Amyloid. VIII. On Strain Variability in Experimental Murine Amyloidosis (33580)

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The role of genetic factors in the development of amyloidosis has been the subject of considerable interest. In the human, familial amyloidosis has been described in association with a number of syndromes including familial Mediterranean fever, polyneuropathy of the Portuguese type, cardiopathy, nephropathy, and medullary carcinoma of the thyroid (1). Although development of spontaneous amyloidosis in mice has been shown to be strain dependent (2), studies on the effect of genetic factors in experimental murine amyloidosis have been few, contradictory and are equivocated by the use of small numbers of strains and the variability and unpredictability of previously employed methods for the induction of this disease (3–7).

We recently described a simple procedure for the rapid induction of amyloidosis in mice, involving a single intraperitoneal (i.p.) injection of Freund's complete adjuvant containing additional heat-killed *M. butyricum* (8). Employing this procedure, the influence of strain and sex on the susceptibility of mice to experimental amyloidosis was investigated, with a view to evaluating the suitability of various available genetic strains of mice in our continuing studies on the pathogenesis of experimental murine amyloidosis. It was found that amyloidosis could be induced in both sexes and all strains of mice so far investigated. However, the genetic makeup of the animal seemed to influence the incidence and severity of the disease.

Materials and Methods. Susceptibility of mice to experimental amyloidosis was investigated in 6–8-week-old male mice of the following strains obtained from the Animal Production Unit of NIH: $AALF_1$, NBL/N, AL/N, A/HeN, C_3H/HeN, AKR/N, DBA/2N, C57BL/6N, STR/N, and general purpose Swiss albino mice (GP). For the effect of sex on the inducibility of amyloidosis, animals of both sexes of a single strain namely C_3H were compared. All animals were identically maintained on standard laboratory diet. Each group under study was initially composed of twelve to fifteen animals.

Amyloidosis was induced with a single i.p. injection of 0.25 ml of an emulsion containing equal volumes of complete Freund's adjuvant and phosphate buffered saline, supplemented with additional heat-killed *M. butyricum* (16.6 mg/ml), as described previously (8). Five uninjected controls were maintained under identical conditions for each group. All the animals were weighed prior to and 2 weeks after the adjuvant injection, just before they were sacrificed by cervical dislocation. The spleens of all the mice were also weighed. Tissues were fixed in 10% neutral formalin for 24–48 hr, paraffin embedded, sectioned, and stained with alkaline Congo red (9) and hematoxylin and eosin. Amyloid was identified by a positive Congo red reaction and the characteristic green birefringence in polarized light.

Results and Discussion. Development of amyloidosis in 10 available strains of mice when injected with a Freund type of emulsion is shown in Table I. All strains under study developed the disease, although strain-dependent differences in the incidence, severity, and organ distribution of the disease were noted. The various strains of mice are arranged in Table I in the decreasing order of their splenic amyloid deposits. It is interesting that $AALF_1$ strain of mice which is a cross between A/HeN and AL/N strains develops amyloidosis to a degree intermediate between the degrees of incidence for its parent strains. BALB/c AnN strain of mice which were previously reported (8, 10) to develop amyloidosis with a lower degree of

TABLE I. Induction of Amyloidosis in Various Strains of Mice.

Strain	Organ distribution of amyloidosis (percentage or no. positive/total)								Incidence of spontaneous amyloidosis
				Kidney:					
	Spleen	Lymph node	Liver	Papillae	Glomeruli	Heart	Tongue	Intestine	
NBL/N	100 (10/10)	100	100	100	56	90	80	100	—[a]
C_3H/HeN	100 (10/10)	100	100	100	20	100	100	100	Low
AKR/N	100 (14/14)	93	100	93	57	93	93	86	Low
GP	100 (14/14)	92	100	85	15	100	83	86	—
DBA/2N	100 (13/13)	92	92	92	0	92	92	89	—
C57BL/6N	100 (10/10)	50	89	45	12	44	25	56	Low
STR/N	92 (11/12)	18	50	8	0	17	12	0	—
A/HeN	90 (9/10)	0	44	30	0	33	30	43	High
$AALF_1$	63 (5/8)	0	25	0	0	0	0	0	High
AL/N	27 (3/11)	10	18	11	0	9	9	20	High

[a] No data available.

incidence than the C_3H strain is not included in this study. Both the males and females of C_3H strain of mice developed amyloidosis following the adjuvant injection and there was no significant difference in the incidence, severity or the organ distribution of amyloid deposits between the sexes.

Williams *et al.* (3), working with an inbred CBA strain and noninbred and hybrid albino strains reported that inbred strains were more susceptible to amyloidosis than noninbred or hybrid strains and that more males than females developed amyloidosis when given multiple casein injections. The present study shows however that the Swiss albino general purpose strain which is a random bred stock is more susceptible to amyloidosis than a variety of inbred strains. Kennedy (4), working with C_3H and AKR strains, found that development of amyloidosis was unrelated to strain or sex.

Table I also includes available information from the literature and personal communication from Dr. T. B. Dunn of the National Cancer Institute on the incidence of spontaneous amyloidosis in the various strains of mice employed in the present study. Strains which are reputed to have a high tendency to spontaneous amyloidosis seem to show a low incidence of experimental amyloidosis and vice versa. It is not clear whether the reciprocal relation noted here between low incidence of spontaneous and high incidence of experimental amyloidosis is fortuitous or has some biological significance.

The spleens of all the mice injected with the adjuvant, irrespective of the strain and the degree of splenic amyloidosis showed a three- to fourfold increase in weight as compared to the spleens of age-matched uninjected controls. The degree of lymphoreticular cell proliferation occurring in the spleens in response to the adjuvant stimulus appears to bear no relation to the amount of amyloid deposited. This is further borne out by the fact that in animals with spontaneous amyloidosis, the spleens are either normal in weight or somewhat smaller, and do not show any evidence of lymphoreticular proliferation. Strain-dependent differences in the activity of the reticuloendothelial cell system in C57BL/Bln Jena and AB/Jena mice have been noted (5, 7) but no definite conclusions relating this activity to the development of amyloidosis could be drawn. Similarly no correlation seems to exist between the capacity for antibody or serum protein production of a given strain of mice and its susceptibility to experimental amyloidosis. In a comparative study of various NIH strains of mice challenged with intraperitoneal injections of complete Freund's adjuvant alone or with ferritin or hemocyanin, Barth *et al.* (11) noted that C57 strain responded with the highest 18S

gamma M globulin production, and GP strain with the highest 7S gamma globulin production, while the AKR strain showed the least change in immunoglobulin production. On the other hand the C57 strain often had lower antibody titers while AKR, AL/N, A/HeN, and GP had higher titers. The susceptibility of these strains to experimental amyloidosis presented in Table I does not seem to correlate with any of the above biological activities of these strains.[1] A similar lack of correspondence between amyloid production and serum protein changes was reported among three inbred strains of mice namely, C57BL—Bln Jena, AB/Jena, and CFW/carw Han Jena (6).

Summary. Variability due to strain and sex in experimental amyloidosis of mice was investigated. When given a single intraperitoneal injection of 0.25 ml of a Freund-type emulsion containing additional heat-killed *M. butyricum* (40 mg/ml), all of 10 strains of mice investigated (NBL/N, C_3H/HeN, AKR/N, AL/N, DBA/2N, C57/6N, STR/N, A/HeN, $AALF_1$, and general purpose Swiss albino) developed amyloidosis. However, strain-dependent differences in the incidence and degree of severity of the disease were note. It was also noted that the strains which are highly susceptible to experimental amyloidosis are the ones reported in literature to be least prone to develop spontaneous amyloidosis and vice versa. Both males and females of a single strain investigated, namely C_3H, developed amyloidosis to a similar degree.

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[1] Similarly, there is no apparent correlation between the susceptibility to induced amyloidosis and the presence or absence of MuBl antigen, reported to be a component of the mouse complement system (12–14). Among the strains employed in this study, NBL, AKR, DBA/2 and A strains were reported to be deficient in this antigen (12) while C_3H and C57Bl strains possessed it.

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