+ T cell				1.6	1.2-3.4				2.4	1.3-4.0	0.20[d]				1.5	1.1-2.4				3.6	2.1-5.8	<0.001[d]*
CD3+CD69+ T cell				2.1	1.0-2.9				2.1	1.6-3.7	0.27[d]				1.9	1.3-2.6				3.1	1.7-4.5	0.02[d]*
CD45+ Cell				95.9	94.5-97.9				97.3	95.1-97.9	0.62[d]				95.9	94.6-97.9				96.2	94.2-98.0	0.81[d]
Lymphocyte**				17.4	12.8-22.3				18.5	14.6-22.4	0.60[d]			16.4±6.5*					23.1±8.0*			0.002[d]*

SCI: Spinal cord injury; SD: Standard deviation; C-IDT: Candida-intradermal test; T-IDT: Tetanus-intradermal test; PPD-IDT: Purified protein derivate-intradermal test; a: Pearson chi-square test with Exact Sig (2-sided); b: Yates Corrected chi-square test results; c: Independent samples t test results; d: Mann-Whitney test results; * Statistically significant; ** Lymphocyte population included CD3+ T cells, CD19+ B cells, and CD3-CD16+56+ natural killer cells.

TABLE 3
In vivo and *in vitro* test results in patients with complete SCI

	T6-↑ Complete patients (n=17)					T7-↓ Complete patients (n=13)					
	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	*p*
C-IDT											0.422[a]
Negative	13	76.5				7	58.3				
Positive	4	23.5				5	41.7				
T-IDT											0.638[b]
Negative	8	47.1				5	38.5				
Positive	9	52.9				8	61.5				
PPD-IDT											0.738[c]
Negative	11	64.7				8	61.5				
Induration 3-9	1	5.9				2	15.4				
Induration 10+	5	29.4				3	23.1				
CD3+			73.5±7.6					70.8±9.2			0.388[d]
CD3+CD25+ T cell			2.2±1.7					1.6±0.9			0.299[d]
CD3+CD69+ T cell				1.9	1.3-2.2				2.1	0.7-2.9	0.916[e]
CD45+ Cell				97.8	95.9-97.9				95.9	94.5-95.9	**0.046[e]**
Lymphocyte*				17.8	14.0-21.5				13.0	11.1-17.0	0.174[e]

SCI: Spinal cord injury; SD: Standard deviation; C-IDT: Candida-intradermal test; T-IDT: Tetanus-intradermal test; PPD-IDT: Purified protein derivate-intradermal test; T7-↓: Injury at or below T7; T6-↑: Injury at T6 or above; Lymphocyte population included CD3+ T cells, CD19+ B cells and CD3-CD16+56+ natural killer cells; a: Fisher exact test; b: Yates corrected chi-square test results; c: Pearson chi-square test with Exact Sig (2-sided); d: Independent samples t-test results; e: Mann-Whitney test results.

not totally blocked. When we compared the results of patients with complete and incomplete injuries, T-cell activation parameters (CD3+CD25+ and CD3+CD69+) in patients with a complete injury were found to be significantly reduced along with the lymphocyte count, and the response to C-IDT was also decreased. These results have revealed that completeness has an important effect on T cell immunity. We could not compare our results with the literature in terms of completeness since, as to our knowledge, this is the first study in chronic SCI dealing with the effect of completeness on immune parameters.

The comparison between patients in Groups 1 and 2 revealed a significant difference only in CD45+ cell percentages (Table 3). Although the results of skin tests and other *in vitro* tests were not significant, autonomic dysfunction is at least an important contributory factor to immunodeficiency in patients with SCI.

We could not find any rationale explaining why there was no significant difference at other *in vitro* tests and skin tests between Groups 1 and 2. The small sample size of our study, the differences in frequencies of autonomic dysreflexia and orthostatic hypotension attacks, and the time between the experimental tests and the most recent prior attack may have had an impact on the results. The discrepancy between *in vitro* and *in vivo* test results presented in this study may be

related to these factors. Another possible explanation for this result may be incomplete SCI, a term which is first described by Dimitrijevic.[13] Residual sensory and motor functions that cannot be detected by physical assessment might cause inappropriate allocation to groups with a resultant bias.

Kliesch et al.[14] reported that after appropriate rehabilitation therapy, T-cell functions recovered to near normal levels and remained in the normal range, whereas natural killer activities returned to prerehabilitation levels after discontinuation of the therapy. They used leucoagglutinin as a leukocyte transformation parameter and IL-2 receptors (sCD25) to determine T-cell activation. They reported the changes in these parameters within a timeline. In our study, all patients were also evaluated in a follow-up period and completed their rehabilitation procedure as a standard of care. However, our findings revealed the existence of impaired T-cell functions in patients who received rehabilitation care and thus did not support the study of Kliesch et al.[14]

Having no healthy control group is a limitation for this study, though the differences between patients with complete and incomplete injury may give an idea. Moreover, the small sample size of this study might have led to a result where possible meaningful results would be neglected.

In conclusion, T-cell activity is impaired in chronic SCI patients with higher levels of injury, and the completeness of injury and autonomic dysfunction gain prominence as important factors affecting immunosuppression. When the close relationship between the autonomic nervous system and the immune system is considered, this result is not surprising. This is most likely a miscommunication between the two systems caused by sympathetic derangement. However, these findings should be supported by future research. We think that future research should focus on how completeness and autonomic dysfunction affect the incidence and severity of infections in patients with SCI with a larger sample.

Ethics Committee Approval: The study protocol was approved by the Kecioren Training and Research Hospital Ethics Committee (date: 13.02.2013, no: B.10.4.ISM.4.06.68.49). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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