tion counting of organ lysates for the assessment of labeled drug uptake, since with the latter technique high uptake by a small number of cells is likely to be masked by the large volume of background activity associated with uninfected brain (*14*).

Our results enhance the prospect of using radioactively labeled antiviral drugs to detect HSV encephalitis in humans. Further studies are required to define conditions optimizing the autoradiographic contrast between infected and background brain tissue. It may also be possible to use the related antiviral pyrimidine nucleoside 2′-fluoro-5-iodo-1-β-D-arabinosylcytosine (FIAC) (*4*) for diagnostic purposes. Incorporation of a positron- or gamma-emitting isotope of iodine into the FIAC might be exploited by positron emission (*15*) or single photon (*16*) tomography, or perhaps even by clinical gamma scanning, to image brain infection in man.

YUTAKA SAITO
RICHARD W. PRICE*
DAVID A. ROTTENBERG
George Cotzias Laboratory of Neuro-Oncology, Sloan-Kettering Institute, New York 10021
JACK J. FOX
TSANN-LONG SU
KYOICHI A. WATANABE
Laboratory of Organic Chemistry, Sloan-Kettering Institute
FREDERICK S. PHILIPS
Laboratory of Pharmacology, Sloan-Kettering Institute

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9. Infection in this model spreads along neural pathways. Characteristically, infection is most prominent in the visual pathway of the inoculated eye, that is, the right optic nerve and the left optic tract along with their target nuclei, including the suprachiasmatic nuclei bilaterally, the left lateral geniculate body, and the left superior colliculus. Infection at times also involves the pineal gland (via the postganglionic fibers of the superior cervical ganglion), the central mesencephalon, and the descending trigeminal tract (Y. Saito and R. W. Price, *Trans. Am. Neurol. Assoc.*, in press).
10. The [2-^{14}C]FMAU was synthesized and its purity assessed in the Laboratory of Organic Chemistry of Sloan-Kettering Institute (T-L. Su, K. A. Watanabe, J. J. Fox, in preparation).
11. The distribution of [^{14}C]FMAU in tissues of uninfected rats has been studied extensively, and the dosage and timing of our study was based on these observations (T-C. Chou, A. Feinberg, F. S. Philips, unpublished data).
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* Address correspondence to R.W.P.

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Type II Collagen Autoimmunity in Otosclerosis and Meniere's Disease

Abstract. *Antibodies to collagen types I, II, and IV were measured in patients with otosclerosis and patients with Meniere's disease. Levels of antibodies to type II collagen were significantly higher in these patients than in control subjects, while no differences were found among levels of antibodies to collagen type I or type IV. These observations suggest a possible role for type II collagen autoimmunity in the etiology of otosclerosis and Meniere's disease.*

The causes of otosclerosis and Meniere's disease are obscure. Histopathologic changes in otosclerosis include spongification of the ossicles and otic capsule (*1*). Meniere's disease is characterized by auditory and vestibular symptoms attributable to the accumulation of excess fluid in the membranous labyrinth of the ear (endolymphatic hydrops) (*2*). The diseases have not been known to be related.

We recently described autoimmune hearing loss, vestibular dysfunction, and otospongiotic lesions in rats. These animals had characteristics of both human otosclerosis and Meniere's disease (*3*). All the abnormalities appeared to be associated with autoimmunity against collagen—in particular, type II collagen. It was, therefore, of interest to test otosclerosis and Meniere's disease patients for the presence of antibodies to collagen.

Serum was obtained from patients at the Shea Clinic, Memphis, Tennessee. Bovine type II collagen and human collagen types I and IV were isolated and purified (*4*). (Bovine type II collagen was used because of the difficulty in obtaining adequate quantities from human cartilage. It cross-reacts extensively with human type II collagen.) The purity of the collagen preparations was determined by amino acid analysis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and uronic acid analysis. In each case there was no detectable contamination by noncollagenous proteins, proteoglycans, or other types of collagen. Immunoradiometric assay was performed by a modification of the procedure described by Clague *et al.* (*5*). Collagen was dissolved in phosphate buffer (*p*H 7.6; ionic strength, 0.4*M*) by stirring overnight at 4°C. The concentration was adjusted to 25 μg/ml based on spectrophotometric determination of specific absorption at 230 nm. To each well of a 96-well polystyrene plate was added 100 μl of the collagen solution. Dynatech Removawell strip holders were used so that individual wells could be easily separated for scintillation counting. (Use of a 96-well plate facilitates the handling of samples during subsequent multiple wash steps.)

After overnight incubation of the plate at 4°C, unbound collagen was removed by washing with 0.15*M* NaCl and 1 percent ovalbumin was added to block nonspecific absorption of antibodies. Incubation was resumed for 1 hour; then the plate was washed three times with 0.15*M* NaCl containing 0.05 percent Tween 20. (All buffers used subsequently also contained Tween 20 to prevent nonspecific absorption.) Dilutions of human serum were added to the collagen-coated plate, the plate was incubated at room temperature for 4 hours, and unbound antibodies were removed by three washes with 0.15*M* NaCl containing 0.05 percent Tween 20. Antibodies bound to the collagen were detected by adding ^{125}I-labeled staphylococcal protein A (Amersham) (10,000 count/min per well). After incubation of the plate for 2 hours, excess protein A was removed by three washes and the wells were separated and placed individually in a gamma scintillation

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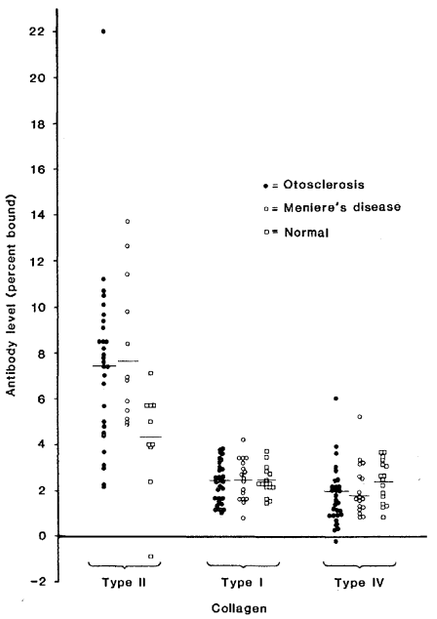

Fig. 1. Levels of antibodies to collagen types I, II, and IV in serum from otosclerosis patients, Meniere's disease patients, and control subjects. Immunoassays were performed with collagen-coated polystyrene microtiter plates. Bound immunoglobulin G was detected with ^{125}I-labeled protein A. Values obtained with wells containing 1 percent ovalbumin but not coated with collagen were subtracted from the former, and the differences were divided by the total isotopically labeled protein A added, giving the percentage bound. In two instances slightly greater binding was detected in the wells not coated with collagen, yielding the negative values shown. Each horizontal line represents the mean binding level for that group.

counter. Each serum sample was added to duplicate collagen-coated wells and to a third well that was treated identically but not coated with collagen. The uncoated wells served as background controls and their mean value was subtracted from the mean for the duplicates. All samples were compared to a standard containing a known concentration of antibodies to collagen.

As shown in Fig. 1 and Table 1, levels of antibodies to type II collagen were higher in patients with Meniere's disease and in those with otosclerosis. Mean binding in serum from otosclerosis patients (1036 ± 99 count/min) was similar to that in Meniere's disease patients (1033 ± 99 count/min); both values significantly exceed the control value of 539 ± 98 count/min ($P < .01$ and $< .025$, respectively). Fifteen of 29 patients with otosclerosis had binding levels > 2 standard deviations above the mean for control subjects. Elevated levels were also found in 5 of 12 patients with Meniere's disease. The reactions were specific for type II collagen, since the mean level of antibodies to collagen types I and IV in Meniere's disease and otosclerosis patients did not differ from the control level.

While the significance of antibodies to type II collagen in these human diseases is not known, it appears that they can cause hearing loss, vestibular dysfunction, and otospongiotic lesions in rodents. In the rodent study (*3*) hearing loss was detected by measuring evoked potentials in the brainstem. Animals immunized with native type II collagen showed decreased amplitudes in response to stimulation and a shift in the latency of the peak. Vestibular dysfunction was evidenced by caloric testing, which showed failure to respond or diminished response. Histopathologic examination showed perivascular abnormalities in the cochlear artery and vestibular artery branches, spiral ganglion cell degeneration, and spongiotic changes in bone. Immunofluorescence testing demonstrated deposition of immunoglobulin and complement in the bone matrix and globular interossei in the enchondral layer of the otic capsule, vessel walls, and perivascular fibrotic areas surrounding cochlear vessels. All affected animals had detectable levels of antibodies in their serum, which was highly specific for native type II collagen. Serum transfer resulted in a significant impairment of hearing. This evidence suggests that a type II or III immunologic injury caused the abnormalities in the rodents.

Several investigators have described "embryonic cartilage rests" in the otic capsules of normal humans (*6*). These rests could provide type II collagen as a site of immunologic attack, resulting in the histopathologic changes characteristic of otosclerosis.

We do not have a complete hypothesis to explain the role of autoimmunity to collagen in Meniere's disease. The disease is clinically different from otosclerosis, and there is little information regarding histopathologic changes early in its course. The characteristic endolymphatic hydrops might represent only the end stage of a prolonged pathologic process involving the inner ear.

Autoimmunity to type II collagen is also found in rheumatic diseases such as rheumatoid arthritis and relapsing polychondritis (*7*). In most of these conditions, however, antibodies to several types of collagen are present, and the reactivity may be due to a more generalized defect in immunoregulation or may be secondary to tissue injury. The collagen reactivity detected in otosclerosis and Meniere's disease, however, appears to be relatively specific for type II collagen. The stimulus for the development of autoimmunity remains unknown. It could be an interaction between infectious agents, such as a virus and cochlear tissue collagen molecules, or it could be genetically controlled. Other investigators have shown that the cellular response to collagen in humans is genetically linked (*8*). Whether the antibodies cause any of the specific abnormalities associated with either of these diseases is unknown. These data do suggest a new avenue of research into the pathogenetic mechanisms for otosclerosis and Meniere's disease in humans.

Table 1. Antibodies to collagen in serum from patients with otosclerosis and Meniere's disease. Values are means ± standard errors.

Group	Number of samples tested	Antibodies bound (count/min)
Type II collagen		
Control	10	593 ± 98
Otosclerosis	29	1036 ± 99*
Meniere's disease	12	1033 ± 77†
Type I collagen		
Control	16	245 ± 16
Otosclerosis	29	246 ± 16
Meniere's disease	17	232 ± 25
Type IV collagen		
Control	16	245 ± 23
Otosclerosis	29	184 ± 24
Meniere's disease	17	207 ± 29

*Significantly different from corresponding control value ($P < .01$, Student's t-test). †$P < .025$.

T. J. Yoo
J. M. Stuart
A. H. Kang
A. S. Townes
K. Tomoda
S. Dixit

Department of Medicine, University of Tennessee Center for the Health Sciences and *VA Medical Center, Memphis 38163*

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Quantitative Electron Microscopic Autoradiography of Insulin, Glucagon, and Somatostatin Binding Sites on Islets

Abstract. *After monolayer cultures of rat islets were exposed to [^{125}I]insulin, [^{125}I]glucagon, and [^{125}I]tyrosinyl somatostatin, specific autoradiographic grains associated with each radioactively labeled ligand were found on B, A, and D cells. The density of labeling of the B, A, and D cells with each labeled ligand correlated well with the known actions of the three hormones on each of the islet cells.*

The islets of Langerhans consist of a heterogeneous population of endocrine cells (*1*). Adjacent islet cells appear to influence each other by direct, local interactions. Evidence for such interactions, which are also called paracrine (*2, 3*), is derived both from morphological studies, showing a nonrandom distribution of the different endocrine cells within a given islet (*1, 4*), and from functional studies, showing that the B, A, and D cells can each influence the secretion of the other two cell types (*5–9*). Furthermore, observations that insulin and somatostatin can themselves inhibit B- and D-cell function, respectively (*6, 10, 11*), suggest that at least these two islet cells are capable of autoregulation by an ultrashort loop feedback mechanism.

The mechanism underlying the paracrine interaction between the adjacent islet cells is poorly understood, but is believed to be mediated by specific cell surface receptors for each hormone. Attempts to characterize these receptors have been made in the case of insulin and somatostatin by means of direct binding studies with whole rat islets and the appropriate radioactive ligands (*12*). By this method, however, one cannot identify binding sites on any given type of islet cell. To overcome this problem and to gain further insight into the functional interactions among the B, A, and D cells, we have studied, by quantitative electron microscopic autoradiography, the distribution of radioactively labeled insulin, glucagon, and somatostatin on B, A, and D cells in monolayer cultures of dissociated rat islets.

Monolayer cultures of dissociated islet cells from the pancreases of 3-day-old Wistar rats were established in 35-mm plastic petri dishes. Such cultures are rich in B, A, and D cells (*13*). The medium consisted of minimum essential medium with 10 percent heat-inactivated fetal calf serum (MEM-FCS). The glucose concentration was maintained at 16.5 m*M* until day 5 of culture when it was reduced to 5.5 m*M*; labeling experiments were then conducted on day 6. Porcine monocomponent insulin (Novo Industrie, Denmark), porcine glucagon (Novo), and synthetic tyrosinyl somatostatin (Tyr-S-14) (Bachem Fine Chemicals) were radioiodinated by the chloramine-T technique (*14*) and purified by gel filtration (Sephadex G-50 superfine for [^{125}I]insulin, Sephadex G-25 superfine for [^{125}I]Tyr-S-14), or ion exchange chromatography with QAE Sephadex for [^{125}I]glucagon), as described (*15*). The specific activities of the three ligands were as follows: [^{125}I]insulin, 87 μCi/μg; [^{125}I]glucagon, 390 μCi/μg; and [^{125}I]-Tyr-S-14, 753 μCi/μg.

Before conducting the labeling experiments we discarded the maintenance media and washed the cells with 2 ml of MEM-FCS. Paired petri dishes were then incubated at 37°C for 5 minutes and 60 minutes with 1 ml of medium (MEM-FCS with 4.5 m*M* glucose) containing radioiodinated hormones at the following concentrations: [^{125}I]insulin, 5.05 n*M*; [^{125}I]glucagon, 0.5 n*M*; [^{125}I]Tyr-S-14, 0.83 n*M*. These concentrations were at least 10 to 100 times greater than the expected concentrations of endogenous insulin, glucagon, and somatostatin secreted under the conditions of the experiment. Control cells were incubated with the same concentrations of labeled hormones in the presence of excess (0.3 to 0.6 μ*M*) unlabeled hormones.

At the end of the incubation period the radioactive media were removed, the petri dishes were washed three times with MEM in phosphate-buffered saline (PBS), and the cultures were fixed with 2.5 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer at room temperature for 4 hours. The fixed cells were then dehydrated with ethanol, embedded in Epon, and sectioned. High resolution autoradiography was performed by coating the sections with a thin emulsion of Ilford L4 according to Caro *et al.* (*16*) and then incubating them at 4°C for 4 to 5 weeks. After development with Microdol X the sections were examined in a Phillips 301 electron microscope. Magnifications were calibrated with a grating replica containing 2160 lines per millimeter. To determine the percentages of islet cells labeled, eight separate well-defined clusters of islet cells were first identified and the total number of B, A, and D cells within each cluster counted at low magnification. Individual B, A, and D cells containing autoradiographic grains were then counted separately at high magnification, and the number of labeled cells of each type was expressed as a percentage

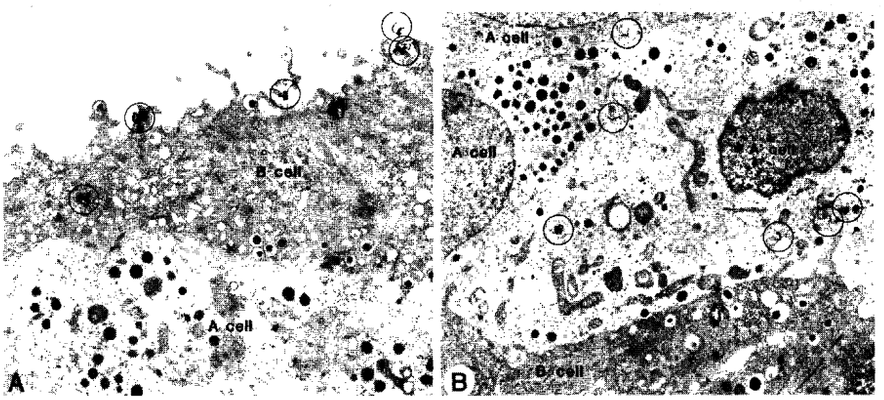

Fig. 1. (A) Monolayer culture labeled with [^{125}I]insulin for 60 minutes at 37°C. The field shows part of a B cell with several autoradiographic grains distributed over its periphery (encircled). The neighboring A cell is not labeled (×16,000). (B) Monolayer culture labeled with [^{125}I]Tyr-S-14 for 60 minutes at 37°C. The field shows the preferential association of autoradiographic grains (encircled) with A cells. The B cell at the bottom of the picture is unlabeled (×12,000). These two examples represent particularly heavily labeled cells for illustrative purposes.

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