Virchows Archiv Abt. A Path. Anat. 353, 261—272 (1971)

Auto-immune Mechanisms as a Probable Aetiology of Behçet's Syndrome, an Electron Microscopic Study of the Oral Mucosa*

Taiichi Saito, Takayoshi Honma, Toshihiko Sato, and Yukio Fujioka
Departments of Pharmacology and Oral Surgery,
Iwate Medical College School of Dentistry, Morioka, Japan

Received February 26, 1971

Summary. A constant and characteristic feature of Behçet's syndrome was the association of macrophages with degenerated prickle cells in the prickle-cell layer of the oral mucosa adjacent to ulcerations. Three types of macrophages could be identified: The *type I* macrophage, rich in cytoplasmic organelles, phagocytosed material from degenerated cells. The *type II* macrophages with scant endoplasmic reticulum came in contact with small lymphocytes which they gave immunological information, inducing them to undergo blastoid transformation. These lymphoblasts produced immunoglobulins. Lymphoid cells that contained numerous ribosomes and polysomes were attached to the prickle cells and probably elaborated a cytotoxic factor, since the cytoplasm of the prickle cells ultimately degenerated and the ulcer expanded. The cytoplasm of prickle cells apparently acts as an auto-antigen. Immune responses against it are mediated by the macrophages and the lymphoid cells. The changes seen are consistent with those of delayed hypersensitivty reactions. *Type III* macrophages had Birbeck granules and were regarded as Langerhans cells. Thymus-dependent *type III* macrophages may have an important role in developing and controlling the ulceration in Behçet's syndrome. The involvement of lysosomes in initiating the ulceration is discussed.

The aetiology of Behçet's syndrome has long been disputed. Viral infection (Behçet, 1937), allergy (Nishiyama, 1959; Shimizu *et al.*, 1965), collagen disease (Shikano, 1960) and other factors were thought to be responsible for this syndrome. Oshima *et al.* (1963) and Shimizu *et al.* (1965) investigated Behçet's syndrome comprehensively and showed a rise in serum globulins, the presence of haemagglutinating antibodies against the oral mucosa and immunofluorescence of the cytoplasm of cells from the oral mucosa. They proposed the involvement of auto-immune mechanisms in the aetiology of the disease.

Lehner (1967a, 1969a, b) suggested an auto-immune aetiology for Behçet's syndrome and recurrent aphthous ulcers. He described an intense lympho-monocytic infiltration of the epithelium and concluded that the changes were consistent with those of delayed hypersensitivity reactions. Although there exists considerable literature on the immunology of Behçet's syndrome, little is known about the cellular mechanisms of the development of the disease. An atempt was made in this paper to reveal the pathogenesis of Behçet's syndrome as a cell-mediated auto-immune disease.

* A preliminary report was given at the 6th Asian-Pacific Dental Congress in Bangkok, Thailand. September 18—21, 1970.

Materials and Methods

Biopsies were taken from three patients with the complete type of Behçet's syndrome with erythema nodosum, ulcers on the oral and genital mucosae and uveitis with hypopion iritis. The specimens were excised from the oral mucosa within 48 hours of the onset of ulceration, and fixed in a cold (4°C) 2 per cent solution of glutaraldehyde in 0.15 M collidine buffer, pH 7.4, for 2 hours, followed by 60 min post-fixation in 1 per cent osmium tetroxide buffered to pH 7.4 with 4 per cent sucrose added. Dehydration was accomplished with graded concentrations of ethanol. They were then embedded in Epon 812 according to Luft's method (Luft, 1961). Ultrathin sections were made with a Porter-Blum microtome, stained with uranyl acetate and lead citrate, and examined under a Hitachi HU-11DS electron microscope.

Results

Corium. Adjacent to the ulcer polymorphonuclear and monocytic infiltrations were predominant features. Plasma cells and mast cells were also seen but eosinophils were rarely found.

Basal-Cell Layer. Two types of cells infiltrated the basal-cell layer of the epithelium through the basement-membrane. One of them was characteristic of macrophages (Fig. 1), which possessed phagosomes, lysosome-like granules, mitochondria and rough-surfaced endoplasmic reticulum (rER). The other (Fig. 2) had the characteristics of lymphocytes and contained smooth-surfaced endoplasmic reticulum (sER), a few rER and sometimes rather large mitochondria. The normal regular configuration of the basal cells was deranged by the invagination of the mononuclear cells, but the basal cells themselves never showed degeneration.

Prickle-Cell Layer. In the prickle-cell layer the most prominent features were the occurrence of condensed degenerated prickle cells and the emigration of macrophages (Fig. 3). The cytoplasm of degenerated cells was condensed and electron dense. The structures of tonofibrils, mitochondria and other organelles were scarcely seen, but vacuoles were frequently noticed. Highly electron dense fine granules were precipitated in the nucleus. These cells, however, were connected to the normal prickle cells with desmosomes. The enlarged intercellular spaces around the degenerated cells were filled with a precipitate which was morphologically similar to that consisting the background of intercellular areas.

Fig. 4 shows a macrophage attached to a degenerated prickle cell. In the intercellular space between these two cells and in the cytoplasm of the degenerated prickle cells there were membrane-bound electron dense granules, 0.15 μ diameter, which were considered to be Odland bodies (Odland, 1960). At the cell surface of the macrophage pinocytotic caveolae were found and intercellular materials were being taken up.

In the prickle-cell layer of the oral mucosa in Behçet's syndrome three populations of macrophages became apparent.—*Type I* macrophages (Figs. 4 and 5), which were provided with numerous vesicular sER, lamellar or tubular rER and many phagosomes, and came in contact with the degenerated prickle cells and there phagocytosed or pinocytosed intercellular material which was probably derived from the prickle cells; *type II* macrophages (Fig. 6), which had some mitochondria, elctron dense granules, poorly developed rER and deeply indented nuclei; and *type III* macrophages (Fig. 7a and b), which encountered infrequently and contained sER and occasional Birbeck granules (Birbeck *et al.*, 1961), and the nuclei showed deep indentations. Adjacent to the *type II* macrophages small lymphocytes were occasionally noted (Fig. 6). These lympho-

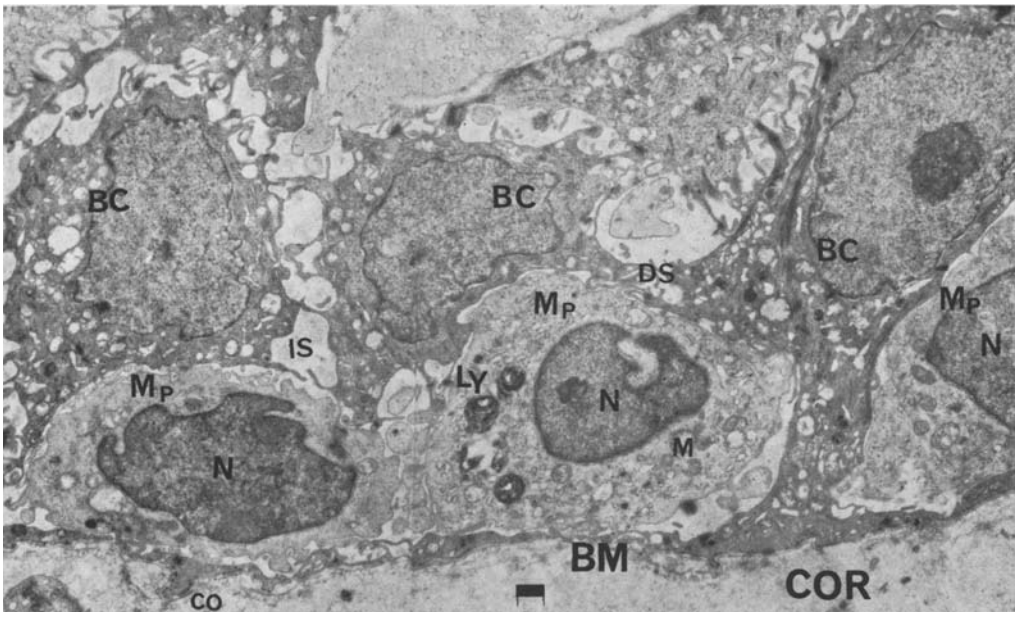


Fig. 1. Macrophages infiltrated in the basal-cell layer of the epithelium of the oral mucosa. ×3700

Abbreviations: *BC* basal cell; *BM* basal membrane; *C* centriole; *co* collagen fibres; *Cor* corium; *DS* desmosome ; *G* Golgi zone; *Gly* glycogen granules; *IS* intercellular space; *L* lymphoid cell; *Ly* lysosome-like granule; *M* mitochondrion; *Mp* macrophage; *N* nucleus; *NO* nucleolus; *PC* prickle cell; *Pv* pinocytotic vesicle; *rER* rough-surfaced endoplasmic reticulum; *sER* smooth-surfaced ER; *TO* tonofibrils; *Vc* vacuoles. Solid bar indicates 1 μ

cytes possessed almost no organelles but numerous free ribosomes in the cytoplasm. Plasma membranes between adjacent cells could not be readily discerned.

Other types of lymphoid cells were also encountered in the prickle-cell layer. There were small lymphocytes and lymphoid cells which had a wider cytoplasmic area, a few mitochondria, Golgi apparatus, but scarce rER and sER. Fig. 8 shows a similar cell but it contained two Golgi zones, more mitochondria and a centriole. Some mononuclear cells had many large mitochondria and vesicular rough-surfaced tubules which occupied the entire area of the cytoplasm (Fig. 9). These three types of lymphoid cells were presumably transformed from lymphocytes.

Quite different kinds of mononuclear cells were also present. These cells might be transformed lymphocytes and had few mitochondria and ER in the cytoplasm (Fig. 10). They had, however, scattered ribosomes and polysomes. The nucleus had a few invaginations and nucleoli. This lymphoid cell attached to a nearly normal prickle cell with interdigitating projections. In the cytoplasm of the prickle cell to which the lymphoid cell adhered, tonofibrils were shortened and became irregular, and vacuoles and clusters of the rosettes of glycogen granules were frequently observed. Sometimes Odland bodies were noticed in the marginal area of the cytoplasm and the cytoplasmic membrane became indistinct. These findings suggest the initiation of a destructive process.

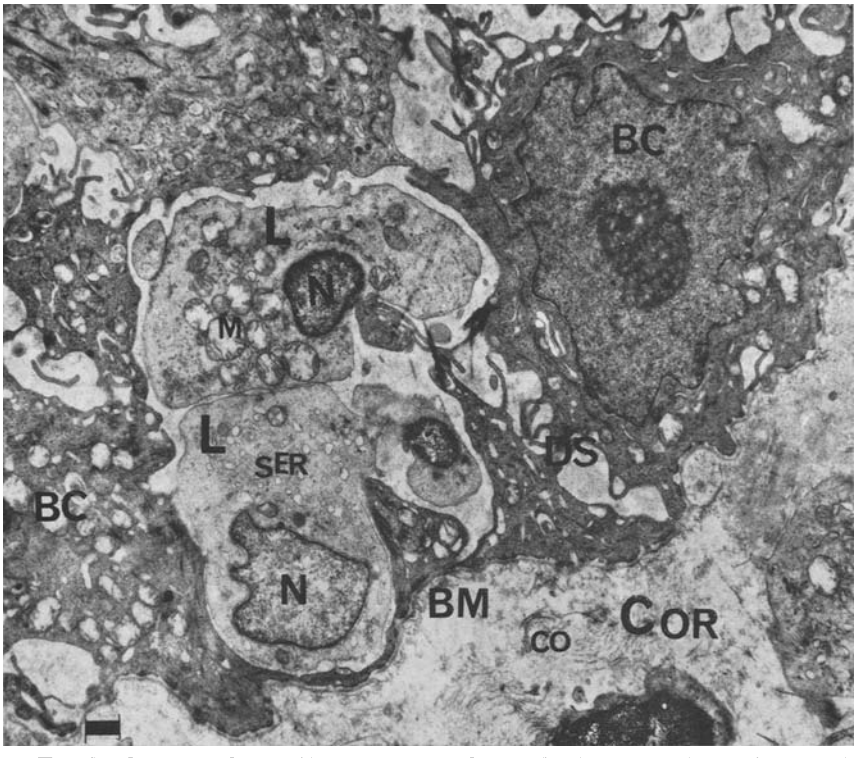


Fig. 2. Two kinds of lymphoid cells infiltrated in the basal-cell layer of the epithelium of the oral mucosa. ×4500

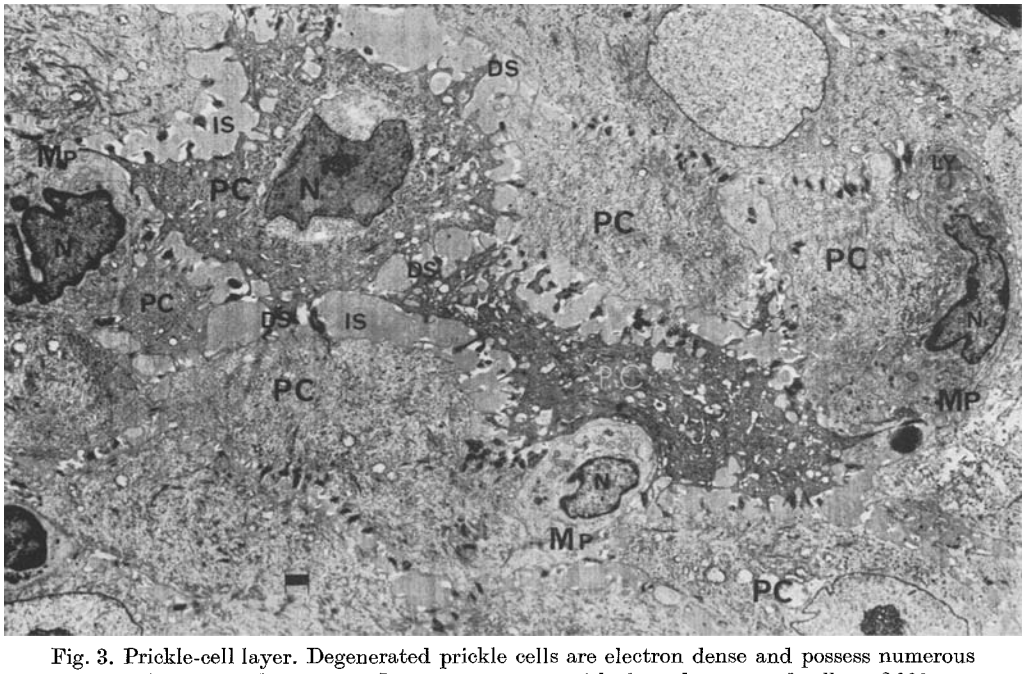


Fig. 3. Prickle-cell layer. Degenerated prickle cells are electron dense and possess numerous vacuoles. Macrophages (*type I*) come in contact with these degenerated cells. ×3000

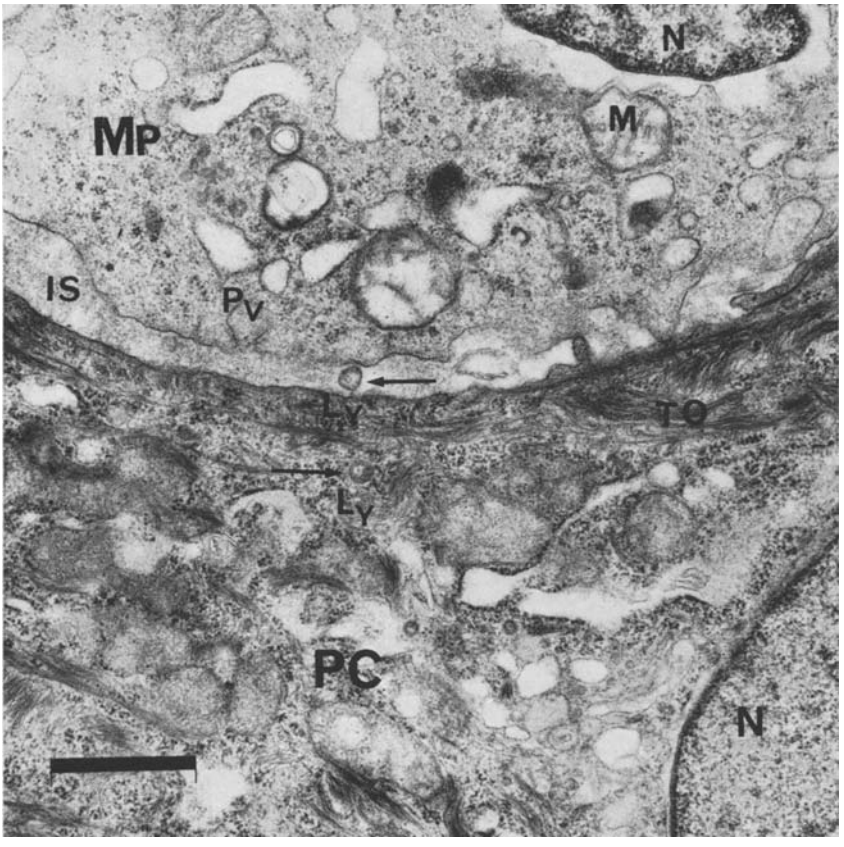


Fig. 4. Electron micrograph showing a close contact of a macrophage with a degenerated prickle cell. Odland bodies (lysosome-like granules) are noticed (arrows). At the cell surface of the macrophage pinocytotic caveolae are observed. ×19000

By fluorescence microscopy using human antisera to IgG, IgM and IgA conjugated with fluorescein isothiocyanate, there was fluorescence in the cytoplasm of the prickle cells for IgM and IgG, but the loci of the latter were different from those of IgM. The intercellular spaces showed neither fluorescence nor positive reactions to PAS staining.

Granulosa-Cell Layer. In the granulosa-cell layer almost no cell infiltrations were observed, and the epithelial cells were flattened and more electron dense than in the prickle-cell layer.

Discussion

Oshima *et al.* (1963), Shimizu *et al.* (1965), Lehner (1967b, 1969a) and Sato *et al.* (1970) have shown the presence of autoantibodies against the oral mucosa and a rise in immunoglobulins in the oral mucosa and the blood of patients with Behçet's syndrome. Lehner (1967a) also suggested that the cytoplasm of prickle cells acts as an antigen but presented no data. An electron microscope study on Behçet's syndrome was done by Lehner (1969b), who demonstrated an intense, early, lympho-monocytic infiltration of the epithelium of oral mucosa, but the

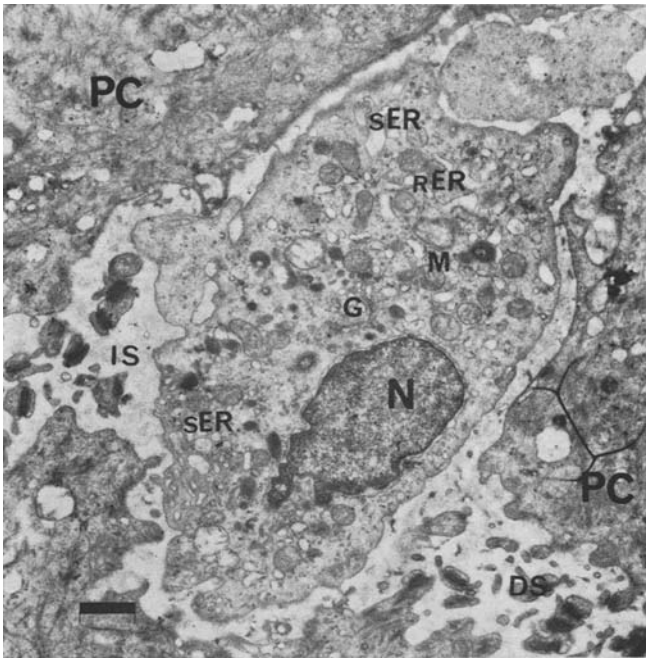


Fig. 5. Macrophage (*type I*) often comes in contact to the degenerated prickle cell and phagocytoses the material presumably derived from the latter cell. ×7200

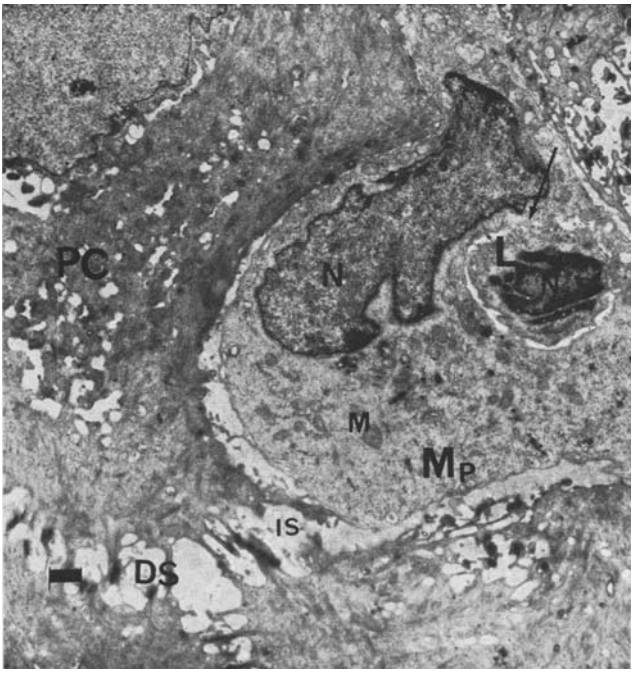
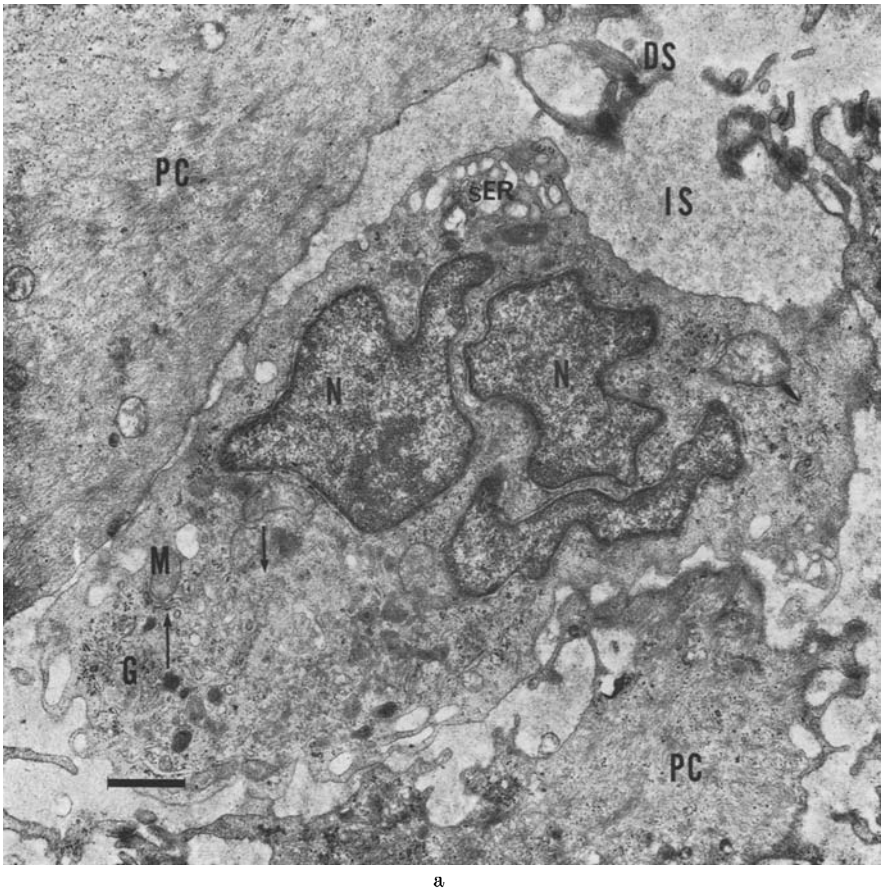
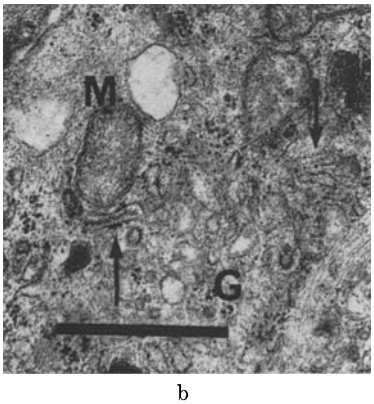


Fig. 6. A macrophage (*type II*) and a small lymphocyte. Note the continuity between two cells (arrow). ×4200



a



b

Fig. 7. a Macrophage (*type III*) possessing Birbeck granules (arrows). ×10000. b A part of 7a showing Birbeck granules (arrows) which have single-layered membrane and fine granules inside. ×22800

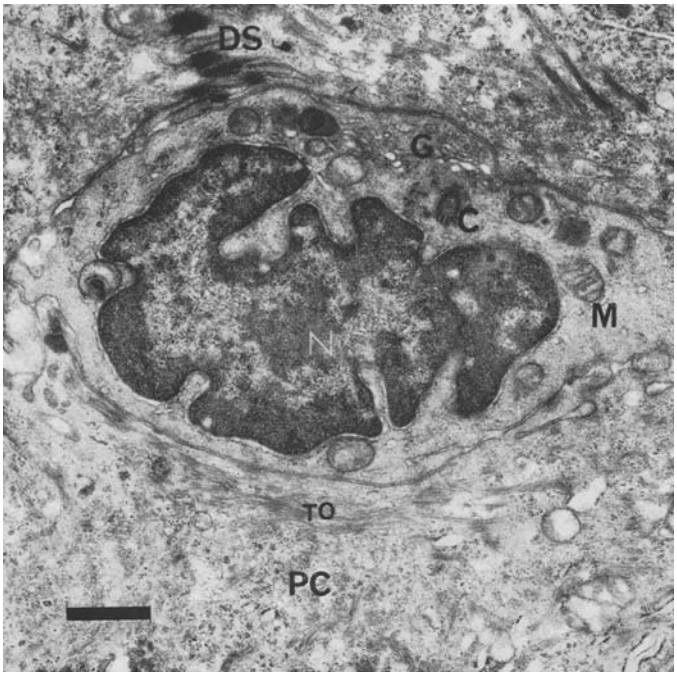


Fig. 8. An active lymphoid cell containing two Golgi zones and a centriole. ×10900

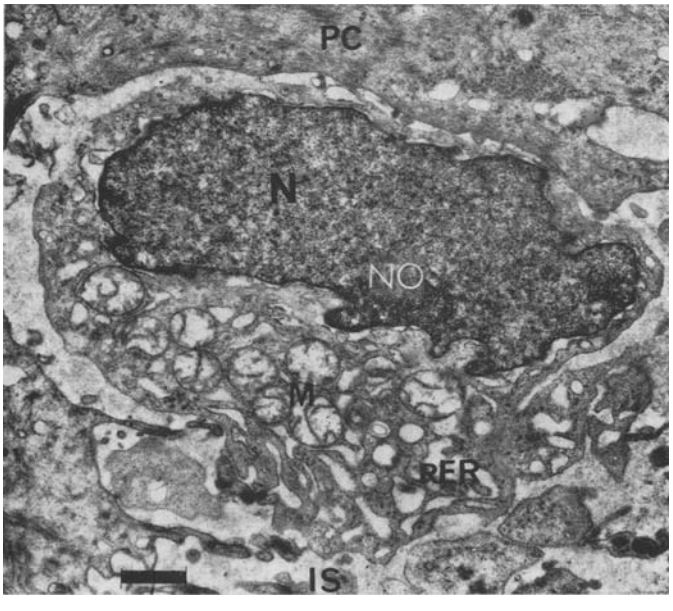


Fig. 9. A mononuclear cell possessing many large mitochondria, rER and a nucleolus, but no phagosomes. This type of lymphoblastoid cell is thought to be in active phase. ×8800

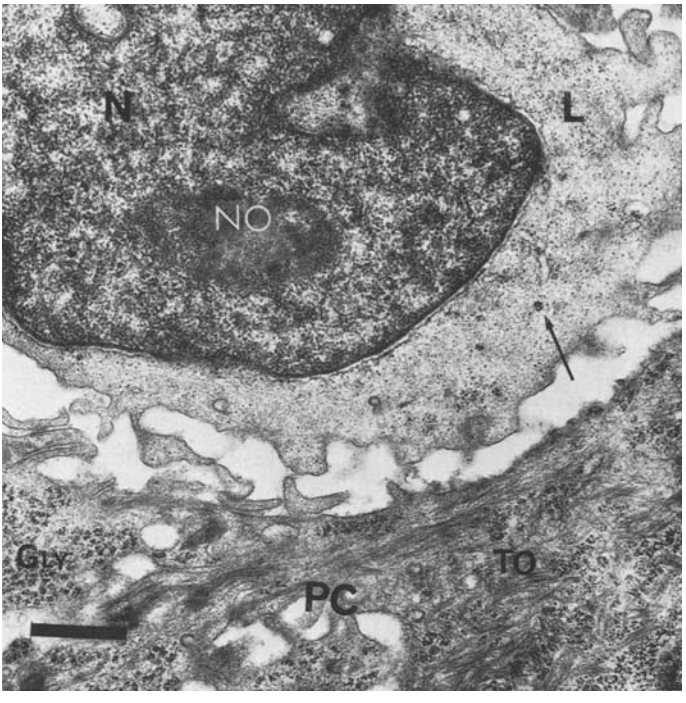


Fig. 10. A lymphoid cell is attached to a prickle cell with spike-like projections. The lymphoid cell has free ribosomes and polysomes (arrow), but no conspicuous organelles in the cytoplasm. The target prickle cell seems to be degenerated. ×12600

literature dealing with the cellular aspects of the pathogenesis of oral ulceration in Behçet's syndrome is otherwise lacking. Intense mononuclear and polymorphonuclear infiltrations of the corium may be attributed to general inflammatory reactions secondary to the ulceration and probably do not contribute much to the pathogenesis of the syndrome.

So far as auto-immune disease is concerned, the formation of antibodies to self-components is necessary. Intracellular substances in the prickle cells might become autoantigens after they degenerate. In the intercellular spaces around the degenerated prickle cells oedema was noticed but neither γ-globulins nor PAS-positive substances were present. Odland bodies, however, were not infrequently observed near the cytoplasmic membrane of the degenerated cells, both inside and out. These bodies are thought of as lysosomes (Wolff and Holubar, 1967), and may have a role not only in digesting the foreign bodies in phagolysosomes, but in degenerating the cytoplasmic self-components. Moreover, lysosomal enzymes can activate the human complement system (Lepow, 1970). Complement has an important role in initiating cellular deterioration (Austen and Cohn, 1963; Müller-Eberhard, 1968). The possibility cannot be excluded that lysosomal enzymes can elicit degeneration of the prickle cells in some fashion.

In the normal epithelium of the human oral mucosa phagocytosing macrophages were never noted (Zelickson and Hartmann, 1962). In Behçet's syndrome,

however, infiltration of macrophages in the prickle-cell layer is one of the striking features. There were different types of macrophages found in the epithelium. They can be classified into three groups in our experiments, but some of them may be transformed into each other. Usually *type I* macrophages (Figs. 3–5) attached to the degenerated prickle cells, phagocytosed the cellular components and processed them in the phagosomes. These macrophages may be transformed into *type II* macrophages. These mononuclear cells may produce a transfer agent of immunity (Osawa *et al.*, 1971) and transfer the information to small lymphocytes. Another possibility is that cytophilic antigens derived from the prickle cells may be caught by the surface of the plasma membrane of the macrophage and transfered to lymphocytes.

The origin and functional differentiation of macrophages is a matter of conjecture. They may be derived from monocytes, lymphoreticular cells or mesenchymal cells. According to Kuwahara (1969, 1970) Langerhans cells are derived from mesenchymal cells, presumably from the thymus cells and they have an important role in immunological responses. *Type III* macrophages had Birbeck granules in the cytoplasm. Birbeck granules are characteristically present in Langerhans cells in the epidermis and the spleen. *Type III* macrophage, therefore, may be equivalent to Langerhans cell and thymus dependent. Langerhans cells which have abundant Birbeck granules have been observed in the epithelium of the normal oral mucosa, especially in the proliferating zones (Listgarten, 1964; Zelickson, 1965; Waterhouse and Squier, 1967; Kuwahara, 1969; Sato, 1970). Birbeck granules can regulate cell division and proliferation (Kuwahara, 1970). The occurrence of macrophages (Langerhans cells) which contain few Birbeck granules suggests that regressive changes have occurred in the epithelium of the patient. *Type III* macrophages may regulate the function of *type I* macrophages or may have an influence upon degeneration of the prickle cells (Fig. 11).

After a small lymphocyte receives an information from the *type II* macrophage, it undergoes a blastoid transformation (Schoenberg *et al.*, 1964), which then is capable of producing an antibody. This type of lymphoblastoid cell (Figs. 8 and 9) has a larger cytoplasm and possesses well developed organelles in the cytoplasm, suggesting hyperfunction of the cells, and may produce immunoglobulins. Another type of lymphoid cell (Fig. 10), which contained poorly developed organelles but many ribosomes and polysomes, did not elaborate antibodies but a cytotoxic factor and destroyed the target cells (Nettesheim and Makinodan, 1965). From which cells these lymphoid cells receive this information is unknown. But the *type II* macrophages (Fig. 6), lymphoid cells or lymphoblastoid cells (Fig. 9) may be the source of the information. Able *et al.* (1970) have reported a similar phenomenon *in vitro* using mouse lymphocytes. They have observed that the sensitized lymphoid cells have a broad close contact with target cell membrane or are attached with interdigitating spike-like projections and destroyed the target cells.

According to Wiener *et al.* (1964), these lymphocytes are thymus-dependent and under the control of the thymus. Thymus dependent-macrophages and -lymphoid cells emigrate into the central nervous system, corneae, skin and mucous membranes in the foetal stage (Breathnach and Wyllie, 1965). Therefore, once an ulcer developed in the oral mucosa of the patient with Behçet's syndrome, tissues

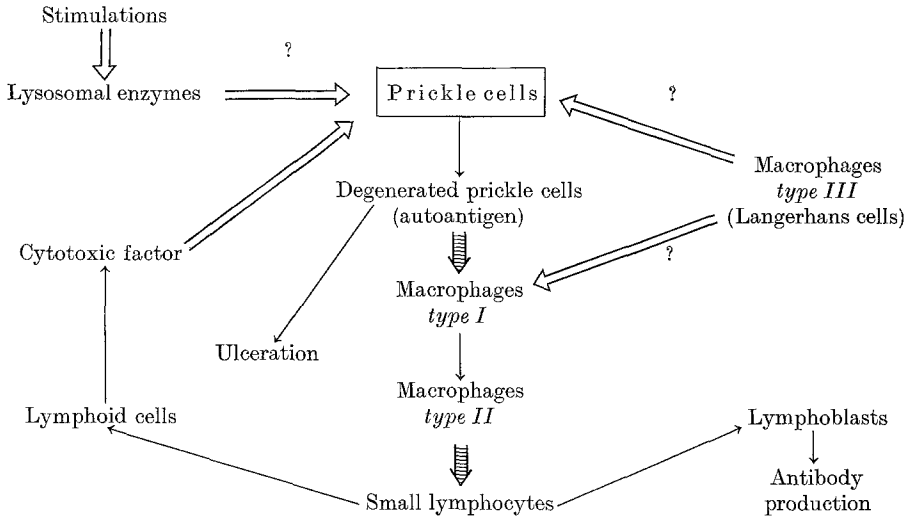


Fig. 11. Schema showing the process of degeneration of the prickle cells of the oral mucosa in Behçet's syndrome mediated by macrophages and lymphoid cells. Lysosomal enzymes are released by stimulation and initiate the degeneration. Macrophages (*type I* and *II*) transfer the immunological information from the degenerated cells to the lymphocytes, which then undergo blastoid transformation. The lymphoblasts synthetize immunoglobulins and the lymphoid cells produce a cytotoxic factor. *Type III* macrophages (Langerhans cells) have an important role in regulating the ulceration, whether directly or indirectly. ↓ Transformation ⇓ action; ⇓ transfer of the immunological information; ? not verified

other than the oral mucosa may be involved successively. During the remittent periods thymus-dependent mononuclear cells may hold the immunological information, and afterwards may exacerbate successive ulcerations. This is not the case in recurrent aphthous ulcers, in which only reticuloid cells were observed (unpublished data) and they are not thymus-dependent, so the lesions are restricted to local regions.

Our hypothesis on the pathogenesis of Behçet's syndrome is summarized in Fig. 11, and these reactions are compatible with those of delayed hypersensitivity reactions and the concept of auto-immune disease.

Acknowledgement. The authors indebted to Dr. Teruo Suzuki, Department of Pathology, the Jikei University School of Medicine, for his valuable criticism.

References

Able, M. E., Lee, J. C., Rosenau, W.: Lymphocyte-target cell interaction *in vitro*. Amer. J. Path. **60**, 421–434 (1970).

Austen,K. F., Cohn, Z. A.: Contribution of serum and cellular factors in host defense reactions. I. Serum factors in host resistance. New Engl. J. Med. **268**, 933–938; 994–1000 (1963).

Behçet, H.: Über rezidivierende, apthöse, durch ein Virus verursachte Geschwüre am Mund, am Auge und an den Genitalien. Derm. Wschr. **105**, 1152–1157 (1937).

Birbeck, M. S., Breathnach, A. S., Everall, J. D.: Melanocytes and high-level clear cells (Langerhans cells) in vitiligo. J. invest. Derm. **37**, 51–64 (1961).

Breathnach, A. S., Wyllie, L. M.: Electron microscopy of melanocytes and Langerhans cells in human fetal epidermis at fourteen weeks. J. invest. Derm. **44**, 51–60 (1965).
Kuwahara, H.: Electron microscopic studies on dendritic cells of the epidermis, especially on relation between melanocytes and Langerhans cells. Jap. J. Derm. **79**, 620–650 (1969).
— Electron microscopic studies on dendritic cells of the epidermis, especially on the function of Langerhans cells and etiology of certain leucodermas. Jap. J. Derm. **80**, 69–112 (1970).
Lehner, T.: Autoimmunity and management of recurrent oral ulceration. Brit. dent. J. **122**, 15–20 (1967a).
— Behçet's syndrome and autoimmunity. Brit. med. J. **1967b I**, 465–467.
— Characterization of mucosal antibodies in recurrent aphthous ulceration and Behçet's syndrome. Arch. oral Biol. **14**, 843–853 (1969a).
— Pathology of recurrent oral ulceration and oral ulceration in Behçet's syndrome. Light, electron and fluorescence microscopy. J. Path. Bact. **97**, 481–494 (1969b).
Lepow, I. H.: In: Teichman, N. S., Mediation on inflammation by the polymorphonuclear leucocyte as a sequela of immune reactions (general discussion, p 232). J. Periodont. **41**, 228–239 (1970).
Listgarten, M. A.: The ultrastructure of human gingival epithelium. Amer. J. Anat. **114**, 49–69 (1964).
Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961).
Müller-Eberhard, H. J.: Chemistry and reaction mechanisms of complement. Advanc. Immunol. **8**, 1–80 (1968).
Nettesheim, P., Makinodan, T.: Differentiation of lymphocytes undergoing an immune response in diffusion chambers. J. Immunol. **94**, 868–876 (1965).
Nishiyama, S.: Klinische Beobachtungen über Behçet'scher Krankheit. Jap. J. Derm. **69**, 1139–1185 (1959).
Odland, G. F.: A submicroscopic granular component in human epidermis. J. invest. Derm. **34**, 11–15 (1960).
Osawa, N., Saito, K., Mitsuhashi, S.: Transfer agent of immunity. V. Antibody formation of cultured macrophages treated with an immune ribonucleic acid preparation. Jap. J. Microbiol. **15**, 29–37 (1971).
Oshima, Y., Shimizu, T., Yokohari, R., Matsumoto, T., Kano, K., Kagami, T., Nagaya, H.: Clinical studies on Behçet's syndrome. Ann. rheum. Dis. **22**, 36–45 (1963).
Sato, S.: Histological and electron microscopic studies of the dendritic cells in human oral epithelium. Jap. J. Derm. **80**, 585–609 (1970).
Sato, T., Kikuchi, M., Hirai, T., Saigo,K., Suzuki, K., Honma, T.: Immunological study on the diseases of oral mucosa: Serum protein patterns by immuno-electrophoresis. Jap. J. Periodont. **12**, 3–6 (1970).
Schoenberg, M. D., Mumaw, V. R., Moore, R. D., Weisberger, A. S.: Cytoplasmic interaction between macrophages and lymphocytic cells in antibody synthesis. Science **143**, 964–965 (1964).
Shikano, S.: Histopathological study on Behçet's disease. Jap. J. Ophthal. **64**, 2341–2368 (1960).
Shimizu, T., Katsuta, Y., Oshima, Y.: Immunological studies on Behçet's syndrome. Ann. rheum. Dis. **24**, 494–500 (1965).
Waterhouse, J. P., Squier, C. A.: The Langerhans cell in human gingival epithelium. Arch. oral Biol. **12**, 341–348 (1967).
Wiener, J., Spiro, D., Russell, P. S.: An electron microscopic study of the homograft reaction. Amer. J. Path. **44**, 319–347 (1964).
Wolff, K., Holubar, K.: Odland-Körper (membrane-coating granules, Kerationosomen als epidermale Lysosomen. Arch. klin. exp. Derm. **231**, 1–19 (1967).
Zelickson, A. S.: The Langerhans cell. J. invest. Derm. **44**, 201–212 (1965).
— Hartmann, J. F.: An electron microscope study of normal human non-keratinizing oral mucosa. J. invest. Derm. **38**, 99–107 (1962).

Dr. Taiichi Saito
Department of Pharmacology
Iwate Medical College
School of Dentistry
Morioka 020, Japan