Clin. exp. Immunol. (1972) **10,** 635–647.

INDIVIDUAL ANTIGENIC SPECIFICITY AND CROSS-REACTIONS AMONG AMYLOID PREPARATIONS FROM DIFFERENT INDIVIDUALS

G. HUSBY AND J. B. NATVIG

Institute of Immunology and Rheumatology, Rikshospitalet and Oslo Sanitetsforenings University Hospitals

(*Received* 15 *September* 1971)

SUMMARY

Amyloid fibrils were isolated from eleven amyloid-laden organs of six patients. By alkaline degradation, soluble units were obtained which gave antibody formation in rabbits. Gel precipitation and haemagglutination inhibition were used to characterize antigens of the amyloid. Evidence was obtained that amyloids from different organs of the same individual were identical in the antigenicity. In contrast, amyloids from different individuals each showed unique individual specificity. Besides this, antigenic cross-reactions were noted between the amyloid preparations. Finally, evidence for antigenic cross-reactivity between certain amyloid preparations and immunoglobulin light chains was obtained.

INTRODUCTION

Although amyloid disease has been known for a long time (von Rokitansky, 1846; Teilum, 1964), the nature and composition of the amyloid substance was not understood until very recently. The first characteristic histochemical property observed was its ability to bind Congo-red (Bennhold, 1922), giving a specific green birefringence in polarized light (Ladewig, 1945, Missmahl & Hartwig, 1953; Missmahl, 1957).

A further major step in characterizing amyloid was achieved by means of electron microscopy which showed characteristic fibrils to be the main component of amyloid (Cohen & Calkins, 1959) with periodic rods another component (Glenner & Bladen, 1968; Pras *et al.*, 1969). The fibrils are unique for amyloid and their presence accounts for the green birefringence after Congo-red staining (Pras *et al.*, 1968; Glenner *et al.*, 1968).

A third step was the recent development of immunological test systems for detection of amyloid (Franklin & Pras, 1969). Immunological techniques enabled some differences to be observed among amyloid preparations from different organs of the same patient, and also from different patients. However, most amyloid preparations showed antigenic similarities by the techniques employed.

Correspondence: Dr G. Husby, Institute of Immunology and Rheumatology, F. Qvamsgt. 1 c, Oslo 1, Norway.

The present report is a further characterization of the amyloid material in various organs from the same individual, and from different individuals. By employing precipitation techniques and sensitive haemagglutination inhibition methods, evidence was obtained that amyloid preparations from different organs of the same individual were identical in the antigenicity. Amyloid from different individuals each showed individual specificity.

Besides this, however, antigenic cross reactions were noted between some of the amyloid preparations. Finally, evidence for partial antigenic cross-reactivity between certain amyloid preparations and immunoglobulin light chains was obtained.

MATERIALS AND METHODS

Isolation of amyloid fibrils

Eleven amyloid organs were obtained by autopsy from six patients. Two patients had primary and four others secondary amyloidosis (Table 1). The diagnosis in each case was confirmed by demonstration of green birefringence, when Congo-red stained sections (see later) from the organs were examined in polarized light. Furthermore, each organ also showed specific staining with a fluorescein-labelled (Munthe & Natvig, 1971) anti-amyloid antiserum.

TABLE 1. Sources of human amyloid

Patient	Diagnosis	Organ	Prep. No
T.H. 9 years	Juvenile rheumatoid arthritis	Liver Kidney	I II
J.R. 61 years	Primary amyloidosis	Spleen Liver	III IV
E.L. 53 years	Hypernephroma, left kidney	Right kidney Liver	V VI
J.L. 50 years	Cirrhosis of the liver Chronic alcoholism	Liver	VII
S.M. age not known	Primary amyloidosis	Liver	VIII*
T.K. 22 years	Ulcerative colitis Carcinoma coli	Spleen Adrenals Kidney	IX X XI

*Lyophylized amyloid fibrils kindly provided by Dr A. J. Pick, Beilinson Hospital, Tel Aviv, Israel.

The isolation of the fibrils was performed according to the method described by Pras *et al.* (1968). Twenty grammes of fresh or deep-frozen tissue was homogenized in 400 ml 0·15 M NaCl in a 45 Virtis homogenizer. The homogenate was spun at 19,600 *g* for 15 min at 4°C. The supernate was discarded and the sediment rehomogenized in 400 ml saline. This procedure was repeated seven to twelve times until no or negligible amounts of protein were detected in the supernate by absorption at 280 nm. The last sediment was rehomogenized in 300 ml of distilled water and spun at 48,200 *g* for 90–120 minutes. This was repeated three times with 150 ml of distilled water. The protein content of the four

supernates was estimated by the micro-Kjeldahl method, and by the modified Folin technique (Lowry *et al.*, 1951). Since the protein concentration in the first supernate was minimal, only supernates 2, 3 and 4 were pooled and lyophylized. The preparations were kept at −20°C until used. The yield of lyophylized amyloid fibrils varied from 80 to 600 mg in the different tissues, but was more or less constant in different preparations from the same tissue.

The lyophylized amyloid fibrils were resuspended in distilled water immediately before use. In some cases suspended fibrils were precipitated in 0·15 M NaCl in the cold for 24 hr, spun and dialysed against distilled water for 7 days. By this procedure a still more purified fibril preparation was obtained.

Control tissues from normal human organs were similarly treated, except that they were washed only five times with saline before extraction with distilled water.

Congo-red staining

Amyloid solution was applied on a glass slide, air-dried, stained with Congo-red and examined in a Leitz Orthoplan polarizing microscope. Specific green birefringence was demonstrated with all amyloid preparations while all the control preparations were completely negative.

Degradation of amyloid

Alkaline degradation of amyloid fibrils was carried out using the method described by Pras *et al.* (1969). Lyophylized amyloid fibrils were incubated in 0·1 N NaOH for 3–72 hr, mostly 24 hr, followed by dialysis against distilled water. After centrifugation at 48,200 *g* for 30 min, a small precipitate occurred. This was discarded, and the supernate used as degraded amyloid (DAM). For control, extractions from normal human organs were similarly treated. Degradation of amyloid was also performed by boiling according to Pick, Lavi & Joshua (1970).

Absorption spectra analysis

The absorption spectra of DAM I and DAM IX were estimated in a Beckman DB-spectrophotometer. For comparison, 0·1 M NaOH-treated extracts from normal liver and spleen were also examined.

Antisera

Antisera were raised in rabbits by weekly intracutaneous injections with alkaline-degraded amyloid (DAM I, III and IX) mixed with equal amounts of Freund's complete adjuvant. Only the degraded products of amyloid were used since native amyloid fibrils are not immunogenic in rabbits (Franklin & Pras, 1969; Ram, de Lellis & Glenner, 1968). The antisera were inactivated at 56°C for 30 min and absorbed with pooled normal human plasma. Except for preliminary experiments, the antisera were also absorbed with one of the following preparations of the corresponding normal human organs: homogenate of fresh tissue or specimens extracted by the same method as for the amyloid organs. These preparations were either used directly, or after treatment with 0·1 N NaOH.

Antiserum against human serum, anti-$F(ab')_2$ anti-light chain, anti-IgG, anti-IgA and anti-IgM were prepared, absorbed and used as previously described (Natvig, 1970).

Immunological tests

Double immunodiffusion and immunoelectrophoresis were performed in 1% agarose or agar with barbital buffer pH 8·6, ionic strength 0·025 as previously described (Munthe & Natvig, 1971). For passive haemagglutination and haemagglutination inhibition tests with alkaline-degraded amyloid, chromium chloride or bis-diazotized benzidine was used as the coupling agent (Gold & Fudenberg, 1967; Natvig, 1970). In control experiments, red cells coated with various immunoglobulin preparations were employed.

Absorption studies were performed by adding native or degraded amyloid fibrils to the

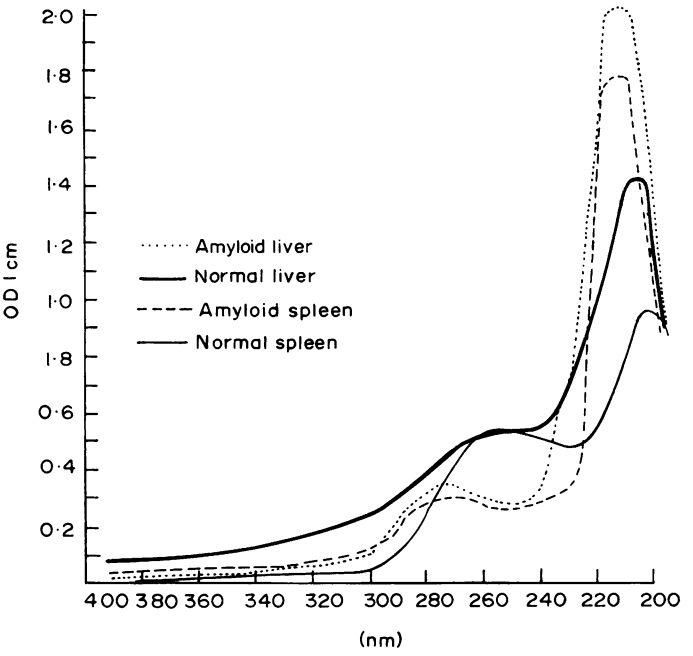

FIG. 1. Absorption spectra of alkaline-degraded amyloid (DAM) I and IX. Normal liver and spleen preparations as controls.

antisera (Franklin & Pras, 1969). The mixtures were kept at room temperature for 1 hr in the cold overnight (4°C), and spun down. The supernates were used in double immunodiffusion and haemagglutination tests against DAM preparations.

RESULTS

Absorption spectra

Results of the absorption spectra analysis are shown in Fig. 1. The absorption spectra of DAM I and IX are strikingly similar, and differ from the spectra of the respective normal organ preparations. With the DAM preparations, there was a significant shoulder at 290 nm, which corresponds well with the absorption maximum for tryptophan. The

absorption spectra was thus typical for amyloid fibrils which have a significant component of tryptophan (Harada *et al.*, 1971).

Immunodiffusion studies

The anti-amyloid antisera gave one single distinct precipitation line in double immunodiffusion against the respective degraded amyloid (DAM) preparations (DAM I and III) employed for immunization (Fig. 2). Anti-DAM I (R 166) showed the best precipitation activity. This antiserum also precipitated with the other amyloid preparations (Fig. 3). No definite spurring could be observed in the precipitation reaction unless partial absorption with various amyloid preparations was utilized (see later). In immunoelectrophoresis

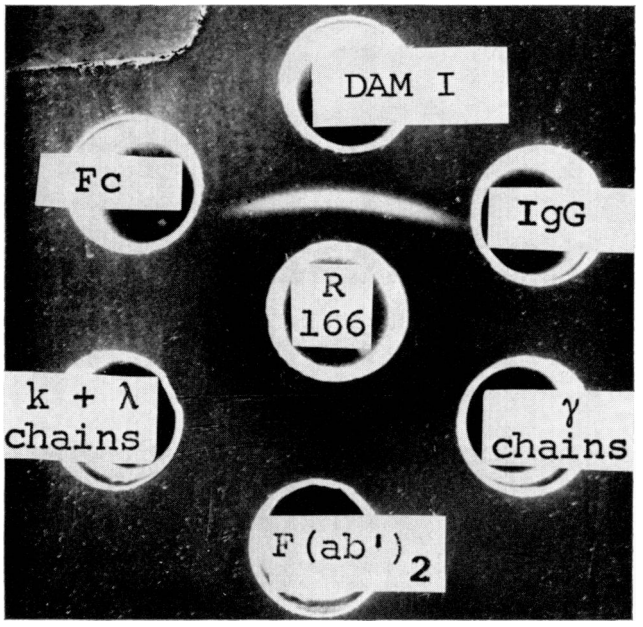

FIG. 2. Double immunodiffusion. Anti-DAM I (R 166) in central well tested against DAM I, IgG, heavy and light chains and fragments of IgG.

with anti-DAM I, the eleven DAM preparations showed a single precipitation line, slightly anodic to the application point (Fig. 4).

In double immunodiffusion and immunoelectrophoresis, the various degraded amyloid preparations showed no reaction against rabbit anti-human serum or plasma. However, unabsorbed rabbit anti-amyloid antisera sometimes had a weak activity against pooled normal human plasma. These other activities were directed mostly against albumin, IgG and transferrin. Thus, although minimal, the contamination of the degraded amyloid preparations with some plasma components was sufficient to induce antibodies in animals, but not to give direct precipitation in agarose.

Some unabsorbed anti-amyloid antisera also showed a faint reaction with alkaline-degraded preparations of normal tissues. However, in double immunodiffusion, there was a reaction of nonidentity with the DAM preparations. The precipitation lines with normal

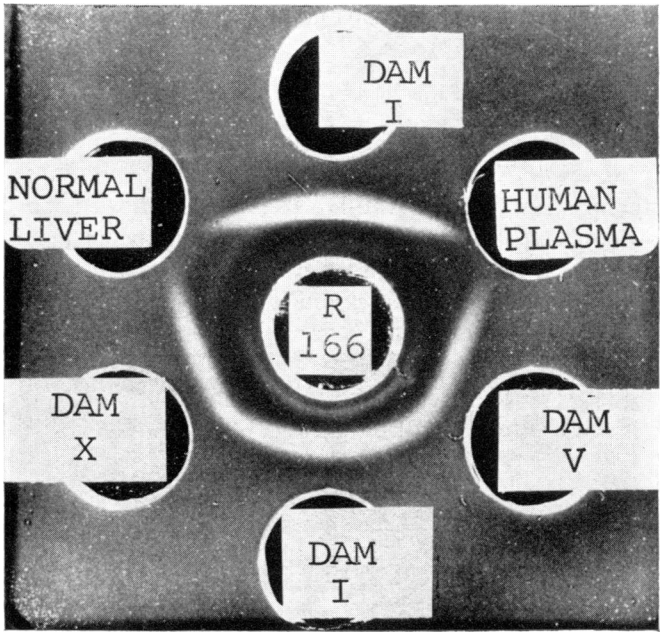

FIG. 3. Double immunodiffusion. Anti-DAM I (R 166) in central well tested against various DAM preparations and normal human plasma and normal liver preparation.

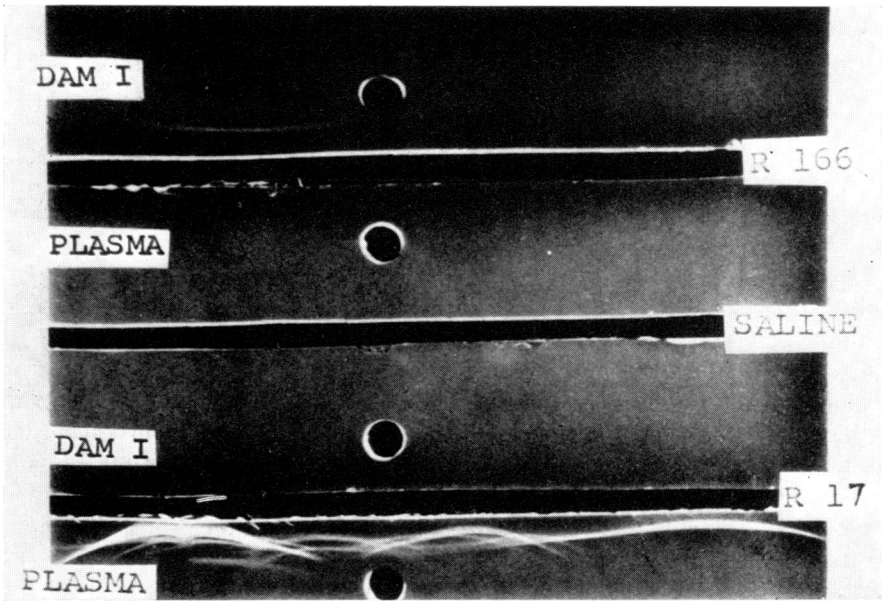

FIG. 4. Immunoelectrophoresis of DAM I and human plasma against anti-DAM I (R 166) and anti-human serum (R 17).

plasma and tissue components were completely abolished by addition of preparations from normal tissue or normal human plasma to the antiserum. Furthermore, these preparations did not even inhibit the specific precipitation line between amyloid and anti-amyloid, which only could be removed by specific absorption with amyloid. The findings demonstrated a specific immunological reaction between amyloid and anti-amyloid antiserum, and cross-reaction between amyloid from different individuals.

Absorption studies

Anti-DAM I (R 166) and anti-DAM III (R 200) were absorbed with varying amounts of native amyloid fibril preparations. The mixtures were left overnight in the cold. After spinning, the supernates were tested against DAM preparations in double immunodiffusion tests. For control, antiserum was incubated with saline, extracts of normal organs or normal rabbit serum. Amyloid fibrils from all the eleven organs affected the precipitating activity against degraded amyloid. There were, however, striking differences among the

TABLE 2. Effect on anti-DAM of absorption with native amyloid fibrils (AM)

	Anti-DAM I (R 166)		Anti-DAM III (R 200)	
Precipitation test* with	DAM I	DAM III	DAM I	DAM III
Absorption with:				
AM I 2 mg/ml	0	0	0	+
AM I 1 mg/ml	0	0	0	++
AM I 0·2 mg/ml	+	0	0	++
AM III 2 mg/ml	++	0	0	0
AM III 1 mg/ml	++	0	0	0
AM III 0·2 mg/ml	++(+)	+	0	+
Control absorption†	+++	++	+	++

*After absorption, the supernates were tested in double immunodiffusion against various DAM preparations.

†In control absorptions, normal plasma and extracts of normal tissue were added.

amyloid preparations. The amyloid fibrils of the autologous preparation used for immunization, or amyloid from other organs of the same patients were most efficient, and in small amounts completely removed the precipitating activity (Table 2).

In contrast, absorption with amyloid from other individuals at approximately 2 mg/ml removed the precipitation against the corresponding DAM preparation, but only to a minor degree affected the precipitation line against DAM used for immunization (Fig. 5). After partial absorption, a definite spurring was observed (Fig. 6). These findings indicated antigenic differences among amyloid preparations from various individuals. These differences were further tested in haemagglutination inhibition tests, which are known to be more sensitive than precipitation.

Haemagglutination and haemagglutination inhibition experiments

Human red blood cells coated with DAM I, II, III and IV, were tested against anti-DAM I and anti-DAM III. The coated cells did not show any reaction with anti-human serum or anti-immunoglobulin antisera, but were clearly agglutinated by antisera absorbed specific-

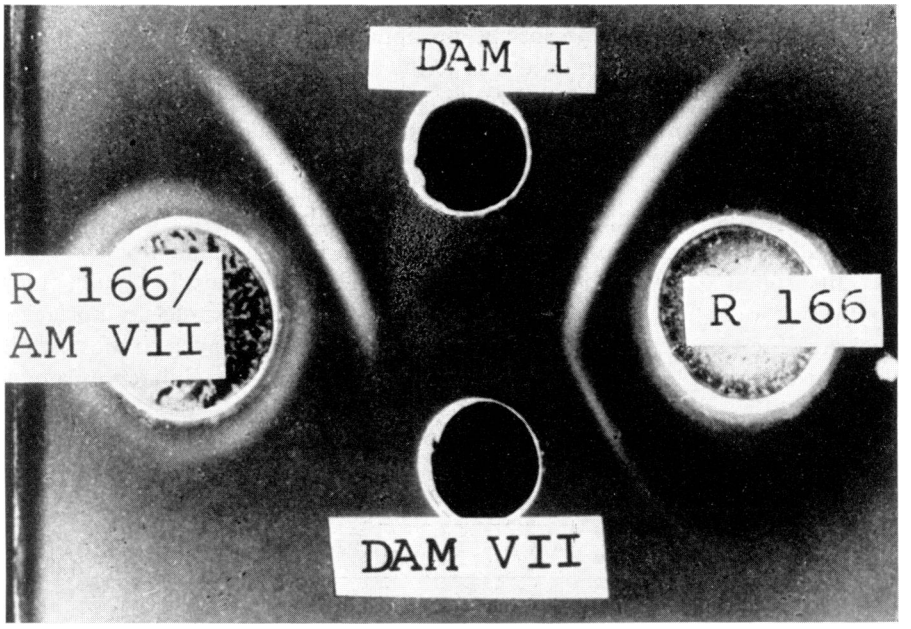

FIG. 5. Precipitation between anti-DAM I (R 166) and DAM I or DAM VII before and after absorption with AM VII.

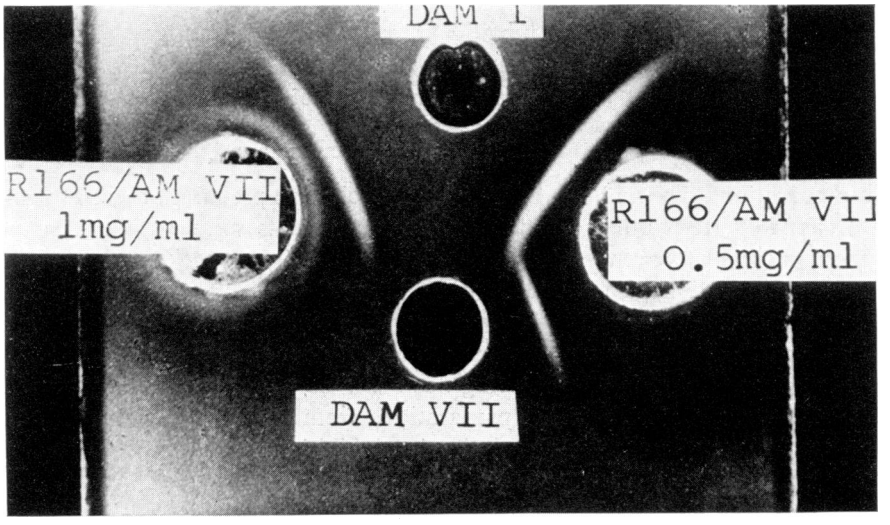

FIG. 6. Precipitation between anti-DAM I (R 166) and DAM I or DAM VII after absorption with various amounts of AM VII.

ally to react with amyloid (Table 3). The reaction with antiserum made against the same amyloid preparations as those used for coating of the red cells gave the strongest reactions. There were no striking differences when DAM preparations from different organs of the same individual were used for coating of the cells.

Furthermore, in inhibition studies, the autologous preparations always gave a definite inhibition, while homologous preparations gave weak or no inhibition. Representative results of several experiments are shown in Table 4. For example DAM I gave a strong inhibition of a test system with anti-DAM I and red cells coated with DAM I. In contrast,

TABLE 3. Haemagglutination reaction with anti-DAM antisera against red cells coated with DAM

	Reciprocal of dilution of antiserum						
	2	4	8	16	32	64	128
Anti-DAM I (R 166)							
DAM I cells	++++	++++	+++	++	++	±	−
DAM III cells	+	−	−	−	−	−	−
Anti-DAM III (R 200)							
DAM I cells	++	+	+	−	−	−	−
DAM III cells	+++	+++	++	++	++	+	−

TABLE 4. Inhibition of anti-DAM by DAM preparations from different individuals

	Anti-DAM I		Anti-DAM III	
Cells coated with:	DAM I	DAM III	DAM I	DAM III
Inhibitor				
DAM I	0·03*	0·015	0·06	>1
DAM II	0·12	0·03	0·12	>1
DAM III	1	0·03	0·06	0·06
DAM IV	1	0·03	0·12	0·12

* Lowest concentration of DAM (mg/ml) giving inhibition.

the test system with anti-DAM III and red cells coated with DAM III was inhibited to a greater degree by DAM III or other DAM preparations from organs or patient J.R. than by DAM from another patient, T.H. Also, when antiserum against DAM I was absorbed with native amyloid III, it still retained activity against red cells coated with DAM I. In contrast, absorption with amyloid from another organ of the same patient completely abolished the agglutinating activity. The findings indicated individual specificity of the amyloid from different patients, while amyloid from different organs of the same patient showed antigenic identity by our techniques.

Comparison between amyloid preparations and light chains

There is similarity between amyloid and immunoglobulin light chains (Pick & Osserman, 1968; Glenner *et al.*, 1971). This was further investigated in haemagglutination in-

hibition experiments. For this study we selected an antiserum (anti-DAM IX), which appeared to show even more cross-reactivity with other amyloid preparations than the antisera previously utilized. Red cells were coated with DAM IX, IgG, γ-heavy chains or various κ and λ Bence-Jones proteins and pooled light chains. Agglutination by anti-DAM IX (R 218) was seen with DAM IX coated cells, and a fairly similar reaction was obtained with the red cells coated by some of the κ Bence-Jones proteins and pooled light chains, but not when IgG, heavy chains or λ light chains were used for coating. Representative examples are shown in Table 5. The agglutination by anti-DAM IX and red cells coated by κ light chains was clearly inhibited by the same κ light chains, but not by λ chains, or intact IgG. Furthermore, a distinct inhibition was also obtained with the autologous amyloid

TABLE 5. Haemagglutination titres of anti-DAM and anti-F(ab')$_2$

	Red cells coated with:			
	DAM IX	κ chains (Ju)	λ chains (Ba)	IgG
Anti-DAM IX (R 218)	32	16	<1	<1
Anti-F(ab')$_2$	<1	256	256	>256

TABLE 6. Inhibition of anti-DAM in reaction with red cells coated by DAM and by κ chains

	Anti-DAM IX	
Coat with:	DAM IX	κ chains (Ju)
Inhibitor		
DAM IX	0·015*	0·06
κ-chains (Ju)	>1	0·015
λ-chains (Ba)	>1	>1
pooled IgG	>1	>1

*Lowest concentration (mg/ml) of inhibitor giving inhibition.

preparation (Table 6). Other amyloid preparations gave a varying degree of inhibition. Agglutination obtained with DAM IX coated red cells was inhibited by the autologous amyloid, and to some extent by another amyloid, but not by κ light chains. The findings could not be explained on the basis of contamination of the DAM preparation with normal light chains, since normal light chains treated with 0·1 N NaOH for 3 hr lost their antigenicity. The data therefore indicate a partial identity between one of the amyloids investigated and certain light chains.

DISCUSSION

In the past, several reports have been published about the antigenic properties of amyloid

(Calkins, 1968). Until recently, however, the specific immunological reactions of the amyloid fibrils could not be investigated (Franklin & Pras, 1969). This is because of the high degree of insolubility of the fibrils and the contamination of the amyloid preparations with non-fibrillar components (Ram *et al.*, 1968).

New methods for isolation and degradation of the fibrils made it possible to obtain purer amyloid preparations and to raise specific antisera in animals against the proteins of amyloid fibrils (Pras *et al.*, 1969; Franklin & Pras, 1969; Glenner *et al.*, 1969; Pick *et al.*, 1970). By employing the isolation and degradation procedures of Pras *et al.* (1969), we obtained amyloid fibrils with a high degree of purity and with a specific absorption spectrum (Glenner *et al.*, 1969). No contaminating proteins were found in double immunodiffusion tests. However, small amounts of plasma proteins, IgG, albumin and transferrin were indicated by weak antibody activities against normal human plasma components in the anti-DAM antisera. After appropriate absorptions, the antisera gave a specific reaction only with amyloid preparations.

In double immunodiffusion precipitation tests there was a high degree of cross-reactivity between amyloid preparations from different individuals. However, after partial absorption with various amyloid preparations, reactions of partial identity were observed. Upon further absorption, antibody activity against the preparation used for immunization could be retained while the activity against the amyloid used for absorption was removed. Franklin & Pras (1969) obtained precipitation in gel only with the autologous amyloid. However, they found evidence for cross-reactivity by quantitative precipitation and complement fixation tests. The individual specificity of amyloid fibrils is comparable to that of isolated antibodies (Kunkel, Mannik & Williams, 1963) and myeloma proteins (Grey, Mannik & Kunkel, 1965).

The antigenic cross-reactivity between amyloid from different individuals was also further supported by our results with immunofluorescence staining technique. Here, antisera made against amyloid from one individual could be utilized to detect amyloid in organ biopsies from other individuals (unpublished observations). The antigenic cross-reactivity between amyloid from different individuals is also supported by the recent immunofluorescence studies by Cathcart, Skinner & Cohen (1971).

It has been suggested that amyloid fibrils are identical with light chains of immunoglobulins, and Glenner *et al.* (1971) have recently obtained biochemical evidence for this hypothesis. We obtained evidence for antigenic similarity between certain light chains and one particular amyloid preparation. Red cells sensitized with a κ Bence-Jones protein were agglutinated by one antiamyloid antiserum. This reaction was inhibited by the same κ light chains and also by the amyloid preparation used for immunization. In contrast, no inhibition was seen with normal human serum, or native IgG. This indicated that certain antigens are shared by this isolated amyloid and κ light chains. The fact that reaction was seen with amyloid and isolated light chains, but not with whole IgG, indicates that amyloid contains some of the specific determinants of free light chains (Tan & Epstein, 1965). That the cross-reaction was most clearly obtained with one particular antiserum may indicate that the corresponding amyloid contained more common determinants, either related to the antigenic subgroups of the variable region or possibly of the constant region. This antiserum also showed more distinct cross-reactivity in haemagglutination inhibition with other amyloid preparations than two other antisera where the individual specificity was more pronounced. The possible existence of identity between monoclonal light chains

and amyloid fibrils in the same patient and the relationship to light chain variable subgroups are at present being studied.

ACKNOWLEDGMENTS

This work was supported by The Norwegian Council for Science and the Humanities. We thank Dr A. I. Pick for providing amyloid material.

REFERENCES

BENNHOLD, H. (1922) Eine spezifische Amyloidfärbung mit Kongorot. *München med. Wchnschr.* **69,** 1537.

BLADEN, H.A., NYLEN, M.U. & GLENNER, G.G. (1966) The ultrastructure of human amyloid as revealed by the negative staining technique. *J. Ultrastruct. Res.* **14,** 449.

CALKINS, E. (1968) Relationship of amyloidosis to immunologic mechanisms. In: *Amyloidosis* (ed. by E. Mandema, L. Ruinen, F. H. Scholten and A. S. Cohen), p. 87. Excerpta Medica, Amsterdam.

CATHCART, E.S., SKINNER, M. & COHEN, A.S. (1971) Immunogenicity of Amyloid. *Immunology*, **20,** 94.

COHEN A.S. & CALKINS, E. (1959) Electron microscopic observations on fibrous components in amyloid of different organs. *Nature* (*Lond.*), **183,** 1202.

FRANKLIN, E.C. & PRAS, M. (1969) Immunological studies of water-soluble human amyloid fibrils. *J. exp. Med.* **130,** 797.

GLENNER, G.G. & BLADEN, H.A. (1968) The relationship of the two fiber types characteristic of human amyloid deposits. In *Amyloidosis* (ed. by E. Mandema, L. Ruinen, F. H. Scholten and A. S. Cohen), p. 216. Excerpta Medica Amsterdam.

GLENNER, G.G., CUATRECASAS, P., ISERSKY, C., BLADEN, H.A. & EANES, E.D. (1969) Physical and chemical properties of amyloid fibers. II. Isolation of a unique protein constituting the major component from human splenic amyloid fibril concentrates. *J. Histochem. Cytochem.* **17,** 769.

GLENNER, G.G., KEISER, H.R., BLADEN, A.A., CUATRECASAS, P., EANES, E.D., RAM, J.S., KAUFER, J.N. & DE LELLIS, R.A. (1968) Amyloid IV. A comparison of two morphologic components of human amyloid deposits. *J. Histochem. Cytochem.* **16,** 633.

GLENNER, G.G., TERRY, W., HARADA, M., ISERSKY, C. & PAGE, D. (1971) Amyloid fibril proteins: proof of homology with immunoglobulin light chains by sequence analysis. *Science*, **172,** 1150.

GOLD, E.R. & FUDENBERG, H.H. (1967) Chromic Chloride: a coupling reagent of passive hemagglutination reaction. *J. Immunol.* **99,** 859.

GREY, H.M., MANNIK, M. & KUNKEL, H.G. (1965) Individual antigenic specificity of myeloma proteins: Characteristics and localization to subunits. *J. exp. Med.* **121,** 561.

HARADA, M., ISERSKY, C., CUATRECASAS, P., PAGE, D., BLADEN, H.A., EANES, E.D., KEISER, H.R. & GLENNER, G.G. (1971) Human amyloid protein: Chemical variability and homogeneity. *J. Histochem. Cytochem.* **19,** 1.

KUNKEL, H.G., MANNIK, M. & WILLIAMS, R.C. (1963) Individual antigenic specificity of isolated antibodies. *Science*, **140,** 1218.

LADEWIG, P. (1945) Double-refringence of amyloid—congored—complex in histological sections. *Nature*, (*Lond.*), **156,** 81.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 256.

MISSMAHL, H.P. (1957) Polarizations optische Beitrag zur Kongorotfärbung des Amyloides. *Ztschr. f. wissensch. Mikr.* **63,** 133.

MISSMAHL, H.P. & HARTWIG, M. (1953) Polarizations optische Untersuchungen an der Amyloidsubstanz. *Virchows Arch. path. Anat.* **324,** 489.

MUNTHE, E. & NATVIG, J.B. (1971) Characterization of IgG complexes in eluates from rheumatoid tissue. *Clin. exp. Immunol.* **8,** 249.

NATVIG, J.B. (1970) Human anti-γ-globulin antibodies specific for γG heavy chain subclasses. *Immunology* **19,** 125.

PICK, A.I., LAVI, G. & JOSHUA, H. (1970) Immunologic and structural properties of amyloid fibrils from

a patient with multiple myeloma. *Proceedings of the XIIIth Int. Congress of Hematol.* Munich, August 1970, (Abstract).

PICK, A.I. & OSSERMAN, E.F. (1968) Amyloidosis associated with plasma cell dyskrasias. In: *Amyloidosis* (Ed. by E. MANDEMA, L. RUINEN, F.H. SCHOLTEN and A.S. COHEN) p. 100. Excerpta Medica, Amsterdam.

PRAS, M., SCHUBERT, M., ZUCKER-FRANKLIN, D., RIMON, A. & FRANKLIN, E.C. (1968) The characterization of soluble amyloid prepared in water. *J. clin. Invest.* **47,** 924.

PRAS, M., ZUCKER-FRANKLIN, D., RIMON, A. & FRANKLIN, E.C. (1969) Physical, chemical and ultrastructural studies of water-soluble human amyloid fibrils. *J. exp. Med.* **130,** 777.

RAM, J.S., DE LELLIS, R.A. & GLENNER, G.G. (1968) Amyloid IV. Is human amyloid immunogenic? *Int. Arch. Allergy,* **34,** 269.

VON ROKITANSKY, C.F. (1846) In *Handbuch der patologischen Anatomie.* Vol. 3, Vienna: Braumüller u. Seidel.

TAN, M. & EPSTEIN, W.V. (1965) A direct immunologic assay of human sera for Bence Jones protein (L-chains). *J. Lab. clin. Med.* **66.** 344.

TEILUM, G. (1964) Pathogenesis of amyloidosis. *Acta. path. microbiol. scand.* **61,** 21.