DIFFERENCES IN COLLAGEN AND CELL DENSITY DURING NORMAL AND DEXAMETHASONE-TREATED SECONDARY PALATE DEVELOPMENT IN TWO STRAINS OF MICE

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One of the main events during morphogenesis of the mammalian secondary palate is the displacement of the palatal processe from an initial vertical position on either side of the tongue to a final horizontal one above it to achieve palate closure.

The synthesis and hydration of glycosaminoglycans have been implicated in the generation of a palatal shelf-elevating force i mammals. Percentage of hyaluronic acid is initially high and decreases after shelf reorientation. Because its ability to bind water, expands and this expansion is modulated by collagen and fibronectin. The rate of collagen synthesis also peaks during the period c shelf reorientation in hamster embryos and inhibition of collagen synthesis by 5-fluorouracil during the development of the palat produces cleft palate suggesting that collagen may play a critical role in shelf reorientation.

An histological and histochemical study analyzing cell density and distribution of collagen in developing secondary palate in tw strains of mice with different H-2 backgrounds was undertaken to investigate differences between one strain susceptible t glucocorticoid-induced cleft palate (A/Sn) and other resistant (C/57BL). In addition, the influence of dexamethasone treatment o collagen was evaluated.

Two experimental and two control groups of pregnant mice were used. At day 12 of gestation, the experimental groups were give a single intramuscular injection of 200μg of dexamethasone (in 200μl of saline). Control groups received 200μl of the saline solution.

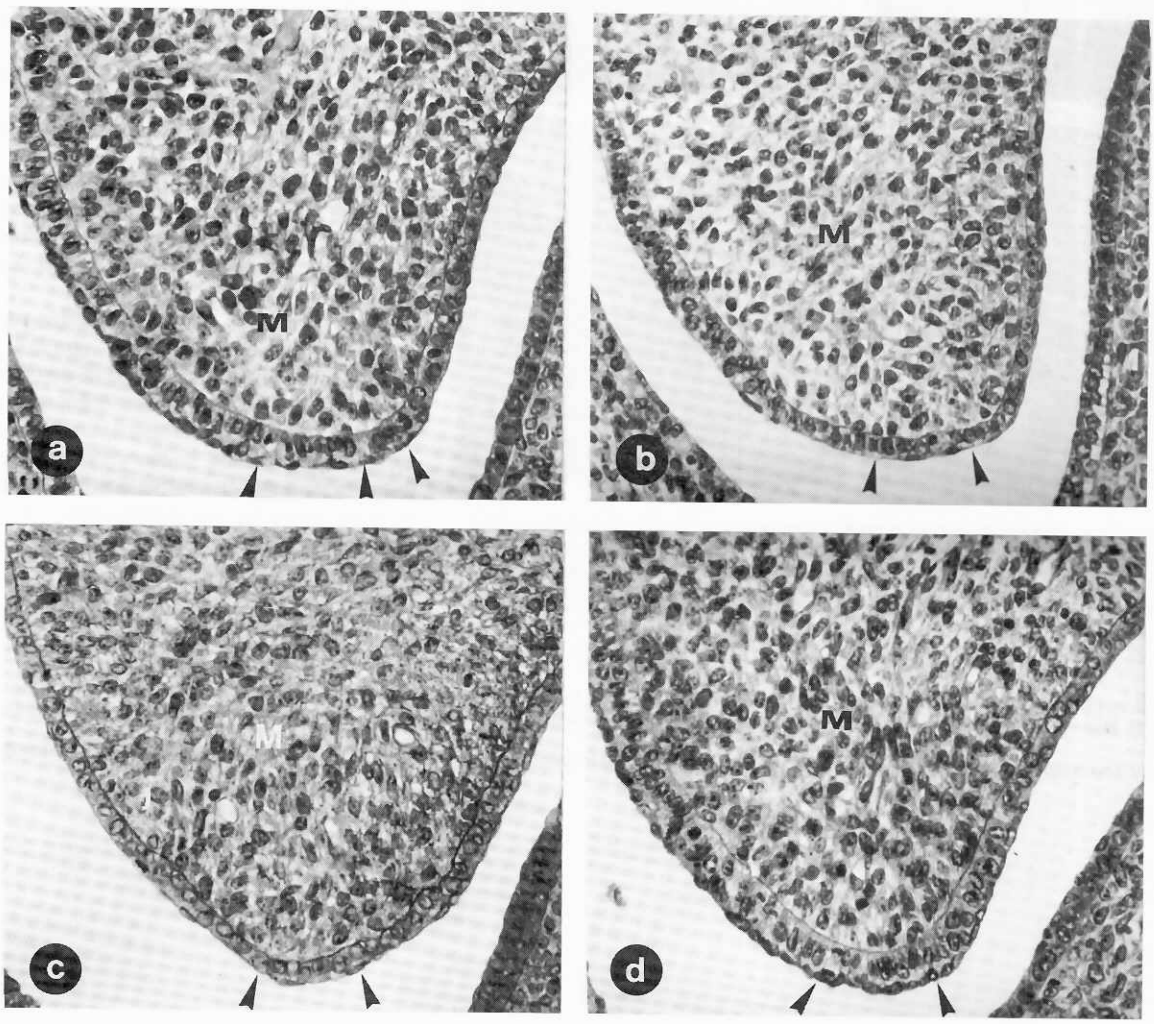

Figure 1.- Frontal section of palatine processes at 13 days of gestation, picrosirius stain. x250. a) Control A/Sn strain; b) Dexamethasone-treated A/Sn strain; c) Control C/57BL strain; d) Dexamethasone-treated C/57BL strain. Arrowheads point to the medial epithelia. Mesenchymal stroma (M).

Both experimental and control litters were recovered by laparotomy at day 13 of gestation. The embryos were removed from the uterus and the heads were processed for histological and immunohistochemical examination. Picrosirius polarization method for selective demonstration of type I and type III collagens was performed. Serial sections (5μm thick) were taken of 5 specimens from at least 3 litters of each mouse strain.

In order to examine the distribution of type IV collagen in the basal lamina of the palatal processes, immunofluorescence method was carried out using a polyclonal antibody with a 1:100 dilution.

Cell density was analyzed by making mesenchymal cell counts of the palatal processes at x400 with an eyepiece micrometer. In control and dexamethasone-treated mouse embryos, the number of mesenchymal cells was counted in 40 sections from each strain of mice.

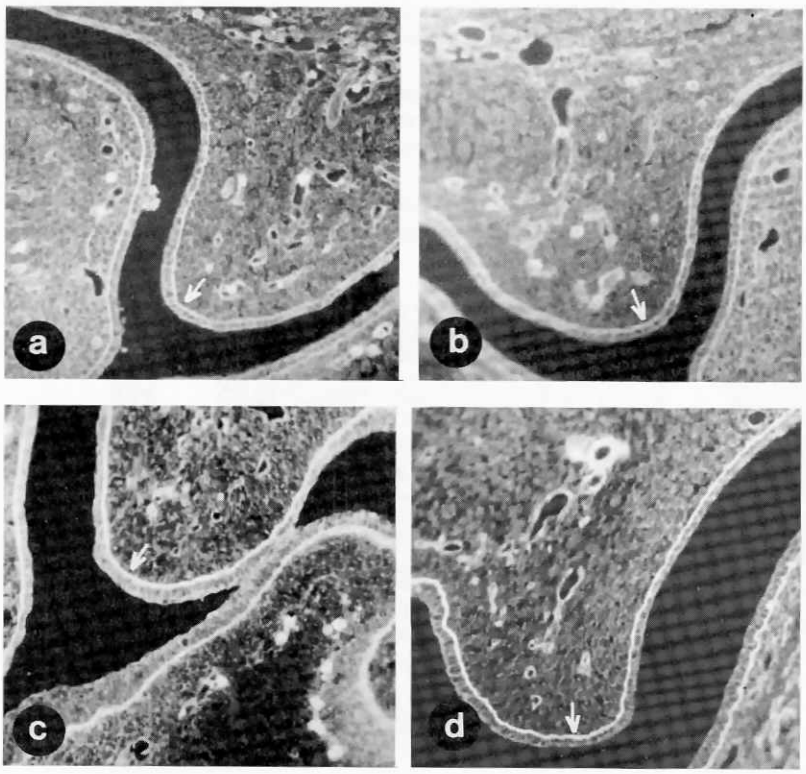

Figure 2.- Immunofluorescence-stain of palatine processes at 13 days of gestation using the policlonal antibody against type IV collagen. x100. Immunostaining was intense in the basal lamina (arrows) in both strains of mice: a) Control A/Sn strain; b) Dexamethasone-treated A/Sn strain; c) Control C/57BL strain; d) Dexamethasone-treated C/57BL strain.

Strains with the A background had significantly higher mesenchymal cell density than their partners with the H-2b haplotype at day 13 of gestation, when the palatine processes are in the vertical position. Dexamethasone did not alter cell density in both strains.

A computer-assisted method utilizing image registration was used to compare the distribution of collagen as judged by the stain intensity. In embryos at day 13, picrosirius staining was present throughout the palatal mesenchyme. This staining was more intense in the C/57BL strain than in the A/Sn strain. Dexamethasone treatment decreased this staining in both strains of mice (Fig. 1). Sections stained with pricosirius and observed with crossed polaroid filters showed mainly type III collagen in the palatine processes of both strains of mice.

Type IV collagen was distributed in the epithelial and endothelial basement membranes of the palatine processes and no differences were observed between both strains at the age studied (Fig. 2).

The differences in collagen staining and in cell density in the two strains studied and the decreasing effects on collagen of dexamethasone, suggest that this extracellular component, may be involved in the different susceptibility to cortisone-induced cleft palate in the mouse. The different patterns in collagen distribution in the two strains of mice could be the consequence of differential gene expression at this critical age of development.

References

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