MYELINATION IN THE MOUSE BY TRANSPLANTED OLIGODENDROCYTES

M. Gumpel[1], F. Lachapelle[1], M. Baulac[2], A. Baron van Evercooren[1], C. Lubetzki[1,3], A. Gansmuller[1], P. Lombrail[3], C. Jacque[1] and N. Baumann[1]

[1] Laboratoire de Neurochimie INSERM U 134,
Hôpital de la Salpêtrière,
47 Boulevard de l'Hôpital, 75013 Paris France

[2] Laboratoire Charles Foix,
Hopital de la Salpêtrière,
47 Boulevard de l'Hôpital, 75013 Paris France

[3] Laboratoire de Neuropathologie Charles Foix,
Hôpital de la Salpêtrière,
47 Boulevard de l'Hôpital, 75013 Paris France

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INTRODUCTION

Numerous observations especially in man in case of multiple sclerosis or after spinal cord trauma, have shown that at adult stage, demyelinated sites can be at least partially spontaneously repaired by Schwann cells migrating into the CNS (14, 16, 18, 20, 24, 46).

These observations have been confirmed experimentally in animals. Experimental demyelinated sites were obtained by various techniques. They could result from chronic experimental allergic encephalomyelitis (EAE), acute thiamine deficiency, spinal cord irradiation, viral infection, local injection of lysolecithine or 6-aminonicotinamide, cuprizone diet (5, 7, 10, 11, 19, 23, 30, 31, 35, 36, 37, 40, 44).

In fact, experimental works have shown that both myelinforming cells (oligodendrocytes and Schwann cells) can participate in the repair of CNS lesions (7, 8, 23). When this double remyelination proceeds the center of the lesion is remyelinated by Schwann cells while oligodendrocytes remyelinate at the periphery of the lesion near to the non affected white matter. It has to be emphasized that astrocytes are present only in the areas where remyelination was due to oligodendrocytes.

Thus in the adult, Schwann cells seem to be very highly competitive in CNS myelin repair, this competitivity being modulated by the presence of astrocytes. A recent work from Blakemore (9) suggests that this competitivity of the Schwann cells could be due to their high motility especially if they move on Extra-Cellular Matrix (ECM) around the blood vessels. Nevertheless oligodendrocytes, even in the adult are able to participate in remyelination (7, 8, 23). It is even possible that their role in remyelination in multiple sclerosis for instance is more important than suggested by the observation: when the process is achieved remyelination by Schwann cells appears to be remarkable and easy to detect because of morphological features including the basal lamina of the myelinating cells and the specific morphology of the PNS myelin. By contrast, it may be difficult to distinguish newly formed CNS myelin from the normal myelin of the brain. When myelination by oligodendrocytes does occur it is not yet clear if it is the fact of undifferentiated, immature oligodendrocytes or if the differentiated oligodendrocytes are able *in vivo* to divide and re-differentiate. However recent works (1, 30) seem to assume that such a possibility could exist.

In the cases discussed above, the myelinating and migrative properties of the myelin-forming cells are studied after the normal process of myelination has occurred and are related to the repair of a demyelinating site.

In the cases reported in this paper, oligodendrocytes contained in fragments of CNS or isolated cells are implanted in new-born host brains.

Thus the transplantation takes place before the moment at which myelination starts in the host. Transplanted oligodendrocytes whatever their stage of maturation are placed in competition with host oligodendrocytes *during the normal process of myelination.*

SHIVERER MODEL AND TRANSPLANTATION TECHNIQUE

The shiverer model (21, 22, 27) was used all along these experiments to distinguish myelin formed by transplanted oligodendrocytes in the shiverer brain. The shiverer mutant mouse (3) is biochemically deprived of Myelin Basic Protein (MBP) (13, 25). Thus by immunohistochemistry using an anti-serum anti-MBP, the MBP positive myelin can be detected in the totally negative shiverer brain (Fig. 1 and 2). This biochemical defect is correlated with the absence of the major dense line (MDL) of the myelin (25). Thus myelin formed by MBP positive oligodendrocytes can

also be recognized at ultrastructural level, using a classical electron microscopy technique (Fig. 3 and 4).

The transplantation technique (Fig. 5), fixation of the samples, sectioning and immunohistochemical techniques have been described in details elsewhere (22, 27). The anti-MBP polyclonal antiserum used for this study was raised in the laboratory against human MBP (12). The specific methodology used in the different series of experiments will be precised in the following chapters.

TRANSPLANTATION OF NORMAL NEW-BORN MOUSE CNS INTO THE NEW-BORN SHIVERER BRAIN

In these series of experiments the transplant was a fragment of olfactory bulb of new-born normal mouse (B6CBA and C57BL6). Exceptionally 2 and 3 day-old animals were used as donors. Dissection of olfactory bulb was described elsewhere (27).

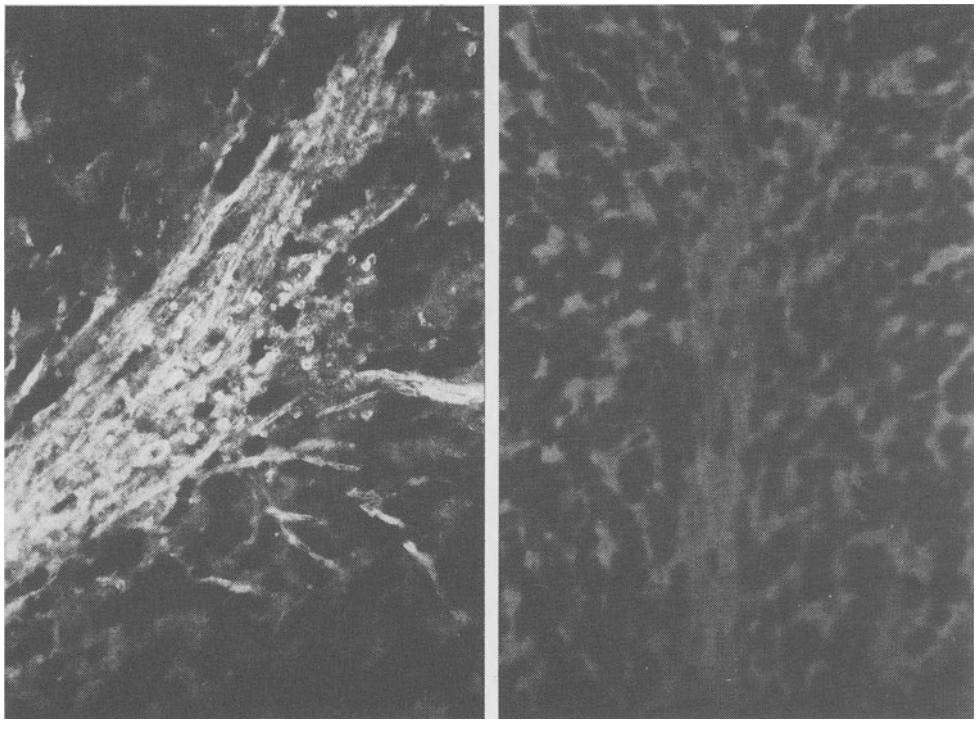

Fig. 1 and 2
The shiverer model: Immunocytochemistry.
1. Myelin basic protein positive myelin detected by immunofluorescence in a normal adult cerebellum.
2. No positive reaction in the white matter of the shiverer adult cerebellum.

MATURATION OF THE OLIGODENDROCYTES IN THE OLFACTORY BULB OF THE NORMAL MOUSE

The timing of myelination in rodents total brain has been studied by biochemical methods (2, 17, 33). From these works, it is clear that myelination starts after birth. However the techniques used did not allow to obtain any information about the possibly different timing of myelination in the various part of the brain. For example nothing was known about the timing of maturation of the oligodendrocytes in the olfactory bulb. This part of the brain being used as implant, experiments were previously designed to follow the timing of MBP expression by olfactory bulb oligodendrocytes *in situ*. Mice of B6CBA stain were used for these experiments. The study (26) was based on the detection of MBP by RIA and of MBP and galactocerebroside (GC) by immunochemistry using dissociated cells and tissue sections. GC positive oligodendrocytes were detected 3 days after birth on dissociated cells from olfactory bulb, while MBP was expressed 4 days later. Myelinated fibers were not detected on cryostat sections of olfactory bulb before 8 days postnatal.

By RIA, a very low amount of MBP was detected in the olfactory bulb from birth up to day 7. From day 8 the amount of MBP increases up to a plateau reached at day 30.

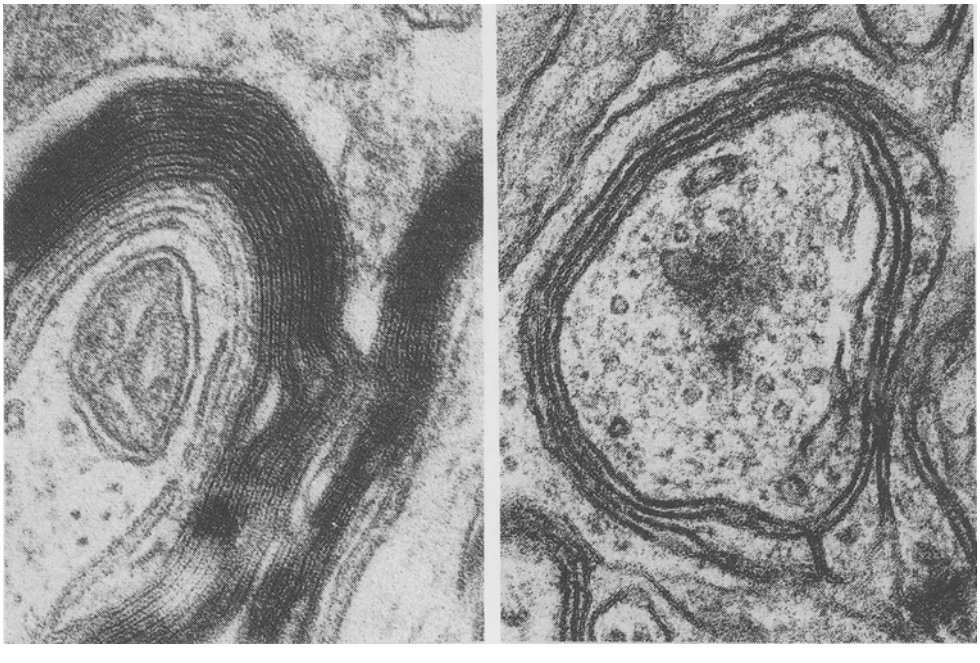

Fig. 3 and 4
The shiverer model: Electron microscopy.
3. Adult normal central myelin with major dense line.
4. Adult shiverer central myelin: absence of major dense line.

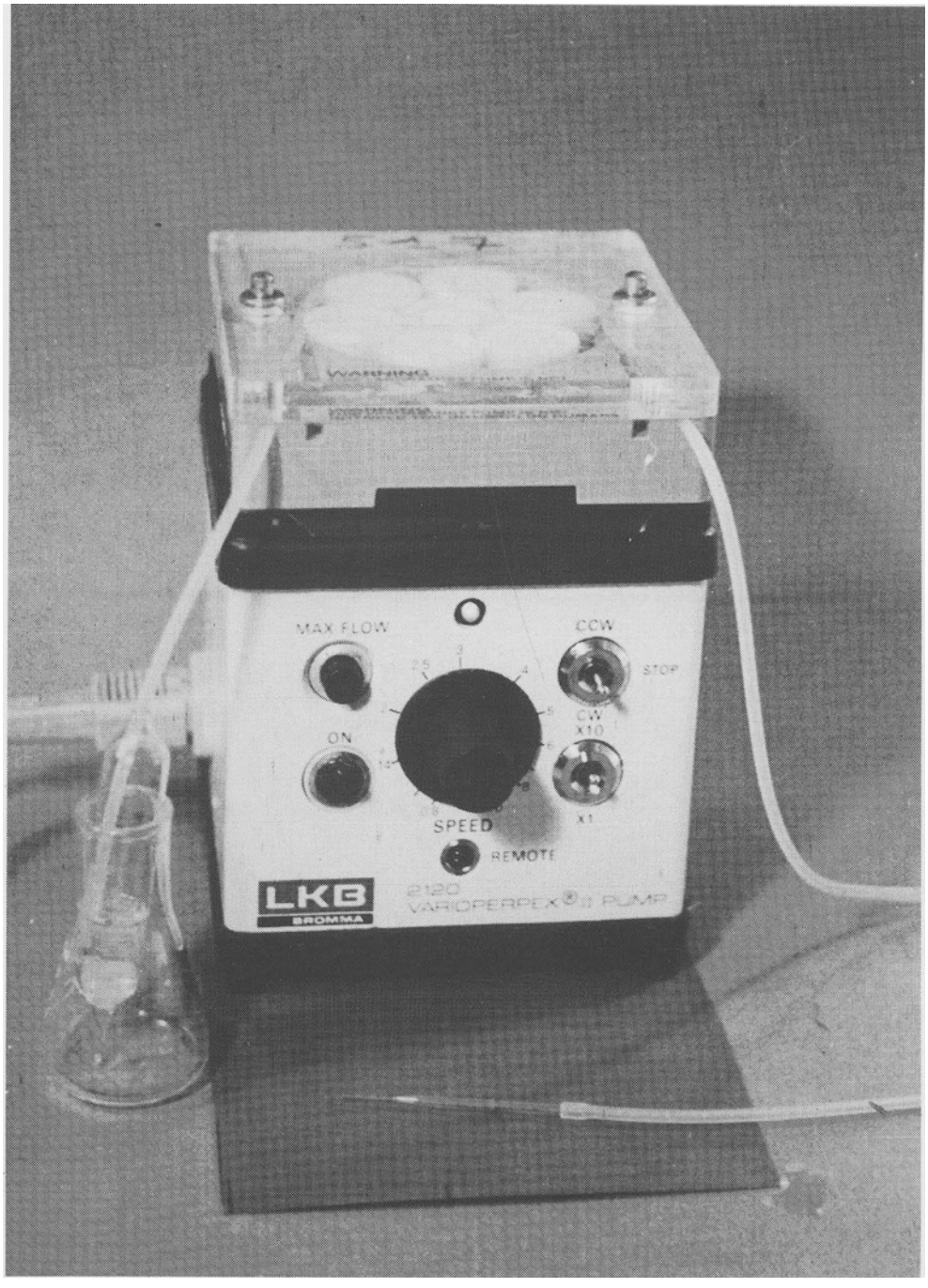

Fig. 5
The system used for transplantation. A micropipette is adapted to a peristaltic pump. The fragment to be grafted can be aspirated into the pipette, placed at its extremity and gently pushed into the host brain.

OBSERVATION OF THE IMPLANTED SHIVERER HOST BRAINS

The brains of the host-animals were studied 6 to 130 days after grafting (120 - 130 days being the limit of viability of the shiverer mutant mouse). After fixation by intracardial perfusion, the site of implantation was detected morphologically by the presence of charcoal used to mark the grafted tissue. The host brain was sagitally cryostat sectioned at the level where charcoal was visible, indicating the location of the graft. It was thus possible to appreciate on the sections, after immunohistochemical treatment, the rostro-caudal extension of myelin formed by implanted oligodendrocytes (MBP positive) in a relatively thin slice of the host brain (27). 6 to 10 days after implantation, the graft was found healthy but no MBP positive myelin was found in the shiverer host brain. From 15 days up to 130 days MBP positive myelin, thus formed by implanted oligodendrocytes was detected in the host brain. If the transplant was in the rostral thalamus, MBP positive myelin appeared as patches disposed along rostro-caudal pathways from the point of implantation to the posterior thalamus, hypothalamus pons and cerebellum. No rejection of the graft was observed.

In a parallel series of "sham" experiments in which shiverer new-born fragments of olfactory bulb were grafted in the rostral thalamus of shiverer new-born mice, no MBP positive myelin was detected in the host brains.

From these series of experiments we could conclude that oligodendrocytes contained in the graft were able to survive in the host brain at least up to 130 days. Moreover they could migrate over long distances in the host brain tissues and myelinate host axons along their pathways of migration.

These results were fully confirmed by electron microscopy (unpublished results). The normal myelin (Fig. 3) appeared to be scattered among the shiverer host one (Fig. 4) in a same axon bundle. Moreover, we were able to demonstrate that a same axon could be myelinated by both types of oligodendrocytes, the node of Ranvier being surrounded by both shiverer and normal myelin.

However from these preliminary investigations many questions remained unelucidated. For example, these experiments did not allow to appreciate the extension of myelination due to the graft in the whole brain. Moreover,the implantation site is always the rostral thalamus and under these experimental conditions the pathways of migration appeared to be comparable from one animal to another. The question arose as to whether the migrative properties of the implanted oligodendrocytes and the pathways of migration were depending on the point of implantation and anatomical environment of the graft.

We designed the following experiments to make an attempt to answer at least these first questions.

EXTENSION OF MYELINATION DUE TO IMPLANTED OLIGODENDROCYTES AND ROLE OF THE SITE OF IMPLANTATION ON OLIGODENDROCYTES MIGRATION

Fragments of olfactory bulb from normal new-born mouse were implanted in various sites of the host new-born shiverer brain. After fixation at 20 to 130 days the *whole* host brains were cryostat sectioned and the sections were treated for immunohistochemical detection of the MBP positive myelin (PAP technique-44).

MBP positive myelin was found in the shiverer brain whatever the site of implantation was. It always appeared as patches on normally myelinated pathways. The lateral and antero-post-

erior extension of these MPB positive patches of myelin was variable but very impressive. As an example in a host brain in which the graft was placed in the anterior cortex near the olfactory bulb, MBP positive myelin was found up to the cerebellum and in both hemispheres (spinal cord was not sectioned). Migration of oligodendrocytes seemed to follow roughly normal axonal myelinated pathways. In the same patch, two axonal directions could be MBP positive myelinated, myelin appearing longitudinally and transversally sectioned. It has to be noted that MBP positive patches of myelin did not appear as gradually degressive with the distance to the graft. The graft being located in the anterior brain, one could observe a very high MBP positive myelination in the caudal structures and a very light myelination in the mid-brain.

As suggested by EM observations and immunohistochemical results the oligodendrocytes could leave out the graft a few days after transplantation. They could migrate interstitially or be passively transported by axons projecting at the same moment. It has to be emphasized that the distance between graft and MBP positive patches of myelin is artificially enlarged by the growth of the host brain from new-born to adult stages.

The first stages of migration including the relationships between the migrating cells and the different cell types of the host brain, the role of extra-cellular matrix and the mitotic activity of pre-myelinating oligodendrocytes are presently under investigation.

The shiverer model is valuable whatever the source of MBP positive oligodendrocytes, the only condition being the possibility to detect the species-specific MBP with the anti-serum we

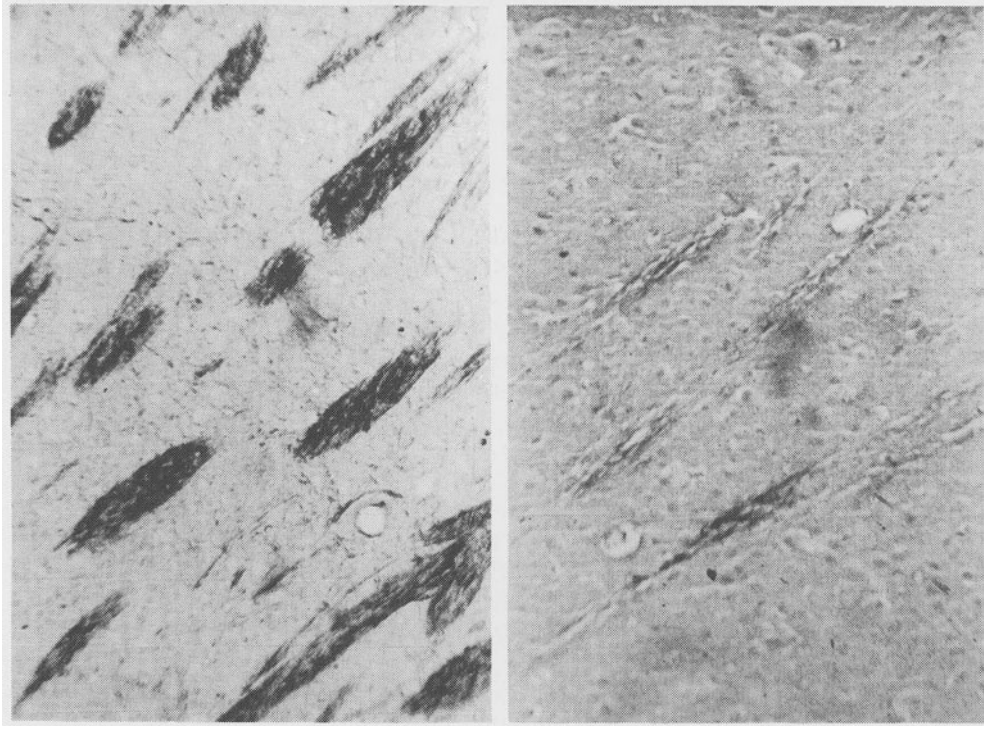

Fig. 6 and 7
Normal and jimpy myelination.
6. Aspects of the striatum in a control normal 25 day mouse.
7. Aspects of the striatum in a 25 day jimpy mouse.
The MBP myelin evidenced by immunocytochemistry (PAP technique) is very rare in jimpy structures.

used. Thus three types of experiments have been attempted: cross-transplantation between jimpy and shiverer mutant mice, transplantation of isolated rat adult oligodendrocytes, transplantation of human embryonic CNS. Most of these results being unpublished they will be briefly summarized in this paper.

CROSS TRANSPLANTATIONS BETWEEN JIMPY AND SHIVERER MICE

Jimpy mutant mouse (34) is very poorly myelinated (12, 15, 35, 39). However its rare (5 % of the axons in the corpus callosum) myelin is MBP positive (Fig. 6 and 7). The phenotype of this mutation (severe tremor) appears only at day 11 - 12. The use of Tabby mutation as a marker allows an earlier detection (absence of post-oral and post-orbital vibrissae at birth). However the presence of some recombinants makes this detection uncertain.

We thus carried out two groups of experiments: transplantations of olfactory bulb from new-born presumably jimpy animals and from 11 - 35 days clearly recognizable jimpy animals.

Unexpectedly, a large amount of strongly MBP positive myelin developed in both series; especially when the donor was 11 - 20 days old (22). It seems unlikely that this high amount of myelin was formed by the few oligodendrocytes which myelinate in the jimpy brain. It is most probable that the non functional jimpy oligodendrocytes which remained immature and proliferating (40)during all the life span of the jimpy mutant were able to express the myelin components in the shiverer environment.

These experiments have to be completed in order to try to precise the role of the shiverer environment in differentiation and expression of myelin components by jimpy oligodendrocytes.

TRANSPLANTATION OF ISOLATED ADULT RAT OLIGODENDROCYTES

The possibility of remyelination by mature differentiated oligodendrocytes is still discussed. However in recent works (1, 3) it has been demonstrated that after a lesion, mature oligodendrocytes in adult rat brains are able to incorporate thymidine. However it is not proved if such oligodendrocytes do divide and remyelinate and even if they do,the events following the possible division (demyelination, disparition of the myelin debris and remyelination) are unknown.

We designed a simplified experiment in which adult rat oligodendrocytes freed of the myelin debris were transplanted into the new-born shiverer brain. Moreover these oligodendrocytes were present in the host brain during the normal process of myelination: thus if an axonal signal was to be postulated to explain the myelination process, the adult oligodendrocytes were placed in the brain at the moment when this signal was fully expressed.

The oligodendrocytes were isolated from forebrains of 4 to 8 weeks old rats (29). The myelin forming cells were isolated according to the procedure described by Lisak et al. (28). After mechanical dissociation followed by trypsin digestion, the homogenate was layered on a Percoll density gradient to isolate the oligodendrocyte-rich fraction. Double labelling indirect fluorescence showed that 90 to 95 % isolated cells co-express galactocerebroside (GC) on their surface and Myelin Basic Protein (MBP) on their cytoplasm; less than 5 % of the cell population were recognized as astrocytes by being GC- and GFAP +. The presence of neurones using anti-$\gamma\gamma$enolase was never detected. Several anti-sera were used for these studies: anti-MBP and GFAP from C. Jacque, anti-GC from B. Zalc and anti-$\gamma\gamma$enolase from A. Keller.

After isolation the oligodendrocyte-rich fraction was centrifuged to obtain a pellet. This pellet was allowed to reaggregate for 1 to 3 hours at 37 C. We thus obtained a tissue which could

be fragmented with scissors and the fragments were implanted according to the method used for CNS fragments. After 40 to 90 days the host brain showed that MBP positive myelination from implanted cells was widely spread in the host brain.

TRANSPLANTATION OF HUMAN EMBRYONIC OLIGODENDROCYTES

We have transplanted fragments of human embryos CNS from stage 16 weeks to stage 24 weeks. We never observed MBP positive myelin in host transplanted with 16 to 22 weeks old CNS. When the transplant was excised from 24 weeks embryos MBP positive myelination due to human oligodendrocytes was observed sometimes in very large areas of the host brain.

At stages anterior to 24 weeks the human oligodendrocytes remained healthy in the shiverer brain but no myelin differentiation was detected. This could mean that when they have not reached a certain stage of development human oligodendrocytes precursor cells need, at least *in vivo,* "homochronic" environment for their different steps of differentiation. This hypothesis has to be tested in mouse by transplantations of early embryonic CNS in the new-born or by tranplantation of new-born CNS in embryo.

DISCUSSION AND CONCLUSION

The existence of a model for oligodendrocytes transplantation in conditions in which the transplanted cells can be followed from the graft up to the differentiated stage opens a large experimental field.

In the experiments summarized in this paper, transplanted oligodendrocytes whatever their genotypic or chronologic characteristics, are placed and followed in the host brain during the normal process of myelination. They are thus in competition with the host oligodendrocytes and this appears to be important to understand some of the normal events of central myelination process.

One of the most important interest of oligodendrocytes transplantations in the shiverer model was to be able to evidence an unexpected long distance migration of oligodendrocytes during the myelination process. Cell motility and plasticity appears to be an essential component of CNS morphogenesis: precocious migration of neuronal and glial cell bodies, projection of sometimes huge neuronal and glial-astrocytic and oligodendroglial-cell processes.

However, it seems to be presently assumed that oligodendrocyte-precursor cells are present in all the brain structures before myelination starts. In these conditions, long migration of myelin forming cells appears to be at least "unnecessary". Nevertheless it is difficult to postulate that implanted oligodendrocytes, especially when they have the same developmental stage as the host ones, (new-born normal olfactory bulb into new-born shiverer brain) have a specific behaviour among the host oligodendrocyte population. Our observations are in favour of the idea of a mixing of both populations, both participating in the myelination of the same axon bundles and even of the same axon. The hypothesis of the existence of such movements of oligodendrocytes in normal post-natal development has thus to be postulated. The causes and conditions of these migrations have to be elucidated by complementary experiments including normal labelled oligodendrocytes grafting in normal mouse and variation of host and donor age. In such experiments, the possibility offered by the shiverer model to distinguish host from donor myelin and MBP+ from MBP- myelin forming cells by electron microscopy could be of great interest to follow cell interaction in the first steps of the migration. The possibility of a passive transportation

by axons due to an early oligodendrocyte precursor cell-axon adhesion is not to be rejected.

Our experiments show that the interaction between axon and oligodendrocytes during the myelination process is not strictly species-specific since myelination proceeds normally when rat and human oligodendrocytes are transplanted into mouse brain. Preliminary experiments seem to indicate that quail oligodendrocytes can also ensheath and myelinate mouse axons. The exchange of signals between cells of phyllogenetically very widely different amniote vertebrates has already been described in several developmental systems. This is consistent with the concept of signal-receptor couple that has been evolutionarily conserved.

Another point which comes out from the results related in this paper is the very important role of environmental factors in myelination process.

Myelination of not yet myelinated axons by oligodendrocytes from adult rats seems to indicate that the developmental stage of the environment is essential for the potential of differentiation of mature cells. Even if oligodendrocytes or oligodendrocyte-precursor cells are able to myelinate in demyelinated sites of the adult brain, the extension of this process is never as important as it is in our experiments. As Schwann cells, mature oligodendrocytes could be reactivated to a certain extend by an environment where the myelination signal is fully expressed. Moreover transplantation experiments prove that environmental factors even if presently not yet elucidated, appear to be one of the most important factor in jimpy myelin deficiency.

It has to be noted that in the experiments we described, massive rejection was never observed: MBP positive myelin was preserved up to the end of the experiments, which implies that the migrating oligodendrocytes survive and the cells of the graft at the site of implantation appeared healthy at electron microscopic observation.

As presented in the introduction, spontaneous remyelination of demyelination sites has been described in pathological diseases or experimental conditions. Schwann cells and oligodendrocytes participate in this remyelination but Schwann cells appear to be more competitive at least in the adults. It has been shown that transplanted Schwann cells were able to repair experimental demyelination (6, 8) and to restore conduction (42, 43).

Our model of oligodendrocytes transplantation makes it possible to compare the efficiency of both types of myelin forming cells during the normal process of myelination on one hand, in demyelination situations on the other hand (survival, migration and myelination capabilities, reaction of the host, restoration of conduction).

This comparison could bring us more informations on the mechanisms of myelination of both types of myelin forming cells and on the best ways to enhance the remyelination process in adult brain.

ABSTRACT

The experiments briefly described in this chapter consist in transplantation of oligodendrocytes into the new-born shiverer mutant mouse brain. In these experiments the transplanted oligodendrocytes are present in the host brain during the normal myelination process.

The shiverer (shi/shi) mutant mouse is completely deprived of Myelin Basic Protein (MBP), one of the major components of the myelin, and presents an abnormality of myelin compaction with the absence of the Major Dense Line (MDL). Thus, myelin due to implanted MBP positive oligodendrocytes can be detected in the host shiverer brain by immunohistochemistry and electron microscopy.

The transplants are:

– solid fragments of the olfactory bulb from normal newborn normal mouse or from jimpy (jp+) mutant mouse containing immature and/or mature myelin forming cells.

– solid fragments of human embryonic CNS with immature oligodendrocytes.

– isolated-reaggregated adult rat oligodendrocytes.

We have shown that in any case transplanted oligodendrocytes were able to survive, migrate and myelinate in the shiverer mouse brain.

The results are discussed with reference to host-graft interactions in the myelination process and cell movements in normal development.

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