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Immune response and immunopathology of the inner ear: an update

José Ramón García Berrocal, M.D., Ph.D., Rafael Ramírez-Camacho, M.D., Ph.D.

Abstract

Immune-mediated inner-ear disease includes clinical conditions associated with unilateral or bilateral rapidly progressive forms of sensorineural hearing loss. A systemic autoimmune disorder can be present in less than one-third of cases.

Because of the lack of well defined detection methods to identify immune-mediated processes within the inner ear, and the fact that the human inner ear is not amenable to diagnostic biopsy, there has been great interest in developing animal models. Experimental models of sterile and virus-induced labyrinthitis support the participation of the immune system in the aetiopathogenesis of inner-ear disorders: interleukin-2 emanates from the endolymphatic sac and assists in changing the spiral modiolar vein, as in the expression of intercellular adhesion molecule 1, which allows the egress of immune cells from the circulation. The formation of a fibro-osseous matrix ultimately results in degeneration of the inner ear.

These investigations have allowed us to alter the immune response for the purpose of regulating its intensity and the subsequent damage to patients.

Key words: Immune system; Ear diseases; Cochlea; Endolymphatic sac; Hearing loss, sensorineural; Labyrinthitis

Introduction

Until relatively recently the inner ear was considered to be isolated from the general immune mechanisms because of its isolation within the otic capsule and its lack of lymphatic drainage. The capacity of immune-mediated involvement of the cochleovestibular peripheral organ is now recognized, although the common clinical expression of these pathological entities and the impossibility of gaining access to the affected organ for morphological investigation without destroying its function make it difficult to delimit these disorders.

As early as the 1970s, and especially during the 1990s, the widespread use of cochlear implants has renewed interest in the study of inner-ear pathology. In the USA four per cent of the population under 45 years of age and 29 per cent of those over 69 suffer a disabling loss of hearing,[1] in Spain, in 1987, there were over 900 000 individuals with hearing problems out of a population of 38 million. Likewise, the incidence of severe hearing loss in the newborn is 1/1000, a rate that increases in the presence of risk factors, 50 per cent of which are genetic in origin. Excluding genetic deafness, immune-mediated hearing loss, with its associated instability, vertigo and tinnitus, represents a field in which therapeutic strategies[2] may achieve the reversal, or at least halt the progression, of one of the most disabling human handicaps.

The purpose of this review is to relate our current knowledge concerning immune-mediated inner-ear disease, discussing its interrelationship with special anatomic and functional conditions.

The immune response in the inner ear

Immunological mechanisms play an aetiological role in diseases of the inner ear,[3,4] despite the fact that this sensorial organ is enclosed with a bony capsule and lacks lymphatic drainage. The labyrinth is separated from the bloodstream by a barrier that helps to maintain the electrolytic features (status quo) of the cochlear environment. This blood–labyrinth barrier presents tight junctions in the stria vascularis (endolymphatic surface) and loose junctions in the spiral ligament (perilymphatic surface).

Immunoglobulins are present in the perilymph at a concentration of approximately 1/1000 of the titre present in serum, similar to the levels in cerebrospinal fluid (CSF).[5] The antibodies present in the perilymph allow complement fixation.

The antigens that reach the cochlea rapidly gain access to the immune system (Table I). Thus, the injection of an antigen into the perilymphatic space is much more effective than its injection into middle

From the Servicio de Otorrinolaringología, Universidad Autónoma de Madrid, Clínica Puerta de Hierro, Madrid, Spain.
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TABLE I
ROUTES OF INNER EAR INFECTION

Systemic infection penetrating via the fluids irrigating the labyrinth (haematogenous route)
Direct extension from the central nervous system via meninges and auditory nerve
Propagation from the cerebrospinal fluid toward the perilymphatic space via the cochlear aqueduct or toward the endolymphatic space via the endolymphatic duct
Through the oval or round window following a middle-ear infection

ear in triggering humoral and cellular immune responses, being equivalent to the peritoneal route.[6] When an antigen is situated in the inner ear of animals that have previously been sensitized by a systemic route, an immune response consisting of cellular infiltration, inflammation and cochlear lesion is produced.[7] In addition, there is an increase in the titre of perilymphatic antibodies and local antibody production.[8]

The endolymphatic sac is involved in this immunological response, as the surgical obliteration or destruction of the endolymphatic duct significantly reduces immune responses and cochlear damage.[9] The endolymphatic sac, surrounded by a rich network of lymphatic vessels, fenestrated capillaries and venules, contains immunocompetent cells, lymphocytes and macrophages (Figure 1) capable of processing and presenting bacterial or viral antigens, potentiating the immune response, attacking the pathogens directly or the infected cells indirectly, and locally producing immunoglobulins.[10] The evidence of secretory IgA and different lymphocyte subpopulations characterizes the endolymphatic sac as a mucosa-associated lymphoid tissue.[11] Within the lymphatic system, memory lymphocytes sensitized to one organ or another are circulating continuously (Figure 2). It has recently been suggested that the lymphoepithelial tissue of the nasopharynx may have a role as a source of immunocompetent cells for the endolymphatic sac.[11]

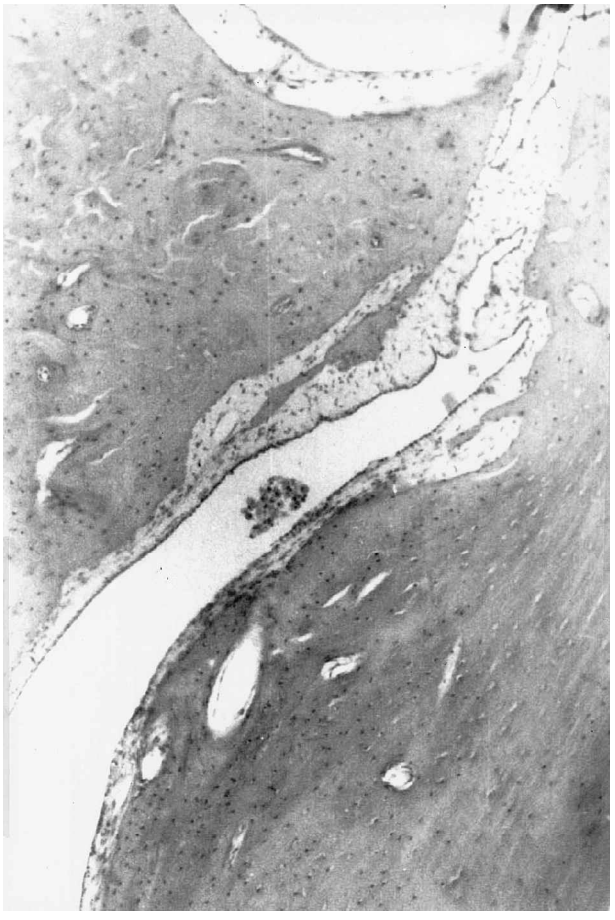

FIG. 1
Photomicrograph of a section of guinea pig cochlea presenting keyhole limpet haemocyanin-induced sterile labyrinthitis showing immunocompetent cells in the endolymphatic duct. (H & E; ×100)

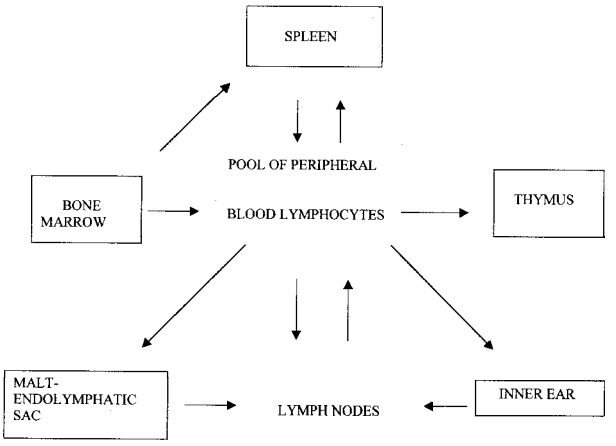

FIG. 2
Lymphocyte traffic. Lymphocytes abandon the circulation through the high endothelial venules of mucosa-associated lymphoid tissues (endolymphatic sac) and lymph nodes, passing through the non-specialized peripheral endothelial in areas of inflammation (the modiolar spiral vein of the inner ear in animal models of experimental immune-mediated labyrinthitis).

Although the endolymphatic sac contains lymphocytes, the normal cochlea does not. The accumulation of leukocytes and the local production of immunoglobulins in the inner ear depends on the entrance of leukocytes from the bloodstream (Figure 3), facilitated by the release of mediators of the immune response such as interleukin-2 (IL-2), which may originate in the endolymphatic sac. This response activates the endothelial cells of the spiral modiolar vein (SMV), which assume features of high endothelial venules,[12] potentiating the expression of the intercellular adhesion molecule 1 (ICAM-1)[13] which recruits leukocytes from the bloodstream. The polymorphonuclear cells, which arrive in 10 minutes, are followed by macrophages 10 hours later and by T, B and NK cells after seven to nine days, with the late phase of specific antibody production.[14] There is a concomitant formation of a dense extracellular matrix with considerable fibrosis[7] which, as it cannot be reabsorbed, triggers ossification (Figure 4). The immune response can provoke the degeneration of the organ of Corti, stria vascularis and spiral ganglion.[7]

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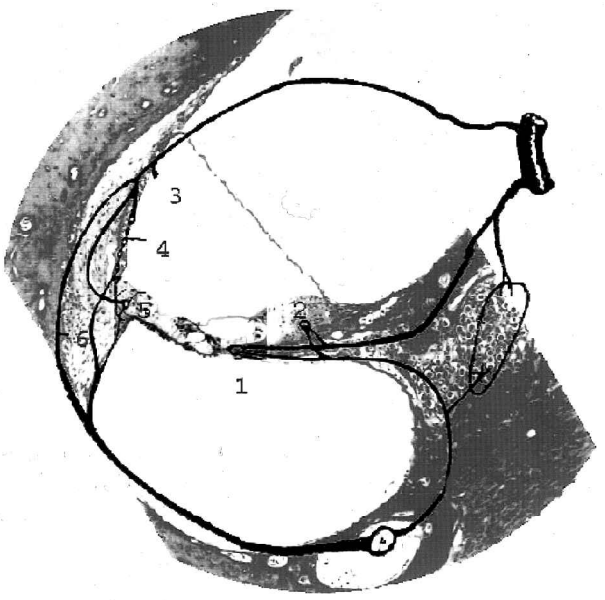

FIG. 3

Cochlear vascularization overlaid on a section of guinea pig cochlea. The cochlear artery, accompanied by nerve fibres, enters the modiolus, issuing branches along its spiralling path through the loose connective tissue between the nerve and the bony wall, toward the limbus (2) and the basilar membrane (1) and over the scala vestibuli. It spreads out toward the spiral ligament (3), stria vascularis (4), spiral prominence (5) and terminal branches deep in the spiral ligament (6) that connect with the collecting venules, receiving branches of the spiral ganglion and coming together in the modiolar spiral vein (*). Inset: the organ of Corti.

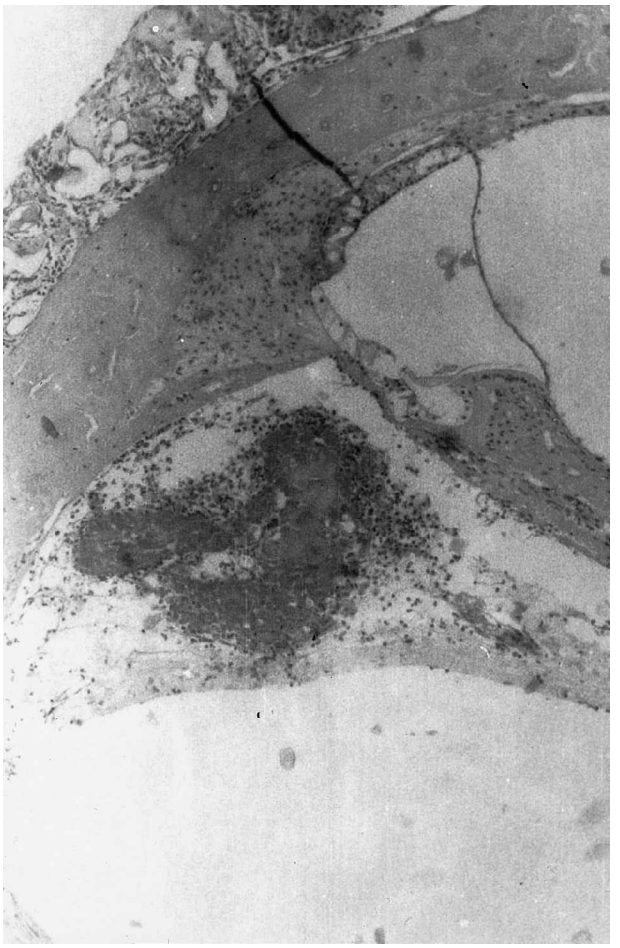

FIG. 4

Photomicrograph of a section of guinea pig cochlea presenting keyhole limpet haemocyanin-induced sterile labyrinthitis showing a neovascularized fibromyxoid matrix that will show new bone formation in six weeks' time. (H & E; ×100)

The inner ear can also be the target for systemic autoimmune diseases, although there is also an autoimmune disorder specific for the inner ear.[7,15]

Experimental models of immune-mediated labyrinthitis

Experimental models capable of altering the afferent and efferent pathways of the immune response have made it possible to change its course and the resulting injury, in addition to deepening our knowledge of these reactions in an organ as inaccessible as the inner ear.

Sterile labyrinthitis

The development of labyrinthitis in rats by inoculation of keyhole limpet haemocyanin (KLH) into the scala tympani has been achieved in animals that had been previously sensitized systematically to this substance (secondary immune response).[13] During inflammation the SMV, the endolymphatic sac and the surrounding region play a critical role in recruiting immunocompetent cells and in producing an immune response.

ICAM-1 plays a fundamental role in immune reactions, acting as a ligand for lymphocytes function-associated antigen-1 receptors on the lymphocytes and Mac-1 receptors on monocytes and polymorphonuclear cells. This binding represents a critical early step in the movement of leukocytes toward the tissues. This receptor can be employed as an endothelial cell activation marker, which increases during inflammation through the mediation of IL-1, tumour necrosis factor (TNF) and interferon-γ (IFN-γ).

The induction of labyrinthitis by means of a secondary immune response was accompanied by an increase in ICAM-1 expression, not only in the endothelial cells of the SMV and its collecting venules, but in the perilymphatic mesothelium, the perineurium of the cochlear nerve, the spiral ligament and the basal cells of the stria vascularis.

After the injection of KLH into the perilymph, some of the antigens may bind immediately to the anti-KLH antibodies present in the perilymph that reach the inner ear via the systemic circulation. The presence of these immune complexes can damage the tissues by their activation of the inflammation pathways: polymorphonuclear cell and macrophage migration and endothelial cell activation. The macrophages phagocytose free antigens, producing IL-1 and TNF and increasing the expression of ICAM-1 in the endothelium.

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The early appearance of ICAM-1 in the SMV correlates with its histological transformation into a high endothelial venule and with the gradual infiltration of inflammatory cells into the inner ear. The spiral ligament contains numerous vessels, including postcapillary venules, and fibrocytes expressing ICAM-1, and thus probably contributes to the pool of immunocompetent cells in the perilymphatic space.

The presence of ICAM-1 in the endolymphatic sac epithelium and in the perisaccular connective tissue is evidence of its contribution to the immune response of the inner ear. The fibrosis and new bone formation resulting from inflammation in the inner ear correlate with the expression of ICAM-1, a phenomenon that might theoretically be applied therapeutically, as the early blocking of the receptor of this molecule could prevent inflammatory cell infiltration, fibrosis and the release of other mediators.

In another model of KLH-induced sterile labyrinthitis in guinea pigs, IgG and albumin from the perilymph enter the spiral ligament through micropores existing between the mesothelial cells surrounding it.[16] The IgG originates in the circulatory system and has been shown to be present in the endolymphatic sac. There is also a reduction in the fibrocytes of the spiral ligament, the subtype II of which is related to active K transport, implicating this structure in the cochlear dysfunction caused by the introduction of an antigen into the perilymph, which has free access to the spiral ligament. Serum antibodies can also reach the spiral ligament, triggering an antigen–antibody reaction that alters the fibrocytes, although the tight junctions between the basal cells of the stria vascularis prevent the penetration of the antigen from the spiral ligament.

The immunohistochemical analysis of the cells that proliferate in experimental sterile labyrinthitis in guinea pigs involved the use of monoclonal antibody Ki-67, which binds to the proliferating cell nuclear antigen.[17] During the early phase the activation of polymorphonuclear cells provokes the secretion of inflammatory mediators that stimulate the endosteal and/or osteoprogenitor cells. These secrete a fibrous matrix into the lumen of the scala tympani, permitting the emigration of inflammatory cells from the bloodstream that predominate six weeks after injection. The inner ear is unable to reabsorb this fibrous material derived from mesothelial or endosteal cells that confine the cochlear scala, pluripotential mesenchymal or fibroblast-like cells that can form osteoprogenitor cells, and can also enter the cochlea from the bloodstream or bone marrow of the otic capsule. Other cochlear sources of the fibre–bone matrix are the pericytes surrounding the blood vessels and the fibroblasts of the modiolus and spiral ligament.

Likewise, proliferating cells can be observed in the apical turns, where ossification has not yet been produced, as well as among the lymphocytes in the endolymphatic sac, a fact that suggests the absence in the inner ear of an immunosuppressive mechanism capable of combating inflammation.

The immunocompetent cells coming from the bloodstream proliferate in the inner ear following antigen presentation by the antigen-presenting cells (APC) or owning to the presence of IL-2. This continued proliferation generates more antigen-specific lymphocytes, sustains the inflammatory response and produces a severe lesion in the organ for hearing and equilibrium, evolving toward the ossification of the inflammatory matrix. Any later exposure to the same antigen provokes a more intense and potentially more damaging response in the inner ear as a consequence of the rapid proliferation of antigen-specific lymphocytes and antigen-specific memory cells following the antigenic stimulation.

New bone is formed six weeks after the inoculation and presents no inner proliferative activity.

Viral labyrinthitis

The creation of a model of experimental labyrinthitis following cytomegalovirus (CMV) inoculation into the scale tympani of guinea pig cochleas has confirmed the role of the SMV in the immune response triggered by the inoculation through the recruitment of lymphocytes and NK cells from the systemic circulation toward the inner ear, the regulation of which depends on different adhesion molecules expressed on the surface of the endothelial cells of the SMV and its collecting venules (ICAM-1, ELAM-1, INCAM-110/VCAM-1).[12]

The morphological changes found in the SMV may be due to the presence of IL-1, IFN-γ and TNF, or to the interactions between endothelial cells and chemoattracted cells, depending on the cytokine gradient, which are detected six hours after inoculation of the antigen (IL-2).

Rather than being a direct result of the cytopathic effect of the CMV, the inflammatory changes are the cause of the sudden hearing loss provoked in animals by the inoculation of live CMV. Thus, the tissue injury responsible for hearing loss during viral labyrinthitis can be prevented by blocking the flow of inflammatory cells with antibodies against the adhesion molecules.

The endolymphatic sac may be the afferent pathway or lymph node of the inner ear, releasing lymphokines that trigger the early changes observed in this structure.

Diagnostic work-up

Immune-mediated inner ear ear disease is diagnosed on the basis of clinical manifestations, immune laboratory tests and favourable treatment response. Numerous attempts have been made to develop assays that might help to confirm the diagnosis and identify patients who are most likely to response to immunosuppressive treatment. However, laboratory testing is not always available and treatment does not invariably produce a favourable response.

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Tests based on reactivity to inner-ear antigens include the lymphocyte migration assay, lymphocyte transformation, indirect immunofluorescence and Western blot analysis. Elsewhere, to determine the participation of subpopulations of lymphocytes, three-colour flow cytometry has been used.

The lymphocyte inhibition assay was proposed by McCabe[18] as a screening tool for autoimmune sensorineural hearing loss. However, the specificity of this test is doubtful. Controls are essential and a positive response may basically be an allogenic reaction. This test has been replaced by the lymphocyte transformation test,[19] which uses unrefined inner-ear membranes as the putative antigen and requires circulating lymphocytes sensitized to inner-ear antigens to respond to the suspected antigen in vitro. Unfortunately, the test cannot be routinely applied and is not widely available. The indirect immunofluorescence test has been used to screen patients with inner-ear disease for autoantibodies, whether specific (directed against inner-ear tissues) or non-specific (directed against various cellular and/or tissue elements). Immunofluorescent labelling identifies antibodies in 18 per cent[20,21] to 71 per cent of patients.[22]

Antibodies against type II collagen were identified in the serum of patients with Menière's disease[23] and in patients affected by idiopathic bilateral progressive hearing loss.

The Western blot technique determines the reactivity of sera from patients with idiopathic progressive sensorineural hearing loss[24] and those with rapidly progressive sensorineural hearing loss[25] against bovine inner-ear material: 32 per cent showed antibody reactivity against a 68 kDa protein. When compared with the results in control subjects the Western blot proved to be highly specific but less sensitive if used in the general population of individuals without hearing loss. Additional inner-ear antigens may be involved in some patients with suspected autoimmune inner-ear disease: some of these patients' sera reacted to inner-ear antigens with molecular masses of 220, 68, 58, 33–35, 32[26] and 30 kDa.[27] Subsequent investigations have identified the 68 kDa protein with the heat shock protein 70.[28,29]

To study the phenotype of peripheral blood lymphocytes in patients with inner-ear disorders three-colour flow cytometry was used. Patients with sudden deafness have shown a decreased number of in vivo naive T cells (CD4RA cells)[4] This abnormality can be explained by several hypotheses: the presence of antibodies to CD4RA molecules, as reported in patients with systemic lupus erythematosus; the in vivo conversion of naive T cells (CD4RA cells) to memory cells (CD4RO cells) as a result of an ongoing immunologic response as described in systemic sclerosis, and finally, the viral infection of these cells. An alternative hypothesis could be a preferential homing of these cells to focal inflammatory sites.

The purpose of future investigations is to develop a clinical profile of high-risk patients with immune-mediated inner-ear disease in whom a presumptive diagnosis can be made. Recently, we proposed the profile of an individual at high risk of developing isolated immune-mediated sudden sensorineural hearing loss: unilateral disease, young/middle aged adult, evidence of antinuclear antibodies in the absence of systemic immunological disorder, decreased number of naive T cells, and response to corticosteroid therapy (recovery rate of hearing $> 80\%$).[30]

Therapeutic approach to immune-mediated inner-ear diseases

Immunomodulating therapy

The first study concerning autoimmune hearing loss, published by McCabe in 1979,[18] was based on a defined clinical pattern and a positive response to treatment with dexamethasone and cyclophosphamide. As it is not possible to correlate the clinical response with the histopathological findings in temporal bone of patients presenting this disease, several experimental models of autoimmune labyrinthitis have been developed in rats and guinea pigs for the purpose of analysing the effects of immunomodulating therapy in the inner ear.

In one of these models, induced with collagen type II, the subsequent administration of methylprednisolone reduced the histopathological changes observed in untreated animals. These alterations consisted of inflammation of the inner sulcus cells and the Claudius cells, degeneration of the cells of the spiral ganglion, mild vacuolar degeneration of the sensory cells in the macula sacculi and the crista ampullaris, and perivascular fibrosis and thickening of the vascular wall, with IgG and C3 deposition in the cochlear vessels. Animals that received no treatment presented high titres of anticollagen type II antibodies, whereas in the treated group the response was less intense.[31]

Histopathological studies showed that these drugs were more effective in reducing the vascular changes than the sensory degeneration, suggesting that it may play a more important role in reducing the primary than the secondary immune injury.

Within eight days the inoculation of CMV into the scale tympani of seronegative guinea pigs produces profound deafness, preceded by inflammatory cell infiltration. Thus, the administration of cyclophosphamide can suppress or reduce the cellular immune response, preventing the hearing loss.[32]

CMV antigens appear in cells beneath the basilar membrane, in mesothelial cells surrounding the scale tympani, and in inflammatory cells present in the perilymph of the scale tympani of the basal cochlear turn. Seven days later a cochlear infiltrate can be observed in the scala tympani. This consists of a great number of neutrophils and a few lymphocytes, plasma cells and macrophages. A fibromyxoid reaction and haemorrhage are detected in the spiral ligament, stria vascularis and Rosenthal's canal.

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These lesions are less severe in the animals treated with cyclophosphamide, coinciding with better auditory nerve action potentials.[32]

On the basis of the histopathological findings a correlation can be established between the degree of inflammatory response in the cochlea and the hearing loss. However, a relationship between the hearing loss and the level of CMV antigen present is not observed.

Cyclophosphamide rapidly crosses the blood–brain barrier and, presumably, the blood–labyrinth barrier as well, exerting its immunosuppressive effect on the immune cell population. The combination of cyclophosphamide and an anti-inflammatory drug may prove to be effective in patients with a recalcitrant immune reactivity, and appropriate in cases of sensorineural hearing loss of viral origin. The use of ganciclovir may be effective during the early phases of the immune response, before irreversible lesions develop.

Recent reports describe the effects of anti-ICAM-1 monoclonal antibodies in rats with KLH-induced labyrinthitis, observing that the inflammatory cell infiltrate is diminished at the level of the scala tympani and perisaccular tissue of the endolymphatic sac, although the endolymphatic fibrosis and hydrops are not. This observation suggests a different aetiopathogenic mechanism for these lesions.[33]

This incomplete immunosuppression may be attributed to the participation of other adhesion molecules (ICAM-2, VCAM-1, VLA-4, CD44, selectins, B7-CD28) in leukocyte traffic out of the SMV and collecting veins toward the inner ear. Likewise, the administration of anti-ICAM-1 monoclonal antibodies fails to alter the perilymphatic concentration of anti-KLH antibodies produced after systemic injection, or the level of KLH antibody immune complexes observed after KLH injection into the scala tympani of animals subjected to previous systemic immunization with this antigen.

Intratympanic corticosteroid therapy

In certain cases of Menière's disease and autoimmune disease of the inner ear causing sudden deafness or rapidly progressive sensorineural hearing loss systemic glucocorticoids improve hearing.[2,34,35] Their administration directly into the inner ear through the membrane of the round window[36] makes it possible to achieve elevated concentrations and avoid the collateral systemic effects of these drugs. The mechanism of action of steroids in the inner ear remains open to speculation: they increase the microvascular blood flow in the cochlea, reduce the inflammation and the onset of endolymphatic hydrops, and lack ototoxic effects in laboratory animals.

Although this therapy is delivered directly to the inner ear, the lack of consensus with respect to the dose to be administered and the duration of treatment, and the presence of a communication through the tympanic membrane that facilitates the presence of micro-organisms from the outer ear in the middle ear, and even the inner ear, increasing the risk of labyrinthitis, has led to the study of designs for drug delivery devices that allow the controlled, chronic administration of different substances.

Drug delivery devices

The development of systems for drug delivery and the administration of antioxidants, growth factors and protease inhibitors, and of heat shock protein (HSP) opens new options in the treatment of hearing loss produced by noise and by ototoxic drugs. This preventive effect was achieved with the administration of certain antioxidants (adenosine agonists, glutathione derivatives) directly into the round window.

The direct administration of immunosuppressive drugs into the inner ear by means of delivery devices (polymers coupled to osmotic pumps) may facilitate the treatment of immune-mediated diseases of the inner ear, especially in patients who do not initially respond to conventional therapy.

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Address for correspondence:
J.R. García Berrocal,
Servicio de Otorrinolaringologia,
Clínica Puerta de Hierro,
San Martín de Porres 4,
28035 Madrid, Spain.

Fax: 34 91 3730535

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