Disseminated Amyloidosis in Germfree Mice

Spontaneous Prevalence, Relationship to Ionizing Radiation and Pathogenetic Implications

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Spontaneous amyloidosis was noted in a significant number of germfree mice in comparison with their conventional contemporaries. The adjusted prevalence of this disease was increased in both groups by whole-body exposure at 6 weeks of age to 700 rad of ionizing radiation. The germfree groups demonstrated persistent hypogammaglobulinemia throughout their lifespans and no evidence of significant inflammatory processes at necropsy. The possible interpretation of these observations is discussed and it is concluded that defective or deficient immunoglobulin production may be the essential prerequisite for the development of amyloidosis. (Amer J Path 65:43–50, 1971)

UNTIL RECENTLY, most investigators have assumed that amyloid production is related to an uncontrolled escalation of the immune response. This assumption was related to several observations: (1) a significant proportion of persons with chronic suppurative inflammatory processes (characteristically osteomyelitis, tuberculosis, bronchiectasis) and with prolonged tissue destruction subsequently develop amyloidosis [1]; (2) amyloidosis may be induced experimentally by repeated injections of select proteins or by hyperimmunization with bacterial vaccines or endotoxin [2,3]; (3) individuals with multiple myeloma and Hodgkin's disease have an increased prevalence of amyloidosis [4]; (4) select autoimmune diseases, most particularly rheumatoid arthritis and parabiosis intoxication, appear to be complicated by amyloidosis at an exceptionally frequent rate.[5] On the basis of these and related reports, it has been postulated that the pathogenesis of amyloidosis involves the diversion of reticuloendothelial cells and/or plasma cells from normal activity (antibody synthesis) to amyloid production, this switch being occasioned by sustained antigenic bombardment or by the malignant transformation of one of the involved cell types.

In the past several years, an apparent association between amyloid and immunologic hyporeactivity has also been noted. Thus, amyloidosis

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Supported in part by grant HD-02194 from the US Public Health Service and in part by research funds from the Albuquerque Veterans Administration.

Accepted for publication May 24, 1971.

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has been reported associated with agammaglobulinemia in humans[6] and occurring after neonatal thymectomy plus appendectomy in rabbits.[7] Moreover, amyloidosis induced by sodium caseinate in mice is promoted by neonatal thymectomy,[8] administration of nitrogen mustard,[9] exposure to ionizing radiation[10] and related immunosuppressive procedures.[11] In the present experiment, the prevalence of amyloid was significantly greater in germfree than in conventional mice and the rate of occurrence was increased in both groups by exposure to ionizing radiation.

Materials and Methods

A total of 331 virgin female mice of the Charles River CD-1 line were divided into experimental groups as shown in Table 1. This colony represents a closed but randomly bred stock originally derived by cesarean section from a HaM/IRC female mouse. The conventional component of the colony is maintained by continuous withdrawal of breeder pairs from the complement of germfree mice.

The conventional and germfree mice were maintained in identical fashion in standard Trexler isolators and fed autoclaved fortified Wayne Lab Blox and sterilized water. Feces and bedding from the germfree mice were cultured aerobically and anaerobically at monthly intervals for evidence of bacterial contamination. Germfree, in the context of this report, indicates that these mice were free of viable bacterial and fungi and did not harbor antibodies to the following murine viruses: PVM, polyoma, K, Sendai, GD VII, rat, H-1, SV_5, mouse adeno, mouse hepatitis and LCM.

The irradiated mice were exposed in whole-body fashion at 6 weeks of age to 700 rad generated by a cobalt-60 gamma source in a sterilized radiation chamber as described elsewhere.[12] Eighteen mice in the conventional, exposed group died within 30 days of exposure and were considered not at risk in the results presented herein. Sixteen mice from each experimental group (64 total) were sacrificed periodically according to a table of random numbers for biochemical studies. The mice in the sacrificed group were examined histologically and therefore were considered at risk.

As noted above, four mice in each group were sacrificed on days 0, 160, 320, and 480 postexposure for the following analyses: serum proteins, blood urea nitrogen (BUN), serum glutamic oxalacetic transaminase (SGOT), lactic dehydro-

Table 1—Prevalence of Amyloid by Experimental Group

Experimental group	No. mice in group	No. dying acutely	Significant amyloid*
Germfree			
Exposed	88	0	38.9
Nonexposed	87	1	55.5
Conventional			
Exposed	100	18	17.2
Nonexposed	88	0	8.6

* Results expressed as percent of mice in group with more than trace or equivocal amount of amyloid

genase (LDH) and renal lysozyme activity. Determinations were performed in duplicate in routine fashion as summarized elsewhere [13]; therefore, each result included herein represents the average of eight determinations.

All mice were observed at least three times daily; animals were necropsied immediately after death and microscopic sections prepared of each major organ in routine fashion. Suspicious infiltrates suggestive of amyloid were confirmed by supravital straining and electron microscopy.

Results

As shown in Table 1, the incidence of amyloid is greater in both the irradiated and nonirradiated germfree groups than in the comparable conventional goups. The occurrence of significant amyloid deposition is more frequent in the irradiated conventional group than in the nonirradiated conventional group; the latter relationship is not evident in the germfree groups. This discrepancy appears to be related to the particularly high frequency of thymic lymphosarcoma in the germfree irradiated group [13]; a significant number of these animals died of this malignancy at a relatively early age and therefore did not survive long enough to develop amyloid. If all mice with thymic lymphosarcoma are excluded from consideration, the incidence of this disorder after irradiation is increased in both conventional and germfree mice, as shown in Table 2. The prevalence in all 4 experimental groups is age-dependent. An additional factor that is not taken into consideration in the data of Tables 1 and 2 is cecal volvulus; torsion of the characteristically dilated caecum is peculiar to germfree mice, which often succumb to this condition at a young age and therefore do not survive until amyloid becomes prevalent. Recognition of this factor in the results of Tables 1 and 2 would serve to accentuate the differences between the conventional and germfree groups.

The results of the serum protein analyses are shown in Table 3. Levels of serum gamma globulin in the conventional group average 2–9 times more than those in the comparable germfree groups. No other consistent discrepancy is noted, although total-protein determinations

Table 2—Significant Amyloid by Experimental Group Excluding Mice with Thymic Lymphosarcoma

Group	Significant amyloid*	
	Nonexposed	Exposed
Conventional	9.0	22.5
Germfree	60.6	63.6

* Results expressed as percent of mice in group with more than trace or equivocal amount of amyloid

Table 3—Serum Proteins as Function of Age*

Protein	Results (g%) Days postexposure at sacrifice 0	160	320	480
Conventional Nonexposed				
Total protein	5.3	5.4	6.1	5.3
Albumin	2.91	2.94	3.41	3.28
α_1 Globulin	1.20	1.24	1.03	0.91
α_2 Globulin	0.45	0.43	0.49	0.35
β Globulin	0.48	0.63	0.67	0.57
γ Globulin	0.26	0.19	0.52	0.20
Conventional Exposed				
Total protein	5.8	5.5	5.3	7.0
Albumin	2.91	3.24	2.81	3.75
α_1 Globulin	1.50	1.10	0.91	1.47
α_2 Globulin	0.51	0.37	0.43	0.56
β Globulin	0.61	0.59	0.72	0.74
γ Globulin	0.27	0.20	0.42	0.42
Germfree Nonexposed				
Total protein	—	5.6	4.7	4.9
Albumin	—	3.47	2.28	2.30
α_1 Globulin	—	1.28	1.27	1.37
α_2 Globulin	—	0.33	0.29	0.55
β Globulin	—	0.51	0.69	0.59
γ Globulin	—	0.03	0.15	0.09
Germfree Exposed				
Total protein	—	5.1	6.0	4.8
Albumin	—	3.49	3.78	2.11
α_1 Globulin	—	0.88	1.10	1.40
α_2 Globulin	—	0.25	0.42	0.53
β Globulin	—	0.48	0.65	0.66
γ Globulin	—	0.07	0.06	0.12

also tend to be greater in the conventional mice than in their germfree contemporaries.

The results of the remainder of the biochemical determinations are summarized in Table 4. The fluctuations in BUN may relate to the prevalence of the renal amyloid, although the average value at the time of the final sacrifice is less than might be anticipated on the basis of this hypothesis. LDH and SGOT levels are also generally highest at the time of the third sacrifice and then drop off; the reasons for this observation are also not clear but may relate to tissue destruction attendant to

amyloid infiltration. Renal lysozyme activity shows the expected rise with age, which generally is accentuated postexposure.

Discussion

As noted above, amyloidosis is particularly associated with chronic inflammatory disease and select immunologic deficiency states. The latter are often complicated by infections and it has been suggested that the attendant tissue destruction, and antigenic stimulation, are the denominators common to the several apparent pathogenetic pathways that lead to amyloid formation. Telium [6] has proposed the following biphasic mode of onset: (1) an initial antigenic stimulation associated with the proliferation of reticuloendothelial and plasma cells and an increase in serum gamma globulins followed by (2) a phase of ex-

Table 4—Serum Enzyme and BUN Determinations as Function of Age

	Results*			
	Days postexposure at sacrifice			
Determination	1	160	320	480
Conventional Nonexposed				
SGOT	51	56	190	268
LDH	1760	2160	4140	2160
BUN	26	22	34	22
Renal Lysozyme	7.19	3.61	3.78	18.92
Conventional Exposed				
SGOT	55	48	140	102
LDH	1200	1680	>6000	960
BUN	37	20	50	40
Renal lysozyme	8.67	6.70	6.48	19.40
Germfree Nonexposed				
SGOT	34	48	190	156
LDH	2650	1350	2940	3600
BUN	21	18	52	38
Renal lysozyme	9.06	7.89	17.50	19.49
Germfree Exposed				
SGOT	34	56	>430	96
LDH	2650	1350	3180	1470
BUN	14	18	48	19
Renal lysozyme	11.17	6.78	6.80	48.89

* Serum glutamic oxalacetic transaminase (SGOT) and lactic dehydrogenase (LDH) expressed in standard units; blood urea nitrogen (BUN) in mg%; renal lysozyme activity as mg lysozyme/g protein.

haustion and immunologic incompetence that occurs in the face of continued antigenic stimulation. Ebbesen, Rask and Nielsen [14] noted that select strains of mice spontaneously develop either paraproteinemia or amyloidosis, suggesting to Mawas *et al* [15] an overlapping genetic predisposition to two apparently related disorders. In a study primarily devoted to an evaluation of the delayed consequences of exposure to ionizing radiation, Walburg and Cosgrove [16] noted an apparently increased prevalence of amyloid in germfree compared with conventional mice, although, as the authors carefully note, the total number of animals involved (5 of 13 in germfree groups with amyloid compared with 0 of 12 conventional mice) is insufficient for definitive comment. The present experiment supports and extends this preliminary observation with a larger study population and complementary chemical analyses.

In the work reported herein, amyloidosis occurred in a significant number of germfree mice in the absence of an inciting inflammatory process and with no evidence of antigenic overload. In conjunction with the experimental evidence alluded to above, the results suggest that deficient or defective immunoglobulin production may represent the common mechanism that triggers amyloid production in the wide spectrum of experimental and clinical situations known to predispose to this disease.

Thus, select colonies of germfree mice would appear to be the experimental counterpart of adult-onset agammaglobulinemia (acquired hypogammaglobulinemia). If insufficient production of gamma globulin *per se* is responsible for the induction of amyloidosis in these persons, the present observations suggest a possible therapeutic approach. Finally, it would be of particular interest to assess the consequences of prolonged immunosuppression with respect to amyloid formation in germfree animals where such an approach is not complicated by overt or subclinical infections.

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Dr. Anderson is a Markle Scholar in Academic Medicine.

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