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REVIEW

New concepts in the pathogenesis of primary and secondary amyloid disease

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SUMMARY

Despite the marked progress obtained in the structural and amino acid sequencing data of amyloid proteins our understanding of the cellular mechanisms causing the deposition of amyloid fibrils is still poor. Some of the questions about the cellular events leading to the synthesis of amyloid fibrils can be approached by evaluating the immune reactivity of animals that develop amyloid after repeated daily casein injections. Recent studies carried out in a mouse model indicate that macrophage activation associated with T–cell suppression and followed by B–cell proliferation appear to be responsible for the immunopathological abnormalities in both primary and secondary amyloid disease.

INTRODUCTION

Amyloid disease in man is either idiopathic (primary form) or associated with certain inflammatory disorders, immunodeficiency states, endocrinopathies or neoplasia (secondary forms). A number of distinct heredofamilial forms have also been described in various parts of the world, and it is now generally believed that amyloidosis may be related to the ageing process (Cohen, 1967). After the discovery that amyloid was not a homogeneous hyaline substance as was originally thought (Cohen & Calkins, 1959), extensive chemical studies demonstrated even greater heterogeneity within the amyloid fibril. Firstly, it was shown that primary amyloid deposits are composed mainly of the variable portions of Bence–Jones proteins (Glenner, 1971) and later it was observed that amyloid fibrils from patients with secondary and heredofamilial types of disease contained a small molecular weight polypeptide, bearing no relation to known immunoglobulin molecules (Benditt & Eriksen, 1971 Levin, Franklin & Frangione, 1972; Husby *et al.*, 1972). Finally, evidence was obtained in this and other laboratories that amyloid fibrils from the monkey, guinea-pig and mouse (Hermodson *et al.*, 1972; Skinner *et al.*, 1974; Eriksen *et al.*, 1976) contain significant amounts of a non-immunoglobulin protein, which is analogous to the AA protein found in secondary amyloid deposits in man.

There is abundant clinical and pathological evidence that dysfunction of the immune system is closely related to the development of amyloid disease in man and other species. Secondary amyloid occurs in a number of conditions associated with excessive immunoglobulin production, including rheumatoid arthritis, tuberculosis and lepromatous leprosy, and primary amyloid disease is frequently accompanied by plasma cell dyscrasias and the presence of serum or urine M components. On the other hand, amyloid has also been described in certain immunodeficiency states and can be accelerated in experimental animals by a number of procedures, including thymectomy (Kellun *et al.*, 1965), irradiation (Druet & Junigan, 1966), and administration of immunosuppressive agents (Hardt, 1971). It should be

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pointed out, however, that many of the immunological disturbances mentioned above may be non-specific, since amyloid deposition is the exception rather than rule in diseases such as rheumatoid arthritis (Ozdemir, Wright & Calkins, 1971) and multiple myeloma (Limas, Wright & Matsuzchi 1973), and since many species and strains of experimental animals fail to develop amyloid, even after prolonged or persistent antigenic stimulation (Scheinberg & Cathcart, 1974).

Recent studies from our laboratory suggest the possibility that disturbances in immunoregulatory function play an essential role in the process of amyloidogenesis, and point to a common pathogenetic pathway in primary as well as secondary human amyloid disease.

A COMMON PATHWAY IN PRIMARY AND SECONDARY AMYLOID DISEASE

Evidence in favour of a more unifying concept of amyloidogenesis has been dervied mainly from experimental work performed in laboratory animals. A series of studies carried out in our laboratory during the past few years firmly established that T-cell function was significantly impaired in amyloidotic mice, as measured by mitogen stimulation of spleen cell suspensions and graft *vs* host reactions (Cohen & Cathcart, 1972; Scheinberg, 1975). Similar results had also been obtained by other investigators, who noted delayed homograft rejection (Ranlov & Jensen, 1966), decreased graft *vs* host reactions and diminished T helper function (Clerici & Franklin, 1974) in casein-induced experimental amyloidosis. Later we became even more impressed by the marked enhancement of B-cell function in similar groups of animals (Scheinberg & Cathcart, 1976; Scheinberg, Goldstein & Cathcart, 1976). Mitogenic responses to polynucleotides (poly I-C), dextran sulphate and pneumococcal polysaccharide were augmented in spleens of amyloid-susceptible CBA/J mice but decreased in the spleens of amyloid-resistant A/J mice. Suitable controls, including non-casein-treated animals and mice receiving multiple injections of a weak amyloidogenic agent (bovine serum albumin), yielded results within the normal range. It was also noted that splenic antibody responses to T-independent antigens (pneumococcal polysaccharide and *E. coli* 055:B5), as determined by the Jerne plaque assay and B-cell antibody-dependent cytotoxicity, were also significantly increased in amyloidotic CBA/J mice as compared to the amyloid-resistant A/J strain and suitable controls. Furthermore, surface membrane characteristics of spleen cell suspensions from amyloidotic CBA/J mice showed an almost complete absence of θ-bearing cells, normal percentages of lymphocytes carrying surface immunoglobulins and decreased percentages of lymphocytes bearing receptors for the third component of complement. Using an *in vitro* approach, Britton (1975) has also found evidence for polyclonal B-cell activation in experimental amyloidosis. The remarkable expansion of the B-cell line in the spleens of pre-amyloidotic and amyloidotic mice is consistent with Teilum's original description of increased pyroninophilia and reticuloendothelial hyperplasia in casein-treated C3H mice (Teilum, 1964).

While the casein experimental model is considered to be analogous to the secondary form of human amyloidosis, no suitable model for human primary amyloid disease is yet available. However, in a few instances, amyloid deposits can be found in the spleens of BALB/c mice inoculated with plasma cell tumours (MOPC 137 cell line) (Baumal, Ackerman & Wilson, 1975). Although the origin and chemical nature of these deposits has not been ascertained, Osserman, Takatsuki & Talal (1964) had previously pointed out the remarkable resemblance between mice bearing plasma cell tumours and patients with multiple myeloma and a myloid disease. It should also be noted that mice receiving intraperitoneal injections of mineral oil not only demonstrate monoclonal B-cell proliferation but also appear to develop significant T-cell suppression. Potter (1973) reports that primary antibody responses to sheep red blood cells (T-helper function) is inhibited in this model and the administration of anti-thymocyte serum markedly decreases the latent period of myeloma induction and increases the yield of solid tumours (Mandel & DeCosse, 1972) (Table 1).

HYPOTHESIS

Most of the substances used to induce both experimental amyloid disease (casein, Freund's adjuvant, endotoxin, etc.) and murine myelomatosis (mineral oils, hydrocarbon conjugates, etc.) are also potent

TABLE 1. Comparison between the immune dysfunction in experimental amyloidosis and murine myelomatosis

Dysfunction	Amyloidosis	Myelomatosis
Macrophage activation	Casein, Freund's adjuvant, Endotoxin	Mineral oils, Hydrocarbon conjugate (Pristane)
T-cell depletion	Thymic involution, Functional impairment	Enhanced by anti-θ sera, Functional impairment
B-cell hyperplasia	Immunoblast proliferation, Polyclonal B-cell activation	Plasma cell proliferation, Monoclonal B-cell activation

macrophage activators (Unanue, 1972). Data have been compiled in the past few years to show that activated macrophages secrete various stimulatory and inhibitory substances which act upon lymphocytes (Calderon, Williams & Unanue, 1974), neutrophils (Parker & Metcalf, 1974) and chondrocytes (Wahl *et al.*, 1974), and that the successful initiation of *in vitro* murine and human plasmacytoma cell lines requires the presence of growth factors derived from tissue macrophages (Namba & Hamaoka, 1972; Sabin, Fahey & Price, 1974). Although much more needs to be learned about the conditions which govern these phenomena, we now propose that macrophage activation and elaboration of one or more of these substances represents the initial step in the genesis of amyloid disease. Massive short term stimulation of the reticuloendothelial system, as in the casein-treated animals, would lead to the rapid accumulation and laying down of AA protein in connective tissues. It would also account for the marked immunoblast proliferation and non-specific T-cell suppression. On the other hand, more sustained but less intensive involvement of tissue macrophages would give rise to the monoclonal proliferation of mature plasma cells and the deposition of L-chain material in the form of amyloid fibrils. We also surmise that normal T-cell function would tend to inhibit amyloid disease by controlling excessive macrophage activation and B-cell proliferation in the face of constant antigenic challenge (Fig. 1).

The new hypothesis brings together and provides a rational explanation for many of the cellular abnormalities that have been described in human and experimental amyloid disease. These include: (a) an inverse relation between the expansion of the total body T-cell pool and the development of casein-induced amyloidosis in AKR mice (Ebbesen, 1974); (b) a direct relation between the development of amyloid disease and the degree of lymphocyte depletion and thymic atrophy (Druet & Janigan, 1966); (c) accelerated amyloid induction in casein-treated mice with congenital aplasia of the thymus gland (nude mice) (Hardt & Claesson, 1972); (d) the development of high-dosage tolerance to casein in amyloidotic guinea-pigs, a phenomenon that appears to be under thymic control (Cathcart, Mullarkey & Cohen, 1971); (e) the recent discovery of a T-cell population in patients with multiple myeloma that is capable of suppressing differentiation of normal lymphocytes into immunoglobulin-secreting plasma cells (Broder *et al.*, 1975); (f) similarities between the surface characteristics of null cells in amyloidotic spleen cell suspensions and premature B cells in the bone marrows of normal mice, i.e. cells lacking θ antigen and receptors for the third component of complement (Ryser & Vassali, 1974); (g) the recent demonstration that simultaneous administration of thymosin (thymic hormone) and casein reduces the incidence and degree of splenic amyloidosis (Scheinberg, 1976); (h) observations by at least two groups of investigators that amyloid formation in casein-treated mice is arrested by simultaneous daily injections of colchicine (Kedar *et al.*, 1974; Shirahama & Cohen, 1974), an agent known to interfere with the fusion of intracellular microtubules and endocytosis by reticuloendothelial cells; and (i) the presence of altered mitogen-induced cellular cytotocicity in amyloidotic mice, an assay previously shown by us to require the presence of macrophages (Yonkovsky, Cuthcart & Scheinberg, 1976; Silverman *et al.*, 1976; Scheinberg, 1978).

Definitive proof of the synthesis of secondary amyloid fibrils by macrophages or B cells is lacking at the present time, and since nude mice are capable of synthesizing amyloid protein SAA, it does not appear to come from T cells (Benson *et al.*, 1977). Amyloid deposits in casein-treated animals have been shown to lie close or adjacent to typical reticuloendothelial cells (Franklin & Zucker-Franklin, 1972;

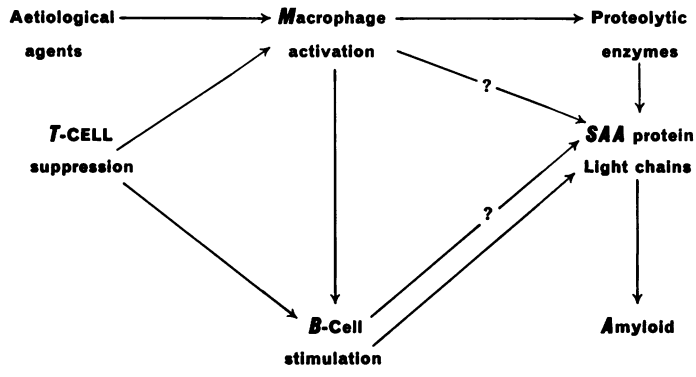

FIG. 1. Cellular events in the pathogenesis of amyloid disease.

Shirahama & Cohen, 1973; Bari, Pettengill & Sorenson, 1969). Lysosomal digestion of immunoglobulin molecules has also been postulated as the final common pathway for amyloid disease in well-defined plasma cell dyscrasias (Glenner, Terry & Isersky, 1973) since typical amyloid fibrils can be obtained *in vitro* from certain Bence-Jones proteins (Glenner *et al.*, 1971; Shirahama, Benson & Cohen, 1973; Linke, Zucker-Franklin & Franklin, 1973). Indeed, the demonstration by Levin, Franklin & Frangione (1972), that AA protein and excessive amounts of immunoglobulin molecules can be isolated from the amyloid deposits from patients with familial mediterranean fever (Benditt & Eriksen, 1971), encourage us to speculate that at least some of the genetic forms of amyloid disease are consequences of both acute and chronic macrophage activation.

CONCLUSIONS

Evidence obtained from studies of experimental amyloidosis on the one hand, and murine plasma cell tumours on the other, raises the possibility that primary and secondary amyloid disease in man share common pathogenetic pathways. Both pathological conditions are, in essence, B-cell dyscrasias and differ only with respect to the intensity and duration of antigen stimulation to which the reticuloendothelial system is exposed. T-cell suppression, which at one time was thought to be central to the pathogenesis of amyloidosis, is now considered to be a consequence rather than a cause of amyloid disease, although impaired immune surveillance probably leads to increased macrophage activation and B-cell proliferation. Recent discoveries that experimental amyloid disease may be arrested or suppressed by colchicine and thymosin provide further clues as to the pathogenesis of this disorder, and raises the possibility that new forms of treatment will soon become available, not only for patients with amyloid disease, but for those other forms of plasma cell dyscrasias as well.

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REFERENCES

BARI, W.A., PETTENGILL, O.S. & SORENSON, G.D. (1969) Electron microscopic and autoradiography of spleen cell cultures from mice with amyloidosis. *Lab. Invest.* **20**, 234.

BAUMAL, R., ACKERMAN, A. & WILSON, B. (1975) Immunoglobulin biosynthesis in myeloma-associated casein and endotoxin murine amyloidosis. *J. Immunol.* **114**, 1785.

BENDITT, E.P. & ERIKSEN, N. (1971) Chemical classes of amyloid substances. *Am. J. Patho.* **65**, 231.

BENSON, M.D., SCHEINBERG, M.A., SHIRAHAMA, T.,

CATHCART, E.S. & SKINNER, M. (1977) Kinetics of serum amyloid protein A in casein induced murine amyloidosis. *J. clin. Invest.* **59**, 412.

BRITTON, S. (1975) Experimental amyloidosis: inducer is a polyclonal B-cell activator to which susceptibility is under genetic control. *J. exp. Med.* **142**, 1564.

BRODER, S., HUMPHREY, R., DURN, M., BLACKMAN, M., MEADE, B, GOLDMAN, C., STROBER, W. & WALDMANN T. (1975) Impaired synthesis of polyclonal immunoglobulins in multiple myeloma. *New Engl. J. Med.* **293**, 887.

CALDERON, J., WILLIAMS, R.T. & UNANUE, E.R. (1974) An inhibitor of cell proliferation released by cultures of macrophages. *Proc. nat. Acad. Sci. (Wash.)*, **71**, 4273.

CATHCART, E.S., MULLARKEY, M. & COHEN, A.S. (1971) Cellular immunity in casein-induced amyloidosis. *Immunology*, **20**, 1001.

CLERICI, E. & FRANKLIN, E.C. (1974) Amyloid workshop report. *Proceedings of the Second International Congress on Immunology*, **4**, 384.

COHEN, A.S. (1967) Amyloidosis. *New Engl. J. Med.* **277**, 522.

COHEN, A.S. & CALKINS, E. (1959) Electron microscopic observations on a fibrous component in amyloid of diverse origins *Nature* (Lond.), **183**, 1202.

COHEN, A.S. & CATHCART, E.S. (1972) Casein-induced experimental amyloidosis. Review of cellular and immunologic aspects. *Meth. Achiev. exp. Path.* **6**, 207.

DRUET, R.L. & JANIGAN, D.T. (1966) Experimental amyloidosis. Rates of induction, lymphocyte depletion and thymic atrophy. *Am. J. Path.* **49**, 911.

EBBESEN, P. (1974) Mutually exclusive occurrence of amyloidosis and thymic leukemia in casein-treated AKR mice. *Brit. J. Cancer*, **29**, 76.

ERIKSEN, N., ERICSSON, L.H., PEARSALL, N., LAGUMOFF, D. & BENDITT, E.P. (1976) Mouse amyloid protein AA. Homology with non-immunoglobulin protein of human and monkey amyloid substance. *Proc. nat. Acad. Sci. (Wash.)*, **73**, 964.

FRANKLIN, E.C. & ZUCKER-FRANKLIN, D. (1972) Current concepts of amyloid. *Adv. Immunol.* **15**, 249.

GLENNER, G.G., EIN, D., EANES, E.D., BLADEN, H. A., TERRY, W. & PAGE, D.L. (1971) The creation of 'amyloid fibrils' from Bence-Jones proteins *in vitro*. *Science*, **174**, 712.

GLENNER, G.G., TERRY, W.D., HARADA, M., ISERSKY, C. & PAGE, D. L. (1971) Amyloid fibrils proteins: proof of homology with immunoglobulin light chains by sequence analysis. *Science*, **172**, 150.

GLENNER, G.G., TERRY, W.D. & ISERSKY, C. (1973) Amyloidosis, its nature and pathogenesis. *Semin. Haemat.* **10**, 65.

HARDT, F. (1971) Acceleration of casein-induced amyloidosis in mice by immunosuppressive agents. *Acta path. microbiol. scand.* **79**, 61.

HARDT, F. & CLAESSON, M.W. (1972) Studies on casein-induced amyloidosis in mice with congenital aplasia of the thymus. *Acta path. microbiol. scand.* **804**, 471.

HERMODSON, M.A., KUHN, R.W., WALSH, K.A., NEURATH, H., ERIKSEN, N., BENDITT, E.P. *et. al.* (1972) Amino acid sequence of monkey amyloid protein A. *Biochemistry*, **111**, 2934.

HUSBY, G., SLETTEN, K., MICHAELSON, T.E. & NATVIG J. (1972) Amyloid fibril protein subunit. Protein AS—disribution in tissue and serum in different clinical types of amyloidosis, including that associated with myelomatosis and Waldestrom's macroglobulinaemia. *Scand. J. Immunol.* **1**, 393.

KEDAR, I., RABID, M., SOHAR, E. & GAFNI, J. (1974) Colchicine inhibition of casein induced amyloidosis in mice. *Israel J. Med. Sci.* **10**, 787.

KELLUN, M. J., SUTHERLAND, D.E.R., ECKERT, E. & GOOD, R.A. (1965) Wasting disease, Coombs-positivity and amyloidosis in rabbits subjected to central lymphoid tissue extirpation and irradiation. *Int. Arch. Allergy appl. Immunol.* **27**, 6.

LEVIN, M., FRANKLIN, E.C. & FRANGIONE, B. (1972) The amino acid sequence of the major non-immunoglobulin components of some amyloid fibrils. *J. Clin. Invest.* **51**, 2773.

LIMAS, C., WRIGHT, J.R. & MATSUZAKI, M.E. (1973) Amyloidosis and multiple myeloma. *Am. J. Med.* **54**, 166.

LINKE, R., ZUCKER-FRANKLIN D. & FRANKLIN, E.C. (1973) Morphological, chemical and immunological studies of amyloid-like fibrils formed from Bence-Jones proteins by proteolysis. *J. Immunol.* **111**, 10.

MANDEL, M.A. & DECOSSE, J.J. (1972) The effects of heterologous anti-thymocyte sera in mice. V. Enhancement of plasma cell tumor formation. *J. Immunol.* **109**, 360.

NAMBA, Y. & HAMAOKA, M. (1972) Immunopathology of cultured IgM-forming cells of mouse. *J. Immunol.* **109**, 1193.

OSSERMAN, E., TAKATSUKI, K. & TALAL, N. (1964) The pathogenesis of amyloidosis. Studies of the role of abnormal gammaglobulin and gammaglobulin fragments of the Bence-Jones (L-polypeptide) in the pathogenesis of primary and secondary amyloidosis and the amyloidosis associated with multiple myeloma. *Semin. in Haemat.* **1**, 3.

OZDEMIR, A.I., WRIGHT, J.R. & CALKINS, E. (1971) Influence of rheumatoid arthritis on amyloidosis of aging. *New Engl. J. Med.* **534**, 38.

PARKER, J. & METCALF, D. (1974) Production of colony stimulating factor in mitogen-stimulated lymphocyte cultures. *J. Immunol.* **112**, 502.

POTTER, M. (1973) The developmental history of the neoplastic plasma cells in mice. A brief review of recent developments. *Semin Haemat.* **10**, 19.

RANLOV, P. & JENSEN, E. (1966) Homograft reaction in amyloidotic mice. *Acta path. microbiol. scand.* **70**, 321.

RYSER, J.E. & VASSALI, P. (1974) Mouse bone marrow lymphocytes and their differentiation. *J. Immunol.* **113**, 3.

SABIN, M.E., FAHEY, J.L. & PRICE, Z. (1974) Long term establishment of a human plasmacytoma cell line derived from a patient with IgG multiple myeloma. Requirement of a plasma cyte stimulatory factor for the proliferation of myeloma cells in tissue culture. *J. exp. Med.* **104**, 494.

SCHEINBERG, M.A. (1975) *The biologic effects of chronic casein administration and its immunopathological significance in experimental amyloidosis.* Ph.D. thesis, Boston University.

SCHEINBERG, M.A. (1978) The immunology of amyloid disease. A Review. *Seminars in Arthritis and Rheumatism* **7**, 133.

SCHEINBERG, M.A. & CATHCART, E.S. (1974) Casein-induced experimental amyloidosis. III. Response to mitogens, allogeneic cells and graft versus host reactions in the murine model. *Immunology*, **26**, 953.

SCHEINBERG, M.A. & CATHCART, E.S. (1975) Casein-induced experimental amyloidosis. Response of lymphoid organs to T and B mitogens. *Lab. Invest.* **33**, 96.

SCHEINBERG, M.A. & CATHCART, E.S. (1976) Casein-induced experimental amyloidosis. VI. A pathogenetic role for B cells in the murine model. *Immunology*, **31**, 433.

SCHEINBERG, M.A., GOLDSTEIN, A.L. & CATHCART, E.S. (1976) Thymosin restores T-cell function and reduces

the incidence of amyloid disease in casein-treated mice. *J. Immunol.* **116**, 156.

SHIRAHAMA, T., BENSON, M.D. & COHEN, A.S. (1973) Fibrillar assemblage of variable segments of immunoglobulin light chains. An electron microscopic study. *J. Immunol.* **110**, 21.

SHIRAHAMA, T. & COHEN, A.S. (1973) An analysis of the close relationship of lysosomes to early deposits of amyloid. *Am. J. Path.* **73**, 97.

SHIRAHAMA, T. & COHEN, A.S. (1974) Blockage of amyloid induction by colchicine in a animal model. *J. exp. Med.* **140**, 1102.

SILVERMAN, S., SCHEINBERG, M.A., YONKOVSKY, D. & CATHCART, E.S. (1976) Monocyte involvement in PHA-induced cytotoxicity of chicken erythrocytes in man. *Cell Immunol.* **22**, 93.

SKINNER, M., CATHCART, E.S., COHEN, A.S. & BENSON, M.D. (1974) Isolation and identification by sequence analysis of experimentally induced guina-pig amyloid fibrils. *J. exp. Med.* **140**, 871.

TEILUM, G. (1964) Pathogenesis of amyloid. The two phase cellular theory of local secretion. *Acta path. microbiol. scand.* **62**, 11.

UNANUE, E.R. (1972) The regulatory role of macrophages in antigenic stimulation. *Adv. Immunol.* **15**, 95.

WAHL, M.L., WAHL, M.S., MERGENHAGEN, S.E. & MARTIN, G.R. (1974) Collagenase production by endotoxin-activated macrophages. *Proc. nat. Acad. Sci. (Wash.)*, **71**, 3598.

YONKOVSKY, D., CATHCART, E.S. & SCHEINBERG, M.A. (1976) B cell activation and macrophage dysfunction in casein-induced experimental amyloidosis. *Arthr. and Rheum.* **19**, 831.