EXPERIMENTAL AMYLOIDOSIS

Rates of Induction, Lymphocyte Depletion and Thymic Atrophy

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While it is widely believed that amyloidosis develops as a direct or indirect consequence of some immunologic disturbance, the necessary conditions for its experimental induction have not yet been defined clearly. Some reasons for this failure have been discussed previously [1]; they include, briefly, the completely unrelated nature of many experimental procedures reported to have induced amyloidosis, the irregular or unpredictable onset of amyloidosis after injections of certain protein antigens, and the uncertainties as to the influences of concomitant bacterial infections or of spontaneous amyloidosis on the results of variously reported experiments.

In an attempt to define necessary experimental conditions for amyloidosis, a modified casein injection method for the reproducible induction of amyloid in young $C_{57}BL/10J$ mice was initially developed.[1] Under similar conditions injections of nonantigenic casein hydrolysate, and of gelatin and bovine serum albumin, relatively weak antigens, failed to induce amyloid.[1,2] When the latter two proteins were injected after coupling them to an azo-hapten, however, amyloidosis resulted.[2] After casein was coupled to the azo-hapten and injected into mice, amyloidosis was induced at a rapid rate, i.e., 10 days as compared to 22 days for casein. When azo-casein was injected in large quantities, amyloidosis developed within 6 to 8 days.[2] The failure of an enzymatic hydrolysate of azo-casein to induce amyloidosis suggested that the enhanced amyloidogenic capacity of these proteins after azotization was due to the known antigen-enhancing effect of this procedure rather than to a simple pharmacologic effect of the azo-hapten.

These experiments [1,2] thus indicated that (1) repeated antigenic challenge is, at least, one reproducible and adequate condition for amyloid

Supported by Grants AM-06626 and 1-F2-GM-23, 666-01, from the United States Public Health Service.

Accepted for publication, May 13, 1966.

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induction; (2) increasing the antigenicity of various soluble proteins by coupling to an azo-hapten increases their capacity to induce amyloidosis, so that there was a nearly direct correlation between antigenicity and amyloid induction times; (3) loss of antigenicity of soluble proteins, through loss of molecular size, results in loss of their amyloidogenic effect and, (4) rapid induction of amyloidosis with large amounts of a strong antigen, azo-casein, may be related to the development of generalized immunologic tolerance. The changes in lymphoid tissues in these experiments were striking and are reported in this paper. They showed a nearly inverse relationship between mature lymphocyte depletion and the induction times for amyloidosis, i.e., the shorter the time of onset of amyloidosis with injection of a soluble protein, the greater the depletion of lymphocytes. The significance of these findings and their similarity to those found in runting or wasting syndromes will be discussed.

Material and Methods

Experimental conditions have been described in detail previously.[1,2] Briefly, groups of inbred $C_{57}BL/10J$ male mice, 6 to 8 weeks of age, were used and given injections of various proteins: gelatin, azo-gelatin, albumen, azo-albumen, casein, azo-casein, enzymatic hydrolysates of casein and azo-casein. All proteins were dissolved in 0.01M $NaHCO_3$ to a final concentration of 10 gm per cent (w/v), with a final pH of 6 to 7. They were injected in 0.3 ml quantities (30 mg), subcutaneously, according to the schedule summarized in Table I, under "routine injection schedule". Three groups of mice were also given daily injections with increased amounts of azo-casein as indicated in Table I under "increased injection schedule". Control mice received 0.01M $NaHCO_3$. A group of 20 untreated mice was used for base line organ weights and histologic structure.

All mice were killed 24 hours after the last injection. At necropsy, splenic weights in all, and thymic weights in all groups with the exception of that receiving azo-gelatin were measured and expressed as the percentage of the body weight at the time of necropsy (Table I). Blocks of all organs, except brain, were fixed 24 hours in 4 per cent formaldehyde in phosphate buffer, pH 7.4, at 2 to 3° C. Particular attention was directed to lymph nodes; the mediastinal mesenteric, lumbar, axillary, brachial and inguinal lymph nodes were always examined and fixed. The sternums from 5 to 12 mice in each group except that receiving azo-gelatin were also fixed. After briefly washing in water, all tissues were embedded in paraffin and sectioned at 4 to 6 μ.

As described in detail previously,[1,2] all tissues were stained with Congo red and examined with a Zeiss polarizing microscope. Selected sections were examined after staining with crystal violet, thioflavine T, the indole method for tryptophane and the periodic acid-Schiff (PAS) technique. In addition, sections of all tissues from 5 to 10 mice in each group were stained with methyl green-pyronine. Bone marrow sections were stained by the Giemsa method. Total and differential leukocyte counts were done in 6 mice, immediately before and 24 hours after 9 daily injections of azo-casein (Table III).

The criteria used for the identification of amyloid as well as the occurrence, incidence and severity of amyloidosis have been reported in detail.[1,2] For present purposes, amyloidosis is indicated in Table I as either present (+) or absent (−).

The histologic changes in lymphoid tissues were assessed and graded by each author on an independent basis. For each animal multiple sections of the entire thymus and spleen, and of the lymph nodes enumerated above, stained as described, were exam-

ined before a final grade was assigned. The grading was done numerically under two categories as follows: (a) *depletion of small lymphocytes:* 0, no recognizable depletion; 1, slight, being just recognizable; 2, moderate, depletion obvious; 3, advanced, with only small numbers present; 4, virtually complete, with difficulty finding lymphocytes; (b) *pyroninophilic cell and hematopoietic cell hyperplasia:* 0, no recognizable increases; 1, slight, being just recognizable; 2, moderate, obvious increases; 3, advanced, i.e., splenic red pulp and medullary cords and intermediate zones of lymph nodes partially replaced by these cells; 4, virtually complete replacement.

TABLE I

AMYLOIDOSIS AND THYMIC AND SPLENIC WEIGHTS. $C_{57}BL/10J$ MICE INJECTED WITH VARIOUS MATERIALS

Material injected *	Amount injected (mg)	Day killed	Organ weights † (% body weight)		Amyloidosis ‡
			Thymus	Spleen	
No injection (20)	—	—	0.213 ± 0.044	0.34 ± 0.02	—
"Routine injection schedule" §					
$NaHCO_3$(30)	—	8–41	0.174 ± 0.04	0.38 ± 0.04	—
Gelatin (14)	750–1800	35–62	—	0.36 ± 0.03	—
Azo-gelatin (6)	630	22	—	0.43 ± 0.045	—
Azo-gelatin (14)	840–960	28–32	—	0.58 ± 0.13	+
Albumen (22)	570–1290	20–47	0.151 ± 0.019	0.35 ± 0.05	—
Azo-albumen (5)	420	15	0.150 ± 0.054	0.62 ± 0.12	—
" " (8)	570	20	0.051 ± 0.02	1.10 ± 0.13	+
Casein (5)	420	15	—	0.54 ± 0.028	—
" (16)	630	22	0.170 ± 0.02	0.54 ± 0.039	+
Azo-casein (8)	150	6	0.066 ± 0.054	0.70 ± 0.28	—
" " (8)	210	8	0.076 ±0.044	1.06 ± 0.11	—
" " (14)	270	10	0.042 ± 0.016	0.81 ± 0.14	+
Casein hydrolysate (5)	1320	62	—	0.35 ± 0.028	—
Azo-casein hydrolysate (13)	270–450	10–16	0.095 ± 0.028	0.59 ± 0.07	—
Increased injection schedule ¶					
Azo-casein (7)	300	4	0.040 ± 0.01	0.41 ± 0.073	—
" " (5)	400	6	0.027 ± 0.004	0.51 ± 0.12	+
" " (8)	500	8	0.022 ± 0.009	0.66 ± 0.66	+

* Number of mice in parenthesis.

† All values represent means with standard deviations.

‡ Amyloid present (+) or absent (—).

§ Mice given one daily injection of 0.3 ml quantities (30 mg), 7 days a week for all groups except gelatin and casein hydrolysate, given 5 days a week. All mice killed 24 hours after last injection.

¶ Mice given two daily injections totalling 0.6 to 1.0 ml (60 to 100 mg respectively) on consecutive days. All mice killed 24 hours after last injection.

RESULTS

The normal histologic structure (Figs. 1, 5 and 6) of the different lymphoid organs was similar to that described in detail by Dunn.[3]

Experimental Changes in Lymphoid Tissues

Within any one experimental group, variations in histologic changes were noted. The differences in these changes between most groups were,

TABLE II

LYMPHOID TISSUES * OF C57 BL/10J MICE FOLLOWING INJECTION OF VARIOUS MATERIALS †

Material injected	Amyloidosis Day of onset	Small lymphocyte depletion			Pyroninophilic cell hyperplasia			Hematopoietic activity	
		Thymus	Spleen	Lymph nodes	Thymus	Spleen	Lymph nodes	Spleen	Lymph nodes
$NaHCO_3$ gelatin, casein hydrolysate, albumen	none	0–1	0–1	0–1	0	0–1	0–2	0	0
Azo-casein hydrolysate	none	2	2	1	0	0–1	0–1	0–1	0–1
Azo-gelatin	28–32	0	1	0–1	0	1	1	1	0
Casein	22	1	1–2	1	0	1	1–2	1	1
Azo-albumen	20	3	3	3	2	4	4	4	2
Azo-casein	10	2–3	3–4	3	2	3–4	3	3–4	2
Azo-casein (increased)	6–8	4	4	3	2	2	2	2	1–2

* Grading of changes—see text.

† Details in Table I.

however, clear-cut (Table II), and their correlation with the absence or appearance of amyloidosis will be emphasized.

Thymus. Amyloidosis was not found after injections of $NaHCO_3$, gelatin, albumen, or hydrolysates of casein and azo-casein (Table I). Thymic weight changes or lymphocyte depletion (Tables I and II) were minor except after azo-casein hydrolysate. Mice given the latter material showed slight to moderate thymic lymphocyte depletion (Table II).

After injection of the proteins causing amyloidosis a gradient of lymphocyte depletion was observed: slight after azo-gelatin; slight to moderate after casein (Fig. 2); advanced after azo-albumen (Table II; Fig. 3). With azo-casein there was virtually complete lymphocyte depletion (Fig. 4) resulting in the lowest thymic weights (Tables I and II). This gradient was the inverse of that for amyloid induction times after injection of these proteins (Table I).

Pyroninophilic cells were observed in the thymic subcapsular areas and there were increased numbers of medullary reticulum cells. Thymic amyloidosis was never found.

Spleen. Changes after $NaHCO_3$, gelatin, albumen, and casein hydrolysate were minor compared to other groups where an inverse correlation between lymphocyte depletion and amyloid induction was again detectable. The depletion ranged from slight to moderate after azo-gelatin and casein (Figs. 7 and 8), advanced after azo-albumen (Figs. 9 and 10), and to virtually complete after azo-casein (Figs. 11 and 12). The depletion of lymphocytes occurred chiefly in the cortical collars of splenic follicles. As a result, the perifollicular zones or mantles were widened and appeared as "clearing zones" (Figs. 7, 9 and 11). Amyloid was first detectable in these zones (Fig. 8). Reticuloendothelial (RE) cells, also in this area, were increased in numbers, and contained prominent PAS-positive cytoplasm that often merged with the amyloid deposits; cytoplasmic pyroninophilia was, however, inconsistent and usually slight.

Pyroninophilic and hematopoietic cell hyperplasia was observed in the subcapsular areas, along periarterial and trabecular sheaths and, less strikingly, in germinal centers (Figs. 7, 9 and 11). These cells, however, were not in contact with amyloid deposits in the "clear" perifollicular zones (Figs. 8, 10 and 12). There was no correlation between amyloid induction times and the severity of the hyperplastic changes.

Because azo-casein hydrolysate was non-amyloidogenic, the inverse correlation of lymphocyte depletion and amyloid induction times did not hold throughout the entire series. In this group, the depletion was greater than that seen after azo-gelatin and casein. Hyperplasia of pyroninophilic and hematopoietic cells after azo-casein hydrolysate was, however, distinctly less than that seen in the latter groups (Table II).

Lymph Nodes. In each group all regional nodes exhibited similar qualitative changes which were, however, more prominent in the inguinal nodes. The gradient of lymphocyte depletion, as well as the variable degrees of pyroninophilic cell hyperplasia among the different groups was virtually identical to that seen in the spleen (Table II). Pyroninophilic cell hyperplasia was most marked in the "intermediate zones"[3] and the medullary cords. Sinusoidal RE cells were increased in numbers. No amyloid was found in the lymph nodes in any group.

Other Organs. No bone marrow amyloid was found. The marrow cellularity in mice given azo-albumen and azo-casein injections was markedly increased, especially among myeloid cells. In the liver, little extramedullary hematopoiesis was found. Kupffer cells in mice with amyloidosis were either increased in number or in size, showed increased PAS-positive cytoplasmic staining and varying degrees of pyroninophilia. Hepatic amyloid deposits were often found in contact with these cells.

Changes in the peripheral blood leukocyte counts in 6 mice receiving azo-casein are listed in Table III. The total counts decreased, with

TABLE III

LEUKOCYTE COUNTS *

Mouse no.		Total/cu mm †	Differential count †		
			Neutrophils	Lymphocytes	Monocytes
1	Azo-casein	12,050	25	69	6
		3,350	85	14	1
2	"	8,700	26	62	12
		2,200	71	25	4
3	"	10,150	12	80	8
		4,050	79	21	0
4	"	9,850	30	58	12
		2,800	81	17	2
5	"	7,000	24	68	8
		5,600	75	25	0
6	"	12,000	18	72	10
		3,450	81	19	0
		Normal mice ‡			
	—	7,610 ± 2,061	20.5 ± 9.7	75.9 ± 10.2	3.5 ± 2.6

* Six mice given daily injections (30 mg) of azo-casein for 9 days and killed 24 hours after last injection. All mice developed amyloidosis.

† Top values, immediately before injections; bottom values, 24 hours after last injections.

‡ Means and standard deviations from 57 untreated mice of same strain, sex and age.

lymphocytopenia and a marked reversal of the lymphocyte–neutrophil ratio.

DISCUSSION

In our previous studies[1,2] with soluble protein antigens, and their hydrolysates, it was apparent that repeated antigenic challenge in mice

was an adequate and reproducible condition for the experimental induction of amyloidosis. With the enhancement of the amyloidogenic capacity of these proteins by azotization (a procedure also known to enhance antigenicity) an inverse correlation between antigenicity and amyloid induction times was also apparent. The greater the antigenicity of the injected protein, the earlier was the time of onset of the amyloidosis.

The present observations extend this relationship by also showing an inverse correlation of mature lymphocyte depletion with amyloid induction times. The subsequent discussion will bear upon (a) the temporal and spatial relationships of these and other lymphoid changes to amyloidosis and (b) the close similarity of the lymphoid changes to those found in experimental states sometimes resulting in wasting syndromes.

Lymphoid Tissue Changes, Soluble Antigens and Amyloidosis

Small Lymphocytes. The times required for amyloid induction by injection of various soluble antigens showed the following decreasing order: azo-gelatin (28 to 32 days), casein (22 days), azo-albumen (20 days), azo-casein (10 days) and increased injections of azo-casein (6 to 8 days). (Table I). As emphasized above, the graded severity of mature lymphocyte depletion (Table II) was related inversely to this time gradient, i.e., the shorter the induction time for amyloidosis, the greater the lymphocyte depletion. Indeed, after the shortest induction time caused by increased azo-casein injections, lymphocyte depletion was virtually complete and there was marked thymic atropy.

Mice given large doses of azo-casein developed extensive subcutaneous fibrosis and small ulcers at injection sites[2]; the stress of these reactions was probably responsible for some portion of the lymphocyte depletion. Azo-casein hydrolysate also produced fibrosis as well as slight to moderate lymphocyte losses. That the latter, therefore, is partly the result of a pharmacologic action of the azo-hapten alone is also possible. If true, however, it must not be a general phenomenon since a similar degree of depletion did not occur with azo-gelatin. Therefore, the nearly direct correlation between the antigenicity of the intact proteins and the lymphocyte depletion strongly suggests that the latter occurs, predominantly, as a result of repeated challenge with these antigens.

Disappearance of lymphocytes after antigen injections has been described recently in detail.[4] It has been suggested or implied[4,5] that these lymphocytes, or lymphocyte-like cells, transform into plasma cells or their precursors, i.e., pyroninophilic cells or immunoblasts. In the present study, despite a similar temporal sequence, hyperplasia of splenic pyroninophilic cells was predominantly located at a distance from the zones of marked lymphocyte depletion.

The close parallel between the onset of amyloidosis and the degree of

lymphocyte depletion appears important, since it suggests some rate limiting influence by the latter on the timing of amyloidosis. Since, however, lymphocyte depletion can occur without amyloidosis, for example, after azo-casein hydrolysate or stress,[3] other cellular changes or factors are probably equally important.

Pyroninophilic Cells. It is now generally believed that plasma cells are concerned with antibody production and that large pyroninophilic cells are their precursors.[6] Plasma cell hyperplasia in experimental amyloidosis has been reported frequently.[7] Some have suggested that amyloid is formed from a contiguous extracellular precipitation of cytoplasmic materials extruded locally from plasma cells; others have reported absence of such a spatial association.

In the present study, relatively few mature plasma cells were found in the spleen or nodes and the degrees of pyroninophilic cell hyperplasia varied quantitatively with induction times in neither a direct nor an inverse manner (Table II). While there was marked hyperplasia of these cells with an intermediate amyloid induction time (after azo-albumen) there were lesser degrees with shorter (after azo-casein) or longer (after azo-gelatin) induction times. These findings are open to at least two interpretations: (a) that some function of these cells may be essential to, but are not rate limiting numerically in, amyloid formation, or (b) that they are not necessary at all. Our data in no way, however, rule out their participation in minimal numbers at some early stage of amyloidogenesis. The failure to find amyloidosis after azo-casein hydrolysate which produced significant lymphocyte depletion but little pyroninophilic cell hyperplasia may support this possibility.

That pyroninophilic cells, i.e., transition forms of plasma cells, do not appear to participate in the final elaboration of amyloid by direct local secretion is suggested by the following: (a) amyloidosis was not found in lymph nodes where pyroninophilic cell hyperplasia was often equivalent to that found in the spleen; (b) early amyloid deposition in the spleen consistently appeared in the widened perifollicular zones at some distance from the hyperplastic foci of pyroninophilic cells. This feature has also been noted in thymectomized rabbits developing amyloidosis.[8] A lack of spatial continuity, while not supporting the concept of local and final amyloid secretion by these cells, does not disprove, however, the suggestions that they might elaborate circulating or diffusible factors indirectly involved in amyloid formation.[9] The lack of correlation between induction times and pyroninophilic cell hyperplasia seems, however, incompatible with this latter premise—at least for such factors participating in the final steps of amyloid synthesis.

Reticuloendothelial Cells. While difficult to grade, increases in the

number of RE cells were noted, especially in those mice developing amyloidosis. In the perifollicular zones of the spleen, these cells were prominent, contained varying amounts of PAS-positive cytoplasm, and were attached to, or abutted upon amyloid deposits, features well described by Teilum.[10,11] Distinct pyroninophilia was, however, not often found. This latter observation conflicted with the increasing pyroninophilia of hepatic Kupffer cells noted in the same mice during amyloid induction.

The very intimate morphologic association between early amyloid deposits and fixed sinusoidal RE cells and with sinusoidal reticulin is well known, and the evidence suggesting that amyloid is elaborated by these cells has been reviewed.[7] Increased cytoplasmic pyroninophilia in RE cells during amyloid induction with casein has been interpreted to be an indication of plasma cell differentiation.[10,11] Without denying this possibility, however, it is worthy of note that pyroninophilia reflects, empirically, RNA content. That an increase in staining intensity necessarily indicates plasma cell differentiation does not follow. Moreover, the ultrastructural evidence for this is inconsistent or contradictory.[7,12,13] Indeed, pyroninophilia may be related to enzyme synthesis, since histochemical evidence of increases of lysosomal enzymes have been observed in Kupffer cells prior to amyloid deposition.[14]

A probable participation of RE cells in humoral antibody formation has become more apparent.[15–17] The nature of this participation is not clear, although it might be involved with "processing" of antigens.[15] Whether or not the above changes in these cells are simply related to antigenic challenge, or, more specifically, to some later stage of amyloid induction, cannot be answered on the basis of our data. Recent work[18] supports the suggestion[7] that RE cells may be the final common pathway of amyloid synthesis.

Hematopoietic Cells. The reason for the increase in the number of these cells in the spleen and lymph nodes or their relationship to amyloidosis are not clear; an association with thymic atrophy or removal and lymphocyte depletion has been reported repeatedly.[19] As with pyroninophilic cells, there was no correlation between amyloid induction times and the degree of hyperplasia.

Lymphocyte Depletion and Amyloidosis

The lymphoid tissue changes accompanying the induction of amyloid with azo-casein are similar to those found in various species developing the wasting syndrome in the "graft-versus-host" state[20–29] or after thymectomy.[8,19,30–32] The syndrome (variously called runting, homologous disease, F_1 disease, secondary disease, depending on the mode of production) is characterized by growth retardation, weight loss, fur

TABLE IV

LYMPHOID CHANGES * AND "AMYLOIDOSIS" IN WASTING SYNDROMES AND IN MICE INJECTED WITH AZO-CASEIN

Model	*Lymphoid organs*				Amyloidosis †	References
	Lymphocytes	Extramedullary hematopoiesis	Plasma cells	Other cells		
Allogeneic spleen cells ⟶ neonatal recipients (mice)	Depletion	—	—	"Lymphoblast" hyperplasia	Liver	22
Parental spleen cells ⟶ F_1 hybrid recipients (mice)						
1	Depletion	—	Hyperplasia (?)	"Mononuclear" cell hyperplasia	Hyaline deposits in spleen—amyloid suggested	27
2	"	—	—	—	"Splenic hyaline"	24
Radiation chimeras (mice)						
1	Depletion	Increase	Hyperplasia	Reticulum cell hyperplasia	Spleen, liver, kidney, intestine, adrenals	25, 26, 28, 29
2	"	—	—	—	Kidneys	21
3	"	No increase	Some hyperplasia	—	"Dense pink-staining material" in spleen	20
4	"	—	—	—	"Peculiar eosinophilic material" in spleen	23
Neonatal thymectomy (rabbits)	Depletion	—	Hyperplasia	Reticulum cell "predominance"	Spleen	32
Adult thymectomy and sublethal x-irradiation						
1 (rabbits)	Depletion	—	Hyperplasia	Reticulum cell "predominance"	Spleen, liver, kidney lymph nodes	8
2 (mice)	"	Increase	—	—	Spleen, liver	30
Azo-casein injections (mice)	Depletion	Increase	Hyperplasia	Reticulum cell hyperplasia	Spleen, liver, kidney, intestine, adrenals	2, present paper

* Not mentioned in cited reference.
† See text.

changes and diarrhea. The common histologic denominator is marked depletion in the number of small lymphocytes in the thymus (when present), lymph nodes, spleen and intestinal tract. This is often accompanied by increased extramedullary hematopoiesis and by hyperplasia of cells variously described as "plasmatoid",[25,28] plasma cells,[8,32] "large mononuclear cells"[27] or "lymphoblasts".[22] Tissue deposits identified as amyloid[8,21,22,30,32] or resembling amyloid,[20,24–27] and later characterized as amyloid,[28,29] have been found in many of these models. These changes, summarized in Table IV, show a similarity to those found in mice receiving azo-casein. Their significance will be discussed in terms of recent concepts concerning the immunity system which have been formulated from studies of thymectomized animals and from graft-versus-host reactions.

Two-Component Immune System

Phylogenetic and ontogenetic studies[19,33,34] indicate that (1) the development of small lymphocytes in lymphoid tissues of most vertebrates is dependent upon a central organ, the thymus, and (2) immunologic competence coincides with appearance of these cells. Thus thymectomy in the neonatal state in appropriate species may result in the changes in the lymphoid organs listed in Table IV. Functionally, there may be complete or partial inability to produce specific antibodies (to many antigens) or to manifest delayed hypersensitivity or allograft rejection.[19,33] Other features of an apparently paradoxic nature, however, have also been observed.[8,32–34] These are specific antibody responses to strong antigens, normal or increased numbers of plasma cells or serum immunoglobulin levels, Coombs-positive red cells and anemia, positive LE cells,[31] "lupus nephritis",[31] prominent RE cells[8,32] and amyloidosis.[8,30,32]

The persistence or increase in the number of plasma cells after thymectomy in the neonate has suggested[33] that these cells either are derived from, or are dependent upon, a central source other than the thymus or that they develop prior to thymectomy. Studies with chickens support the former possibility since, in this species, the development of plasma cells is dependent upon the bursa of Fabricius,[35,36] a cloacal lymphoid organ. From these and clinical observations, a two-component system for the immunologic apparatus has been proposed.[33] The first is a thymic-dependent recognition system (for which the central primordium is the thymus) which is responsible for small lymphocyte development, recognition of antigenicity, delayed hypersensitivity and allograft rejection. The second component is an immunoglobulin-system which is responsible for the development of plasma cells and immunoglobulin production.

The central primordium in mammals is unknown but in the chicken it is the bursa of Fabricius.

How the two systems integrate for specific antibody production is not yet known. The over-activity of the immunoglobulin-system (i.e., plasma cell hyperplasia, elevation of immunoglobulins) observed in thymectomized states, suggests a negative-feedback inhibition of this system by the thymus in the intact animal.[33] The Coombs-positive anemia [8,32] and lupus-like phenomena [31] and, perhaps, the wasting have also suggested that with removal of the thymus, antibodies to self-components may result.

Graft-versus-Host Reactions

A variety of immunologic phenomena collectively referred to as graft-versus-host reactions are becoming well known.[29] As this operational term implies pathologic changes, i.e., the wasting syndrome, occur in a host animal following the transplantation of immunologically competent cells (graft) from a genetically different donor. Recent studies have suggested that the grafted cells may attack the host's lymphocytes, and that the wasting syndrome develops when the latter are depleted. The syndrome can be prevented by the infusion of lymphocytes from genetically identical donors into the host.[25,26,29] The changes in lymphoid tissues, including amyloidosis, in the graft-versus-host states, are very similar to those seen in wasting following thymectomy (Table IV). The clinical features are also identical.[19,29]

These morphologic analogies suggest additional systems for the study of specific factors functionally related to amyloid induction by soluble antigens. The mechanisms proposed by the two-component concept of immune functions may be utilized tentatively to interpret these observations. According to this concept it is possible that the final mechanism responsible for all changes is related to the overactivity of the host's immunoglobulin-system. That amyloidosis is the result of this overactivity or of autoimmune processes does not necessarily follow and this is not our contention. Rather, the association of amyloidosis with states of thymic destruction and lymphocyte depletion or generalized tolerance is, we feel, the important consideration in these models. At the present time, the functional analogies of our observations with those just described must be made with caution; our studies on specific antibodies and immunoglobulin levels are not yet completed. The morphologic similarities are, however, striking. One may therefore conjecture that a state of impairment or destruction of the thymic-recognition system with lymphocyte depletion may be a phase in the immunologic response to soluble antigens necessary for amyloidosis. The experimental proofs de-

manded by this hypothesis have been partially fulfilled here and elsewhere.[2] There is an inverse correlation between the rates of thymic atrophy and lymphocyte depletion on the one hand and the time of onset of amyloidosis on the other.

Summary

Mice were given injections of various non-amyloidogenic and amyloidogenic soluble protein materials. Analysis and correlation of the histologic changes in lymphoid organs with the time of onset of amyloidosis induced by these various proteins showed a striking inverse correlation between mature lymphocyte depletion and the length of amyloid induction times. As a result it has been suggested that lymphocyte depletion might have a rate limiting influence on the timing of the appearance of amyloidosis during experimental induction.

Pyroninophilic and hematopoietic cell hyperplasia were observed in lymphoid organs after injection of soluble proteins, but there were no temporal or spatial correlations between the appearance, severity or location of the changes and the development of amyloidosis. On the basis of these variabilities it is proposed that neither pyroninophilic cells (plasma cells or their precursors) nor hematopoietic cells are directly responsible for the final step in amyloid elaboration.

Close morphologic similarities between the lymphoid changes induced by soluble protein antigens and those found in wasting syndromes following thymectomy or graft-versus-host states have been noted. These have been related to newer concepts of immune mechanisms.

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[*Illustrations follow*]

Legends for Figures

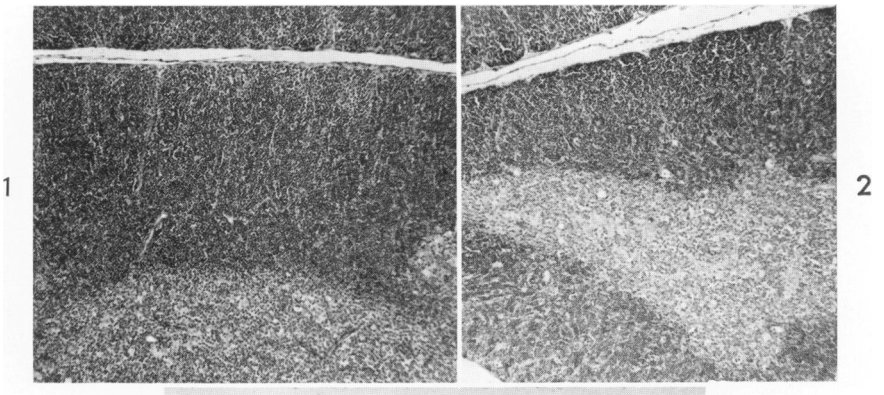
All photomicrographs were prepared from formaldehyde-fixed tissues; Figures 1 to 4 were stained by Lillie's PAS method, and Figures 5 to 12 by methyl green-pyronine.

Fig. 1. Thymus, normal mouse. × 63.

Fig. 2. Thymus, mouse given injections of casein for 21 days. The cortex is slightly reduced in thickness because of an irregular depletion of small lymphocytes. This is shown more prominently in the cortex at the lower left. × 63.

Fig. 3. Thymus, mouse given injections of azo-albumen for 19 days. There is advanced depletion of cortical lymphocytes with virtual loss of cortico-medullary zonation. The PAS-positive material in the medullary vessels is probably serum. × 63.

Fig. 4. Thymus, mouse given injections of increased amounts of azo-casein for 7 days. Virtually complete depletion of cortical lymphocytes is accompanied by absence of a cortico-medullary boundary. Attention is called to the difference in magnification when comparing this illustration with previous figures. × 100.



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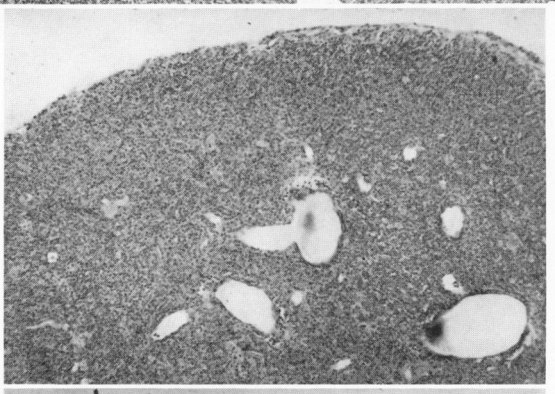

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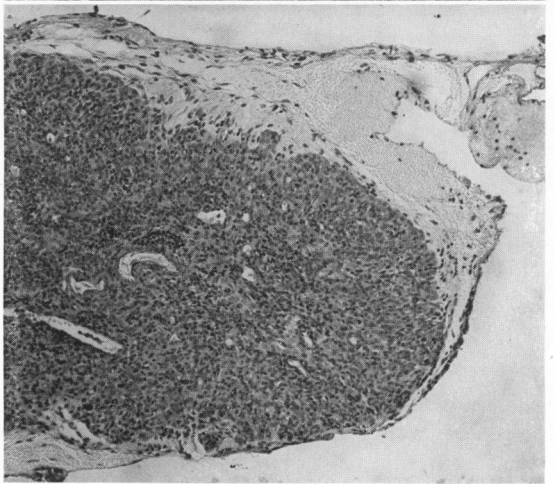

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FIG. 5. Normal spleen. × 67.

FIG. 6. Normal spleen. One half of a splenic follicle with a distinct collar of small cortical lymphocytes is shown at the left. Near the middle is the perifollicular or mantle zone containing reticuloendothelial cells and few lymphocytes. On the right is a trabecula (arrow) surrounded by small numbers of lymphocytes, pyroninophilic cells and a megakaryocyte. × 175.

FIG. 7. Spleen, mouse treated 21 days with casein. Splenic follicles are enlarged. Cortical lymphocytic depletion is apparent and the perifollicular zones are widened or "cleared". Interfollicular areas are not widened but contain increased numbers of pyroninophilic and hematopoietic cells. Early amyloid deposits are barely discernible in the clear zones of the two follicles on the right at this magnification. × 67.

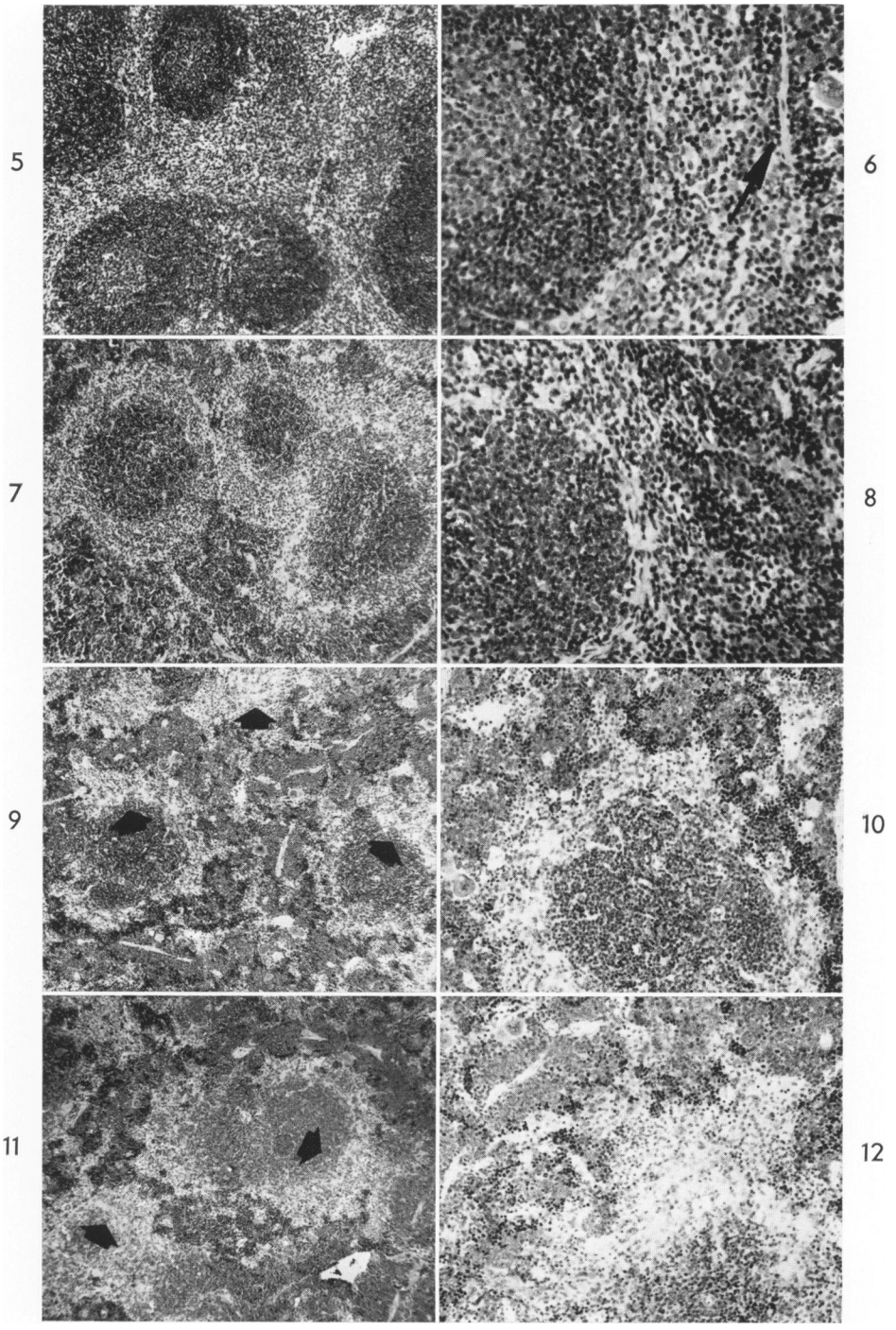
FIG. 8. Spleen shown in Figure 7. A portion of a follicle with lymphocyte depletion appears on the left and is immediately bordered by a rim of unstained amyloid which occupies the inner part of the widened perifollicular zone. The darkly stained cells on the right of this zone and at a distance from the amyloid are mixtures of hematopoietic and pyroninophilic cells. × 175.

FIG. 9. Spleen, mouse treated 19 days with azo-albumen. There is advanced depletion of follicular lymphocytes and the perifollicular "clear" zones are widened. Unstained areas around depleted follicles are amyloid deposits (arrows). The widened interfollicular areas are occupied by sheets of pyroninophilic cells with lesser degrees of hematopoiesis. These cells exhibit an association with the trabeculae. × 50.

FIG. 10. Spleen shown in Figure 9. A follicle with lymphocytes depleted is surrounded by a rim of unstained, pale appearing amyloid in the widened perifollicular zone. Peripheral to this zone are sheets of pyroninophilic and hematopoietic cells. × 125.

FIG. 11. Spleen, mouse treated 7 days with azo-casein. Features are similar to those shown in Figure 12. Note the virtually complete depletion of small lymphocytes chiefly from the cortical collars of the splenic follicles. Amyloid (arrows) appears around the follicle at the right and that at the lower left which is sectioned paracentrally. × 44.

FIG. 12. Spleen shown in Figure 11. Part of a follicle near the lower left is barely recognizable. The perifollicular zone is largely occupied by unstained, pale amyloid deposit. Peripherally there are interfollicular accumulations of pyroninophilic and hematopoietic cells. A trabecula appears at the upper left. × 125.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