Specialised contacts of endoneurial fibroblasts with macrophages in Wallerian degeneration

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INTRODUCTION

Subplasmalemmal linear density is the term first introduced by Yajima, Fletcher & Suzuki (1977) to describe membrane specialisations of globoid cells in globoid cell leukodystrophy. It is characterised by subplasmalemmal cytoplasmic condensations which have a relatively constant thickness and varied length. The linear, dense structures are always associated with an electron-dense coating along the extracellular surface. On occasions, the linear densities are often found on the apposing plasma membranes of adjacent globoid cells, forming specialised cell contacts. Similar membrane specialisation is a common feature in the cells of the mononuclear phagocytic system (Kawanami, Ferrans & Crystal, 1980) and the occurrence of specialised cell contacts with adjacent homogeneous cells has also been reported in a variety of granulomatous lesions (Ebner & Gebhart, 1971; Judd, Finnegan & Curran, 1975; Caputo & Gianotti, 1979; Kawanami *et al.* 1980). Kawanami *et al.* (1980) have suggested that the ability to form subplasmalemmal linear densities is a specialised property of the cells of the mononuclear phagocytic system. Recently, however, Takahashi & Suzuki (1982) have reported the occurrence of specialised contacts of similar nature between cells of different embryonic origins, i.e. between the astrocyte, which is of ectodermal origin, and the globoid cell which has recently been shown to be of monocytic lineage (Kobayashi *et al.* 1985).

During the study of Wallerian degeneration of peripheral nerves (Ohara, Takahashi & Ikuta, 1986), a membrane specialisation was observed of a similar nature in the apposing plasma membranes of the macrophage and the endoneurial fibroblast. Although fibroblasts are known to have several types of intercellular contact with each other, a contact with the macrophage, which is morphologically and functionally different from the fibroblast, has, to the authors' knowledge, never been documented previously.

In this communication, the ultrastructural features of specialised cellular contacts between fibroblasts and macrophages are described and their possible functional significance is discussed.

MATERIALS AND METHODS

A total of 52 adult BALB/c mice was used. Under Nembutal anaesthesia, the right phrenic nerve was crushed at the neck with a tip of well fitting watchmaker's forceps. The crush lesion, a semitranslucent cleft 0·2–0·3 mm in width was made at the level of the upper margin of the brachial plexus. The procedures were carried

out in sterile conditions under a dissecting microscope. At intervals of 1, 2, 3, 4, 5, 6 7, 8, 9, 12, 16, 21, 35 and 56 days after the operation, groups of three animals were anaesthetised and killed by perfusion through the heart with phosphate buffered 3 % glutaraldehyde–1 % paraformaldehyde, pH 7·3, for 10 minutes at room temperature and further fixed in the same fixative for 6 hours. The phrenic nerves were then removed and cut into segments at constant distances (6, 11 and 16 mm) from the site of crush.

These segments were postfixed with 1 % osmium tetroxide, dehydrated in graded ethanol and embedded in Epon 812. The unoperated, contralateral phrenic nerves as well as the ipsilateral ones from four untreated animals served as controls. Sections 1 μm thick from the distal end of each segment were stained with toluidine blue for light microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a HITACHI 11B electron microscope at 75 kV.

RESULTS

In the control phrenic nerves, the endoneurium contained several fibroblasts and infrequent resident macrophages scattered in the interstices of the nerve fibres. Their cytological features were in accordance with previous reports (Leibovich & Ross, 1975; Oldfors, 1980). In fibroblasts, the nuclear chromatin was finely dispersed and the cytoplasm contained relatively well developed granular endoplasmic reticulum often in a parallel array. Other cell organelles such as the Golgi complex and mitochondria were less conspicuous. The cell surface often showed many pinocytotic vesicles and a few coated pits. On the other hand, the resident macrophages were characterised by peripherally condensed nuclear chromatin and relatively electron-dense cytoplasm which contained numerous free ribosomes and several dense bodies of lysosomal origin. Coated pits and vesicles were common features but, in contrast to fibroblasts, pinocytotic vesicles were never seen. Macrophages were further characterised by possessing many pseudopodial processes.

The occurrence of subplasmalemmal linear density has been reported in mature macrophages (Kawanami *et al.* 1980). In the present study, however, such structures could not be found in the macrophages present in the control.

Following Wallerian degeneration, both fibroblasts and macrophages developed prominent modifications but the cell types were easily distinguishable from each other. In fibroblasts, the nucleoli became apparent and the cytoplasm showed prominent expansion and elongation containing well developed Golgi complexes as well as extensively dilated granular endoplasmic reticulum (Fig. 1). Lipid droplets were occasionally seen but no phagocytosed materials such as myelin debris were observed in the cytoplasm. In addition, microfilaments, about 6 nm in diameter, and microtubules became prominent especially at the periphery. The subplasmalemmal linear densities (Figs. 1, 3) appeared to increase in number with time, although they were also noticed in the control. They were always associated with extracellular fine granular materials which were separated from the plasma membrane by a thin translucent layer. The resulting complex thereby gave some structural resemblance to hemidesmosome-like structures (Gabbiani *et al.* 1972).

The number of macrophages had greatly increased, most of which were probably haematogenous in origin. Their cytoplasm now contained prominent Golgi complexes and many coated vesicles and showed extensive microvillar projections. The presence of much myelin debris and many phagolysosomes were prominent features
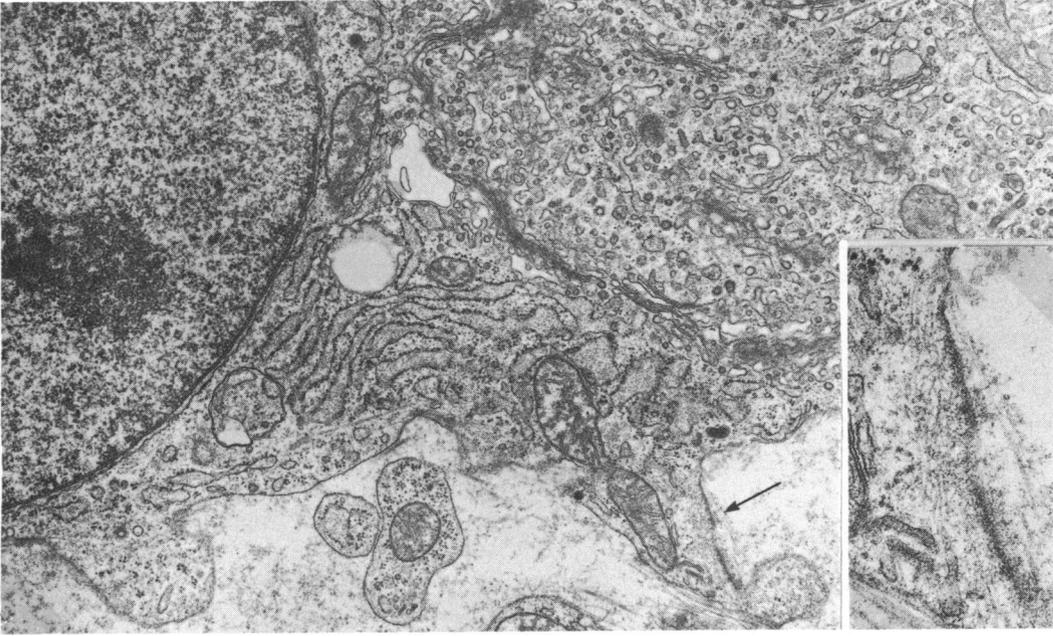
Fig. 1. Electron micrograph of hypertrophied fibroblast on Day 7. Part of the large nucleus and dilated granular endoplasmic reticulum as well as a prominent Golgi complex are seen in the relatively electron-lucent cytoplasm. An area of membrane specialisation, subplasmalemmal linear density, is also seen on its surface (arrow). × 16000. Inset: higher magnification of the area indicated by the arrow. Many microfilaments are seen converging at the site of subplasmalemmal density which is associated with fine granular coating materials along its external surface. × 43000.

Fig. 2. Electron micrograph showing subplasmalemmal linear density (arrow) of a macrophage which contains several disintegrated myelin sheaths and lipid droplets. *A*, regenerating axon. Day 12. × 28 500.
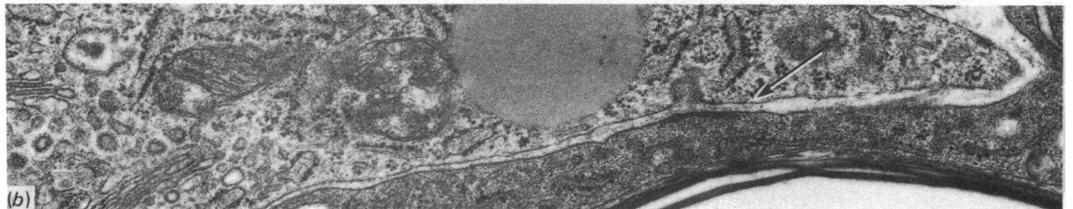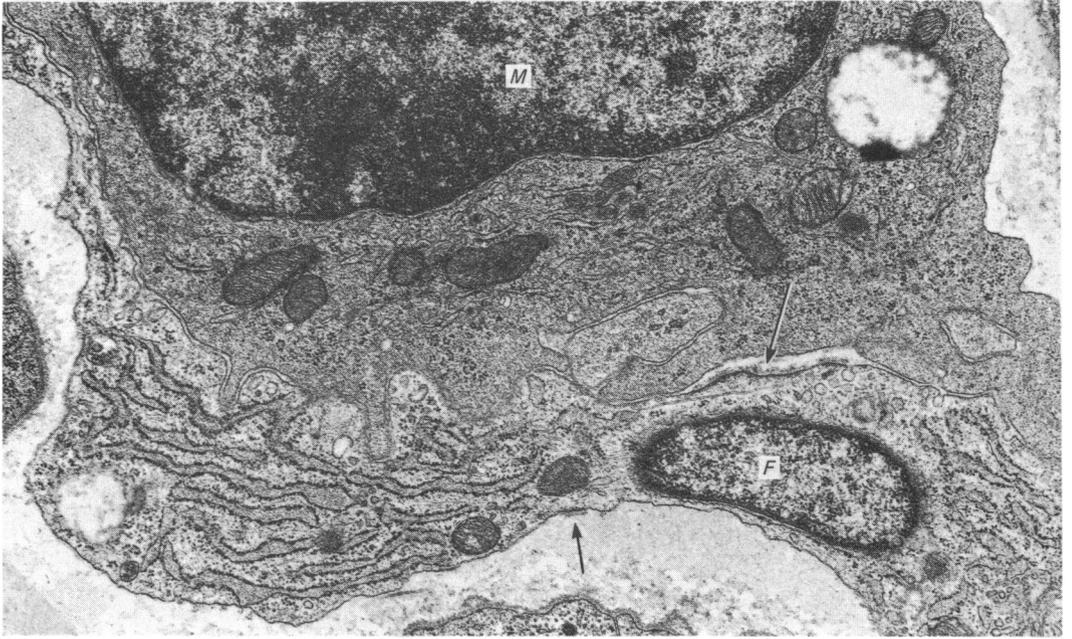
Fig. 4.
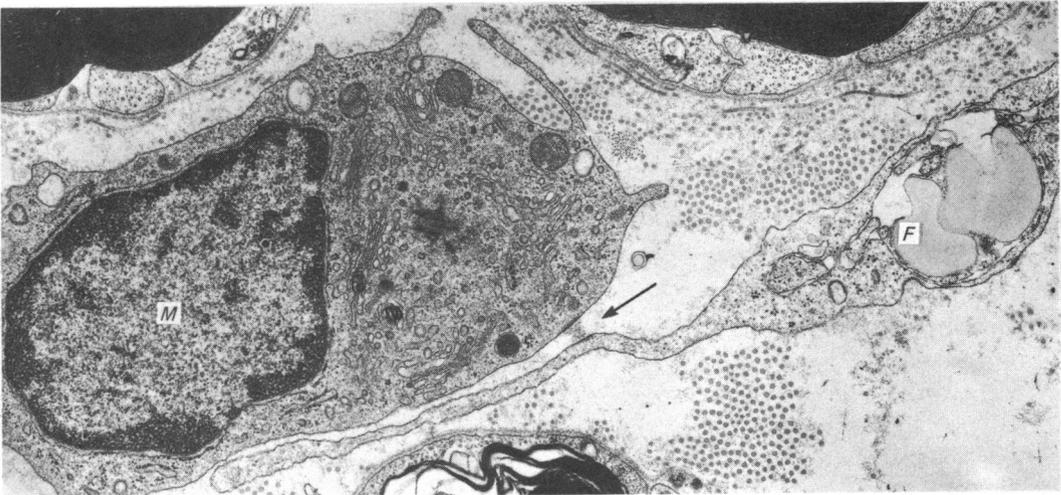
Fig. 6.

Fig. 3. Electron micrograph showing close apposition between a fibroblast (*F*) and a macrophage (*M*) without any specialised contact. The fibroblast shows two subplasmalemmal linear densities of varied length (long and short arrows). Day 9. × 17500.

Fig. 4(*a–b*). (*a*) This electron micrograph demonstrates a specialised cell contact comprising a paired symmetrical subplasmalemmal linear density (arrow), between the fibroblast (*F*) and the macrophage (*M*) which contains a large mass of myelin debris. The fibroblast also shows several unilateral and varied lengths of subplasmalemmal densities (arrowheads). Day 12. × 28000. (*b*) Serial section reveals direct continuation of a coated pit with subplasmalemmal linear density forming a specialised cell contact which is asymmetrical. × 28000.

Fig. 5. Another example of a paired subplasmalemmal linear density (arrow) between a macrophage (*M*) and a slender cytoplasm of a fibroblast (*F*). Note the shorter length of the membrane specialisation compared to those of Figure 4. Day 12. × 29000.

Fig. 6. Short specialised contact (arrow) between a macrophage (*M*) and a slender cell process of a fibroblast (*F*) with a wider intercellular distance. Day 5. × 15500.

in their cytoplasm. In addition, subplasmalemmal linear densities of various lengths were often observed (Fig. 2). While the subplasmalemmal linear densities of the fibroblast were morphologically indistinguishable from those of the macrophage, their sites of occurrence showed some differences. Those in the fibroblast were evenly distributed over the entire cytoplasmic surface, including the slender cell processes. On the other hand, those in the macrophage were always found on the cytoplasmic surface but not on the pseudopodial cell processes. On occasion, a fibroblast and macrophage were found in close contact with each other over large areas of their cell membranes (Fig. 3).

Besides the unilateral occurrence of subplasmalemmal linear densities of fibroblasts and macrophages, specialised intercellular contacts of varied length were often found between these two different kinds of cells (Figs. 4–6). In these specialised cell contact areas, the interspace of the apposing plasma membranes of fibroblast and macrophage was filled with fine granular materials and the cytoplasm immediately subjacent to these materials constantly showed fine granular condensations identical to those occurring singly. The intercellular space was bisected by a denser midline stratum (Figs. 4, 5). The intercellular distance was by no means constant (Fig. 6) although it was usually about 38 nm wide. Furthermore, the subplasmalemmal linear densities forming cell contacts were not always symmetrical (Fig. 4b).

The presence of coated pits and vesicles was often a prominent cytoplasmic feature of both fibroblast and macrophage, forming subplasmalemmal linear densities (Figs. 4, 6). They were often observed on the cell surface in direct association with subplasmalemmal linear densities (Fig. 4b).

The specialised cell contacts between fibroblasts and macrophages could not be found in the later stage of Wallerian degeneration, when myelin debris was largely removed with the disappearance of macrophages from the endoneurium.

DISCUSSION

The present study shows clearly that a specialised intercellular contact develops between endoneurial fibroblasts and macrophages in the course of Wallerian degeneration of peripheral nerves. The occurrence of such contacts between these two morphologically and functionally different kinds of cells does not appear to have been documented previously.

The specialised contact reported here ultrastructurally consists of paired subplasmalemmal linear condensations in the apposing fibroblasts and macrophages with fine granular materials filling the intercellular space. This space is bisected by a denser midline stratum. These features are essentially identical to those of the specialised contacts described previously between cells of the mononuclear phagocyte system in a variety of granulomatous lesions of the skin (Ebner & Gebhart, 1971; Judd et al. 1975; Caputo & Gianotti, 1979) and of the lung (Kawanami et al. 1980). According to Kawanami et al. (1980), the specialised contact is apparently formed when subplasmalemmal linear densities occur in pairs and, when they occur unilaterally, it is associated with a basal lamina-like external coating layer. Furthermore, microfilaments are selectively associated with these specialised cell membrane areas.

The unilateral subplasmalemmal linear density observed in the endoneurial fibroblast (Fig. 1) seems to be consistent with the previously known hemidesmosome-like membrane specialisations of the activated fibroblast (Gabbiani et al. 1972).

Furthermore, it is also indistinguishable from the unilateral subplasmalemmal linear density which occurs in the macrophage (Fig. 2). It seems reasonable to assume that the paired subplasmalemmal linear densities forming the specialised contacts between fibroblasts and macrophages have apparently originated from unilateral subplasmalemmal linear densities of the fibroblast and the macrophage respectively.

The specialised cell contacts reported here also show close resemblance to those reported by Takahashi & Suzuki (1982), who first described the occurrence of specialised contacts between cells of different embryonic origin. In their study using the twitcher mouse, an authentic murine model of globoid cell leukodystrophy, the globoid cell, which belongs to the cells of the mononuclear phagocytic system (Kobayashi *et al.* 1985), was found to have specialised contacts with the astrocyte. Taken together with the present observations, it strongly suggests that the ability to form this type of intercellular contact is not a specific property of the cells of the mononuclear phagocytic system.

In the present study, many coated vesicles were often seen in the cytoplasm of both fibroblast and macrophage, and some of them were found in close association with the subplasmalemmal linear densities forming the specialised contacts (Fig. 4). A similar close association of coated vesicles to paired or unpaired subplasmalemmal linear densities has been reported in the cells of the mononuclear phagocytic system (Caputo & Gianotti, 1979; Kawanami *et al.* 1980) and in the astrocyte in the twitcher mouse brain (Takahashi & Suzuki, 1982). Caputo (1979) suggested that coated vesicles released from the Golgi apparatus migrate to the cell surface and release materials which would form the 'dense material area', favouring the attachment of adjacent histiocytes. Since such a close association of coated vesicles with specialised contact areas has never been mentioned previously in any other types of intercellular 'junctions' (Farquhar & Palade, 1963; McNutt & Weinstein, 1973), it seems permissible to say that this feature is unique to this type of intercellular 'contact'.

It has been reported that fibroblasts have several forms of intercellular contact with each other. They include gap junctions (Gilula *et al.* 1972; Pinta da Silva & Gilula, 1972), close contacts (Shore, Berkovits & Moxham, 1981) and intermediate or simplified desmosome-type junctions (Ross & Greenlee, 1966; Greenlee & Ross, 1967; Parry, 1970; Gabbiani, Ryan & Majno, 1971; Shore *et al.* 1981; Sima & Westfall, 1982). Among these examples, the intercellular contacts between fibroblasts and macrophages presented in this study seem to bear some superficial resemblance to the intermediate or simplified desmosome type junction. However, they are quite different from each other in certain respects. The former type of contact has a wider and often more variable intercellular distance, with the presence of a denser midline stratum. Furthermore, subplasmalemmal linear densities are sometimes asymmetrical. By contrast, the latter type of contact is characterised by an increased density of the apposing cell membrane and underlying cytoplasm of the fibroblast as well as a narrow intercellular distance of about 10 to 20 nm which also shows an increase in density. It is always symmetrical.

There have been several reports suggesting functional interaction between fibroblasts and macrophages in certain pathological conditions. For example, fibroblasts together with macrophages are known to participate in granuloma formation in wound healing (Leibovich & Ross, 1975). It was suggested that macrophages secrete substances which regulate the proliferation of fibroblasts (Leibovich & Ross, 1976; Korn, Haluschka & Leroy, 1980; Martin, Gimbrone, Unaue & Cotran, 1981). Fibroblasts, on the other hand, have been shown to secrete a substance, fibronectin,

which may promote macrophage migration and facilitates its phagocytic activity (Van de Walter, Shroeder, Crenshaw & Hynes, 1980). However, so far there has been little morphological evidence of cellular interaction between these two different kinds of cells. The specialised cell contact described here clearly indicates an occurrence of cell-to-cell interaction between the fibroblast and macrophage, although its precise role has yet to be determined.

In the present study, it is noteworthy that the development of the contact is apparently closely related to the time course of Wallerian degeneration, being most prevalent at the stage when myelin debris removal by macrophage is most prominent in the endoneurium. Macrophages are well known to behave as active phagocytic cells in the early stage of Wallerian degeneration (Gibson, 1979) and eventually disappear from the endoneurium. However, little attention has been paid to the role of the endoneurial fibroblast. The present observations suggest strongly that the pre-existing fibroblasts also play an important role in this particular condition, through the formation of specialised cell contacts with infiltrating macrophages. The specialised contact is of apparently a short lived nature.

SUMMARY

Wallerian degeneration was induced by crushing the mouse phrenic nerve at the neck. During a chronological study, a specialised cell contact was often observed between the activated endoneurial fibroblast and the macrophage at the period when the removal of myelin debris by macrophages was prominent in the endoneurium. The specialised contact was characterised by paired subplasmalemmal linear condensations with a relatively constant thickness and varied length. It was sometimes asymmetrical. In these specialised cell membrane areas the intercellular space was filled with fine granular material showing a midline denser stratum. Coated vesicles were occasionally found in association with the subplasmalemmal densities. The specialised contacts with these features are quite different from any type of previously described cell contact between fibroblasts but are morphologically identical to those reported between cells of the mononuclear phagocytotic system. The significance of specialised contacts between the fibroblasts and macrophages in Wallerian degeneration is discussed.

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