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Amyloid-like protein in children with rhinoscleroma

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SUMMARY

Mucosal specimens from two children with rhinoscleroma have been investigated by light-, fluorescent- and electron microscopy. Amyloid-like protein was found, located not only in the vessel walls but also impregnated in the basic substance of the connective tissue. In this paper the genesis of this protein is discussed, assuming that it could be a result of auto-immune reactions.

INTRODUCTION

The scleroma is a chronic granulomatous disease which occurs predominately in the upper respiratory tract. The causative agent is Klebsiella rhinoscleromatis. The first symptoms appear in childhood, but due to the lack of specific characteristics it is diagnosed later, after the typical granulomatous formations appear. A number of publications refer to pathological specifications in scleroma. Welsh et al. (1963) were the first to publish electron-microscopic observations concerning the disease. A number of works were published later on the ultrastructure of the Frisch-bacilli and the Mickulicz-cells (Gonzales-Angulo et al., 1965; Woyke et al., 1969; Hoffmann et al., 1973; Gaafar et al., 1979). Review of the available literature indicates that only a few investigators have focused their attention on the condition of the connective tissue in the scleroma process. In 1947 Eggeston and Wolff indicated the presence of various stages of sclerosis. In 1981 Toppozada et al. for the first time mentioned the existence of deposits of amyloid under the endothelial cells of the capillaries in rhinoscleroma, although they did not comment on this phenomenon.

Over the last ten years systematic research has been performed on the rhinoscleroma-patients in the Scientific Institute of Otorhinolaryngology of the Medical Academy of Sofia. This present paper discusses the result from our observations on the connective tissue in rhinoscleroma.

MATERIAL AND METHODS

Specimens from the nasal mucosa of two children with rhinoscleroma, aged 11 and 14, have been studied and the following methods were used:

1. Light microscopy on samples, coloured by using standard histological methods with Hemalaun-eosin, after Van Gieson; modification after Holuska and after Weigert.
2. Fluorescent microscopy, coloured with Thioflavin "S". First paraffin was removed from histological slices with water and then coloured with Hemalaun and after a new washing coloured again with 1% water solution of Thioflavin "S". After differentiation in 80% alcohol and water washing they were included in glycerine and observed under a polarized microscope.
3. Electron microscopy. Specimens were fixed in 1,6% glutaraldehyde immediately after they had been obtained. After postfixation in phosphate-buffered serum with O_sO_4 and dehydratation in alcohol the specimens were embedded in Durkopan (Fluka). After cutting on a ultramicrotome (LKB), they were contrasted with uranilacetate and leadcitrate. Observations were made under a Hitachi HS-7S electron microscope.

RESULTS

Light-microscopic observations:

Figure 1 (x 80, treated with Hemalaun-eosin) shows deposits of abnormal amyloid-like (ALP[1]) among the cells of the scleroma infiltrate. Figure 2 (x 320, Hemalaun-eosin) ALP impregnates the basic substance of the connective tissue and small cleft-like lumens with the formation of basal membranes (—>) are observed in these areas.

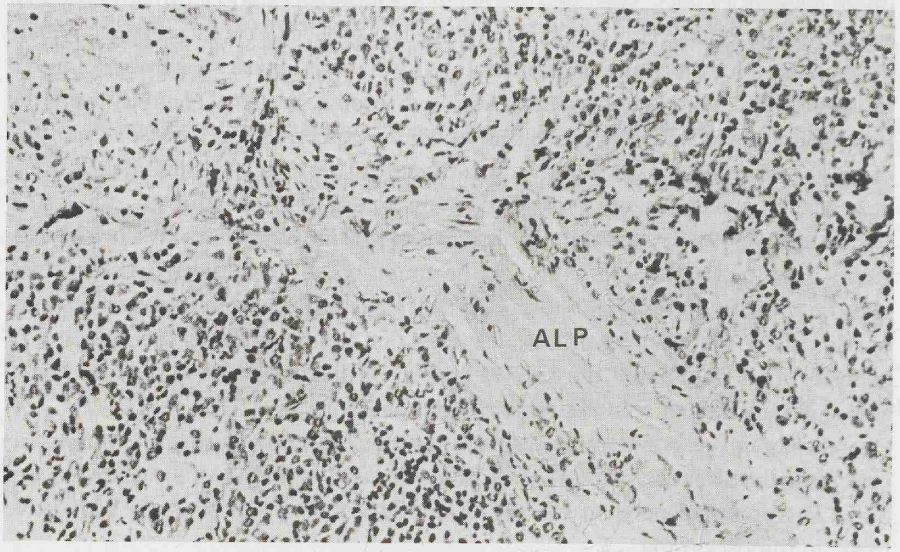

Figure 1. (x 80, Hemalaun-eosin): Deposits of ALP among the cells of the scleroma infiltrate.

[1] ALP shows negativ reaction with Van Gieson, modification after Holuska and Weigert.

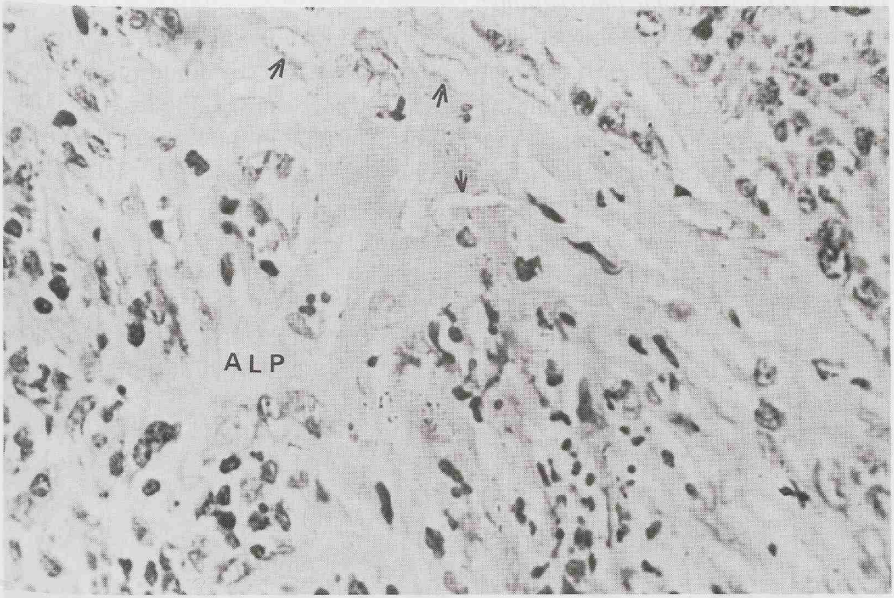

Figure 2. (x 320, Hemalaun-eosin): ALP impregnates the basic substance of the connective tissue. Small cleft-like lumens with the formation of basal membranes (→).

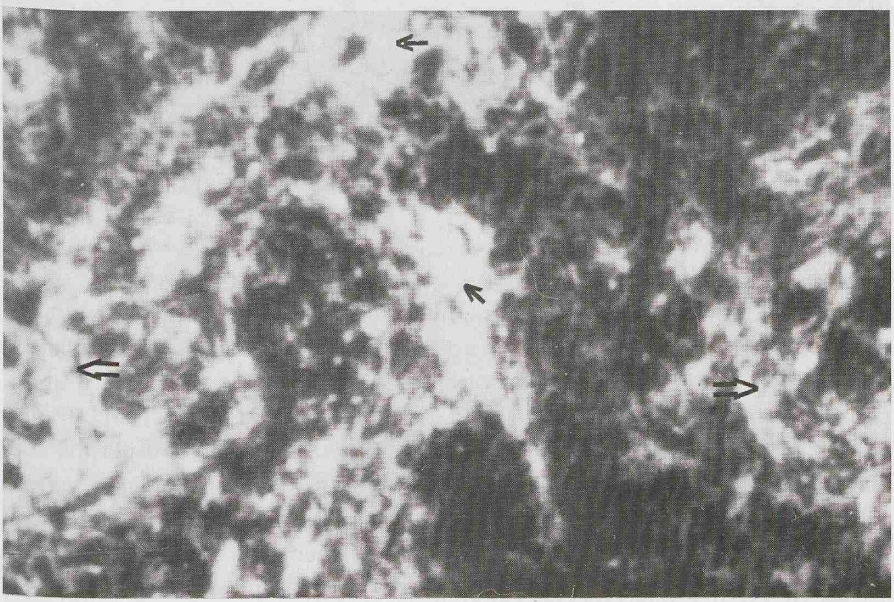

Figure 3. (x 320, Thioflavin'S'): ALP deposits concentrated in the walls of the blood vessels (→) and in the perivascular spaces (⇒).

Fluorescent-microscopic observations:
Fluorescent microscopy showed (Figure 3, treated with Thioflavin"S", x 320) that the ALP deposits are mainly concentrated in the walls of the blood vessels (—>) and like irregular masses in the perivascular spaces (=>). Figure 4 (x 320), Thioflavin "S") indicates that besides deposits of ALP in the walls of the vessel (—>) there is also a tendency of irregular deposits of ALP along the tract of some fibre connective tissue structures (=>).

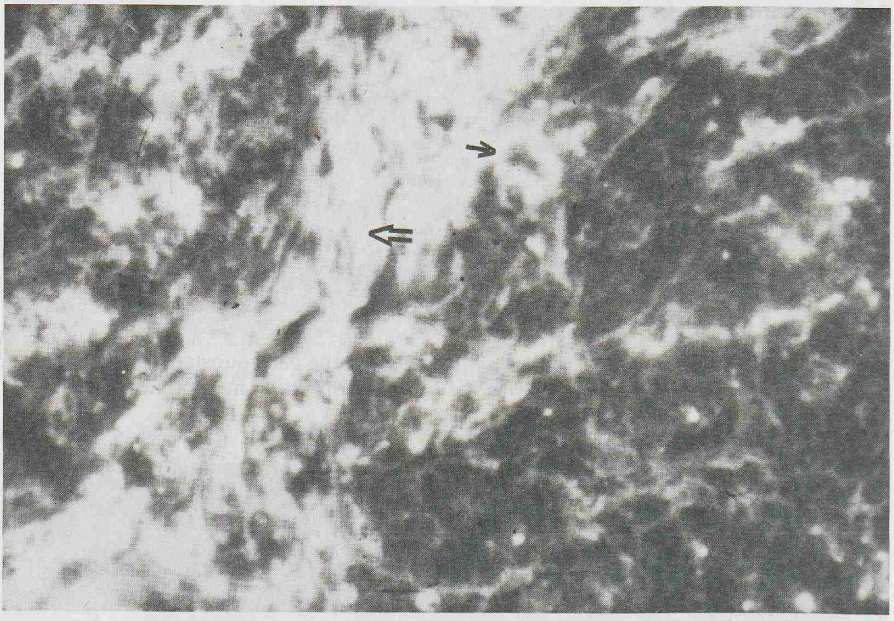

Figure 4. (x 320, Thioflavin'S'): ALP in the walls of the vessels (→) and along of some fibre connective tissue structures (⇒).

Electron-microscopic observations:
The presence of bundles of collagene fibres, scattered indiscriminately among the cells which are intact and their debris have been observed under the electron microscope. In the near proximity of the collagene fibres there are areas, consisting of thinner fibre structure, cut longitudinally and transversally (Figure 5, x 17,250). Figure 6 (x 17,250) shows part of the cytoplasm of a plasma cell, characterized by the presence of rough endoplasmic reticulum and cysterns and filled of with electron-dense material. In the near proximity of the plasma cell there are indiscriminately scattered parts of collagene fibres and ALP, "swimming" in the inter-cellular fluid. Figure 7 (x 8630) presents areas in which ALP structures like a basal membrane intimately intervene with the collagene fibres. In other areas the collagene fibres are formed inside the mass of the ALP.

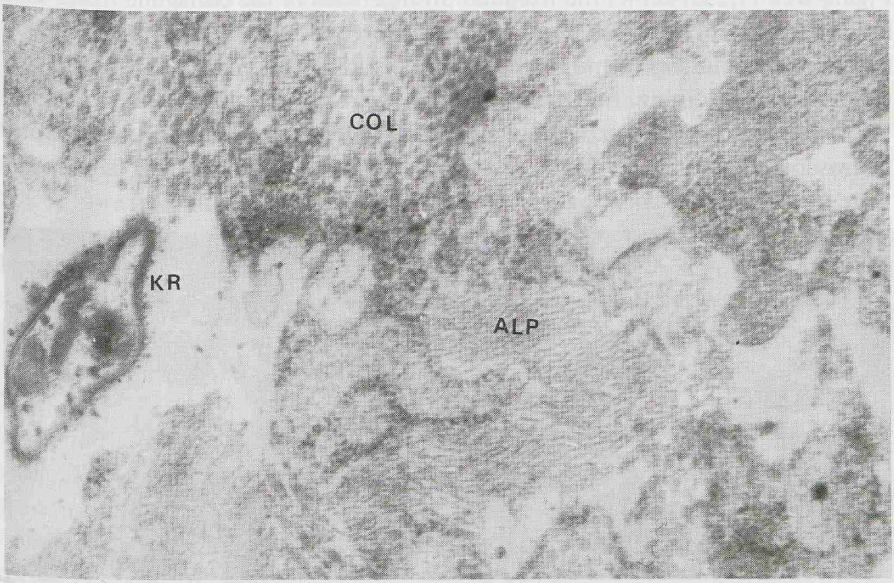

Figure 5. (x 17,250): Thinner fibre structures, cut longitudinally and transversally, in the near proximity of the collagene fibres.

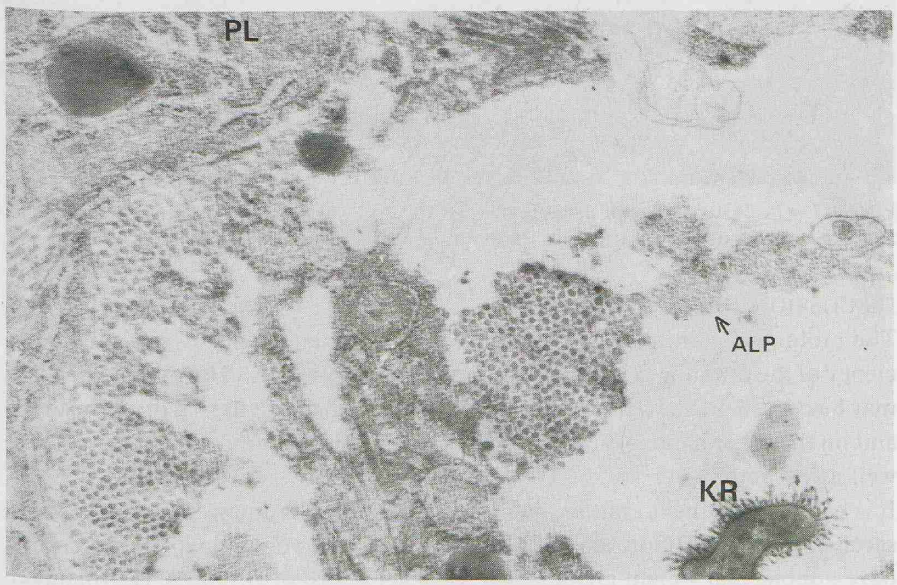

Figure 6. (x 17,250): Part of a plasma cell with rough endoplasmic reticulum and cysterns, filled in with electron-dense material. In the near proximity with the plasma cell there are indiscriminately scattered parts of collagene fibres and ALP, 'swimming' in the inter-cellular fluid.

Figure 8 (x 17,250) shows the irregular arrangement of the collagene fibres in a longitudinally cut bundle. There are clear differences not only in the position of the individual bundles (some of which mutually intersect one another) but also in their transversal lining.

There are Frisch-bacilli in different stages of their development on all electron-microscopic figures.

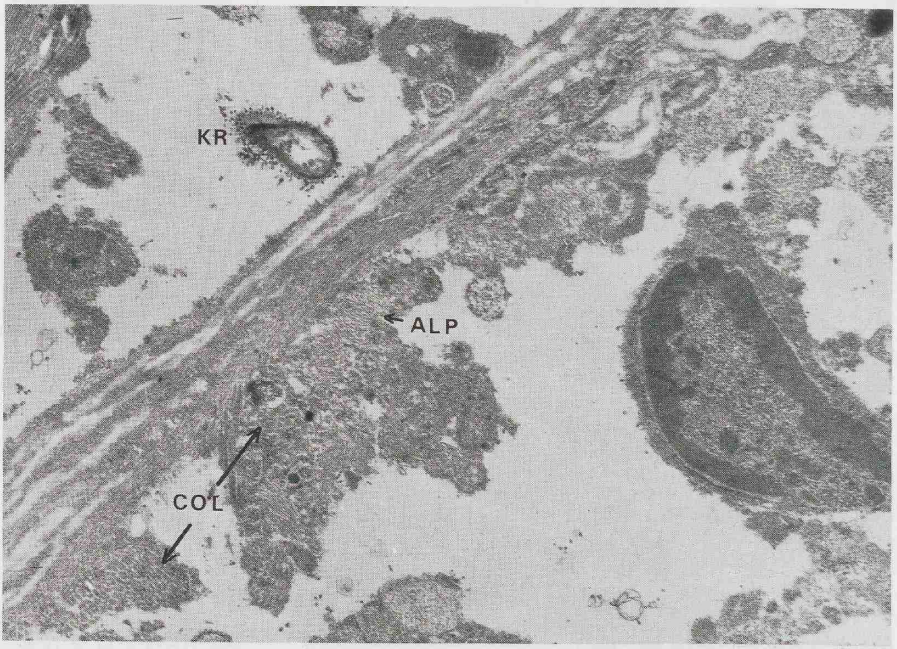

Figure 7. (x 8630): Areas of arrangement of the ALP-structures like a basal membrane and the collagene fibres intimately intervening with them.

DISCUSSION

The prolonged continuation of the disease, scleroma is indicative of the inefficiency of the immune system regarding the destruction of the Frisch-bacilli. This may be caused on one hand by the vitality and resistance of the bacilli themselves and on the other hand, by insufficient activity of the immunocompetent cells, as well as by both.

It is known from the literature that Klebsiella rhinoscleromatis has a dense polysaccharide capsule (Gonzales-Angulo et al., 1965) and the mucopolysaccharides have a molecular weight greater than 300,000 (Wheat et al., 1965). Hoffmann et al. (1973) assumed that it was the mucopolysaccharide layer round the bacilli which caused the formation of vacuoles in the Mickulicz-cells. This theory was supported by the observation of Cohn and Plarks (1967) who found that in

experimental animals macrophages from vacuoles in the presence of mucopolysaccharides.

It is possible that the osmotic attraction of water in the vacuoles leads to its prompt bulging and following rupture before the destruction of the embraced bacilli under the activity of the lysosomal enzymes takes place. In other words, it appears that the Mickulicz-cells are macrophages in which no stable phagosome can be formed. This assumption proves that the antigene stimulation of the immune-competent cells is insufficient.

The lysosomal enzymes being released from the Mikulicz-cells in the intercell space can cause cell necrosis and release of the matrix lysosomes, which is a prerequisite for maintaining the inflammation. The lysosomal enzymes can also lead to changes in the fibrillar elements of the connective tissue (Mann et al., 1980).

In the initial stage of scleroma there are a number of plasma-cells in an active phase. Therefore it is likely that in the area where the Frisch-bacilli are developed specific immunoglobulines are produced. However, for no clear reason the infection persists. The continuous antigene irritation (by spontaneous destruction of the bacilli) leads to constant activation of the oncoming B-cells and their trans-

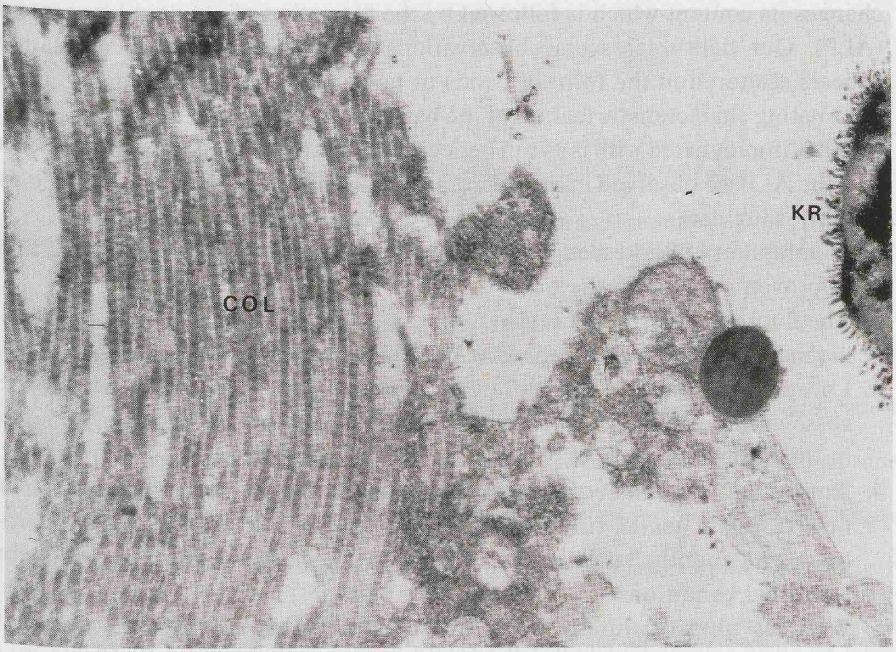

Figure 8. (x 17,250): Irregular arrangement of the collagene fibres in a longitudinally cut bundle. There are clear differences in the position of the individual bundles as well as in their transversal lining.

formation in plasma cells. From our observations it becomes clear that in this stage of the disease, it is essential that the number of lymphocytes is relatively small. It is possible that this process, as described above exists for many years before the formation of the specific granulomas begins.

The prologed activity of the disease can be a result of antigene blocking of the control mechanism for fibroblast activity resulting either in an increase of production or a decrease of destruction of a new collagene (Schiff et al., 1980).

The scleroma process should probably be connected with the kind of diseases characterized by a deficiency of collagene destruction like for instance liver cirrhosis, sclerodermia, tympanosclerosis, etc.

We assume that the formation of granulomes is preceded by an insufficiency in the function of the plasma cells, which causes the production of abnormal proteins. The presence of similar amorphous material in plasma cells is pointed out by different investigators (Unna, 1903; Woyke et al., 1969; Friedmann, 1971).

A number of investigators connect the Russel-bodies with the abnormal function of the plasma cells (Welsh et al., 1963; Toppozada et al., 1981). Toppozada et al. found that the Russel-bodies are formed inside the plasma cells after which they can be released in the extra-cellular spaces. We believe that the accumulation of abnormal protein in the basic substance of the connective tissue probably changes its content which is followed by the formation of amyloid-like protein (ALP). Our light-microscopic observations indicate that such accumulation appears scattered in the form of bands or polygonal fields but generally it is a dominating characteristic feature of the basic substance of the connective tissue, which is impregnated with is as can be seen with fluorescent microscopy. The fact that the ALP we observed in the scleroma granulome is not amyloid is proved by the following data:

1. Suppression of fluorescency exposure is considerably faster compared to that in proven amyloid in the tissues.
2. The fluorescent character is also different – this refers to the paler, yellowish-green fluorescence, rather than the bright green colour of the typical amyloid.
3. Location in tissues is also different: this refers not only to deposits in the vessel walls and basal membranes (amyloid) but also to an even impregnation in the basic substance of the connective tissue (ALP).
4. Among the impregnated vast connective tissue spaces, there is a formation of new vessels – gentle, tiny capillaries with gradual formation of basal membrane and endothelial cells. We assume that this is a process of reparation in abnormal conditions.
5. Whereas ultrastructurally the fibres of the typical amyloid are scattered chaotically and cross one another, the abnormal ALP, observed by us, has fibrillar structures which are regularly arranged.

Our observations under the electron-microscope allow us to assume that the

scleroma granulome has also disturbances in its collagene structure. The observations of the presence of collagene fibres inside the ALP are particularly important. It cannot be eliminated that during the changes of the basic substances of the connective tissue and collagene synthesis in scleroma there are conditions for the formation and development of auto-immune reactions. We admit that they should also be taken into consideration in the explanation of the continuation and the clinical picture of the disease.

There are grounds for such assumptions from the analogy with other chronical granulomatosis like leprosy, tuberculosis, etc. with proved auto-immune mechanism (Vulchanov et al., 1972). Naturally, further immunological surveys are needed to prove the presence of auto-immune reactions in scleroma in which infection with Klebsiella rhinoscleromatis serves as a trigger mechanism and the following pathological dynamics have an autonomous character.

It is probable that the abnormal amyloid-like structures in scleroma which we have found are also a result of auto-immune reactions against the normal collagene type of connective tissue in respiratory mucosa. This can explain the diffuse sclerosis of mucosa in the upper as well as in the lower respiratory tracts.

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