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Report

Evidence for the Neuroectodermal Origin of the Human Lacrimal Gland

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Immunohistochemical staining with antineuronal-specific enolase antibodies showed a positive reaction product in the acini and in the nerves of human major and accessory lacrimal glands. The fibrous tissue interstitium, septae, capsule, and blood vessels reacted negatively. Our findings indicate that the acinar portion of the lacrimal gland, but not the fibrous tissue component, is derived from neuroectoderm. We propose that the acini of the human lacrimal glands develop, not as an ingrowth into the mesenchymal stroma from the surface ectoderm that forms the conjunctiva, but as an outgrowth from the embryonic neuroectoderm, and more specifically, from cells that have migrated from the neural crest. Invest Ophthalmol Vis Sci 31:393–395, 1990

Up until now, both the major lacrimal glands and the minor ectopic lacrimal glands of Krause and Wolfring in human eyes were thought to represent end-stage differentiations of conjunctival epithelium that invaginates the mesenchymal stroma.[1-5] The conjunctival epithelium is derived embryonically from the surface ectoderm. Furthermore, studies of developing avian eyes have suggested that the fibrous stroma of the lacrimal gland originates from the cells of the neural crest.[6] In order to investigate whether the lacrimal gland in human eyes is derived from the neural crest, we localized neuronal-specific enolase (NSE) in this tissue with an immunohistochemical technique.

Materials and Methods. Five normal, adult human eyes and adnexa obtained at surgery for maxillary carcinoma (patient age range, 31–72 yr), and five normal globes enucleated at autopsy within 2–10 hr after death (subject age range, 40–79 yr), were fixed in 10% buffered formalin solution and embedded in paraffin wax. Neither the eyes nor the orbits had undergone previous surgery or radiation treatment, and none of the subjects had a clinical history of diabetes, hypertension, autoimmune disease, or metastatic systemic disease.

Sections of the globes and adnexa, 6–8 μm thick, were deparaffinized by warming for 30 min at 55–57°C and by placing them successively in xylene, descending grades of ethanol, and finally in distilled water baths at room temperature. Next, we treated the sections with 0.3% hydrogen peroxide for 15 min at 25°C to block endogenous peroxidase activity, and incubated them for 2 hr at room temperature with a rabbit antihuman NSE polyclonal antibody (Biogenex Laboratories, Dublin, CA). Negative control sections were incubated with nonimmune normal rabbit serum.

All sections were incubated subsequently with biotinylated goat antirabbit immunoglobulins (Biogenex) for 20 min at room temperature, and then were treated with peroxidase-conjugated streptavidin, also for 20 min at room temperature. Finally, a freshly prepared solution of 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO) chromogenic substrate was applied to the specimens for 40 min at room temperature. The sections were rinsed with distilled water, mounted in glycerol, and examined by light microscopy. A positive result was indicated by a deep pink to reddish brown reaction product in the tissue structures.

Results. Sections of the major and accessory lacrimal glands which had been stained with anti-NSE antibodies showed a positive reaction product in the cytoplasm of the acinar cells of the glands (Fig. 1). The nerves in the lacrimal glands also stained positively. In contrast, no staining was observed in the connective tissue interstitium, septae, capsule, or in the endothelial lining of the blood vessels (Fig. 1). The negative controls showed no staining of any tissue in the lacrimal gland (Fig. 2). Positive control sites, such as the retina and corneal endothelium, in the same tissue sections that contained the lacrimal glands, showed an intense reddish brown reaction product.

Discussion. NSE, which is the gamma-gamma isoenzyme of the glycolytic enzyme enolase, is confined normally to neurons and to cells of neuroectoderm origin.[7] Thus, the expression of NSE by normal cells is believed to indicate their differentiation from neuroectoderm.[8,9] With immunohistochemical techniques with anti-NSE antibodies, it has been possible to map out the derivatives of the embryonic neural

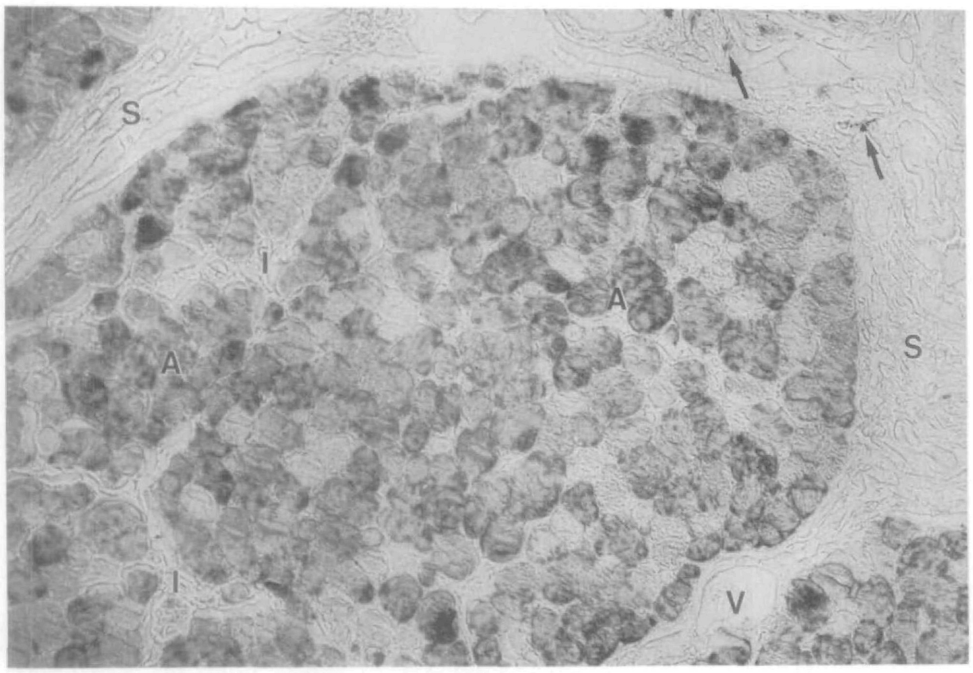

Fig. 1. Section of human lacrimal gland stained with anti-NSE antibodies by the avidin–biotin-amplified, peroxidase–antiperoxidase technique. The cytoplasm of the acinar cells (A) shows a positive reaction, whereas the fibrous tissue of the interstitium (I), the septae (S), and the endothelium of blood vessels (V) show no staining. Arrows point to NSE-positive nerves. (Light micrograph, ×220)

crest in humans.[10] Our current findings indicate that the acinar portion of the normal human lacrimal gland expresses NSE and that it is derived probably from cells that have migrated from the neural crest.

In humans, the major lacrimal gland is located in the upper temporal quadrant of the orbit and secretes a watery fluid. The minor glands of Krause are located in the substantia propria of the conjunctival fornices of the upper and lower eyelids, and the glands of Wolfring are located near the anterior borders of the tarsal plate in the upper and lower eyelids.[4,11] The structure of all of these glands is tubuloacinar in humans, but is alveolar in some other mammals.[3] The human lacrimal gland is innervated by the sympathetic and parasympathetic nervous systems.[3,12] Positive staining of these nerve fibers with anti-NSE antiserum is a normal finding, and in the current study, served as an internal positive control.

The lacrimal gland in higher vertebrates is believed to have evolved from the Harderian gland.[3,12,13] In

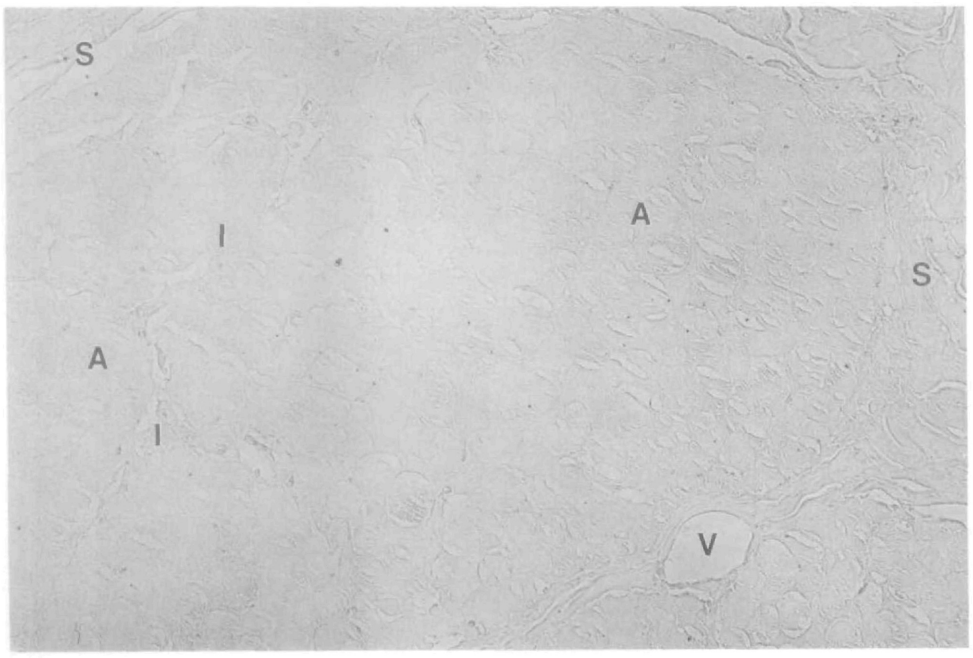

Fig. 2. Section of human lacrimal gland adjacent to that shown in Figure 1 and used as negative control. None of the structures shows any staining. A, acinar cells; I, fibrous tissue of the interstitium; S, septae; V, blood vessel. (Light micrograph, ×220)

lower vertebrates, the Harderian gland is tubuloacinar, and the primary functions of its mucinous secretion are to keep the cornea moist and to lubricate the nictitating membrane.[3,12,13] In humans and other primates, this gland is rudimentary and may be present only as a transitory fetal structure in the inferolateral fornix.[3,12] It would be of relevance, therefore, to investigate whether the acini of the Harderian gland also develop from neural crest cells.

Our current investigation shows that in humans, the acinar portion of the lacrimal gland stains positively for the neuroectodermal marker NSE, whereas the fibrous stroma does not. This result is in contrast to that in the earlier report on the avian eye,[6] and it provides a basis for further investigations into the embryonic development of lacrimal glands. In view of our findings, we postulate that the acinar portion of the human lacrimal gland does not develop as an invagination of the conjunctival epithelium, but that it is an outgrowth of the neuroectoderm, most likely from the cells of the neural crest that have migrated and are known to give rise to many ocular and facial structures.[10,14] Perhaps some diseases of the human lacrimal gland should be regarded as manifestations of neurocristopathy.

Key words: acini, conjunctiva, embryology, Harderian gland, immunohistochemistry, neural crest, neuronal specific enolase

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