Role of HLA antigens in Rh (D) alloimmunized pregnant women from Mumbai, Maharashtra, India

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Immunogenetic studies in various diseases provide potential genetic markers. We have studied the incidence of HLA A, B, C, DR and DQ loci antigen in Rh (D) antigen isoimmunized mothers compared to those nonimmunized isoimmunized Rh negative mothers. Seventy six mothers who were immunized to Rh (D) antigen due to pregnancy (responders) and fifty four mothers who did not develop Rh (D) isoimmunization despite positive pregnancies (nonresponders) were selected for the study. Standard methods of serological HLA typing, ABO and Rh (D) groups, and screening for Rh D antibodies were used. 392 unrelated individuals from the population were compared as controls. In addition 45 unrelated individuals from the same population were typed for HLA DRB and DQB gene using PCR-SSP kits. The genotype frequencies of HLA A2, A3, A28, B13, B17, B35, B52, B60, Cw2, Cw6, DR4, and DQ3 were significantly increased, while the frequencies of the HLA A11, A29, A31, B7, B37, B51, Cw1 and DR9 were decreased in the responder women when compared to the non-responder women. HLA A30 (19) split antigen was not identified in immunized women while HLA A23 (9) split antigen was not identified in non immunized women. HLA A3, B17, Cw2 and DR4 showed a significant relative risk among the immunized responder women. When compared with Rh immunized women (responders) reported from USA, England and Hungary the phenotype frequencies of HLA A11, A24, A28, B5, B17, B40, DR2 and DR5 were increased while HLA A23, B8, B18, and DR6 were decreased in the Indian Rh immunized women. Two locus haplotype frequency analysis observed among the responders women revealed that among the significant haplotypes expressed A2–B5, B7–Cw1, DR2–DQ1 were highly significant haplotypes in positive linkage, while A1–B5, and A1–B7 were in significant negative linkage disequilibrium. The haplotype frequencies were ≤ one when these common haplotypes were compared with control population. Thus in the present study it is evident that the inheritance of HLA A3, B17, Cw2 and DR4 increases the relative risk factor by 2.6 times among Indian Rh isoimmunized women. Further, it is evident that there are significant differences in the observed HLA antigen frequencies and two locus haplotypes in Rh isoimmunized women when compared to women from USA, UK and Hungary due to extreme HLA polymorphism in different populations of the world.

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1. Introduction

The Rh (D) antigen is a potent immunogen but only a small portion of Rh (D) negative pregnant individuals respond to it (Mollison et al 1993). Several factors are known to influence Rh immunization. In our earlier report (Gupte and Kulkarni 1994) we suggested that ABO incompatibility and Rh-negative pregnancies have lesser impact than the parity of the women in Rh immunization. Transplacental hemorrhage is the major

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source of Rh immunization (Woodrow 1970; Clarke and McConnel 1972). Before the discovery of anti D immunoglobulins, Walker (1958) and Boggs et al (1963) reported a 4.8% incidence of Rh immunization among Rh negative mothers. In India, about 5% of the population are Rh (D) negative and 1.7% of Rh (D) negative pregnant women develop Rh antibodies (Gupte and Kulkarni 1994). Since majority of the patients are not covered by health insurance and some Rh (D) negative mothers are unable to buy costly anti D immunoglobulins, we are still far from eradicating Rh alloimmunization from India.

Immunogenetic studies in many diseases have provided potential genetic markers for immune response studies (Tiwari and Terasaki 1985). During the international workshop held in 1972 no association between HLA and immune response to Rh (D) antigen was reported (Petrayani et al 1974). Later studies have revealed positive as well as negative correlation with class I and class II antigens (Van Rood et al 1975; Murray et al 1976; Darke 1977; Darke et al 1983; Kruskall et al 1990) with Rh (D) isoimmunization from different parts of the world. Further, HLA phenotyping of women known to have anti-D antibodies early in pregnancy seems to be an effective way to assess the probability of severe haemolytic disease of the New Born (Hilden et al 1995; Neppert et al 1999). As there is a fairly high incidence of haemolytic disease of the new born among Indian women and a significant difference in the HLA antigen and haplotype frequencies in different populations, and a paucity of data on the impact of Rh (D) isoimmunization in Indian women we carried out the present study.

2. Materials and methods

2.1 Samples

One hundred and thirty Rh (D) negative women attending the antenatal clinic at Nowrosjee Wadia Maternity Hospital, Mumbai between 1994–1998 were selected for this study. They were divided into responders and non-responders for the Rh antigen D depending on whether they developed demonstrable anti-D antibody after one or more pregnancies with Rh (D) positive fetus. They were immunologically separated into two groups as follows: Group I: Seventy-six Rh (D) immunized women (responders) were referred to the hospital from small towns and the mothers were not aware of their Rh status. Group II: Fifty four non-immunized pregnant women were retrospectively selected from the hospital out of which 41 had more than 2 Rh (D) positive pregnancies but were not administrated prophylactic anti-D and yet did not respond to D antigen. Thirteen women who had large (> 10 ml) fetal cell leakage during the first pregnancy formed the remaining of non-responders group. These patients also did not receive anti-D immunoglobulin and they were assessed for isoimmunization every week up to 3 months after delivery. From the same ethnic population 392 unrelated individuals were compared as control (Shankarkumar et al 1999).

2.2 Serology

ABO and Rh (D) grouping were performed in the samples collected by the standard method (Bhatia 1977). The enzyme (papain-cysteine) technique was used to detect the Rh antibodies. Detection of fetal cell leakage on stained EDTA blood smear was done by Nierhaus Betke technique (Dacie and Lewis 1967). The amount of fetal cell leakage was calculated using the standard graph published by Mehta et al (1976).

2.3 HLA typing

Ten to fifteen millilitres of venous blood (in heparin 50 IU/ML) was collected in a sterile tube from each pregnant mother. The lymphocytes were isolated by density gradient centrifugation on Histopaque (Boyum 1968). HLA A, B, and C locus antigens were identified by NIH two stage microlymphocytotoxicity assay (Terasaki and McCelland 1964). HLA DR and DQ locus typing were performed on B-lymphocytes isolated using a miniature nylon wool column (Manikasundari et al 1984) and a long incubation method. A total of 238 HLA antisera were used for defining 16 specificities of HLA A locus, 22 for HLA B locus, 5 for HLA C locus, 10 for HLA DR locus and 3 for HLA DQ locus antigens. These antisera were commercial (Biotest, Germany; Pelfreez, USA); gifted (NIH, Bethesda) as well as indigenous (Shankarkumar et al 1998) in origin. The typing tray included a minimum of 3 antisera for each serotype specificity.

2.4 Statistical analysis

The phenotype frequency (PF); genotype frequency (GF); two locus haplotype frequency (HF); coefficient of linkage disequilibrium (delta) and ‘t’ value were calculated following the methods described by Baur and Daniloves (1980).

3. Results

3.1 HLA gene frequencies in Rh negative women

The percentage gene frequencies of HLA A, B, C, DR and DQ loci antigens among the 76 immunized

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