Transfer Amyloidosis

I. Studies on the Transfer of Various Lymphoid Cells from Amyloidotic Mice to Syngeneic Nonamyloidotic Recipients and II. Induction of Amyloidosis in Mice with Spleen, Thymus and Lymph Node Tissue from Casein-Sensitized Syngeneic Donors.

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Lymphoid cells from various lymphoid organs were transferred from amyloidotic mice to normal syngeneic recipients. The recipients were treated with nitrogen mustard. Only the recipients of spleen cells developed amyloidosis. Furthermore, slices of spleen, thymus and lymph node from casein-sensitized mice were transplanted to kidneys of normal syngeneic mice. The recipients were treated with ten casein injections, and they all developed amyloidosis. The results from both experiments indicate that amyloid is formed by macrophages due to a stimulation of these cells with both antigen and an amyloid-inducing factor released from the pyroninophilic lymphoid cells. (Amer J Path 65:411–424, 1971)

AMYLOIDOSIS IN MICE can be induced by a prolonged antigenic stimulus.[1–3] In the course of this induction the histologic appearance of the spleen changes from a marked perifollicular pyroninophilia, after about 10 days of antigen treatment, to a phase in which after continued antigenic challenge, the amyloid is deposited perifollicularly by cells from the reticuloendothelial system (RES).[4]

Transfer studies of experimental amyloidosis have been performed with spleen tissues, cells and homogenates, using either spleens in the pyroninophilic phase[5] or spleens in the amyloidotic phase.[6,7] When spleens from the pyroninophilic phase are used as donor material, further antigenic treatment of the recipients is necessary.[8] Transfer of amyloidotic spleens and spleen elements demands a nonspecific depression of the immune apparatus of the recipients (*ie,* application of nitrogen mustard) before the formation of amyloid can take place.[6,9]

In the present transfer experiments, lymphoid cells from various organs (spleen, thymus, lymph node, bone marrow, peritoneum and

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blood) were transferred to normal recipients from donor animals in the amyloidotic phase; the recipients were treated with nitrogen mustard in order to accelerate a possible transferred amyloidosis.[4] Furthermore, tissues from spleen, thymus and lymph node of donor mice with spleens in the pyroninophilic phase were grafted onto normal recipients kidneys and the recipients received successive injections of antigen after the grafts were transplanted. The local as well as the general effect of the graft was studied.

Materials and Methods

An equal number of male and female, 8–10-week-old, 23–27 g mice were randomly selected from our inbred colony of the C3H strain. The mice were fed on oats.

Experiment I

Donors. Donors were given a total of 17 daily subcutaneous injections of 0.5 ml of a 5% solution of sodium caseinate. Untreated control donors were included. The day after the last casein injection, the animals were killed with ether and cell suspensions were prepared from various organs as described below. From the spleens, thymi and lymph nodes, small specimens were taken and fixed in neutral 10% formalin for histologic examination.

Preparing Suspension of Bone Marrow Cells. The femurs and tibiae were cleaned of muscle tissue and the marrow cells were washed out with ice-cold Hanks' solution. The cells were then washed three times and adjusted to a final concentration of 200×10^6 nucleated cells/ml.

Preparing Suspensions of Thymus, Spleen and Lymph Node Cells. The spleens, thymi and the lymph nodes were homogenized in a Potter Elvehjem homogenizer, and the cells were washed three times in ice-cold Hanks' balanced salt solution (Hanks' solution). The final suspensions of donor cells were adjusted with Hanks' solution to a concentration of 200×10^6 nucleated cells/ml.

Preparing Suspensions of Macrophage-rich Cells. Five days before the last casein injection was given, each mouse (experimental as well as control mice) was injected intraperitoneally with 1 ml of 10% sodium caseinate in order to increase the amount of macrophages in the peritoneum.[10] The day after the last casein injection, the animals were killed with ether. The peritoneum was washed carefully with 5 ml of Hanks' solution. The cells obtained were predominantly macrophages (70%) and the number of cells harvested per mouse was approximately 5×10^6. The cells were washed three times in Hanks' solution and adjusted to a concentration of 50×10^6/ml.

Preparing Suspensions of Peripheral Lymphocytes. Three days before the last casein injection was given, each mouse (experimental as well as control) was injected intravenously with 1 ml of pertusis vaccine in order to increase the number of circulating lymphocytes.[11] The day after the last casein injection, about 1 ml of blood was drawn by means of heparinized Carlsberg pipets from the retroorbital plexus of anesthetized mice. The blood from all sensitized donors was pooled as

was that from control donors. The number of lymphocytes was calculated as 125,000/μl.

The transfer of the cell suspensions was performed as outlined in Table 1. All recipients were subcutaneously injected three times with 0.05 mg of nitrogen mustard (Erasol) in 0.5 ml of saline, on day 1, 3 and 5 after the transfer of the cells. Nitrogen mustard was applied in order to accelerate the presumed formation of amyloid.[4] The day after the last injection of nitrogen mustard, all recipients were killed. Liver, lung and spleen were fixed in neutral formalin and embedded in par-

Table 1—Transfer of Various Lymphoid Cells from Amyloidotic Donor Mice to Normal Syngeneic Mice

No. of animals	Degree of amyloid in donor spleen	No. and type of cell transferred intravenously	Treatment of recipients*	Incidence of amyloid	Degree of amyloid in recipient spleen
		Experimental			
37	2	100×10^6 spleen cells	HN2 × 3	37/37	2 (1–3)
9	2	100×10^6 thymus cells	HN2 × 3	0/9	0 (0–0)
5	2	50×10^6 bone marrow cells	HN2 × 3	0/5	0 (0–0)
9	2	50×10^6 thymus cells and 50×10^6 bone marrow cells	HN2 × 3	0/9	0 (0–0)
10	2	100×10^6 lymph node cells	HN2 × 3	0/10	0 (0–0)
11	2	25×10^6 peritoneal macroph.	HN2 × 3	0/11	0 (0–0)
17	2	100×10^6 peripheral lymph.	HN2 × 3	0/17	0 (0–0)
		Controls (All Donor Mice Untreated)			
39	None	100×10^6 spleen cells	HN2 × 3	0/39	0 (0–0)
11	None	100×10^6 thymus cells	HN2 × 3	0/11	0 (0–0)
3	None	50×10^6 bone marrow cells	HN2 × 3	0/3	0 (0–0)
9	None	50×10^6 thymus cells and 50×10^6 bone marrow cells	HN2 × 3	0/9	0 (0–0)
10	None	100×10^6 lymph node cells	HN2 × 3	0/10	0 (0–0)
8	None	25×10^6 peritoneal macroph.	HN2 × 3	0/8	0 (0–0)
7	None	100×10^6 peripheral lymph.	HN2 × 3	0/7	0 (0–0)

* Nitrogen mustard × No. of days.

affin. Sections were cut 5 μ thick and stained with haematoxylin-eosin (H&E), methyl green–pyronin, alkaline Congo red, and the periodic acid–Schiff stain. Amyloid was identified by its morphology and its birefringence with Congo red under crossed polarizing lenses. The degree of amyloidosis, if any, was evaluated in sections of spleens, thymi and lymph nodes on a scale ranging from 0 to 6, according to the semiquantitative method, described by Christensen and Hjort.[12]

Experiment II

Donors. The donor mice were given a total of ten daily subcutaneous injections of 0.5 ml of a 5% solution of sodium caseinate. Untreated control donors were included.

Transplantation. Slices from the spleens, thymi and the axillary lymph nodes (in the region of the casein injections) were removed from donors anesthetized with Avertin and immediately transferred to the lateral margins of the right kidneys of the anesthetized recipient mice, according to the method described by Wheeler *et al.*[13] These recipients will be referred to as the experimental group. The remaining parts of the spleens, thymi and lymph nodes were kept for histologic examinations. Similarly, untreated control donors supplied grafts of spleen, thymus and lymph node for recipients, which will be referred to as the control group. Likewise, the remainder of spleens, thymi and lymph nodes from control donors were kept for histologic examination.

Recipients. The recipients were treated with various doses of casein after the transplantation (see Table 2). Eleven animals from the experimental group and 8 from the control group died during the experiment and were excluded. All animals were killed with ether the day after they had received their last casein injection, or at the time given in Table 2. The right kidney carrying the graft was fixed in neutral formalin and embedded in paraffin separately. The left kidney, liver and spleen were similarly fixed and embedded in paraffin. Sections were prepared as described in experiment I.

Results

Experiment I (Table 1)

All donor spleens from the experimental groups showed grade 1–2 amyloidosis in the spleens, whereas none of the control donor spleens showed amyloid formation.

The animals receiving spleen cells from amyloidotic mice all developed amyloidosis of the usual distribution pattern: the amyloid was most abundant in the spleen, less abundant in the liver and only sparse in the kidneys. No recipient of the following cells from amyloidotic mice developed amyloidosis: lymph node, thymus and bone marrow cells or combinations of thymus and bone marrow cells, macrophage-rich cell suspensions or peripheral lymphocytes.

The mice in the control groups, which received normal lymph node cells, spleen cells, thymus cells, bone marrow cells, combination of thymus and bone marrow cells, peritoneal macrophages or peripheral lymphocytes, all failed to develop amyloid deposits.

Table 2—Transfer of Various Lymphoid Tissue from Mice Treated with Casein for 10 Days to Normal Syngeneic Mice

No. of animals	Treatment of donors × No. of days	Donor spleen amyloid	Tissue transplanted	Treatment of recipients × No. of days	Incidence of amyloid	Amyloid in recipients spleens
			Experimental			
6	Casein × 10	0	Thymus	Casein × 4	0/6	0 (0–0)
6	Casein × 10	0	Spleen	Casein × 4	0/6	0 (0–0)
3	Casein × 10	0	Thymus	Casein × 6	0/3	0 (0–0)
4	Casein × 10	0	Spleen	Casein × 6	0/4	0 (0–0)
3	Casein × 10	0	Thymus	Casein × 8	3/3	2 (1–2)
3	Casein × 10	0	Spleen	Casein × 8	3/3	3 (2–3)
3	Casein × 10	0	Thymus	Casein × 10	3/3	3 (2–3)
3	Casein × 10	0	Spleen	Casein × 10	3/3	2 (1–4)
8	Casein × 10	0	Lymph node	Casein × 10	8/8	3 (2–3)
			Control			
6	Casein × 10	0	Thymus	None †day 5	0/6	0 (0–0)
6	Casein × 10	0	Spleen	None †day 5	0/6	0 (0–0)
4	Casein × 10	0	Thymus	None †day 7	0/4	0 (0–0)
4	Casein × 10	0	Spleen	None †day 7	0/4	0 (0–0)
2	Casein × 10	0	Thymus	None †day 9	0/2	0 (0–0)
4	Casein × 10	0	Spleen	None †day 9	0/4	0 (0–0)
3	Casein × 10	0	Thymus	None †day 11	0/3	0 (0–0)
3	Casein × 10	0	Spleen	None †day 11	0/3	0 (0–0)
5	None	0	Thymus	Casein × 10	1/5	0 (0–2)
7	None	0	Spleen	Casein × 10	1/7	0 (0–2)
10	None	0	Lymph node	Casein × 10	1/10	0 (0–2)
6	None	0	Thymus	None †day 11	0/6	0 (0–0)
4	None	0	Spleen	None †day 11	0/4	0 (0–0)

† Indicates day recipient was sacrificed.

Experiment II (Table 2)

In the donor mice treated with casein for 10 days, the spleens showed an increased cellularity, particularly perifollicularly, and many of these cells appeared to be of the pyroninophilic variety. In none of the donor spleens, however, could amyloid be detected. The axillary lymph nodes used as donor organs all showed hyperplasia with a marked pyroninophilia; no amyloid was found. The transplanted thymi were all histologically normal.

In all animals, the grafts of spleen, thymus and lymph node survived and were easily recognized macroscopically as well as microscopically: All mice that received a spleen graft from a casein-treated donor and given eight or ten daily injections of casein showed severe amyloid formation in the host spleen as well in the spleen graft. The kidney bearing the grafted spleen showed infiltration of amyloid in the area close to the

graft bed. (Fig 1 and 2). No amyloid was found in recipients that received either a casein-sensitized spleen slice and less than eight daily injections of casein, or a nonsensitized spleen slice and treatment after the transfer. In the last-mentioned group, however, an insignificant amount of amyloid was seen in the spleen of 1 animal. The mice receiving a thymus graft from a casein-treated donor showed significant amyloid formation in the host spleen, if the recipients were treated with eight or ten injections of casein. No local effect of the thymus graft on the kidney was observed. The recipients with thymus grafts from sensitized donors and receiving less than eight injections of casein showed, as was the case of the spleen transfer, no amyloid formation; nor could transfer of untreated thymi stimulate amyloid production in recipients receiving ten injections of casein as the treatment after transfer, except in 1 case where small amyloid deposits were found in the spleen. Also, transplantation of casein-stimulated regional lymph nodes did lead to amyloid formation when the recipients were treated additionally with at least ten casein injections. No amyloid was found in the grafted lymph node and no local reaction in the graft-bearing kidney was observed. The recipients of normal lymph node tissue and injected ten times with casein failed to develop amyloidosis, except in 1 animal.

Discussion

The results of the present transfer experiments will be discussed in the light of the following hypotheses:

1. *Letterer (1934).*[14] "The amyloid is a precipitate of antibody-antigen complexes."

The suggestion that the amyloid substance is composed of a precipitate of antibodies with the corresponding antigens has not been confirmed. On the contrary, it has been impossible to demonstrate any quantitative or qualitative alterations in the serum levels of immunoglobulins that could be correlated to amyloid formation.[15,16] Likewise, it has been shown that immunoglobulins are not an integral component of the amyloid substance.[17,18]

2. *Teilum (1964).*[19] "The process of amyloid formation is dependent on a biphasically developed aberration of the protein synthesizing function of mesenchymal (reticulo-endothelial) cells. Namely (a) an initial, pyroninophilic phase with proliferation of reticulo-endothelial pyroninophilic cells and plasma cells and a rise in serum gamma-globulin, (b) an amyloid phase depending on a supression of proliferating pyroninophilic cells, leading to local amyloid formation from now P.A.S.

positive cells, and associated with a decrease in the gamma globulin level."

This hypothesis has proved to be a very inspiring working model, as it points out the sequence of cellular events in amyloid formation. Furthermore, it points out that amyloid is formed *in situ* by cells belonging to the RES, which has been confirmed by electron microscopy.[20,21] In these respects, this hypothesis can be said to have fruitfully spearheaded most current research on amyloid disease. However, it confines itself mainly to the morphology of amyloidogenesis and does not visualize in detail the dynamics of the processes involved.

3. *Ranløv (1968).*[22] "In principle amyloid formation involves two entirely different series of events: (1) A pyroninophilic tissue phase which can be caused by a number of different stimuli, though mainly (probably, only) antigenic, acting via cell-mediated immunity. (2) An actual amyloid phase, during which the amyloid substance is being deposited in the tissues by active secretion from cells belonging to the reticulo-endothelial system. The link between these two phases should be a "transfer factor" of nuclear nature released from the pyroninophilic cells, either directly or as the result of cell fragmentation. This transfer factor will impose an aberrant protein synthesis on R. E. cells resulting in amyloid formation."

Ranløv brought forth this hypothesis mainly as a result of transfer experiments.[6,9] In these experiments, Ranløv was able to transfer amyloidosis with spleen cells, with homogenates of spleen cells and even with a nuclear fraction. In the syngeneic combination employed, the donor mice received 17 injections of casein and were early in the amyloid phase. The transfer should, according to Ranløv, be brought about by a factor derived from pyroninophilic cells; this factor within the recipients should instruct the macrophages to form amyloid. In the present work, too, it has always been possible to transfer amyloidosis with spleen cells, and in similar experiments performed by Hardt and Hellung-Larsen, amyloidosis has been transferred between syngeneic mice with spleen cell homogenates and with crude nuclei, using the same treatment of donor and recipients that Ranløv used. However, the suggestion made by Ranløv that amyloid formation should depend *solely* on a factor from the pyroninophilic cells is not supported by the present work in which the transfer of cells from highly pyroninophilic regional lymph nodes failed to induce amyloid formation in recipients given nitrogen mustard alone as post-transfer treatment.

4. *Cathcart et al (1970).*[23] "Amyloid formation could be a positive expression of tolerance (*ie,* increased cellular metabolism within

the macrophage-lymphocyte axis). As long as the specific antigenic challenge is sustained, amyloid production would be enhanced; but once the antigenic stimulus is removed, amyloid production would cease."

Impaired cellular immunity as a possible pathogenic mechanism in amyloidosis was first proposed by Ranløv and Jensen,[24] and was based on the findings of prolonged survivals of allogeneic skin grafts in amyloidotic mice. Further support is found in the observation of amyloid formation in mice undergoing graft-versus-host reactions,[25] and in the recent results of Hardt and Claësson,[26] who found spleen cells from casein-treated parental-strain mice totally incapable of eliciting a graft-versus-host reaction in F_1 hybrids, thus indicating that a functional impairment of the immune apparatus must involve the cellular part of it. This dysfunction is evident even before amyloid is formed. Using an *in vitro* test system, Cathcart *et al* [17] found that guinea pigs chronically treated with large amounts of casein initially developed a cell-mediated immune response to this protein. However, the response waned as treatment continued, whereas the animals throughout the study showed a positive cell-mediated immune response to other antigens. The amyloid, it was postulated, should then be the result of a specific state of tolerance towards casein. These findings are not in accordance with the above mentioned work by Hardt and Claësson,[26] who, under similar conditions, found an unspecific impairment of the cellular immune response (as did Ranløv and Jensen [24]). Furthermore, in the present work, it has been possible to induce amyloid formation very rapidly by transferring spleen, thymus and lymph node grafts from mice that had received ten injections of casein (the mice thereby being in the pyroninophilic phase). The grafted animals all developed amyloid after ten injections of antigen, which corresponds to the amount of amyloid found in mice that have a total of 25 injections of casein. This enhancing effect of the grafts could be explained as a transfer of highly antigen-sensitive cells without a simultaneous transfer of specific humoral antibody, which, by means of a feed-back mechanism, is known to inhibit the proliferation of the antigen-sensitive cells.[27] The antigen-sensitive cells might thus be allowed to undergo a full-scale immune response when exposed to the specific antigen, resulting in a heavy pyroninophilia. This situation seems to be contrary to one of tolerance.

5. Amyloid formation could be due to the release of an amyloid-inducing factor from antigen-stimulated pyroninophilic cells. A prerequisite for the amyloid-promoting performance of this particular factor should be the sustained presence of the specific antigen.

According to this hypothesis, the macrophages should at the same

time be stimulated both by the antigen and by the unknown amyloid-inducing factor (AIF) derived from the pyroninophilic cells, resulting in the formation of amyloid by the macrophages. The positive transfer experiments with spleen cells could thus be explained as a transfer of (1) the AIF from overstimulated pyroninophilic cells and (2) macrophages containing antigen in a "highly immunogenic complex with RNA."[28] Under these conditions, the post-transfer treatment with nitrogen mustard could act through the release of these immunogenic complexes of RNA and casein. Thus, the macrophages of the recipients would be stimulated both with the AIF and the antigen, resulting in the formation of amyloid.

The hypothesis of release of an AIF from the pyroninophilic cells as a possible pathogenetic factor in amyloidosis has also been lent support by some recent findings by Claësson and Hardt,[29] in which a significant decay of lymphoid cells in spleen and thymus, prior to the formation of amyloid, was observed.

The negative effect of transfer of thymus cells, peritoneal exudate cells and peripheral lymphocytes can be taken as evidence against any concept of autoimmune mechanisms operating in amyloidogenesis, as these cells all are known carriers of this particular type of immune response.[30–32] Furthermore, the present results are somewhat in line with the negative transfer experiment of Clerici *et al*[33] using cells from the thoracic duct, and those of Lüders,[34] using dead leukocytes and lymph node cells. These negative transfer experiments, together with the present ones, can be explained by a lack of specific antigen in the transferred material (thymocytes, bone marrow cells, peripheral lymphocytes, peritoneal exudate cells). This is, however, not obvious when speaking about the lymph node cells, as lymph nodes are known to trap antigen. It appears though, from the work mentioned above, that the decay of lymphoid cells within the lymph nodes (regional, stimulated) remains on a steady level throughout the induction of amyloidosis. To account for this, it might be argued that the overstimulated lymphoid (pyroninophilic) cells containing the AIF are rapidly removed and transported from the lymph nodes to the spleen and the liver. Transfer of lymph node cells would hence be transfer of antigen only, plus cells without significant amount of AIF.

6. The active uptake of antigen by the macrophages is the first step in an immune response to a weak antigen. An overloading of the macrophages with antigen might lead to a dysfunction of these cells, with production of amyloid as a result. The pyroninophilia could then be the normal response to an antigen when the macrophages are normally functioning. When this function of the macrophages is disturbed, the pyro-

ninophilia decreases as the antigen-sensitive cells are not stimulated in the normal way (via macrophages). At the same time, amyloid is formed by such impaired macrophages.

This hypothesis has much in common with the tolerance hypothesis put forward by Cathcart *et al*, but whereas they operate with a concept of specific alteration of the response of the macrophages toward casein alone and not toward other antigens, this hypothesis suggests that amyloid formation is caused by an unspecific, general interference with the macrophages due to overloading with antigen. The transfer should then be possible only with "damaged" macrophages which are known to concentrate in the spleen, thereby spotlighting the conspicuous and specific role of this organ in amyloidosis, in general, and in transfer amyloidosis, in particular. More in line with current views on immunity, this hypothesis seems to assign the macrophages to the role of nonspecificity, leaving the specificity to the lymphocytes.

The failure to mediate a graft-versus-host reaction with spleen cells from mice undergoing amyloid induction might thus be due to an impairment of macrophages function. Arguing against the theory of damage of the macrophage due to overloading with antigen is a recent experiment by Hardt and Ebbesen, where transfusion of fresh normal peritoneal macrophages from late in the pyroninophilic phase into the amyloidotic phase failed to alter the course of amyloid formation.

Conclusion

Available data from current research into the pathogenetic mechanisms of amyloidosis can be interpreted as indicating a cooperation between an amyloid-inducing factor, operating intercellularly, and a sustained presence of specific antigen. As a result of these combined activities, an aberration of macrophage function occurs, leading to synthesis of the abnormal protein, amyloid.

References

1. Kuczynski MH: Edwin Goldmanns Untersuchungen uber zellulare Vorgange im Gefolge des Verdauungsprozesses auf Grund nachgelassener Praparate dargestellt und durch neue Versuche erganzt. Virchow Arch [Path Anat] 239:185–302, 1922
2. Jaffé RH: Experimental amyloidosis in mice: effect of different forms of diet. Arch Path 2:149–160, 1926
3. Letterer E: Studien über Art und Entstehung des Amyloids. Beitr Path Anat 75:486–588, 1926
4. Teilum G: Studies on pathogenesis of amyloidosis; effect of nitrogen mustard in inducing amyloidosis. J Lab Clin Med 43:367–374, 1954

5. Hardt F, Ranløv P: Transfer amyloidosis, local and systemic amyloidosis in recipients of syngeneic spleen grafts from non-amyloidotic, casein-sensitized donor mice. Acta Path Microbiol Scand 73:549–558, 1968
6. Werdelin O, Ranløv P: Amyloidosis produced in mice by transplantation of spleen cells from casein-treated mice. Acta Path Microbiol Scand 68:1–18, 1966
7. Hultgren MK, Druet RL, Janigan DT: Experimental amyloidosis in isogeneic x-irradiated recipients of sensitized spleen tissue. Amer J Path 50:943–955, 1967
8. Hardt F: Transfer of experimental mouse amyloidosis with whole-spleen grafts between syngeneic animals. Acta Path Microbiol Scand 72:446–447, 1968
9. Ranløv P: The adoptive transfer of experimental mouse amyloidosis by intravenous injections of spleen cell extracts from casein-treated syngeneic donor mice. Acta Path Microbiol Scand 70:321–335, 1967
10. Weir DM: Handbook of Experimental Immunology. Oxford and Edinburgh, Blackwell Scientific Publications, 1967, p 1022
11. Morse SI: Studies on the lymphocytosis induced in mice by *Bordetella pertussis*. J Exp Med 121:49–68, 1965
12. Christensen HE, Hjort GH: X-irradiation as accelerating factor in caseinate-induced amyloidosis in mice. Acta Path Microbiol Scand 47:140–152, 1959
13. Wheeler HB, Corson JM, Dammin GJ: Transplantation of tissue slices in mice. Ann NY Acad Sci 129:118–129, 1966
14. Letterer E: Neue Untersuchungen über die Entstehung des Amyloids. Virchow Arch [Path Anat] 293:34–72, 1934
15. Giles RB, Calkins E: The relationship of serum hexosamine, globulins and antibodies to experimental amyloidosis. J Clin Invest 37:846–857, 1958
16. Willerson JT, Asofsky R, Barth WF: Experimental murine amyloid. IV. Amyloidosis and immunoglobulins. J Immun 103:741–749, 1969
17. Cohen AS: The constitution and genesis of amyloid. Int Rev Exp Path 4:159–243, 1965
18. Cohen AS, Paul WE: Relationship of gamma-globulin to the fibrils of secondary human amyloid. Nature 197:193–194, 1963
19. Teilum G: Pathogenesis of amyloidosis: the two-phase cellular theory of local secretion. Acta Path Microbiol Scand 61:21–45, 1964
20. Ranløv P, Wanstrup J: Ultrastructural investigations on the cellular morphogenesis of experimental mouse amyloidosis. Acta Path Microbiol Scand 71:575–591, 1967
21. Zucker-Franklin D, Franklin EC: Intracellular localization of human amyloid by fluorescence and electron microscopy Amer J Path 59:23–41, 1970
22. Ranløv P: Immunology and pathogenesis of experimental amyloidosis. Danish Med Bull 15:289–296, 1968
23. Cathcart ES, Mullarkey M, Cohen AS: Amyloidosis: an expression of immunological tolerance? Lancet 2:639–640, 1970
24. Ranløv P, Jensen E: Homograft reaction in amyloidotic mice. Acta Path Microbiol Scand 67:161–164, 1966
25. Fiscus WG, Morris BT, Session J, Tretin JJ: Specificity, host-age effect, and pathology of homologous disease induced in unirradiated F_1 hybrid mice by

transplantation of parental lymphoid tissue. Ann NY Acad Sci 99:355–373, 1962

26. Hardt F, Claësson MH: Graft-versus-host reactions mediated by spleen cells from amyloidotic and non-amyloidotic mice. Transplantation 12:36–39, 1971
27. Möller G, Möller E: Immune cytotoxicity and immunological enhancement in tissue transplantation, Antibodies to Biologically Active Molecules. Proceedings of the Second Meeting of the Federation of European Biochemical Societies. Vol 1. Oxford and New York, Pergamon Press, 1966, pp 349–407
28. Askonas BA, Rhodes JM: Immunogenicity of antigen-containing ribonucleic acid preparations from macrophages. Nature 205:470–474, 1965
29. Claësson MH, Hardt F: Quantitative studies on the decay of lymphoid cells during the development of casein-induced murine amyloidosis. Acta Path Microbiol Scand (in press)
30. Chase MW: The cellular transfer of cutaneous hypersensitivity to tuberculin. Proc Soc Exp Biol Med 59:134–135, 1945
31. Hess EV, Ashworth CT, Ziff M: Transfer of an autoimmune nephrosis in the rat by means of lymph node cells. J Exp Med 115:421–450, 1962
32. Claman HN, Chaperon EA, Triplett RF: Immunocompetence of transferred thymus-marrow cell combinations. J Immun 97:828–832, 1966
33. Clerici E, Mocarelli P, de Farrari F, Villa ML: Studies on the passive transfer of amyloidosis by cells. J Lab Clin Med 74:145–152, 1969
34. Lüders K: Leukozytentransfer und Amyloidose. I. Übertragung avitaler Leukozyten der Blutbahn. II. Übertragung vitaler Lymphozyten von Lymphknoten innerhalb eines Mäuseinzuchtstammes. Z Immunitaetsforsch 137:474–491, 1969

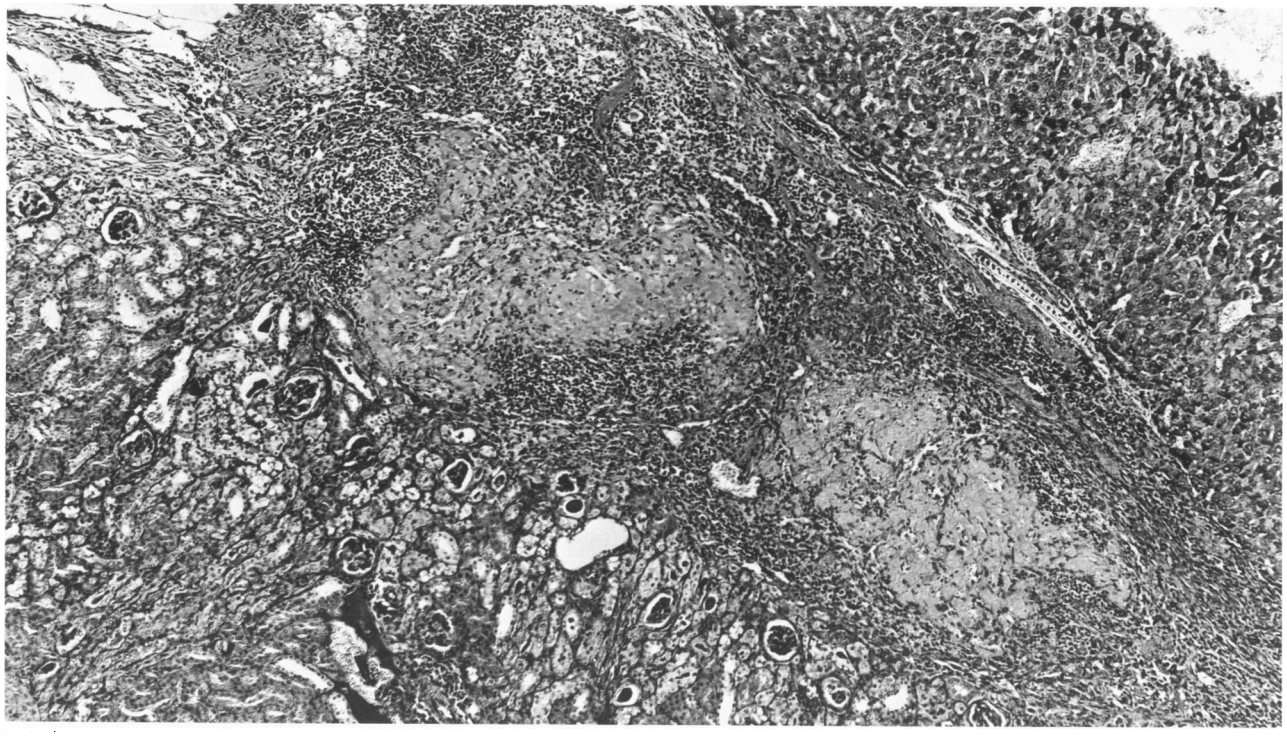

Fig 1—Right kidney of recipient, carrying spleen graft from sensitized donor. After 10 days of casein treatment, the graft shows massive amyloid formation (H&E, × 56).

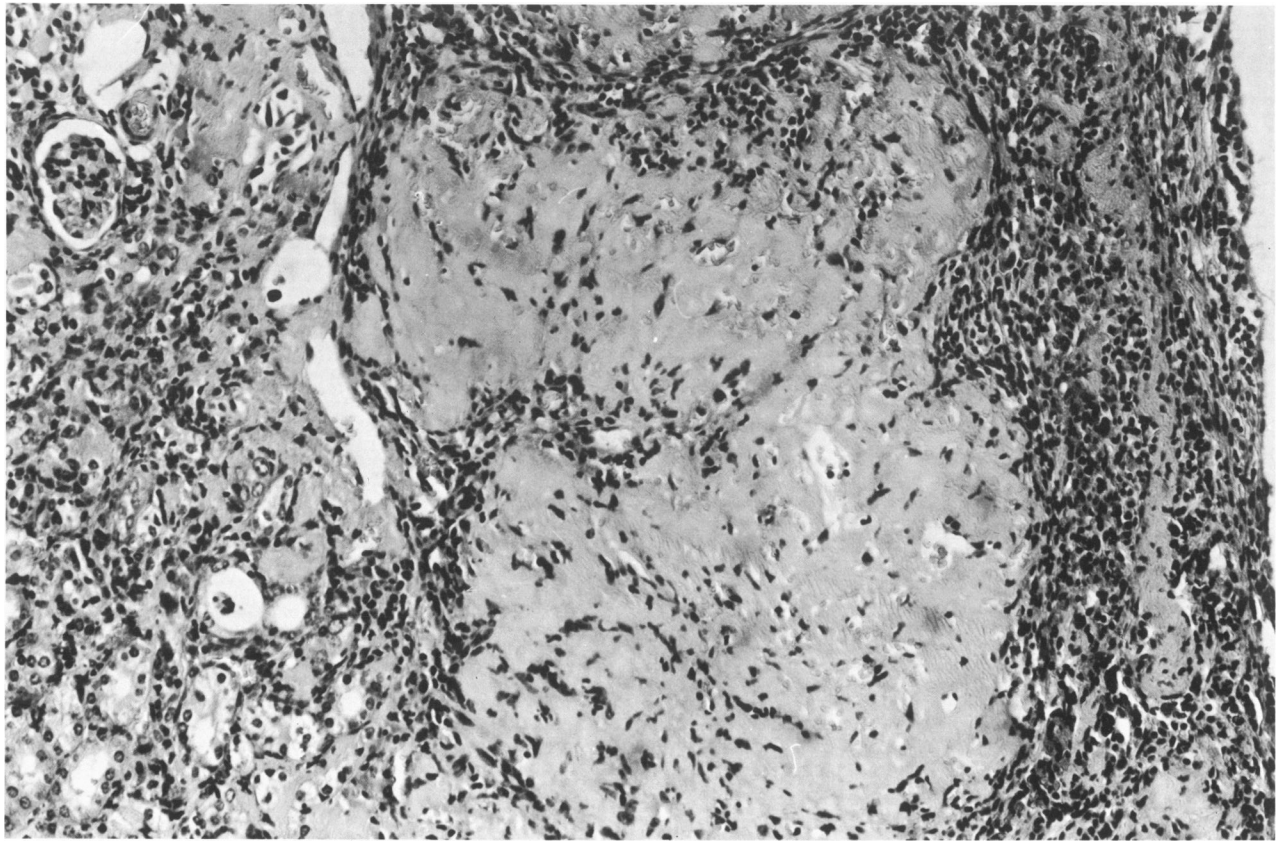

Fig 2—The amyloid substance of the spleen graft is seen extending into the adjacent host kidney, involving the tubular interstitial tissue and a few glomeruli. No amyloid could be demonstrated in the remaining parts of this or the contralateral kidney (H&E, × 140).