KAWASAKI MED. J. VOL. 14 : NO. 2, 63-74 (1988)

Fibroblast-Lymphocyte-Macrophage Interaction in Erythema Nodosum-like Lesions of Behçet's Syndrome: An Ultrastructural Study

Takayoshi HONMA, Dongsik BANG, Taiichi SAITO, Shojiro NAKAGAWA* and Hiroaki UEKI***

Department of Pharmacology,
**Department of Dermatology,*
Kawasaki Medical School, Kurashiki 701-01, Japan
***Department of Dermatology,*
Yonsei University Wonju College of Medicine,
Wonju, Korea

Accepted for publication on April 30, 1988

ABSTRACT. In this study biopsy specimens of erythema nodosum-like lesions from eighteen patients with Behçet's syndrome were examined with the electron microscope to elucidate the close relationship between lymphocytes and/or macrophages and fibroblasts in the perivascular regions of the dermis and subcutaneous fat accompanied by prominent edematous changes and extensive depletion of collagen fibers. Electron microscopic evaluation revealed lymphocyte emperipolesis as a special pattern of adhesion of lymphocytes to fibroblasts in 3-4 days after the onset of the skin lesions. The emperipoletic lymphocytes as well as the lymphocytes adhering to fibroblasts contained a number of polyribosomes and few organelles in the cytoplasm. The fibroblasts with these lymphocytes showed remarkable development of rough endoplasmic reticulum and any other cytoplasmic organelles, indicating activated cellular function as collagen synthesis. Adhesion of lymphocytes to fibroblasts was followed by adhesion to macrophages. In the triads of lymphocyte-fibroblast-macrophage, morphological profiles of the fibroblasts showed involutional changes suggestive of suppressed cellular function through their cytoplasmic lobulation. Our findings indicate that fibroblasts are regulated directly by adhesion of lymphocytes and/or macrophages in fibrous repair of the erythema nodosum-like lesions of Behçet's syndrome.

Key words : Behçet's syndrome — erythema nodosum-like lesion — fibroblast activation — emperipolesis — cell-cell interaction — electron microscopy

The etiology of Behçet's syndrome remains unclear.[1] The infiltration of lymphocytes around small blood vessels in mucocutaneous lesions during the onset of symptoms, has lead to speculation that a delayed-type hypersensitivity reaction is involved.[2-6] These perivascular infiltrating lymphocytes have been shown to be subsets of T lymphocytes by immunocytochemical techniques employing monoclonal antibodies.[7,8] T lymphocyte subpopulations respond to a class of soluble mediators defined as lymphokines,[9] which may play an important role in mobilizing inflammatory cells in aphthous ulcers and in erythema nodosum-like lesions.

本間隆義，方　東植，斎藤泰一，中川昌次郎，植木宏明

In our previous reports,[6,10] we described proliferation and degeneration of endothelial cells in the dermal and subcutaneous microvasculature in the erythema nodosum-like lesions of Behçet's syndrome and suggested a close relationship between these phenomena and the pathogenesis of vascular changes in these lesions. It is probable that these microvascular changes associated with the appearance of the degenerated dark endothelial cells and endothelial cell necrosis in erythema nodosum-like lesions are accompanied by extensive tissue edema and intervascular deposition of fibrin derived from circulating fibrinogen in the dermis and subcutaneous fat.[11] In fact, it has been reported that fibrin deposits in the connective tissues of erythema nodosum-like lesions subsequently cause fibrinoid degeneration, disintegration, and decomposition of collagen fibers.[12] However, this type of tissue damage, with fibrinoid degeneration and necrosis of collagen fibers, is repaired rapidly by regeneration and replacement of collagen fibers during the healing process.[12] Tissue repair by collagen deposition is based on fibroblast proliferation and collagen synthesis, and fibroblastic growth and function are known to be regulated by T lymphocyte-derived lymphokines and macrophage and/or monocyte-derived monokines.[13] Since inflammatory sequelae such as fibrosis or atrophy are not usual clinical findings associated with the erythema nodosum-like lesions in Behçet's syndrome, some type of homeostasis may be involved. To obtain morphological evidence that fibroblasts are regulated not only by the stimulatory and inhibitory lymphokines and/or monokines, but also by direct interaction with lymphocytes and/or macrophages in erythema nodosum-like lesions, we studied the connective tissues from the dermis and subcutaneous fat with extensive depletion of collagen fibers and perivascular cell infiltration by electron microscopy. We obtained findings suggesting a close relationship between perivascular infiltrating lymphocytes and/or macrophages, and fibroblasts.

MATERIALS AND METHODS

Biopsy specimens from eighteen patients with Behçet's syndrome were examined. The criteria for the diagnosis of Behçet's syndrome were the presence of at least three of the four cardinal manifestations of this disease : aphthous stomatitis, genital ulcerations, uveitis and skin lesions. All patients had the mucocutaneous type with skin manifestations. Details of the patients were listed in our previous paper.[14] The biopsy specimens were obtained within one to four days after the initial clinical manifestations of the cutaneous eruptions that were observed on presentation as rounded or ovoidal dermohypodermic nodules that varied in size from 1 to 5 cm in diameter, were bright red, tender, slightly painful and on the lower extremities.

The specimens were fixed for two hours in a chilled solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Before fixation, the tissues had been washed in 0.1 M phosphate buffer (pH 7.4) and were cut into about 1 mm thick slices perpendicular to the epithelial surface. These slices were postfixed at 4°C for two hours in 1% osmic acid buffer solution (pH 7.4). Following dehydration in ethanol, the slices were embedded flat in Epon 812. One micron thin sections were cut from all slices and stained using toluidine blue. Ultrathin sections were cut and stained with uranyl acetate - lead citrate. The sections were examined under an electron microscope.

RESULTS

1) Light microscopic examination

All toluidine blue sections displayed perivascular lymphocyte-mononuclear cell infiltrates in the dermis and subcutaneous fat of erythema nodosum-like lesions. Occasionally, polymorphonuclear leukocytes also were observed both inside and outside blood vessels.

2) Electron microscopic examination

Infiltrating lymphocytes and fibroblasts were observed in the edematous perivascular connective tissues accompanied by extensive depletion of collagen

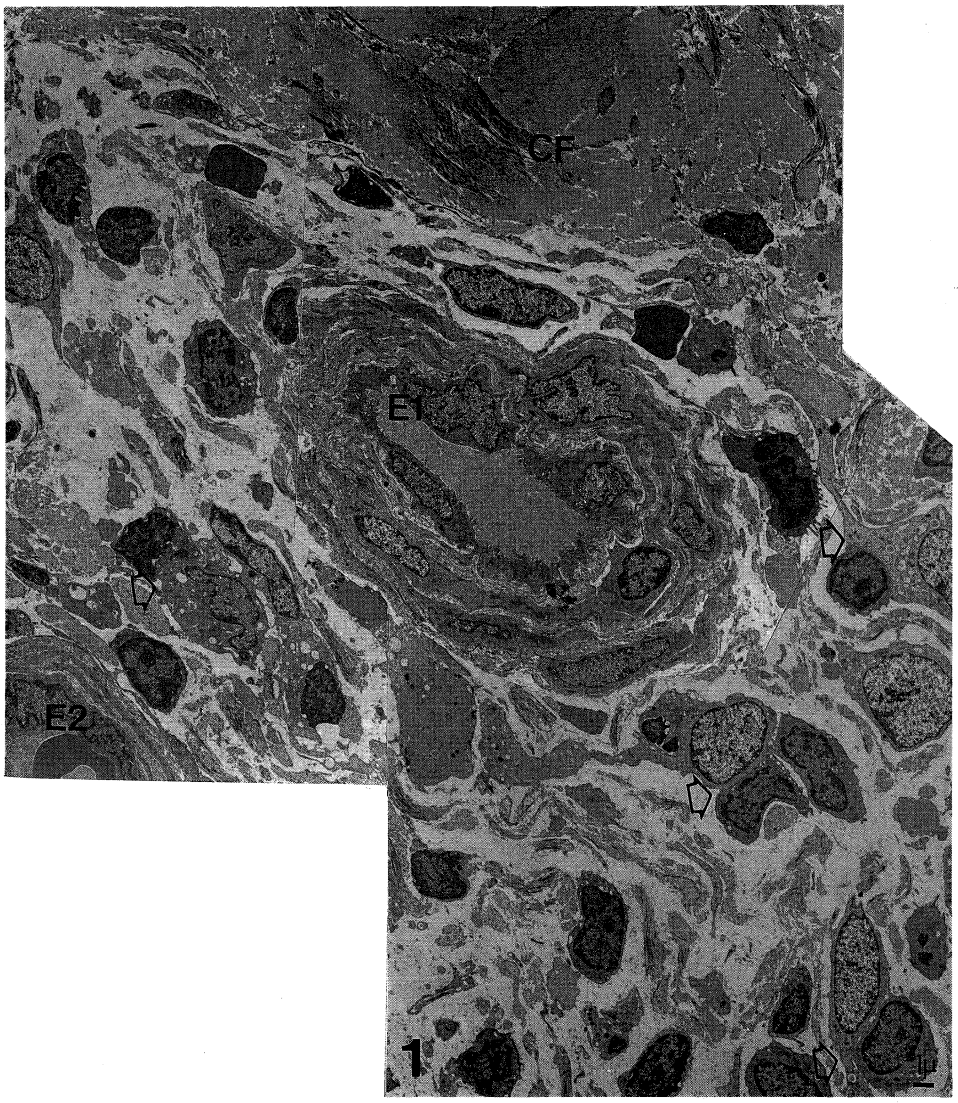

Fig. 1. Lymphocyte-fibroblast clusters (arrows) are seen around small blood vessels (El and E2) in the dermis of the erythema nodosum-like lesion of Behçet's syndrome. The edematous connective tissue with depletion of collagen fibers in the perivascular areas is bordered with collagen-rich connective tissue (CF).

fibers. Perivascular infiltrating lymphocytes often were intermingled with fibroblasts forming adhesions (Fig. 1). There were several types of adhesion : 1. The cell membranes of adjacent cells were arranged in parallel close, to each other (Fig. 2), 2. Cytoplasmic projections from the lymphocytes deeply penetrated into the cytoplasm of the fibroblasts (Fig. 3), 3. The lymphocytes were

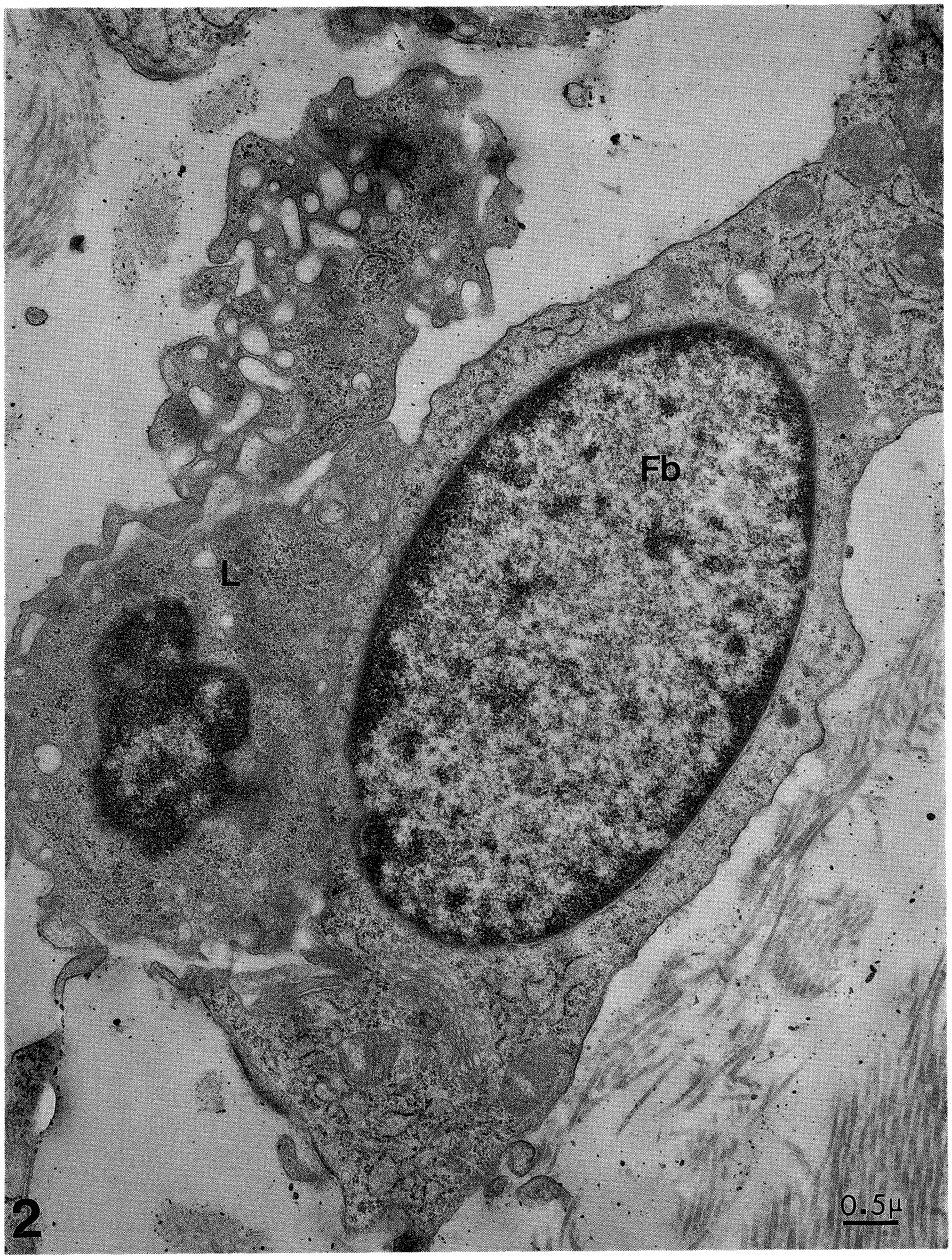

Fig. 2. Adhesion of a lymphocyte (L) to a fibroblast (Fb) is maintained by the close apposition of the two cells. The fibroblast contains well-developed rough endoplasmic reticulum and the Golgi apparatus with membranous system in the cytoplasm.

enveloped by cytoplasmic projection from the fibroblasts (Fig. 4), and 4. Lymphocytes penetrated into the cytoplasm of fibroblasts completely (Fig. 5) (this phenomenon is lymphocyte emperipolesis).

Lymphocytes which adhered to fibroblasts had only mitochondria and Golgi apparatus in the cytoplasm, but were rich in polyribosomes. This is a characteristic morphologic feature of lymphocyte activation (Figs. 3 and 4). No special

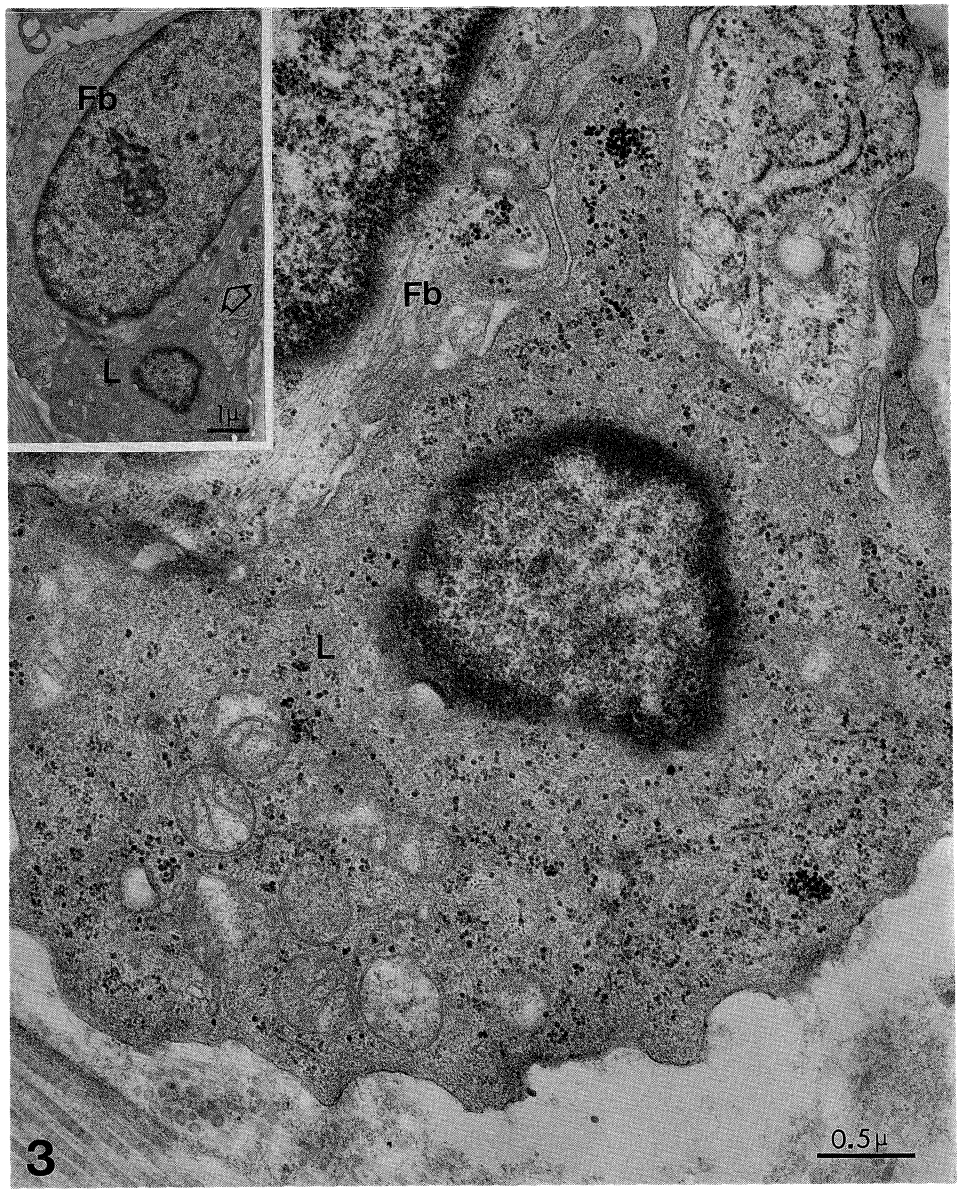

Fig. 3. A lymphocyte (L) is linked to a fibroblast (Fb) by several long, slender, cytoplasmic processes from the lymphocyte. Inset: Lower magnification of the two cells shows the close apposition of them (arrow). The lymphocyte contains numerous free ribosomes, clusters of polyribosomes and large mitochondria, but there are no prominent organelles in the cytoplasm.

apparatus for adhesion was observed in the cell membrane at the site of adhesion of either cell. Even when lymphocytes had penetrated completely into the cytoplasm of the fibroblasts, the cell membranes of both cells were preserved with a slight gap between them. Fibroblasts which were attached to lymphocytes showed marked development of rough endoplasmic reticulum and dilated

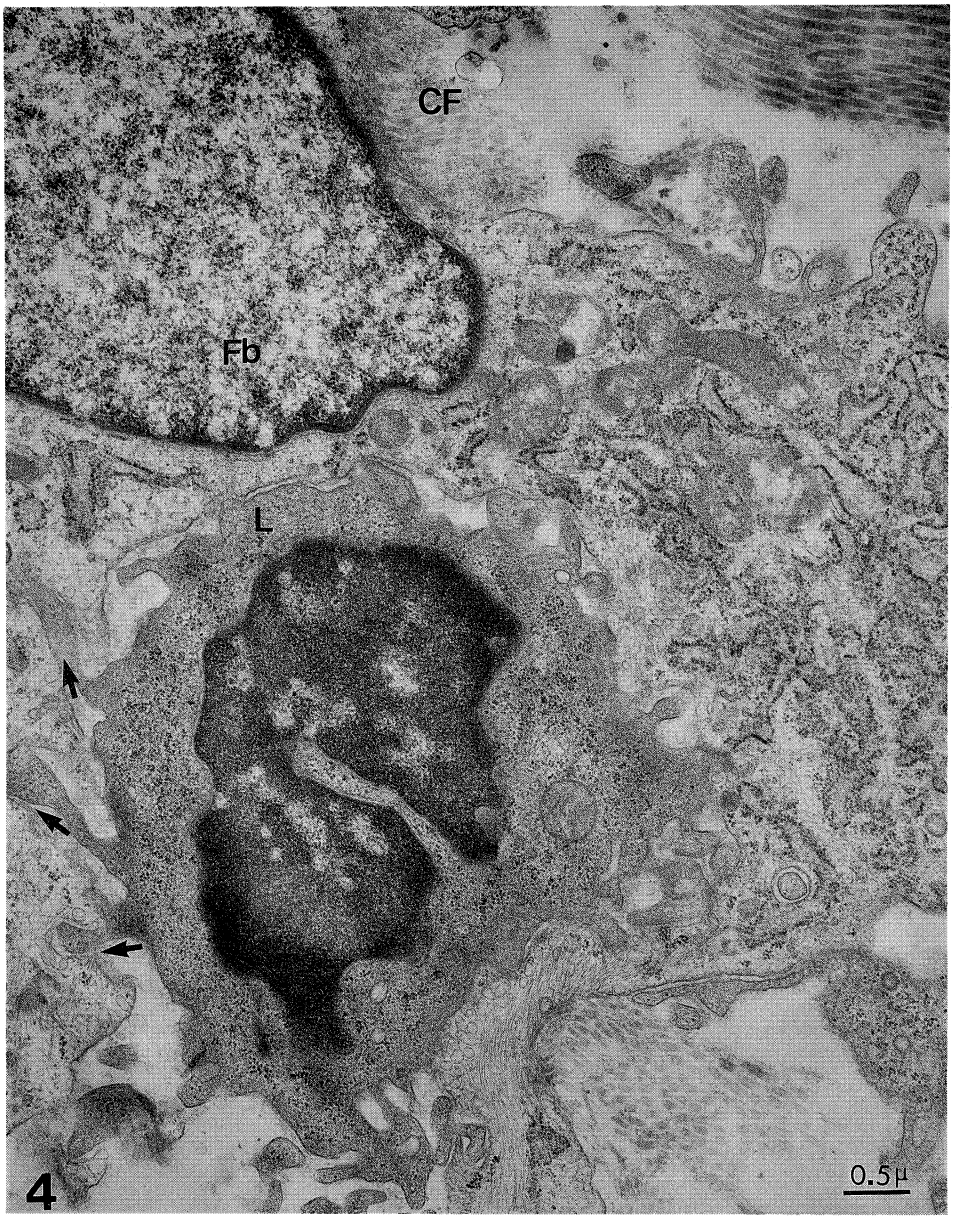

Fig. 4. A lymphocyte (L) is entering into a fibroblast (Fb). It attaches to the surface of the fibroblast by many villous cytoplasmic projections, insinuating themselves into the latter (arrows). The lymphocyte is studded with numerous clusters of polyribosomes in the cytoplasm. The fibroblast contains well-developed rough endoplasmic reticulum, and collagen fibers (CF) surround the cell membrane.

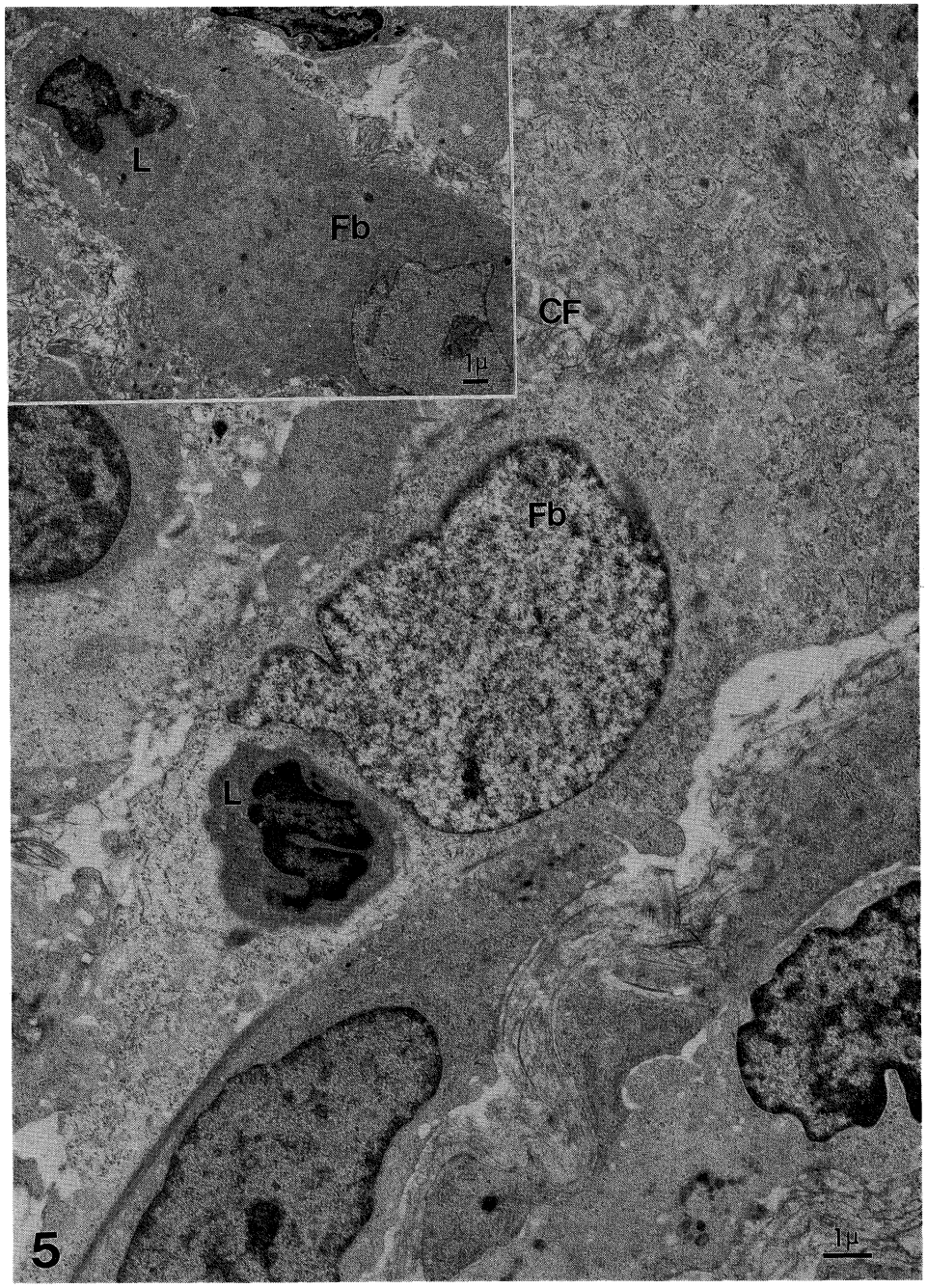

Fig. 5. A lymphocyte (L) is seen within the cytoplasm of a fibroblast (Fb) with extensive rough endoplasmic reticulum. This is emperipolesis. Collagen fibers (CF) surround the cell membrane of the fibroblast. Inset: An emperipoletic lymphocyte (L) is almost completely contained within the cytoplasm of a fibroblast (Fb). The fibroblast is also studded with extensive rough endoplasmic reticulum in the cytoplasm.

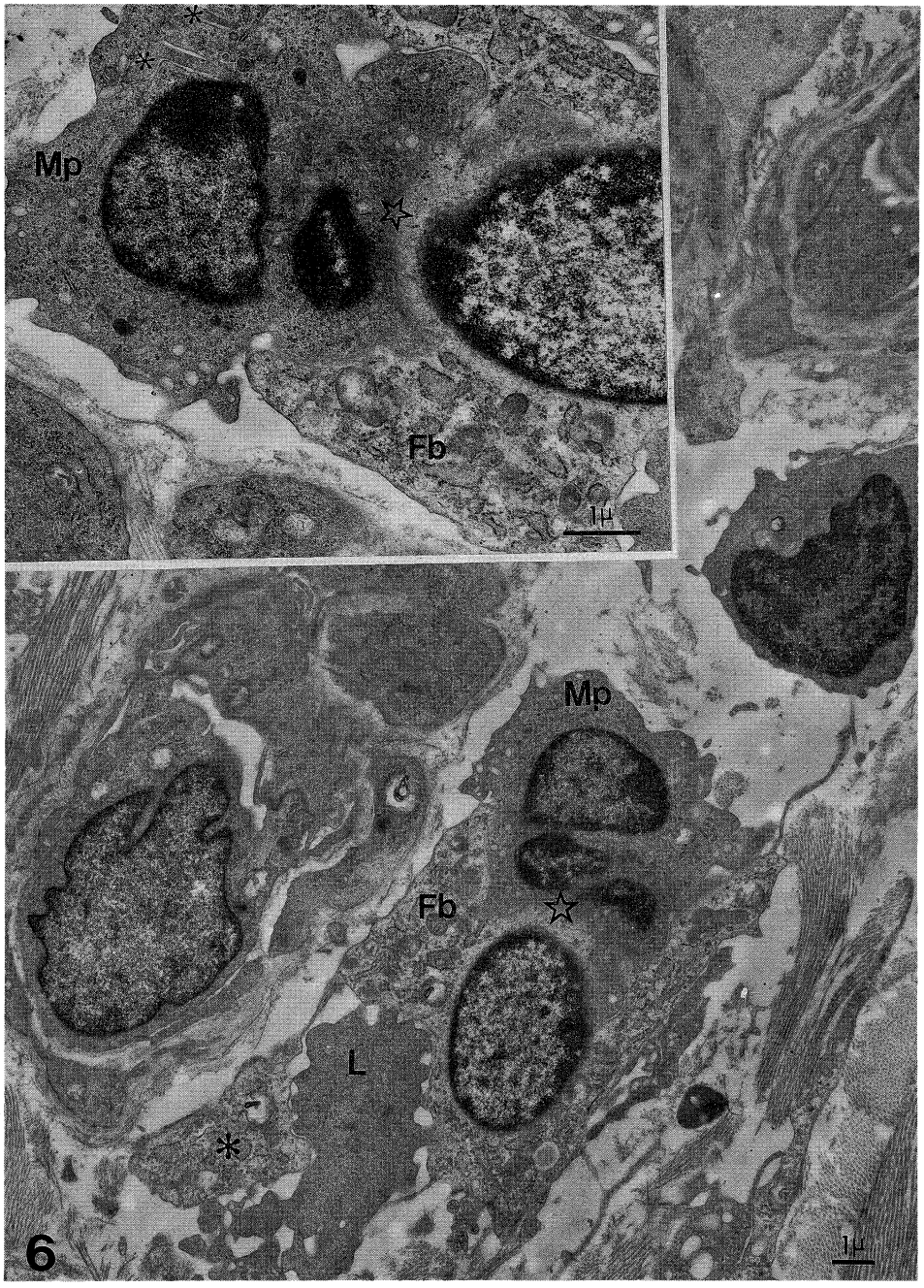

Fig. 6. Adhesion of a macrophage (Mp) to a fibroblast (Fb) adhering to a lymphocyte (L) is also observed in the perivascular area of edematous connective tissue. The nucleus of the lymphocyte is not present in this plane of section. The fibroblast with dilated cisternae of the rough endoplasmic reticulum shows cellular lobation (asterisk) and is in close contact with the macrophage. Inset: The cytoplasm of the macrophage contains distinct cytoplasmic processes unidentified (asterisks). The position of the star corresponds to that in inset.

cisternae of them, but no degeneration or involutional changes suggestive of suppressed cellular function. Instead, numerous collagen fibers were observed on the surface of the cell membrane (Figs. 4 and 5), indicating active collagen synthesis. Since both the lymphocytes which had adhered to and penetrated

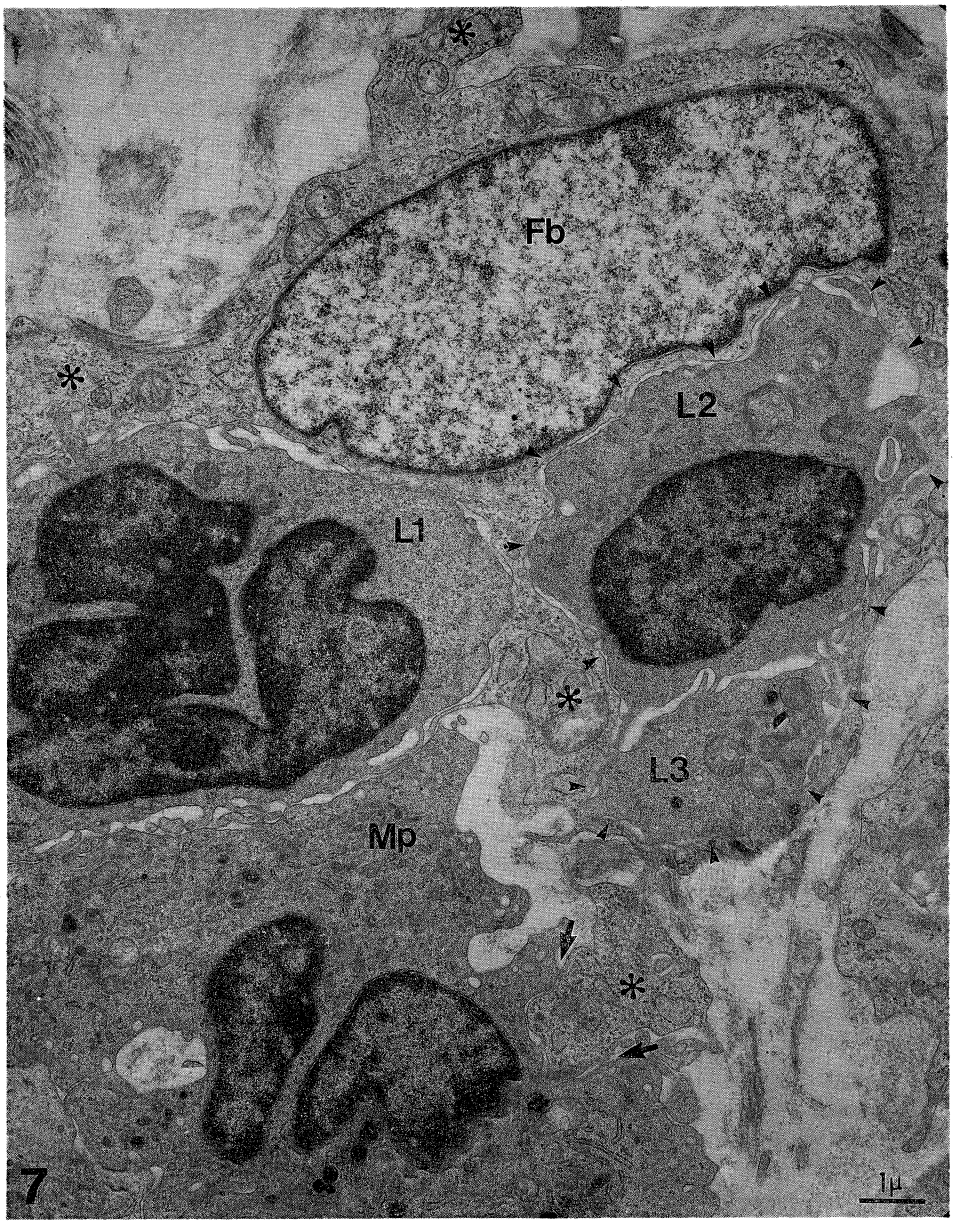

Fig. 7. A fibroblast (Fb) with a closely neighboring lymphocyte (Ll)-macrophage (Mp) cluster contains two lymphocytes (L2 and L3) within an intracytoplasmic membrane-bound space (arrowheads). The fibroblast shows cellular lobation or fragmentation (asterisks) and a relative lack of intracellular organelles such as rough endoplasmic reticulum. A portion of the cytoplasmic lobulation of the fibroblast attaches to the adjacent macrophage (arrows).

fibroblasts and the lymphocytes which were involved in emperipolesis had scarce intracytoplasmic organelles but abundant polyribosomes, they were considered to be cells of the same subset.

Macrophages as well as lymphocytes have also a tendency to invade into the cytoplasm of fibroblasts adhering to lymphocytes (Fig. 6). Moreover, the adhesion of macrophages to fibroblasts along with lymphocytes formed clusters, or "triads", with the fibroblasts (Fig. 7). In the clusters, the entire fibroblast become lobulated showing an increase in the number and size of cytoplasmic projections (Fig. 7). The fibroblasts lobulated do not manifest degenerative changes of cellular and nuclear configuration, but show a decrease of the intracytoplasmic organelles.

Adhesion of lymphocytes and/or macrophages to fibroblasts were observed in eleven of the eighteen patients in the studies, and six of the eleven patients were collected three to four days after the onset of the skin lesions. Emperipoletic lymphocytes in lymphocyte penetration of fibroblasts were seen in four of these six patients.

DISCUSSION

The ability of both T lymphocytes and macrophages to regulate fibroblast proliferation and collagen synthesis has been reviewed recently.[13] Lymphocyte products can stimulate fibroblast migration,[15] proliferation,[16] and collagen synthesis.[17] A close relationship between lymphocytes and fibroblasts has been suggested also by adhesion between these cells as observed by electron microscopy.[18] We observed adhesion primarily between perivascular infiltrating lymphocytes and fibroblasts in edematous connective tissues with depletion of collagen fibers in the dermis and subcutaneous fat at the sites of the erythema nodosum-like lesions in patients with Behçet's syndrome. By this adhesion, lymphocytes tended to penetrate into the cytoplasm of the fibroblasts. Since both the lymphocytes which were involved in adhesions to fibroblasts and the lymphocytes which had completely penetrated fibroblasts had scarce intracytoplasmic organelles but abundant polyribosomes, they were considered to be cells of the same subpopulation. Their penetration is probably the inherent nature of this subpopulation of lymphocytes and the process was termed lymphocyte emperipolesis by Humble *et al.*[19] in 1956. Emperipolesis has been described in a number of cases, primarily in tissue culture. In most instances, normal lymphocytes or neoplastic lymphoid cells were apparent within the cytoplasm of normal or neoplastic monolayer-forming cells.[19–22] In vitro penetration of lymphocytes into fibroblasts derived from normal lymph nodes of dogs has already been reported by Moore and Hlinka[23] in 1964. In vivo demonstration of lymphocyte emperipolesis in this study reports a new context in which this process occurs. However, there is another reason for reporting the ability of lymphocytes to penetrate fibroblasts. In the erythema nodosum-like lesions of Behçet's syndrome, lymphocyte emperipolesis was observed frequently three to four days after the appearance of the skin eruptions and their fibroblasts with a powerful affinity for activated lymphocytes appeared in a possibly healing stage accompanied dominantly by fibroblast proliferation.[12] This finding seems to be closely associated with the clinical finding that erythema nodosum-like

lesions heal with few sequelae. Speculation on the significance of lymphocyte emperipolesis in the evolution of these lesions is based on the observation that penetrated fibroblasts showed morphologic features suggestive of cell activation, such as well-developed rough endoplasmic reticulum and numerous collagen fibers on the cell membrane. Since, in the present study, it is conceivable that fibroblasts could be activated not only by the stimulatory lymphokines and/or monokines, but also by direct interaction with lymphocytes.

Another significant aspect of lymphocyte emperipolesis is that it has been assumed to be a morphologic index of the cytotoxicity of killer cells and natural killer cells. These cells are FC receptor-bearing, and they attack target cells such as tumor cells by mechanisms other than phagocytosis.[22,24] However, lymphocyte emperipolesis seen in erythema nodosum-like lesions seems to reflect direct activation of fibroblasts and not cytotoxicity as when killer cells and natural killer cells attack target cells, otherwise, we would not expect to see fibroblasts with such well-developed cytoplasmic organelles. Natural killer cells is the name given to large granular lymphocytes with electron-dense granules.[25–28] Lymphocytes which adhered to fibroblasts lacked the characteristics of natural killer cells. Therefore, emperipoletic lymphocytes observed in this study represent a morphologically distinct lymphocytic subpopulation.

On the other hand, macrophage and/or monocyte products which suppress fibroblast chemotaxis, proliferation, and collagen synthesis also have been identified, suggesting the presence of homeostatic control of fibroplasia and fibrogenesis.[13] Adhesion of lymphocytes to fibroblasts was followed by adhesion to macrophages. The fibroblasts adhering to lymphocytes are also possessed of a powerful affinity for the macrophages. In the triads of lymphocyte-fibroblast-macrophage, the cytoplasm of the fibroblasts adhering to macrophages tended to be lobated by intracytoplasmic lymphocytes penetrating. Such phenomena of the triads have not yet been reported in pathologic conditions accompanied by fibroplasia and fibrous repair. Although the mechanism of their cytoplasmic lobulation is unknown too, lymphocytes and macrophages adhering to and penetrating into the cytoplasm of fibroblasts, it is probable that the fibroblasts themselves become to slim as lobulating their cytoplasm. Consequently, the following adhesion to macrophages might be induced to dampen the intrinsic multifunctions in the fibroblasts through their cellular lobation and indiscriminately to suppress collagen synthesis. It is proved morphologically by our present data which show a decrease in the number of dilated rough endoplasmic reticulum in their cytoplasm (see Fig. 7).

In conclusion, our findings indicate that the homeostatic balance of connective tissue repair in the destructive process of erythema nodosum-like lesions is also maintained by cellular regulation of fibroblastic activity through the adhesion of lymphocytes and/or macrophages.

REFERENCES

1) Bang, D., Honma, T., Saito, T., Nakagawa, S., Ueki, H. and Lee, S.: Electron microscopic observation on dark endothelial cells in erythema nodosum-like lesions of Behçet's disease with ultrastructural X-ray spectroanalysis. J. Toxicol. Sci. **12** : 321-328, 1987
2) Nazzaro, P.: Cutaneous manifestations of Behçet's disease: Clinical and histopathological findings. *In* Behçet's disease, ed. by Monacelli, M. and Nazzaro, P. Basel, Karger. 1966, pp. 15-41
3) Lehner, T.: Pathology of recurrent oral ulceration and oral ulceration in Behçet's syndrome: Light, electron and fluorescence microscopy. J. Pathol. **97** : 481-494, 1969

4) Honma, T., Saito, T. and Fujioka, Y.: Ultrastructural observations on small blood vessels of gingiva in aphthous lesions. J. Jpn. Stomatol. Soc. **28** : 217-225, 1979
5) Kaneko, F., Takahashi, Y., Muramatsu, Y. and Miura, Y.: Immunological studies on aphthous ulcer and erythema nodosum-like eruptions in Behçet's disease. Br. J. Dermatol. **113** : 303-312, 1985
6) Bang, D., Honma, T., Saito, T., Nakagawa, S., Ueki, H. and Lee, S.: The pathogenesis of vascular changes in erythema nodosum-like lesions of Behçet's syndrome. Hum. Pathol. **18** : 1172-1179, 1987
7) Yamana, S., Jones, S.L. and Aoi, K.: Lymphocyte subsets in erythema nodosum from patients with Behçet's disease. *In* Studies on Behçet's disease, ed. by Behçet's disease research committee of Japan, Ministry of Welfare, Japan 1984. Tokyo. 1985, pp. 116-121
8) Savage, N.W., Seymour, G.J. and Kruger, B.J.: T-lymphocyte subset changes in recurrent aphthous stomatitis. Oral Surg. Oral Med. Oral Pathol. **60** : 175-181, 1985
9) Altman, L.C.: Chemotactic lymphokines: A review. *In* Leukocyte chemotaxis: Methods, physiology, and clinical implications, ed. by Gallin, J.I. and Quie, P.G. New York, Raven Press. 1978, pp. 267-287
10) Bang, D., Honma, T., Saito, T., Nakagawa, S., Ueki, H. and Lee, S.: Ultrastructure of vascular changes in cutaneous manifestations of Behçet's disease. Acta Derm. Venereol. (Stockh) **68** : 33-40, 1988
11) Honma, T., Bang, D., Saito, T., Nakagawa, S., Ueki, H. and Lee, S.: Appearance of membranocystic lesion (Nasu)-like changes in Behçet's syndrome. An electron microscopic study of erythema nodosum-like lesions. Acta Pathol. Jpn. **38** : 1001-1010, 1988
12) Shikano, S.: Ocular pathology of Behçet's syndrome. *In* Behçet's disease, ed. by Monacelli, M. and Nazzaro, P. Basel, Karger. 1966, pp. 111-136
13) Wahl, S.M.: Inflammatory cell regulation of connective tissue metabolism. Rheumatology **10** : 404-429, 1986
14) Honma, T., Bang, D., Saito, T., Nakagawa, S., Ueki, H. and Lee, S.: Ultrastructure of lymphocyte-mediated fat-cell lysis in erythema nodosum-like lesions of Behçet's syndrome. Arch. Dermatol. **123** : 1650-1654, 1987
15) Postlethwaite, A.E., Snyderman, R. and Kang, A.H.: The chemotactic attraction of human fibroblasts to a lymphocyte-derived factor. J. Exp. Med. **144** : 1188-1203, 1976
16) Wahl, S.M. and Gately, C.L.: Modulation of fibroblast growth by a lymphokine of human T cell and continuous T cell line origin. J. Immunol. **130** : 1226-1230, 1983
17) Johnson, R.L. and Ziff, M.: Lymphokine stimulation of collagen accumulation. J. Clin. Invest. **58** : 240-252, 1976
18) Wahl, S.M., Hunt, D.A., Allen, J.B., Wilder, R.L., Paglia, L. and Hand, A.R.: Bacterial cell wall-induced hepatic granulomas: An in vivo model of T cell-dependent fibrosis. J. Exp. Med. **163** : 884-902, 1986
19) Humble, J.G., Jayne, W.H.W. and Pulvertaft, R.J.V.: Biological interaction between lymphocytes and other cells. Br. J. Haematol. **2** : 283-294, 1956
20) Ioachim, H.L.: Emperipolesis of lymphoid cells in mixed cultures. Lab. Invest. **14** : 1784-1794, 1965
21) Hughes, D., Raine, C.S. and Field, E.J.: Invasion of neurones in vitro by non immune lymphocytes: An electron microscopic study. Br. J. Exp. Pathol. **49** : 356-359, 1968
22) Sandilands, G.P., Reid, F.M., Gray, K.G. and Anderson, J.R.: Lymphocyte emperipolesis revisited: 1. Development of in vitro assay and preliminary characterisation of the lymphocyte subpopulation involved. Immunology **35** : 381-389, 1978
23) Moore, A.E. and Hlinka, J.: Activities of lymphocytes inside fibroblasts. Proc. Am. Assoc. Cancer Res. **5** : 46, 1964 (abstract)
24) Burns, E.R., Zucker-Franklin, D. and Balentine, F.: Cytotoxicity of natural killer cells: Correlation with emperipolesis and surface enzymes. Lab. Invest. **47** : 99-107, 1982
25) Timonen, T., Ortaldo, J.R. and Herberman, R.B.: Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. J. Exp. Med. **153** : 569-582, 1981
26) Grossi, C.E., Cadoni, A., Zicca, A., Leprini, A. and Ferrarini, M.: Large granular lymphocytes in human peripheral blood: Ultrastructural and cytochemical characterization of the granules. Blood **59** : 277-283, 1982
27) Huhn, D., Huber, C. and Gastl, G.: Large granular lymphocytes: Morphological studies. Eur. J. Immunol. **12** : 985-988, 1982
28) Manara, G.C., De Panfilis, G. and Ferrari, C.: Ultrastructural characterization of human large granular lymphocyte subsets defined by the expression of HNK-1 (Leu-7), Leu-11, or both HNK-1 and Leu-11 antigens. J. Histochem. Cytochem. **33** : 1129-1133, 1985