The finding that dopa and 5-S-cysteinyldopa levels in the serum reflect the pigmentation of guinea pigs will certainly facilitate studies on pigment metabolism.

5-S-cysteinyldopa has now for many years been considered a specific melanocyte amino acid. Dopa is formed in many organs where it is a precursor of catecholamines. The present findings on dopa make it likely that the serum levels of this amino acid mainly reflect melanocyte metabolism—at least in pigmented animals.

Since dopa is rapidly metabolized in the body, investigations into dopa metabolites such as dopamine may also prove helpful in the study of pigment metabolism.

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REFERENCES

1. Breuer, M. M.: Binding of aromatic compounds by hair. Nature Phys Sci *229:* 185, 1971.
2. Hansson, C., Edholm, L.-E., Agrup, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: The quantitative determination of 5-S-cysteinyldopa and dopa in normal serum and in serum from patients with malignant melanoma by means of high-pressure liquid chromatography. Clin Chim Acta *88:* 419, 1978.
3. Harrison, W. H., Gray, R. M. & Solomon, L. M.: Incorporation of L-dopa, L-α-methyldopa and DL-isoproterenol into guinea pig hair. Acta Dermatovener (Stockholm) *54:* 259, 1974.
4. Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Determination of 5-S-cysteinyldopa in melanomas with a fluorimetric method. Yale J Biol Med *46:* 516, 1973.

C3b Receptor in Normal Human Oral Mucosa

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Abstract. The presence of a receptor for C3b in normal human oral mucosa was studied using C3b produced by treating normal human serum with cobra venom factor. Cryostat sections of normal human oral mucosa were incubated with C3b, followed by a direct immunofluorescence technique using monospecific goat-antihuman C3. The histologic localization of C3b fluorescence was determined by fixing cryostat sections with glutaraldehyde and staining with hematoxylin and eosin. The endothelial cells of the capillaries and smaller venules in the oral mucosa showed staining by anti-C3. C3b did not bind to the intercellular substance nor to the basement membrane zone of normal human oral mucosa. Normal human serum treated with EDTA was negative, thus indicating that native C3 did not bind to the receptor. The endothelial C3b receptor of the oral mucosa may have an important function in the localization of immune complexes that occur in systemic diseases, as well as in diseases restricted to the oral mucosa.

Key words: C3b receptor; Oral mucosa; Endothelial cell

Direct immunofluorescence of oral lesions is of great diagnostic value in bullous and ulcerative diseases involving the oral cavity (4, 5). Findings of immunoglobulins and complement components have been interpreted as evidence of immunologic pathogenesis mediated either by autoimmune antibodies or by immune complexes (1, 2, 6).

Thyresson et al. (7) reported the presence of an endothelial C3b receptor in normal human skin. This endothelial C3b receptor was located in the capillaries of the papillary dermis and dermis and on the endothelial cells that line the lumina of arterioles and venules. C3b receptors were not found in the intercellular substance or in the basement membrane zone of normal human epidermis.

In an effort to further investigate normal human tissue for the presence of a similar C3b receptor, normal human oral mucosa was examined.

METHODS

Tissue. Normal human oral mucosa was obtained from healthy patients undergoing elective dental surgical procedures. The mucosal specimens were immediately snap-frozen in liquid nitrogen and stored at −70°C until used. Serial cryostat sections 4 μm thick were cut fresh each working day. No air drying or fixatives were used.

Preparation of C3b. Fluid-phase C3b was prepared accordnig to the method of Thyresson et al. (7). Normal human serum was obtained from whole blood of healthy donors and was allowed to clot at room temperature for 60 min, after which the serum was separated by centrifugation, aliquoted in 0.1 ml volumes, and stored at −70°C, until use. All sera had normal levels of total complement and C3. To produce C3b, a mixture of cobra venom factor (1 : 2) (Cordis Laboratory, Miami, FL) and normal human serum (1 : 2) was allowed to react for 30 min at 37°C. The

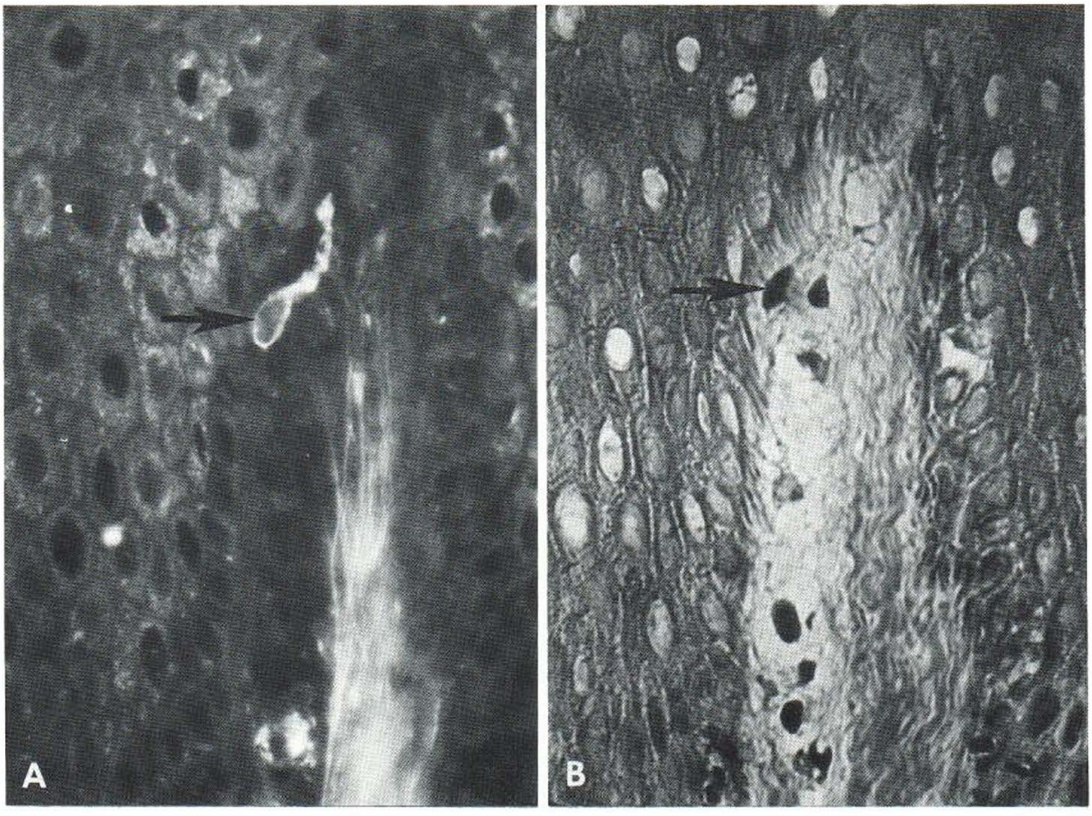

Fig. 1. Localization of C3b receptor in capillary endothelium of oral mucosa. (A) Section of oral mucosa incubated with cobra venom-treated human serum, followed by FITC-anti-C3. *Arrow* indicates endothelial staining (×500). (B) Same section stained with hematoxylin and eosin. *Arrow* indicates endothelium (×500).

pH was then adjusted to between 7.1 and 7.3, and the material was immediately used on the tissue sections.

Immunofluorescence. Cryostat sections were incubated for 20 min at 25°C, with a source of C3b at pH between 7.1 and 7.3. The slides were washed for 10 min in phosphate-buffered saline (PBS), pH 7.2, followed by an addition of fluorescein-conjugated anti-C3. After additional washing, the slides were mounted with coverslips in 10% PBS and glycerin and were examined the same day. Monospecific fluorescein-conjugated goat anti-C3 (Hyland Laboratory, F/P weight ratio 8.3 μg/mg, F/P molar ratio 3.4, specific antibody concentration, 2.2 mg/ml was used in a dilution of 1:60. The localization of specific immunofluorescence could be directly evaluated by fixing selected cryostat sections in 3% glutaraldehyde for 30 min, followed by staining with hematoxylin and eosin. Photographs of the exact same tissue sites were thus obtained utilizing immunofluorescence and tissue sections stained with hematoxylin and eosin.

Microscope. A Leitz Ortholux II UV-Epiluminator microscope was used. A UG1 (300 to 400 nm) excitor filter and (430 nm) barrier filter were used. A 25× fluoride water-immersion objective was used for screening, and a 50× fluoride water-immersion objective was used for photographing.

Buffers. The following buffers were used in the study: glucose-gelatin Veronal buffer (gl-gvb^{++} 0.075), pH 7.4, for dilution of normal human serum and cobra venom factor, and PBS, pH 7.2 to 7.3, was used for the washing of tissues as one of the controls and to dilute fluorescein-conjugated antibody.

Controls. Phosphate-buffered saline and normal human serum treated with 0.01 M EDTA were used as controls.

RESULTS

C3b did not bind to the intercellular substance or to the basement membrane zone of intact normal human oral mucosa (Table 1). The endothelial cells in the capillaries and small venules of the oral mucosa showed binding of C3b, which was demonstrated by the positive immunofluorescence by anti-C3. Fluorescence of the endothelial cells was seen in the capillaries (Fig. 1 A). The endothelial cell iden-

Table I. *Immunofluorescent staining of normal human oral mucosa after incubation with serum treated with cobra venom factor*

Structure/cell	Result[a]
Intercellular substance of epithelium	Neg.
Basement membrane zone	Neg.
Endothelial cells of capillaries–venules	++/+++

[a] Result of staining with goat FITC anti-C3 (1 : 60).

tification is demonstrated by comparing the section stained with anti-C3 (Fig. 1A) with the same one stained with hematoxylin and eosin (Fig. 1B). The pattern of immunofluorescence consisted of linear and wavy fluorescent streaks that appeared to be located on the surface of the endothelial cells. It was not possible by means of an immunofluorescence histologic study to localize the staining precisely. Oral mucosa incubated with normal human serum treated with 0.01 M EDTA did not show any positive immunofluorescence with anti-C3, thus indicating that native C3 did not bind to the oral mucosa. The goat anti-C3 conjugate showed no binding by itself to the tissues (Table II).

DISCUSSION

Our study of normal human oral mucosa demonstrated the presence of an endothelial C3b receptor. In an *in vitro* technique, C3b (produced by treatment of normal human serum with cobra venom factor) was bound to the capillaries and small venules in cryostat sections of normal human oral mucosa. Recently, Thyresson et al. (7) reported the presence of a similar endothelial C3b receptor in the capillaries, venules, and arterioles of normal human skin. Our study of human oral mucosa had findings parallel to those in normal human skin: (1) the presence of an endothelial C3b receptor, (2) no binding of C3b by either the intercellular substance or the basement membrane zone of normal human skin and oral mucosa, and (3) no binding of native C3.

We have proposed (7) that the endothelial C3b receptor could serve as the anchor by which immune complexes localize to the surface of endothelial cells, followed by subsequent release of C3a and C5a, with attraction of granulocytes and liberation of histamine, leading to tissue injury. The same pathogenic event is likely to occur in the vessels of the oral mucosa in diseases mediated by immune complexes. Indirect evidence for such a mechanism is the report by Ullman & Gorlin (8) of positive immunofluorescence in aphthous stomatitis, showing IgA, IgM, and C3 in the capillaries of oral mucosa.

We did not find in vitro binding of C3b to the intercellular substance or basement membrane zone of normal human oral mucosa. This is in contrast to the frequent finding of in vivo deposition of C3 in these locations in various bullous and ulcerative diseases of the oral mucosa. The consistent occurrence of *in vivo* bound C3 to the intercellular substance and to the basement membrane zone in bullous and ulcerative diseases of the oral mucosa might be caused by (1) the binding of C3b to opened sites in these structures by a preceding disease mechanism, or (2) the fixation of C3 to immunoglobulin molecules already bound to the intercellular substance and the basement membrane (3).

The present study demonstrated an endothelial C3b receptor in the oral mucosa—a finding similar to that previously described for normal human skin. The endothelial C3b receptor might serve as a binding site for immune complexes, which will evoke an immunologic response leading to tissue injury.

Table II. *Control studies of immunofluorescent staining of normal human oral mucosa by anti-C3 to establish tissue binding of C3b—but not C3*

Normal human oral mucosa incubated with:	Goat FITC anti-C3 (1 : 60)
Normal human serum 1 : 4 (0.01 M EDTA)	Neg.
Phosphate-buffered saline	Neg.
Normal human serum 1 : 4 treated with cobra venom factor	+ +/+ + +

REFERENCES

1. Chorzelski, T., Jabłońska, S., Blaszczyk, M. & Jarzabek, M.: Autoantibodies in pemphigoid. Dermatologica *136*: 325, 1968.
2. Jordon, R. E., Beutner, E. H., Witebsky, E., Blumenthal, G., Hale, W. L. & Lever, W. F.: Basement zone antibodies in bullous pemphigoid. JAMA *200*: 751, 1967.

3. Jordon, R. E., Sams, W. M., Jr & Beutner, E. H.: Complement immunofluorescent staining in bullous pemphigoid. J Lab Clin Med *74:* 548, 1969.
4. Jordon, R. E., Schroeter, A. L., Good, R. A. & Day, N. K.: The complement system in bullous pemphigoid. II. Immunofluorescent evidence for both classical and alternate-pathway activation. Clin Immunol Immunopathol *3:* 307, 1975.
5. Lever, W. F.: Pemphigus and Pemphigoid, 266 pp. Charles C Thomas, Publisher, Springfield, IL, 1965.
6. Rogers, R. S., III, Perry, H. O., Bean, S. F. & Jordon, R. E.: Immunopathology of cicatricial pemphigoid: studies of complement deposition. J Invest Dermatol *68:* 39, 1977.
7. Thyresson, H. N., McDuffie, F. C. & Schroeter, A. L.: Unpublished data.
8. Ullman, S. & Gorlin, R. J.: Recurrent aphthous stomatitis: an immunofluorescence study. Arch Dermatol *114:* 955, 1978.

Subpopulations of T Lymphocytes in a Patient with Fulminant Mycosis Fungoides

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Abstract. A patient with fulminant Mycosis fungoides was found to have a very high percentage of T lymphocytes with Fc receptors for IgG and a low percentage of T lymphocytes with Fc receptors for IgM. The total number of T lymphocytes was normal, but the in vitro function of the lymphocytes was depressed in short-term cultures, though not in cultures of 5 days' duration. T lymphocytes with Fc receptors for IgG are considered to have suppressor functions, and the immmunological changes in this patient may be explained by an increased suppressor cell activity. Other investigators have proposed that the abnormal lymphocytes in Mycosis fungoides are T helper cells. After treatment with electron beam and transfer factor, our patient developed uraemia due to uric acid crystallization in the kidneys. This complication to the treatment given seems not to have been reported before.

Mycosis fungoides is characterized by an infiltration of lymphocytes in the skin. The cells are mostly large lymphocytes, immunoblasts, or Sézary cells (6). There is reason to believe that the 'mycosis cell' is derived from the thymus-derived (T) lymphocyte population, and that the T-lymphocytes must be T helper lymphocytes characterized byan Fc receptor for IgM on their surface (2, 7).

We report here our studies on subpopulations of T lymphocytes and lymphocyte reactivity in vitro in a patient with a fulminant form of Mycosis fungoides.

CASE REPORT

A 73-year-old man was admitted with a universal erythrodermia and an itching eruption of brownish-red infiltrated patches and tumours. The symptoms were of 6 months' duration. The inguinal lymph nodes were enlarged, but histological investigation of these glands and of bone marrow showed no sign of involvement. A skin biopsy confirmed the diagnosis of Mycosis fungoides, stage III. The patient had light crural oedema, but apart from the skin changes the clinical findings were normal. Laboratory investigations showed a slight increase in the erythrocyte sedimentation rate, normal haemoglobin and a normal white blood cell count. The serum creatinine content was normal (1.0 mg per 100 ml of serum), serum uric acid was 8.4 mg per 100 ml of serum.

The patient was treated with potent steroid ointment, electron beam and transfer factor. Initially, he responded well, but a few months later, one week after a new course of electron beam therapy, he was admitted with uraemia, which apparently developed within the course of a few days. Serum creatinine was around 20 mg per 100 ml of serum, serum uric acid was 24 mg per 100 ml of serum, there was polyuria, proteinuria and erythrocyturia. The situation deteriorated in spite of treatment, and the patient died from bronchopneumonia and uraemia. Because of excessive cadaverositas a search for uric acid crystals in the kidneys failed.

Immunological investigations using our routine techniques (4) showed a normal number and percentage of T lymphocytes forming rosettes with sheep erythrocytes (Table I). However, the percentage of T lymphocytes carrying an Fc receptor for IgM was low. Actually, the ratio between these two populations was completely reversed at all investigations. Table II shows the results of the in vitro function of the lymphocytes. In unstimulated cultures the DNA synthesis was high during the first 3 days of culture after which it fell to normal levels at the 5-day culture. The PHA-stimulated cells