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NEUROIMMUNOMODULATIN BY DORSOLATERAL HIPPOCAMPUS – ROLE OF MACROPHAGES, T AND B CELLS

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Abstract : In dorsolateral hippocampal lesioned, Sham and control animals, the total number of splenocytes was determined and macrophages, B cells and T cells were isolated and their percentage distribution in total splenocytes were determined. The leukocyte migration inhibition was studied in these groups using different fractions of splenocytes namely whole splenocytes, macrophage removed fraction, and nylon wool eluted T cell population. The same groups were also studied after an antigenic challenge. The results indicate alterations in the macrophages, adherent cell population as well as T cell population in lesioned and Sham animals and also in their immunized groups. These altered cell sub-populations may be responsible for the altered migration.

Key words : dorsolateral hippocampal lesion, leukocyte migration inhibition index, neuroinmmunomodulation, cell mediated immunity

INTRODUCTION

The central and peripheral lymphatic tissue are composed of cells that are not permanently anchored to stromal or structural components as in many other organs. Lymphoid cell migration, re-circulation and interaction among them help or suppress the immune response. So an effective immune response depends on the highly mobile population of precursor and effector cells accumulation in the lymphoid system. The spleen is the key lymphoid organ for generating humoral immunity.

Our earlier work on neuroimmuno-modulation in bilateral dorsolateral hioppocampal formation (DLH) lesioned albino rats (1) revealed an enhanced migration index, a decrease in plaque forming cells (PFC) associated with a fall in antibody titre levels. To understand the cause behind this, an attempt was made to isolate and study the number of total splenocytes, macrophages, nylon wool adherent cell population (B cells) and eluted cells (T cells). Since the changes observed in migration may be mediated via the cytokines (cell mediators) which are known

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to affect the migration of these populations, the isolated fractions were also subjected to migration inhibition test in the present study.

METHODS

All experiments were performed under the guidelines prescribed for the care and use of animals in the field of physiological sciences as per International regulations.

Male Wistar strain albino rats (180–200 g) were housed under standard laboratory conditions and fed *ad libitum* with standard laboratory feed and water. The animals were divided into six groups. Group 1-control rats (n=8) to provide normal base line level of the immune parameters studied after handling and subjecting to anaestheticss similar to the test groups to rule out the changes induced by them. Group 2-Immunized control rats (n=8), treated similar to the control rats, to determine the normal immune response for the particular dose of antigen used. Group 3-DLH lesioned animals (n=8), to detect the change in basal immune status due to lesion. Group 4-DLH lesioned and immunized animals (n=8), to detect the effect of lesioning during an immune response. Group 5-DLH Sham operated animals (n=8). Group 6-DLH Sham operated and immunised animals (n=8). The Sham groups 5 and 6 were included to elucidate the effect of non-specific stress and surgical injury influencing the immune mechanism.

The bilateral hippocampal lesion and Sham operation were performed according to the co-ordinates of rat brain atlas (2) (anterior 2.58 mm, lateral 4.3 mm and depth from dura 5 mm) under pentathol sodium (40 mg/kg body weight, i.p) anaesthesia. Direct current of 2 mA for 45 sec was delivered through a coated stainless steel electrode (0.22 mm dia.). In the Sham animals, the electrodes were lowered till the DLH was reached, then removed without any passage of current. This served as the site-specific Sham group.

The animals were immunized intraperitoneally with 1 ml of sheep red blood cells (5×10^9 cells per ml). The day of immunization was day zero. In Sham and lesioned animals the immunization was carried out on the 10th day after surgery. The parameters were studied (on the 15th day after surgery) in Sham and lesioned animals and on the 5^{th} day after immunisation in control animals. The control and experimental animals were killed between 0800 and 0900 h using quick deep ether anaesthesia as this technique produces no stress as judged by corticosteroid levels (3). The spleen and the thymus were removed aseptically from these animals. The brains of lesioned group animals were preserved in Bodian fixative and site of lesion was confirmed by paraffin histological technique using cresyl fast violet. The data from properly lesioned animals were only considered for analysis.

Isolation of splenic cell subpopulation was performed as follows. Spleen and thymus cell suspensions were prepared by teasing in minimum essential medium (MEM AT045). From the splenocytes the red blood cells were removed by using haemolytic Gey's solution and cell counts

were determined. A small portion of this (0.2×10^8 cells/ml) formed the total splenic population (fraction I) and stored at 4°C until subjected to migration along with other groups. The remaining splenocytes were incubated with carbonyl iron powder (Sigma, USA) at concentration of 40 mg for $2–3 \times 10^8$ cells at 37°C for 30 minutes (4). After 30 minutes macrophages were removed using power magnet and the remaining population of cells were harvested. A small portion (0.2×10^8/ml) from this was preserved as macrophage removed fraction (fraction II). The remaining cells suspension was loaded in a nylon wool column (Fenal Laboratories, Morton Grove II, USA) and incubated at 37°C for 30 minutes to remove the adherent B cell population (5). The T cell enriched population was eluted using warm medium (37°C) (fraction III). The various fractions were also analysed for their cell count. The degree of separation and purity of the isolated population was already established by specific markers and is comparable to many other techniques available (6). Since the cells separated were subjected to further investigations usage of markers was spared.

Splenocytes can produce migration inhibition and stimulation factors. The thymocytes were used as the migrating population. The various splenocyte fractions were mixed with thymocytes at a ratio approximately 3:1 to 5:1 so as to provide a migrating cell density of 80×10^6 cells per ml (7). Leucocyte migration inhibition (LMI) Index (7) was studied in all fractions in presence and in absence of antigen. From the area of migration, LMI Index was calculated as below.

$$\text{Migraton Index [MI]} = \frac{\text{Area of migration of the cells in presence of antigen (SRBC)}}{\text{Area of migration of the cells in absence of antigen}}$$

The total splenic cell population, macrophages, B cells and T cell population were counted using haemocytometer.

All results were analysed in various combinations using ANOVA followed by Tukey's multiple comparisons when there is a significant F test ratio. The significance was fixed at $P<0.05$. To understand which cell population is responsible for enhanced MI, index from total splenocytes (fraction I), macrophage removed fraction II and column eluted cells (fraction III) were compared in each group namely controls, Sham and lesioned groups. To differentiate the lesion and Sham effects the migration indices (MIs) from them were compared among themselves and along with controls.

RESULTS

The migration index of various population are summarised in Table I and their cell counts with percentage distribution are given in Table II. Total splenocyte fractions of isolated cell populations were converted to their respective percentage. As the alterations in the cell count may be responsible for the altered migration, the migration and the cell population are discussed together.

The total splenocyte count was markedly elevated in both Sham and lesioned animals compared to controls (F [df 2, 19] = 11.8). But the MI of total splenocytes was markedly elevated in DLH lesioned animals

TABLE I: Migration Index of various fractions (Mean±SD).

	Migration index	
	Unimmunized	*Immunized*
Control		
Fraction I	0.893±0.035	0.39±0.067
Fraction II	0.768±0.081	0.688±0.10
Fraction III	0.962±0.117	0.68±0.15
Sham		
Fraction I	0.929±0.053	0.30±0.068
Fraction II	0.923±0.058	0.714±0.082
Fraction III	0.939±0.099	0.754±0.124
Lesion		
Fraction I	1.29±0.229	0.58±0.095
Fraction II	1.48±0.17	0.769±0.048
Fraction III	1.38±0.16	0.876±0.115

TABLE II: Splenocytes and their various populations (Mean±SD).

	Splenocytes/spleen	*Macrophages/Spleen*		*B cells/Spleen*		*T cells/Spleen*	
	x 10^8 cells	*x 10^8 cells*	*%*	*x 10^8 cells*	*%*	*x 10^8 cells*	*%*
Control	3.98±0.76	1.28±0.5	35±5.9	1.32±0.48	34.5±5.95	1.116±0.19	29.7±6.29
Sham	5.59±1.22	1.62±0.44	29.6±3.4	2.51±0.82	46.11±5.78	1.16±0.24	21.8±3.4
Lesion	6.58±1.14	3.2±0.59	49±0.30	2.28±0.47	34.58±3.36	1.04±0.22	15.5±2.5
Imm. control	4.81±1.18	2.56±0.84	48.3±2.3	1.44±0.43	27.25±3.49	1.11±0.32	23.12±3.2
Imm. Sham	4.6±0.86	2.35±0.64	47.8±6.12	1.32±0.33	27.21±4.5	1.11±0.19	24.3±2.11
Imm. lesion	4.17±0.35	1.76±0.15	37.2±2.3	1.9±0.51	43.7±2.8	0.84±0.10	18.21±1.9

compared to Sham and control animals (F [df 2, 19] = 20). This difference in migration indicates that elevated population of splenocyte may not be same cell type and it is not due to surgical procedures alone.

In lesioned animals, the percentage distribution of macrophages was significantly more compared to controls and Sham animals (F [df 2, 18] = 37.9). Removal of macrophages did not affect the MI in Sham animals. However, controls showed a marked decrease in MI and lesioned animals showed a significant increase in MI (F [df 2, 19] = 18.9) compared to Sham. These results indicate that the enhanced migration resulted in lesioned group could not have been contributed by macrophages alone.

One of the significant observation noticed in Sham group was the elevation of adherent cell population (B cell) (F [df 2, 19] = 11.76) compared to control and lesioned animals. The elevated adherent cell population in Sham animals could not alter the MI. Moreover, removal of adherent cell population in lesioned animals did not alter the elevated index (F [df 2, 19] = 27.6) suggest that these adherent population could not play a role in migration.

The T cell (column eluted fraction–III) cell count was significantly less in lesioned animals than that of the controls and Sham animals (F [df 2, 19] = 18.5). However, the Sham animals showed a marked decrease from control animals in the T cell fraction. The MI of this fraction was still significantly elevated in lesioned animals compared to Sham and control animals (F [df 2, 19] = 27.6) revealing that these T cells may be responsible for the altered cell mediated immunity observed. This suggests the release of migration inhibitory factor from T cells in Sham and controls.

After immunisation the total splenocyte count increased in controls and the MI was decreased compared to macrophage and adherent cell removed fractions (F [df 2, 21] = 18.3). Similar decrease in MI in total splenocyte population was observed in immunised lesioned (F [df 2, 18] = 18.4) and immunised Sham animals (F [df 2, 18] = 49.2). However, the MI of total splenocyte was significantly elevated in immunised lesioned animals compared to immunised controls and Sham (F [df 2, 10] = 24.6).

Removal of macrophages elevated the MI in immunised controls and Sham animals but not in immunised lesioned animals. In immunised lesioned animals, there was a decrease in macrophages (F [df 2, 18] = 19.27) and this may be the cause for the enhanced MI of the total splenocytes observed in these animals. The present results also indicate that macrophages can also secrete migration inhibitory factors as migration inhibition was observed in immunised Sham and controls.

There was a significant increase in adherent cell population in immunised lesioned animals (F [df 2, 18] = 47.5). Removal of adherent population could not produce any change in MI in all the immunised groups under investigation suggesting these cells may not be releasing any mediators that affect the migration.

The T cell count was reduced in immunised lesioned animals (F [df 2, 18] = 10.7) compared to immunised control and Sham animals. The more striking observation is removal of any of the isolated cell fractions (macrophage or B cell) could not restore the migration index to control levels indicating the role played by the T cells in migration inhibition in all these immunised groups. The enhanced migration observed in immunised lesioned animals may be due to decreased macrophages and T cell counts.

DISCUSSION

Our earlier work in bilateral lesion at dorsolateral hippocampal formation (DLH) in albino rats revealed an enhanced migration index, a decrease in plaque forming cells and associated with a fall in antibody titre levels (1). The enhanced migration seen in lesioned immunised animals may be due to decreased macrophages, T cells and increased adherent cell population. Because the various mediators (cytokines or lymphokines) that are released from these cell types determine the course of cell mediated immunity.

The significant changes observed between the lesion and Sham indicates the specific lesion effects and can not be interpreted as a non specific CNS lesion effect as the changes observed in hippocampal lesioned animals were not seen in ventromedial hypothalamic lesioned animals (8). The MI in lesioned animal's

fraction I was probably due to mediators from macrophages as removal of macrophages increases the index in the next fraction. Macrophage count was significantly higher in lesioned animals; the change in MI was elevated after removal of macrophages. Buiting et al (9) reported that lymphoid organs are populated by four different types of macrophage sub-populations. He further reported a strong decrease in the number of antibody secreting cells when marginal metallophilic macrophages were removed. The increased macrophage population in DLH lesion may be of a different sub-population which emphasises the need for further investigation on the sub type analysis of concerned cells in these animals.

After an antigenic challenge, in controls and Sham animals, macrophages increased significantly compared to lesioned animals. After an immune challenge recruitment of macrophages is a normal phenomenon. Fraction I in immunised control and Sham animals exhibited a greater migration inhibition index and removal of macrophages elevated the index, indicating that these macrophages could have released the migration-inhibiting factor. Report by Gordon et al (10) indicates that macrophages can release stimulating and inhibiting factors. Further it appears that release of inhibition factor may be an important phenomena for an increase in the phagocytic activity of macrophage and leukocytes during an immune response (11). From these findings it is clear that such type of secretary macrophages were absent in lesioned animals and it might be one of the possible causes for the enhanced migration observed.

Removal of macrophages could not reverse the migration inhibition index back to control level (unimmunized state) in immunised groups studied, indicating that the inhibition was not offered by macrophages alone.

The Sham effects could not be neglected as it was reported to be region specific (12). In this study the striking observation was the increase in adherent cell population in sham animals and after an immune challenge, this population reverted back to control levels, whereas in lesioned animals, this adherent cells significantly elevated after the immune challenge. However, we have reported earlier (1) that there was an increase in PFC in spleen in DLH immunized Sham animals, and it was also associated with increase in antibody titre against the antigen used and this increase was confined to hippocampal Sham and not to other areas like VMH (8). A similar rise in antibody was also reported in Sham animals that were subjected to Sham surgical procedure simulating hypophysectomy in rats (13).

It may be possible that the increased transformation of B cells to plasma cells in the immunized Sham animals could have lead to the formation of more antibodies. This was well supported by the fact that in immunized Sham animals and immunized control animals, there was a decrease in the B cell number when compared with their unimmunized state. Further increase in adherent population (B cell count) seen in immunised lesioned animals support this concept.

A common observation noticed in all the immunized groups studied was the inhibition in the MI, even in the isolated T cell population. Hence it appears that T cell population can also release migration

inhibitory factor. Fox and Rajaraman (14) showed the release of migration stimulation factor from the suppressor cell and migration inhibition factor from the helper cells and they also demonstrated the release of these factors from the splenic mixed cell population. As the immunized lesioned animals of DLH exhibited a decreased antibody level with a marked decrease in plaque forming cells (1), it appears that this immuno-suppressive state is either due to the dominant suppressor cells or deficiency of helper cell number and function. It is natural in such a situation the animal will show a suppressed immune response with an enhanced migration.

Thus the present study reveals that enhanced migration observed is due to altered macrophage and T cells, which affect migration by their cytokines. The increased and accumulated adherent B cell population indicates an inadequate transformation to plasma cells as indicated by fall in antibody titre. This also suggests that reduction in T cell count may be a result of decreased T helper cells. Fall in helper cell may be one of the important contributing factors as the antigen used is a T dependent one.

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