Smallpox and the ABO Blood Groups in Brazil

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SUBSTANCES SEROLOGICALLY RELATED to the human ABO groups are found in a variety of organisms, including bacteria. This has led Mourant (1954) and Livingstone (1960), among others, to speculate that the antigens of infecting organisms are the primary factors responsible for the ABO polymorphism in man. Vogel, Pettenkoffer, and Helmbold (1960) have elaborated this suggestion by proposing that smallpox selects against blood groups A and AB. As evidence, they cite the predominance of group B over A in central Asia, the alleged greater antiquity of smallpox in Asia than in the rest of the world, the presence of A activity in cowpox–chick embryo homogenates, and a personal communication from Livingstone of a possible disturbance in blood groups of multitribal West Africans with severe smallpox scars. These arguments have been criticized by Springer and Wiener (1962), who reject correlation of present-day *in vitro* findings with historically remote epidemics. They question whether the one tested cowpox strain is sufficient for extrapolation to other strains of cowpox and smallpox. They also point out that chickens possess A-specific activity, so that the A-like antigen detected by Pettenkoffer might have derived from the chicken egg rather than the cowpox virus. Finally, they quote the conclusion of Muschel and Osawa (1959) that the blood-group antibodies are "relatively insignificant in the presence of an antiserum directed against the entire antigenic mosaic of the organism." In rebuttal, Pettenkoffer *et al.* (1962) suggest that their cowpox strain contained attenuated smallpox virus and refer to preliminary, unpublished observations of a significant association with groups A and AB of severe smallpox scars in India and of postvaccination reactions in Germany. They defend the thesis that the A-like antigen in their experiments was viral rather than avian, but Harris, Harrison, and Rondle (1963) have found that egg materials possess A-like substance while the Lister strain of cowpox does not. Thus the unpublished epidemiological studies are the principal evidence in favor of a simple relation between the A gene and susceptibility to smallpox.

MATERIAL AND METHODS

We have recently completed observations at the Hospedaria de Imigrantes in São Paulo on more than 1,000 families migrating from northeastern Brazil,

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mostly from the interior of the states of Bahia and Minas Gerais (Morton, 1964). In the course of this study, 6,414 persons submitted to a medical examination in which the adults were asked if they had ever had smallpox (*bexiga*) or had ever been vaccinated against smallpox. Persons responding affirmatively were asked if they retained the scars and, if so, to demonstrate them, while persons answering negatively were questioned about the cause of suggestive scars. Smallpox or vaccination was recorded only when history and scars were concordant. Thus a history of smallpox without multiple facial scars was considered negative as was a vaccination that left no scar, while multiple scars without a history suggestive of smallpox were neglected. Young children were examined, but their medical history was obtained from their parents and older sibs. In this way, we believe that no case of acne and few if any cases of chickenpox (*catapora*) were confused with smallpox. There were 87 cases of smallpox (1.4%) and 1,407 vaccinated persons (22%).

Blood drawn during the medical examination was kept at 4° C and typed blind the next day. Anti-A titers were determined for 638 medically examined O and B mothers, of whom 15 had smallpox scars and 192 were vaccinated. Titer was recorded as the number of tubes in a threefold dilution showing agglutination of A_1 cells. The tests were all read microscopically after one hour at room temperature, a weak reaction being considered positive and a trace reaction negative. The cells were then incubated 15 minutes at 37° C and scored for hemolysis.

These data have been analyzed at the Computing Center of the University of Hawaii by a general multiple regression program (MULREG) for the IBM 7040. All estimates and probability levels are based on multiple regressions from which nonsignificant variables, other than the relevant smallpox, vaccination, or A + AB frequency, were excluded.

RESULTS

Among unvaccinated subjects, the frequency of smallpox scars increased with age ($P < 10^{-9}$) and with Negro ancestry ($P < 10^{-5}$), which was estimated by the medical examiner (E.A.) from physical appearance on a 7-point scale. The racial association was unexpected, although a sixteenth century smallpox epidemic in Recife was especially severe among Negroes and Indians (Freitas, 1945), the morbidity (but not mortality) from smallpox is higher in Africa than in other continents (Morais, *et al.*, 1962), and casual observations have suggested a greater susceptibility in Negroes than in whites (Cecil and Loeb, 1959). We have no evidence to indicate whether the observed racial effect is genetic or environmental, nor whether it is due to greater morbidity or scarification in Negroes. There was no significant effect of social level, literacy, inbreeding, sex, geographic area, or population density. Allowing for age and race, the incidence of smallpox scars was 1.45% among persons with the A gene and 1.91% in O and B individuals. This nonsignificant difference ($-.46 \pm .38\%$) is opposite in direction to the unpublished observations from India. The fatality rate from variola is low in Brazil (Morais,

1955), so differential mortality of persons carrying the A gene is not a likely explanation of the discrepancy.

The vaccination rate is significantly dependent on literacy, age, and geographic area and decreases with inbreeding ($P < .001$), suggesting that Brazilian estimates of consanguinity effects may be biased by social factors (Krieger and Freire-Maia, 1962). The partial regression on race is nonsignificant.

The mean anti-A titer was 5.31, decreasing with age ($P < .01$) and higher in O than B mothers ($P < .01$). The difference between mothers with smallpox or vaccination scars and unscarred mothers was .097 ± .078, which is not significant. Hemolysis was visible for 64% of O mothers and 41% of B mothers ($P < 10^{-6}$). Allowing for this, the difference in percentage of visible hemolysis between scarred and unscarred mothers was 2.4 ± 4.1. Excluding vaccinated cases, the difference between mothers with smallpox scars and unscarred mothers remains nonsignificant for both titer and hemolysis.

SUMMARY

In Brazilian material, vaccination and smallpox did not significantly affect the titer of A antibodies or the frequency of anti-A hemolysins. Smallpox scars were not more frequent in persons with the A gene. Our observations do not support the hypothesis that an A-like antigen alleged to be common to cowpox and smallpox has been a selective force acting on the *ABO* locus.

NOTE ADDED IN PRESS

Dr. James Garlick (personal communication, September 2, 1964) found no association between ABO type and smallpox incidence or severity in a Nigerian sample. He noted "a suggestion of increased average severity from M through MN to N." We therefore tested this association and found that the dosage of the *N* gene is positively but not significantly correlated with smallpox incidence in our sample ($t = 1.81$, $P = .07$, by a two-tailed test).

While this may merit further study, it seems safe to conclude that no association between smallpox and any human polymorphism has been demonstrated.

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