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Transmission studies from blood of Alzheimer disease patients and healthy relatives

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ABSTRACT **The etiology of Alzheimer disease (AD) is unknown. To investigate the transmissibility of AD, the buffy coat of the blood from 11 relatives of AD patients, including 2 with suspicious or early signs of AD, was inoculated intracerebrally into hamsters. In these pilot experiments, 5 individuals produced histologically documented spongiform encephalopathy on primary passage in recipient hamsters. Material from 3 of these positives was serially transmitted in a second passage. The histological alterations observed in the brains of positive hamsters were similar to those seen in experimental Creutzfeldt–Jakob disease (CJD). These transmission results raise the intriguing possibility that CJD-like agents may be involved in at least some forms of AD.**

Alzheimer disease (AD), the most common and devastating dementia of humans, with catastrophic effects for patients and their families, still remains a disease of unknown etiology. Investigators have advanced several hypotheses on the nature of AD, and for several years the working hypothesis in our laboratory has been that AD may be a transmissible dementia not unlike Creutzfeldt–Jakob disease (CJD) of humans. CJD is a dementia of unquestionable infectious etiology; it was successfully transmitted several years ago to subhuman primates and monkeys (1, 2) and, in our laboratory, to several species of rodents (3, 4).

Similarities between AD and CJD have been noted by several investigators, and these similarities served as an impetus to experimentally reevaluate the possible transmissibility of AD. It has been stated that the clinical similarities between AD and CJD may be so great in some cases that only autopsy can clarify the situation (5). Overlapping of lesions between AD and CJD have also been noted in rare instances (6–10). Reference is made to the concomitant occurrence in the same individual of plaques and tangles, which are characteristic of AD, with spongy changes, which are the most prominent histological lesions of CJD. These cases present neuropathologists with diagnostic challenges. Furthermore, AD and CJD are known to occur, although rarely, in members of the same family (11–13). It should be emphasized that all above citations (5–13) must be considered as stimulating but anecdotal. Firm evidence for an infectious etiology in AD up to the present time is entirely lacking. Indeed, attempts to transmit AD from patients to convenient laboratory animals from brain tissue of two sporadic cases in our laboratory failed (14). Similar negative results were obtained in the laboratory of Gajdusek and Gibbs, where numerous transmissions were attempted with brain material from AD patients (15). The widely quoted report of successful transmission of two familial cases of AD has been proven to be irreproducible (15). However, all of these negative attempts on transmission of AD to animals have not entirely deterred us. Notably, all negative AD transmission experiments have been undertaken with human brain tissue at terminal stages of AD.

We considered that we were looking at the wrong end of the human disease (14). Perhaps it would be rewarding to check infectivity at early stages of AD, when patients have only mild signs or no symptoms at all. We reasoned that in AD, as in some conventional viral encephalitides—e.g., polio—the titer of the virus at end stages of disease might be so low as to be undetectable in animal transmission experiments (14). With these thoughts in mind we began in 1984 pilot experiments with human volunteers.

To obtain human brain tissue for experimentation from apparently healthy humans would have been both impossible and unethical. However, we were the first to demonstrate viremia in experimental and human CJD by using the buffy coat of the blood (16, 17). We speculated that viremia might transiently occur during some early stage of AD.

MATERIALS AND METHODS

Eleven human volunteers, members of families in which at least two relatives (siblings or parents) had AD, generously cooperated and were psychiatrically and psychologically examined by one of us (J.M.d.F.). With two exceptions, all volunteers were healthy and still continue to be free of any signs and symptoms 3 years after the commencement of our studies. The two exceptions were volunteer no. 5 (Table 1), diagnosed as having early AD when blood was drawn, and no. 8, who at the time of blood donation had suspicious signs of AD. Now, 3 years later, volunteer no. 8 has unquestionable manifestations of the disease. The following relationship existed between our human volunteers: nos. 5 and 6 are brother and sister, nos. 8 and 9 are mother and daughter, and nos. 10 and 11 are sisters (Table 1). The preponderance of female subjects was merely circumstantial or accidental: no attempt was made to select subjects for either age or gender. In reality, we gratefully accepted anyone who was willing to cooperate with us. Twenty milliliters of blood was drawn from each volunteer in a heparinized tube, the buffy coat was collected and homogenized in a 1:1 volume of saline, and 0.05 ml of this homogenate was injected intracerebrally into each of six young (6–8 weeks) LVG (outbred) hamsters per human buffy coat sample by techniques described in detail elsewhere (16, 17). Always, three hamsters were kept in a numbered cage and were observed daily for any unusual signs by at least two technicians and ourselves.

RESULTS

The buffy coat of five volunteers (nos. 2, 7, 8, 9, and 11; Table 1) produced an unquestionable spongiform encephalopathy in hamsters (Figs. 1 and 2). Nine animals (Table 1) developed the spongiform encephalopathy between 196 and 517 days

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Abbreviations: AD, Alzheimer disease; CJD, Creutzfeldt–Jakob disease.

Table 1. Buffy coat infectivity in AD patients and in their healthy relatives

Volunteer no. (Exp. no.)	Sex	Age, yr	Passage 1
1 (2756)	F	51	1− /5 f.d.
2 (2757)	F	42	**4+** /1− /1 f.d.
3 (2758)	F	59	1± /1− /4 f.d.
4 (2759)	F	46	1− /5 f.d.
5 (2760)	M	68	1± /5 f.d.
6 (2761)	F	57	1± /1− /4 f.d.
7 (2762)	F	37	**1+** /2− /3 f.d.
8 (2763)	F	77	**2+** /4 f.d.
9 (2764)	F	44	**1+** /1− /4 f.d.
10 (2765)	F	50	2− /4 f.d.
11 (2766)	F	53	**2+** /1± /3 f.d.

+, Positive; −, negative; ±, questionable; f.d., found dead.

after inoculation with an average incubation of 352 days. One additional animal had to be sacrificed 104 days after inoculation because it was severely wounded by cage mates: even at this relatively early postinoculation time, this hamster showed positive lesions of a spongiform encephalopathy (Fig. 2). The histological changes in the positive hamsters consisted of spongy changes in the neuropil, mild astrocytosis, and neuronal loss. They were similar to the histological alterations described in experimental CJD in our laboratory in guinea pigs, hamsters, and mice (18, 19). There are two criteria in our laboratory in evaluating positive animals, and these are positive histology and successful transmission. Only animals with well-preserved brains showing unquestionable vacuolization of the neuropil were labeled as positive. When the spongy changes in the brains of animals were not clear-cut (i.e., questionable), they were scored as ±. In such instances, a second passage from first passage material was undertaken. In case of doubtful histological evaluation, a second passage in our laboratory is a must. Nine animals were found dead shortly after inoculation as a result of inoculation trauma or fighting with cage mates prior to 103 days and were discarded. In addition, 33 animals were found dead long after they survived intracerebral inoculation, at times coinciding with the incubation periods of our positive animals. Some of these animals were also wounded by cage mates (after 103 days). Although postmortem changes prevented any histological evaluation of the brains of these animals, no pathological changes at autopsy were found in the viscera in these animals that survived up to 512 days. Since the clinical signs in first passage animals were subtle or difficult to score, their cause of death was ambiguous.

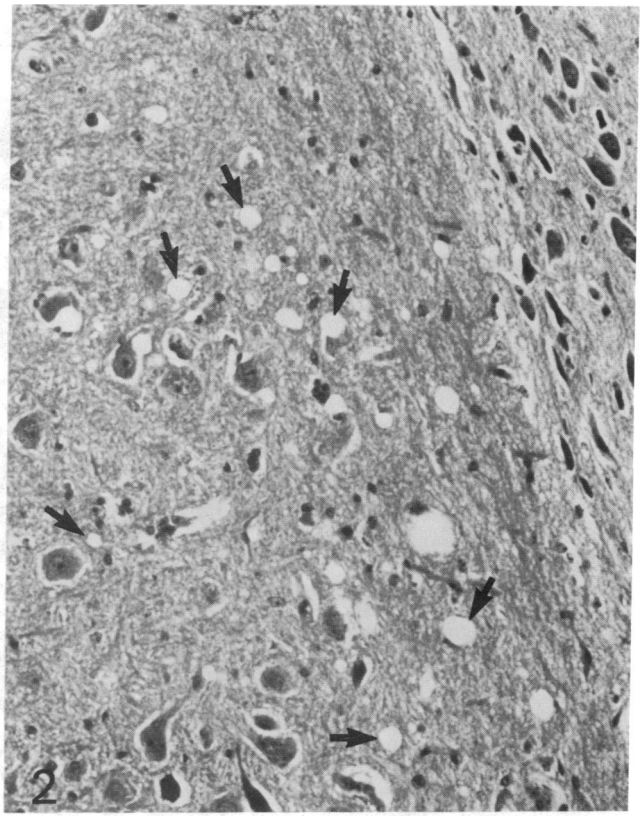

FIG. 2. Passage 1. Volunteer no. 8 (Table 1). Hamster no. 4 (sacrificed at 104 days). Midbrain. Isolated vacuoles of various sizes are present in the neuropil (arrows). (Hematoxylin/eosin stain; ×200.)

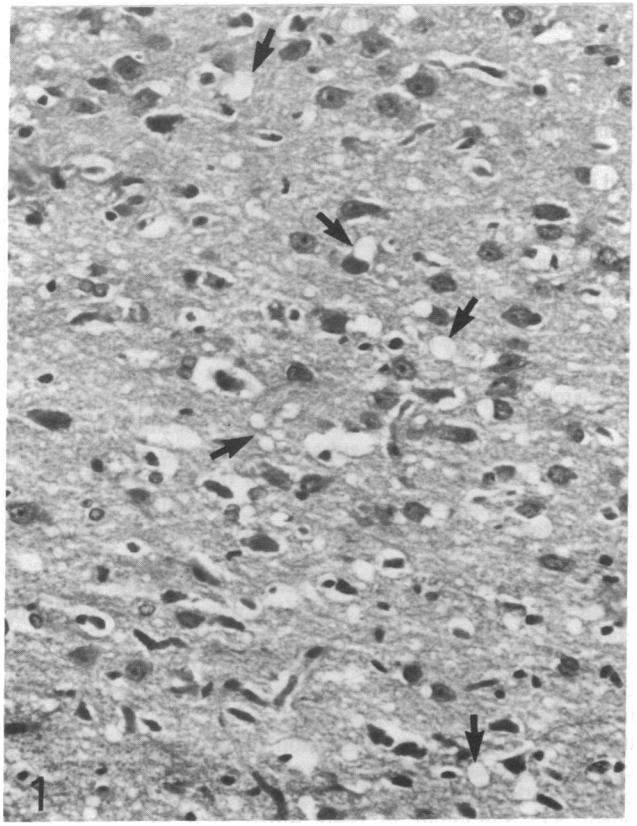

FIG. 1. Passage 1. Volunteer no. 2 (Table 1). Hamster no. 6 (sacrificed at 517 days). Cerebral cortex. Several vacuoles of various sizes are shown (arrows). (Hematoxylin/eosin stain; ×300.)

A second passage attempted from first passage hamster brain material in three cases (nos. 2, 8, and 11), done to determine serial transmission, resulted in all three cases in a greater number of positive transmissions and in florid more marked spongy changes in the brain (Fig. 3). With material from nos. 2 and 8, four of six animals in each case were positive and two were found dead; with no. 11, five hamsters were positive and one was found dead. In the second serial passage, incubation periods were somewhat shorter—e.g., 299 days. It has been reported that in experimental CJD the incubation period during the second passage may be reduced by half (18). Also during the second passage the detection of clinical signs of the experimental disease was more evident. During the first passage the animals were thin and scruffy but otherwise alert and without any obvious neurological signs. During the second passage, however, the positive animals revealed clinical signs very similar to those of experimental CJD. The hamsters were thin, slow moving, depressed with hunched trunks, jumpy to noises, and ultimately immobile and moribund with closed eyes. These signs lasted from 3 to

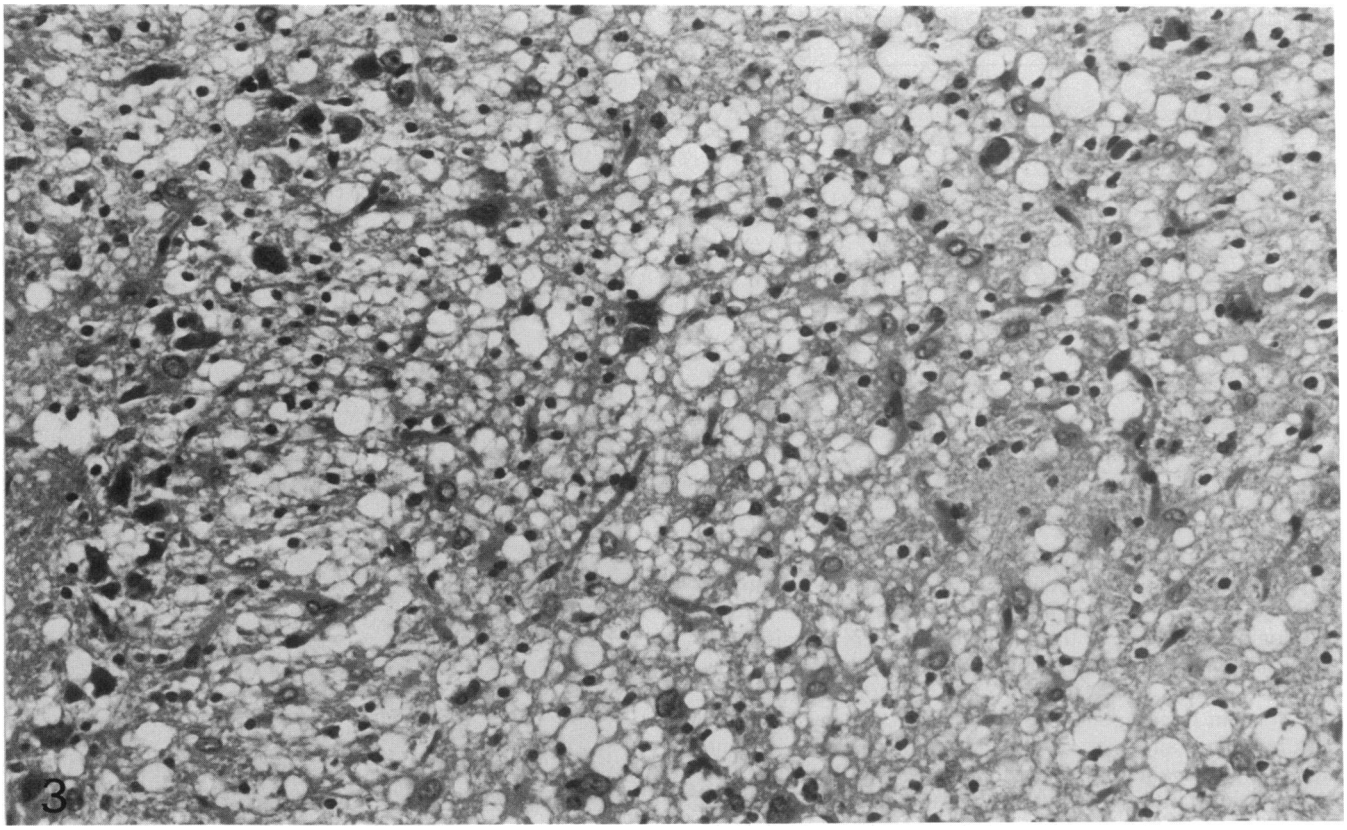

FIG. 3. Passage 2. Volunteer no. 11 (Table 1). Hamster no. 5 (sacrificed at 432 days). Ammon's horn. Very severe vacuolation is seen. (Hematoxylin/eosin stain; ×330.)

4 days in some animals and up to 15 days in others. With the exception of volunteer no. 1, all remaining material from primary transmissions (positive, questionable, and negative) in Table 1 has also been recently inoculated for second passages and are pending. No material for second passage was available from volunteer no. 1.

The more ideal controls for this survey would have been healthy age- and gender-matched subjects who had been evaluated psychiatrically/psychologically. These were not available to us at the time of writing, but buffy coat material from as many such subjects as possible will now be collected for testing. Random samples from healthy age-matched subjects, although somewhat less ideal as controls, were not included in this pilot study by reason of expense. However, in addition to the three negative "internal controls" cited in Table 1, one human buffy coat from a healthy (not psychiatrically evaluated) middle-aged person* served as a control; this buffy coat material was injected into 36 guinea pigs, 36 hamsters, and 36 mice, none of which developed an encephalopathy. Moreover, intracerebral injection of several normal guinea pig buffy coat samples has failed to induce encephalopathy in guinea pigs (16). In addition, uninfected rodent or human central nervous system samples have failed to induce similar lesions in guinea pigs, hamsters, or mice.

DISCUSSION

Our data on the infectivity of the buffy coat from one patient with AD and four healthy relatives of AD patients open several avenues for research and raise numerous challenging questions to be answered by tedious and very time-consuming experiments. In our laboratory, waiting for 2 or 3 years before an experiment or animal is declared negative is the rule rather than the exception. The first question that may be raised is whether a mix-up did occur in our laboratory. However, as is evident in Table 1, the experiments were consecutive (Table 1, experiment numbers), the animals from each patient were housed in the same cages (three per cage) and in the same racks; they were inspected and counted daily including weekends by two highly trained attendants. The organization of our P3 facility, the strict control imposed by our biohazard committee, and the small number of easily identifiable numbered animals in this rack make a single missing animal noticeable within 12 hr. We consider such an error in our facility to have been very unlikely. Furthermore, the 10 positive first-passage animals are from five different experiments and therefore cannot be due to a mix-up of one or two animals, and at least three of these human samples were clearly positive on a subsequent second serial passage. Although in rare instances CJD can be clinically confused with AD in humans, it is also unlikely that all five positively transmitted AD relative cases here represent misdiagnosed CJD.

One might question whether the injection of the buffy coat might somehow activate or cause the phanerosis of an endogenous virus. However, in preliminary electron microscope studies no virus particles were detected in these hamster brains. Furthermore, such activation of an endogenous virus in hamsters was never observed, regardless of the type of the inocula in numerous transmission experiments with CJD or control material. With these reservations, we tentatively conclude that relatives of AD patients and one patient with early AD contained in their buffy coat an infectious or transmissible agent causing a deadly encephalopathy in animals. Furthermore, the encephalopathy caused by the human buffy coat is similar, if not identical, to that caused by human

*This sample was obtained from the Connecticut Red Cross and all data on this patient (including sex and exact age) are considered confidential (not available).

CJD samples (including buffy coat) in rodents. The fewer positive takes from a human source during the first passage in rodents, the long incubation periods, and the large numbers of unexplained "found dead" animals are reminiscent of our experience with experimental CJD in guinea pigs, hamsters, and mice. Notably, all of the human donors are still alive 3 years after the onset of our study.

We are aware of both the importance as well as the numerical shortcoming of our pilot experiments, which should and will serve as stimulus for additional research. Because of the implications of our preliminary results, the desirability of its confirmation in other laboratories, and because of the great length of the latent period after inoculation, we are publishing these data without the extensive controls that will ultimately be necessary and that we are now preparing. We expect these results to be controversial. However, if the initial observations are confirmed by us as well as by others, which can take 3–4 additional years, it would indicate that at least some AD cases may have an infectious etiology comparable to CJD.

In the absence of any additional hard data, we would like to amplify on one possible hypothesis. Several years ago, we parenthetically expressed the view that the low incidence of 1–2 per million per year of human CJD might be only "the tip of the iceberg" of a widespread asymptomatic infection (20). Could it conceivably be that a CJD-like agent is a silent resident in the human body, and that host factors (e.g., genetic, immunologic, humoral, including aging itself), as well as exogenous factors encountered during the lengthy human life span, elicit CJD in rare instances, whereas the same class of agent in more numerous instances induces a dementia of the AD type? It is conceivable that the same agent, when transferred from the human host to a different host—e.g., the hamster—can in turn cause a more simplified picture with only the expression of CJD spongiform changes—i.e., the hamster may be incapable of expressing the long-term AD neuropathological changes seen in humans. Indeed there are numerous examples of conventional viruses in which viral expression and pathology is markedly affected by species. Finally, transmission in our laboratory of infantile CJD (Alpers disease) as well as familial CJD (including those with a well-documented dominant inheritance pattern) suggests that CJD-like agents might at least in rare instances be integrated into the germ line, and this concept may have relevance for AD (21). We hope in the ensuing years to have some answers to at least some of these puzzling questions.

Note Added in Proof. One patient (volunteer no. 5) in the interim died. Autopsy limited to the brain showed lesions of AD but no pathological changes of CJD.

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