Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 4263–4267, June 1985
Medical Sciences

Specific proteins associated with Creutzfeldt–Jakob disease and scrapie share antigenic and carbohydrate determinants

(slow virus/glycoproteins/abnormal fibrils/membranes)

LAURA MANUELIDIS, SUSAN VALLEY, AND ELIAS E. MANUELIDIS

Yale University School of Medicine, 310 Cedar Street, New Haven, CT 06510

Communicated by Lewis Thomas, March 8, 1985

ABSTRACT **Small amounts of brain tissue (2 g) infected with Creutzfeldt–Jakob disease (CJD) can be fractionated by using a simple 1-day method that includes lysis with *N*-lauroylsarcosine. Unique fibrils have been identified previously in scrapie- and CJD-infected tissue. These fibrils were abundant in final fractions. Preparations from human CJD autopsy material and from experimental hamster and guinea pig CJD all displayed readily identifiable fibrils that were not seen in control preparations. Thus, these methods appear to be of value in biopsy diagnosis of suspected human cases of CJD. Lysis with *N*-lauroylsarcosine quantitatively solubilized infectivity from membrane-rich fractions. Significant infectivity was recovered in microfractionations. After proteinase K digestion, a diffuse band at 29 kDa was detectable on NaDodSO$_4$/PAGE. This 29-kDa material was not present in uninfected control brain and was similar to that seen in scrapie. Protein blots of human, guinea pig, and hamster CJD fractions were tested with an antibody raised against a 29-kDa band from mouse scrapie; 29-kDa proteins were labeled in all CJD and scrapie fractions but not in controls. These results indicate that specific proteins in both these diseases share common antigenic determinants. Ricin and wheat germ agglutinin, but not concanavalin A, also labeled a portion of the 29-kDa band from hamster CJD and hamster scrapie fractions, but they did not label any bands in normal hamster fractions at the same gel protein loads. When proteinase K treatment was omitted, specific bands of ≈35 kDa were detected in CJD samples. These results are consistent with the idea that some CJD- and scrapie-specific proteins are glycoproteins or sialoglycoproteins that can reside in or possibly derive from cell membranes.**

Scrapie, Creutzfeldt–Jakob disease (CJD), and kuru are slow progressive neurological diseases. They elicit little appreciable inflammatory response and are caused by infectious agents that are not well delineated (for reviews, see refs. 1–3). In our laboratory, CJD was transmitted to small rodents (4–6) rather than primate hosts in order to facilitate pathological and molecular studies of this human disease. Experimental CJD in mice results in a clinical syndrome that mimics scrapie (6, 7). However, with the exception of one unusual CJD isolate (8), inoculation from other cases of human CJD results in an extremely slow disease in mice, with incubation periods >180 days. We have found that the shortest incubation period (≈125 days) and highest end-point titer (≈10^8 infectious units/gram of brain) are reproducibly obtained in hamsters (ref. 9 and unpublished data). In comparison, hamster scrapie strain 263K, isolated by Kimberlin and Walker, has been reported to have a very short incubation period and up to 1000 times more infectivity per gram of brain than any typical CJD isolate (10, 11).

Given this experimental disadvantage, we set out to find specific marker proteins for the CJD agent. Our initial studies indicated that the synaptosomal/mitochondrial fraction (SM), which is enriched in neuronal membranes, contained ≈40% of the starting infectivity; infectivity remained in SM membranes when they were stripped of surface proteins (9), and we suspected that the CJD agent might carry specific glycoproteins that could be integrated into cell membranes. SM also contained unique fibrils called scrapie-associated fibrils (SAF; refs. 9 and 12), which were initially described by Merz *et al.* in scrapie-infected tissue (13). SAF were not observed in a variety of control preparations (12).

Significant purification of SAF was achieved by Diringer *et al.* (14) and shown to coincide with high titers of infectivity in scrapie-infected hamsters. NaDodSO$_4$/PAGE of these SAF-enriched fractions revealed a broad band of material at ≈29 kDa that corresponded to the "prion protein" described by Prusiner *et al.* (15). That group, however, used 1000 brains in each preparation and a much more complex purification scheme (15, 16), which was not feasible for simple, inexpensive, reproducible preparations. Polyclonal antibodies to the 29-kDa protein(s) in scrapie have been reported recently (17, 18), and a third group (P. Merz, R. Kasak, and R. Rubinstein) generously donated antibody as well as a 263K scrapie fraction for the present studies.

We here show that several membrane-disrupting detergents can be used for effective solubilization of CJD infectivity, with no apparent loss of infectious titer. Using one of these detergents and small amounts of starting material, we obtained fractions highly enriched in SAF from both human and experimental CJD brain; this rapid simple microfractionation (19) can be performed in 1 day and should be of value as an additional diagnostic assay in human biopsy specimens. CJD fractions after proteinase K digestion (used to facilitate comparisons with scrapie) also reveal 29-kDa material on polyacrylamide gels. We show that proteins of similar size in scrapie and experimental CJD share common antigenic determinants. Furthermore, we also demonstrate that these same 29-kDa proteins probably contain specific sugar residues, as determined by binding to specific lectins. This latter finding is compatible with the idea that scrapie and CJD contain specific glycoproteins or sialoglycoproteins that can derive from, or reside in, cell membranes.

MATERIALS AND METHODS

SM fractions were prepared as described (9) from clinically ill hamsters with experimental CJD. Aliquots were mixed, with a Vortex, in buffer A (9, 20) with 0.5 mM phenylmethylsulfonyl fluoride. A 10% (wt/vol) detergent solution was added dropwise to the sample with swirling, and the mixture

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CJD, Creutzfeldt–Jakob disease; SM, synaptosomal/mitochondrial; SAF, "scrapie-associated fibril(s)" characteristic of scrapie and CJD; SB, sulfobetaine; WGA, wheat germ agglutinin.

was allowed to lyse for 5–10 min on ice or at 22°C (final concentrations: 1% detergent, 1.6 mg of protein/ml). Samples were layered on a 50-μl 60% (wt/vol) sucrose cushion (for pellet resuspension) and centrifuged in 1.5-ml polypropylene tubes at 21,500 × *g* for 40 min at 4°C. Supernatants were removed, and pellets were resuspended by mixing with a Vortex and then placing the tubes in a sonication bath for 20 sec. Serial 10-fold dilutions were made in phosphate-buffered saline (P_i/NaCl: 20 mM phosphate buffer, pH 7.4/0.15 M NaCl) with either 0.1% sarkosyl (*N*-lauroylsarcosine) or 0.1% sulfobetaine (SB) 3-14 (Calbiochem–Behring) so that 50 μl of a 10^{-1} dilution would contain 4.2 μg of protein/μl. Four hamsters each were inoculated with serial dilutions (10^{-4}–10^{-7}) of each fraction for end-point titrations of infectivity.

For isolation of SAF from small amounts of brain, minor modifications of the methods of Hilmert and Diringer were used (19). Brain from hamsters and guinea pigs with CJD, serially passaged from one human with CJD (3, 7), and an autopsy specimen of brain from another human (R.L.) with CJD were studied; the latter case was also serially transmissible to rodents. Frozen brain (2 g), equivalent to two hamster brains, was homogenized in 25 ml of 1% or 10% sarkosyl/10 mM Tris Cl, pH 7.4. The homogenate was kept at 22°C for 30 min with 4 drops of 1-octanol and then centrifuged at 22,000 × *g* for 30 min at 6°C. Pooled supernatants were centrifuged at 215,000 × g_{max} for 150 min at 10°C. The pellet (p215) was resuspended in 4 ml of buffer B (1.7 M NaCl/10 mM Tris Cl, pH 7.4/1% sarkosyl) by mixing with a magnetic stirring bar for 2 hr at 37°C; p215 contained ≈1% of the starting protein. Resuspended p215 was spun at 13,000 × *g* for 15 min at 22°C. The resulting pellet (p215/salt) contained ≈10% of the p215 protein. p215/salt was again suspended in buffer B, and proteinase K was added to a final concentration of 5 μg/ml. The mixture was incubated, with stirring, at 37°C for 2.5 hr, and then centrifuged for 15 min at 13,000 × *g*. The resulting pellets (pPK) contained 10–20% of the protein of the p215/salt pellet, equivalent to 0.01% of the starting homogenate protein. For further purification, pPK fractions were suspended in 500 μl of buffer B, and 100 μl was layered on each sucrose step gradient (300 μl each of 30% and 10% sucrose in buffer B). The 2-ml straight wall tubes were spun at 13,000 × *g* for 15 min at 22°C. The 30% sucrose pellets (p30%) were resuspended in buffer B and stored at 4°C. Each of the sucrose layers was pooled, diluted with buffer B, and pelleted at 40,000 × *g* for 30 min to determine SAF losses; few SAF were detected. p30% has been stored for ≥5 months with no apparent changes in SAF. [For electron microscopy (EM), material was deposited on carbon films and stained with phosphotungstic acid (12)]. Fractions from one preparation homogenized in 1% sarkosyl were used to inoculate hamsters (6–12 per fraction) for assays of infectivity by incubation period. Donated strain 263K scrapie was digested as described (21).

After $NaDodSO_4$/12% PAGE (22), proteins were electro-eluted onto nitrocellulose (23) for 1.5 hr at 0.7 A. For monitoring of elution, gels were stained with silver (24), destained, and restained for increased sensitivity. Eluted proteins on nitrocellulose were visualized with india ink (25). Before detection with affinity reagents, nonspecific binding sites on blots were blocked by incubation for 45 min at 43°C in P_i/NaCl/3% bovine serum albumin/1% Ficoll/1% polyvinylpyrrolodine followed by washing in P_i/NaCl. All affinity reagents were diluted in 1% bovine serum albumin. Biotinylated lectins (Vector Laboratories, Burlingame, CA) were applied at 50 μg/ml in buffer C (100 mM Tris Cl, 7.4/50 mM NaCl/2 mM $MgCl_2$) for 40 min at 22°C. Washes (4 × buffer C) included 0.1% Tween 20. Blots then were exposed for 30 min at 37°C to streptavidin (1 μg/ml), washed, and incubated in biotinylated alkaline phosphatase (1 μg/ml; Bethesda Research Laboratories) for 30 min at 37°C. Alkaline phosphatase was detected as described (26). Comparable experiments with peroxidase reagents (27) showed low sensitivity (see Fig. 3*B*, lane 4). For detection with antibody, all incubations were done with P_i/NaCl/0.1% Tween 20. After an additional blocking step in 2% normal goat serum for 15 min, blots were incubated in anti-scrapie antibody (1:1000) for 1 hr at 37°C. Blots then were washed and exposed to biotinylated goat anti-rabbit IgG antibody (1:450, Vector) for 1 hr at 37°C. Streptavidin and alkaline phosphatase incubations were done as for lectins.

RESULTS AND DISCUSSION

There are essentially no reports on the effect of detergents on CJD infectivity. We wanted to gently solubilize SM membranes, rich in infectivity, without reducing the titer. Aliquots of an SM fraction from CJD hamster brain were therefore treated with various detergents. Solubilized and insoluble (sedimented) material were serially diluted at identical protein concentrations for end-point titrations of infectivity. The infectivities are presented in Table 1 and compared to that of the starting (non-detergent-treated) SM membranes. In most cases, nearly 100% of the initial SM infectivity was recovered. The milder detergents (e.g., Brij 58) yielded less solubilization of infectivity, whereas the nonionic, membrane-disrupting detergents Triton X-100 and Nonidet P-40 solubilized >50% of the infectivity. SB 3-14 did not effectively solubilize infectivity in this fraction. The most effective detergent was the ionic detergent sarkosyl, which, when used at 22°C, resulted in ≈90% solubilized infectivity.

Table 1. Detergent solubilization of CJD infectivity

Fraction	% of SM protein in fraction	log titer per 2 mg of protein	% of SM infectivity in fraction
SM (A)	100	6	100
Brij 58			
Supernatant (A)	75	5.25	13
Pellet (A)	25	6.5	80
Pellet (B)		6.5	80
n-Octylglucoside			
Supernatant (A)	75	5.5	24
Pellet (A)	25	6.25*	45
Triton X-100			
Supernatant (A)	85	6	85
Pellet (A)	15	6	15
Pellet (B)		5.75	7.5
Nonidet P-40			
Supernatant (A)	85	6	85
Pellet (A)	15	6	15
Pellet (B)		6	15
SB 3-14			
Supernatant (A)	90	5.25	16
Pellet (A)	10	7*	100
Sarkosyl (4°C)†			
Supernatant (B)	82	5.5	26
Pellet (B)	18	6.5	57
Sarkosyl (22°C)†			
Supernatant (B)	90	6	90
Pellet (B)	10	6	10

Equal aliquots of a single SM preparation were used to assess solubilization and recovery of infectivity with different detergents (1% final concentration). Dilutions for inoculation were made in 0.1% SB 3-14 (A) or 0.1% sarkosyl (B) in P_i/NaCl for end-point titrations; thus in some cases, A and B are replicate values for each fraction. This particular SM preparation had ≈10% of the infectivity of typical preparations per mg of protein.

*Non-end-point calculation.

†Lysis temperature in parentheses.

These CJD-infectivity recoveries with sarkosyl are different than one report for scrapie (28) but consistent with scrapie results reported by two other groups (19, 29). Our solubilization of CJD agent by use of SB 3-14 is in contrast with solubilities suggested for scrapie (15).

We were interested in high recoveries of infectivity as well as in preparations that were significantly enriched in SAF, since SAF may represent infectious agent; alternatively, SAF may be unique pathological products of these diseases that could be useful as a specific marker (12). To date, we have not been able to recover large amounts of purified SAF from SM fractions, possibly due to losses during differential centrifugation. Hilmert and Diringer (19) reported using a roughly equivalent SM fraction (a postnuclear 22,000 × *g* pellet) from scrapie-infected brain for purification of SAF but obtained significantly improved SAF recoveries by using lysis of whole brain. We therefore modified their short, high-recovery procedure and used 1% sarkosyl lysis of whole brain since the data above indicated that this concentration of sarkosyl does not reduce CJD infectivities. Subsequent experiments have been done with 10% sarkosyl, which gives similar fractions, as judged by electron microscopy, but higher yields of SAF. In all cases (human, hamster, and guinea pig CJD), with either detergent concentration, significant enrichments for SAF were obtained. No SAF were detected in any of three uninfected hamster brain preparations. SAF were easily visualized in the proteinase K-treated pellets, but a few SAF could also be detected in the p215/salt pellets. Representative p30% preparations are shown in Fig. 1. SAF recovered from different hosts with CJD (hamster, guinea pig, and human) were all similar, except somewhat thicker, tighter fibrils were seen in the human material (Fig. 1). The SAF shown are longer than those depicted by Diringer *et al.* (14), probably because we did not use extensive probe sonication for resuspension of pellets.

Titration results indicate quantitative recovery of infectivity in p215/salt. Total recoveries were as follows: homogenate (53 mg of protein) = 7.9 logs; p215 = 7.6 logs; p215/salt = 8.1 logs; pPK = 7.7 logs; p30% = 6.7 logs. Unlike other fractions, p30% was stored at 4°C prior to inoculation, which may have contributed to poorer (factor of 10 lower) recoveries in this fraction. We do not know whether extensive proteinase K digestion leads to loss of infectivity. However, purifications of ≥500-fold (p215/salt) or >1000-fold (pPK) of CJD infectivity with respect to protein were obtained, similar to those achieved for the scrapie agent (21).

$NaDodSO_4$/PAGE followed by silver staining revealed a diffuse band centered at ≈29 kDa in CJD preparations lysed with 10% sarkosyl and digested with proteinase K. We compared this CJD preparation to preparations from scrapie-infected and uninfected hamster brain. Scrapie and CJD preparations showed bands of similar mobility and diffuseness at 29 kDa (Fig. 2*C*, lanes 8, 10, and 12), whereas uninfected hamsters yielded no significant material of this size (Fig. 2*C*, lane 9). Some of the larger species detected on silver-stained gels are RNA, as determined by analysis on glyoxal-containing gels (unpublished data).

To further test the similarity of the scrapie and CJD isolates, we used immunoblotting with an anti-scrapie antibody. This antibody clearly stained the 29 kDa smear as well as lower molecular mass bands in both hamster CJD and hamster scrapie samples (Fig. 2*A*, lanes 1, 3, and 5). Faint but visible bands at 29 kDa or less were also labeled in guinea pig and human CJD fractions (Fig. 2*A*, lanes 4 and 6). These bands were not present in the normal hamster fractions at equivalent protein loads (Fig. 2*A*, lane 2; Fig. 3*C*, lane 2). The antiserum also labeled bands near 50 kDa that were present in both the normal and infectious material. Many sharp, higher molecular mass bands also were detected with this antibody in p215/salt (Fig. 2*A*, lane 7). This polyclonal serum therefore may bind to at least one other antigenic determinant that can contaminate the digested 29-kDa material.

Blots of scrapie samples were also tested with several biotinylated lectins. At 50 μg/ml, ricin and wheat germ agglutinin (WGA) showed reproducible binding to the 29-kDa smear in scrapie isolates (Fig. 3*B*, lanes 5 and 6). Even at 250 μg/ml, concanavalin A showed no binding to this material, but it appropriately labeled ovalbumin at 43 kDa in the marker lane (data not shown). Addition of 0.2 M D(+)-galactose to ricin prior to incubation with the blot caused an ≈70% reduction in labeling of the 29-kDa material, as determined by the most sensitive alkaline phosphatase detection procedures. The lack of complete inhibition by a simple sugar is not surprising in view of the complex affinities of

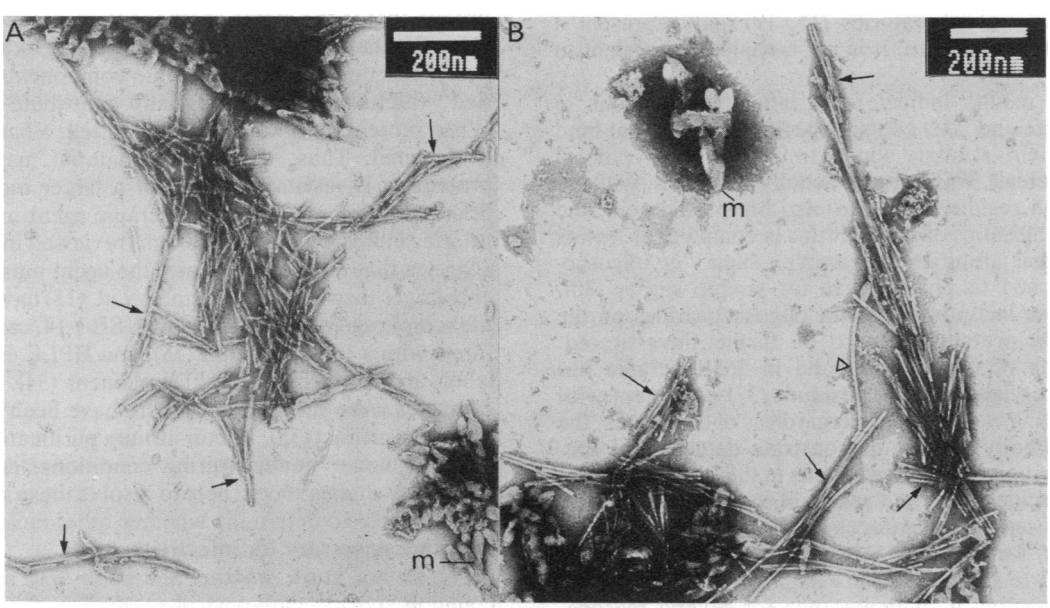

FIG. 1. Electron micrographs of SAF in p30% prepared from hamster (*A*) and human (*B*) CJD-infected brain. Arrows show aggregates of typical SAF. A few fibers in *B* are not SAF (e.g., see Δ); they were distinguished from SAF on higher magnification by their thickness, curvature, and lack of a double helix-like substructure (12). Ferritin and small membrane-like structures were also observed. CJD guinea pig SAF were indistinguishable from hamster SAF depicted in *A*. m, Detergent micelles. (Bar = 200 nm.)

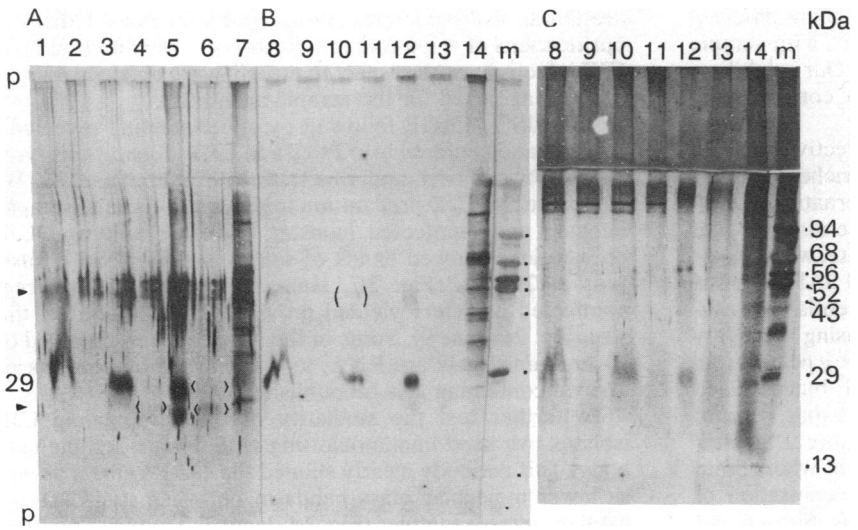

FIG. 2. (*A* and *B*) NaDodSO$_4$/PAGE analyses of proteins from various stages of preparation based on sarkosyl lysis. Protein electrophoretically transferred to nitrocellulose and labeled with anti-scrapie antibody (*A*) or ricin (*B*). (*C*) One half of the gel (corresponding to blot *B*) stained with silver after electroelution. Lanes: 1 and 8, p30% from CJD hamster; 2 and 9, pPK from normal hamster; 3 and 10, pPK from CJD hamster; 4 and 11, pPK from CJD guinea pig; 5 and 12, strain 263K scrapie; 6 and 13, p30% from human CJD; 7 and 14, p215/salt from CJD human; m, marker proteins. Lanes 1 and 8 show salt and detergent artifacts. Visible bands that do not reproduce well are shown in parentheses. Arrowheads indicate stained bands other than the 29-kDa one. p denotes positions of pyronin Y dye.

these lectins (see below). The procedures used were capable of detecting 1–3 pg of biotinylated lectin on a dot blot (Fig. 3*A*). It was notable that both ricin and WGA delineated a less diffuse band of material at 29 kDa than was detected with anti-scrapie antibodies (Fig. 3*B*, lanes 3, 5, and 6). They also did not strongly label bands at <29 kDa that were labeled clearly by antibody (Fig. 3*C*, lanes 1, 4, and 8). Thus, lectins could clearly discriminate different molecular species with similar mobilities. On occasional blots, a faint CJD- and scrapie-specific band at ≈50 kDa was detected with ricin (Fig. 2*B*, lanes 8, 10, and 12), which probably represents incompletely dissociated aggregates. WGA bound to a CJD-specific band at ≈35 kDa in p215/salt. We thus made additional parallel isolations with normal and CJD hamster brain to monitor degradation by proteinase K. In CJD, two sharper bands at 34–36 kDa were visualized by silver staining and these bands reacted with antibody and lectins. Again, considerably more diffuse staining was observed with polyclonal antibodies than with lectins (Fig. 3*C*, lanes 3, 6, and 10). Labeled bands were completely absent in analysis of p30% and p215/salt from uninfected brain (Fig. 3*C*, lanes 2, 5, 7, 9, and 11). Thus, it is likely that the 29-kDa smear detected in several laboratories (15, 19) may represent a breakdown product(s); i.e., it is not completely resistant to proteolysis.

The above results indicate that infectious fractions in hamster scrapie and CJD contain specific proteins that bind to lectins. Ricin strongly binds to β-D-galactopyranosyl residues, whereas WGA preferentially interacts with β-glycopyranosyl residues and can also bind sialic acid; the carbohydrate affinities of these lectins is complex, as lectins can show higher affinities for complex sugars or oligosaccharides attached to protein than for simple sugars (30). Details of these lectin affinities for oligosaccharides on the related 29-kDa proteins need to be further investigated. Direct analysis of the 29-kDa band in 263K scrapie has revealed *N*-acetylglucosamine residues (37) consistent with the WGA binding we observed. Regardless of the details, the lectin and antibody binding demonstrate detection of specific, common molecular determinants in these two diseases, and such molecules may well reside in, or derive from, cell membranes. Complex oligosaccharides residues may partially account for the protease resistance and apparent hydrophobicity (e.g., aggregation in high salt) of these agents.

The lectin binding is of importance for several reasons. First, lectin binding may be useful as a simple reproducible marker for specific proteins in these diseases. Second, there are strain and host differences in both scrapie and CJD (5, 31). It is possible that sugar residues may help to distinguish some of these isolates in different hosts. Third, modifications of sugar or sialic acid residues might be used to alter infectivity of the agent or the course of the disease.

Finally, lectin-affinity chromatography could be useful in the isolation of nondegraded, highly purified infectious fractions. Such purification is essential in order to resolve several major issues concerning these diseases. The first is that of nucleic acids. To our knowledge, no infectious fractions have been reported that are devoid of nucleic acid. Although picogram amounts of DNA have been studied in some detail in CJD with specific labeling techniques (32), careful examination of specifically labeled RNAs has not been reported for either CJD or scrapie. Since the isolated 29-kDa material is not significantly infectious (16), the lack of nucleic acid in this material (33) cannot be used to exclude a nucleic acid component. We think it premature to classify these agents as infectious proteins or "prions" (34), although they may well represent a class of subviral pathogens or "unconventional agents" (1, 2, 28). Second, the size of the infectious agent is not well defined. It has been claimed that the diffuse 29-kDa band seen after proteinase K digestion represents a purified protein that is the major component of infectivity in scrapie (16). We have demonstrated that the 29-kDa material is heterogeneous; other, less visible components in the "purified" fraction might substantially contribute to infectivity. When proteinase K treatment is omitted, a band at ≈35 kDa is detected. Thus, the 29-kDa material may represent a proteinase K-resistant portion of a larger molecule. Membrane-filtration studies of the scrapie agent in several laboratories indicate a size of ≥30 nm (reviewed in ref. 1), which suggests that in its native state, the agent must be considerably larger than 29 kDa. Prusiner *et al.* (15) have claimed that the scrapie agent, when mixed with SB 3-14, can pass through filters with a 100-kDa cutoff (15), and HPLC estimates in his laboratory suggested a 50-kDa element (34). These HPLC estimates were shown by others to have been the result of a detergent artifact (35). Lectin-affinity purification, which can be done under nondenaturing conditions, combined with infectivity studies should help to resolve these size discrepancies and help to determine whether other molecular components are necessary for infectivity.

We do not know whether the large ≥30 nm SAF are a common specific pathological product in these diseases or whether they represent the infectious agent. There is also no direct, conclusive evidence (i.e., by reconstitution) that SAF are composed of the 29-kDa protein. Although SAF and infectivity can copurify (14), initial reports showed no SAF-

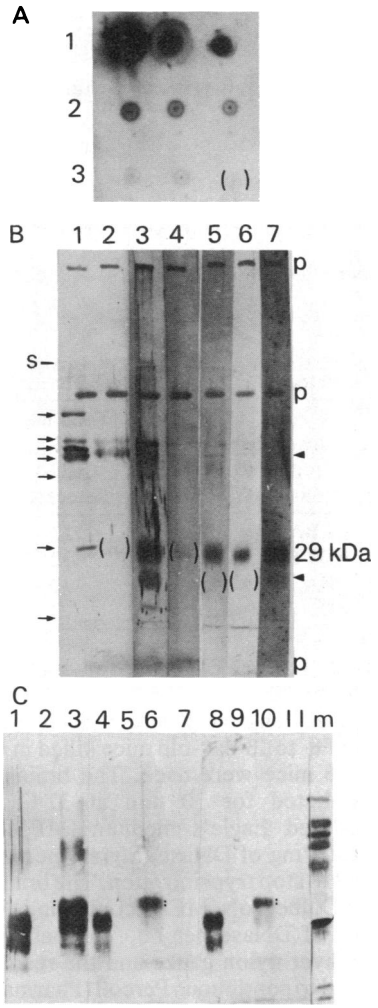

FIG. 3. (*A*) Dot blot detection of biotinylated lectin diluted in 1% bovine serum albumin. Row 1: 80 μg, 8 μg, and 800 pg. Row 2: 270 pg, 90 pg, and 30 pg. Row 3: 10 pg, 3 pg, and 1 pg. With development for 16 hr, 1 pg was barely detected (parentheses). (*B*) Electrophoretic-transfer blot of strain 263K scrapie after $NaDodSO_4$/PAGE. Lane 1: marker proteins, stained with india ink. Lanes 2–7; equal amounts of 263K scrapie. Staining with india ink (lane 2) showed a very faint band at 29 kDa (parentheses). Anti-scrapie antibody (lane 3) stains the 29-kDa material as well as species both of higher and of lower mobilities (arrowheads at right). WGA (lanes 4 and 5) and ricin (lanes 6 and 7) show mainly 29-kDa material. Peroxidase detection of WGA (lane 4) was less sensitive than alkaline phosphatase detection (lane 5). Tween 20 was omitted from the sample in lane 7. p, Pyronin Y; s, top of separating gel. (*C*) Blot of preparations from normal and CJD-infected hamster brains. Equal amounts of protein (as judged by silver staining, not shown) were present in each gel lane. Detection was with anti-scrapie antibody (lanes 1–3), WGA (lanes 4–7), or ricin (lanes 8–11). Lanes 1, 4, and 8: p30% from CJD brain. Lanes 2, 5, and 9: p30% from normal brain. Lanes 3, 6, and 10: p215/salt from CJD brain; note sharp bands at 35 kDa (dots). Lanes 7 and 11: p215/salt from normal brain. More labeling is seen with antibody than with lectins. Lane m: markers stained with india ink. Arrow at right indicates 29-kDa position.

like "rods" or any unique particles in highly infectious fractions (15). More recently, such rods were reported to be amyloid-like aggregates of the 29-kDa protein (16). Labeled lectins can be used for electron microscopic detection of specific glycoproteins (36). If SAF are found not to bind these reagents, SAF are unlikely to be composed of the 29-kDa protein(s). Monoclonal antibodies will be useful for sorting out the complex binding of polyclonal antibodies shown here. Such approaches should help to delineate both the size and molecular composition of these infectious agents.

We thank Ruth Chesney for preparing the manuscript. This work was supported by National Institutes of Health Grants AG03106 and NS12674.

1. Gajdusek, D. C. (1977) *Science* **197**, 943–960.
2. Manuelidis, L. & Manuelidis, E. E. (1979) *Prog. Neuropathol.* **4**, 1–26.
3. Prusiner, S. B. & Hadlow, W. J. eds. (1979) *Slow Transmissible Diseases of the Nervous System* (Academic, New York), Vols. 1, 2.
4. Manuelidis, E. E., Kim, J., Angelo, J. N. & Manuelidis, L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 223–227.
5. Manuelidis, E. E., Gorgacz, E. J. & Manuelidis, L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3432–3436.
6. Manuelidis, E. E., Gorgacz, E. J. & Manuelidis, L. (1978) *Nature (London)* **271**, 778–779.
7. Manuelidis, E. E. & Manuelidis, L. (1979) in *Slow Transmissible Diseases of the Nervous System*, eds. Prusiner, S. B., & Hadlow, W. J. (Academic, New York), pp. 147–173.
8. Tateishi, J., Ohta, M. & Kuroiwa, Y. (1978) *J. Neuropathol. Exp. Neurol.* **37**, 699.
9. Manuelidis, L. & Manuelidis, E. E. (1983) in *Biological Aspects of Alzheimer's Disease*, Banbury Report 15, ed. Katzman, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 399–412.
10. Kimberlin, R. H. & Walker, C. A. (1978) *J. Gen. Virol.* **39**, 487–496.
11. Marsh, R. F., Malone, T. G., Lancaster, W. D., Hanson, R. P. & Semancik, J. S. (1978) in *Persistent Viruses*, ICN–UCLA Symposium on Molecular and Cell Biology, eds. Stevens, J. G., Todaro, G. & Fox, C. (Academic, New York), Vol. 2, p. 581.
12. Merz, P. A., Somerville, R. A., Wisniewski, H. M., Manuelidis, L. & Manuelidis, E. E. (1983) *Nature (London)* **306**, 474–476.
13. Merz, P. A., Somerville, R. A., Wisniewski, H. M. & Iqbal, K. (1981) *Acta Neuropathol.* **54**, 63–74.
14. Diringer, H., Gelderblom, H., Hilmert, H., Ozel, M., Edelbluth, C. & Kimberlin, R. H. (1983) *Nature (London)* **306**, 476–478.
15. Prusiner, S. B., Bolton, D. C., Groth, D. F., Bowman, K. A., Cochran, S. P. & McKinley, M. P. (1982) *Biochemistry* **21**, 6942–6950.
16. Prusiner, S. B., McKinley, M. P., Bowman, K. A., Bolton, D. C., Bendheim, P. E., Groth, D. F. & Glenner, G. G. (1983) *Cell* **35**, 349–358.
17. Diringer, H., Rahn, H. C. & Bode, L. (1984) *Lancet* **ii**, 345.
18. Bendheim, P. E., Barry, R. A., DeArmond, S. J., Stites, D. P. & Prusiner, S. B. (1984) *Nature (London)* **310**, 418–421.
19. Hilmert, H. & Diringer, H. (1984) *Biosci. Rep.* **4**, 165–170.
20. Manuelidis, L., Sedat, J. & Feder, R. (1980) *Ann. N.Y. Acad. Sci.* **342**, 304–325.
21. Diringer, H., Hilmert, H., Simon, D., Wermer, E. & Ehlers, B. (1983) *Eur. J. Biochem.* **134**, 555–560.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Towbin, H., Staehelin, T. & Bordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
24. Morrissey, J. H. (1981) *Anal. Biochem.* **117**, 307–310.
25. Hancock, K. & Tsang, V. (1983) *Anal. Biochem.* **133**, 157–162.
26. Leary, J. J., Brigati, D. J. & Ward, D. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4045–4049.
27. Manuelidis, L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3123–3127.
28. Millson, G. C., Hunter, G. D. & Kimberlin, R. H. (1977) in *Slow Virus Diseases of Animals and Man*, ed. Kimberlin, R. H. (North-Holland, Amsterdam), pp. 243–266.
29. Prusiner, S. B., Groth, D. F., Cochran, S. P., Masiarz, F. R., McKinley, M. P. & Martinez, H. M. (1980) *Biochemistry* **19**, 4883–4891.
30. Goldstein, I. J. & Hayes, C. M. (1978) *Adv. Carbohydr. Chem. Biochem.* **35**, 127–340.
31. Dickinson, A. G. & Fraser, H. (1977) in *Slow Virus Infections of the Central Nervous System*, eds. Ter Meulen, V. & Katz, M. (Springer, New York), pp. 3–14.
32. Manuelidis, L. & Manuelidis, E. E. (1981) *Virology* **109**, 435–443.
33. Prusiner, S. B., Groth, D. F., Bolton, D. C., Kent, S. B. & Hood, L. E. (1984) *Cell* **38**, 127–134.
34. Prusiner, S. B. (1982) *Science* **216**, 136–144.
35. Diringer, H. & Kimberlin, R. H. (1983) *Biosci. Rep.* **3**, 563–568.
36. Manuelidis, L., Tomita, M. & Manuelidis, E. E. (1977) *Experientia* **33**, 949–952.
37. Multhaup, G., Diringer, H., Hilmert, H., Prinz, H., Heukeshozens, J. & Beyreuther, K., *EMBO J.*, in press.