treated with NE and flurbiprofen. It is possible that the early rise of IOP may diminish or postpone the later hypotensive response, resulting in the appearance of adaptation to NE. In our experiments the IOP did not usually drop from the 5 to the 7 hr reading, indicating that the maximal hypotension occurred before 7 hr. Therefore a direct relationship may exist between the degree of early IOP rise and the subsequent diminishing IOP fall.

Waitzman et al. blocked the hypertensive response to NE with indomethacin. After sympathectomy in rabbits, indomethacin prevented the ocular hyperemia that is associated with degeneration release of NE and also inhibited the enhanced outflow facility. These studies suggest that prostaglandins or other cyclooxygenase products mediate various actions of NE, including the IOP rise and subsequent adaptation of the IOP fall associated with repetitive NE administration.

Bhattacherjee and Hammondobserved that indomethacin inhibited the ocular hypotensive effect of epinephrine in rabbits. However, the hypotensive effect of NE is probably not caused by cyclooxygenase products, since neither flurbiprofen nor indomethacin blocked the hypotension.

Finally, other possible mechanisms for IOP modification include changes in the ocular penetration, neuronal uptake, or degradation of NE. However, cyclooxygenase products are not known to affect these processes. The IOP could also be modified by ocular inflammation mediated by prostaglandins. Nevertheless, suppression of the inflammation by a cyclooxygenase inhibitor would not establish a cause-effect relationship.

In summary, topical flurbiprofen was found to inhibit adrenergic adaptation to daily NE administration in the rabbit eye. The maximal effective concentration of flurbiprofen for this purpose is probably between 0.01% and 0.1%. Although not conclusive, our data strongly suggest that a cyclooxygenase product mediates this adaptation phenomenon. The mechanism may be related to postsynaptic inhibition of NE or to the early IOP hypertensive response.

Key words: adrenergic adaptation, cyclooxygenase inhibitor, flurbiprofen, intraocular pressure, norepinephrine, prostaglandin, rabbit eye

REFERENCES


Langerhans cells in the normal conjunctiva and peripheral cornea of selected species. MERLYN M. RODRIGUES, GEOFFREY WODDEN, JOSEPH HACKETT, AND IRENE BAKOS.

The distribution of Langerhans cells (LCs) in human corneal and conjunctival epithelial sheets was investigated by histochemical, immunofluorescence, and immuno-electron microscopic methods. The LCs stained positive with ATPase and with antibodies to HLA-DR antigen and were negative to DOPA-oxidase. Human conjunctiva showed 250 to 300 LCs/mm² compared to 15 to 20/mm² in the peripheral third of the corneal epithelium; approximately similar numbers of LCs were present.
in the Lewis rat, fewer cells in guinea pigs and mice, and no detectable cells in the chick. (INVEST OPHTHALMOL VIS SCI 21:759-765, 1981.)

Epidermal Langerhans cells (LCs) may be distinguished from keratinocytes and also from other epidermal dendritic cells by their unique expression in the epidermis of Fc and C₄ receptors and immune response-associated antigens (Ia). It has recently been shown that they are derived from bone marrow and probably represent antigen-presenting cells of the macrophage/monocyte series. There have been numerous studies employing either heavy metal or ATPase staining techniques to quantify the LCs in cutaneous specimens in various mammalian species. In a few of the cases electron microscopy has also been applied to these investigations in order to confirm the identity of stained cells. At present, it is necessary to identify Birbeck granules in the cytoplasm of LCs before the name may be applied to the cell. Quantitation at the transmission electron microscopic level is, however, both tedious and, at present, unreliable for LCs. It is becoming clear that considerable species and regional variations exist with respect to numbers of LCs per square millimeter of surface epithelium.

Dendritic cells in corneal and conjunctival epithelia have been investigated by enzyme histochemical techniques, transmission electron microscopy, and immunofluorescence techniques. A description of LCs in a single case of human corneal epithelium comprises the total available information of the ultrastructure of the cell. Similarly, descriptions of the LC and the Birbeck granule in guinea pig conjunctiva are controversial and need clarification. It is generally accepted that LCs are present in epithelia of the conjunctiva and peripheral third of the cornea but are absent in the central corneal region. These statements are based on ATPase staining, mainly with more recent support from staining for human HLA-DR antigens. We have investigated the distribution of LCs in human corneal and conjunctival epithelia by means of a combination of histochemical techniques, immunofluorescence techniques, TEM, and immunoelectron microscopy (IEM). Besides quantifying the numbers of LCs in the various regions in man, a comparison to distribution patterns in other species was made. Finally, proof of the staining of LCs with anti-HLA-DR antisera was achieved by means of ferritin-labeled antibodies applied at the electron microscopic level.

Materials and methods. Conjunctival and corneal epithelial sheets were separated after ½ to 1 hr incubation in EDTA. Portions of the sheets were fixed and stained for ATPase and DOPA-oxidase.
Immunofluorescence staining was carried out on acetone-fixed preparations by using either rabbit anti-HLA-DR antibodies (kindly provided by Dean Mann, National Institutes of Health) for human and rodent specimens or alloantisera for appropriate anti-la antigens in certain mouse strains. Routine TEM and IEM investigations with ferritin labeling were carried out as described previously. Specimens of human tissue were obtained from eye bank specimens enucleated up to 6 hr after autopsy. Corneal and conjunctival specimens were also obtained from surgically enucleated globes in an 11-month infant.

Animal species studied included Lewis rats, Hartley guinea pigs, and Swiss albino, BALB/cJ, ATL, ATH, BIOs, and BIOBr strain mice. Chick corneas and conjunctivas were also studied by ATPase and TEM. Control incubations carried out for the immunofluorescence studies included omission of primary antiserum for indirect studies, staining for endogenous immunoglobulins, and specific immune blocking for the direct staining. Sections were viewed with a Zeiss Photomicroscope III.

Results. LCs were demonstrated by ATPase staining in the conjunctival and peripheral corneal (limbus) tissue sheets in all species except the chick (Fig. 1). Quantitative counts were made by using a reticle fitted on an eyepiece of the microscope. Human conjunctiva showed 250 to 300 LCs/mm² compared to 15 to 20/mm² in the peripheral third of the corneal epithelium. Occasional positive cells were noted, however, in the central corneal sheet, and these cells generally lacked the extensive dendrites seen in the LCs elsewhere. Counts made on the same material stained for HLA-DR antigens demonstrated a slightly reduced population of positive cells; that is, counts were 75% to 80% of the ATPase figures (Fig. 2). Once again, a few scattered HLA-DR-positive cells were noted in the otherwise negative central corneal sheets.

LCs were present in approximately similar numbers in the Lewis rat specimens, i.e., 260 to 290 LCs/mm² in the conjunctiva and 9 to 10/mm² in the limbus. Guinea pig and mice specimens showed essentially similar numbers, i.e., 130 to 150/mm² and 9 to 10/mm² in cornea and limbus, respectively. No significant variations were noted in the various murine strains mentioned, in contrast to strain variations in various murine epidermal specimens. The LCs were negative to staining with DOPA-oxidase. Ultrastructural localiza-
Fig. 3. Transmission electron micrograph of an LC in human conjunctiva. Dense nuclear chromatin (N) and cytoplasm lacking filaments and having Birbeck granules (circle). (×14,400.)
Inset, Higher magnification of Birbeck granules (arrows). (×30,900.)

Condition of the ATPase staining reaction was examined with TEM, and the precipitate obtained was confined to the surface of LCs as has been reported previously. The Birbeck granules were present in the cytoplasm of LCs in all species examined (Fig. 3) except in chicks. Dendritic cells were not identified by ATPase staining in this case. No desmosomal attachments were noted between LCs and epithelial cells, and no Birbeck granules were identified in either keratinocytes or goblet cells.

IEM of both human and murine material with
either anti-HLA-DR or appropriate anti-\(\text{Ia}\) alloantibodies localized the antigens on the surface of dendritic cells to the basal or suprabasal layers (Fig. 4). In most cases, one or more Birbeck granules were present in the cytoplasm of the positive cells. It was, however, often necessary to examine multiple sections before an identification of the characteristic granule could be assured. Stepped-sectioning revealed occasional cells in which no Birbeck granules were present, yet surface ferritin label was positive. The occasional cells staining for ATPase and HLA-DR noted in the central corneal
region were extremely difficult to locate in either TEM or IEM specimens. However, those that were seen lacked cytoplasmic filaments, desmosomal attachments, and Birbeck granules. Positive membranes staining in the IEM study permitted their classification as indeterminate cells.12

Discussion. The ontogeny of Langerhans cells has been the subject of earlier controversy.3 Recent studies have established that LCs are mesenchymal rather than neural or melanocytic in origin.4 LCs apparently function as allogeneic stimulatory cells and antigen-presenting cells to primed T lymphocytes.5 In the epidermis of all mammalian species so far examined, Ia antigens or their molecular equivalents are expressed only on LCs or on indeterminate cells.4,12 The Ia antigens are cell membrane glycoproteins determined by genes on chromosomes 17 in the mouse; their equivalents in man, the HLA-DR antigens, are coded for by genes on chromosome 6. Light and electron microscopic studies of LCs have mostly been performed on sectioned materials,7,8 which lack sufficient surface area for reliable quantification of LCs. Adequate documentation of numbers of cells present in epithelial specimens may only be obtained with the separate sheets, as has been employed herein.

In normal human epidermis the population of LCs is approximately 500/mm². In contrast, in our study of normal human conjunctiva, LCs were fewer and were much more reduced in the peripheral third of the corneal epithelium. A slight difference was determined in the staining results obtained with either ATPase or HLA-DR methods. The difference was, however, not significant. Similarly, although lower numbers were noted on the older patients (80 years) and the most numerous were seen in the infant, these figures require further investigation before any age dependency may be suggested.

Our findings support and extend the observations of Klareskog et al.,7 who demonstrated LCs in the peripheral third of guinea pig cornea. Although grafting in corneas is clearly not directly comparable to skin grafting, owing to the semipriviliged nature of the recipient grafting site, the assumption that the central cornea is completely devoid of LCs and may thereby fail to initiate graft rejection needs further investigation.13 Individual cells bearing HLA-DR or Ia antigens have been demonstrated in this region in this study and have received comment by previous investigators.8 In view of studies suggesting that the population of LCs in the corneal epithelium may undergo quite dramatic variations in numbers,9 some circumstence may be advisable before any corneal specimen is considered as representing a uniformly LC-deficient epithelium.

No evidence was found in this study to support earlier statements that cells other than LCs in the conjunctival epithelium contained Birbeck granules.8 Indeed, the numbers of such granules was significantly less than that reported for a single human case.7 Many cells that qualify as indeterminate cells were detected in both the conjunctival and corneal epithelia. The argument has been made that meticulous serial sectioning often reveals Birbeck granules in such cells.14 Such achievements are scarcely practical in any extensive comparative study. However, step-sectioning carried out on selected blocks consistently failed to show any Birbeck granules in a small number of HLA-DR-positive dendritic cells. In the absence of any such characteristic granule, the positive staining must be interpreted as evidence for a relationship to LCs, hence the classification as indeterminate cells. Several authors have commented in the past on the relationship between numbers of Birbeck granules and the stage of differentiation of both the LCs and the keratinocytes.6 The suggestion that newly immigrated LCs are poor in Birbeck granules may imply that the turnover of such cells may be quite rapid in the epithelia associated with the eye.

A target role for LCs has been proposed in cutaneous contact hypersensitivity reactions15 and in T-cell lymphomas such as mycosis fungoides.16 Whether LCs in conjunctival and corneal epithelia are involved in similar processes is not as yet known. Their very position in such epithelia might further support the proposals of immune surveillance of chemical, microbial, or viral intrusions, since reactions to the above agents are of varying degrees of intensity in different parts of the orbital epithelial surfaces.

Further investigations of the movement of LCs in these regions, as, for example, in the face of vitamin A deficiency or after chemical or haptenic stimulation,17 not only may prove to be important in determining the role of local immune surveillance, inflammation, and other processes of interest in ophthalmic pathology but may also clarify the suggested connections between LCs and the degree of keratinization achieved by the epithelium in which they are found.

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Key words: Langerhans cells, cornea, conjunctiva, HLA-DR, immunofluorescence, immuno-electron microscopy, ATPase

REFERENCES


Temporal aspects of the dark-adapted cone a-wave in retinitis pigmentosa. MICHAEL A. SANDBERG, PETER L. SULLIVAN, AND ELIO L. BERSON.

Cone electroretinograms were elicited with a full-field red flash after 45 min of dark adaptation from 16 patients with retinitis pigmentosa and from 17 normal subjects. The average latency and implicit time of the cone a-waves recorded from the patients were each significantly delayed (p < 0.005) compared with the average normal values. In addition, the patients' waveforms typically showed a loss of two oscillations on the rising slope of the cone b-wave. These delays and waveform changes could be simulated in normal subjects by reducing the luminance, but not the diameter, of the stimulus. These studies in normal subjects suggest that shortening of outer segments of remaining cones and not simply loss of cones could account for the waveforms observed in patients with retinitis pigmentosa. The results also suggest that remaining cones generate an a-wave in the dark that is normal for a reduced cone visual pigment density. (Invest Ophthalmol Vis Sci 21:765-769, 1981.)

Ultrastructural studies of postmortem eyes from patients with hereditary retinitis pigmentosa have revealed retinal areas where cones were reduced in number and had shortened outer segments. Patients with early retinitis pigmentosa have shown reductions in the amplitude of the cone-dominant early receptor potential and elevations of dark-adapted cone thresholds. These physiologic findings can be explained, at least in part, by reduced numbers of cones or shortened outer segments or both. A reduction solely in the number of cones would be expected to result in a reduction in the amplitude of the cone a-wave without a change in its latency and implicit time. If remaining cones had shortened outer segments but were otherwise functioning normally, then cone a-wave responses should be slowed as well as