Otosclerosis—an inflammatory disease of the otic capsule of viral aetiology?

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Abstract

Fragments of otospongiotic and otosclerotic footplates were investigated by immunohistochemical methods. Antibodies IgG, IgA, IgM were found to be bound to the vascular connective tissue of the resorption lacunae, IgG also to osteocytes. The application of antibodies against mumps, measles and rubella antigens showed the expression of the relevant viral antigens in the large cells of the resorption lacunae, in the vascular connective tissue, and in osteocytes, osteoclasts and chondrocytes, present in or around the otospongiotic areas. In the sclerotic stage only the perivascular connective tissue and chondrocytes have expressed viral antigens whereas IgG was restricted to the osteocytes of the sclerotic focus and to the residual perivascular tissue. Two footplates with postinflammatory sclerosis serving as controls revealed only IgG in some chondrocytes. Healthy footplates showed neither a deposition of antibodies nor any expression of viral antigens. These results favour a viral aetiology of otosclerosis as an inflammatory vascular reaction of the otic capsule initiated or caused by the viruses of measles, rubella and mumps.

Introduction

The aetiology of otosclerosis has remained a mystery. The histological frontline of reconstruction of the otosclerotic focus is formed by the border between the enchondral and periosteal otic-capsule (Wittmaack, 1926; Nager and Meyer, 1932; Bast, 1933; Nager, 1969). The pathological process seems to be linked to the coexistence of an apparently incompletely reconstructed enchondral and a completely reconstructed periosteal layer. It begins with lacunary resorption of the bone by the proliferating active connective tissue of the vessels, probably from the mucosa of the tympanic cavity in the region of the stapes footplate. In the course of this process thickening or the vascular basement membrane and endothelial oedema is observed; some vessels show features of obliteration and fibroblasts of the vascular connective tissue are converted into functional osteoclasts (Arnold and Plester, 1974, 1975, 1977; Arnold, 1976). The mechanism triggering the osteolytic process of the proliferation of the osteoclasts is unclear (Holtrop *et al.,* 1978). Lymphocytes, granulocytes and mast cells are present in the osteolytic zones and could provide the mediators stimulating the osteoclasts; macrophages may act as precursor cells (Wright, 1977 Horton *et al.,* 1978; Neiders and Nisengard, 1978; Tashjian, 1978; Lim, 1985; Lim *et al.,* 1987; Quaranta *et al.,* 1987; Yoo *et al.,* 1987).

The familial incidence of the disease has suggested hereditary and genetic factors which might account also for an enhanced affinity or acceptance of viruses by immunologically unstable cells. The affinity (organotropism) of certain viruses for the cochleo-vestibular system is well recognized. There is some microscopical evidence of a viral infection of otosclerosis. Nucleocapsids resembling the measles virus have been noted under the electron microscope in osteocyte-like cells in the vascular spaces of the otosclerotic lesion (McKenna *et al.,* 1986). Deposits of IgG and IgA were found in the enchondral zone of ossification and immunohistochemical tests have determined the expression of measles and rubella antigens in otosclerotic lesions (Arnold and Friedmann, 1987, Lim *et al.,* 1987).

The present paper is based on our investigation of the otosclerotic footplate with the aim of confirming or otherwise the observations mentioned above; with particular reference to the distribution and classification of specific antibodies (IgG, IgA, IgM and IgD) and of the presence and distribution of the viral antigens of mumps, measles and rubella.

Materials and methods

The examined material consisted of parts of the footplates at various histological stages of otosclerosis from 42 patients, 27 to 76 years of age. Nine patients had a family history of otosclerosis (21, 24 per cent).

The surgical specimens were fixed either in Bouin's fixative or in sublimate-formaldehyde for three hours (formaldehyde 40 per cent, 10 ml; mercuric-(II)-chloride, 6 g; glacial acetic acid, 5 ml; distilled water, to a total volume of 100 ml), rinsed overnight in ethyl alcohol (70 per cent) and decalcified in 10 per cent EDTA for

Fig. 1

P

Fig. 2

Fig. 3

Fig. 1

Undecalcified postinflammatory sclerosis of the stapes footplate in a case of severe tympanosclerosis. Note IgG-positive chondrocytes (indicated by arrows). HE, 100×

Fig. 2

Semithin section—after EDTA decalcification—of an otospongiotic area in a footplate abutting on the perilymph (P). There are two multinucleated giant cells near a congested capillary. Toluidin blue, 570×

Fig. 3

Undecalcified semithin section of an otospongiotic footplate. There is a mast cell in the perivascular tissue of a small capillary. Toluidin blue, 1500×

two to three days. Following dehydration in ascending concentrations of alcohol, the specimens were embedded in Paraplast. Serial sections (4 μm thick) were cut and every 10th section was stained with haematoxylin-eosin. The remaining sections were stained for immunohistochemical demonstration of the immunoglobulins (IgG, IgM, IgA, IgD) and of the viral antigens of measles, rubella and mumps, using the Avidin Biotin Complex (ABC) method. Goat anti-measles, rubella and mumps sera were obtained from C.M.D. (UK) Ltd. Bournemouth, rabbit-anti-goat serum from Northeast Biomedicals, Denham, Bucks, U.K.

The control material consisted of footplates with post-inflammatory sclerosis and of normal stapedes (kidney donors). Additional specimens that had been used in earlier morphological studies (Arnold and Plester, 1974, 1975, 1977; Arnold and Friedmann, 1987) were included for comparison.

Results

Normal footplate

The specimens of normal footplates showed no deposits of antibodies of the immunoglobulin classes G, A, M or D and expressed no viral antigen of measles, rubella or mumps.

Post-inflammatory sclerosis

There were antibodies of the G class present in occasional osteocytes and chondrocytes but no antibody of the classes A, M or D and no viral antigens were expressed (Fig. 1).

Active phase of otosclerosis

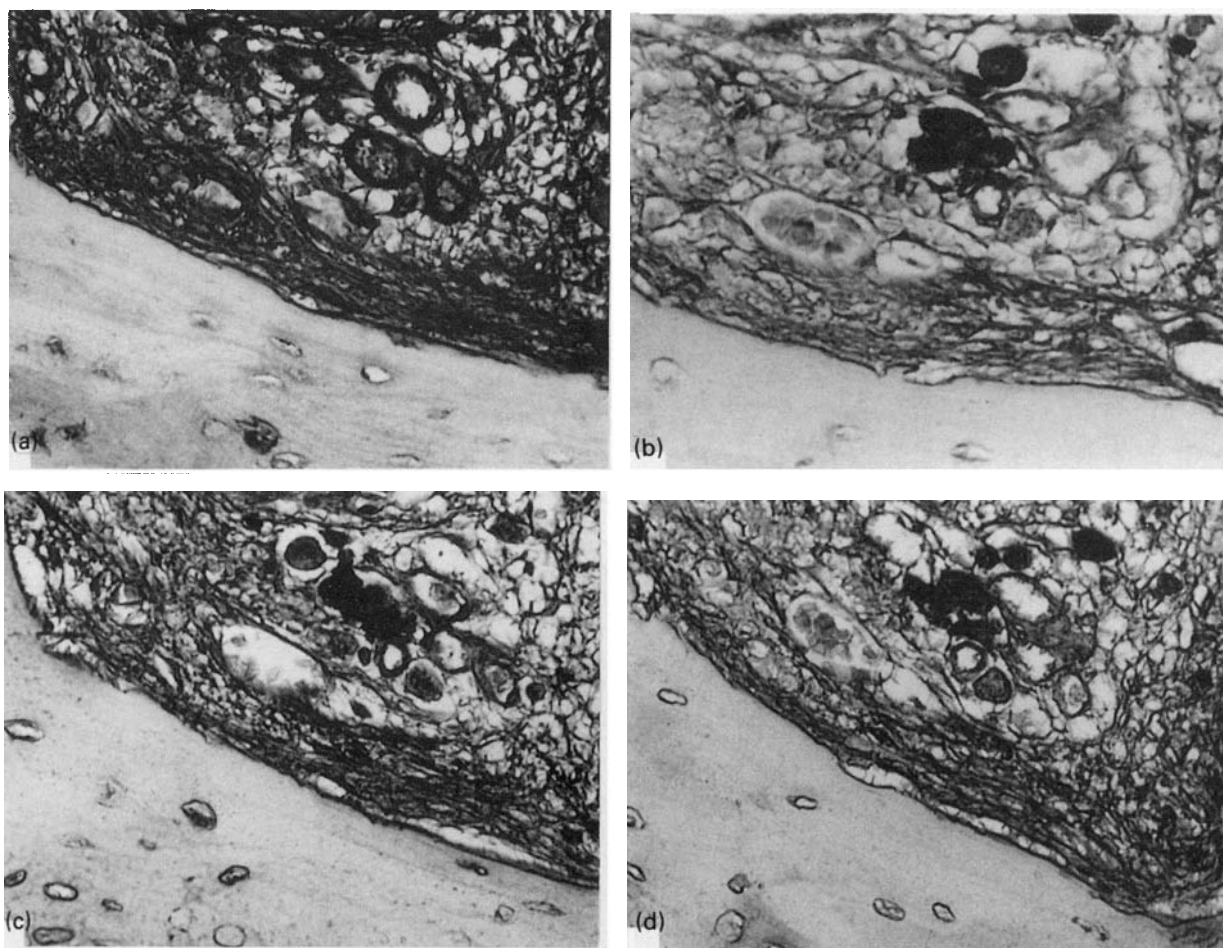Light microscopy showed in the perivascular spaces

FIG. 4

Serial sections through a resorption lacuna of an active otosclerotic area of the footplate. The connective tissue and capillary walls show heavy deposits of IgG-antibodies (a). There is intensive expression of the viral antigens measles (b), Rubella (c), Mumps (d) by large cells (macrophages?) in the perivascular tissue.
ABC, no contrast staining, 380×

some lymphocytes, monocytes, macrophages, giant cells and mast cells (Fig. 2, 3). This phase was characterized by the deposition of antibodies of class G in the vessel walls, supporting cells and fibres of the resorption lacunae; also on osteocytes, and osteoclasts at the periphery of the lacunae, but not on the chondrocytes of the residual enchondral layer. IgA was occasionally found to be bound to the supporting cells of the lacunae, rarely to vessel walls or to surrounding osteocytes, but not to osteoclasts or chondrocytes. IgM reacted weakly in some vessels walls and supporting cells of the vascular spaces. IgD was negative in all cases.

Viral antigens (mumps, rubella and measles) were expressed most strongly in some large cells (probably pericytes or fibroblasts which may be transformed into functioning osteoclasts) lying in close proximity of the vessels of the 'resorption-lacunae' and in their tissues. Serial sections have shown that, on the one hand, the same cell can contain all three viral antigens; on the other, there are some cells expressing only a single antigen. The examined viral antigens have been identified also in fibrocytes, osteocytes and osteoclasts in the vicinity of the resorption lacunae (Figs. 4 and 5).

Inactive phase of otosclerosis

In the inactive (mineralized) phase of otosclerosis

only IgG was found in the osteocytes and in the remaining supporting tissue of the greatly reduced vascular lacunae, whilst IgA and IgM were lacking in his phase. Mumps antigen is still strongly expressed by the fibrocytes of the residual perivascular spaces, and by osteocytes and chondrocytes. Measles and rubella antigens appeared to be restricted to chondrocytes (Fig. 6 and Table 1).

Discussion

What is the factor stimulating the proliferation and aggressive infiltration by the connective tissues of the blood vessels into the bone of the otic capsule, accompanied by a variety of inflammatory cells including lymphocytes, granulocytes, macrophages and occasional mastcells (Quaranta *et al.*, 1987; Wright, 1977)?

Hereditary and genetic factors have been widely considered and the multiple incidence in certain families supports this theory. A genetic proneness, stimulated by some unidentified factors, might lead to the development of the characteristic otosclerotic process of reconstruction in the previously healthy border region between bone and cartilage of the otic capsule. Genetic proneness might account for an enhanced affinity or
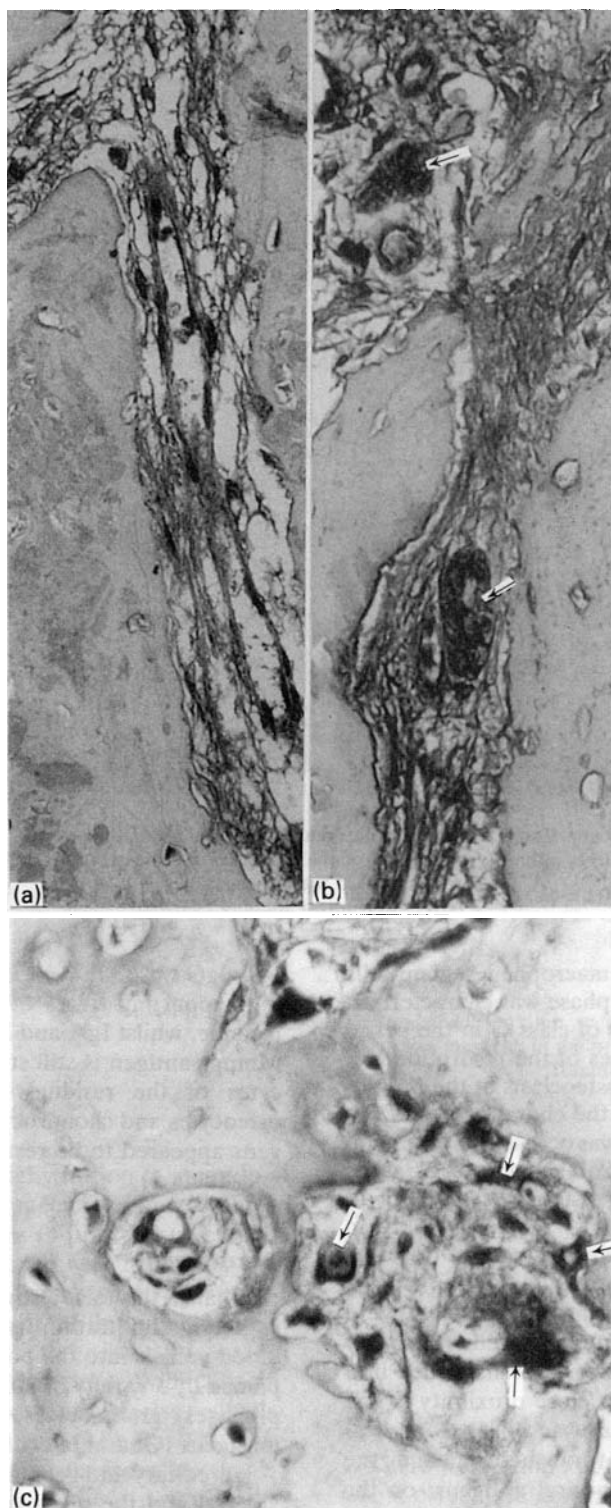
FIG. 5

Viral antigens to measles, mumps and rubella are expressed by various cells in the resorption lacunae of the active otosclerotic lesion. ABC, no contrast staining.

a: Measles antigen in the connective tissue, 420×.

b: Mumps antigen in the cytoplasm of large cells (indicated by arrows), 490×

c: Rubella antigen in large macrophage-like cells. Arrows indicate the heavily stained cytoplasm whereas the nuclear region remains unstained, 680×.
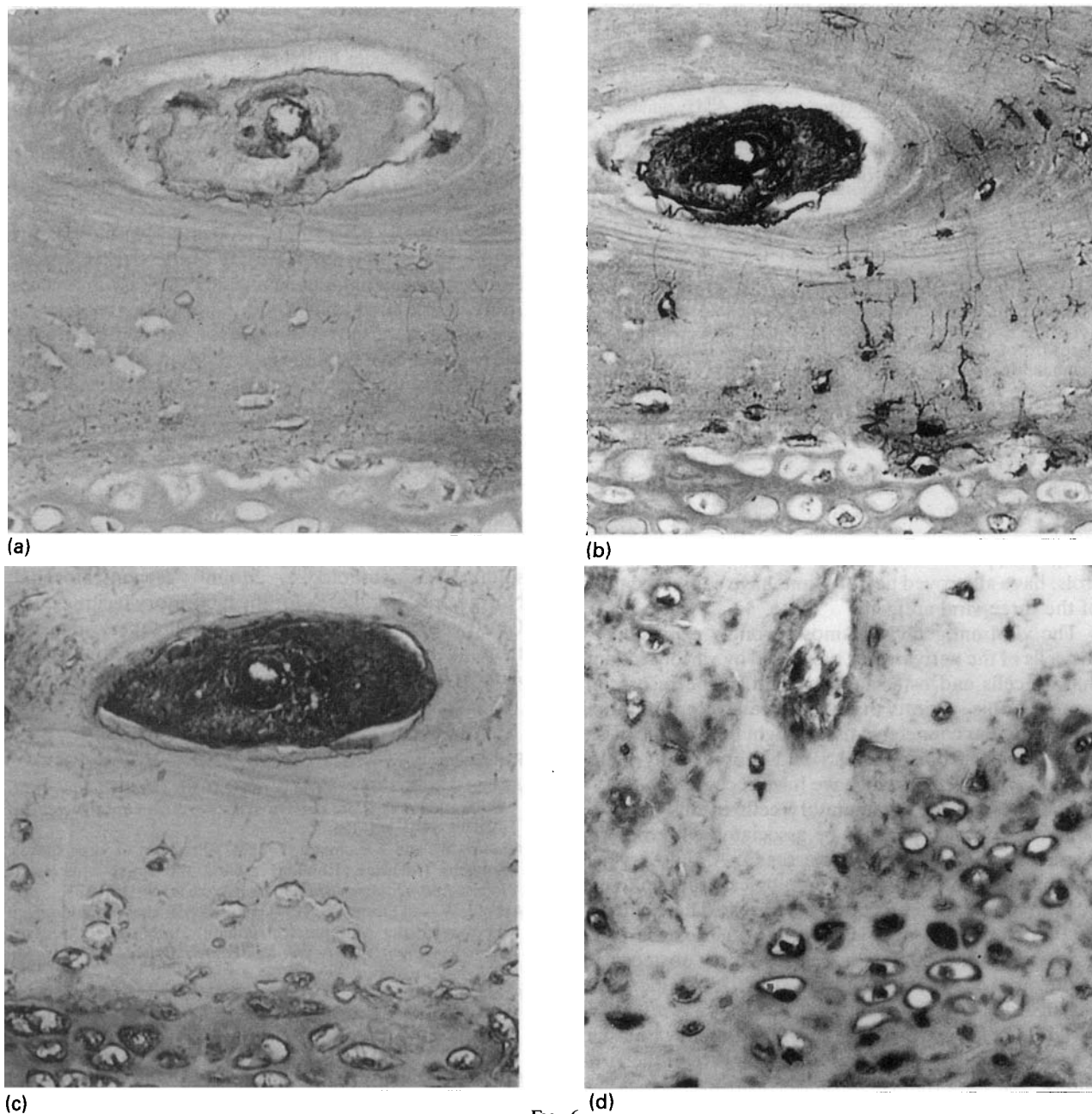
FIG. 6

Inactive stage of otosclerosis: IgM antibodies are absent in the perivascular connective tissue of a residual lacuna. (a) In the same lacuna heavy deposits of IgG-class antibodies are bound to the perivascular connective tissue and to some osteocytes nearby. (b) The expression of mumps antigen is restricted to the perivascular connective tissue of the lacuna. (c) A strong reaction for rubella antigen is displayed by the chondrocytes in the enchondral layer (d). ABC, no contrast staining, 480×.

acceptance of viruses by cells or tissues and certain cellular components which may be lacking in immunological stability. However, since the incidence of otosclerosis of the cases is nonfamilial in about 50 per cent an individually enhanced susceptibility to an unknown causative agent would have to be considered. Furthermore, such an agent might itself possess an enhanced affinity for certain tissues.
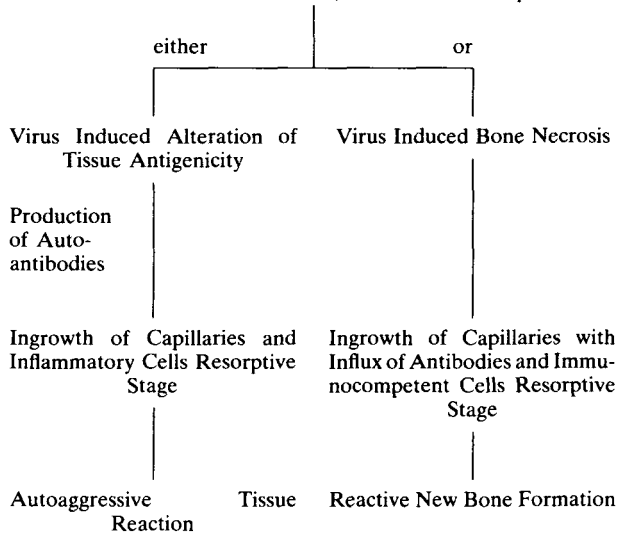
This investigation has provided evidence of the presence of three viral antigens-mumps, rubella and measles, in all the otosclerotic specimens studied. In contrast, the non-otosclerotic specimens and also the

TABLE I

IMMUNOHISTOCHEMISTRY OF FOOTPLATE OTOSCLEROSIS, POSTINFLAMMATORY FOOTPLATE SCLEROSIS AND OF THE NORMAL FOOTPLATE

Stapes footplate	IgG	IgA	IgM	IgD	Measles	Rubella	Mumps
Otosclerosis resorptive stage	+++	+	+	−	+++	+++	+++
Sclerotic stage	++	−	−	−	+	++	+
Postinflammatory sclerosis	+	−	−	−	−	−	−
Normal footplate	−	−	−	−	−	−	−

TABLE II
VIRUS INDUCED BONE REACTION IN OTOSCLEROSIS
Viral infection of Measles, Rubella and Mumps

either	or
Virus Induced Alteration of Tissue Antigenicity	Virus Induced Bone Necrosis
Production of Auto-antibodies	
Ingrowth of Capillaries and Inflammatory Cells Resorptive Stage	Ingrowth of Capillaries with Influx of Antibodies and Immunocompetent Cells Resorptive Stage
Autoaggressive Tissue Reaction	Reactive New Bone Formation

unaffected parts of the otosclerotic stapes, used as controls, have all proved negative and have expressed none of the three viral antigens.

The viral antigens were most strongly expressed by the cells of the perivascular tissue and by various inflammatory cells and osteoclasts present in the resorption lacunae. This suggests that the aggressive proliferation of the vascular connective tissue might be initiated in the early stages of otospongiosis and subsequently maintained by the viruses which we have recognized.

The presence of inflammatory cells in this phase of bone resorption appears to be associated with the production of antibodies of the classes G, A and M which may be interpreted as an immunological reaction to the inflammatory stimulus of the viruses. These immunocompetent cells are participating in the resorptive process by activating the proliferating osteoclasts (Holtrop et al., 1978, Horton et al., 1978; Neiders and Nisengard, 1978; Tashjian, 1978; Quaranta et al., 1987; Wright, 1977). The presence of antibodies may be a sign that the organism (body) has become aware of the virus and/or that the products of the reconstructed bone had acquired some antigenic properties. A secondary auto-immune reaction may be activated by the immunological system of the body reacting to the tissues affected by the viruses as 'not self' in producing antibodies against them (epiphenomenon). This view is supported by the continued presense of IgG-antibodies in the otosclerotic focus so that the disease could be interpreted as a localized autoimmune process of viral aetiology (Table II). However, this is contradicted by the finding of the heaviest concentration of G, A and M type antibodies in the vessel walls and in the perivascular structures during the early osteolytic phase, being greatly reduced in the course of advancing sclerosis. In the sclerotic stapes, IgG was found only in the remaining perivascular tissue and in some osteocytes and the viral antigens were expressed mainly at the margin of the otosclerotic area by some chondrocytes and scanty osteocytes.

Conclusions

The presented observations indicate that the patho-

genesis of otosclerosis may be associated with an infection by certain organotropic viruses. Mumps, rubella and measles have been incriminated as playing a role in the causation of various diseases of the inner ear and appear to be involved also in the initiation and maintenance of the otosclerotic process.

The root of the infection may be vascular or tympanic. The closeness of the middle ear mucosa to the resorption lacunae of the stapes footplate and also to the regions most affected by otosclerosis i.e. oval and round window region, suggest a tympanic route of the viral infection. The frequent familial incidence may be due to a genetically determined enhanced affinity of the tissues to a viral infection at the border of bone and cartilage in the otic capsule. This hypothesis is supported by the observation that otosclerotic patients display regionally different but significant HLA patterns (Wayoff et al., 1979; Gregoriadis et al., 1982; Dahlqvist et al., 1985).

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