Section - 7

Summary and Conclusions

7. SUMMARY AND CONCLUSIONS

Schizophrenia is a devastating mental illness characterized by debilitating hallucinations, paranoia and delusions, that affect one percent of the world's population. Current researches primarily focus on the neurochemical and biological pathology of schizophrenia. But a subset of research has taken a decidedly different approach. This research postulates that, in at least some cases the immune system causes the disastrous psychotic symptoms of schizophrenia. This autoimmune hypothesis describes that somehow the immune system is triggered to attack the brain producing neurodegeneration and inflammation. For nearly a century ago the autoimmune basis for the onset of schizophrenia and progression have been proposed. This hypothesis continues to grow stronger as more markers of immune dysfunction are linked to schizophrenia.

Schizophrenia as an autoimmune disease was first theorized based on observed commonalities between the onset and progression of well-known autoimmune diseases. In this study we have investigated the circumstantial evidence of autoimmunity in schizophrenia. For this purpose we have selected certain parameters of the immune system like HLA system, serum IL , C-reactive protein and CD4 and CD8 cells. Along with this, we have studied the demographic characteristics and tried to correlate it with the disorder.

HLA study

HLA system comprises polymorphic class I and class II loci. The peculiarity of these regions is the high degree of polymorphism of most loci, that is, these locus encode a multitude of alleles which have evolved to fight a multitude of microbial factors. In the present investigation HLA-Class I genes were studied because HLA-A loci is juxtaposed to a non-classical HLA locus i.e., HLA-G which is involved in the protection of the tropoblast. Besides, many of the proven autoimmune disorder have shown association with HLA.

In the present investigation 136 schizophrenic patients and 150 unrelated controls were included. We have studied the frequency HLA Class I genes in all the cases. Among them the patients comprising of different subtypes were as follows: 118

Paranoid, 9 Disorganised, 1 Catatonic, 5 undifferentiated and 3 Residual. All the subjects were India born schizophrenic patients of West Bengal and recruited from the outpatient department (OPD) of Psychiatry, North Bengal Medical College and Hospital, Siliguri. The typing of the HLA was done with the help of serological as well as PCR SSP method.

The findings of the HLA Class I study yielded the interesting results. The result showed a significantly higher frequency of HLA-A*03 in patients than the control groups. On the other hand HLA-A*31 and HLA-B*51 showed the decreased frequency in the patient groups. At this moment it is difficult to propose the mechanism of association of HLA with schizophrenia. Correlations with several other factors are also need to be established such as low birth weight, viral infections, prenatal infections etc. which are at least common in Indian population to further shed light in this respect. Nevertheless, our result provides the evidence for the possible existence of a susceptibility locus for schizophrenia within the HLA region.

Interleukin study

Several circulating cytokines have been identified that mediate immune response. Cytokines have multiple roles including induction of an antiviral state. Abnormalities in cytokine concentrations many reflect the presence of an infectious or modulation in the immune process. To date, the most frequently studied cytokines in schizophrenia are IL-2 and IL-6. IL-2 is a T-cell growth factor and has been shown to modulate some neurotransmitter systems including dopamine metabolism within the central nervous system. More interestingly, a range of psychiatric manifestations including delusions, delirium, paranoia, hallucinations and lethargy have been observed in patients receiving IL-2 immunotherapeutically. These findings suggest that IL-2 may contribute to the pathophysiology of schizophrenia. IL-6 exerts trophic effects on glial cells, including oligodendroglia themselves, producing increased expression of glial fibrillary-acidic protein. Paradoxically, IL-6 increases intracellular calcium levels during N-methyl-D-aspartate receptor (NMDA-receptor) activation, enhancing neurotoxicity and cell death in granular neurons. Thus IL-6 can have both neurotrophic and neurotoxic effects in different neuronal types and at different developmental stages. This dual role that IL-6 appears to play in the CNS may explain the wide range of psychiatric disorders. Therefore, the present preliminary study was

undertaken to investigate the serum levels of IL-2 and IL-6 in the Indian schizophrenic patients.

For the study of serum level of Interleukins, 50 schizophrenic patients were considered for the present study. They were further classified into two groups, 20 schizophrenic patients who had stopped taking antipsychotic drugs for at least 6 weeks were considered as psychotropic medication free, rest 30 schizophrenic group were under antipsychotic medication. The study comprises of 44 Paranoid, 1 Residual, 4 Disorganized and 1 Catatonic schizophrenic patient. A total number of 30 unrelated, ethnically matched healthy individuals were considered as controls. The assay was carried out with the help of ELISA kit.

The result of the interleukin assay showed the lower level of IL-2 and IL-6 in the patient group. The unique finding of the present study is the significantly lower level of IL-6 in the schizophrenic subjects. To our knowledge this is the first report of lower level of IL-6 in the schizophrenic patients and nowhere else have been reported previously. The immunosuppressive and cytokine modulating effects of the antipsychotic drugs have been found by different studies. In the present study only atypical antipsychotics were prescribed to the patients. Therefore the lower levels of IL-2 and IL-6 observed among the medicating patients in the present study suggest the cytokine modulating activity of atypical antipsychotics. Thus, our finding supports the earlier findings that treatment with antipsychotic drugs affects the cytokine network. On the other hand our findings are not in agreement with the exhaustion theory of schizophrenia, also our findings do not fit well into the Th1/Th2 paradigm or with the Th2 shift hypothesis. Moreover the present findings strengthen the previously reported studies of immune system dysregulation in schizophrenia which may be one of the etiological factors for the disorder.

C- reactive protein study

The roles of immune dysfunction and inflammation have been described in schizophrenia. One of the well known inflammatory marker is C-reactive protein (CRP). We have studied the CRP as it has been hypothesized that some kind of inflammatory process is involved in schizophrenia.

Sera level of CRP were measured for 64 schizophrenic patients. Out of them, 57 were paranoid, 2 residual, 3 undifferentiated and 2 were disorganized type. Latex agglutination test was followed to detect the serum level of CRP.

The elevated level of CRP was observed in 3 patients and 61 patients were found to have normal CRP. All the three elevated cases were found to be of paranoid type. No differences were found in CRP levels among different subgroups of schizophrenia. Further, when the level of CRP was compared to the other demographic variables, only the drug naïve status of the patients showed statistically significant value. The study provides further evidence that some kind of inflammatory process may play a role in the etiopathology of schizophrenia.

CD4+ and CD8+ study

Investigations of lymphocytes in patients with schizophrenia started as early as 1900 AD. Advances in immunologic techniques, as well as a deepening understanding of lymphocyte function have opened the way towards the quantitation of specific, functionally distinct lymphocyte subsets. Initially, these studies focused on T lymphocytes such as CD4 and CD8. The CD4+ T lymphocytes, also known as T helper cells facilitate both humoral and cell-mediated immune processes. In contrast, CD8 cells act to shut off CD4 cell activity when sufficient antibodies have been produced. Changes in T helper/inducer (CD4) and T cytotoxic/ suppressor (CD8) cells are related to a variety of illnesses. The appropriate CD4/CD8-lymphocyte ratio is expected to be 2:1. Ratios below 1:1 indicate serious disorder of the immune system. It has also been suggested that changes in the T-lymphocyte ratio reflects changes in the metabolism of central nervous system cells and it can be used as neural markers in the analysis of psychiatric disorders.

The CD4+ and CD8+ ratio in the blood were estimated by flow cytometry in 20 patients and compared with the same number of control. Although the mean percentage of CD4+ cells were found to be little higher in the patients, it was not significantly higher than the control groups. Also the mean percentage of CD8+ cells is not found to be significantly deviated in patients and control groups. On the other hand the CD4+ and CD8+ subset ratio was found to be normal in both patients and the control. When we compare our results with our own findings on IL-2 and IL-6 it is

a complete contrast in the sense that the lower level of IL-2 and normal CD4+ cell percentage, which is unlike the findings by Zhang *et al.*, 2002 who reported lower number of CD4 cells and lower IL-2. Our results suggest the abnormal production of ILs is not due to the abnormal number of CD4 cells but it may by due to some abnormality in the CD4 cells or else there are other factors responsible for this phenomenon. Moreover, the knowledge about Th17 cells and Treg are emerging. As more and more knowledge about the mechanism of their function would become available, the role of CD4+ and CD8+ in schizophrenia will be understandable in a better way.

Demographic study

During the three year period of study, it was observed that more number of male patients were attending the OPD than the females. Therefore the present study consists of more number of male schizophrenics and they were found to have long duration of illness compared to females. Although the observation hinted the higher vulnerability of men to this disorder in this region, a more comprehensive study is needed in this respect before concluding any remarks. One of the interesting observations of the present study is that, the majority of the schizophrenic patients were not the first child of the family. This finding corroborated with of the study of Sham *et al.*, (1993). Thus the present study strengthens the hypothesis "younger children in a family has a significantly increased risk of later developing schizophrenia". The study also suggests that in addition to the genetic predisposition some environmental factors such as viral infection may play a pivotal role on the onset of the disorder and the older children in the family may act as a source of viral infection for developing fetus in the mother. These infections may alter the neurodevelopmental process leading to schizophrenia to the unborn child in the later years.

Suggestive conclusion

At this moment it is not clearly understood whether the changes in the immune system is the byproduct of the pathophysiology of schizophrenia or directly contributes to the clinical manifestations of the disorder. Moreover it is too early to speculate the autoimmune hypothesis of schizophrenia. On the other hand the results of our present

investigation definitely suggest some kind of immune dysregulation in schizophrenia which may be one of the etiological factors for the disorder. Overall, from our study we can make the following specific concluding remarks:-

1. HLA-A*03 gene may contribute to the risk of the disease or else that there might be a separate gene in strong linkage disequilibrium with HLA-A*03 gene.

2. HLA-A*03 gene may be used as genetic marker for schizophrenia.

3. HLA-A*31, B*51 may act as the protective markers for schizophrenia.

4. The older children in the family may be the source of viral infections, which they transmit to their pregnant mothers and these infections may alter the neurodevelopmental processes leading to schizophrenia.

5. The inflammatory process may play a role in the etiopathology of schizophrenia.

6. The study suggests that some kind of dysfunction in immune system may be involved with schizophrenia.