Antiserum to Scrapie-Associated Fibril Protein Cross-React with *Spiroplasma mirum* Fibril Proteins

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Protease-resistant fibril proteins purified from *Spiroplasma mirum* and from Creutzfeldt-Jakob disease-infected brain tissues reacted with antisera to scrapie-associated fibrils on Western immunoblot analysis. These data suggest that there are conformational similarities among spiroplasma proteins and infection-specific proteins of the transmissible spongiform encephalopathies.

Two protein components that relate to infectivity have been purified from brain tissues of a rodent infected with experimental scrapie and from human Creutzfeldt-Jakob disease (CJD)-infected tissues (11). Fibrillar proteins of the synaptosomal-mitochondrial fraction of scrapie-infected brains (scrapie-associated fibrils [SAFs]) (14) were visualized by transmission electron microscopy as fibrillar structures, 4 to 5 nm in width, often arranged in twisted, intertwined collections. Morphologically identical and immunologically related structures have been identified in CJD-infected brain homogenates (12) (CJD-associated fibrils [CJDAFs]). A second protein that relates to scrapie infectivity is present as the major component of purified fibril preparations (16). That protein, PrP27-30, is a sialoglycoprotein which is purported to polymerize into SAF rods. It has been emphasized that reconstitution of the rods has not been sufficiently demonstrated (10, 11). Moreover, the purity of PrP27-30 is suspect since a recent study indicated that the protein contains at least three different peptide moieties (19). At least one peptide in PrP27-30 has been shown to possess an amino acid sequence which is transcribed in normal brain tissues (6). SAFs or CJDAFs have not been found in normal brain tissues (11) and may be the only protein components that are pathognomonic for scrapie or CJD. SAFs, CJDAFs, and PrP27-30 are immunologically related (13) and are considered to be noninfectious (15).

We have previously noted the morphological similarity between CJDAFs or SAFs and the fibrillar cytoarchitecture of *Spiroplasma mirum* (3, 4; F. O. Bastian and R. A. Jennings, Infect. Dis. Newsl. 5:1-3, 1986), a helical-cell wall-less procaryote in the class *Mollicutes* (24). Spiroplasma fibrils (SpFs) have been shown to be unique to that class of microorganisms (21). A variety of spiroplasma strains have been isolated from hematophagous arthropods (24). Strain GT-48 of *S. mirum* was originally isolated from ticks and was shown to experimentally produce a persistent and fatal brain disease in rodents (3, 23). GT-48 consistently produces microcystic encephalopathy during peak infection (3); this observation is intriguing since several independent laboratories have reported helical membranous inclusions in CJD-infected brain tissues (1, 2, 7, 17). Preliminary studies in our laboratory indicated that SpFs, like CJDAFs, were resistant to nonionic detergents and proteinase K. The present study was designed to investigate the immunological reactivity of rabbit anti-SAF serum to the fibrillar proteins of *S. mirum* and CJD.

Purification of fibrils from spiroplasma- and CJD-infected brains was accomplished by a previously described method (8). Briefly, equivalent volumes of CJD-infected brain homogenate or packed-broth-cultured *S. mirum* cells (strains GT-48 and SMCA, provided by J. G. Tully, National Institutes of Health Mycoplasma Laboratory, Frederick, Md.) were homogenized in 10% *N*-lauroylsarcosine (sarcosyl) (Sigma Chemical Co., St. Louis, Mo.) and passed through a series of differential centrifugation steps incorporating sarcosyl to render the fibrils sedimentable at $215,000 \times g$ (P_{215}). Further purification was achieved by treatment of P_{215} with proteinase K (Sigma) followed by centrifugation for 5 h at $215,000 \times g$ on a 20% sucrose cushion. The final pellet was suspended in Tris-buffered saline containing 0.1% Zwittergent (Calbiochem-Behring, La Jolla, Calif.) and stored at 4°C.

Fibril protein suspensions were dialyzed against distilled water, and protein concentrations were determined by protein assay (Bio-Rad Laboratories, Richmond, Calif.). Proteins were solubilized at 100°C for 5 min in 1% lauryl sulfate-8 M urea (Sigma) because of their resistance to dissociation in nonionic detergents (25). Approximately 200 ng of protein was added to each lane, and the protein was electrophoresed on a 12.5% Laemmli gel (9). Proteins were electrotransferred to nitrocellulose by using the Towbin Tris-glycine-methanol buffer system (20) at 200 mA of constant current for 1 h at 4°C. Western immunoblot staining was carried out by using an immunogold procedure with silver enhancement (Janssen Life Science Products, Beerse, Belgium). Transferred proteins were blocked with 5% bovine serum albumin (BSA) in 20 mM Tris buffer (pH 8.2) for 1 h at 37°C followed by a 2-h incubation at 37°C in either rabbit antiserum to fibrils of the ME7 strain of experimental mouse scrapie (8, 13) (provided by R. Rubenstein, Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y.) or normal rabbit serum (NRS). The ME7 antibody has been previously characterized (18) and was shown to be specific for the detection of SAF proteins obtained by the extraction of scrapie-infected brain tissue with sarcosyl followed by proteinase K digestion. A 1:500 dilution of antiserum or NRS in 0.1% BSA-20 mM Tris (BSA-Tris) (pH 8.2) containing 1% normal goat serum was used. After extensive washing with BSA-Tris, the transfer membrane was agitated overnight at 37°C in 10-nm colloidal-gold-conjugated goat anti-rabbit immunoglobulin (Janssen) diluted 1:50 in BSA-Tris containing 20 mM sodium azide-0.4% gelatin (wt/vol). After successive washes in BSA-Tris-distilled water-2 M citrate buffer, the detection of immunoreactive epitopes was improved by silver enhancement of the gold decoration.

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FIG. 1. Western blot analysis of detergent-protease-treated SpF and CJDAF protein. SpF protein (lanes 1 and 3) or CJDAF protein (lanes 2 and 4) was electrophoresed and electrotransferred to nitrocellulose paper. SpFs and CJDAFs were treated with proteinase K at 100 μ g/ml for 2 h at 37°C before electrophoresis. Antiserum to SAFs was used at 1:500 for lanes 1 and 2, and NRS was used at 1:500 for lanes 3 and 4. Blots were stained with 5-nm colloidal-gold-conjugated goat anti-rabbit immunoglobulin and enhanced with silver lactate.

SAF antiserum reacted with four SpF proteins at 28, 30, 66, and 76 kilodaltons (kDa) (Fig. 1). Unresolved CJDAF proteins showed reactivity in a region from 40 to 80 kDa, with an epitope at 29 kDa. No reactions were detected in control blots against NRS. Enzyme-treated normal human and rat brain preparations showed no reactivity with SAF antiserum. The reactivity of anti-SAF serum to SpFs and CJDAFs suggests conformational similarities between spiroplasma proteins and CJD-infection-specific proteins.

Recent studies have identified and characterized spiroplasma fibrillar proteins. Numerous polypeptides from whole spiroplasma cells were extracted with alkyl-*N*-sulphobetaine (25). The major component was shown to be spiralin, a fibrillar protein consisting of 26- to 30-kDa polypeptides. Spiralin has been visualized as a fibrillar protein localized to the plasma membranes and as extracellular strands (22). A second fibrillar protein of spiroplasma cells, an internal structure presumed to be involved in motility, has not been as efficiently extracted by detergent (5) and has been shown to compose only 3% of the cell (21). The molecular size of those internal fibrils before protease digestion is 55 kDa (21, 22). It is these internal fibrils (SpFs) that morphologically resemble and show the same measurements as SAFs. Ongoing studies will attempt to determine which of the spiroplasma fibril polypeptides immunoreact with SAF antiserum.

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