We have investigated normal human corneas for the presence of T6-marker on Langerhans cells. With the exception of one pair of newborn corneas and two pairs of very young infant corneas, all HLA-DR-positive cells in central and peripheral corneal epithelium were T6-negative by double-labeled immunofluorescence. In contrast, epidermal sheets from normal human eyelid skin displayed positive staining for T6 on most of the HLA-DR-positive Langerhans cells. Since T6 antigen is considered to be a specific Langerhans cell differentiation marker, we interpret this finding to indicate a nonactivated or undifferentiated state of Langerhans cells in normal human corneas. Invest Ophthalmol Vis Sci 28:1719–1722, 1987

Studies of normal human epidermal Langerhans cells have demonstrated heterogenic populations of such cells. HLA-DR+/T6−, HLA-DR+/T6+, and HLA-DR+/T6− Langerhans cells have all been detected in human skin.4–6 The discovery of human thymocyte antigen (T6) on Langerhans cell membranes has led to subsequent convictions that T6 is a more specific marker for human Langerhans cells than previously used markers such as 1a antigen, ATPase, and Fc and C3b receptors.4–5 One study has shown T6 antigen to be present on Langerhans cells of normal human conjunctiva.6 However, there have been no published studies as yet on the presence or absence of this marker on human skin epidermis.5 We have investigated normal human corneas of donors of various ages for the presence of T6 antigen. All corneas, with the exception of one pair of newborn corneas and two pairs of young infant corneas, contained HLA-DR-positive Langerhans cells that were T6 negative.

**Materials and Methods.** Whole globes were obtained from the University of Washington Lions Eye Bank and University of Wisconsin Eye Bank within 6 hr of the donor’s death. Corneas were dissected with approximately 2 mm of scleral rim. After a brief rinse in normal saline, the corneas were incubated in 20 mM EDTA in phosphate buffered saline (PBS, pH 7.3) for 45–60 min at 37°C. The epithelial sheets were separated from the corneal stromata with fine dissecting forceps and fixed in cold acetone for at least 10 min. Eyelid epidermal sheets used as controls were similarly isolated from tissue obtained from blepharoplasties. Prior to staining, each epithelial sheet was washed and rehydrated in PBS containing 1% bovine serum albumin (BSA-PBS) for 10 min. The tissues were then incubated in suspension with a 1:50 dilution of OKT6 monoclonal antibody (Ortho Diagnostic Systems, Raritan, NJ) at 4°C overnight. Following this incubation the epithelial sheets were washed twice in BSA-PBS for 10 min and incubated in a 1:50 dilution of rhodamine-conjugated sheep antirabbit IgG (H&L) (Cappel Laboratories, West Chester, PA) at 4°C overnight. The tissues were then incubated in suspension with a 1:50 dilution of fluorescein-conjugated anti-HLA-DR antibody (Becton Dickinson, Mountain View, CA) at 4°C overnight. After two final washes in BSA-PBS, radial incisions were made through each epithelial sheet to allow a flat mount onto a microscopic slide. The tissues were coverslipped with Aquamount (Lerner Laboratories, New Haven, CT) and the edges of the coverslip sealed with clear nail polish to prevent evaporation. Additionally, a number of corneas and human skin epidermis specimens were stained in a one-step process with only a fluorescein-conjugated OKT6 (Ortho Diagnostic Systems).

**Results.** Twenty-six pairs of corneas were examined by double fluorescent-labeled antibody staining for HLA-DR and T6. The age ranges of these corneas are given in Table 1. Staining of these corneas was compared to that of identically processed human skin epidermis. Of these 26 pairs of corneas, 23 pairs were completely negative for T6 antigen (Fig. 1a) but dis-
Table 1. Age range of corneas examined by double fluorescent-labeled antibody staining for HLA-DR and T6 antigen

<table>
<thead>
<tr>
<th>Age range</th>
<th>Number of corneas</th>
</tr>
</thead>
<tbody>
<tr>
<td>newborns-20 yr</td>
<td>10 pairs</td>
</tr>
<tr>
<td>20-40 yr</td>
<td>3 pairs</td>
</tr>
<tr>
<td>40-60 yr</td>
<td>6 pairs</td>
</tr>
<tr>
<td>60-80 yr</td>
<td>7 pairs</td>
</tr>
<tr>
<td>Total</td>
<td>26 pairs</td>
</tr>
</tbody>
</table>

displayed normal densities of HLA-DR-positive Langerhans cells near the limbus (Fig. 1b). Human skin epidermis, by comparison, displayed positive staining on its Langerhans cells for T6 as well as HLA-DR (Fig. 2a, b).

One pair of newborn infant corneas and two pairs of young infant corneas (3-day-old, 8-day-old) did exhibit some double immunocytochemical staining of T6 on HLA-DR-positive Langerhans cells (Fig. 3a, b). As reported earlier, Langerhans cells in infant corneas are numerous not only in the periphery of the tissue but centrally as well.7

An additional four pairs of normal corneas were stained with OKT6-FITC alone, without subsequent staining for HLA-DR. The ages of the corneas used were 5 days, 64 years, and 76 years old. An epidermal sheet was also stained in this one-step process. None of the corneas examined demonstrated any positive fluorescence for T6, whereas the skin epidermis displayed positive staining of Langerhans cells at a density similar to that observed with double-stained epidermal sheets (described above).

Discussion. The surface antigen identified by OKT6 is recognized to co-exist with, but differ from, the Ia antigen found on Langerhans cell membranes.4,8 In addition, many investigators feel that T6 is a specific differentiation antigen of these cells in the epidermis.2,5,9,10 Others hypothesize that the HLA-DR+/T6+ subset of Langerhans cells may indicate an activated state of a functionally distinct subset.1

Conjunctival Langerhans cells have been reported to express T6; this has been observed in our laboratory as well. Our study demonstrates the absence of this antigenic marker on Langerhans cells of normal corneas. Only three pairs of infant corneas displayed positive staining for this thymocyte antigen. However, one pair of a 5-day-old infant’s corneas were negative for T6 antigen.

If T6 is a differentiation antigen, as others believe,
this would seem to indicate that Langerhans cells in a normal cornea may exist in an undifferentiated or nonactivated state. The reasons for this are unknown thus far; however, the fact that the cornea differs markedly from other sites in the body where Langerhans cells are found (e.g., skin, lymph node paracortex, thymus, etc.) may be a contributing factor. Although Langerhans cells are bone marrow-derived, T6+ cells are not found in bone marrow aspirates or peripheral blood monocytes. Perhaps corneal Langerhans cells have not been subjected to thymic influences. Expression of T6 may be induced by factors in certain epithelial microenvironments after the Langerhans cells localize there. Recent investigations by others seem to support this contention. Corneal Langerhans cells, situated in a normally avascular tissue different from that of the skin, may not be exposed to these factors and therefore remain undifferentiated. Migrating Langerhans cells, or "streamers," reported by some investigators, were not observed in the normal corneas in this study, either by HLA-DR staining or by T6 staining.

Alternatively, epidermal Langerhans cells may acquire T6 antigen exogenously from the milieu of the skin. The unique characteristics of corneal tissue may prevent such exogenous acquisition by its Langerhans cells.

The presence of T6 antigen on the three pairs of newborn and young infant corneas may be due in part to the differences between infant and adult corneas. Langerhans cells are found uniformly across the central regions of infant corneas but are absent in the central corneas of normal adults. It may be that factors that prevent Langerhans cells from existing in the central portion of normal adult corneas also operate to hinder the expression of T6 on the Langerhans cells at the periphery, even if they are derived by migration of HLA-DR+/T6+ conjunctival Langerhans cells.

Clearly, the dilemma of why T6 was not detected requires further investigation, including the possibility that T6 expression can be modulated by exogenous mediators, much like la induction on certain cells by gamma interferon. Further, it is not yet known whether T6 expression is induced during certain diseases involving the ocular surface.

Key words: cornea, langerhans cell, HLA-DR positive, T6 negative, epithelium

References

7. Chandler JW, Cummings M, and Gillette TE: Presence of
The effects of oral immunization with a recombinant vaccine expressing chlamydial lipopolysaccharide (LPS) on subsequent ocular challenge with *Chlamydia trachomatis* were studied in cynomolgus monkeys. Groups of four or five monkeys were given an oral vaccine containing $5 \times 10^8$ parent or recombinant *Escherichia coli* on days 0, 14, and 35 and were challenged with either $2 \times 10^3$ or $5 \times 10^3$ inclusion forming units of viable purified elementary bodies on day 42. On clinical and microbiologic grounds, oral immunization failed to protect monkeys against subsequent ocular challenge. Antichlamydial IgG or IgA antibodies were not induced by oral vaccination, and the antibody response following oral challenge was similar in vaccinated and nonvaccinated animals. Paradoxically, however, while nonvaccinated control animals developed antibodies against chlamydial LPS detectable by immunoblotting after chlamydial challenge, the LPS vaccinated animals did not. This study demonstrates that the oral recombinant vaccine expressing chlamydial LPS was ineffective in protecting against chlamydial eye infection and strongly suggests that chlamydial LPS may not be an important antigen for protective immunity against chlamydia. Invest Ophthalmol Vis Sci 28:1722–1726, 1987

It is generally assumed that an effective vaccine against trachoma will stimulate protective immunity at the mucosal surface of the eye. Previous studies have shown that the oral administration of an antigen can prime the eye to subsequent challenge with that antigen, and preliminary vaccine trials in a monkey model of trachoma have shown that protection equivalent to that seen after ocular infection can be induced by oral immunization with viable whole *Chlamydia trachomatis* elementary body (EB) vaccines. However, some of these oral preparations as well as systemic vaccines can also induce a hypersensitivity response with more severe disease occurring after ocular challenge. It seems clear that the immune response to *C. trachomatis* can be both protective and deleterious and that different chlamydial antigens may be responsible for the different types of immune response. For this reason, subunit vaccines may offer the most appropriate mechanism of stimulating protective immunity without inducing harmful sensitization. The first subunit chlamydial antigen preparation to be available in sufficient quantities for study is chlamydial lipopolysaccharide (LPS) expressed by a recombinant *Escherichia coli*. This report presents the first studies on the efficacy of a recombinant chlamydial LPS oral vaccine tested in the monkey model of trachoma.

Materials and Methods. Animals: Groups of young adult cynomolgus monkeys were obtained from Charles River Primates (Boston, MA). There were five monkeys in each group unless otherwise specified. All procedures described herein conform to the ARVO Resolution on the Use of Animals in Research.

Oral vaccination: Recombinant *E. coli* harboring plasmid pFEN 207 which expresses chlamydial LPS group antigen on its surface and the parent *E. coli* harboring only the pUC8 plasmid vector were cultured in LB medium and adjusted to contain $1 \times 10^8$ organisms per ml. Monkeys were fasted overnight; and immediately after neutralization of gastric contents with 5 ml of sodium bicarbonate solution, the enteric dose of 5 ml suspension ($5 \times 10^8$ organisms) was administered via a gastric tube.

Oral vaccine, or the parent *E. coli*, was given on days 0, 7, 14, and 35; and the ocular challenge was given on day 42.

Ocular challenge with *C. trachomatis*: *C. trachomatis* serovar B (TW-5) was grown in mass tissue culture. Purified EBs were prepared by centrifugation through renograin and resuspended at various dilutions in phosphate-buffered saline. Ocular inoculations were adjusted to $1 \times 10^5$, or $2.5 \times 10^5$ infection