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HLA antigens and responses to rubella vaccination

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SUMMARY

Attempts were made to correlate virus excretion, joint symptoms and antibody response with human leukocyte antigens (HLA) in seronegative adult women given attenuated rubella vaccine. No association was shown between HLA antigens of the A and B loci and excretion of either high or low titres of RA27/3 vaccine among 26 volunteers. However, virus excretion was influenced by such factors as the time of day at which specimens were collected and the method of virus isolation. Our study therefore failed to confirm the hypothesis that certain persons are good 'spreaders' of rubella virus and that this capacity is associated with HLA-A1 and B8.

The study of joint symptoms following vaccination with Cendehill, HPV77.DE-5, RA27/3 or To-336 vaccines showed no association between such symptoms and HLA antigens. However, joint symptoms occurred within 7 days of the onset of menstruation in 33 of 47 (70 %) vaccinees ($P < 0{\cdot}01$) and it is therefore suggested that hormonal factors must play a role. No association between HLA antigens and haemagglutination inhibition (HAI) antibody titres, 8 weeks after vaccination with RA27/3, was found amongst 34 volunteers.

INTRODUCTION

A considerable number of diseases, including rubella, have been reported to be associated with particular HLA antigens (Dausset & Svejgaard, 1976). Thus, Honeyman *et al.* (1975) reported an increased prevalence of HLA-A1, A3, B5 and B8 among patients with congenital rubella in Australia. In most urban populations the proportion of women of child-bearing age who are immune to rubella is 80–85 %. Honeyman & Menser (1974) showed an association between the frequency of HLA-A1 and B8 in a population and the percentage of young adults seropositive to rubella. These authors suggested that those with HLA-A1 and B8 have an increased susceptibility to infection and a high potential for spreading rubella virus. The mean gene frequency of HLA-A1 and B8 in a population might therefore be related to the efficiency of transmission of rubella in that community

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and could be the reason for the comparatively lower proportion of women immune to rubella (28–52%) on islands such as Hawaii, Jamaica, Trinidad and rural Japan (Rawls *et al.* 1967; Halstead, Diwan & Oda, 1969; Dowdle *et al.* 1970), where the mean gene frequency of HLA-A1 and B8 is low. In a previous study on rubella vaccination (Best, Banatvala & Bowen, 1974), it was shown that although the amount of virus excreted by the majority of vaccinees was $\leqslant 10^{0.5}$TCD 50/0·1 ml, 11 of 55 (20%) samples had titres of $\geqslant 10^{1.5}$TCD 50/0·1 ml. We have therefore looked for an association between rubella virus excretion and HLA antigens and also between HLA antigens and two other responses to vaccination; severe joint symptoms and antibody response.

MATERIALS AND METHODS

Study population

Seronegative volunteers (HAI < 1/4) were nurses, medical students and physiotherapists at St Thomas' Hospital. Unless otherwise stated, they were given RA27/3 vaccine subcutaneously. Although 67 of our 75 volunteers were of European origin, 5 were of Asian, 2 of Chinese origin, and 1 was negroid.

Techniques

Virus excretion. Swabs were taken from the nose and throat of 26 volunteers between 9 and 13 days after vaccination, since at that time virus excretion is most likely to be detected (Best *et al.* 1974). Both swabs were immersed in virus transport medium and shaken for 20 min at 4 °C on a mechanical shaker. All samples were titrated in vero cell cultures and incubated for 8–10 days at 37 °C. Rubella virus titres were determined by passage into RK13 cell cultures. Viruses isolated were confirmed as rubella virus by neutralization tests using hyperimmune rabbit serum.

Severe joint symptoms. Volunteers were asked to keep a daily record of symptoms including joint pains, for 30 days after vaccination with one of four rubella vaccines (Cendehill, HPV-77.DE-5, RA27/3 or To-336). Those with joint pains were examined clinically. Vaccinees with arthritis (joint swelling or limitation of movement or both) or arthralgia involving three or more joints, were considered to have severe joint involvement. Twenty of these volunteers, and 20 with no joint symptoms were HLA typed.

Antibody response. Blood samples were obtained from all vaccinees 8 weeks after vaccination. These were tested for rubella antibodies by HAI, using a modification of the method described by Cooper *et al.* (1969).

HLA typing was carried out using a microcytotoxicity technique (Welsh & Batchelor, 1978). Fisher's 'Exact' Method was used to look for statistically significant differences between groups.

Table 1. *Comparison of HLA antigens of volunteers excreting high titres of rubella virus with those excreting low titres*

	No. of volunteers	Locus A									
		1	2	3	9	10	11	28	29	w31	w32
High titre $\geqslant 10^{2\cdot5}$TCD 50/0·1 ml	9	3	2	4	1	1	2	—	1	1	2
Low titre $< 10^{1\cdot5}$TCD 50/0·1 ml	9	3	4	4	2	1	1	1	—	—	—
Total tested	26*	10	7	11	4	2	5	2	1	1	2

	Locus B									
	7	8	12	14	15	w16	17	w21	w35	40
High titre	1	3	3	1	2	—	1	1	3	1
Low titre	5	1	2	1	1	1	—	—	—	2
Total tested	11	8	6	2	5	1	3	1	3	3

* Eight volunteers excreted between $10^{1\cdot5}$ and $10^{2\cdot2}$ TCD 50/0·1 ml.

RESULTS

Virus excretion

Rubella virus was isolated from 24 of the 26 (92 %) vaccinees, and from 34 of the 44 (77 %) swabs obtained. Titres ranged from $10^{0\cdot5}$ to $10^{3\cdot5}$TCD 50/0·1 ml. Nine volunteers excreting high titres ($\geqslant 10^{2\cdot5}$TCD 50/0·1 ml) and nine excreting low titres ($< 10^{1\cdot5}$TCD 50/0·1 ml) were HLA typed. There was no increased frequency of any HLA antigen in either group when compared with the total group tested (Table 1), nor was there any evidence that the eight volunteers with haplotype A1 and B8 were excreting high titres, since the geometric mean titre (GMT) of virus excreted by this group was $10^{1\cdot56}$TCD 50/0·1 ml, and the GMT for those without A1 and B8 was $10^{1\cdot41}$TCD 50/0·1 ml.

Since the titre of virus detected might depend on other factors such as the vigour with which vaccinees were swabbed, specimens were taken from four RA27/3 vaccinees by the same person on four occasions between 10 and 11 days after vaccination. Variations in titre as large as a thousandfold over a 4 h period were obtained (Table 2). The shaking of swabs for 20 min at 4 °C produced a tenfold increase in titre.

Severe joint symptoms

When the tissue types of the 20 vaccinees with severe joint symptoms were compared with the 20 without joint symptoms, no correlation was found between the occurrence of joint symptoms and HLA antigens (Table 3).

However, when the records of 249 women vaccinated during the last five years were analysed, 68 (27 %) had recorded joint symptoms. A significant association was noted between the occurrence of joint symptoms and time of onset of menstruation among 47 vaccinees with complete records. Thus joint symptoms

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Table 2. *Amount of virus excreted over 30 h period (titres expressed per TCD50/0·1 ml)*

Volunteer	Day 10 (10 a.m.)	Day 10 (4 p.m.)	Day 11 (10 a.m.)	Day 11 (4 p.m.)
1	$10^{1.5}$	$10^{2.5}$	$10^{1.2}$	$10^{1.5}$
2	10^{3}	0	0	0
3	0	0	$10^{1.5}$	$10^{1.2}$
4	$10^{1.2}$	$10^{1.2}$	*NT	NT

* NT = Not tested.

Table 3. *HLA antigens of those with and without joint symptoms*

	Locus A									
	1	2	3	9	10	11	28	29	w32	w19*
Joint symptoms	7	13	3	2	—	4	1	1	1	2
No joint symptoms	8	8	4	6	1	2	3	2	2	—

	Locus B														
	5	7	8	12	14	18	27	15	w16	17	w21	w22	w35	37	40
Joint symptoms	2	5	4	7	1	—	4	4	—	2	—	1	4	1	—
No joint symptoms	2	7	4	4	1	1	3	3	1	2	1	1	3	—	2

* Aw19, excluding A29 or Aw32.

occurred within 7 days of the onset of menstruation in 33 of 47 (70%) vaccinees ($P < 0{\cdot}01$).

Antibody response

When tested 8 weeks after vaccination, the GMT of HAI antibodies of 34 RA27/3 vaccinees was 1/64. When the HLA types of 11 volunteers with titres higher than the mean (> 1/64) and 15 volunteers with titres less than the mean (< 1/64) were compared with those of the total group tested, no correlation was observed between the high and low titres and any HLA antigen (Table 4).

Records of previous vaccine trials in which Cendehill, HPV-77.DE-5, and RA27/3 vaccines were used, showed that nine vaccinees, all of European origin, failed to develop an antibody response detectable by HAI or radioimmunoassay (Sugishita *et al.* 1978), when tested 12 weeks or more after primary vaccination. However, no significant differences were detected when the HLA types of these volunteers (Table 5) were compared with the distribution of HLA antigens in our total population and in a group of Caucasian blood donors from southern England ($P > 0{\cdot}3$).

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Table 4. *Comparison of the HLA antigens of vaccinees with high HAI titres with those of vaccinees with low HAI titres 8 weeks after vaccination*

	No. of volunteers	Locus A											
		1	2	3	9	10	11	28	29	w23	26	w32	w19*
High titre (H1 > 1:64)	11	6	5	4	1	1	2	2	—	—	—	—	—
Low titre (HI < 1:64)	15(2)†	7	6	5(2)	—	1	1 (1)	—	2	—	—	2	2
Total tested	34(4)‡	15	13	13(2)	4 (1)	3	6 (2)	4	2	1	1	3	3(1)

	Locus B												
	5	7	8	12	13	14	27	15	w16	17	w21	w22	w35	40
High titre	—	5	5	—	1	1	—	1	—	—	1	—	2	—
Low titre	—	4(1)	4	4	—	1	2	2	1	2(1)	—	1	2(1)	1
Total tested	2	14(1)	11	5	1	2	3	6(1)	1	4(2)	1	2	5(2)	5

* Aw19, excluding A29 and Aw32.
† Number of volunteers of non-European origin in parentheses.
‡ Eight volunteers had titres = 1/64.

Table 5. *HLA antigens of nine vaccinees who failed to seroconvert*

Vaccinee	Locus A	Locus B	Locus C
1	2, 9	40	*NT
2	3, 11	12, 27	NT
3	1, 28	8, 12	NT
4	2, 3	40, 27	NT
5	1, 3	5, 7	NT
6	1, 2	15, w22	Cw3
7	2, 26	7, 12	NT
8	2	8, 15	Cw3
9	1, 3	5, 8	NT

* NT = Not tested.

DISCUSSION

Certain island populations have a greater susceptibility to rubella than mainland populations but the reason for this is not fully understood (Dowdle *et al.* 1970). We have been unable to show any correlation between HLA antigens and the excretion of the RA27/3 strain of rubella virus, indicating that the HLA antigens studied do not play a role in the spread of this attenuated strain. However, attenuated rubella differs from the wild virus in its lack of communicability. We have previously suggested that attenuation may alter the biological properties of the virus in such a way that it is not able to replicate in the respiratory mucosa of susceptibles as readily as the wild virus (Best *et al.* 1974). We therefore cannot exclude the possibility that excretion of wild virus may be related to genetic factors.

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The amount of virus detected in pharyngeal swabs is likely to depend on many variables, for example; the expertise and vigour with which swabs were taken, the rapidity with which specimens are inoculated into cell cultures, the efficacy of elution of virus from the swab into the transport medium and the sensitivity of the cell cultures employed for virus isolation. In this study we showed that the amount of virus recovered may vary at different times of day, since, when swabs were taken from four vaccinees by one person, the titre of virus recovered from one volunteer was shown to vary by as much as 10^3 TCD 50 within 6 h.

In addition to the genetics of the host, the immunity of a population to virus infection may also be influenced by factors such as whether different strains of virus exist, population density, opportunities for reintroduction of the virus into the community, climate and mode of transmission of the virus (Lang, 1975). Although Japanese and U.S. strains of rubella virus have been reported to differ in such biological characteristics as teratogenicity (Kono *et al.* 1969) and their capacity to induce interferon in human placental cultures (Potter, Banatvala & Best, 1973), there is no evidence that rubella virus strains differ antigenically (Best & Banatvala, 1970).

Cockburn (1969), Pitts, Ravenel & Finklea (1969), and Dowdle *et al.* (1970) suggested that population density and geographical location were more likely to influence the spread of rubella than ethnic factors, since the populations of Jamaica, Trinidad and Hawaii studied are of mixed ethnic origin. Black (1966) has shown that measles does not become endemic in island populations of less than 500000. In such populations there will be breaks in the transmission of measles virus either when the susceptibles are exhausted or, in a disperse population, when the susceptibles are too far apart for virus transmission. Rawls *et al.* (1967) suggested that the minimum population required to maintain the endemicity of rubella is greater than that required for measles. This is probably because closer personal contact is required for transmission of rubella than required for measles, which is spread more efficiently by aerosol formation during the sneezing and coughing phase, the most infectious stage of the disease (Black, 1976).

A comparison of the spread of rubella on tropical islands with Iceland, which has a temperate climate, indicates that climate may also influence rubella virus transmission. It has been shown in Taiwan, some areas of which are densely populated, that transmission of rubella is interrupted during the hottest summer months (Gale *et al.* 1969). A similar phenomenum probably occurs on tropical islands such as Jamaica, Trinidad and Hawaii. On these islands only 28–52 % of women of child-bearing age are immune, which suggests that rubella ceases to spread before susceptibles are exhausted. In contrast, epidemics in Iceland continue throughout the year and since in urban areas approximately 80 % of women of child-bearing age are immune, virus spread apparently continues until available susceptibles are exhausted (Tómasson & Ögmundsdóttir, 1975). The break in transmission in the tropics, during the most hot and humid months, may be due to loss of stability of the virus or perhaps to social factors, for example, spending less time indoors, during this time.

Our results suggest that the occurrence of severe joint symptoms is related to

hormonal factors and is not associated with HLA antigens. Griffiths *et al.* (1977) and Spencer *et al.* (1977) were also unable to show an association of any HLA antigen with arthralgia and arthritis in children following rubella vaccination. Since joint symptoms usually occur between 13 and 21 days after vaccination in adult women (Best *et al.* 1974), it should be possible to reduce the incidence of joint symptoms by vaccinating women in the last 7 days of their cycle.

Although there was no apparent correlation between antibody response and HLA antigens in our study, Spencer *et al.* (1977) in a much larger group of 232 RA27/3 vaccinees in California showed that HLA-A28, B14 and Bw22 and AB blood type were associated with high convalescent HAI antibody titres and HLA-Bw17 with failure to seroconvert. Kato *et al.* (1978) in a study of 172 Japanese schoolgirls given To-336 vaccine also showed an association of antibody titres with HLA antigens. However, these differed from those shown by Spencer *et al.* (1977), HLA-A11 and B15 being associated with high convalescent HAI titres and HLA-Aw24 and HLA-B5 with low convalescent titres. In both these studies sera were tested only 6 weeks after vaccination. We have found that maximum HAI titres are sometimes not obtained for more than 8–12 weeks after vaccination (Best & O'Shea, unpublished observations). Perhaps these studies should be repeated with sera collected 3 months or more after vaccination.

It was only possible to include a small number of volunteers in this study since most of the available volunteers are now vaccinated at school and the number of seronegative adult women who were available for vaccination and subsequent study was therefore limited.

We have only looked at the HLA antigens of the A and B locus, for which antisera are readily available. It is possible that virus excretion and antibody response may be related to the more recently described antigens of the D locus, D-related antigens or to genetic factors not yet described.

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