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PERIPHERAL IMMUNE RESPONSE IN ALBINO RATS FOLLOWING CEREBROVENTRICULAR AND INTRAPERITONEAL ANTIGEN CHALLENGE

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Abstract: Success in neural tissue transplants at central nervous system suggest that the site may be immunologically privileged. However, this experimental study in which an antigen (Sheep Red Blood Cells) was administered into the third ventricle does not support the above concept. The antibody titre and soluble immune complex levels seen in these animals are similar to the levels seen in animals immunized with the same amount of antigen through the intraperitoneal route. Intraventricular immunization is rather a more potent modulator in decreasing the total WBC count ($P<0.05$) and neutrophils ($P<0.001$). Further a marked increase in lymphocytes ($P<0.01$) in peripheral blood was observed in these animals. Intraventricular immunization also increased the killing power (NBT reduction) of the neutrophils ($P<0.05$).

Key words: central immunization peripheral immunization immune response

INTRODUCTION

The therapeutic application of heterospecific fetal neural tissue transplantation has gained much importance all over the world to alleviate human neurological disorders related to neural degeneration, like Parkinson's disease (1, 2, 3). Further, numerous studies have shown that it is possible to graft embryonic neural tissue among different immunologically defined strains (4). Non-neuronal adult tissue such as adrenal medulla (5) and endocrine pancreas (6) have also been transplanted in brain successfully. The success of such neural transplantation has been largely attributed to the privileged nature of the implantation site as well as due to lack of immunogenecity of the tissue transplanted. The traditional explanation for this include the presence of blood brain barrier, barriers at choroid plexus that permits the selective secretion of cerebrospinal fluid (CSF) and unidirectional re-entry of CSF into blood through arachnoid villi. The absence of conventional lymphatic drainage from CNS added further support for the prevention of antigen entry into the peripheral lymphoid tissue.

The reported lack of immunogenecity after the introduction of heterospecific tissue (which may act as antigen) in central nervous system (CNS) needs reanalysis as neural tissue is also proved to be antigenic. As little as 1 mg of myelin base protein in Freund's adjuvant inoculated peripherally has been reported to induce allergic encephalomyelitis in Rhesus monkeys (7).

However, it must also be remembered that an immune response offers protection against the invading antigen for the whole body, which includes the brain also. In other words it has not been demonstrated conclusively that brain is excluded from the systemic host resistance mechanism. Moreover, the success in rat neural tissue transplant is supported by the fact that rat brain cells (oligodendrocytes and schwann

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cells) do not have immune response gene associated antigen (Ia) on their surface (8) which provokes one's curiosity to ask the question, whether antigen (Sheep red blood cells - T cell dependent) challenged at CNS (intraventricular) could provoke an immune response in the periphery? It is also known that the magnitude of an immune response for a particular antigen depends on the route of administration. Hence this study was undertaken to determine whether an antigen, when administered into the CNS could provoke an immune response at the periphery and if so, what is its magnitude compared to the same antigenic challenge through intraperitoneal route.

METHODS

Experimental animals were all healthy inbred Wistar strain albino rats weighing approximately 180-200 g maintained under standard laboratory conditions with food and water *ad libitum*. The study comprised 4 major groups namely:

Control group :

This group (n=15) was included in this study to assess the base line preimmunized values of the parameters studied.

Experimental groups :

Group - 1 : Control animals (n=10) immunized to elucidate the normal immune response for the particular dose of antigen used in this study.

Group - 2 : Animals (n=7) with cannula implanted into third ventricle and immunized intraperitoneally were used as sham animals. This group was used to judge the influence of surgical trauma and stress on the elicited immune response.

Group - 3 : Animals (n=7) with cannula implanted into third ventricle and immunized through third ventricle formed the test group.

Implantation of the cannula :

Cannula implantation was performed for precise administration of antigen into the third ventricle and also to avoid any accidental blood vessel rupture during antigen administration whereby the antigen could directly enter into the peripheral circulation. The stainless steel guide cannula (21 gauge) of 2 cm length with appropriate injector cannula (27 gauge) was prepared (9). Keeping the bregma as reference point the guide cannula was inserted into the third ventricle at an angulation of 10 degree using the following coordinates: 1.5 mm lateral, 1 mm below the bregma and approximately 9 mm depth from dura (10). The appearence of CSF in the cannula forms the confirmatory evidence. The cannula was closed by a cap to avoid contamination. The animals of group 2 and 3 were implanted thus and allowed to recover for a week. All animals appeared healthy and no mortality was seen among the among the groups studied.

Immunization :

Sheep red blood cells (SRBC) washed in saline were used as antigen. 20 µl of antigen in saline (30% cells) was injected intraperitoneally in group I & II and into 3rd ventricle in group III. In group II & III the immunization was performed on 7th day after surgery and the day of immunization was considered as "zero" day. All groups were studied simultaneously 5 days after immunization.

Parameters studied :

1. Total WBC count and Differential count (Standard technique)
2. Neutrophil function test :

   Candida phagocytosis -

   Phagocytic index (PI) and avidity index (AI) (11).

   Nitroblue tetrazolium reduction test (NBT) (12).
3. Serum Antibody titre (Direct agglutination)
4. Serum level of Soluble immune complex (SIC) (13).

To avoid circadian rhythm induced variations, the entire sample collection and animal sacrifice were performed between 8.00 and 9.00 am.

Statistics :

To reveal the effectiveness and adequacy of the dose of injected antigen to elicit an immune response, the preimmunized values were compared with the control immunized group of animals by Students' 't' test.

One way analysis of variance (ANOVA) (14) was performed to find out the overall group differences in experimental group of animals. When there was a significant 'f' test ratio, Tukey's multiple comparison was performed to find out the significance between the groups.

RESULTS

The results of various parameters are given in Table I, II and III.

After immnunization the immunized control rats exhibited a decrease in total WBC count (P<0.001), neutrophils (P<0.001), SIC (P<0.01) with an increase in lymphocyte count (P<0.001), PI (P<0.001), AI (P<0.01), NBT (P<0.001) and antibody titre (P<0.001). This indicates that the dose of antigen used in the present study is

TABLE II : Neutrophil function tests in control and immunized groups.

Groups	*Phagocytic index*	*Avidity index*	*NBT*
Control	76.1 ± 6	2.9 ± 0.7	10.7 ± 3
Control Immunized	92.4 ± 4.9	3.7 ± 0.5	21.5 ± 5
Sham Immunized	98.0 ± 2.2	4.0 ± 0.5	12.3 ± 2
CNS Immnunized	97.5 ± 1.9	4.6 ± 0.4	18.0 ± 2
Control vs Control Immnunized	P<0.001	P<0.01	P<0.001
Sham Immunized vs CNS Immunized	NS	NS	P<0.05

All the values are expressed as mean ± SD.

TABLE III : Soluble immune complex and antibody titre in controls and immunized groups.

Groups	*SIC index*	*Antibody titre*
Control	22.27 ± 8.1	1.5 ± 0.93
Control Immunized	12.28 ± 7.7	8.4 ± 0.7
Sham Immunized	19.42 ± 7.1	8.1 ± 2.1
CNS Immnunized	11.35 ± 4.2	7.5 ± 1.6
Control vs Control Immunized	P<0.01	P<0.001
Sham Immunized vs CNS Immunized	NS	NS

All values are expressed as mean ± SD;
NS = Not significant.

TABLE I : Total WBC count and differential count in control and immunized groups.

Groups	*Total WBC count per cubic mm*	*Neutrophils %*	*Lymphocytes %*	*Eosinophils %*	*Monocytes %*	*Basophils %*
Control	16706±1669	27±5	67.9±6.4	3.4±2.58	1.3±0.8	0.3±0.5
Control Immunized	11271±1495	15±2.5	78.4±3.3	3.4±2.5	2.5±1.5	0.5±0.5
Sham Immunized	13035±3208	19±2.7	75.5±3.3	2.1±1.7	3.1±1.2	0.4±0.5
CNS Immunized	7757±1306	12±2.0	83.5±3.1	1.7±1.7	1.7±1.2	0.5±0.5
Con.vs Con.Imm	P<0.001	P<0.001	P<0.001	NS	NS	NS
Sham vs CNS	P<0.05	P<0.001	P<0.001	NS	NS	NS

All the values are expressed as mean ± SD; NS = Not significant

adequate and it alters the baseline levels of all the parameters studied.

Total WBC count :

The intraventricular immunized (IVI) animals showed a significant decrease (P<0.05) from control intraperitoneal immunized (CIPI) and Sham intraperitoneal immunized (SIPI) animals (f=10.63, df 2,18) whereas CIPI and SIPI did not differ among themselves suggesting that intraventricular immunization can reduce total WBC count more effectively compared to intraperitoneal immunization.

Differential count :

The peripheral neutrophil count in CIPI (P<0.05) and IVI (P<0.001) animals showed a marked decrease from SIPI animals (f=11.74 df 2, 18).

The peripheral lymphocytes of IVI animals showed marked increase from CIPI (P<0.05) and SIPI (P<0.001) animals (f-10.84 df 2, 18) suggesting that the route of administration of antigen does play a role in modulating peripheral distribution of leucocytes.

No distinct change in the distributions of eosinophil, monocyte and basophil cells were observed in this study.

Neutrophil function test :

Two independent tests were used to assess the neutrophil functions. The phagocytic activity was assessed by killed Candida phagocytosis and intracellular killing ability by nitroblue tetrazolium (NBT) reduction. The phagocytic ability of neutrophils is expressed as phagocytic index (PI) and avidity index (AI). Phagocytic index was given by the number of Candida positive cells per 100 neutrophils. Avidity index or mean particle number was calculated by counting the number of Candida particles within 100 Candida positive neutrophils and taking the mean for a single neutrophil.

Phagocytic index :

Both SIPI and IVI animals showed a significant increase (P<0.05) from CIPI animals (f 6.13, df 2, 18) and both these groups did not vary among themselves suggesting that this could be due to non-specific cerebral trauma by the surgical procedure of cannula implantation.

Avidity index :

The control immunized and SIPI did not show any variation in their avidity index, whereas IVI immunized animals showed a marked increase in avidity index (P<0.01, F 6.67 df 2, 18) from CIPI animals. As the SIPI animals did not show a significant variation from the IVI animals, this could not be considered as the effect of immunization by different routes.

NBT :

The NBT reduction test in SIPI animals showed a significant decrease from CIPI (P<0.01) as well as from IVI animals (P<0.05, F 14.61 df 2, 18).

Antibody titre and SIC :

None of the groups studied showed any significant variation among themselves in their antibody titre, suggesting that antigen presented at the intraventricular (CNS) route could also provoke a similar response as that by peripheral intraperitoneal antigen administration.

DISCUSSION

The results of this study have been statistically compared in all possible combinations. The comparison of preimmunized animals with immunized control animals revealed that the dose of antigen used was adequate. Leucopenia was characteristically seen in all the immunized groups studied compared to preimmunized animals. This is the effect of immunization and such a characteristic leucopenia after parenteral administration of foreign protein has been reported by Wintrobe (15).

As the capillary surface in 1 gm of brain tissue is approximately 240 sq cm (16), the cannula implantation procedure was adopted to

avoid any accidental fresh damage to the blood vessels. The micro environment of glial cells is influenced by cerebrospinal fluid and neither the pia-glial surface nor ependymal cells are connected by bands of tight junctions (17). The antigen administrated into the third ventricle could easily percolate the brain parenchymal tissue.

During cannula implantation at 3rd ventricle some of the brain regions were necessarily damaged. As neuroimmuno modulation by lesioning procedure has been well established (18) to avoid such interference, sham cannula implanted animals were considered as strict controls to evaluate the effect of immunization through various routes. In the present study SIPI animals showed a significant increase in the neutrophil count with associated fall in lymphocytes. The neutrophils also showed an increased phagocytic index with a decrease in their killing ability compared to the CIPI animals, suggesting that neuroimmunomodulation is also possible by surgical trauma. Existence of immunomodulation due to lesion at various neural structures have been reported earlier (19, 20, 21).

Electrical stimulation of hypothalamus (22), lesion of anterior hypothalamus (23), or cannula implantation (present study) could produce a similar increase in phagocytosis suggesting that this could be due to brain insult or cerebral trauma. The IVI caused a pronounced fall in total WBC count with a decrease in neutrophils compared to sham. When antigen reaches a node in primed animals, there is a dramatic fall in the output of cells in the efferent lymphatics, a phenomenon described variously as 'cell shut down' or 'leucocyte trapping' (24). According to Ackerman et al (25), the major site of norepinephrine innervation in spleen and lymphnode occur within the sites of entry, antigenic capture and exit of lymphocytes. This accumulated evidence suggests that immunization at ventricle could possibly enhance the leucocyte trapping at the lymphoid organs via their innervation whereby the drastic fall in peripheral leucocyte count could be explained.

Differential count, the neutrophil and lymphocytes are always reciprocally related to each other. The altered cell pattern observed in the peripheral blood of the IVI, could also be due to a redistribution of circulating cells as suggested by Selye (26) or it could be due to an altered rate of production of WBC from the bone marrow. In addition, it has also been shown that the neutrophil functions could be modified by the CNS. This modulation could be mediated through serum factors (27). At cellular level neutrophil functions could be influenced by second messengers like cAMP, cGMP etc which regulate the extrusion of lysozyme (28). Though in this study the neutrophil functions were altered, it is not possible to identify the exact component responsible with the available evidence.

For an immune response to occur, either the antigens should come out of the CNS to reach the immunologically competent cells in the periphery or the immunologically competent cells must enter in to the CNS and reach the antigen injected site and produce antibodies *in situ* which must then percolate into the peripheral circulation. In this connection it is pertinent to point out that entry of antigens themselves from the CNS to the periphery has been well documented. Adams and Prawirohardjo (29) found accumulation of erythrocytes in arachnoid villi after injection of such cells into the CNS. According to Davson (30) the red cells are eliminated from the subarachnoid space without the preliminary hemolysis. Moreover, the arachnoid cells display the pinocytic and phagocytic activity and this can transport antigen (31), suggesting the entry of antigen as big as erythrocyte from the CNS to the peripheral circulation is possible. The studies of Jankovic et al (32) have shown that injection of antigen into the CSF causes appearence of antibody in serum impart further evidence for antigen entry into the peripheral circulation.

Hochwald et al (33) had reported a significant number of antibody forming cells in deep cervical lymphnodes of rats after

intraventricular injection of antigen including trinitrophenylated hemocyanin, trinitrophenylated abortus and sheep erythro-cytes. By comparing the dose response in spleen, after intravenous antigen injection they suggested that 20% of intraventricularly injected immunogen drained into the peripheral blood.

Neither the antibody titre nor soluble immune complex level showed any significant change among the groups studied, suggesting that the antigen entry as well as the clearance in intraventricular route is almost equal to intraperitoneal route. According to Hochwald et al (33) in intraventricular injection, the deep cervical lymphnode response is more important than that in spleen. However, these authors did not measure antibody level in serum. Hence it can be concluded from this study that the magnitude of antibody titre level tend to remain the same irrespective of the site of antibody production.

Since the antibody titre is similar in all the groups studied, this study does not support the report of Kaplan and Stereilein (34) who have reported that introduction of antigen into brain might induce tolerance (induction of T suppressor) instead of immune response of rejection (deviation of immunity).

Thus the present study confirms that the privileged nature of CNS is incomplete. Immunization at CNS could provoke a change in the peripheral innate immunity in different magnitudes without altering the specific immunity. Hence it appears that survival of neural transplant depends largely on the local factors, inspite of antigen entry into the peripheral circulation. Further in-depth study is required to confirm this concept.

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