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Morphology and Functional Roles of Synoviocytes in the Joint

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Summary. **The joint capsule exhibits a unique cellular lining in the luminal surface of the synovial membrane. The synovial intimal cells, termed synoviocytes, are believed to be responsible for the production of synovial fluid components, for absorption from the joint cavity, and for blood/synovial fluid exchanges, but their detailed structure and function as well as pathological changes remain unclear. Two types of synoviocytes, macrophagic cells (type A cells) and fibroblast-like cells (type B cells) have been identified. Type A synoviocytes are non-fixed cells that can phagocytose actively cell debris and wastes in the joint cavity, and possess an antigen-presenting ability. These type A cells, derived from blood-borne mononuclear cells, can be considered resident macrophages (tissue macrophages) like hepatic Kupffer cells. Type B synoviocytes are characterized by the rich existence of rough endoplasmic reticulum, and dendritic processes which form a regular network in the luminal surface of the synovial membrane. Their complex three-dimensional architecture was first revealed by our recent scanning electron microscopy of macerated samples. The type B cells, which are proper synoviocytes, are involved in production of specialized matrix constituents including hyaluronan, collagens and fibronectin for the intimal interstitium and synovial fluid. The proliferative potentials of type B cells *in loco* are much higher than type A cells, although the transformation of subintimal fibroblasts into type B cells can not be excluded. In some mammals, type B cells show features suggesting endocrine and sensory functions, but these are not recognized in other species. The synoviocytes, which form a discontinuous cell layer, develop both fragmented basement membranes around the cells and junctional apparatus such as desmosomes and gap junctions. For an exact understanding of the mechanism of arthritis, we need to establish the morphological background of synoviocytes as well as their functions under normal conditions.**

Joints that permit free movement of the bones are termed diarthroses. In the diarthrodial joints, there is a liquor-containing cavity enveloped by a stuff fibrous sheath. The wall of the joint capsules is composed of two fairly distinct layers: an external consisting of a thick, dense connective tissue called the fibrous layer (stratum fibrosum), and an inner one which is the synovial membrane or synovium, this being more cellular in nature and thought to secrete the lubricant fluid of the joint cavity.

The synovial membrane contains in its superficial layer a unique cellular lining which is one to three cells deep and is called the synovial intima. The thickness and morphology of the synovial membrane differs considerably according to its types, which were originally classified by KEY (1928) as areolar, adipose and fibrous (cf. WYSOCKI and BRINKHOUS, 1972). These three types have been differentiated on the basis of the composition of the connective tissues underlying the intimal cellular lining. The areolar synovial membrane is composed of a considerably thick cellular intima and subintimal loose connective tissue, rich in blood vessels. The adipose type has a single flattened cell layer and subintimal adipose cell layer. In the fibrous type, a thin cellular lining directly contacts dense collagenous tissue. Mixed forms are present in the junctional region between the different types of synovium. In the areolar type, the synovial membrane possesses an undulating or folded surface. When these folds, conspicuously tall in large sized animals, protrude into the cavity, they are interpreted as synovial villi (SHIVELY and VAN SICKLE, 1977; LEACH et al., 1988; SHIKICHI et al., 1999) (Fig. 1). This region, rich in cellular elements and blood vessels, may be responsible for homeostasis of the joint cavity such as the production of synovial fluid, clearance of intra-articular debris, and regulation of

Fig. 1. Scanning electron microscopy of synovial villi. In the horse joint the articular surface is covered by densely arranged villi showing various shapes and lengths. ×25

immunological events. Therefore, the areolar synovial membrane has represented a favorite sample for the investigation of synoviocytes. In this paper, we review the morphological aspects of synoviocytes in consideration of their functions.

Cell types in the synovial intima

Identification and characterization of synovial lining cells, frequently called synoviocytes, have been performed mainly by transmission electron microscope (TEM). Although there has been controversy regarding the classification of synoviocytes, it is generally accepted that the synovial intima contains two morphologically different types of cells: macrophages (type A cells) and fibroblast-like cells (type B cells) (BARLAND et al., 1962; GRAABAEK, 1982, 84; EDWARDS, 1994) (Figs. 2, 3). The intermediate type (transitional cells) had been identified as the third type of synoviocyte (BARLAND et al., 1962; SHANNON and GRAHAM, 1971; FELL et al., 1976; NISHIJIMA, 1981), leading us to the idea that these three types of cells are merely functional variants of the same cell lineage. However, the existence of the third type was refuted by detailed TEM observations of rat joints using serial sections (GRAABAEK, 1982, 1984).

Some previous TEM studies documented the predominancy of type A cells in the human (BARLAND et al., 1962), guinea pig (WYLLIE et al., 1964), rat (ROY and GHADIALLY, 1967a), cat (GROTH, 1975) and cattle (LEACH et al., 1988), in disagreement with histochemical and other TEM studies (KREY and COHEN, 1973; JOHANSSON and REJNÖ, 1976; OKADA et al., 1981b; GRAABAEK, 1982, 1984; HORKY, 1984; EDWARDS, 1994; KITAMURA et al., 1999). This discrepancy may be explained by the uneven distribution of type A cells as well as the misinterpretation of many type B cells as intermediate cells (cf. GRAABAEK, 1982). On the other hand, JILANI and GHADIALLY (1986) insisted on the existence of an age-associated change in synoviocyte population: a significant reduction in number of type B cells and a relative increase of type A cells in older animals. At present, it is generally believed that type B cells are the predominant and proper cells in the synovial membrane. According to KREY and COHEN (1973) and OKADA et al. (1981b), type B synoviocytes occupy 75% and 80% of all synoviocytes in the rabbit and mouse, respectively. In organ cultures of the rabbit synovial membrane, macrophagic, type A cells disappeared early from the synovial lining, while type B cells came to occupy more than 90% of cells by the 3rd day of culture (KREY and COHEN, 1973). Mitosis was associated predominantly with type B cells in culture and *in vivo* (KREY and COHEN, 1973; GHADIALLY, 1983; LEACH et al., 1988). The decreased percentage of macrophagic synoviocytes in the organ culture can be explained not only by the proliferation of type B cells but also by the movement of macrophages into the culture medium. This suggests the active migration of type A cells into the articular cavity *in vivo*, in agreement with the rich existence of macrophagic cells in the normal synovial fluid (CURTISS, 1964).

The nomenclature of synovial intimal cells has been confusing. For macrophagic synoviocytes such names as type A cells, synovial macrophages, M cells, A (absorptive) cells and V (vacuole) cells have been given, whereas for fibroblast-like cells, type B cells, synovioblasts, F cells, S (secretory) cells and ER (endoplasmic reticulum) cells are found. In this review article on synoviocytes, the classical terms "type A and type B synoviocytes" have been adopted.

Morphology and function of type A cells

Type A synoviocytes are immunoreactive to several monoclonal antibodies against macrophages or macrophage-derived substances (FORRE et al., 1982; BURMESTER et al., 1983; HOGG et al., 1985; IZUMI et al., 1990). They also express a major histocompatibility class (MHC) II molecule and Ia antigen which play key roles in antigen-presentation in the initial stages of the immune response (SHIOZAWA et al., 1983; IZUMI et al., 1988, 1990; ATHANASOU, 1995; NOZAWA-INOUE et al., 1998). Lysosomal enzymes, such as non-specific esterase (NSE), acid phosphatase and cathepsins B,

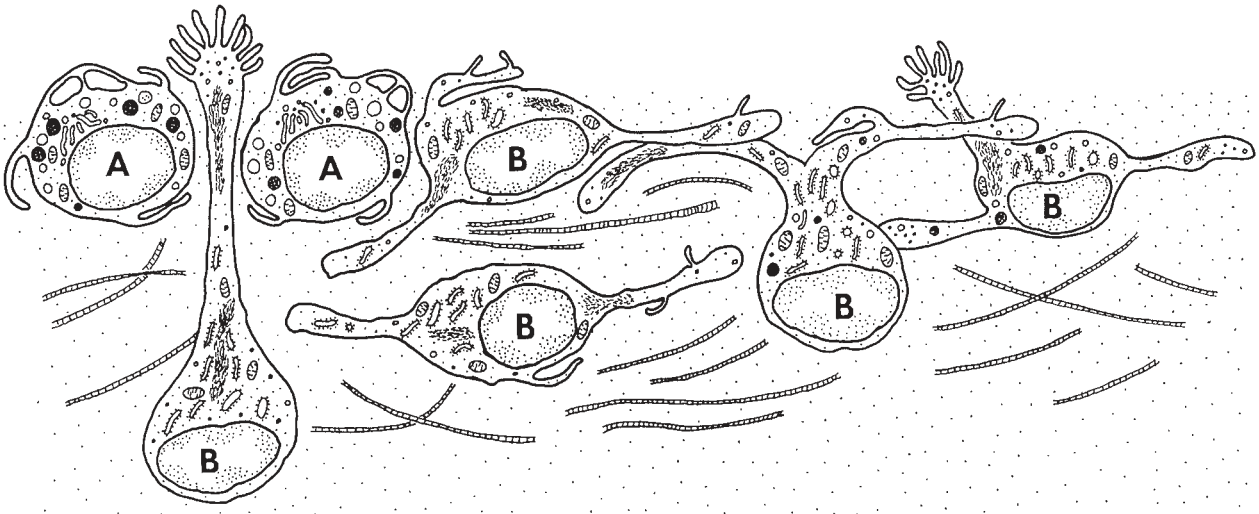


Fig. 2. Schematic drawing showing the location and structure of two types of synoviocytes. Type A cells (*A*) are located at the superficial layer of the synovial intima, while type B cells (*B*) characterized by the cytoplasmic processes, are present at various depths, frequently in the deeper layer of synovial intima.

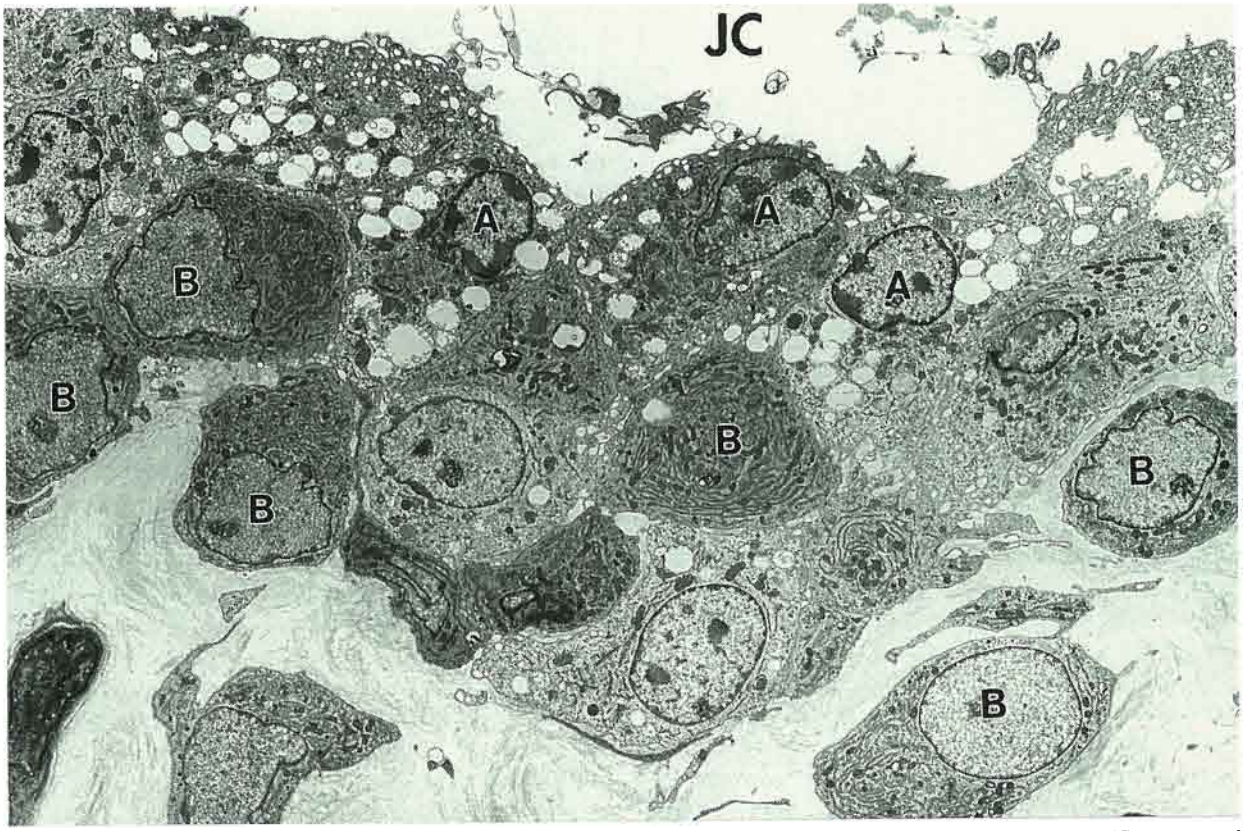


Fig. 3. Ultrastructures of two types of synoviocytes in the temporomandibular joint of the rat (Courtesy of Prof. T. MAEDA, Niigata University School of Dentistry). Type A cells (*A*), rich in vacuoles, occupy the superficial layer of the intimal cell lining. Type B cells (*B*), which contain developed endoplasmic reticulum, are located far from the joint cavity (*JC*). ×2,800

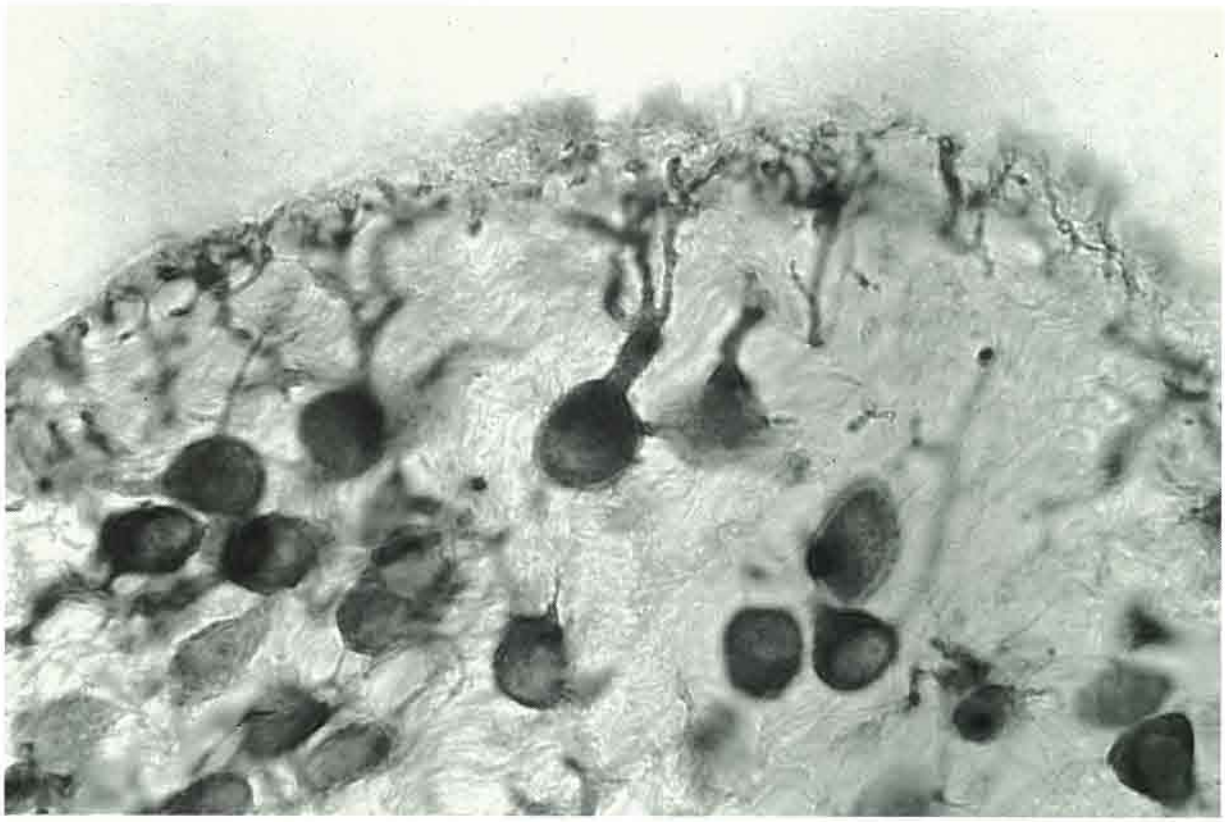
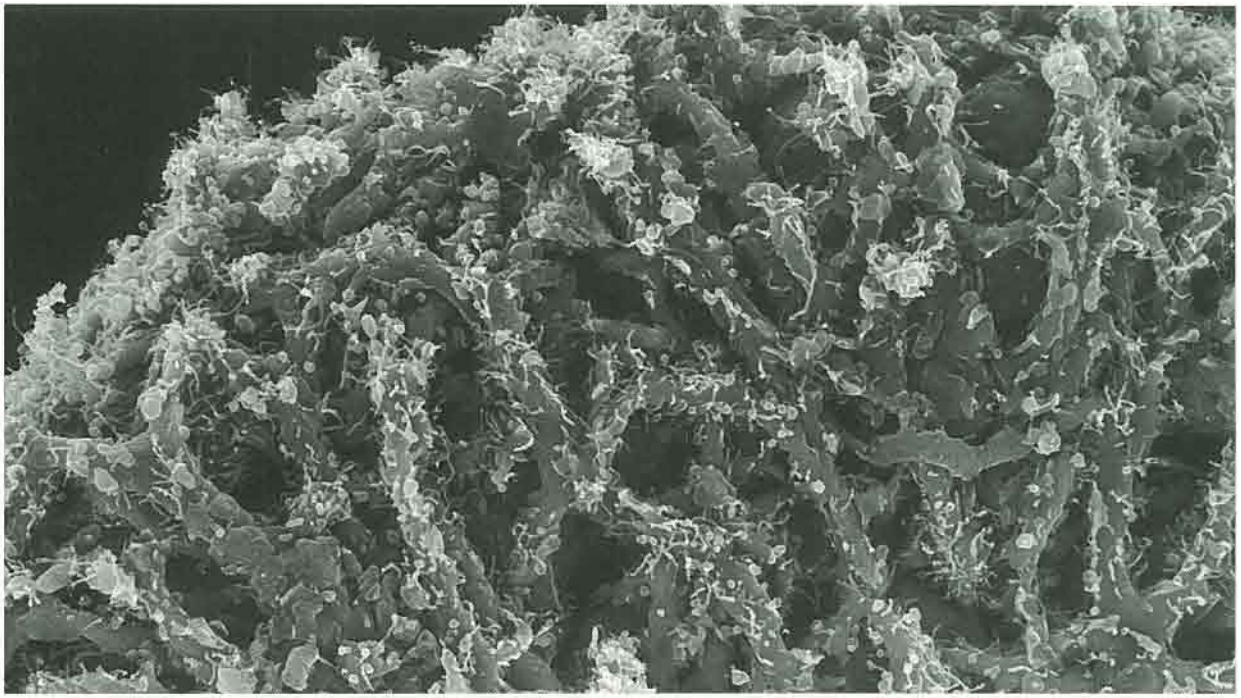


Fig. 4. Immunostaining of synoviocytes in the horse joint by use of an anti-PGP 9.5 serum. Immunoreactive cells, which are localized in the deeper layer of the synovial intima, extend processes toward the joint cavity and ramify in a dendritic fashion forming a plexus of fine processes on the intimal surface. ×1,200

D, and L, are also useful for the cytochemical detection of type A cells (GRAABAEK, 1985a; KIYOSHIMA et al., 1993, 1994; EDWARDS, 1994). There is a possibility that, under inflammatory conditions, the production of these enzymes, especially cathepsins, is elevated and they are released into the extracellular matrix to induce tissue damage (KIYOSHIMA et al., 1994). According to ^{3}H-thymidine labeling of the synovial membrane, the labeling index of macrophagic cells is extremely low or null, as compared with non-macrophagic synoviocytes (IZUMI et al., 1990). In developing stages of the rat knee joint, macrophagic, type A cells emerge only after the appearance of blood vessels in the articular interzone (IZUMI et al., 1990). Observations of the mouse synovial membrane at the neonatal stage have also demonstrated the frequent occurrence of type A cells around capillaries (OKADA et al., 1981b). The macrophagic origin of type A cells was supported by the fact that in macrophage-deficient mice (osteopetrotic *op/op* mice), the synovial membrane also lacks this type of synoviocyte (NAITO et al., 1991). Under inflammatory conditions, a great number of macrophagic cells derived from the blood are introduced into the synovial intima (HOGG et al., 1985; NOZAWA-INOUE et al., 1998).

Type A cells are usually round and located in the upper part of the synovial intima; about half of the cell bodies are projected over the surface line of the synovial membrane (Fig. 6). Type B cells are commonly found further from the joint lumen, when the synovial lining is more than one cell thick (CUTLIP and CHEVILLE, 1973: LEACH et al., 1988). The rat synovial membrane observed by MURASHIGE (1981) contained distinct two-cell layers comprising an adluminal layer consisting mostly of type A cells and a deeper layer consisting of type B cells (Fig. 3). The surface of type A cells is covered by microvilli and microplicae (filopodia or lamellipodia) which are structures unique to typical macrophages (Fig. 6). Thus, type A cells exhibit cauliflower-like features under the scanning electron microscope (SEM) (DATE, 1979). The ultrastructure of type A cells under the TEM is characterized by the rich existence of



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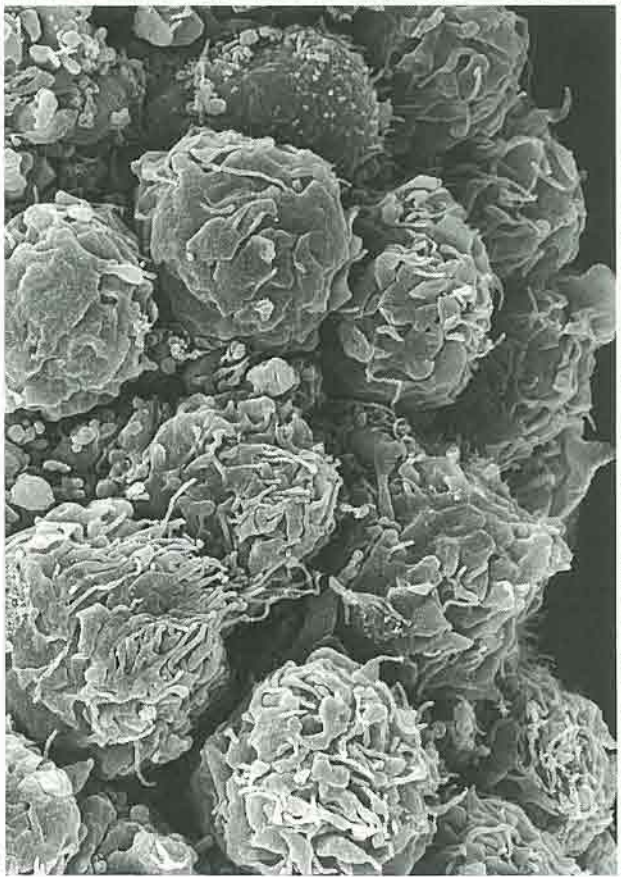

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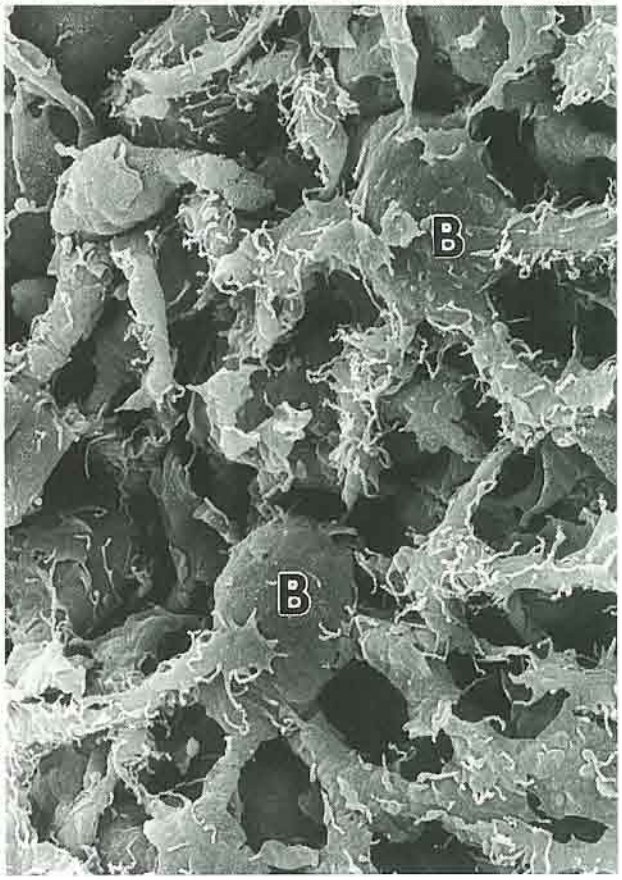

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Figs. 5–7. Scanning electron microscopic observation of synoviocytes in macerated samples from the horse joint. Type B cells (*B*) extend several thick primary processes radially (**Fig. 7**) and form a coarse network of processes covering the intimal surface (**Figs. 5** and **7**). **Fig. 6** shows aggregated type A cells which are equipped with microplicae and microvilli on their cell surface. Fig. 5: ×2,300, Fig. 6: ×3,400, Fig. 7: ×2,500

lysosomes and large empty-appearing vacuoles as well as pinocytotic vesicles, often in close proximity to the joint cavity (SOUTHWICK and BENSCH, 1971) (Fig. 3). They possess a prominent Golgi complex but little rough endoplasmic reticulum (WYLLIE et al., 1964; ROY and GHADIALLY, 1967a; SHANNON and GRAHAM, 1971). Although the content and function of the vacuoles remains unknown, intraarticularly injected horseradish peroxidase (HRP) is incorporated by the pinocytotic (coated) vesicles, and subsequently by the vacuoles, suggesting that the vacuoles grow up through the fusing of small vesicles.

Type A synoviocytes are active in the uptake of foreign substances injected into the joint cavity such as iron dextran, colloidal carbon, colloidal gold, latex particles or HRP (KEY, 1926; BALL et al., 1964; SHANNON and GRAHAM, 1971; SOUTHWICK and BENSCH, 1971; OKADA et al., 1981b; GRAABAEK, 1982, 1985b; SENDA et al., 1999). According to GRAABAEK (1985b), the uptake of HRP by type A cells was most vigorous at 5 min after injection at the injection of a low dose of HRP (0.1 mg/ml); HRP reaction products were localized in coated pits/vesicles, smooth-walled vesicles and large vacuoles at the periphery of the cells. The number of HRP-positive structures decreased as early as 15 min. On the other hand, uptake by type B cells was only seen at intraarticular injections of higher concentrations of HRP. Physiologically, the type A cells may absorb and degrade extracellular constituents, cell debris, microorganisms, and antigens in the synovial fluid and intimal matrix, by the use of well-developed vesicular and lysosomal systems. Since the synovial cells take up significant amounts of proteins, they may be capable of modifying the protein composition of the synovial fluid.

Although there are few reports in the literature on the distributional density of type A cells, these cells may be unevenly distributed in the synovial membrane. It is supposed that the distribution of type A cells is denser in the areolar synovial membrane than in other types of the synovial membrane. In the horse joint, type A cells tend to gather at the tips of synovial villi and are free from both the basal part of the villi and the fibrous synovial membrane (SHIKICHI et al., 1999). The rich existence of type A cells in villous tips is reasonable in consideration of their contacting and uptaking substances and cellular elements wandering in the joint cavity. They can carry ingested materials to lymphatic channels of the subintima, and eventually remove them from the joint (KEY, 1926; AHLBERG et al., 1969; MORAWSKI et al., 1995; ITONAGA et al., 1997). In contrast, SENDA et al. (1999) observed a gathering of latex particles-laden type A cells in the synovium-cartilage junction, which is rich in vascularity and corresponds to the areolar synovium, and concluded that intra-articular particles might be drained via the blood circulation.

Staining characteristics of type B cells

The cell lineage of type B cells as well as their function remain unknown, possibly due to the lack of specific cell markers. For the cytochemical detection of type B cells, the following antisera and markers have been reported to be available. A monoclonal antibody termed Mab 67 was able to stain the cell surface of type B synoviocytes, but also reacted with connective tissue fibers and vascular elements in humans (STEVENS et al., 1990). Antisera against an enzyme prolyl hydroxylase which is involved in collagen synthesis labeled type B synovial lining cells but could not distinguish these from subintimal fibroblasts (WILKINSON et al., 1992). Vascular cell adhesion molecule-1 (VCAM-1) antibody could label intensely type B cells, but stained, to a lesser extent, some macrophages (WILKINSON et al., 1993). Another immunohistochemical study demonstrated that synoviocytes in the limb joints express heat shock proteins (Hsp) which protect cells against irreversible protein damage and are involved in adaptive responses to stress lesions (DA SILVA, 1991). Recently, NOZAWA-INOUE et al. (1999b) showed that Hsp 25 is a useful marker substance for the identification of type B cells in the rat temporomandibular joint, with a still broad distribution in chondrocytes and vascular endothelial cells. Uridine diphosphoglucose dehydrogenase (UDPGD), which is a rate-limiting enzyme for hyaluronan (hyaluronic acid) synthesis, appears to be more specific to type B cells than Mab 67 antigen and other substances mentioned above (WILKINSON et al., 1992). However, histochemistry for UDPGD can not stain the entire extension of cells because of lower reactivity and limited intracellular localization (EDWARDS, 1994).

On the other hand, our immunostaining of the horse synovial membrane for protein gene product (PGP) 9.5, a neuron-specific ubiquitin C-terminal hydrolase, demonstrated the selective localization of the immunoreactivity in type B synoviocytes (KITAMURA et al., 1999) (Fig. 4). PGP 9.5 is a cytosolic protein diffusely distributed throughout the cytoplasm and may exist richly in a cell, as suggested by the fact that PGP 9.5 is a major protein component of the neuronal cytoplasm (DORAN et al., 1983). Therefore, immunohistochemistry for PGP 9.5 revealed the entire shapes of type B synoviocytes, including characteristic dendritic processes, at both light and electron microscopic levels (KITAMURA et al., 1999). A

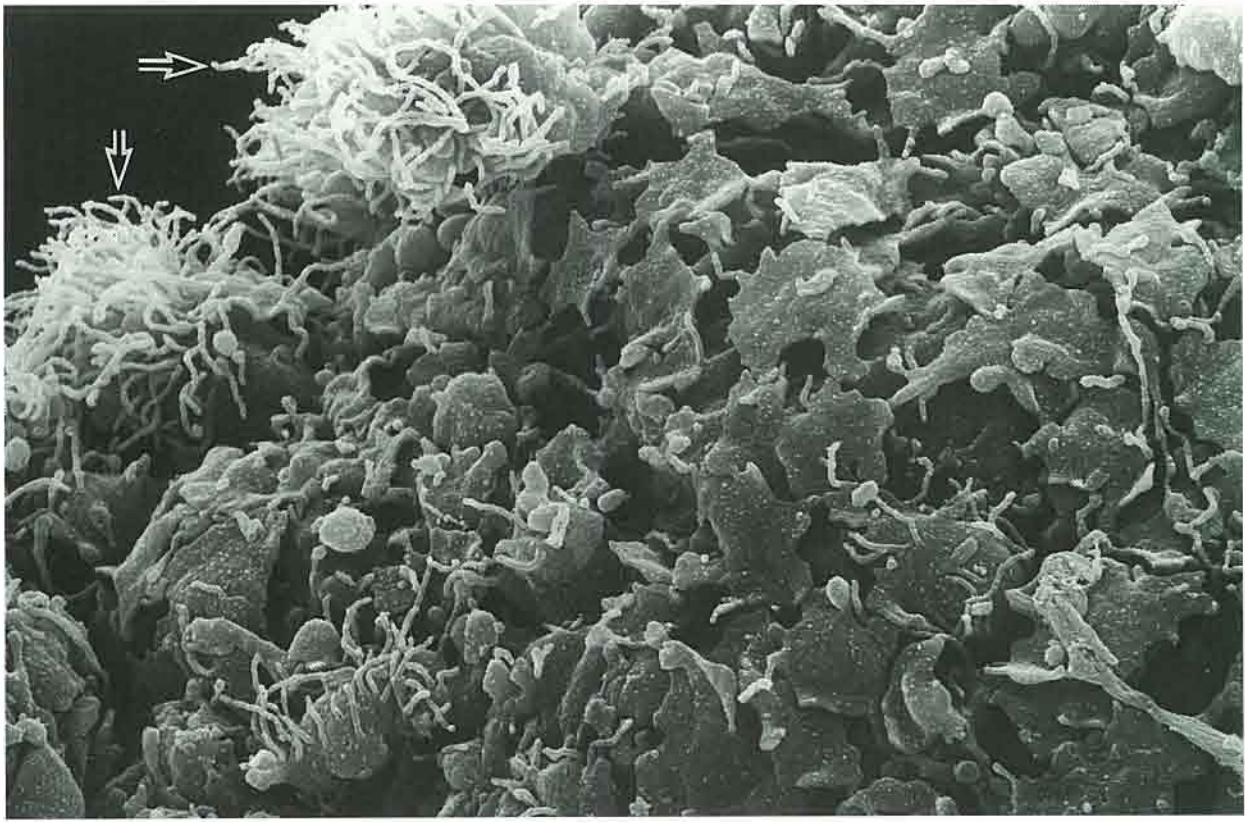


Fig. 8. A closer view of the intimal surface by SEM (macerated sample from the horse joint). Type B cells develop fine membranous processes and cover the intimal surface, although the covering is fragmental. *Arrows* indicate intraarticular projections of type B cells covered by long microvilli. ×62,500 (From SHIKICHI et al., 1999)

similar stainability with the PGP 9.5 antiserum was obtained in the synovial bursae and synovial tendon sheath of the horse. Thus, we can conclude that PGP 9.5 is the most reliable marker for the cytochemical detection of type B cells, at least in the horse. However, we failed to confirm these staining results in other animals, except for the detection of only a few immunoreactive synoviocytes in the joints of cattle and sheep. Further studies will be required to determine whether the existence of PGP 9.5 in synoviocytes is unique to the horse, or whether related substances, which can not be detected by our antiserum against the human molecule, are generally present in the synoviocytes of other mammals.

Ultrastructure and secretory function of type B cells

Ultrastructural characteristics of type B cells have been well-established by many TEM studies. These cells possess a relatively large nucleus, often deeply indented, compared with the small amount of surrounding cytoplasm. One of the most conspicuous features of type B cells is the development of rough endoplasmic reticulum distributed throughout the cytoplasm, suggesting active protein synthesis. Thus, SOUTHWICK and BENSCH (1971) termed these synoviocytes ER cells against V (vacuole) cells for type A cells. Some degree of cisternal dilatation of the rough endoplasmic reticulum is frequently encountered. Libosomes are easily identified on the membrane of the rough endoplasmic reticulum and also as clumps lying free in the cytoplasm. Unlike type A cells, the type B cells have few vesicles and vacuoles and a less prominent Golgi apparatus in the cytoplasm (SHANNON and GRAHAM, 1971; JOHANSSON and REJNÖ, 1976; JILANI and GHADIALLY, 1986). In contrast, OKADA et al. (1981a) and GRAABAEK (1982, 1984) documented a well-developed Golgi complex in type B cells rather than type A cells in the mouse and rat. Type B cells contain a small number of large vacuoles at the apical end of their processes. Since the vacuoles are an important ultrastructural marker for type A cells, this leads us to a misinterpretation

of type B cells as so-called intermediate cells (GRAABAEK, 1982).

The plasma membrane of type B cells reveals numerous, small smooth-walled invaginations, 60–90 nm in diameter (WYLLIE et al., 1964; KREY and COHEN, 1973; GRAABAEK, 1984; NOZAWA-INOUE et al., 1998; SHIKICHI et al., 1999), similar to caveolae which develop in smooth muscle cells, although they might be often identified as pinocytotic vacuoles (GHADIALLY and ROY, 1966; GHADIALLY et al., 1978). Type B cells are known to have well-developed longitudinal bundles consisting of microfilaments and intermediate filaments (10 nm filaments) in their cytoplasm (FELL et al., 1976; OKADA et al., 1981b; SHIKICHI et al., 1999). Histochemistry for the detection of actin demonstrated that the tufts of filaments may contain actin filaments (our unpublished data). The development of actin filaments and caveola system in type B synoviocytes is reminiscent of their relation to smooth mucle cells or myofibroblasts. Furthermore, type B cells frequently contain a single cilium which usually projects from the bottom of a deep invagination of the cell membrane (WYLLIE et al., 1964; SCHUMACHER, 1975; GRAABAEK, 1984; SHIKICHI et al., 1999).

A secretory function for type B cells has been long suggested. Type B cells secrete collagens, fibronectin (MATSUBARA et al., 1983; MAPP and REVEL, 1985), hyaluronan (hyaluronic acid) (YIELDING et al., 1957; ROY and GHADIALLY, 1967b) and other glycosaminoglycans into the intimal interstitium and joint cavity. Hyaluronic acid is a representative proteoglycan which is present in relatively high concentrations in the synovial fluid of the normal joint (3–4 mg/ml) (HAMERMAN and SCHUSTER, 1958). The type B cells are also considered to be involved, directly or indirectly, in the control of the protein composition of synovial fluid. Interestingly, PGP 9.5 contained in the cytoplasm of type B cells is detectable in the synovial fluid, and its concentration changes under inflammatory conditions, suggesting the direct release of this protein into the joint cavity (our unpublished data). However, most protein in synovial fluids as well as small molecules, such as glucose, urea, and electrolytes, are derived from plasma within subintimal vascular beds. The relative concentrations of proteins with greater molecular weight are different between plasma and synovial fluid; their concentrations are usually less in the synovial fluid than those in the serum. In contrast, plasma proteins of lower molecular weight are present in synovial fluid in relatively greater concentrations, although certain exceptions have been described (CURTISS, 1964; SHANNON and GRAHAM, 1971). Thus, a barrier in the synovial membrane, designated the blood-joint barrier, offers resistance to a passive and free exchange of some substances. This may be not attributed to the blood vessels, since fenestrated capillaries occur in any region of the synovial membrane (KREY and COHEN, 1973; SCHUMACHER, 1975; HAMANISHI, 1978; NISHIJIMA, 1981). Although the barrier function is established partially by the preferential permeability of the ground substance in the synovial intima, the involvement of synoviocytes is undeniable, as mentioned below.

Unique cytoplasmic processes at the luminal surface of synovial membrane

One of morphological characteristics of type B synoviocytes is the development of cytoplasmic processes showing a variety of shape and extension. Previous histochemical and electron microscopic studies have noted the irregular, frequently dendritic, shape of type B synoviocytes in various animals (BARLAND et al., 1962; SOUTHWICK and BENSCH, 1971; GRAABAEK, 1985a; WILKINSON et al., 1992). Most of cells contain one or more large cytoplasmic processes which project toward the surface of the synovial membrane. BARLAND et al. (1962), who observed synoviocytes in the human joint by TEM, presented a three-dimensional drawing of synoviocytes in which the cells extended long cytoplasmic processes overlapping with those from other cells, thus forming a plexus of processes in the synovial intima. This image was partially confirmed by our recent immunohistochemical study for PGP 9.5 in the horse joint (KITAMURA et al., 1999) (Fig. 4).

A three-dimensional view of the network of processes at the synovial surface was clearly demonstrated by our recent SEM observation (SHIKICHI et al., 1999). SEM is one of the most powerful tools for the three-dimensional viewing of whole cells, being especially effective for complicated structures. There have been many SEM observations on the synovial membrane, but these were restricted to viewing the synovial surface (FUJITA et al., 1968; WOODWARD et al., 1969; WYSOCKI and BRINKHOUS, 1972; SHIVELY and VAN SICKLE, 1977; DATE, 1979; MCDONALD and LEVICK, 1988; MURASHIGE et al., 1999). In order to obtain SEM images of cells embedded in the tissue, researchers have developed various maceration techniques that remove the intercellular matrix using enzymes or alkaline solutions (EVAN et al., 1976; MILLER et al., 1982; TAKAHASHI-IWANAGA and FUJITA, 1986). Using such macerated samples, our first attempt to demonstrate the entire shape of type B synoviocytes successfully revealed: 1) a prominent surface meshwork

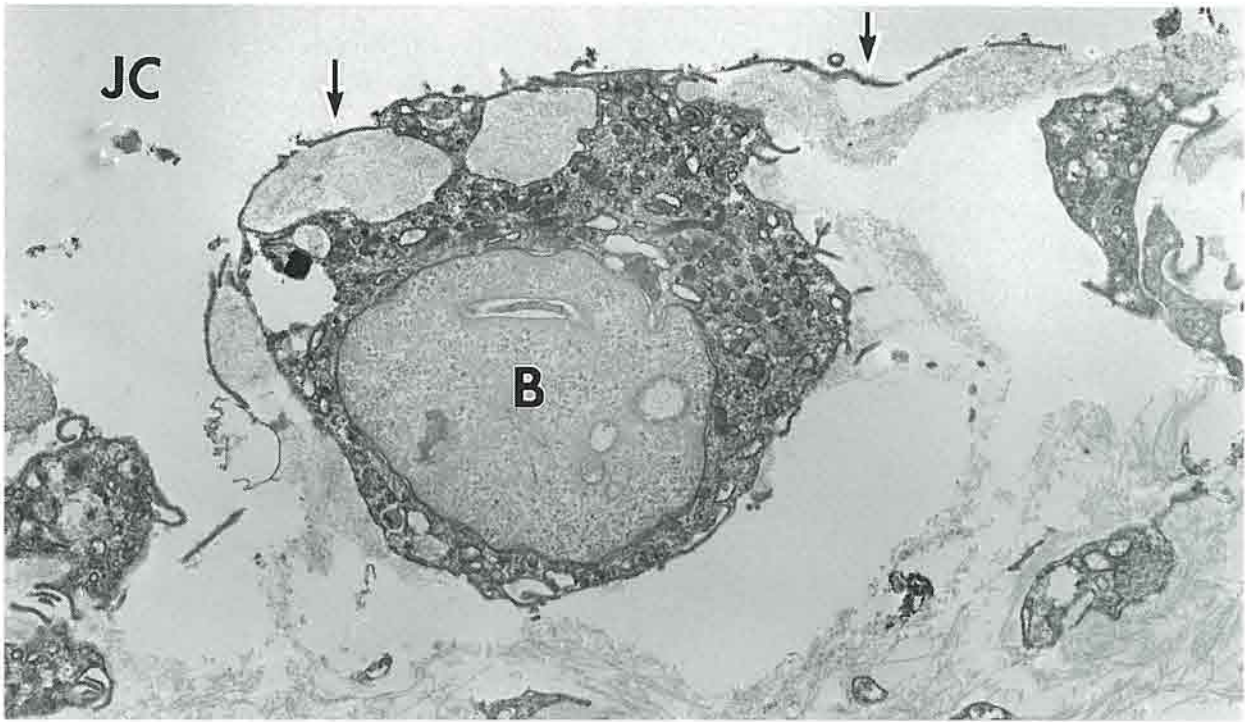


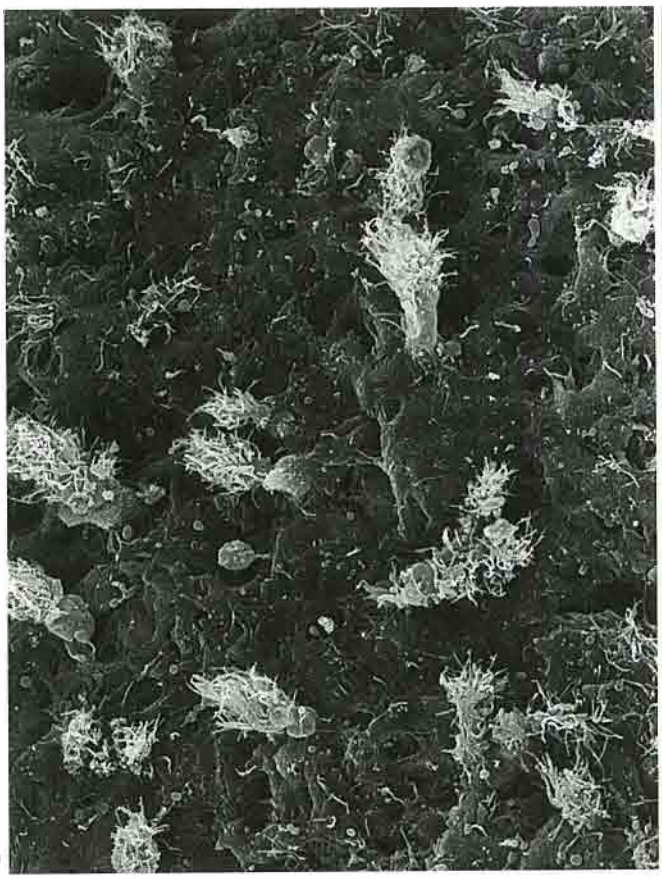
Fig. 9. Electron microscopic observation of a PGP 9.5-immunoreactive type B cell in the horse joint. The immunoreactivity for PGP 9.5 is distributed diffusely throughout the cytoplasm of type B cells (*B*). The cell delicately covers the intimal surface by the membranous processes (*arrows*). *JC* joint cavity. ×7,200

of cytoplasmic processes, 2) antenna-like microvillous crowns projecting into the joint cavity, and 3) membranous processes covering the synovial surface (SHIKICHI et al., 1999).

Type B cells located near the joint cavity issue several long, rod-shaped cytoplasmic processes radially along the surface of the synovial membrane (Figs. 5, 7). When type B cells exist in the deeper layer of the synovial intima, they extend an apical process toward the joint cavity; their processes reach the surface and ramify in a dendritic fashion (Fig. 4). These cytoplasmic processes form considerably regular networks at the superficial layer of the intima, although the density differs from place to place; they frequently exhibit a densely arranged plexus on the surface, like a lamina limitans (KITAMURA et al., 1999). Unfortunately, the functional significance of the characteristic networks is unclear. Since type B cells are engaged in the secretion of components contained in the synovial fluid, their contact with the joint cavity via the broad cell surface may be advantageous. BARLAND et al. (1962) thought that the passage of proteins and small molecules from the intimal capillaries to the joint cavity was influenced by a physical barrier of the overlapping cytoplasmic processes of lining cells. However, the network composed of the primary processes of synoviocytes is too rough to regulate the fluid transportation. On the other hand, the membranous processes, as first shown by our SEM and TEM study in the horse joint (SHIKICHI et al., 1999), tend to cover delicately the surface of the synovial intima and demarcate it against the joint cavity, being reminiscent of the glial limiting membrane composed of astrocytes' processes (Figs. 8, 9). It appears more likely that the membranous processes may function as cellular elements of the blood-joint barrier, rather than the coarse network of processes.

Endocrine and sensory functions of type B synoviocytes

Earlier ultrastructural observations of rodent synoviocytes, which focused on the rich existence of cytoplasmic granules, have indicated the endocrine nature of type B synoviocytes (LINCK and PORTE, 1978; OKADA et al., 1981a; GRAABAEK, 1984). Mouse synoviocytes in particular contain many membrane-bounded, electron-dense granules approximately 250 nm in diameter, which resemble those of pituitary



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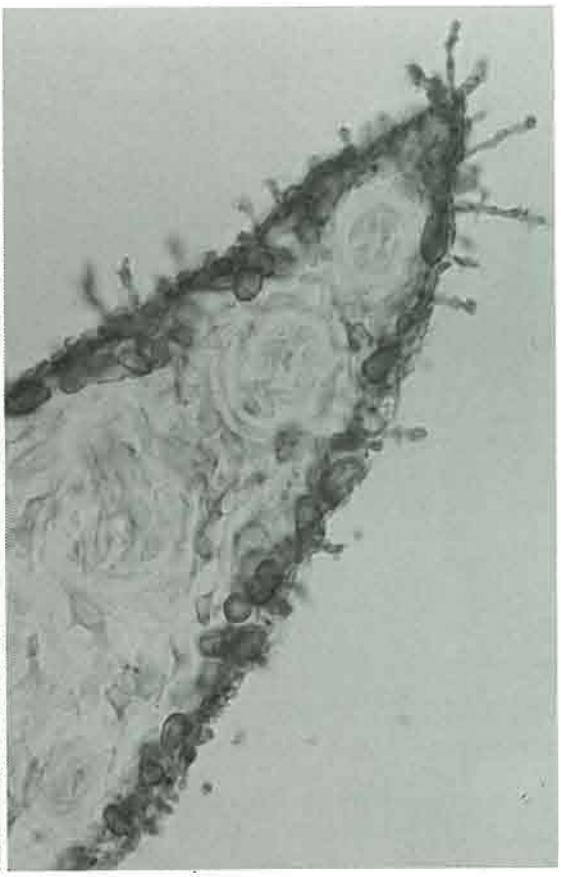

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Figs. 10 and **11.** Intraarticular processes of type B cells projecting over the synovial intima in the horse joint. The processes are recognizable as microvillous crowns under SEM (**Fig. 10**). Immunostaining for PGP 9.5 (**Fig. 11**) can demonstrate the rod-shaped processes at a light microscopic level. Fig. 10: ×1,800, Fig. 11: ×550

and pancreatic endocrine cells. The cluster formation of granulated synoviocytes close to fenestrated capillaries and their rapid degranulation in response to stimuli noxious to the synovial membrane (LINCK and PORTE, 1981) suggest their function as receptosecretory cells, a characteristic shared by paraneuron members (FUJITA et al., 1988). LINCK and PORTE (1981) also demonstrated a conspicuous increase in number of secretory granules in the hypophysectomized or thyroid inhibitor-treated mice, indicating that their secretory activity is influenced by hormones directly or indirectly. The secretory granules, distributed throughout the cytoplasm, are more often concentrated at the periphery without obvious polarity toward the synovial cavity. The random distribution of the granules suggests rather a diffuse discharge of secretory products into the intimal layer. Since the type B cells are always near the fenestrated capillaries, one might also envisage a possible hormonal function. Cytochemical studies have showed that secretory granules consist of a carbohydrate-containing matrix and proteinaceous cores, and lack an acid phosphatase activity (LINCK and PORTE, 1978; OKADA et al., 1981a). Although the chemical identification of granular contents is scanty, until now no one has succeeded in isolating substances contained in the granules as well as determining their biological function. One of reasons for the stagnant approach may be the limitation of available species. In many species, including the guinea pig, rabbit, horse and human, type B cells lack such cytoplasmic granules (LINCK and PORTE, 1978).

Our recent study demonstrated the existence of PGP 9.5 in the type B synoviocytes in the horse joint (KITAMURA et al., 1999). PGP 9.5, a neuronal marker, is shared by several sensory cells including gustatory cells, olfactory receptor cells, and Merkel cells (IWANAGA et al., 1992). The selective localization of PGP 9.5 in horse type B cells brings us to a consideration of their sensory function. Gut endocrine cells,

which also contain PGP 9.5, essentially reach the apical cytoplasm to the lumen and are designated as open-type cells (FUJITA and KOBAYASHI, 1977). Tips of the apical processes are endowed with a characteristic tuft of microvilli that function as a receptor site for chemical information in the gut lumen (FUJITA and KOBAYASHI, 1977; HASHIMOTO et al., 1999). The antenna-like processes demonstrated by us in the horse joint protrude into the joint cavity and are covered by long microvilli (Figs. 8, 10, 11), quite similar to the open-type gut endocrine cells. These morphological findings support the hypothesis that type B synoviocytes are sensory in nature. Possible sensations by synoviocytes are not transmitted to the brain, due to a lack of synaptic contact with sensory neurons. In response to conditions of the joint cavity such as pressure, viscosity, and change in chemical composition, the synoviocytes may autoregulate the secretion of components into the intimal matrix and joint cavity.

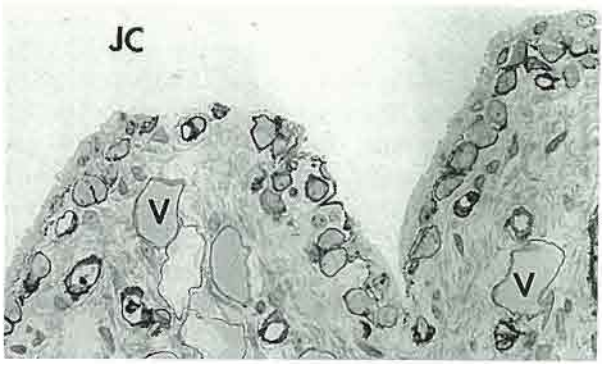

Fig. 12. Immunostaining of laminin in the rat temporomandibular joint. Immunoreactive products, linear in appearance, envelop the cell bodies, predominantly the basolateral face, of type B synoviocytes. Type A cells located at the surface of intima are free of the immunoreactivity. *JC* joint cavity, *V* blood vessels. ×100 (From NOZAWA-INOUE et al., 1999a)

Epithelium-like nature of synovial intima

The synovial lining cells exhibit a more or less epithelium-like arrangement, especially in the areolar synovial membrane, along the luminal surface (Fig. 3). For early histologists, the synovial membrane, which faces a liquor-containing cavity, was interpreted as an endothelial layer or a continuous cellular layer covering the articular lumen. Electron microscopically, however, it became clear that the synovial lining consists of a discontinuous layer of cells, which possess sufficient intercellular spaces (Fig. 2). Thus, the articular lumen is in direct contact with the matrix of the synovial intima; the exposed interstitium constitutes roughly 20–50% of the surface area (MCDONALD and LEVICK, 1988). Intimal synoviocytes are surrounded by a specialized amorphous or fibrillar matrix relatively free of collagen fibers in the areolar synovial membrane. In spite of this consensus, many TEM observations have shown a frequent occurrence of cell-to-cell contacts and the existence of the basement membrane-like structures between synoviocytes in the joint of various animals. Generally, basement membrane and cell junctions are well-developed in the epithelium, but not specific to the epithelium. The basement membrane is formed around muscle cells and adipose cells, and similar cell linkages appear in fibroblastic cells including interstitial fibroblastic cells in the renal medulla (MAJACK and WILLIAM, 1980; MCAULIFFE, 1980) and fat-storing cells in the fish liver (FUJITA et al., 1980).

LANGER and HUTH (1960) stressed the existence of a coherent basement membrane separating the synovial intima from deeper connective tissue, but many electron microscopists agree that there is no linear basement membrane between the synovial cell layer as a whole and the underlying connective tissues; cell bodies of synoviocytes are surrounded only by fragmented or dotted basement membrane-like materials (WYLLIE et al., 1964; GROTH, 1975; LINCK and PORTE, 1978). Some immunohistochemical studies demonstrated the existence of laminin, one of predominant elements of the basement membrane, around synoviocytes in humans (POLLOCK et al., 1990; RITTIG et al., 1992). Ultrastructurally, immunoreactive products for laminin were deposited around type B cells in the rat temporomandibular joint (NOZAWA-INOUE et al., 1999a) (Fig. 12). The laminin immunoreactivity was not distributed uniformly around the cell membrane with the most intense immunoreactivity being present on the basal part of cell surface (NOZAWA-INOUE et al., 1999a). The covering of type A synoviocytes by the basement membrane-like materials is less prominent than that of type B cells (GROTH, 1975) or completely lacking at all (NOZAWA-INOUE et al., 1999a). Furthermore, the extension of the pericellular basement membrane-like materials differs according to species; no basement membrane appears around synoviocytes in the human (BARLAND et al., 1962; GHADIALLY, 1983). MAEDA et al. (1991) investigated the multi-layered basal lamina around periodontal Ruffini nerve endings and hypothesized that the multi-layered basal lamina is induced by intense and consistent mechanical stimulation. This idea may hold true for the basement membrane-like structures among synoviocytes.

Desmosomes and gap junctions of synoviocytes have been repeatedly reported in the synovial cells, for example, of the rat (ROY and GHADIALLY, 1967a; NISHIJIMA, 1981; NOZAWA-INOUE et al., 1998), cat (GROTH 1975), mouse (LINCK and PORTE, 1978) and rabbit/guinea pig (LINCK and PORTE, 1978). Even if desmosomal junctions are formed among synoviocytes, an interstitial space with fine collagenous fibrils always separates the clustered cells, quite different from the image of the authentic epithelium. We often encountered two opposing cells linked via desmosomes in areas of the horse synovial membrane where the synoviocytes are dispersed. Moreover, invagination (interdigitation) of adjacent cell surfaces without any membranous specialization was a common feature in the synovial intima (ROY and GHADIALLY, 1967a). GROTH (1975) reported the development of cell junctions in relation to cell types, although he classified synoviocytes into three types, namely, A, B and intermediate cells. These junctional devices appear to be found irrespective of cell types. The frequent occurrence of desmosomes between two A cells (GROTH, 1975) may be contrary to the notion of the free movement of macrophagic type A cells. No cell junctions have been found in normal human synovial membrane (BARLAND et al., 1962; GHADIALLY, 1983; HENDERSON and PETTIPHER, 1985), but they are often seen in pathological states associated with hyperplasia of the synoviocytes (GHADIALLY et al., 1978). GHADIALLY et al. (1978) proposed that the desmosome-like structures are formed in response to fibrin trapped between the synovial intimal cells, and their appearance possibly precedes the formation of coated vesicles for the endocytosis of fibrin.

In the present review article we have mainly dealt with the cytochemical and ultrastructural features of synoviocytes. The origin and function of type B synoviocytes remain unclear as compared with type A synoviocytes. At present, we can recognize the type B synoviocytes to be specialized fibroblasts which are active in secretory functions and possess sensory and contractile abilities. In spite of the advance of selective staining methods for type B cells, we still eagerly await more specific and universal staining methods, such as immunohistochemistry using monoclonal antibodies raised against the cells. Scanning electron microscopy of macerated samples might promote a morphological analysis of synoviocytes, and we hope that our method will be applied to other mammals and pathological samples. In this review, we have omitted a description of the vascular system and innervation in the synovial membrane. Some researchers have noted the uniqueness of blood vessels, represented by convoluted or helically running venules or arterioles in the synovial membrane. These vessels may be comparable to helicine arteries in the uterine endometrium or the arteriovenous anastomosis. Clinically, it is well known that the joint is a painful tissue in inflammatory states. Many studies have dealt with the distribution of nerve fibers including substance P-containing fibers in the joint capsule, but the previous reports have been insufficient to explain the severe pain generated in the joint. The relationship of the innervation and the inflammatory pain sensation is one subject for future study.

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