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Increased Expression of HLA DR2 in Acquired Aplastic Anemia and its impact on response to Immunosuppressive Therapy

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Abstract

Objective: To study the frequency of HLA DR2 status of patients with aplastic anemia and their response to immunosuppressive therapy at a tertiary care hospital.

Methods: Thirty eight consecutive patients of acquired aplastic anemia were evaluated with respect to demographic features, severity of HLA DR2 status and response outcome to immunosuppressive therapy.

Results: The mean age of the patients was 24.6 years + 16.4 with a male to female ratio of 2.8:1. Positivity of HLA DR2 was markedly high in acquired aplastic anemia patients. Twenty four (65%) out of 38 patients as compared to 45 (15%) of 300 healthy controls ($p < 0.0001$) were positive for HLA DR2. Response to immunosuppressive therapy, which included antilymphocyte globulin, cyclosporin and methylprednisolone, was available in sixteen HLA DR2 positive patients and was found satisfactory in 12/16 (75%) patients.

Conclusion: HLA DR2 was significantly higher in patients with acquired aplastic anemia and favourable response to immunosuppressive therapy was also associated with HLA DR2 positivity (JPMA 54:251;2004).

Introduction

By definition aplastic anemia is described as pancytopenia with empty bone marrow in which the marrow is replaced by fat cells. The etiology of acquired aplastic anemia is largely unknown, however in one third of the patients it has been associated with viruses, drugs and chemicals.¹ The way in which these agents produce aplastic anemia is unknown but it has been suggested that both stem cells and microenvironmental factors play a part. Activated CD8+ lymphocytes may play a role in inducing the production of gamma interferon, which lead to nitrous oxide synthesis in stem cells causing apoptosis.²

It has been shown that many autoimmune disorders are strongly associated with certain HLA DR phenotype. Since acquired aplastic anemia has an immune etiology, and it was noted that HLA DR2 phenotype is more common in patients with aplastic anemia³, its expression has been suggested to predict a response to anti lymphocyte globulin and cyclosporin. When aplastic anemia patients were assessed for response to cyclosporin; the response rate in patients with HLA DR2 was significantly higher.^{4,5} Based on these informations, we studied the frequency of HLA DR2 status of patients with acquired aplastic anemia and their response to immunosuppressive therapy.

Methods

This retrospective cross-sectional study was conducted at the Aga Khan University Hospital on patients who presented to the hematology outpatient or inpatient in the hospital during the period of January 1993 to July 2001. The medical record department of the hospital retrieved the medical records of the patients and the data was recorded on a preformed questionnaire. The demographic features including age, sex, month and year of presentation were noted along with severity of aplastic anemia.

Patients with acquired aplastic anemia were diagnosed according to the International Study of Aplastic Anemia Group.⁶ The severity was classified according to the Camitta classification.⁷ Patients of Fanconi's anemia, hypoplastic myelodysplastic syndrome and hypoplastic acute lymphoblastic leukemia was excluded.

Patients received immunosuppressive therapy which consisted of injection anti lymphocyte globulins 10 mg/kg daily for 5 days, injection methylprednisolone 2mg/ kg for 4 days and then gradual tapering off with oral steroids within two weeks and cap. cyclosporin 10 mg /kg per OS daily for six months. However, the dose of cyclosporin was titrated according to the serum level. Patients who received immunosuppressive therapy were assessed for response at 6 months.

Response criteria was defined as follows:

- a) Complete response: Transfusion independence to both packed red cell and platelets.
- b) Partial response: Transfusion independence to one component, either packed red cells or platelets.
- c) No response: Transfusion dependence to both packed red cells and platelets.

Methodology for HLA DR analysis

HLA DR analysis by polymerase chain reaction-sequence specific primer

We applied low-resolution HLA typing results for class II using sequence specific primer, a form of polymerase chain reaction (One Lambda, USA) The final PCR product was visualized on a 1.5% agarose gel and the presence or absence of appropriately sized bands was assessed. The results of typing were then converted to already established HLA phenotypic equivalents and HLA DRB 1*15 and DRB1*16 were analyzed as HLA DR2. The control group (n=300) comprised of healthy individuals of Pakistani population belonging to all ethnic groups, the majority being donors for bone marrow and renal transplantation.

Statistical data was analyzed using statistical package SPSS for Window's version 10.0. Chi-square test was used to analyze p value. Statistical differences between various groups and a p value of <0.05 was consistent as significant.

Results

Thirty eight consecutive patients meeting the criteria for the diagnosis of aplastic anemia were evaluated for HLA typing. Further characteristics of these patients are given in Table. The male to female ratio was 2.8:1. The mean age was 24.6 years and ranged between 4 years and 70 years. Five patients were less than 14 years of age.

Table. Characteristics of patients.

Total number of patients	38
Male to female ratio	2.8:1
Mean age (years)	24.6±16.4
Median age (years)	21 (range 4-70)
HLA DR2 positive	25 (65.7%)
HLA DR2 negative	13 (34.2%)
Severity of aplastic anemia	
Severe	27
Very severe	11

Twenty five (65.7%) of 38 patients were HLA DR2 positive. The frequency of HLA DR2 positivity in the control group (n=300) was 45 (15%) indicating higher frequency of HLA DR2 in patients with aplastic anemia ($p < 0.0001$).

Out of the total thirty eight patients, the response to immunosuppressive therapy was evaluable in nineteen patients. Sixteen and three patients belonged to HLA DR2 positive and negative groups respectively. Out of the sixteen HLA DR2 positive patients, thirteen had severe aplastic anemia whereas the other three belonged to very severe group. Among the three HLA DR2 negative patients, two were severely aplastic and the remaining one patient was suffering from very severe aplasia. All of them had received anti lymphocyte globulin, steroids and cyclosporin as described above.

The response rate was 12/16 (75%) evaluated at six months in HLA DR2 positive patients. Eleven patients had complete response whereas one had partial response. Ten of the thirteen severely aplastic and two of the three very severely aplastic patients responded to immunosuppressive therapy respectively.

In HLA DR2 negative group, two out of three responded to immunosuppressive therapy.

Discussion

Keeping in view the pathogenesis of acquired aplastic anemia, there is little doubt that the bone marrow stem cells are destroyed by autoimmune process², which is also supported by the evidence of response to immunosuppressive therapy.⁸ Genetic susceptibility and association of HLA types with certain autoimmune diseases has been reported.⁹ Increase in frequency of HLA DR2 expression was noted in patients with aplastic anemia.^{3,4,10} The expression of HLA DR2 varies significantly in different ethnic groups. It was reported as 25.3% for North American Caucasians (n=1145), 14.8% for Hispanics of Mexican origin (n=88), 33.9% for American Blacks (n=168), 18.3% for Orientals (n=536), 17.9% for Italy, 26.5% for Germany and 21.2% for France.^{4,11}

Those patients who have been diagnosed as a case of acquired aplastic anemia, the frequency of HLA DR2 positivity was found as 31/75 (41.3%), 23/44 (44.2%) and 104/242 (43%)^{3,4,10} in various studies. Similarly, in this study, a significantly higher expression of HLA DR2 when compared to unrelated healthy controls ($P = 0.0001$).

HLA DR2 status of the aplastic anemia patients have been examined for the ability to predict a clinical response to immunosuppressive therapy, however no definitive statement could be made regarding the predictive value of HLA DR2 positive status in predicting the outcome. Conflicting results have been reported so far and it was found to be predictive for immunosuppressive therapy in some studies

when comparing the HLA DR2 positive and negative group as reported by Osman et al⁴ 73.3% versus 30%, Shinji et al⁵ 71% versus 35% and Jaroslaw et al¹⁰ 34% versus 50%. Similarly response rates to immunosuppressive were not significantly different in HLA DR2 positive and negative patients in other studies.³

In our study, we only focused on the response of combination of immunosuppressive in HLA DR2 positive subjects and we found that HLA DR2 positivity is significantly associated with response to immunosuppressive therapy. However no definitive statement could be made regarding the predictive value of HLA DR2 positive status in predicting the outcome in our study because of smaller number of patients in HLA DR2 negative group.

It is now accepted that more than 50% of patients with aplastic anemia have an immunological basis of the disease and it is postulated that the autoreactive T cells that escape negative selection are normally kept under control by largely unidentified regulatory mechanisms. And when these mechanisms fail, the products of certain HLA molecules with particular peptides can activate self-reactive T cells.¹² These particular groups of patients who are HLA DR2 positive showed an excellent response to combination immunosuppressive therapy and further support the immunological basis of the disease.

At present, however, it is difficult to decide regarding the use of immunosuppressive versus transplantation in aplastic anemia patients solely on the criterion of HLA status because a substantial proportion of HLA DR2 negative patients also responded to immunosuppressive therapy suggests some other mechanisms also play their role in the pathogenesis of aplastic anemia.

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