The 200- and 150-kDa neurofilament proteins react with IgG autoantibodies from chimpanzees with kuru or Creutzfeldt–Jakob disease; a 62-kDa neurofilament-associated protein reacts with sera from sheep with natural scrapie

(immunoblot/cytoskeleton/spongiform encephalopathies)

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ABSTRACT Sera from 46 chimpanzees with spongiform encephalopathy (18 kuru, 28 Creutzfeldt–Jakob disease) and sera from 12 sheep with natural scrapie were tested for reactivity with immunoblots of neurofilament preparations obtained from mouse brain. The sera from the chimpanzees reacted mainly with the 200- and 150-kDa proteins of the neurofilament triplet and less frequently with the 70-kDa component of the triplet and with a 62-kDa neurofilament-associated protein. In contrast, the sera of sheep with natural scrapie reacted exclusively against the 62-kDa protein. The specificity of the reactions was established by comparison of sera reactivities with those of rabbit and mouse polyclonal antibodies and mouse monoclonal antibody to neurofilament proteins.

In this work, the specificity of the antineurofilament autoantibody in chimpanzees with experimental kuru and Creutzfeldt–Jakob disease and in sheep with natural scrapie (1) was investigated by immunoblotting studies with sera from 46 chimpanzees with spongiform encephalopathy (18 kuru, 28 Creutzfeldt–Jakob disease), 16 chimpanzees inoculated with human material from patients with other neurologic diseases, 12 sheep with natural scrapie, and 8 normal sheep. The sera of chimpanzees with spongiform encephalopathy reacted mainly with the 200- and 150-kDa proteins of the neurofilament triplet, whereas the sera of sheep with natural scrapie reacted exclusively with a 62-kDa neurofilament-associated protein. Bhamyayar et al. (2) have shown previously that the serum of one chimpanzee with Creutzfeldt–Jakob disease reacted mainly with the 200-kDa, and less strongly with the 150-kDa neurofilament protein.

MATERIALS AND METHODS

Sera. Paired pre- and post-inoculation sera from 62 chimpanzees were examined for reactivity with immunoblots of neurofilament-enriched preparations from mouse brain. The chimpanzees comprised 28 that had been inoculated with suspensions of Creutzfeldt–Jakob disease-infected human and animal brain tissues, 18 that had been inoculated with suspensions of kuru-infected human and animal brain tissues, and 16 that had been inoculated with suspensions of brain tissues from human patients with other central nervous system diseases (4 patients with amyotrophic lateral sclerosis, 2 patients with Parkinsonism-dementia, 2 patients with Alzheimer disease, 1 patient with subacute sclerosing panencephalitis, 1 patient with striatal degeneration, 1 patient with Schilder disease, and 2 patients with progressive supranuclear palsy). Also examined were sera from 12 sheep with natural scrapie and from 8 normal sheep.

Neurofilament Preparation. The method for obtaining preparations from mouse brains enriched for the 200-, 150-, and 70-kDa neurofilament proteins and the 62-kDa neurofilament-associated protein has been described (3, 4). Briefly, whole mouse brains were homogenized at 4°C in phosphate-buffered saline containing 0.6 M KCl and 0.5% Triton X-100 (wt/vol), centrifuged at 16,319 × g in a Sorval GSA rotor, for 10 min, resuspended in the same buffer without Triton X-100, and centrifuged again to obtain a pellet enriched for neurofilament protein.

Immunoblotting. The immunoblotting technique used has been described (4, 5). Briefly, the proteins in the mouse neurofilament-enriched preparation were separated by NaDodSO4/PAGE (6) in slab gels and then electrophoretically transferred to nitrocellulose paper. The transferred proteins were incubated with chimpanzee or sheep sera followed by peroxidase-labeled goat anti-human or anti-sheep IgG. Immunoreactive bands were visualized with 4-chloro-1-naphthol and hydrogen peroxide (7).

Specificity Studies. To establish specificity of the autoantibody reaction for neurofilament proteins, immunoblot reactivity obtained with chimpanzee sera was compared to those obtained with rabbit and mouse polyclonal antibodies (8), with mouse monoclonal antibody (characterization to be described elsewhere), and with a human autoantibody (4) to neurofilament proteins. The appropriate peroxidase-labeled goat anti-IgG was used as second antibody in these experiments.

To demonstrate species non-specificity of the autoantibody reactions, immunoblots carried out with mouse neurofilament-enriched preparations were compared to immunoblots with chimpanzee neurofilament-enriched preparations and with neurofilament preparations from bovine spinal cord.

RESULTS

The results summarized in Tables 1 and 2 show that chimpanzee sera reacted most frequently with the 200- and 150-kDa proteins and less frequently with the 70- and 62-kDa proteins. In contrast, the sera of sheep with natural scrapie
reacted exclusively with the 62-kDa dalton neurofilament-associated protein (Fig. 1).

The incidence of autoantibody to neurofilament proteins was higher in post-inoculation sera than in pre-inoculation sera from chimpanzees inoculated with kuru or Creutzfeldt-Jakob disease-infected material, but an increase in autoantibody inoculated with material from patients with other neurologic diseases.

The chimpanzee autoantibody reacted with the same proteins that were recognized by rabbit and mouse polyclonal antibodies, by mouse monoclonal antibody, and by a human autoantibody to neurofilament proteins (Fig. 2). The autoantibody, in preinoculation sera when present, was directed against the same neurofilament proteins as the antibody in postinoculation sera (Fig. 3). The chimpanzee autoantibody reacted not only with mouse neurofilament preparations but also with neurofilament preparations from chimpanzee brain and from bovine spinal cord, although reactivity with the corresponding proteins in the different species was not always obtained (Fig. 4).

**DISCUSSION**

In a companion study (4), we showed that the human anti-neurofilament autoantibody in patients with kuru and Creutzfeldt-Jakob disease (9-12) is directed mainly against the 200- and 150-kDa proteins of the neurofilament triplet. The present study shows that the anti-neurofilament autoantibody previously reported in chimpanzees with experimental kuru and Creutzfeldt-Jakob disease (1) is also directed mainly against the same proteins, reaffirming the association between the anti-200-/150-kDa protein autoantibodies and the spongiform encephalopathies. We have previously speculated (4) that these autoantibodies may be directed against antigen(s) common to both the 200-/150-kDa proteins and the infectious "scrapie-associated fibrils" of kuru and Creutzfeldt-Jakob disease (13, 14). Gajdusek (15-17) has suggested that autoantibody-producing B-cell clones are activated as a result of release of neurofilament proteins after neurolysis due to a block in axonal transport of these proteins.

We found that the anti-neurofilament autoantibody reported previously in sheep with natural scrapie (1) is not directed against the 200- and 150-kDa proteins but exclusively against a 62-kDa protein. This 62-kDa protein is not part of the neurofilament triplet but is probably associated with it: this protein disappears together with the triplet proteins after wallerian degeneration (18). This difference in specificity of autoantibodies in kuru and Creutzfeldt-Jakob disease, on the one hand, and in sheep with natural scrapie, on the other, should be further investigated.

![Fig. 1. Immunoblots of neurofilament-enriched preparations of mouse brain reacted with sera from six sheep naturally infected with scrapie and a rabbit antiserum against the 200-kDa neurofilament protein (anti-200). Four of the sera from scrapie-infected sheep recognized the 62-kDa neurofilament-associated protein, a pattern not seen for human Creutzfeldt-Jakob disease or kuru sera or for serum from a sheep experimentally infected with scrapie.](image-url)
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Fig. 2. Immunoblots of neurofilament-enriched preparations of mouse brain reacted with 1:50 dilutions of sera from a chimpanzee with kuru and from five chimpanzees and a human patient with Creutzfeldt-Jakob disease (CJD), with a mouse monoclonal antibody to the 200-kDa neurofilament protein (anti-200), or with rabbit polyclonal antibody that recognizes the 200- and 70-kDa neurofilament proteins (anti-200/70).

Fig. 3. Immunoblots of neurofilament-enriched preparations of mouse brain reacted with paired pre- and post-inoculation sera of four chimpanzees with Creutzfeldt-Jakob disease (CJD).
