AUSTRALIA ANTIGEN IN SINGAPORE—I FREQUENCY IN BLOOD DONORS

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SYNOPSIS

2,563 of 2,604 (98.4%) Singapore blood donors presenting during the month of January, 1971, were screened for the presence of Australia antigen. The antigen was detected in 79 of these apparently normal subjects (3.08%). The frequency was significantly higher in Chinese compared to Malays and Indians. The significance of these findings to the acceptance of donors at the Singapore Blood Transfusion Service is discussed.

INTRODUCTION

Australia antigen (Au) is the name given to a factor first identified in the serum of an Australian aborigine (Blumberg *et al*, 1965a). Subsequent studies have revealed an association between Au and long-incubation hepatitis (serum hepatitis; type B hepatitis; or MS-2 hepatitis), giving rise to the alternative name of Hepatitis-associated antigen (HAA). Experience in Singapore inclines us to accept the non-commital name of Australia antigen (Fung *et al*, 1971). The literature in this field has recently been reviewed (WHO, 1970; Shulman, 1970).

The association of Au with serum hepatitis is a finding of considerable practical importance in relation to transfusion of blood and blood-derived products. Several reports have documented the transmission of Au-positive blood (Okochi and Murakami, 1968; Gocke *et al*, 1969). In Western countries the incidence of positive blood donors is of the order of 0·1% or less. The frequency is much higher in the tropical countries of Asia (Blumberg *et al*, 1965b) and Africa (Bagshawe *et al*, 1971). In a previous limited study of 109 Singaporeans only 1 subject was Au positive, as detected by the relatively insensitive technique of double diffusion in agar (Blumberg *et al*, 1965b).

In order to assess the incidence in Singapore, sera of blood donors were screened for Au using an electro-osmodiffusion technique (Yap, Ee and Simons, 1971). The results are reported in this paper.

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SUBJECTS AND METHODS

2,563 of the 2,604 (98·42%) apparently normal blood donors presenting during January, 1971, were studied. Serum samples were obtained from clotted venous blood and held at 4°C for up to 48 hours. Samples not tested by 48 hours were frozen at −20°C until later study.

An electro-osmodiffusion technique was employed as described elsewhere (Yap, Ee and Simons, 1971). Briefly, this technique involves the electrophoresis in agar of 10 lambda (0·01 ml.) of test serum against 10 lambda of serum containing antibodies to Au. The antiserum was obtain in the first instance from Dr. J. D. Mathews and later from a multiply-transfused haemophiliac patient in Singapore. The immuno-chemical relations between these two and other antisera are discussed separately (Yap, Ee and Simons, 1971). Using LKB equipment 90 sera can be tested for Au on each tray. Four trays can be run at one time. Thus 360 sera can be screened by one technologist in a day. The tests were performed at the WHO Immunology Research and Training Centre. Following identification of Au-positive donors the Blood Transfusion Service was notified and a check made on the name and location of the recipient. The transfused patients were then followed closely by frequent examination of blood samples for evidence of Au, of an immune response to Au, and of liver dysfunction. The follow up study will be the subject of a subsequent report.

RESULTS AND DISCUSSION

The distribution of blood donors according to ethnic type is shown in Table I. The classification of ethnic groups is that adopted for the 1970 Singapore census (see reference). Table I also

TABLE I

INCIDENCE OF AUSTRALIA ANTIGEN (Au) IN SINGAPORE BLOOD DONORS JANUARY 1971

	Blood Group								Total	
	A		B		O		AB			
Chinese	470	23 (4·89%)	414	14 (3·38%)	667	29 (4·35%)	81	3 (3·70%)	1,632	69 (4·23%)
Malay	91	2 (2·20%)	118	2 (1·69%)	204	2 (0·98%)	32	1 (3·13%)	445	7 (1·57%)
Indian	55	0 —	77	1 (1·30%)	102	1 (0·98%)	14	0 —	248	2 (0·81%)
Others	77	1 (1·30%)	35	0 —	112	0 —	14	0	238	1 (0·42%)
TOTAL	693	26 (3·75%)	644	17 (2·64%)	1,085	32 (2·95%)	141	4 (2·84%)	2,563	79 (3·08%)

shows the distribution of ABO blood groups amongst the donors, and the frequency of Au according to blood group.

Au was detected in a total of 79 subjects (3·08%). The frequency in Chinese was significantly greater than that in Malays ($X^2 = 6·99$; $0·005 > p < 0·01$) and in Indians ($X^2 = 6·93$; $0·005 > p < 0·01$). There is a lesser, but still statistically significant, difference between the frequency in Chinese and that in the total non-Chinese subjects studied ($X^2 = 3·84$; $p < 0·05$). The numbers in each of the non-Chinese groups are too small for statistical analysis one to the other. There is no difference between the distribution of Au positivity and the frequency of the ABO blood groups in Chinese. Once again the frequencies amongst the other groups are too small for blood group analysis.

In view of the previous report of 1 positive in 109 (Blumberg *et al*, 1965b) it is interesting to analyse the distribution of numbers of positive sera in successive groups of 100 Chinese subjects. This is shown in Table II. The incidence of Au ranges from 1% to 7%, indicating the importance of studying large numbers when the incidence of the marker being sought is only a few per cent.

TABLE II

INCIDENCE OF AUSTRALIA ANTIGEN IN SINGAPOREAN CHINESE BLOOD DONORS

1 - 100	7
101 - 200	2
201 - 300	4
301 - 400	1
401 - 500	6
501 - 600	6
601 - 700	4
701 - 800	2
801 - 900	4
901 - 1,000	4
1,001 - 1,100	4
1,101 - 1,200	2
1,201 - 1,300	3
1,301 - 1,400	7
1,401 - 1,500	7
1,501 - 1,600	3
1,601 - 1,632	3
1,632	69

In addition to sampling size another source of variation is the sensitivity of the technique employed. The initial method of double diffusion in agar is known to be insufficiently sensitive for use as a screening technique (Shulman, 1970). The application of electrophoresis to diffusion in agar (Pesendorfer *et al*, 1970; Yap, Ee and Simons, 1971) increases the sensitivity from 5 to 10 fold. Four other types of technique are more sensitive again than electro-osmodiffusion. They are complement fixation, haemagglutination, immune adherence and radioimmunoassay. Obviously the number of positive sera that are identified will be related to the sensitivity of the technique employed. In order to determine the number of sera in which Au can be detected by complement fixation and immune adherence, but which are negative by our technique, all 2,563 sera have been sent to Drs. Nishioka, Okochi and Murakami in Tokyo for further testing. It will be interesting to compare the results with those of Shulman and his colleagues (1970) who find that the method which they employ, and which involves the same principles as electro-osmodiffusion, detects the same number of Au carriers as complement fixation.

The significance of Au is not yet clear. It is not established whether the antigen is part of a virus particle, whether it represents an incomplete virus particle, or whether it is a virus-coat protein (Dane *et al*, 1970). Even if the antigen is not associated structurally with a virus, the important question is whether antigenaemia is indicative of viraemia. If the presence of Au in the blood merely represents the existence of virus localized to the tissues and not freely circulating then the implication for blood transfusion practice would be different from that if antigen was a direct manifestation of circulating virus. Another factor of major importance is that the carrier state

persists for long periods. Au has been found to persist for up to 18 years (Zuckerman and Taylor, 1969) and carriage for a number of years has been recorded in hepatitis patients (see WHO, 1970).

These facts raise two important issues in connection with the identification of Au-positive subjects. Firstly, is there any evidence of liver or other organ dysfunction in these apparently normal people, and if so what recommendations and management are indicated? Secondly, do they have the potentiality for transmitting disease, and in particular for transmitting hepatitis?

Our studies on the liver function of the 79 positive blood donors were commenced when they were recalled for investigation, and are not yet complete. Serum glutamic-pyruvic transaminase and iso-citric dehydrogenase enzymes are being quantitated, and serum bilirubin levels being estimated. Serum enzyme studies by another group (Deutsch and Wewalka, 1970) revealed that approximately three-quarters of their Au-positive donors had normal enzyme levels, and would not have been detected by enzyme studies alone. Of 18 of our 79 donors studied so far, there is an increase in serum glutamic-pyruvic transaminase in 8. Asymptomatic subjects with normal liver function tests and no history of antecedant hepatitis, but who are positive for Au, have been found to have histological evidence of chronic liver disease (Wright *et al*, 1969; Peters *et al*, 1970; Prince *et al*, 1969). The complete investigation of these subjects may therefore involve liver biopsy in addition to blood tests. With an incidence of 80 odd carriers a month this would entail approximately 1,000 subjects per year in Singapore.

The potentiality of these 79 and other Au-positive donors for transmitting disease can only be precisely determined by a longitudinal study of the corresponding recipients. Although we are still awaiting this definitive information from our Singapore study we might reconsider the current practice for the detection of unsuitable donors. This involves questioning the potential donor as to whether jaundice has occurred in the past. If there is a positive history but the episode occurred more than 10 years ago the donor is accepted. As mentioned before, it is now known that the Au carrier state can persist for 20 or more years, so the 10 year period currently accepted may have to be revised. Secondly, donors implicated retrospectively following the development of transfusion hepatitis are recalled and investigated. Thirdly, serum samples of all potential donors are assessed visually for the presence of bilirubin. If suspected, biochemical tests are undertaken. None of the 79, in fact none of the 2,604 donors presenting during January, were suspected by visual examination of sera.

In a previous study, 2 of 7 subjects whose sera were jaundiced had Au (Fung *et al*, 1971a). Since the majority (5/7) lacked detectable Au, visual examination must be continued. Serum enzyme studies are only performed on jaundiced sera. In a study undertaken in Taiwan an elevation of SGPT was found in 2·3% of apparently healthy subjects (81/3,529). Thus ideally, all sera should be screened for transaminase levels, but in practice this is rarely possible.

Finally all sera are screened by the V.D.R.L. test. If positive, the F.T.A. test is performed. In January 29 subjects were considered unsuitable as donors on this ground, and were referred for investigation.

It is also important to remember that Au is associated with long-incubation hepatitis. The contribution of the agent responsible for short-incubation infectious hepatitis is not known and hence not being sought. The relative importance of Au to these other putative agents is not known because the incidence of short versus long incubation post-transfusion hepatitis has not been established as yet. Early indications are that Au is rarely associated with non-transfusion hepatitis in Singapore. Only one of 41 jaundiced patients had Au (Fung *et al*, 1971b). If this frequency is confirmed in a prospective study currently being undertaken, it would suggest that the agent or agents responsible for most cases of hepatitis is not transmitted by injection. If this is so then one possible explanation for the high frequency of the Au in normal subjects can be discounted. Other sources of Au infection will have to be discerned.

Despite these unknowns, it does appear to be important to identify Au carriers in Singapore. The frequency is high relative to other parts of the world so at least some percentage of transmission risk can be assumed until the actual risk is established. Secondly, the technique is so relatively simple that it can be introduced for routine screening of all potential blood donors. It would make a further contribution towards the goal of safe and effective transfusion. The usual laboratory precautions should be taken. There is no cause for concern at handling sera containing a known infectious agent. Such sera have been processed for an indefinite time in the past, and a proportion of Au negative sera are almost certain to contain some as yet undefined infectious agent.

The frequency of Au in the general population in Singapore can only be roughly estimated. A

significant proportion of donors are members of a unit (e.g. National Servicemen, Police etc.) who are not living under domestic conditions in a family domicile. The projection can therefore only be approximate until studies of other groups are completed. A frequency of 4·23% corresponds to roughly 66,000 Singapore Chinese, while there are of the order of 6,000 other Singapore residents who are Au positive. The possible influence on the health status of the population, and perhaps on work performance, remain to be investigated. The problem offers a very important challenge to specialists in social, medical and in health economics. Just as important is the problem of the source of the infection. This is currently being investigated.

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