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CELLULAR SENSITIZATION IN KURU, JAKOB-CREUTZFELDT DISEASE AND MULTIPLE SCLEROSIS:

with a Note on the Biohazards of Slow Infection Work

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ABSTRACT

Following intramuscular injection of Kuru and Jakob-Creutzfeldt brain material into chimpanzees, circulating lymphocytes became sensitized to scrapie mouse brain (and spleen) to a greater degree than to normal tissue. This sensitization subsided after about a month, to be followed some 90 days later by a secondary peak attributed to establishment of changes in the nervous system. Special sensitization to scrapie material occurs in Kuru and Jakob-Creutzfeldt disease, but is not specific to them. The immunological evidence suggests that parenchymatous destruction may precede astroglial hypertrophy in these diseases. An early peak occurred in animals inoculated with multiple sclerosis brain and normal brain, but neither showed a delayed second peak. Thus there was no evidence of establishment of infection, even though all four animals were in intimate contact for over 200 days, and no evidence of the injection of MS material itself having established an infection. Biohazards in Kuru, Jakob-Creutzfeldt and MS work appear to be very low. The significance of the increased sensitization to scrapie material in Kuru and Jakob-Creutzfeldt disease, especially in relation to normal ageing (where it also increases), is discussed.

It is widely accepted that a characteristic feature of scrapie, kuru and Jakob-Creutzfeldt (J-C) disease, (though not of other typical slow infections like visna), is the absence of any detectable immunological response (*Chandler* 1959, *Gajdusek* 1973). However, *Gardiner* (1965) reported that scrapie tissue showed enhanced antigenic activity when injected into rabbits, resulting in higher titres of antibody to a wider range of test antigens. With the development of a reliable and highly discriminatory method of measuring lymphocyte sensitization to antigenic determinants (*Field & Caspary* 1970, *Caspary & Field* 1971, *Carnegie et al.* 1973) it seemed appropriate to re-study scrapie, kuru, J-C disease and multiple sclerosis (MS) for evidence of accompanying

immunological change of any sort. Because certain resemblances between the changes of the normal ageing process and those seen in the young animal with scrapie had been remarked (*Field* 1967), the study was linked with that of antigenic changes in the tissues in old age (*Field & Shenton* 1973b). Here development of lymphocyte sensitization in four experimental chimpanzees will be presented and considered in relation to other findings.

MATERIAL AND METHODS

Four young male chimpanzees were inoculated in the right quadriceps femoris muscle with 10 per cent sterile saline suspension of (1) third passage kuru chimpanzee brain (the second having been accomplished in Newcastle in 1971 and the original by Dr. Gajdusek in Washington)—chimpanzee Tim: (2) brain from a histologically confirmed case of classical J-C disease—chimpanzee Butch: (3) brain from the case SELL from which scrapie was reported to have emerged in Icelandic sheep (*Palsson et al.* 1965)—chimpanzee Peter: (4) brain from a middle-aged woman who died from non-neurological disease without clinical or pathological evidence of brain changes, i.e. normal brain—chimpanzee Andy. Inoculations were made under light sernylan anaesthesia (induced by oral administration in black-currant juice) and were preceded by withdrawal of 10 ml blood from the femoral vein. Subsequent bleedings were similarly carried out.

The youngest animal was chosen for injection with normal brain since it was planned to leave it longest, the objective of the experiment being broadly two-fold: (a) to determine the point at which reactivity of the animal's lymphocytes to scrapie as opposed to normal tissue (i.e. the scrapie-normal difference or SND) became increased as had already been reported for the late stages of kuru and J-C disease (*Field & Shenton* 1973 b); to find out if a similar change occurred in an animal injected with MS material; and (b) to determine whether, if all four animals were allowed to run freely together, infection (either kuru or J-C disease) might pass from the injected animals to the normal or MS inoculated ones—it being confidently expected, in view of the extensive American experience, that inoculation of MS material would not, of itself, produce disease.

Some knowledge of the contagious infectivity of these diseases was regarded as important, if only because J-C disease is currently nursed in open wards (see below). The animals were accordingly caged in a single large room and shared food and drink. At intervals of the points in the accompanying figures blood was withdrawn for measurement of lymphocyte reactivity.

Lymphocytes were prepared by the method of *Coulson & Chalmers* (1967) as slightly modified by *Hughes & Caspary* (1970), and the cells were either examined the same day or were found to keep perfectly in 10 per cent autologous serum in medium 199 overnight at 4° C for study next day.

Lymphocyte sensitization was measured by the macrophage electrophoretic mobility (MEM) test referred to above, of which full experimental protocols have been given by *Caspary & Field* (1971) and further details by *Shenton et al.* (1973). In this method the percentage slowing of macrophage migration speed is a measure of the lymphocyte sensitization to the antigen with which the cells have been brought into contact. If t_e = macrophage migration time when antigen is present;

and t_c = time when no antigen is present, the $t_e > t_c$ and $\frac{t_e - t_c}{t_c} \times 100$ is the percentage slowing and measures lymphocyte sensitization to that antigen.

The slowing produced by scrapie tissue (brain or spleen) with any given specimen of lymphocytes is always greater than that with the same normal tissue, so that the scrapie-normal difference (SND) is uniformly positive. It is this SND which is found to rise as the incubation of the diseases goes on.

Antigens used for testing were encephalitogenic factor (EF) (*Caspary & Field* 1965) and 10 per cent suspensions of scrapie mouse brain and normal mouse brain of the same age. (The latter was from a mouse which had been injected intracerebrally with normal bi

RESULTS

The sensitization of the lymphocytes is shown in Figure 1.

(1) Andy—injected with normal brain.

In the month following inoculation there was a well-marked peak in the sensitization to both scrapie and normal brain though the SND remained small and within normal limits. This is most clearly seen in Figure 2, where SND is plotted against time. From 49 days onwards the sensitization remained at the original low level. Sensitization to EF likewise after the initial peak fell to the initial level and the curve as a whole followed that of scrapie and normal brain. Sensitization to scrapie and normal spleen also showed a small initial peak. The response was thus a monophasic one consequent upon injection of brain material quite comparable to that we have found in guinea-pigs.

(2) Peter—injected with MS brain (SELL).

Whilst the general form of the lymphocyte sensitization curves was similar to that of Andy (normal brain), in that no second peak occurred once the original response had died away, there were differences in detail in the first peak. Reactivity to both scrapie brain and spleen was increased as compared with the normal tissues, so that the SND was elevated (Figure 2). This has likewise been found in a whole series of experiments (unpublished) in which gliosed human nervous tissue injected into guinea-pigs resulted in an elevated SND. Indeed injection of mouse brain in which gliosis has been induced by feeding cuprizone (*Pattison & Jebbett* 1971) also leads to a large SND (*Field & Shenton* 1973 b). This response in the chimpanzee is thus not specific but results from injection of gliotic brain material. Precisely the same curves are, therefore, obtained with the kuru and J-C injected animals so far as the first peak is concerned. It may be noted that elevated SND in respect of both brain and spleen is always due to increased sensitivity to the scrapie material, that to the normal remaining about the same as in the normal animal.

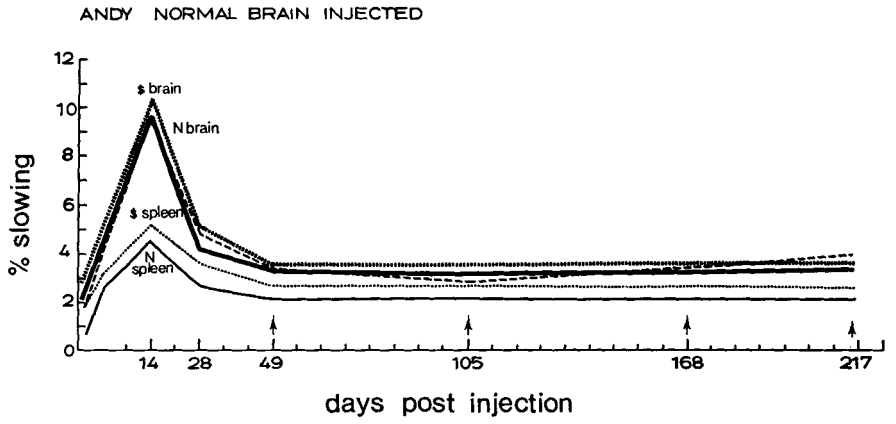

Figure 1 a.

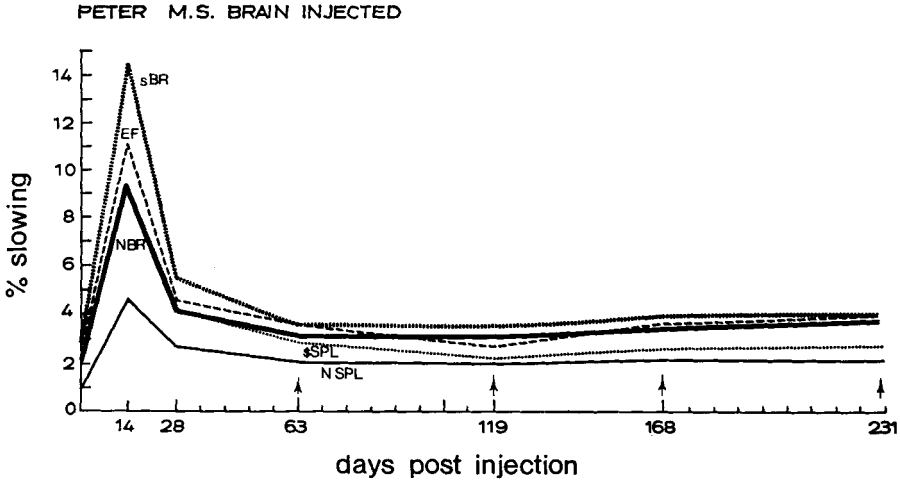

Figure 1 b.

(3) Tim—injected with kuru brain.

The initial peak resembles that of Peter (MS injected) with increased SND due to the high result given by the scrapie material. However, between the 63rd and 119th day after injection, sensitization to EF has risen in a way which did not occur in Andy (normal) or Peter (MS). Sensitization to EF is a delicate indicator of parechymal brain destruction (*Caspary & Field* 1970). At 119 days SND is still at a normal level, but by 168 days has risen markedly (Figures 1 c, 2). The secondary peak indicates change going on in the brain leading to self-sensitization of the lymphocytes.

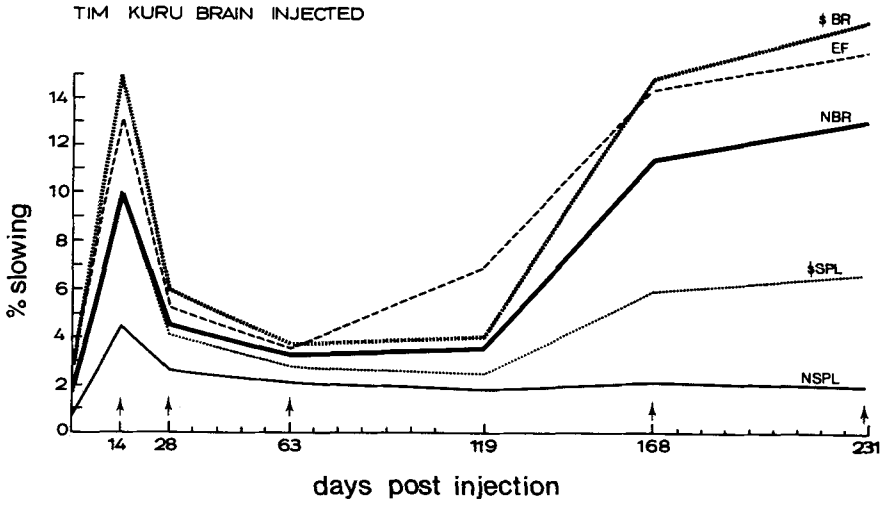

Figure 1 c.

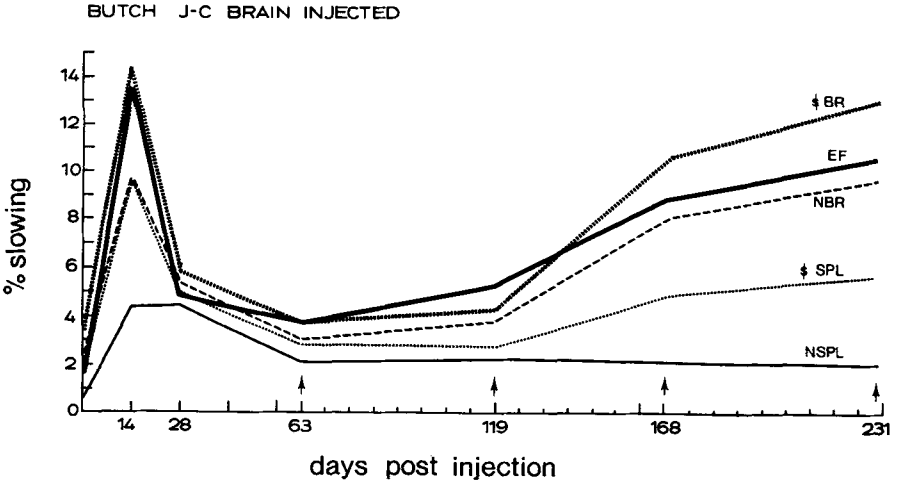

Figure 1 d.

Figure 1. Sensitization of lymphocytes to scrapie (and normal) brain and spleen and to EF in chimpanzees following intramuscular inoculation of 10 per cent suspension of brain material. Ordinate = percentage macrophage slowing in MEM test (an index of lymphocyte sensitization), abscissa = days after inoculation. (a) Andy—normal brain injected. (b) Peter—MS brain injected. (c) Tim—Kuru brain injected. (d) Butch—J-C brain injected. Note second rise in sensitization to scrapie tissues long after the initial response has died away in (c) and (d) but not in (a) and (b). Note rise in EF sensitization anteceding that to scrapie tissues in (c) and (d).

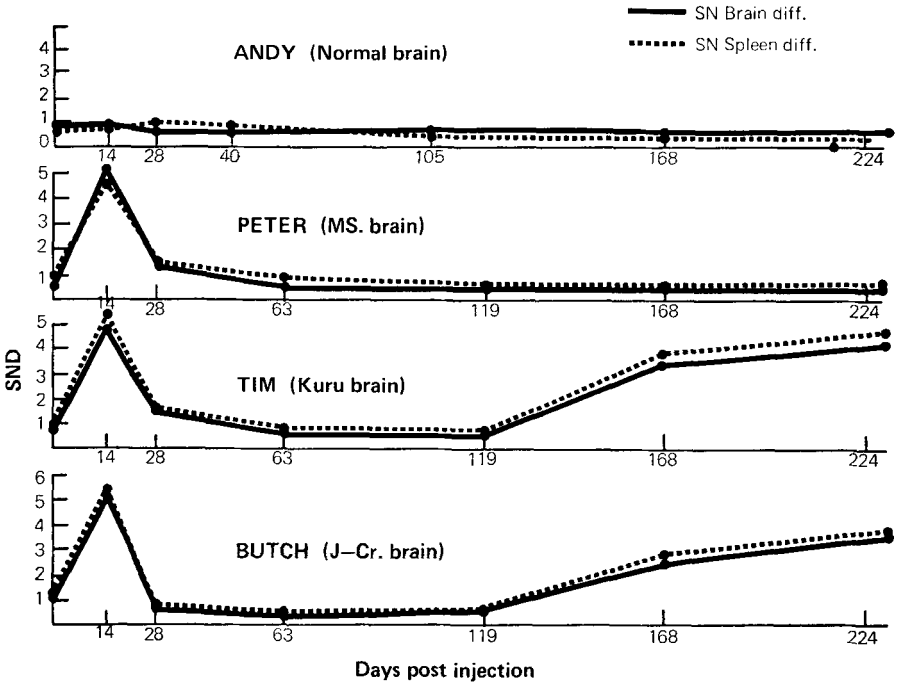

Figure 2. Difference in scrapie brain (or spleen) sensitization and sensitization to corresponding normal tissue (SND). Note no SND after injection of normal brain: peak in (b), (c) and (d). No second rise in (b) but late rise in kuru and J-C animals as infection becomes established and secondary "internal" sensitization of lymphocytes occurs.

(4) Butch—injected with J-C material.

The form of the curves is similar to that shown by Tim (kuru), though the increased response to EF at 119 days is not so marked. Again beyond 119 days there is a second peak long after the initial response has subsided.

Changes subsequent to the initial response have occurred in the J-C and kuru injected animals but not in the normal or MS. Moreover, the latter have not responded over the period of 200 days for which they have been in contact with animals incubating disease.

DISCUSSION

The establishment of kuru and J-C disease in chimpanzees was recorded by the Washington group headed by *D. C. Gajdusek* in 1966 and 1967, and was first confirmed outside America in Newcastle in 1971 (a brief illustration of the histology being given by *Field & Shenton* 1973). The

resemblance between these two diseases and scrapie has long been recognized (*Hadlow* 1959, *Klatzo et al.* 1959). Increased SND is found in established scrapie both in the mouse and sheep, and indeed offers a ready means of immunological diagnosis in place of lengthy bioassay (*Field & Shenton* 1972, 1974) in the absence of any other known gliosing disease in these animals. Moreover, after intracerebral inoculation of scrapie in the mouse SND elevation only begins after 50 days, though scrapie agent has been proliferating there long before (*Field & Shenton* 1973 c). The increased reaction to scrapie brain does not, therefore, simply result from the presence of the agent itself, i.e. the increased sensitization to scrapie brain measured is not due to sensitization of lymphocytes to the agent itself. There is evidence that it is associated with gliosis (itself perhaps a manifestation in the brain of some subtle bio-physico-chemical structural alteration occurring widely in membranes (*Gibbons & Hunter* 1967, *Field & Shenton* 1973 b) and possibly representing the fundamental pathogenetic change in scrapie—and possibly ageing). There is now experimental evidence that the antigenicity appearing in scrapie tissues also appears gradually in normal ageing mice and humans (*Field & Shenton* 1973 a) and this is particularly interesting in view of the similarities between these diseases and the ageing process (*Field* 1967) and the occasionel overlapping between Alzheimer features and those of J-C disease (*Hirano et al.* 1972). It may be that the emergence of scrapie-like antigen(s) in the brain (they have not been sought in other tissues) may constitute a unifying factor of pathogenetic significance, and may represent a step in the evolution of pathological changes.

The rise in sensitization to EF preceding that in the SND was unexpected, since the scrapie—J-C—kuru group is regarded as characterized by a precocious astroglial hypertrophy which is characteristic of the pathological reaction. The EF findings, however, indicate that subtle degenerative changes in the parenchyma, whilst not readily recognisable in the microscope, occur early and are sufficient to cause lymphocyte sensitization. If so, then the pronounced astroglial changes may be of secondary and not of the primary significance usually accorded them (Field 1969). In this regard, it is of interest that, contrary to what had been expected, *Narang et al.* (1972) reported a higher titre of scrapie agent in the neuronal than in the glial compartment of mouse brain with early scrapie infection (before signs).

The secondary peak occurring in Butch (J-C) and Tim (kuru) appears to result from sensitization occurring "internally" (i.e. from the development of the pathogenic process) as opposed to the first peak which resulted from "external" immunization. It would be reasonable

to suppose this associated with gliosis and a study of the early morphological changes in relation to the immunological ones in these diseases would be rewarding.

The absence of the secondary rise in Peter (MS) and Andy (normal) must mean that the pathogenetic mechanisms which lead to disease are not occurring.

The question of biohazard in relation to kuru and J-C disease (and perhaps also MS) may be considered. The bizarre occurrence of MS in four out of seven research workers in Cambridge who had been studying swayback in lambs (*Campbell et al.* 1947) is still unexplained, and the significance of the well-known "clustering" of MS cases remains unresolved. The hypothesis that MS is contracted by exposure to some exogenous agent about puberty (*Schapira et al.* 1963) rests on no support other than circumstantial. On the other hand, studies of the type reported by *Müller* (1973) on the detailed distribution of MS with special reference to possible vectors in nature deserve more attention than they have received.

Gajdusek & Gibbs (1973), from their unparalleled experience of kuru and J-C disease both in the field and in the experimental animal house, are of the opinion that "the epidemiology of the disease gives no cause for suspicion that the diseases are naturally contagious". Thus, no Caucasian who has come into contact with kuru in the Okapa region of New Guinea has ever developed anything resembling kuru or J-C disease, and the former seems restricted to the Fore area. One case of a non-Fore patient has been reported in a Rabaul (New Guinea) newspaper, but this remains unconfirmed (*C. J. Gibbs*—personal communication). It may reasonably be concluded that under ordinary conditions kuru is non-infective.

In the case of J-C disease, infectivity is again very low under ordinary conditions. An example of conjugal disease has been reported and J-C disease seems also to have occurred in a 54-year-old neurosurgeon in whom a diagnosis of papulosis atrophicans maligna had been made (Kohlmeier-Dagos disease), but possible occupational exposure to J-C disease material is not established. More significant (and indeed predictable) is the reported development of J-C disease in a 55-year-old female recipient of a corneal graft from a 55-year-old man with a two months' history of inco-ordination, memory deficit, involuntary movements and myoclonia and whose brain at autopsy showed the changes of J-C disease (*Duffy & Wolf* 1974). Other than by direct introduction of tissues or by ingestion, there would appear to be only very low grade, if indeed any, infectivity.

"As regards animal caretakers, laboratory technicians, professional

and non-professional personnel working with human cases of kuru and J-C disease and experimentally infected animals—we have no evidence even remotely suggesting infection. In the case of kuru, this would be more than 18 years that non-Fore Negroids and Caucasians have been in close contact with materials now known to be highly infectious for animals." (*C. J. Gibbs*—personal communication).

Clearly contagion is of very low order and the material comes into the "low risk category—meaning that careful handling ... in a well-trained virus laboratory is acceptable".

Animal to animal contagion is unknown to the American workers despite extensive experiments in Washington, Louisiana and California in which primates have been housed together. No instance of "cross-infection" has ever been seen and this applies also to serial passages of the agents in animal hosts which might be expected to enhance virulence. Over the relatively short period during which chimpanzees infected with kuru and J-C disease were in intimate contact at Newcastle with a normal and an MS injected animal, neither of the latter developed immunological reactions suggestive of the establishment of an infection, though clearly it would have been more informative if they had been allowed to survive longer.

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