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Interactive effect of Gm allotypes and HLA-B locus antigens on the human antibody response to a bacterial antigen

SENGA WHITTINGHAM, J. D. MATHEWS, M. S. SCHANFIELD, JULIE V. MATTHEWS, B. D. TAIT, P. J. MORRIS & I. R. MACKAY *The Clinical Research Unit, The Walter and Eliza Hall Institute of Medical Research and the Royal Melbourne Hospital, Victoria, Australia; The Department of Medicine, University of Melbourne, Australia; American National Red Cross, Washington, USA; the Tissue Typing Laboratory, The Royal Melbourne Hospital, Victoria, Australia and the Nuffield Department of Surgery, Radcliffe Infirmary, England*

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SUMMARY

Two hundred healthy adults were immunized with 1 μg of the bacterial antigen monomeric flagellin from *Salmonella adelaide*, and grouped as responders and non-responders on the basis of a rise in titre of antibody 2 weeks after immunization. Immunoglobulin allotypes G1m(a), G1m(z) and G3m(g) were more frequent among responders who made immunoglobulin (Ig)G antibody ($P<0{\cdot}02$), and HLA-B12 was more frequent among responders who made IgM antibody ($P<0{\cdot}05$). The mean log titre of IgG antibody was higher in females ($P<0{\cdot}001$), in subjects with G1m(a), G1m(z) and G3m(g) allotypes ($P<0{\cdot}05$), and in Gm heterozygotes ($P<0{\cdot}01$). The mean log titre of the IgG antibody response in subjects with particular Gm phenotypes was also dependent on the HLA-B locus phenotypes HLA-B7, B8 and B12 ($P<0{\cdot}005$); for example, among those with the phenotype Gm(a−x−b+), subjects with HLA-B7 were low responders and those with HLA-B8 were high responders. These findings are consistent with the hypothesis that there are immune response genes within the major histocompatibility complex (MHC) which interact with Gm-linked genes in determining levels of serum antibodies of different isotypes and specificities.

INTRODUCTION

Much of the variation in the antibody response in mice can be attributed to genetic control. With immunogens of restricted heterogeneity, recognition depends on immune response genes that reside in the major histocompatibility complex and exert their effect through T lymphocytes (Paul & Benacerraf, 1977). Another set of immune response genes linked to immunoglobulin structural genes appears to exert an effect through B lymphocytes (Blomberg, Geckeler & Weigert, 1972; Eichmann, 1972; Pawlak & Nisonoff, 1973; Sher & Cohn, 1972). With immunogens that are more heterogeneous genetic control is more complex; for example, the immune response to multideterminant antigens such as sheep red blood cells depends on several sets of genes controlling the proliferation and differentiation of antibody-forming cells (Feingold *et al.*, 1976). X-linked genes

Correspondence: Dr Senga Whittingham, Clinical Research Unit, The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia.

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can also affect the murine immune response (Amsbaugh *et al.*, 1972). Analogous mechanisms would be expected to control the immune response in man although genetic analysis is complicated by the genetic heterogeneity of human populations. We sought evidence for genetic control of the human antibody response by immunizing healthy adult volunteers with a low dose (1 μg) of a purified bacterial antigen from flagellin of *Salmonella adelaide*, and by relating the primary response to Gm and HLA phenotypes, and to sex.

MATERIALS AND METHODS

Immunization. Two hundred unrelated healthy Caucasian adult volunteers gave informed consent to immunization with 1 μg of flagellin. Previously, 5 μg flagellin was used for studies of the human antibody response (Rowley & Mackay, 1969), but for this study a low dose was chosen so that subjects could be more clearly grouped as antibody responders and non-responders. There were 102 men and ninety-eight women whose ages ranged from 17 to 65 years (mean 30·6 years). Polymeric flagellin was prepared from *Salmonella adelaide*, strain SW 1338, as described by Ada *et al.* (1964), depolymerized to monomer, and injected as monomer subcutaneously into the forearm in a volume of 0·1 ml immediately after depolymerization. Serum samples were obtained before and 2 weeks after immunization, and antibody to flagellin was measured by haemagglutination using sheep red blood cells coated with polymerized flagellin (Rowley & Mackay, 1969; Wistar, 1968). Adults have natural antibody to flagellin and preliminary studies had established that this natural antibody present before immunization was IgM, and that the peak antibody response to 1 μg of flagellin occurred at 2 weeks. Sera were titrated in doubling dilutions from a starting dilution of 1/5 and the titre of total antibody was expressed as the reciprocal of the dilution. Sera were also titrated after addition of 2-mercaptoethanol (2-ME), and 2-ME-resistant antibody was regarded as IgG antibody (Rowley, Wistar & Mackay, 1972). Subjects were defined as 'IgM antibody responders' if the titre of total antibody at 2 weeks was 2 or more dilutions greater than the titre of natural antibody and there was no increase of IgG antibody; subjects were defined as 'IgG antibody responders' if the titre of IgG antibody at 2 weeks was 10 or greater irrespective of the titre of total antibody.

Gm allotyping. The Gm phenotypes of the 200 subjects were established by testing their sera for the allotypic markers G1m(f,z,a,x) and G3m(b0,b1,b3,b5,c3,s,t,g) by the passive haemagglutination inhibition procedure described by Schanfield (1978). The known antigenic and linkage relationships within the Gm complex in Caucasian populations (Schanfield, 1978) enable maximum statistical information to be obtained by analysis of a restricted set of allotypes: G1m(f,z,a,x), G3m(b,g), and a simplified set of phenotypes: Gm(a−x−b+), Gm(a+x−b−), Gm(a+x+b−), Gm(a+x−b+) and Gm(a+x+b+). Apart from minor discrepancies due to missing data or equivocal typing reactions, all Gm(a) subjects typed as Gm(z,a,g) and all Gm(b) subjects typed as Gm(f,b0,b1,b3,b5).

HLA typing. Lymphocytes from 181 of the 200 unrelated subjects were separated from heparinized blood by centrifugation through Isopaque–Ficoll and tested by a standard microlymphocytotoxicity procedure (Mittal *et al.*, 1968; Ting & Morris, 1971) to establish the HLA phenotype for A and B locus antigens. For statistical analysis, only the HLA-B locus antigens were examined, these being chosen *a priori* because of their reported association with immunologically mediated diseases in Caucasian populations (Svejgaard & Ryder, 1977). High frequency antigens (HLA-B7, B8 and B12) were chosen to obtain maximum statistical information from the analysis.

Statistical analysis. Contingency tables were compiled to summarize the frequencies of males, females, the Gm allotypes G1m(f,z,a,x), G3m(b,g) and the HLA-B locus antigens 7, 8 and 12 among non-responders, IgM antibody responders and IgG antibody responders. Significance levels were derived by chi-square (χ^2) analysis.

Analysis of variance (Kim & Kohout, 1975) was used to estimate whether factors based on Gm and HLA-B locus phenotypes could explain a significant proportion of the variation in the natural logarithm of the IgG antibody titre between subjects. For the main analysis, all subjects were assignable to one or other of five common Gm phenotype classes (a−x−b+), (a+x−b−), (a+x+b−), (a+x−b+) and (a+x+b+) as five levels of a single factor and to one or other level

of three dichotomous factors corresponding to the presence or absence of HLA-B7, 8 and 12 respectively. The nineteen subjects not phenotyped for HLA were categorized as negative for HLA-B7, B8 and B12. Sex was treated as a covariate in the main analysis, and as a factor in several subsidiary analyses. Examination of residuals showed that the usual assumptions for analysis of variance were satisfied. The ANOVA sub-programme of the SPSS package (Kim & Kohout, 1975) was used to analyse the data; computer and data limitations precluded the testing of models involving larger numbers of HLA antigens.

RESULTS

Natural antibody

Before immunization, antibody was detected in sera from 167 of the 200 subjects with titres ranging from 5 to 5,120. This pre-immunization 'natural' antibody was free of any IgG components.

Response to immunization

At 2 weeks, the time when the titre is known to peak, 125 of the 200 subjects had responded to immunization with 1 μg flagellin. Twenty-five subjects who produced total antibody with titres from 20 to 20,480 were found to produce no IgG antibody and were defined as IgM responders. One hundred subjects who produced IgG antibody with titres from 10 to 40,960, and total antibody with titres from 40 to 163,840, were defined as IgG responders. Antibody titres were higher in women than in men (Fig. 1).

Frequency of genetic markers in responders and non-responders

Sex differences between the responder groups were not significant (Table 1). Males comprised forty-two (56%) of the seventy-five non-responders, sixteen (64%) of the twenty-five IgM responders, and forty-four (44%) of the 100 IgG responders. Allotypes G1m(a), G1m(z) and G3m(g) occurred more frequently among IgG antibody responders ($P<0{\cdot}02$), and HLA-B12 occurred more frequently among IgM antibody responders ($P<0{\cdot}05$) (Table 1).

Relationship of genetic markers to IgG antibody titre

Analysis of the IgG antibody titres showed that for females the mean log titre of IgG antibody of 3·90 was significantly higher than the mean titre of 2·69 for males ($P<0{\cdot}001$) (Table 1); the mean log

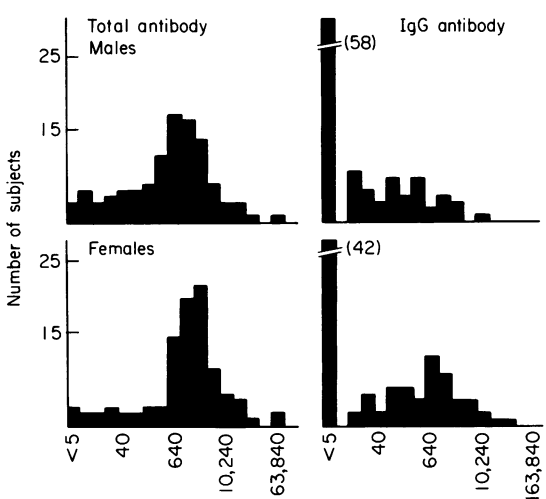

Fig. 1. The total and IgG antibody titres in 200 subjects 2 weeks after injection with 1 μg flagellin were significantly higher in females than in males. Numbers in brackets are IgG antibody non-responders.

Table 1. Responder status and IgG antibody response to flagellin related to sex, Gm and HLA markers

Genetic markers	Number (%) positive for marker*						IgG antibody response	
	Non-responders	IgM responders	IgG responders	Total	χ^2	*P*	Mean log titre	*P*
Sex								
Male	42 (56)	16 (64)	44 (44)	102 (51)	4·40	n.s.	2·69	0·001
Female	33 (44)	9 (36)	56 (56)	98 (49)			3·90	
Glm(a)	35 (47)	7 (28)	60 (60)	102 (51)	9·75	0·01	3·69	0·025
Glm(f)	61*(84)	23 (92)	81*(83)	165*(85)	1·20	n.s.	3·29	n.s.
Glm(x)	18 (24)	3 (12)	20 (20)	41 (20)	2·30	n.s.	3·19	n.s.
Glm(z)	35 (47)	7 (28)	59*(60)	101 (51)	8·78	0·02	3·64	0·05
G3m(b)	63 (84)	23 (92)	83 (85)	169 (85)	0·79	n.s.	3·28	n.s.
G3m(g)	35 (47)	6*(25)	58*(59)	99*(50)	9·63	0·01	3·66	0·04
HLA-B7*	15 (21)	8 (32)	24 (29)	47 (26)	1·88	n.s.	3·32	n.s.
HLA-B8*	17 (24)	5 (20)	24 (29)	46 (25)	1·06	n.s.	3·49	n.s.
HLA-B12*	17 (24)	11 (44)	17 (20)	45 (25)	5·93	0·05	3·24	n.s.
Total	75*(100)	25*(100)	100*(100)	200 (100)			3·28	

* Numbers and percentages based on smaller totals for some markers (discrepancies are due to missing information and/or equivocal typing reactions).

See Materials and Methods section for definition of responder status.

n.s. = Not Significant.

titres in subjects with G1m(a), G1m(z) and G3m(g) allotypes were significantly higher than in subjects lacking these allotypes ($P<0·05$); and the mean log titres were higher in presumptive heterozygotes, Gm(a+x+b−), Gm(a+x+b+), and Gm(a+x−b+), than in presumptive homozygotes, Gm(a−x−b+) and Gm(a+x−b−) ($P<0·01$) (Table 2). Within Gm phenotype subgroups the mean log titre of the IgG antibody was significantly related to HLA type (Table 3). These non-additive interactions of the Gm phenotype with HLA-B7, B8 and B12 (Table 2) indicate that the effect of Gm phenotypes on the IgG antibody response was influenced by the HLA phenotype, and vice versa; thus, the mean log titre in subjects with Gm(a−x−b+) varied from 3·93 in seventeen subjects with HLA-B8 to 1·86 in eleven subjects with HLA-B7, and the mean log titre in subjects with Gm(a+x+b+) varied from 0·92 in five subjects with HLA-B12 (all non-responders) to 5·17 in seven subjects with other HLA antigens. The magnitude of the sex difference in the IgG antibody response was similar in most genetic subgroups; however, for HLA-B12 there was suggestive evidence ($P<0·02$) for non-additive interactions with female sex as well as with Gm phenotype in the determination of antibody titre (Table 2—detailed ANOVA results are not shown for this comparison).

DISCUSSION

Human adult volunteers immunized with a low dose (1 μg) of monomeric flagellin from *Salmonella adelaide* were grouped as responders or non-responders on the basis of a rise in titre of serum antibody demonstrated 2 weeks after injection. Responders fell into two groups: a group of twenty-five who produced flagellin antibody exclusively of the IgM class, and a group of 100 who produced IgG antibody. The substantial IgG antibody component of the primary response suggests that the immune response to flagellin in man exhibits a T cell dependence similar to that reported in mice (Feldmann & Basten, 1971).

The relationship in humans of the IgG antibody response to Gm phenotypes, HLA phenotypes

Table 2. Mean natural log of titre of IgG antibody to flagellin according to Gm, HLA phenotype* and sex (number in each group)

HLA-B locus phenotype*	Gm phenotype					
	a−x−b+	a+x−b−	a+x+b−	a+x−b+	a+x+b+	Total
B7						
Male	1·78 (8)	3·00 (1)	6·12 (2)	3·46 (3)	7·85 (1)	3·18 (15)
Female	2·07 (3)	1·96 (2)	0·92 (1)	5·77 (4)	7·16 (1)	3·75 (11)
Total	1·86 (11)	2·30 (3)	4·38 (3)	4·78 (7)	7·50 (2)	3·42 (26)
B8						
Male	3·52 (8)	4·38 (1)	—	3·69 (1)	0·92 (4)	2·85 (14)
Female	4·31 (9)	—	3·69 (3)	5·21 (5)	3·69 (3)	4·35 (20)
Total	3·93 (17)	4·38 (1)	3·69 (3)	4·96 (6)	2·11 (7)	3·73 (34)
B12						
Male	1·67 (11)	1·61 (1)	5·77 (1)	3·00 (3)	0·92 (2)	2·03 (18)
Female	5·60 (8)	3·00 (2)	—	5·77 (3)	0·92 (3)	4·43 (16)
Total	3·32 (19)	2·53 (3)	5·77 (1)	4·38 (6)	0·92 (5)	3·16 (34)
B7/B8						
Male	1·09 (4)	5·77 (1)	—	2·65 (2)	—	2·20 (7)
Female	3·69 (2)	—	—	—	5·77 (1)	4·38 (3)
Total	1·96 (6)	5·77 (1)	—	2·65 (2)	5·77 (1)	2·85 (10)
B7/B12						
Male	0·92 (1)	—	—	1·38 (3)	—	1·04 (4)
Female	1·26 (2)	—	8·54 (1)	5·08 (3)	7·16 (1)	4·78 (7)
Total	1·15 (3)	—	8·54 (1)	3·23 (6)	7·16 (1)	3·50 (11)
Other†						
Male	2·49 (19)	3·00 (2)	2·30 (5)	3·41 (15)	4·61 (3)	2·95 (44)
Female	2·94 (23)	0·92 (2)	2·48 (4)	4·38 (8)	5·60 (4)	3·34 (41)
Total	2·73 (42)	1·96 (4)	2·38 (9)	3·75 (23)	5·17 (7)	3·13 (85)
All						
Male	2·22 (51)	3·46 (6)	3·69 (8)	3·10 (27)	2·72 (10)	2·69 (102)
Female	3·56 (47)	1·96 (6)	3·38 (9)	5·07 (23)	4·33 (13)	3·90 (98)
Total	2·86 (98)	2·70 (12)	3·52 (17)	4·00 (50)	3·63 (23)	3·28 (200)

Two subjects were HLA-B8/B12 and were included in the B8 category.

Note that apart from minor differences due to missing or equivocal typing reactions, all Gm(a) were actually Gm (z,a,g) and all Gm(b) were actually Gm(f,b0,b1,b3,b5).

* Exclusive phenotypic classes (e.g. 'B7' includes all subjects with B7 who did not also have B8 or B12).

† Also includes unknown HLA phenotypes for nineteen subjects.

and to female sex suggests that at least three sets of genes influence the primary response to flagellin; one set being in linkage disequilibrium with genes coding for Gm allotypes, one in linkage disequilibrium with genes coding for HLA-B locus antigens and one related to the X chromosome. Studies of antibody responses to multideterminant antigens such as sheep erythrocytes in selectively outbred mice show that genes concerned with the regulation of the rate of multiplication and differentiation of antibody-producing cells are also of major importance in the response (Biozzi *et al.*, 1971; Feingold *et al.*, 1976).

The present association of G1m(a) with the immune response to 1 μg flagellin from *Salmonella adelaide* confirms a previous study of the immune response to 5 μg flagellin (Wells, Fudenberg & Mackay, 1971). The allotype G1m(a) has also been associated with high responder status following immunization with *Salmonella typhi* (Nevo, 1975); in contrast, allotypes G1m(f) and G3m(b) are associated with thyroid autoimmunity in Graves' disease (Farid *et al.*, 1977), and the Km(1)

Table 3. Summary of effects described in Table 2

	Sum of squares (SS)	Degree of freedom (df)	Mean square (MS)	F	*P*
Covariate					
Sex	73·31	1	73·31	12·22	0·001
Main effects*	54·29	7	7·76	1·29	0·270
Gm	51·97	4	12·99	2·16	0·08
B7	0·08	1	0·08	0·01	n.s.
B8	2·27	1	2·27	0·38	n.s.
B12	0·03	1	0·03	0·01	n.s.
Interactions*	213·53	15	14·24	2·37	0·005
Gm × B7	70·37	4	17·59	2·93	0·02
Gm × B8	64·14	4	16·03	2·67	0·04
Gm × B12	77·39	4	19·35	3·22	0·02
B7 × B8	5·57	1	5·57	0·93	n.s.
B7 × B12	7·85	1	7·85	1·31	n.s.
B8 × B12	7·00	1	7·00	1·17	n.s.
Residual	1056·29	176	6·00	—	—
Total	1397·42	199	7·02		

* Classical method of decomposition of SS (Kim & Kohout, 1975).

Significance of saturated model: explained SS = 341·13 on 23 df; MS = 14·83; F = 14·83/6·00 = 2·47 on 23, 176 df; $P < 0·001$.

immunoglobulin light chain allotype is associated with higher immune responses to *H. influenzae* and meningococcal C polysaccharides in white children (Pandey *et al.*, 1979). The association of high titres of flagellin antibody of IgG class with G1m(a), G1m(z) and G3m(g) allotypes suggests that haplotypes with these alleles also carry genes augmenting the immune response to flagellin; however, as the log titre of antibody in presumptive homozygotes for Glm(a) was generally less than the titres in presumptive heterozygotes, it seems likely that any effect of Gm(a)-carrying haplotypes on the antibody response is also influenced by non-additive interactions with Gm haplotypes carrying different allotypic markers. Thus the overall association of G1m(a) with the IgG antibody response to flagellin may merely reflect the fact that most individuals with G1m(a) are heterozygous. Functional interactions within a single gene complex, namely H2, have been shown to be important in immune regulation in the mouse (Dorf & Stimpfling, 1977); analogous interactions in man would explain the present finding of higher titres of flagellin antibody in G1m(a) heterozygotes. If, as seems likely, higher titres of antibody confer a survival advantage during Salmonella infection, this phenomenon would exemplify heterozygote advantage or heterosis (Cavalli-Sforza & Bodmer, 1971).

Within Gm phenotype subgroups, HLA-B7, B8 and B12 had significant effects on mean log titres of IgG antibody to flagellin. For example, amongst subjects with Gm(a−x−b+) those with HLA-B7 tended to be low responders while those with HLA-B8 tended to be high responders; amongst subjects with Gm(a+x+b+), those with HLA-B12 were low responders. We postulate that the interactive effects of Gm and HLA on antibody titre reflect the recognition of determinants on the flagellin molecule by different populations of lymphocytes. As the efficiency of this dual recognition is presumed to depend on particular combinations of lymphocyte phenotypes recognizing combinations of determinants on the flagellin molecule, it is plausible that an HLA phenotype which confers a high responder state when combined with one particular Gm phenotype could confer a poor responder state when combined with another Gm phenotype. At the cellular level the interactions related to HLA phenotype would reflect the effects of MHC-linked gene products expressed on either helper T cells, suppressor T cells or both. Thus, the high frequency of HLA-B12 in IgM responders may indicate an association of HLA-B12 with lack of T cell help or with

overriding T cell suppression (Katz & Armerding, 1976). Although HLA and Gm both appear to be located on chromosome 6 (Lamm *et al.*, 1974; Smith & Hirschhorn, 1978), there is no evidence for close linkage in family studies (Weitkamp, May & Johnston, 1975) and no evidence of significant phenotypic association in the present data. This makes it unlikely that the interactive effect of Gm and HLA phenotypes on antibody production is an artefact arising from their common chromosomal localization. Antigen recognition by T cells could be related to Gm allotype in view of the evidence for linkage between T cell idiotypes and Ig heavy chain allotypes in the rat (Binz, Wigzell & Bazin, 1976).

One possible interpretation of the sex-related differences in antibody response is that there are sex-linked alloantigen systems in man which are analogous to those on murine T cells (Zeicher, Mozes & Lonai, 1977) and which are involved in T–B collaboration. Although there was some evidence for interaction of sex with specific HLA and Gm phenotypes, we cannot exclude the simpler explanation that the effect of sex chromosomes on antibody production is indirect and mediated through sex-hormones (Eidinger & Garrett, 1972).

Our evidence for non-additive interactions involving HLA and Gm is a novel result with important implications. Interactions between sets of polymorphic genes may not only provide a mechanism for increasing the specificity of antibody reactions, but also provide a more flexible means of evolutionary adaptation to newly encountered and potentially harmful infections.

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