

Immunogenetic Studies of Respiratory Anaphylaxis in Selectively Bred Guinea Pigs

Inheritance of Atopy, Linkage to MHC-Complex

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The genetic and immunological basis of respiratory anaphylaxis was studied in two strains of guinea pigs selectively bred for either high or low respiratory anaphylactic response to inhalation of ovalbumin. Individuals of the parental strains, F1-hybrids and backcross offspring were examined for class II MHC type and asthmatic response to ovalbumin. Analysis of the results revealed an association of asthma phenotype and MHC class II type. Apart from the gene(s) in the MHC several other genes seem to be involved in the control of guinea pig asthma.

Key words: asthma; heredity; immune response; major histocompatibility complex.

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Bronchial asthma and other human atopic diseases are influenced by heredity, and multiple genes seem to be involved (21). Several studies have been performed to elucidate a possible association between the human MHC system and atopic diseases. There have been conflicting reports concerning the association between disease and single HLA-antigens or distinct haplotypes (6, 7, 8, 11, 15, 16, 23, 25, 26, 27, 29, 33, 34, 35). Most of these studies included only testing for the class I antigens. Recent reports including class II typing (20, 22, 24) have shown evidence of a strong correlation between susceptibility to atopic disease and the class II region. Genetic studies in humans are, however, complicated because of the unfeasibility of controlled breeding.

In order to investigate the genes which control the asthma phenotype in a system allowing controlled breeding, two strains of guinea pigs have been selectively bred for either high or low propensity for responding to repeated inhalations of ovalbumin by respiratory anaphyl-

axis (18). The inbreeding has been maintained for more than 25 generations now, and has resulted in the establishment of five strains which respond with strong asthma to inhalation of ovalbumin (OA) and three strains which do not respond at all. The official name of the high asthma strains and of the low asthma strains are IMM/S and IMM/R, respectively. The strains have been further characterized with respect to rejection of skin grafts, erythrocyte enzyme polymorphism (32), production of homocytotrophic antibodies to OA (9), and immune responsiveness to certain synthetic antigens (19) which can be used as markers of class II MHC genes. Collectively, the results indicate that the members of each of the strains are homozygotes.

The purpose of the work reported in this paper was to investigate if class II MHC genes are involved in defining the anaphylactic phenotype in one high asthma and two low asthma strains of guinea pig. The approach has been to determine in individuals of the strains, and

their F1-hybrids and backcross offspring, both respiratory anaphylaxis and MHC type in order to see if any correlation exists. We report that class II MHC genes are involved in defining the asthmatic phenotype but that several non-MHC genes are involved too.

MATERIAL AND METHODS

Animals

The guinea pigs used were of the high asthma strain IMM/S 740 and the low asthma strains IMM/R 201 and IMM/R 203, and of the NIH strains 2 and 13. All animals were bred and kept under identical conditions.

Cross-breeding

The two strains IMM/R 201 and IMM/R 203 were crossed with the IMM/S strain 740 to produce the F1-hybrids F1 (740 $\times$ 201) and F1 (740 $\times$ 203). F1-animals were back-crossed to their respective parental strains, giving the (F1 $\times$ 740), (F1 $\times$ 201), and (F1 $\times$ 203) backcross animals.

Antigens, immunization, and skin testing

Ovalbumin, crystallized five times, was obtained from Sigma. The following synthetic polypeptides were obtained from the Miles Yeda Company: the copolymer of L-glutamic acid and L-tyrosine 1:1 (GT) of an average mol. wt. of 30,800, the copolymer of L-glutamic acid and L-alanine 6:4 (GA) of an average mol. wt. of 144,500, and the homopolymer of L-lysine, hydrobromide, of an average mol. wt. of 77,000. Poly-L-lysine (PLL) was used conjugated with dinitrophenyl using DNCB as described by Eisen et al. (12). The degree of dinitrophenyl conjugation was calculated to 10 DNP groups/molecule of PLL.

Immunization with DNP-PLL, GT, and GA and skin testing for delayed hypersensitivity responses were carried out as described by Lundberg et al. (19).

Inhalation of ovalbumin

The inhalations of OA were performed before the guinea pigs were immunized with synthetic antigens. The guinea pigs were exposed to inhalation of OA once a week for 6 weeks according to the method described by Lundberg (18). Briefly, up to 30 animals at a time were deposited in a box, and aerosolized OA (0.5%) in saline solution was introduced into the box at a constant pressure during the exposure. Each exposure took 50 min, during which the animals were under constant observation. The severity of the asthmatic response (forced breathing, coughing, shock), graded on a scale from 0 to 5, and the time elapsing from the beginning of the inhalation to the occurrence of distinct signs of respiratory anaphylaxis, were recorded for each animal by a trained observer who had no knowledge of the genetic make-up of the animals. The scale used for quantitating the asthma was as follows. 0: no signs of reaction; 1: incidental or slightly forced respiration; 2: clearly forced respiration; 3: strongly forced respiration and fits of coughing; 4: as 3, but animal is shocked and in poor condition; 5: as 4, but interruption of the inhalation and treatment with aerosolized adrenalin is necessary.

Calculation of "anaphylactic index"

The recorded grades of the asthmatic responses from the fourth, fifth, and sixth inhalations of OA were multiplied by 10 and added up for each animal to give the value R, ranging from 0 to 150. Likewise the time in minutes elapsing from the beginning of the inhalation to the occurrence of distinct signs of respiratory anaphylaxis during the fourth, fifth, and sixth inhalations were added to give the value E, also ranging from 0 to 150 (50 min being the observation time). There was a close negative correlation between R and E. The index was calculated as R-E, ranging from -150 to +150, and gave a somewhat better discrimination between the various groups than did either R or E used alone. For the purpose of illustration (Fig.

1) the abscissa was divided linearly into 10 intervals.

Tissue typing

Tissue typing of the animals was performed with a microcytotoxic assay, employing lymphoid cell suspensions enriched in B lymphocytes as described by Clausen et al. (10). Alloantisera were produced by cross-immunization of the different guinea pig strains with lymphoid cells, according to Geczy et al. (13, 14).

Statistical analysis

The significance of differences between the various crosses was evaluated by using Wilcoxon's two sample test. Frequencies of non-responders were compared by using Fisher's exact test. The possible number of genes contributing to the variation observed were calculated with the method described by Lande (17), which is based on an analysis of the mean values and variances within crosses.

Table 1

Correlation between immune response, phenotype and serologic tissue type

Strain/ Cross	Serologic tissue type		Immune response phenotype			No. animals
	Ia 1.3	Ia 2.4	GA, DNP- PLL	GT		
201	+	-	-	+		128
740	-	+	+	-		60
(201 × 740) F1	+	+	+	+		137
(201 × F1)	+	-	-	+		38
(201 × F1)	+	+	+	+		44
(740 × F1)	-	+	+	-		16
(740 × F1)	+	+	+	+		16
203	+	-	-	+		72
(203 × 740) F1	+	+	+	+		86
(203 × F1)	+	-	-	+		59
(203 × F1)	+	+	+	+		51
(740 × F1)	-	+	+	-		19
(740 × F1)	+	+	+	+		16

Tissue typing by microcytotoxic assay, +: 75–100% killing; -: 0–10% killing. Immune response phenotype by skin testing, +: positive skin test; -: negative skin test.

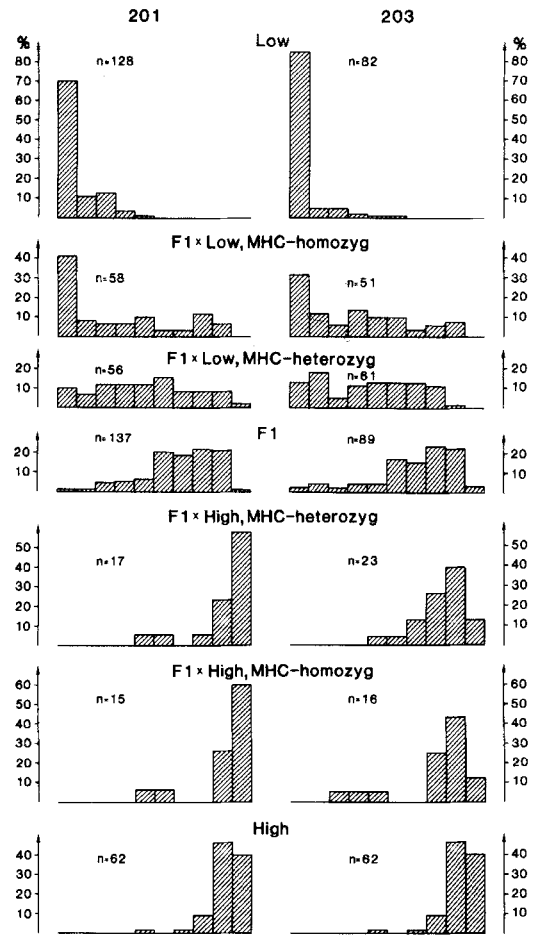


Fig. 1. The distribution of asthmatic response in two low asthma strains, 201 and 203, and the high asthma strain, 740, their F1-offspring, and the backcross offspring from the cross of F1-animals to the parental strains. The ordinate represents the percentage of the total number of animals in the group, the abscissa, divided in 10 intervals, represents the degree of asthmatic response expressed as an anaphylactic index (see text).

RESULTS

Correlation between immune response phenotype and tissue type

A large number of guinea pigs of strains 740, 201, and 203, their F1-hybrids, and backcross offspring were immunized with the synthetic antigens DNP-PLL, GT, and GA, and were tested for delayed hypersensitivity reactions to all

three antigens. The serological class II MHC type was also determined. The results (Table 1) demonstrate a complete correlation between immune response phenotype and serological tissue type. All strain 740 animals were Ia2.4 and were high-responders to DNP-PLL and GA but low-responders to GT; all strain 201 and 203 animals were Ia1.3 and were high-responders to GT but low-responders to DNP-PLL and GA. The F1-hybrids were high-responders to all three antigens and were Ia2.4 and Ia1.3 positive. The backcross individuals fell into two groups, one in which the animals were responders to all three antigens and also possessed both parental tissue types (MHC-heterozygotes), and another in which the animals were responders to only some of the antigens and possessed only one of the parental tissue types (MHC-homozygotes). This confirms what has been demonstrated in other guinea pig strains, namely that the serologic MHC class II type and the immune response phenotype are correlated in the guinea pigs, and that these traits can be used as genetic markers and as substitutes for each other (4, 5).

Correlation between MHC type and asthma phenotype

The possible association between the MHC class II genes and asthma phenotype was examined by testing the F1 and backcross animals for severity and time of onset of the asthmatic response to OA-inhalation, and relating the findings to immune response phenotype and serological tissue type. The asthma response was expressed as an index incorporating the severity and time factors. A total of 795 animals were tested. Fig. 1 shows the result. The animals have been grouped according to their tissue type/immune response phenotype. The asthma responses in the high and low asthma strains differed greatly. The F1-hybrids had an intermediate asthma response with a tendency towards the high asthma strain, indicating that one or more dominant genes may be involved. Backcross of the F1-hybrids to the high asthma strain produced asthma responses not much different from the high asthma parental strain,

and there were no significant differences between those guinea pigs that were homozygous and those that were heterozygous with respect to MHC type.

The backcrosses of the F1-hybrids to the low asthma strains, however, showed a significant difference between MHC homozygous and heterozygous in the frequency of the lowest degree of asthma response. By using Fisher's exact test $P = 0.0002$ was found for $F1 \times 201$ and $P = 0.029$ for $F1 \times 203$. For the two types of backcrosses combined, the differences between MHC homozygotes and heterozygotes gave a P -value as low as 0.00002 (39 of 109 versus 14 of 117). Hence, in these $F1 \times$ low asthma crosses there is a clear tendency for the higher asthma response phenotype to segregate in association with the MHC class II genes of the high asthma strain.

Estimation of the number of genes

The distribution of the asthma responses in the backcross offspring indicates that this character depends on the activity of multiple genes. An attempt was made to estimate the minimum number of genes involved. The commonly used method described by Lande (17) assumes additive effects of unlinked genes and, if standard errors are to be derived, normal distributions of the observations. Furthermore, the variance of the F1-hybrids should be of the same magnitude as the variances in the parent population. These assumptions are not well met in the present study, and it was not possible by transformation of the data to improve the situation very much, especially not the effect of the relatively high variance in the F1-group. Application of the method therefore gave a very uninformative result, namely that two or more genes were estimated to be involved.

A closer inspection of the distributions shown in Fig. 1 may, however, proceed along the following lines: If k genes, additive or dominant, are fixed in the low asthma strain, a proportion $(1/2)^k$ of the backcross to the high asthma strain will be identical to the F1-hybrids and another equally large proportion will be identical to the high responders. This argument assumes un-

linked genes. It is seen that *k* can hardly be less than 2 if the (*F1* × IMM/S) distribution is to contain these proportions. By the same argumentation if *g* genes (apart from the MHC gene(s) or MHC-linked gene(s)) are additive or d^2 minant in the high asthma strain a proportion $(1/2)^g$ of the (*F1* × IMM/R) offspring, homozygotes, will be identical to the low responders, and the same proportion of the (*F1* × IMM/R) offspring will be identical to the *F1*-hybrids. It is seen that *g* can hardly be less than two.

Some of the *k* and *g* genes might, however, if additive, be alleles of the same loci, and therefore it can not be claimed with certainty that more than two genes, apart from the MHC gene(s) or MHC-linked gene(s) in the high

asthma strain, have been demonstrated. It can be concluded, therefore, that the total number of genetic loci influencing the asthmatic response is at least three.

Asthma to ovalbumin in NIH strain 2 and 13

Since NIH strain 2 shares at least some MHC genes with the high asthma strain 740, and the NIH strain 13 shares some with the low asthma strains 201 and 203, we tested the asthma responses in 10 animals of each of the NIH strains. The results, listed in Table 2, indicate that the members of each strain differ in the response to OA-inhalations. The distribution of animals responding strongly or not responding at all was, however, the same in the two strains.

DISCUSSION

Our guinea pig asthma model consists of one inbred strain with high and one with low susceptibility to respiratory anaphylaxis to ovalbumin. The two strains differ in class II MHC type, and it was obvious to look for an association between this trait and the trait of anaphylactic susceptibility. Individual guinea pigs of the parental strains, their *F1*-hybrids, and backcross offspring were MHC-typed by serological methods and by testing their immune responses to synthetic antigens. As shown previously (7, 8, 10) and confirmed here, the serological MHC class II-type and the immune response phenotype are correlated in the parental strains and can be used as genetic markers and as substitutes for each other. All of the animals were, before immunization and testing with the synthetic antigens, exposed repeatedly to inhalation of OA, and their asthma responses were recorded. An analysis of the data indicates that the high asthma phenotype tends to segregate together with the MHC class II-type of the high asthma strain. The degree of MHC control of the asthma was moderate, but statistically highly significant. This suggests that the class II MHC genes themselves, or closely linked genes, play a role in the control of guinea pig asthma. It has not yet been possible to identify the particular allele(s) responsible.

Table 2

Respiratory anaphylactic reaction to repeated inhalation of ovalbumin in the strains NIH 2 and NIH 13

Strain	No.	Inhalation no.					
		1	2	3	4	5	6
NIH 2	701	0	0	0	2	0	0
	— 702	0	0	0	2	2	2
	— 703	0	0	0	0	0	0
	— 706	0	0	0	0	1	0
	— 709	0	0	0	3	0	2
	— 713	0	0	0	4	4	3
	— 714	0	0	0	5	3	0
	— 715	0	0	0	3	0	3
	— 710	0	0	0	3	5	2
	— 716	0	0	0	4	2	3
NIH 13	355	0	0	0	4	0	2
	— 356	0	0	2	3	0	2
	— 357	0	0	1	3	2	0
	— 359	0	0	2	3	4	5
	— 360	0	0	2	0	0	0
	— 361	0	0	2	2	2	0
	— 364	0	0	0	3	0	0
	— 365	0	0	5	5	5	5
	— 366	0	0	2	4	4	5

Evaluation of the anaphylactic reaction in the bronchial provocation test. 0: no signs of reaction; 1: incidental or slightly forced respiration; 2: clearly forced respiration; 3: strongly forced respiration, fits of coughing; 4: as 3, but in addition the animal is shocked and in poor condition; 5: as 4, but interruption of the inhalation and treatment with aerosolized adrenalin is necessary.

To our knowledge this is the first demonstration of MHC control in experimental respiratory anaphylaxis in the guinea pig. There are reports (20, 22, 24) of an association between asthma and MHC class II type in humans.

It is likely that the MHC class II genes which maximize the asthma response to OA have become sequestered in the high asthma strain used here during the long history of inbreeding (more than 25 generations) for the asthma trait. On the other hand, it is clear that the MHC genes of the high asthma strain do not solely control the expression of the asthma trait, since one strain (201,4b) out of the three independent low asthma strains raised by Lundberg (18) apparently has the same MHC genes as the high asthma strain. Furthermore, guinea pigs of the NIH strain 2, which share most of their MHC class II genes with the guinea pigs of the high asthma strain 740, display only sporadic and weak respiratory anaphylaxis to OA. Moreover, F1-hybrids between the high and low asthma parental strains which carry a complete set of MHC genes from the high asthma parental strain give asthma responses to OA which are intermediate between the high and low asthma strains.

A guinea pig of the high asthma strain can give an asthmatic response to OA only after it has been exposed to the antigen at least once. After an inhalation of OA these guinea pigs develop homocytotrophic anti-OA antibodies of the IgG1a and IgG1b isotypes, whereas IgE antibodies cannot be detected during the first 7 weeks after inhalation (9). If serum from OA-exposed guinea pigs from the high asthma strain are injected into normal unsensitized guinea pigs of the same strain these now respond to a first inhalation of OA (18). Thus, the homocytotrophic antibodies appear to be pathogenic. Such antibodies are not produced by guinea pigs of the low asthma strain, even after several inhalations of OA. This points to genetic differences between the two strains with respect to the production of the pathogenic antibodies. The class II MHC genes may exert their control of the asthma by regulating the production of these antibodies. The class II genes are immune response genes and are involved in the control

of antibody responses to protein and polypeptide antigens (2), although the mechanisms of the control are poorly understood. According to the "determinant selection" idea (1, 30) these genes collectively control which of several epitopes on an antigen the animal's antibody response shall be directed against (3). One may therefore hypothesize that the class II genes of the high asthma strain provide for an immune response to a set of epitopes on the ovalbumin molecule which favours the production of large amounts of homocytotrophic antibodies against ovalbumin.

However, the genes which control the production of homocytotrophic antibodies against ovalbumin in the high asthma strain cannot all be MHC genes, since guinea pigs of the low asthma strain 201, 4b (not used in the experiments reported here) share MHC genes with the high asthma strain, but do not produce homocytotrophic antibodies after OA-inhalation. This raises the question of whether the high asthma strain possesses non-MHC genes, which enable them to produce such antibodies. A gene or set of genes controlling the production of homocytotrophic antibodies of the IgE isotype have been identified in mice and shown to be independent of the MHC genes (28, 36). A homologous gene or set of genes is involved in human asthma (21). It is therefore tempting to postulate that guinea pigs of the high asthma strain possess a similar non-MHC gene or set of genes, which enable them to produce IgG1a and IgG1b homocytotrophic antibodies in response to ovalbumin inhalation.

In addition to the MHC gene(s) and the putative gene(s) controlling the production of homocytotrophic antibodies there is experimental evidence for the involvement of at least one more gene in guinea pig asthma. This is suggested by the finding (18) that injection of sera from OA-sensitized high asthma guinea pigs into low asthma guinea pigs did not prepare the recipients for an asthma response to a first inhalation of OA – contrary to the effect of serum injection in high asthma guinea pigs. This suggests that there are differences in the effector organ, i.e. the lung, in the two strains. Lundberg & Diamant (unpublished) have

shown that the histamine content of the lung tissue from untreated animals is far higher in the high asthma strain than in the low asthma strain. This difference, which is likely to be genetically determined, may be important in the respiratory anaphylaxis.

Our calculations suggest that, apart from the MHC gene(s), at least two other genes may be involved in the control of guinea pig asthma. A more precise estimate may result from an analysis of the asthma trait in the F2 generation of crosses between the high and low asthma strains. Our attempts to identify these non-MHC genes will concentrate on genes controlling the production of homocytotropic antibodies and genes controlling histamine production and release in the lung. We believe that the guinea pig model, which allows controlled cross-breeding between stocks of homozygous animals differing in the extreme with respect to asthma phenotype, is suitable for such studies.

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