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Influence of Mouse Genotype on Passive Systemic Anaphylaxis by Immune Complexes

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The influence of mouse genotype on passive systemic anaphylaxis (PSA) by immune complexes was studied. PSA was induced by using *Brucella abortus* endotoxin as the antigen and rabbit anti-*Brucella* endotoxin antisera. Experiments using syngeneic mice as well as mice congenic for *H-2* showed that the *H-2* haplotype influenced the sensitivity of mice to PSA. Among the *H-2* haplotypes studied, *H-2*^{b} was the most sensitive, followed by *H-2*^{k} and *H-2*^{d}. Experiments using passive transfer of serum as well as the complement inhibitors suramin and flufenamic acid indicated that variations in complement levels under control of *H-2* may be responsible for the effects described. Cyproheptadine, a blocker of serotonin and histamine receptors, and imidazol-α-ketoglutarate, an inhibitor of thromboxane synthesis, inhibited PSA, indicating that platelet aggregation, possibly mediated by activated components of the complement cascade, is an important feature in the development of PSA reactions in this system. Differences between strains for protection by cyproheptadine and for the effect of complement inhibitors indicated a role of early components of the classical pathway in this model.

The process of active immune-complex deposition in vessels and tissues appears as a pathogenic mechanism of neuritis, vasculitis, and glomerulonephritis, with a possible anaphylactic origin (5, 12, 18, 26). The size of immune complexes is a critical factor in determining their clearance from the circulatory system (24). It has been proposed that the size of immune complexes mainly depends on the antigen-antibody ratio, the number of antigenic determinants, and the affinity between antigen and antibody (7, 12, 26). Some relationships between the size of the immune complexes and the heterogeneity of antibodies have also been described (15), and we have used passive systemic anaphylaxis (PSA) by immune complexes as a model to study atherogenic processes in mice through the vascular deposition of the complexes (21).

In this work, we report the influence of the genetic background of mice on their responses to PSA produced by immune complexes. Clear differences between mice bearing the *H-2*^{b}, *H-2*^{d}, and *H-2*^{k} haplotypes were found. In addition, cyproheptadine chlorohydrate (CY), a blocker of serotonin and histamine receptors (3), ticlopidine (TC), an inhibitor of platelet aggregation (17, 25), and imidazol-α-ketoglutarate (α-IK), which inhibits thromboxane synthesis (11), were used to ascertain possible differences in the pathogenic mechanisms of PSA among strains.

MATERIALS AND METHODS

Antigen. The f5 endotoxic fraction (f5) obtained from *Brucella abortus* 2308 by the Westphal method (2) was used as the antigen. Cultures grown for 48 h at 37°C in a rotary shaker on Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) were used as the cell source for obtaining endotoxin.

Antiserum. Rabbit anti-f5 endotoxic fraction antibody was prepared essentially as previously described (21). High-titered rabbit antisera were obtained 1 month after injecting intravenously (i.v.) 2×10^9 bacteria in 0.5 ml of phosphate-buffered saline.

Drugs. CY (Merck Sharp & Dohme, Madrid, Spain), TC (Arcor, Toulouse, France), and α-IK (UCB Laboratorios, Barcelona, Spain) were assayed as protective drugs in PSA responses. Suramin (Germanine Bayer, Leverkusen, Federal Republic of Germany) and flufenamic acid (Laboratorios Hubbert S.A., Barcelona, Spain) were used as pharmacological inhibitors of complement-mediated effects. Ovalbumin (Sigma Chemical Co., St. Louis, Mo.) was used in rabbit immunizations to obtain antisera for ovalbumin-antiovalbumin complexes. Human immunoglobulin (Cohn fraction II and III; Sigma) was used to prepare heat-aggregated globulin.

Animals. All experiments were performed with 8- to 10-week-old male mice from the congenic strains BALB/c, BALB.B, and BALB.K or the strains C3H, C57BL/6, and DBA/2. All mouse strains were from the Animal Housing Department of our center and were initially obtained from Olac Ltd. Animals were housed in plastic cages with water and food ad libitum with a 12:12 day-to-night photoperiod.

PSA response. The procedure used to elicit PSA by immune complexes in mice has been described before (20). Briefly, mice were sensitized by an i.v. injection of 0.2 ml of a rabbit antiserum raised against endotoxin (f5) from *B. abortus* 2308 (2). After 3 h, the sensitized mice received i.v. injections of 0.75 mg (two times the 50% lethal dose) of the same endotoxin in 0.2 ml of saline. Lethal anaphylactic shocks were recorded at different times after the second injection (see below). Under these experimental conditions, animals affected by anaphylactic reaction died sooner (before 24 h) and showed classic specific symptoms; after 36 h, we considered they had overcome the anaphylactic phenomenon and were variably protected from the toxicity of f5 by the anti-f5 antiserum. As previously reported (8), the dif-

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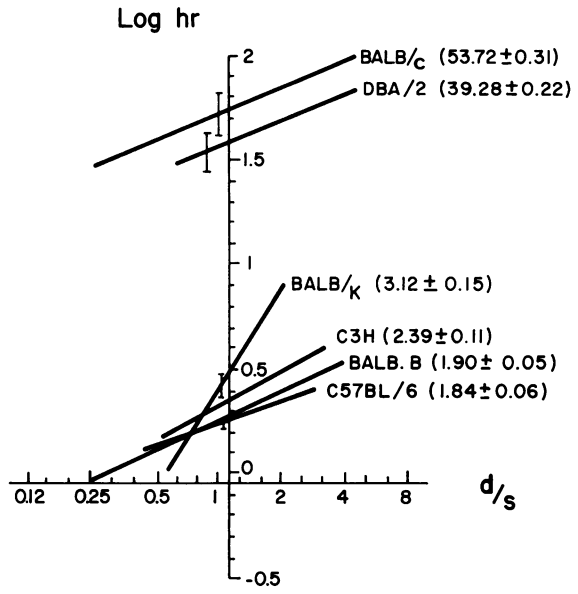

FIG. 1. MTD in mice after induction of PSA by immune complexes. Groups of 10 to 12 mice of the indicated strains were sensitized by i.v. administration of 0.2 ml of rabbit antiserum against *B. abortus* 2308 endotoxin. PSA was induced 3 h later by i.v. injection of 0.75 mg of the antigen (*B. abortus* 2308 endotoxin [f5]) in 0.2 ml of saline. Abscissa, ratio of dead to surviving mice; ordinate, log of MDT time after antigen administration. Intersections with ordinate indicate $\log_{10}$ hour of populational MTD calculated by nomograms (20) from the origin (death of the first mouse) and for the time of death of all other mice. MTD ± the standard deviation in hours was calculated as the antilogarithm from these values and is represented between brackets.

ferent effects of endotoxin are perfectly distinguishable in the mouse model proposed.

To investigate the possible influence of complement in the PSA response, pharmacological inhibition of complement activity by suramin (10) and flufenamic acid (P. F. Kohler and J. S. Martinez, Fed. Proc., p. 472, 1971) was studied. For passive transfer of serum, either 0.25 ml of normal mouse serum or the same volume of complement-depleted serum was administered i.v. Complement-depleted serum samples were prepared as described previously (16); briefly, antigen-antibody complexes were obtained with ovalbumin and a rabbit antiovalbumin serum mixed at the equivalence point and incubated at 4°C for 24 h. The immune complexes were washed three times with cold saline, and equal samples of these antigen-antibody complexes were mixed with 2 ml of mouse serum. The sera obtained were used after 24-h incubations at 4°C. In some cases, drugs (CY, TC, and α-IK) were perorally administered as a suspension in 0.5% carboxymethyl cellulose 2 h before serum sensitization and their effects were compared to groups of mice receiving carboxymethyl cellulose only.

Data analysis. A computer-assisted BASIC program was used for analysis and comparison of mean time of death (MTD). The relationship between the survival index and time was fitted by means of a regression line. The significance of differences between data was calculated by using a Student's *t* test. Differences were considered significant when $P < 0.02$.

RESULTS AND DISCUSSION

In previous studies with several strains of mice, we observed marked differences in PSA responses among strains (data not shown). Consequently, we performed experiments in which groups of 10 to 12 mice from inbred strains were tested for their sensitivity to PSA. The results were recorded as MTD calculated from nomograms (20) (Fig. 1). It was clear from the data obtained that C3H, BALB.K, and especially C57BL/6 and BALB.B mice had MTDs shorter than those of BALB/c and DBA/2 mice. The susceptibilities to PSA of the different strains of mice in relation to some of their genetic traits are shown in Table 1. The data show that sensitivity to PSA by immune complexes could be correlated with the *H-2* haplotype of the mice, since strains BALB.B and C57BL/6 ($H\text{-}2^b$) were very sensitive to PSA; BALB.K and C3H were slightly less sensitive to anaphylaxis, and BALB/c and DBA/2 ($H\text{-}2^d$) were markedly resistant. The hypothesis that there is a relationship between the *H-2* haplotype and sensitivity to PSA is supported by the fact that BALB.B and BALB.K are strains selected for differences in *H-2* haplotype in a BALB/c background.

One possible mechanism by which the *H-2* haplotype could influence PSA is through complement, whose functional levels in relation with *H-2* have been studied (6), especially for the early components of the complement cascade (13). It has been reported (14) that the formation of immune complexes causes platelet aggregation and histamine release by complement-mediated mechanisms. In addition, there are indications that stimulation of phagocytes through C3b receptors induces the release of platelet-activating factor (22). In mice, the *Ss* locus of *H-2* determines high or low levels of the *Ss* protein, which was later identified as C4 (23). Our data show that PSA-susceptible strains can be either Ss^h or Ss^l (Table 1). However, Démant et al. (6) reported that Ss^h mice have high levels of functional complement only when they express a certain *Slp* allotype (Slp^a) but not when they express other allotypes (Slp^o) (6) (Table 1). Surprisingly, we found that all the PSA-susceptible strains had low levels of complement as defined by Démant et al. (6) and PSA-resistant strains had high levels of complement. Although these data indicate that there is no

TABLE 1. Susceptibility to PSA of several strains of inbred mice

Mouse strain	*H-2* haplotype	*Ss–Slp* region variant	Complement level[a]	MTD (h ± SD)[b]	Susceptibility to PSA
BALB.B	*b*	Ss^h–Slp^o	Low	1.90 ± 0.05	High
C57BL/6	*b*	Ss^h–Slp^o	Low	1.84 ± 0.06	High
BALB.K	*k*	Ss^l–Slp^o	Low	3.12 ± 0.15	High
C3H	*k*	Ss^l–Slp^o	Low	2.39 ± 0.11	High
BALB/c	*d*	Ss^h–Slp^a	High	53.72 ± 0.31	Low
DBA/2	*d*	Ss^h–Slp^a	High	39.28 ± 0.22	Low

[a] As described by Démant et al. (6).
[b] Evaluated by nomograms (20) by using a computer-assisted program.

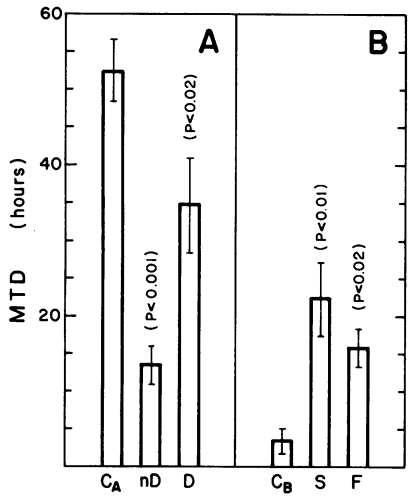

FIG. 2. Variations in the MTD after serum transfer assays (A) and after treatments with complement-inhibitory drugs (B). (A) MTD of normal BALB/c mice (C_A) or BALB/c mice injected i.v. with 0.25 ml of normal BALB.B serum (nD) given 30 min before sensitization with *B. abortus* endotoxin are depicted. Another group of BALB/c mice received BALB.B serum depleted of complement (D) as described in Materials and Methods. (B) The effect of suramin (S) (i.v. dose, 10 mg/kg, 2 h before sensitization) or flufenamic acid (F) (oral dose, 200 mg/kg, 2 h before sensitization) on the MTD of BALB.B mice (C_B). The statistical significance of the difference from the control is given in brackets.

correlation between PSA sensitivity and high complement levels, results from additional experiments suggest an important role of complement in PSA. To study this, two experimental approaches were followed: (i) passive transfer of normal mouse serum from a high-susceptibility strain to a low-susceptibility strain before or after complement depletion by antigen-antibody complexes (16) and (ii) treatment of mice with complement-inhibitory drugs to depress complement-mediated effects. Results of these experiments are presented in Fig. 2, in which the variations of PSA responses in the resistant BALB/c mice subsequent to serum transfer from sensitive strains are indicated (Fig. 2A). As can be seen, high susceptibility to PSA in H-2^d mice could be obtained by passive transfer of normal serum from the susceptible H-2^b mice. In these cases, the MTD of BALB/c mice was more than four times lower, but this effect was drastically decreased when BALB.B serum samples were depleted of complement by treatments with ovalbumin-antiovalbumin immune complexes. Similar results were obtained by using heat-aggregated (63°C, 20 min) human immunoglobulin to deplete complement activity (data not shown).

On the other hand, the high susceptibility of BALB.B mice to PSA diminished when animals were treated with the complement-inhibiting drugs suramin and flufenamic acid; MTD was augmented more than six times by treatment with suramin (10 mg/kg) and more than four times by flufenamic acid (4 mg/kg). This inhibitory effect indicates a role for classical pathway activation in the PSA response and may be similar to inhibition of the Arthus reaction in rabbits treated with suramin or flufenamate (for a review, see reference 1).

The mechanism by which *H-2* influences complement levels is not clear, although there are some indications that *H-2* may control the levels of other components of the complement system that are different from C4. Ferreira and Nussenzweig (9) reported that AKR/J mice (*H-2*^{k}) have serum C3 levels higher than DBA/2J mice (*H-2*^{d}). Studies with hybrids and backcrosses to the parental strains showed that the levels of C3 were controlled by a gene(s) linked to the *H-2* complex. Higher levels of C3 in H-2^k than in H-2^d mice fit with the high or low sensitivity to PSA of mice from these haplotyes (Table 1), but the complexity of the system requires a careful analysis at the genetic level.

Another aspect studied was the implication of platelet aggregation in our model of PSA. As mentioned above, there is evidence for complement-mediated mechanisms of platelet activation (14, 22). Consequently, several treatments with drugs that prevent platelet aggregation were established in order to examine the mechanism of PSA. Variations in PSA by immune complexes after treatment with CY, TC, and α-IK are presented in Table 2 for each of the strains of mice. CY, which blocks serotonin and histamine receptors (3), was protective for PSA, especially in mice of the *H-2*^{k} haplotype, pointing to a role of platelet aggregation in PSA. Another drug assayed was α-IK, an inhibitor of thromboxane synthesis (11), which protected the animals from PSA to some degree. The third drug under study was TC, which alters arachidonic acid metabolism in platelets, augmenting prostaglandin E_2 (PGE_2) and PGD_2 synthesis and lowering thromboxane synthesis without diminishing endoperoxide production (17). Thus, TC could favor PGI_2 synthesis by the vascular endothelium and, consequently, inhibit platelet aggregation. TC, however, did not show any protective effect under the experimental conditions used (Table 2).

Taken together, these results suggest that PSA is mediated by the aggregation of platelets and release of vasoactive amines. In addition, we observed in a previous study (21) that etofibrate, an antiatherogenic drug which inhibits platelet aggregation (19), can also inhibit PSA in mice. It is interesting that there are some differences among strains in protection by CY and α-IK (Table 2). BALB/c and DBA/2 mice were comparatively less protected by these treatments than were the other strains, indicating that several factors could influence the outcome of PSA responses. In addition, a differential behavior in CY-treated H-2^k and H-2^b mice was

TABLE 2. Variations in survival of different strains of mice after single-dose drug treatments

Mouse strain	PSA responses (MTD in h ± SD) with and without[a]:			
	Without drug	CY (0.2 mg/kg)	TC (7.5 mg/kg)	α-IK (25 mg/kg)
BALB.B	1.92 ± 0.05	5.85 ± 0.19	2.93 ± 0.06	4.86 ± 0.17
C57BL/6	1.81 ± 0.04	5.00 ± 0.11	3.44 ± 0.11	3.78 ± 0.08
BALB.K	3.09 ± 0.12	26.95 ± 0.13	2.67 ± 0.03	7.82 ± 0.15
C3H	2.26 ± 0.09	11.89 ± 0.06	2.20 ± 0.01	6.52 ± 0.09
BALB/c	54.32 ± 0.33	60.42 ± 0.15	29.21 ± 0.09	74.99 ± 0.52
DBA/2	39.35 ± 0.25	57.85 ± 0.23	26.77 ± 0.12	43.31 ± 0.38

[a] Drugs were orally administered as suspensions in 0.5% carboxymethyl cellulose 2 h before serum sensitization.

observed, since H-2^k animals were more effectively protected than were H-2^b mice by CY. One of the factors influencing protection by CY could well be the levels of the early components of the classical activation pathway, because, according to Goldman and Goldman (13), H-2^b mice of the have higher levels of C1, C4, and C2 than H-2^k mice. Thus, the protection by CY is more effective when the levels of these components are low, stressing the importance of the classical pathway in the outcome of the PSA response in our model.

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