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Scanning electron microscopy observations of nasal mucosa in patients affected by retinitis pigmentosa

V. Petrozza[1], E.M. Vingolo[2], J.L. Pacini Costa[3], G. de Ruggieri[2], F. Carpino[1] and M. Melis[1]

[1]Dipartimento Biopatologia Umana, [2]Istituto di Oftalmologia, Roma, Italy and [3]Immunologia Cellular, Universidade de Brasilia, Brasil

Summary. A controlled study of the ciliated epithelium of olfactory mucosa was performed on 25 patients affected by retinitis pigmentosa and in 5 healthy subjects. Scanning Electron Microscopy showed a significant lack of ciliated epithelial cells. These cells probably represent undifferentiated ciliated cells. These morphological alterations of the olfactory mucosa might correspond to similar changes in the outer segment of retinal photoreceptors in retinitis pigmentosa.

Key words: Retinitis pigmentosa, SEM, Nasal mucosa, Eye

Introduction

Retinitis pigmentosa (RP) is a group of illnesses which share a similar retinal pigmentary degeneration pathway. Two thirds of the affected people have a family history consistent with genetic inheritance (Vingolo et al., 1989a,b,c).

This disease has a slowly progressive evolution over many decades and can lead to total loss of vision by complete destruction of retinal photoreceptors. Its pathogenesis is unknown and there is no efficient method to cure or prevent blindness (Vingolo, 1987).

It remains unclear whether the primary abnormality in RP is in the photoreceptor or in the pigmentary epithelium of the retina (Heckenlively, 1988).

Offprint requests to: Dr. V. Petrozza, Dipartimento Biopatologia Umana, V.le Regina Elena 324, Università «La Sapienza», 00161 Roma, Italy

RP clinical findings are characterized by a progressive decrease of visual field, nictalopia and good visual acuity until late in the course of the disease. Opthalmoscopy shows peripheral retinal «bone-spicule» pigmentation and narrow retinal vessels which usually spread centrifugally to the macula and finally involve all the retina. Optic atrophy normally appears late. The electroretinogram (ERG) is decreased or absent (Vingolo, 1987; Heckenlively, 1988).

RP pigmentosa appears as an isolated finding or part of many systemic syndromes like Usher's, Edward's and others. Hearing loss has always been associated with RP especially in Usher's syndrome which is characterized by a profound hearing defect from an early age. In these cases an alteration of Corti's organ and nasal mucosa are also present (Usher, 1914; Finkelstein, 1982; Hersch, 1983).

Ciliated neuroepithelium of neuroreceptors of retina, coclea and olfactory mucosa have a similar embryonic origin. The retinal photoreceptors seem to be modified cilia and neuronal hearing loss in RP could be a ciliary defect (De Robertis, 1956; Arden and Fox, 1979; Fox, 1980; Hersch, 1983).

The aim of this study is to observe superior olfactory mucosa epithelium by Scanning Electron Microscopy (SEM) in patients with RP in order to foresee possible alterations concerning the ciliated cells of the same origin of retinal photoreceptors.

Materials and methods

Our observations were performed on bioptic samples of nasal mucosa obtained from 25 patients affected by RP. Patients in which there were either diseases in their upper respiratory ways or eventually

showed casual alterations due to other pathological or environmental factors (smoke, congenital and acquired nasal cavity alterations, recent antibiotic treatment, etc) were excluded.

Moreover, biopsies from five normal healthy volunteers were performed. These results were casually mixed with the other ones of patients with RP to perform a double blind control. In all patients a full informed consent according to the research procedure was obtained.

Drawings were obtained using microsurgical techniques, reaching homogeneity of samples at nasal vault level in correspondence of the lamina cribrosa of ethmoid and upper face of superior horn.

The bioptic fragments were immediately fixed in 2.5% glutaraldehyde (cacodylate buffer 0.1 M, pH 7.4) for 24 hours; postfixed in OsO_4; washed in buffer and dehydrated in acetone at decreasing concentrations; treated according to the techniques of CO_2 critical point; coated with gold and observed by S.E.M. 15-25 KW Cambridge stereoscan 150 (Anderson, 1951).

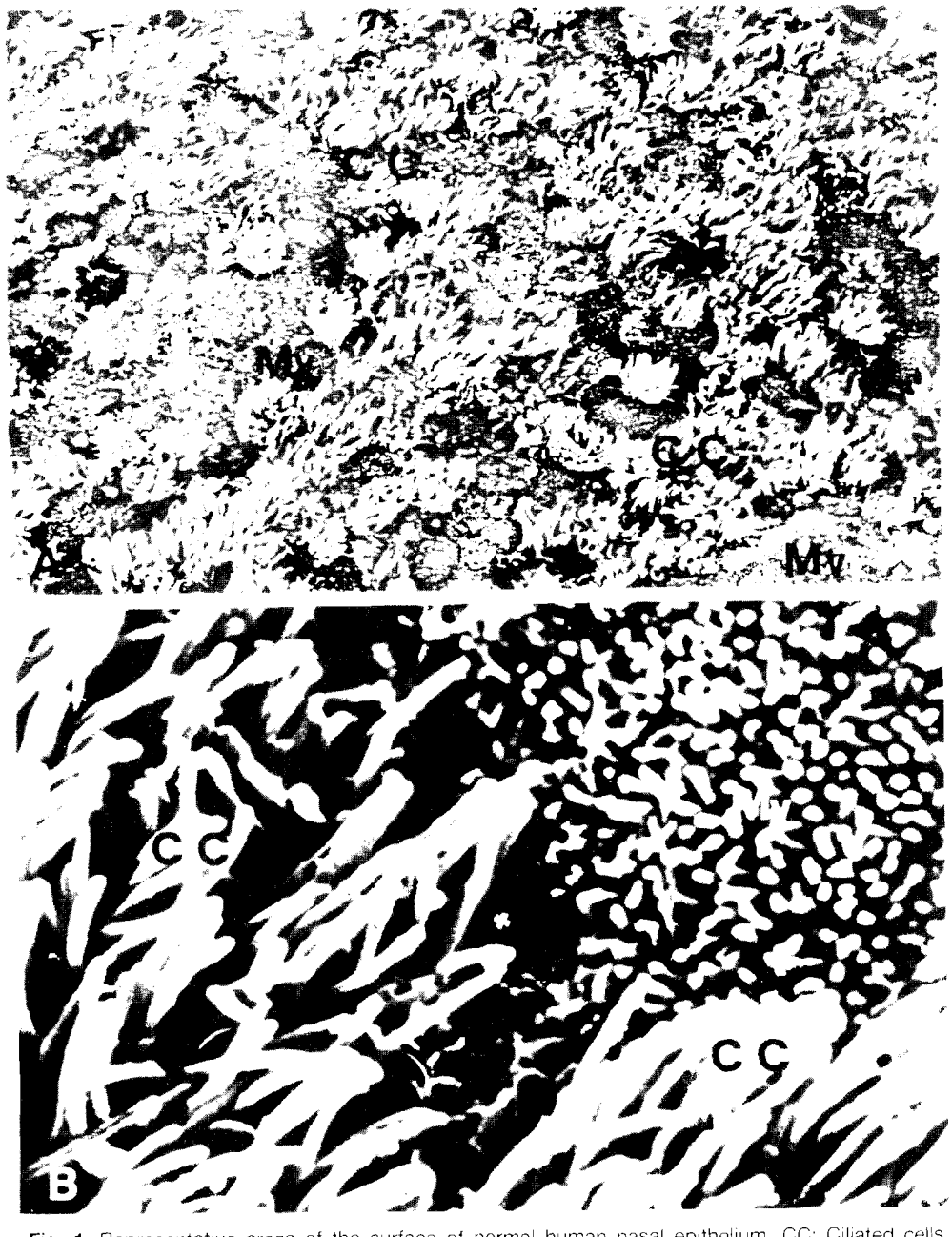

Fig. 1. Representative areas of the surface of normal human nasal epithelium. CC: Ciliated cells. Mv: Microvilli. *: Bowman glands. A × 1,000. B × 10,000

Results

The epithelial lining of the nasal mucosa of normal patients, examined by S.E.M. was composed of two cellular types: polygonal cells with several long cilia (5-6 μm), and cells that showed a number of short microvilli on the free surface with a regular distribution (Figs. 1A,B).

S.E.M. observations of bioptic samples from subjects with RP revealed significant morphological alterations of the epithelium inasmuch that the cell surfaces appeared almost completely devoid of cilia.

Furthermore, a great number of cells characterized by rare microvilli and few short cilia with altered cytoplasmic membranes were observed. Many lymphocytes were present over this mucosa surface (Figs. 2A, B).

These morphological alterations and the presence of lymphocytes in the olfactory mucosa were found in different places in all the RP patient samples, but not in the control group.

The statistical analysis, performed with χ^2 text 2 x 2 table for one degree of freedom, showed high significance levels between two groups ($p < 0.0001$).

Discussion

Cilia have a primary role in respiratory and reproductive function and also in sensorial receptors. Systemic defects of cilia are exemplified in Kartagener's

syndrome (Kartagener, 1933; Lupin, 1978) and Immotile Cilia Syndrome (ICS) (Afzelius et al., 1975; Afzelius, 1976, 1979). So the possibilities of systemic involvement of cilia RP seem to be real and led to several studies.

In early studies (Arden and Fox, 1979; Fox, 1980) a considerably high and statistically significant number of cilia ultrastructural anomalies of the epithelium of nasal mucosa was reported in 14 patients with RP.

These data were also confirmed by Finkelstein (1982) who described an alteration in the arrangement of microtubuli forming the internal architecture of nasal mucosa cilia. Therefore, they supposed that the reduced ciliary motility was related to an ultrastructural alteration.

Other studies (Charles, 1979; Hersch, 1983) pointed out very extensive ciliary alterations in ciliated cells so that one could presume to find characteristics of immotile cilia syndrome (ICS) in RP.

Further studies (Afzelius et al., 1975; Afzelius, 1976, 1979) on 9 patients affected by nasal mucosa alterations also reported spermatozoa immotility, chronic sinusitis and bronchiectasies which make these results comparable to an incomplete Kartagener syndrome.

However, more recent studies distinguished RP from ICS because no alterations of statistically significant motility of spermatozoa in subjects affected by retinal pigmentation were observed (Pedersen, 1975; 1976; Schneeberger, 1980).

Moreover, patients with ICS did not show that many morphofunctional changes (retinic pigmentation, electron-retinographic reductions, hemeralopia) to suggest that an involvement of the retinal structures may be similar to RP.

In another study (Finkelstein, 1982) on nasal mucosa biopsies from 15 patients, ciliar abnormalities, and the presence of ciliogenetic cells were attributed to altered ciliogenesis phenomena caused by the lack or the wrong synthesis of external arms of dinein, or by rays that join external tubular couples to two central tubulies of the axonem.

The current hipothesis is that a very extensive involvement of ciliated cells in RP may occur, although

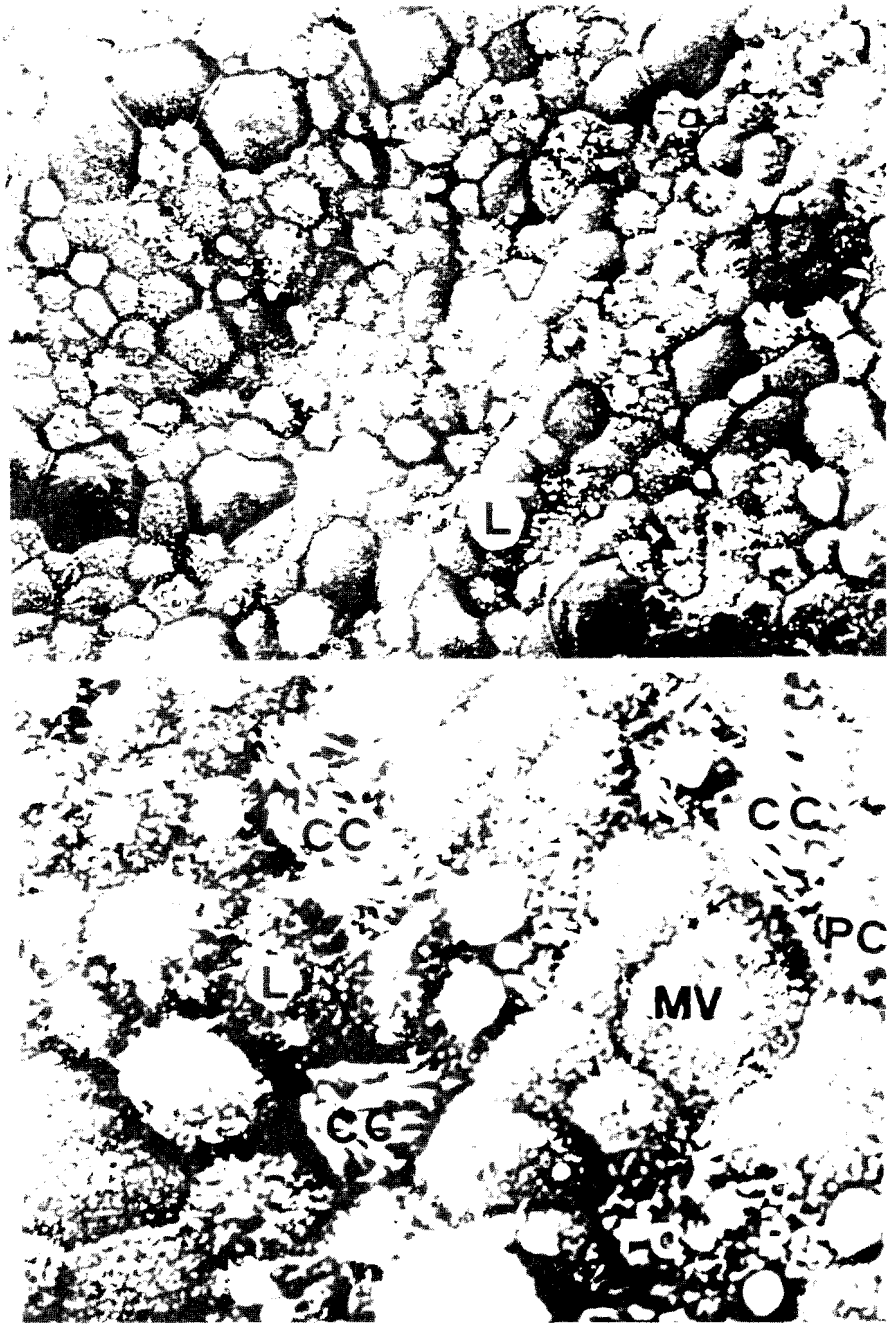

Fig. 2. Nasal epithelium from subjects affected by Retinitis Pigmentosa. The epithelial surface appears almost devoid of cilia. Ciliated cells (CC) show morphologically altered cilia, irregularly distributed. Non-ciliated cells, populated with microvilli (Mv) exhibit variation in the length and distribution of microvilli. Cells with few microvilli and some rather short cilia, perhaps representing a progenitor type (PC) are clearly visible. L: Lymphocytes. A × 1,000. B × 2,000

related cases in the literature only showed alterations in the inferior horn of the nasal cavity and inner ear.

Our SEM study at the olfactory-mucosa (OM) level in RP showed a lack of ciliated epithelial cells. The remaining cells only had rare microvilli or a few short cilia. A lack of cilia orientation was also noted.

It seems that the compound cilia are the result of an

abnormal regeneration very similar to that seen in local damage by external agents or infections. Our samples were obtained from patients without pathology which might justify a similar histopathological aspect.

The cellular type described in our study may represent an undifferentiated (progrenitor) ciliated cell (Friedman, 1971). Altered ciliogenesis phenomena are, perhaps, caused by the lack or wrong synthesis of external arms of dinein, or by lack of radial spokes that join external tubular couples to the two central tubules of the axoneme (Warner, 1974; Pedersen, 1975,1976; Marinozzi and Crifo, 1979; Finkelstein, 1982; Hunter et al., 1986).

The presence of lymphocytes at the sensorial mucosa level in RP patients which was not seen in controls is very interesting. We were not able to explain the role of lymphocytes in this degenerative tissue. The lymphocytes may be involved in an immune mechanism related to the alterated OM or they may play some role in the degenerative process of ciliated cells in the olfactory mucosa.

In conclusion, our study on the olfactory mucosa of the superior horn of the nasal cavity supports the hypothesis of ciliogenesis defects noted before by Fox (1980) and Finkelstein (1982) in the inferior nasal mucosa in RP patients.

The morphological defect in the olphactory cells suggests that a similar process may also occur in the outer segment of photoreceptors thus emphasizing the hypothesis that in RP the primary alteration is in the photoreceptor and not in the retinal pigmented epithelium (Heckenlively, 1988).

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