Original Articles

Varied distribution of RhD epitopes in the Indian population

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ABSTRACT

Background. Inhabited by more than 4000 caste and tribal groups, India has an extremely heterogenous population. For thousands of years many tribal groups have practised endogamy and are practically genetically isolated. Traditionally, polyclonal anti-D reagent has been used for RhD typing; though monoclonal antibodies are increasingly being used. As a result, blood banks find it difficult to assign the RhD status to an increasing number of people. As monoclonal anti-D typing reagents may not detect all RhD antigen epitopes, we studied the RhD antigen epitope heterogeneity in different population groups in India.

Methods. Red cells of 5315 RhD-positive individuals belonging to different castes and tribes of India were tested with 30 different epitope-specific monoclonal anti-D antibodies.

Results. No single monoclonal antibody could detect all RhD-positive red cells detected by polyclonal antisera. The highest proportion of D antigen was detected by LHM 76/55 and BRAD-8 (98%) monoclonal antibodies.

Conclusion. We need to determine the correct mix of monoclonal antibodies that will detect nearly all RhD antigens detected by polyclonal anti-D sera. Similarly, before accepting monoclonal anti-D for therapeutic use, it would be necessary to determine the appropriate ones for use in the Indian population.

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INTRODUCTION

The Rh antigen varies quantitatively and qualitatively.[1] It has been recognized that the D antigen is not a single entity but made up of antigenic determinants.[2] Red cells of rare D-positive people who lack part of the D mosaic are called partial D variants.[1] If exposed to the appropriate red cells these individuals can produce antibodies against the missing part of their D antigen. RhD antigen status is usually determined by testing with either polyclonal or monoclonal anti-D. The advent of human monoclonal anti-D with a unique specificity led to the concept of epitopes.[3] Monoclonal antibodies (MAbs) provide unlimited supplies of reagents of identical specificity which are ideal for definition of partial D antigen.

Since the time MAb reagents became available in the Indian market, blood banks have been encountering more cases with doubtful RhD status. We have also observed an increasing number of cases referred to us for confirmation of the RhD group. It is possibile that the reagents produced in western countries may not be suitable for India as D antigen is genetically controlled and major variations may exist in the D antigen profile of Caucasians and Indians.[4] The incidence of different partial D variants in our population is at variance from what has been reported from western countries.[5-8] Major differences between D variants in western and African populations have also been reported.[9]

The administration of prophylactic anti-D immunoglobulin to RhD-negative women after delivery of an RhD-positive infant has been successful in reducing the incidence of Rh alloimmunization.[10,11] Trials using monoclonal anti-D prophylaxis are ongoing in western countries.[12,13] Would these be suitable for anti-D immunoglobulin prophylaxis in the Indian population as the profile of D antigen epitopes can vary from population to population. The Indian population is extremely heterogenous and is distributed among no less than 4000 castes and tribes. These castes and tribes are still largely genetically isolated because of the practice of endogamy for thousands of years. Hence, it is expected that the RhD epitopes may be distributed differentially among different caste or tribal groups. Due to these variations, we felt a need to study the D antigen epitope in an Indian population. It is vital for the safe and efficient practice of transfusion that Rh typing reagents used in India are reliable and suitable for our population.

Different epitope-specific monoclonal anti-D produced in other countries (UK, Germany, France, USA and Japan) were evaluated for reactivity with our population. Some of these MAbs are available commercially as RhD typing reagents. We studied 100 samples each from different castes and communities, as a representative sample of the population to detect the presence or absence of reactivity with MAb.

METHODS

Thirty MAbs, 27 of IgG type and 3 of IgM type were used. LHM 76/55, LHM 77/64, LHM 70/45, LHM 76/58, LHM 169/80, LHM 76/59, LHM 174/102, LHM 59/19, LHM 59/20, LHM 50/3.7 and ESD-1 were obtained from Dr Robin Fraser, Scottish National Blood Transfusion Service; OSK-3, OSK 3–1, OSK 3–3 from Osaka Red Cross Blood Center, Japan; BRAD-1, BRAD-2, BRAD-3, BRAD-4, BRAD-5, BRAD-6, BRAD-7, BRAD-8, H 27, 2B6,

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RUM-1 and AB5 from the International Blood Group Reference Laboratory (IBGRL), UK; AR and Co88 from Centre Regional de Transfusion, France; and GLR-02 and SF11D8 from Stanford University Medical Center, USA.

Blood samples of different Indian castes, communities and tribal groups were obtained largely from among the 14 million population of Mumbai city and were screened with 30 monoclonal anti-D culture supernatants. The samples collected were from various camps organized by our institution, blood banks and the antenatal outpatient department. All cells were tested with standard polyclonal anti-D antibody using standard techniques[14] and the Rh-positive samples were tested with a battery of 30 epitope-specific MAbs. To one volume of papainized red cells, one volume of MAb was added, mixed well in micro tubes and incubated for 45 minutes at 37 ºC for IgG MAb. For IgM MAb, one volume of 2% red cells suspension was added to MAb and kept for 45 minutes at room temperature (22 ºC). The tubes were then centrifuged at 1000 rpm for a minute and the results recorded.

RESULTS

Blood samples of 5315 RhD-positive subjects of different castes and communities were screened using a panel of 30 epitope-specific MAbs. A negative reaction with any monoclonal anti-D was repeated and if consistent was considered as an absence of that particular epitope. A negative reaction with 1 to 8 monoclonal anti-D was observed in 1339 samples (25.2%), of which 70 (5.22%) samples gave negative results with 5–8 MAbs. The communities which predominantly showed a negative reaction with some monoclonal anti-D were *Bhandari* (55%), *Vatalia Prajapati* (70%), *Halai Lohana* (38%), *Hindu Mahadeo Koli* (51.5%), *Thakar* (70%), *Halai Memon* (75%), Protestant Christians (40%) and Parsees (41.6%). A positive reaction with all monoclonal anti-D was obtained in 30%–92.5% of samples. In *Vaishnavi*, *Boudha*, *Kshatriya* and *Sanchor Jain Samaj* communities, about 90% of subjects showed a positive reaction with MAb.

Thirty-six per cent and 41.7% of blood samples from Parsee and Christian communities, respectively, showed a negative reaction with MAb. *Boudha* community had the lowest incidence of negative reaction with MAb. BRAD-3, BRAD-7, H 27, SF11D8 and LHM 59/20 frequently had a negative reaction in the Parsee community while BRAD-4, BRAD-8, H 27, GLR-02, AR and LHM 77/64 frequently had a negative reaction in the Christian community. The negative reaction with some MAbs was more frequently seen in tribal groups (34.65%) compared with non-tribal subjects (23.61%) indicating the absence of these epitopes in tribal populations. Chi-square test revealed there was significant difference between these two populations ($p<0.001$). Among the tribals, a negative reaction with MAb was more prominently seen in the *Hindu Mahadeo Koli* and *Thakar* communities.

The reactivity of the 30 epitope-specific MAb panel in RhD-positive Indian population is shown in Fig. 1. The positive reaction rate of BRAD-3, BRAD-7 and H 27 was lower than that for other MAbs. None of the MAbs had a 100% positive reaction in all blood samples. BRAD-1, BRAD-6, BRAD-8, 2B6, LHM 70/45, LHM 76/55, LHM 50/3.7 and LHM 59/19.5 showed a 97%–98% positive reaction rate.

Figure 2 shows the percentage of negative reactions with MAb among subjects with absence of reactivity to 5–8 monoclonal anti-D more frequently. The negative reactions were more often seen in the *Vatalia Prajapati*, *Hindu Mahadeo Koli* and *Halai Memon* communities.

DISCUSSION

Our study is likely to be beneficial in choosing an appropriate prophylactic monoclonal anti-D for antenatal patients in India. The screening of samples with 30 epitope-specific MAbs revealed that none of the culture supernatants reacted with all the RhD-positive red cells. This shows that no single monoclonal anti-D would give correct results in the whole population. Some MAbs such as BRAD-1, BRAD-6, BRAD-8, 2B6, LHM76/55, LHM 50/3.7, LHM 59/19.15 and LHM 70/45 showed 97%–98% reactivity with RhD-positive individuals. A blend of few of these MAbs might be able to detect all RhD-positive Indians but this will require further studies in different population groups. Of a total of

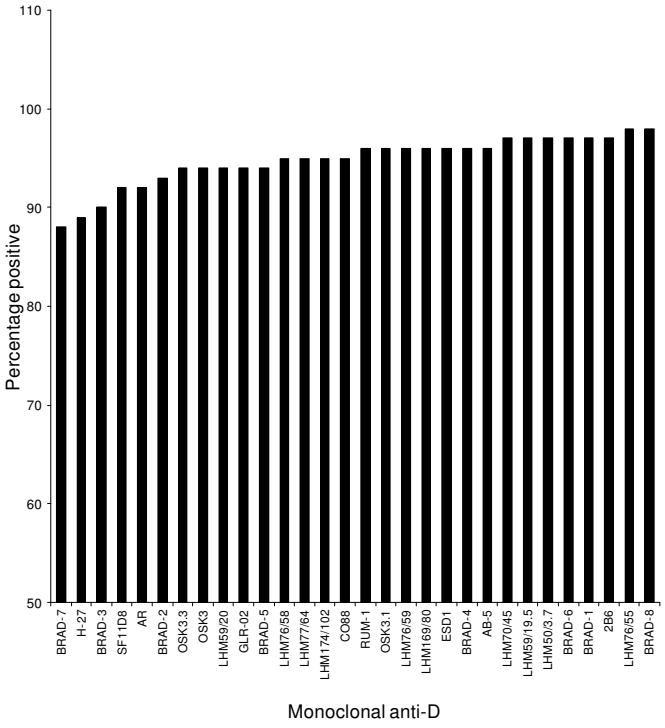

FIG 1. Percentage of population positive with epitope-specific monoclonal anti-D. None of the monoclonal antibodies reacted with all RhD-positive individuals tested in the study. The negative reaction rate of BRAD-7, H 27 and BRAD-8 was less than that for other monoclonal antibodies.

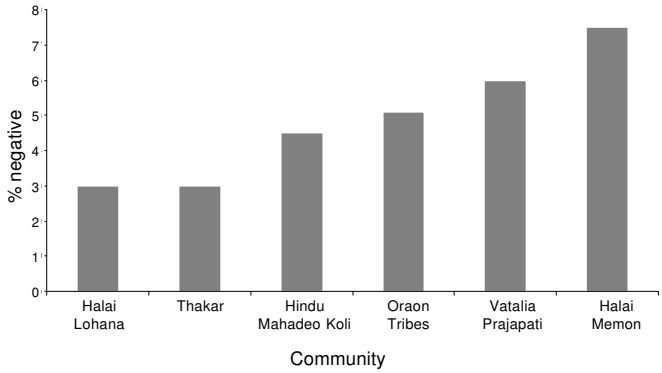

FIG 2. Percentage negativity with 5–8 epitope-specific monoclonal anti-D in selected communities. The negative reaction with monoclonal antibodies was more frequently seen in *Vatalia Prajapati* and *Halai Memon* communities.

5315 subjects tested, 74.8% showed a positive reaction with all MAbs. A negative reaction with 1 to 8 MAbs was seen in 25.2% of subjects.

As India has enormous genetic, cultural and linguistic diversity, our population is ideal for genetic studies. We included various castes, tribes and communities among our sample. There was variation in reactivity with MAbs in various communities. The communities, which predominantly showed negative reactions with MAbs were *Bhandari*, *Vatalia Prajapati*, *Halai Memon*, Protestant Christians and Parsees. Analysis of subjects of different religions such as Parsees and Christians showed a negative reaction with MAbs more frequently. Both the communities are known to have a higher incidence (15%–17%) of RhD-negative group.[15] If MAbs of different epitope specificities used in our study are employed as reagents to test the blood samples of the above communities, more discrepant results will be obtained. As more than 90% of samples of the remaining communities showed appropriate results, there would be comparatively less problem if these MAbs are selected as reagents. The percentage of subjects showing positive reactivity with BRAD-1, BRAD-7 and H 27 anti-D was less compared with other MAbs used in our study. Only 15 of 30 MAbs showed >95% positive reaction with Rh-positive subjects in our study population. Hence, our study shows that if these MAbs are used as reagents for testing in the Indian population more discrepancies in D typing will be observed.

It is believed that tribal people who constitute about 8% of the total population are the original inhabitants of India.[16] The reactivity of D antigen with MAbs was also studied in some tribal groups and compared with data from non-tribal groups. A negative reaction with MAbs was found more frequently in the tribal population than in non-tribals ($p<0.001$). The endogamous nature of tribal groups may explain this observation as other genetic markers also show significant difference in prevalence in the tribal groups.[17]

Trials have been done in UK using BRAD-3 and BRAD-5 as anti-D prophylaxis.[18] These antibodies efficiently clear D-positive cells from D-negative subjects.[19] Prophylactic anti-D prepared from BRAD-5 and BRAD-3 may be marketed in the near future. Hence, culture supernatants of these MAbs were included in our study. BRAD-5 reacted with 94% and BRAD-3 with 90% of the RhD-positive population studied**.** These results indicate that this prophylactic anti-D will fail to provide protection to a mother if her RhD-positive foetus has epitopes against which these antibodies have not been raised**.** It would be necessary to conduct clinical trials in the Indian population before accepting these for therapeutic use. MAbs raised against the epitopes of D antigen predominantly found in our population would be ideal for anti-D prophylaxis in India**.**

The incidence of weak D varies from 0.3% to 0.7% in UK and the USA and is reported as 0.016% in Chinese donors in Hong Kong.[6–8,20] Muller *et al.*[21] found significant differences in the regional distribution of the 3 most common weak D types by PCR screening in Germany. Okubo *et al*.[22] reported the incidence of partial D to be 0.0005% in Japanese; this is lower than that in western countries**.** The incidence of D^u was found to be 1.7% in blacks, 0.3% in whites and 0.3%–0.5% in a western Indian population.[23,24] Our study shows that a single MAb against the RhD epitope will miss a large number of RhD-positive individuals in India. A mix of MAbs to different epitopes of RhD antigen will have to be worked out so that a majority of D-positive Indian population (about 99%) will be assigned the correct RhD status. Using this mix, a study on the Indian population needs to be done to assess whether a particular reagent can be used for all castes and communities in India. It would be necessary to conduct a clinical trial of prophylactic MAbs in India before accepting it for therapeutic use.

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