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Ciliary ultrastructure and nasal mucociliary clearance in chronic and allergic rhinitis

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SUMMARY

The authors have studied nasal specimens collected by means of nasal brushing in eight patients affected by allergic rhinitis and in eight affected by chronic rhinitis, while in other four patients affected by allergic rhinitis a lower turbinate biopsy was performed. All twenty patients showed an increased mucociliary clearance time and a reduced velocity regardless to the pathology during a previously performed saccharine test.

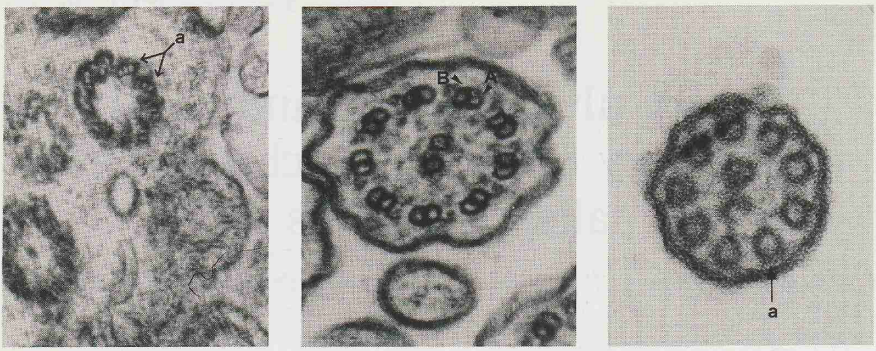
Different ultrastructural alterations have been observed, such as: a) both central and peripheral microtubules alterations; b) absence of dynein arms; c) absence of radial spokes; d) ciliary membrane alterations; e) "compound" cilia; f) disorientation of central tubules. These alterations have been observed variously associated in both allergic and chronic rhinitis patients groups.

Basing on their data, the authors state that ciliary abnormalities cannot be considered specific of a particular pathology but they can coexist in different situations. They also think that the mucociliary clearance parameters determination represents the only method to evaluate, even if in an indirect fashion, the percentage of ciliary abnormalities, as no direct quantitative method has been described. Ciliary ultrastructural alterations can be of diagnostic value only if associated with mucociliary clearance time and velocity determination.

INTRODUCTION

The rheological properties of mucous, that acts as a "carrier band", and the ciliary beats that allow its movements, are both fundamental for the mucociliary transport, one of the main aspecific defensive mechanisms of the respiratory tract (Satir, 1974; Warner and Satir, 1974; Mygind, 1979; Proctor, 1983).

The ultrastructural characteristics of normal cilia have been described in a previous paper (Paludetti et al., 1983); they are cellular processes, ranging in number from 50 to 250 per cell depending on the site and protruding into the nasal cavity. The complex structure that characterizes their intermediate part (Figure 1b), changes at the basal body (Figure 1a) and at the apex (Figure 1c) (Freedmann and



a. nasal biopsy b. nasal biopsy c. nasal brushing

Figure 1. Normal ciliary structure. The intermediate portion of the cilium is characterized by a double membrane wall, which surrounds the axoneme. The 9 peripheral doublets of microtubules are made up by two sub-units (A-B); dynein arms connect each A sub-unit to the consequent B one. The two central microtubules are connected by a sheat. Radial spokes converge to this sheat from the peripheral A sub-unit (X = 305,000) (1b).
At the basal body level the central microtubules disappear, and the peripheral doublets become triplets (a) (X = 183,000) (1a). At the apex the 9 peripheral doublets loose the B sub-units and only 9 single microtubules can be appreciated (X = 335,000) (1c).

Bird, 1971). In order to allow an optimal ciliary beat the orientation axis of the central tubules of adiacent cilia has to be as parallel as possible, the difference never exceeding 25°.
Disruption of ATP, due to an adenosintriphosphatase present within the dynein arms, produces the energy for the sliding of microtubules one on the other; this sliding together with the simultaneous stability of the basal body allows the methacronal ciliary beat that, with a frequency of 800–1000 beats/min promotes the mucous movement towards the rhinopharynx.
The aim of the present paper is to study ultrastructural ciliary abnormalities in subjects with different nasal pathology (chronic rhinitis, allergic rhinitis) but all presenting prolonged nasal mucociliary transport parameters and therefore to define, if present, a correlation between functional and morphological characteristics.

MATERIAL AND METHODS

Nasal mucociliary clearance parameters have been evaluated by means of the saccharine method (Andersen et al., 1971; Puchelle et al., 1981) in twelve subjects with allergic rhinitis and eight with chronic rhinitis. Reference values of the time and velocity of nasal mucociliary transport obtained in our laboratory have been reported elsewhere (Paludetti et al., 1983).
Nasal mucosa specimens have been then collected by means of "nasal brushing"

(Rutland and Cole, 1980) in eight allergic rhinitis patients and in eight with chronic rhinitis, while in four patients with allergic rhinitis the specimens were collected by means of a biopsy from the lower turbinate. Concerning the first method, a microbrush such as those used for bronchial "brushings" has been repeatedly passed upon the inferior turbinate to collect, in a relatively harmless fashion, specimens of nasal mucosa. The microbrush was then washed in 2.5% glutaraldehyde and the specimens fixed in the same solution. After six hours, glutaraldehyde was eliminated and the specimens stored in cachodylate buffer (pH 7.4, 0.1 M), until an inclusion in EPON 812 was performed. The sections, obtained with an ultramicrotome, were observed at the transmission electron microscope Philips ME 300 and then photographed. The same fixation and inclusion procedures were adopted for the biopsies.

RESULTS

Concerning nasal mucociliary clearance, the values of time and velocity of the mucociliary transport exceeded in each subject one normal standard deviation. Velocity ranged in the different subjects between 1.6 mm/m and 2.5 mm/m (normal values: 5.7 ± 3 mm/m for the right, and 6.0 ± 3 mm/m for the left nasal cavity). All the subjects showed therefore an impaired nasal clearance function.

One or more of the following ultrastructural alterations have been observed in each of the examined subjects: a) both central and peripheral microtubular anomalies (Figures 2, 3, 4, 5); b) absence of dynein arms (Figures 3, 5); c) absence of the radial spokes (Figures 3, 4, 5, 6); d) irregular contours of the cellular mem-

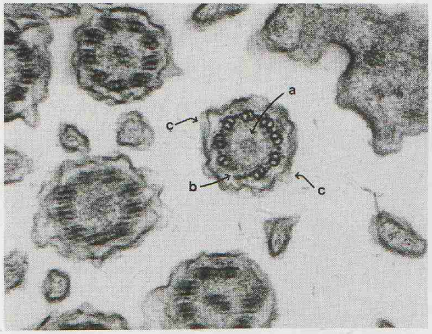


Figure 2.
Nasal brushing. Central microtubules (a); and one peripheral doublet (b) are absent (8 + 0); furthermore ciliary membrane alterations (c) are visible (X = 220,000).

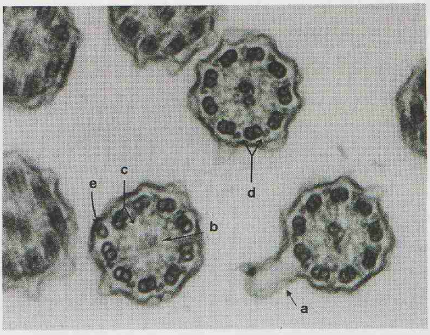


Figure 3.
Nasal brushing. Ciliary membrane alteration (a); loss of central microtubules (b); absence of radial spokes and dynein arms (c); which, on the contrary, are clearly identifiable in (d); eccentric microtubule (e); central microtubule loss (9 + 1, 9 + 1) but in eccentric position (X = 276,000).

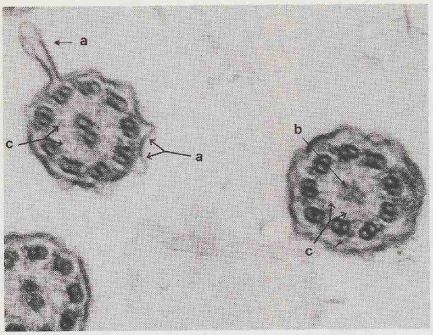


Figure 4.
Nasal brushing. Ciliary membrane alterations (a); absence of central microtubules (b); loss of radial spokes (c) (X = 280,000).

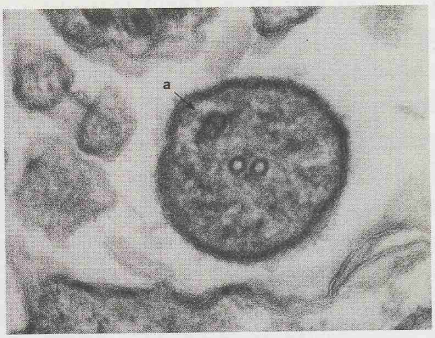


Figure 5.
Nasal brushing. Only one doublet of peripheral microtubules is present (a); radial spokes and dynein arms are absent (X = 400,000).

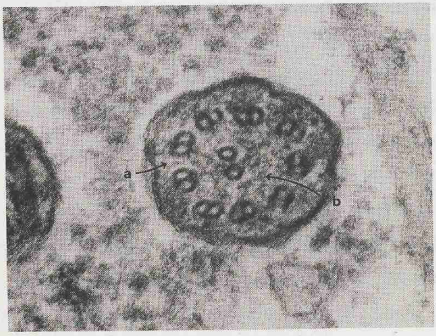


Figure 6.
Nasal brushing. Radial spokes (a) and dynein arms (b) are absent (X = 400,000).

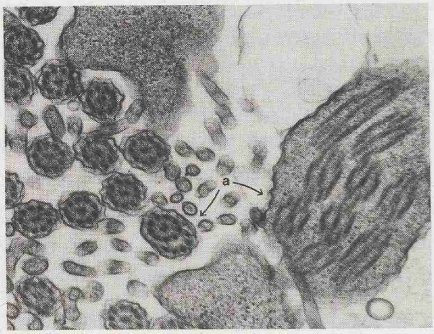


Figure 7.
Nasal biopsy. Ciliary membrane alterations, with presence of "compound" cilia (a) (X = 120,000).

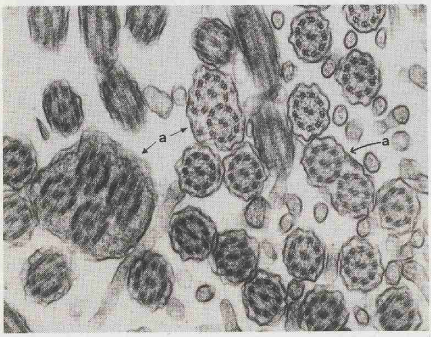


Figure 8.
Nasal biopsy. Ciliary membrane alterations, with presence of "compound" cilia (a) (X = 120,000).

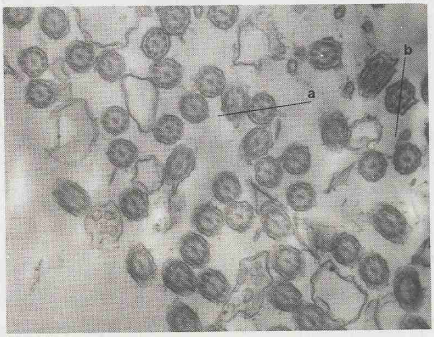


Figure 9.
Disorientation of central tubules exceeding 25° (a, b) (X = 80,000)

brane (Figures 2, 3, 4, 7, 8); e) compound cilia (Figures 7, 8); f) central microtubules disorientation (Figure 9).

A. The usual 9 + 2 figure of microtubules was often altered and figures such as 8 + 0, 9 + 1, 9 + 1 (but in anomalous position), 9 + 0, 9 + 0, 1 + 2, 8 + 2 were observed.
B. The absence of dynein arms is often associated with the absence of radial spokes and with anomalies of the cellular membrane.
C. The absence of radial spokes is often associated with the absence of central microtubules.
D. The cellular membrane contour abnormalities, observed by some authors in chronic bronchitis patients, resulted to be extremely frequent.
E. The compound cilia are characterized by a single cellular membrane that encloses several normal and abnormal ciliary structural units.
F. In only two of the cases in whom a biopsy of the lower turbinate has been performed a disorientation exceeding 25° of the central tubules has been observed.

Specimens obtained with "nasal brushing" and with biopsies resulted to be suitable for ultramicroscopic examination. Qualitative ciliary characteristics can be reliably investigated with both methods. The only limit of nasal brushing technique vs biopsy concerns the quantity of collected cilia, that is obviously greater when using biopsies. The main advantage of the nasal brushings is its harmlessness.

DISCUSSION

Ciliary motility is directly related to the normality of the structures that make up the cilium. Ultrastructural abnormalities rarely cause a total ciliary immobility, while often a reduction greater than 50% of the rhythm of their beat or, finally, a total desynchronization of their movement, that becomes vibratory, rotatory or vortical (Wayoff et al., 1982).The final result is that the mucous film covering the ciliary apex and forming the first defensive mechanism of the upper respiratory tract, slows down or stops.

Some syndromes involving especially the respiratory airways are probably due to the reduction of this defensive mechanism. The most well known and described of them is the Kartagener's syndrome (situs viscerum inversus, bronchiectasis, sinusitis), in which various kinds of ciliary abnormalities have been recognized (Afzelius, 1976; Pedersen and Mygind, 1976; Katz et al., 1977; Lupin and Misko, 1978; Rott, 1977; Rossman et al., 1980a; Schidlow et al., 1982); the absence of dynein arms that, according to Herzon (1981) is the only ciliary abnormality able to cause a ciliary immotility, seems to be its main characteristic feature.

Various ciliary abnormalities such as absence of radial spokes, ultrastructural pathology of microtubules, presence of compound cilia, orientation of central

tubules and even to the absence of dynein arms, have been observed in several other syndromes, involving only the respiratory tract, without association of other pathologies. The syndromes have been called "immotile cilia syndromes", to differentiate them from the Kartagener's syndrome (Satir, 1962; Afzelius, 1963; Ailsby and Ghadially, 1973; Konradovna et al., 1975; McDowell et al., 1976; Eliasson et al., 1977; Boat et al., 1979; Forrest et al., 1979; Jahrdoerfer et al., 1979; White et al., 1980; Rooklin et al., 1980; Rossman et al., 1980b; Veerman et al., 1980; Gilbert et al., 1981; Herzon, 1981; Teichberg et al., 1982). Similar ciliary abnormalities have been observed in subjects affected by allergic or chronic rhinitis (Sturgess et al., 1979, 1980; Takasaka et al., 1980; Dundley et al., 1982).

Ciliary abnormalities described in the present paper have been observed in patients selected not only according to clinical data but also to mucociliary clearance time and velocity data, exceeding in all subjects the $M \pm 1SD$ range. They have been observed both in subjects with allergic and chronic rhinitis. None of the subjects has been diagnosed as affected by Kartagener's syndrome.

This seems to demonstrate the relative aspecificity of the ciliary ultrastructural abnormalities that rarely can be correlated to a specific airway pathology.

Abnormal cilia can, in our opinion, be present in several pathologies and are able to evoke clinical symptoms when their quantity exceeds a certain percentage. In fact, according to Wiessemann et al., (1981), 5% of abnormal cilia are present in normal children. The mucociliary clearance parameters evaluation can represent an indirect but still valid and necessary test in order to correlate the qualitative and quantitative abnormalities of nasal mucosa as no direct quantitative methods are yet available.

RÉSUMÉ

Les auteurs ont étudié l'ultrastructure de la muqueuse nasale chez douze sujets atteints de rhinite allergique et huit de rhinite chronique. Les fragments de muqueuse ont été obténus à l'aide d'une biopsie du cornet inférieur chez quatre sujets atteints de rhinite chronique, tandis-que chez les autres ils ont été obténus à l'aide d'un "brushing" nasal. Tous les sujets étaient caractérisés par un temps de clearance muco-ciliaire nasale augmenté et par une velocité réduite, sans rapport avec la pathologie. Cettes donnes avaient été obténues avec un test à la saccharine.

On a observé des altérations ultrastructurales différentes: a) altérations combinées des microtubules centraux et des doublets périphériques; b) absence des bras de dynéïne; c) absence des ancres radiaires; d) altérations de la membrane ciliaire; e) "compound cilia"; f) désorientation des microtubules centraux. Cettes altérations ont été observées associées de façon différente chez les sujets atteints de rhinite allergique et chronique.

Sur la base des données obténus, les auteurs affirment que les altérations ciliaires

ne peuvent pas être considérées spécifiques d'une pathologie particulière mais, au contraire, elles peuvent coexister en des conditions différentes. Les auteurs affirment aussi que la détermination des paramètres de la clearance muco-ciliaire représente la unique méthode connue pour évaluer, aussi si d'une façon indirecte, le pourcentage d'abnormalités ciliaires; en effet, on n'a jamais décrit, dans la littérature, une méthode quantitative directe. Les altérations ultrastructurales ciliaires peuvent aider le diagnostic seulement si ells sont évaluées en association avec la détermination de la vélocité et du temps de clearance muco-ciliaire nasale.

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