

Adenovirus-Directed Ocular Innate Immunity: The Role of Conjunctival Defensin-like Chemokines (IP-10, I-TAC) and Phagocytic Human Defensin- α

Stephen A. K. Harvey,¹ Eric G. Romanowski,^{1,2} Kathleen A. Yates,^{1,2} and Y. Jerold Gordon^{1,2}

PURPOSE. Adenovirus (Ad), a major cause of viral conjunctivitis worldwide, is controlled initially by the innate immune response on the ocular surface. In the present study, cultured human conjunctival epithelial cells were used to identify host genes responsive to infection by a clinical isolate of Ad5, and the antiviral activity of some of their peptide products was investigated.

METHODS. Primary human conjunctival epithelial cells in culture were infected with Ad5 at high (1.0), low (0.1), or zero (0.0) multiplicities of infection (MOI) and harvested at different times after infection (1.5, 6, and 16 hours). Host gene expression was profiled with oligonucleotide microarrays (GeneChips; Affymetrix, Santa Clara, CA). Peptide products of interest were tested for antiviral activity in direct inhibition assays against respiratory serotypes (Ad3, Ad5), ocular serotypes (Ad8, Ad19), and HSV-1.

RESULTS. At high MOI, viral infection suppressed the interferon (IFN)-mediated host antiviral response seen at low MOI. At 16 hours after infection, 63 unique characterized transcripts were identified that were robustly and significantly suppressed by high MOI, (i.e., MOI [0.1]/MOI [0.1] ≥ 2 , $P < 0.006$). Of these, 29 (46%) transcripts are involved in IFN signaling or are IFN or virus induced. This subset included CXCL10 and CXCL11, encoding IP-10 and I-TAC, respectively. These defensin-like chemokines and human α -defensin-1 directly inhibited Ad3 and Ad5 but not Ad8 or Ad19.

CONCLUSIONS. In response to low-level Ad5 infection, conjunctival epithelial cells showed upregulation of IFN-associated genes. The peptide products of two of these, IP-10 and I-TAC, are directly active against Ad3, and IP-10 is active against Ad5. The ocular tropism of Ad8 and Ad19 may be due in part to their resistance to these defensin-like chemokines. (*Invest Ophthalmol Vis Sci.* 2005;46:3657-3665) DOI:10.1167/iovs.05-0438

Innate immunity evolved approximately 2.6 billion years ago as a rapid, nonspecific, redundant, multifunctional, "fail-safe" host defense system.^{1,2} A plethora of effector molecules

including antimicrobial peptides (AMPs: defensins, defensin-like chemokines, and cathelicidin) and proteins (CAP-37), alone and in combination, demonstrate inhibitory activity against a wide variety of pathogens, including bacteria, fungi, parasites, and some viruses.^{3,4} In addition, AMPs serve as important effector molecules in inflammation, immune activation, and wound healing.^{5,6} Naturally occurring AMPs are secreted by various epithelia in skin and mucosal surfaces, including the ocular surface,^{7,8} and are also found in circulating phagocytes. Modified and synthetic AMPs comprise a potential new class of drugs and are in various stages of development for a variety of topical and systemic applications.^{9,10}

Microarray technology has facilitated the monitoring of host cell responses to viral infection, including adenovirus,¹¹ HSV-1,¹² hCMV,¹³ HIV,¹⁴ KSHV,¹⁵ human papilloma virus,¹⁶ hepatitis C virus,^{17,18} and dengue virus.¹⁹ Collectively, these studies have advanced our understanding of host interaction with these important human pathogens. Of particular interest is the balance between host defense and viral immune evasion during acute and chronic infections.²⁰⁻²² Elucidation of the virus lytic cycle, viral perseverance, and latency are also topics of interest.

However, with one exception,¹¹ the infection of human ocular cells by wild-type adenovirus remains unaddressed by microarray analysis. Studies of nonocular cells or ocular cell lines²³⁻²⁶ may incompletely reflect host cell response in vivo. In the literature, studies of recombinant adenovirus vectors²⁴⁻²⁶ are favored over those of pathogenic strains, because the former have potential use in gene therapy. Moreover, in most studies, unphysiologically high infectivity titers^{23,26} were used, and so these studies do not accurately model naturally occurring eye infections.

In the present study, we focused on infection at the ocular surface by treating primary cultured human conjunctival epithelial cells with a pathogenic clinical ocular isolate of Ad5. This was accomplished at multiplicities of infection (MOI) sufficiently low (0.1 or 1.0) to mimic clinical infection closely. Host expression was characterized using oligonucleotide microarrays (GeneChips; Affymetrix, Santa Clara, CA), and transcripts that were elevated at MOI = 0.1 (host defense predominates) relative to MOI = 1.0 (viral control predominates) were designated as host antiviral genes. Based on the existing literature, we hypothesized that β -defensins, which are active against HIV,²⁷⁻²⁹ would appear in this host antiviral group and would show direct antiviral effects against Ad5. This was not the case, however. The speculated role of β -defensins was filled by IP-10 and ITAC, defensin-like chemokines with direct antiviral effects.

METHODS

Cell Lines

A549 cells, an epithelium-like continuous cell line derived from human lung carcinoma, were grown and maintained in Eagle's minimum

From the ²Charles T. Campbell Laboratory and the ¹Department of Ophthalmology, University of Pittsburgh, Pittsburgh, Pennsylvania.

Supported by National Eye Institute Grants EY008227 (YJG) and EY008098 Core Grant For Vision Research (to Robert L. Hendricks), The Eye and Ear Foundation of Pittsburgh, and Research to Prevent Blindness.

Submitted for publication April 6, 2005; revised May 24 and June 21, 2005; accepted August 26, 2005.

Disclosure: S.A.K. Harvey, None; E.G. Romanowski, None; K.A. Yates, None; Y.J. Gordon, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Stephen A. K. Harvey, Department of Ophthalmology, University of Pittsburgh, EEINS 911, 203 Lothrop Street, Pittsburgh, PA 15213-2588; harveysa@msx.upmc.edu.

essential medium with Earle's salts (Cell Culture Reagents; Sigma-Aldrich, St. Louis, MO), supplemented with 10% fetal bovine serum (Harlan Bioproducts for Science, Indianapolis, IN), 2.5 $\mu\text{g}/\text{mL}$ amphotericin B, 100 u/mL penicillin G, and 0.1 mg/mL streptomycin (Sigma-Aldrich), hereafter known as OG.

All primary cell cultures were prepared in the departmental Core Hybridoma and Tissue Culture Module by a dedicated technician. Human donor eyes deemed unsuitable for transplantation were obtained from the Center for Organ Recovery and Education (CORE, Pittsburgh, PA), in accordance with the provisions of the Declaration of Helsinki for research involving human tissue. Conjunctival tissue was harvested under sterile conditions by an experienced corneal surgeon (YJG). Conjunctival epithelial cells were grown in primary culture over 1 to 3 weeks by using established procedures^{30,31} and then were used for gene array experiments.

Virus Serotypes and Peptides

Adenovirus serotype 3 (Ad3) was purchased from the American Type Culture Collection (ATCC; Manassas, VA). Adenovirus serotypes 5, 8, and 19 were clinical isolates cultured from patients who presented with typical adenovirus ocular disease at the Eye and Ear Institute of Pittsburgh and were typed by serum neutralization. The isolates were grown in A549 monolayers at 37°C, in a 5% CO₂-water vapor atmosphere, harvested, aliquoted, and frozen as a virus stock at -70°C. HSV-1 McKrae was grown in Vero cell monolayers and processed the same as the adenovirus stocks. Before use, the stock viruses were titered with a standard plaque assay.

Human defensins $\alpha 1$, $\beta 1$, and $\beta 2$ were purchased from Peptides International (Louisville, KY), whereas IP-10 and I-TAC were purchased from Cell Sciences (Canton, MA). All peptides were stored at -20°C before use and reconstituted according to the manufacturers' instructions on the day the experiment was performed.

Gene Microarray Analysis

Cultured primary human conjunctival epithelial cells were treated with the clinical isolate of Ad5 at multiplicities of infection (MOI) of 0, 0.1, or 1.0 and harvested at 1.5, 6, or 16 hours after infection. Total RNA was isolated from cultured cells (RNeasy/Qiashredder systems; Qiagen Inc., Valencia, CA), with a yield of $46 \pm 14 \mu\text{g}$ (mean \pm SD, $n = 9$) RNA per 60-mm culture dish. Total RNA was processed and analyzed (HU133A GeneChips; Affymetrix Inc., Santa Clara, CA). The mRNA component of the total RNA was reverse transcribed (SuperScript system; Invitrogen Corp., Carlsbad, CA) in the presence of a T7-(dT)₂₄ primer. The resultant cDNA was extracted with phenol-chloroform, ethanol-precipitated from the aqueous phase, and transcribed in vitro in the presence of biotin-labeled ribonucleotides using (BioArray kit; Enzo Biochem, Inc., Farmingdale, NY). The biotinylated RNA was separated from cDNA and other transcription components (RNeasy; Qiagen), 20 μg was fragmented (35 minutes at 94°C in Affymetrix sample fragmentation buffer), and the fragments were hybridized to a microarray (GeneChip; Affymetrix). The chip was then washed and developed before it was read in a scanner (ChipScanner; Affymetrix Inc.). Raw data were processed and analyzed on computer (Microarray Analysis Suite [MAS] ver. 5.0; Affymetrix) with software that uses nonparametric algorithms to determine (1) whether a transcript is present or absent on a single microarray ($P < 0.04$) and (2) in comparing any two microarrays, whether the signal change is significantly increased or decreased ($P < 0.006$).

Real-Time PCR Analysis

Quantitative real-time PCR (QRT-PCR) assays were performed with a sequence detector (Prism 7700 Sequence Detector; Applied Biosystems, Inc. [ABI], Foster City, CA) running 96-well plates at 50 $\mu\text{L}/\text{well}$ final volume. Instrument control and data analysis were made using the default settings of the supplied software (Primer Express v. 1.5a; ABI). Each assay comprised triplicate measurements of cDNA generated by the microarray protocol, using ~10 ng nucleic acid per well and

duplicate measurements of (minus RT) control. Samples were mixed with PCR master mix (*TaqMan* Universal; Roche, Branchburg, NJ) and the appropriate primer-probe kits (Assays-on-Demand; ABI). The following kits were used (HGNC symbols and ABI catalog numbers): CXCL10 (as SCYB10), Hs00171042_m1; CXCL11 (as SCYB11), Hs00171138_m1; DEFB1, Hs00174765_m1; RSAD2/viperin (as cig5), Hs00369813_m1; RRM2, Hs00357247_g1; ACTB, Hs99999903_m1. The relative level of transcripts was calculated using the $\Delta\Delta C_t$ method using the housekeeping gene β -actin (*ACTB*) as endogenous control.

Direct Antiviral Inhibition Studies

Stock solutions of defensins (400 $\mu\text{g}/\text{mL}$) or IP-10/I-TAC (222.22 $\mu\text{g}/\text{mL}$) were made in distilled water with a subsequent twofold dilution using 2 \times PBS. Frozen viral stocks were thawed in a 37°C water bath and diluted in serum- and antibiotic-free OG to yield approximately 10² and 10⁵ pfu/mL. Virus and peptide stocks were mixed to produce final peptide concentrations of 100 $\mu\text{g}/\text{mL}$ and 10² and 10⁵ pfu/mL. The virus/peptide combinations were incubated in a 37°C water bath for 60 (defensins) or 75 (IP-10/I-TAC) minutes to assess the direct antiviral effect. After incubation, each virus/peptide mixture and control was either added directly to A549 monolayers (defensins) or diluted in ice-cold tissue culture medium containing 10% fetal bovine serum before addition (IP-10/I-TAC). After viral adsorption for 3 hours at 37°C and 5% CO₂ with constant rocking, the appropriate overlay medium was added to the monolayers. The plates were incubated at 37°C, 5% CO₂ and examined daily for plaques (viral cytopathic effect). When plaques were visible, the multiplates were stained with gentian violet and counted with a 25 \times dissecting microscope. In the IP-10/I-TAC assays unincubated virus-PBS standards were used to check the viral plaque-forming units for each assay, and if the plaque-forming units recovered from incubated virus-PBS controls was <20% of the unincubated virus-PBS standards, then data from that assay were discarded. The plaque-forming unit titer of each virus/peptide combination was reported as a fraction of the matched virus-PBS control for two to four independent experiments. Virus/peptide data were first compared with incubated virus-PBS control using Student's *t*-test. Because multiple comparisons (one per peptide) are made with each PBS control, we also checked using the more appropriate Dunnett's test, which confirmed the *t*-test results.

RESULTS

Identification of Antiviral Genes in Cultured Human Conjunctival Epithelial Cells by Gene Array Analysis

We reasoned that a high MOI (1.0) would lead to successful infection of a substantial proportion (~60%) of conjunctival cells in the culture and a robust, targeted downregulation of antiviral genes would occur as a part of a viral immune evasion strategy. In contrast, infection at a lower MOI (0.1) would lead to successful infection of a smaller percentage of cells and that these infected cells would signal adjacent, uninfected cells via interferon, to activate antiviral genes, including secreted innate immune proteins. Of the 22,215 host cell panels on the microarray chip (U133A; Affymetrix), 9,068 were absent in all nine samples and were excluded from further consideration. In the remaining panels, 1294 (~10%) showed at least one twofold change. From these we selected panels in which the host defense response evoked by low MOI was robustly and significantly suppressed by high MOI (MOI [0.1]/MOI [1.0]) ≥ 2 , $P < 0.003$, MAS ver. 5.0). These criteria were met by 37, 17, and 57 microarray panels at 1.5, 6, and 16 hours after infection, respectively. We focused on the largest group (16 hours), which contained a high percentage of interferon-dependent transcripts. Accordingly, the 16 hours samples were reassayed

using U133B chips and another 23 panels were found fulfilling the same criteria. Of the total of 80 panels, 10 were characterized only by their panel numbers (see legend to Table 1). The remaining 70 characterized panels represented 63 unique transcripts that were divided by function into nine groups: secreted products ($n = 5$, 8%), IFN- or virus-induced ($n = 21$, 33%), IFN signaling ($n = 2$, 3%), transcriptional control ($n = 7$, 11%), receptor and integral membrane proteins ($n = 3$, 5%), apoptosis ($n = 4$, 6%), enzymes ($n = 2$, 3%), unassigned ($n = 15$, 24%), and unknown ($n = 4$, 6%). For brevity we included in Table 1 only the 49 panels for which one or both of the infected samples' value was also significantly different from the uninfected sample. The 21 panels omitted from Table 1 include the toll-like receptor 3, IFN-induced *IFIT2*, and an additional panel for *RSAD2/viperin*.

Transcripts in Table 1 that are marked with an asterisk also were suppressed twofold by an MOI of 1.0 at the earliest time studied (1.5 hours after infection), confirming that Ad5 attempts to suppress antiviral host cell defenses very early in the infection process. Thirteen (72%) of these 18 suppressed transcripts are IFN dependent. Of the secreted chemokines, two (*CXCL10* and *CXCL11*) are IFN dependent, whereas two (*CXCL2* and *CXCL5*) are TNF- α dependent.

Expression data were examined for other innate immune genes. α -Class defensins (*bDEF1A*, *bDEF4A*, *bDEF5A*, and *bDEF6A*) are present in circulating phagocytes but absent from conjunctival epithelia and were not detected in the present study. Transcripts of the CAMP gene encoding cathelicidin (*LL37*) also were undetectable. We have shown previously⁵⁷ that CAMP transcripts are detectable by RT-PCR in both corneal and conjunctival epithelial cells, so their apparent absence here is probably due to the known lower sensitivity of gene arrays relative to RT-PCR. Similarly, *CAP37* reported as a corneal AMP⁵⁸ was not detected in the present study. However, for transcripts present at higher concentrations, gene arrays and quantitative RT-PCR (QRT-PCR) gave comparable results (Fig. 1).

Time Course of Innate Immune Gene Expression in Conjunctival Epithelial Cells

The gene array data provided a time-course of expression for each MOI. For selected genes, we confirmed the time course using QRT-PCR (Figs. 1). Transcripts of the genes *CXCL10*, *CXCL11*, and *RSAD2*, respectively encode the defensin-like chemokines IP-10 and I-TAC and the intracellular antiviral protein viperin. QRT-PCR data confirmed the early (1.5 hours) downregulation of all three transcripts in infected cells, as well as the upregulation at 16 hours, when MOI = 0.1. Figure 1D summarizes the expression of β -defensin-1 (*DEFB1*) which was suppressed at 6 and 16 hours. Qualitatively similar gene array data (not shown) were obtained for β -defensin-2; however, at the time of the study, no QRT-PCR kit was available to confirm the *DEFB2* changes.

The modest (~2-fold) changes shown in Figure 1 led us to confirm examples from other data groups that showed more substantial changes. Members of the carcinoembryonic antigen cell adhesion molecule (CEACAM) family, *CEACAM5* and *CEACAM7* are presumptive innate immune molecules⁵⁹ in epithelial cells. Gene array data showed both were substantially increased by viral infection at 1.5 hours, an increase that was confirmed by QRT-PCR for *CEACAM7* (Fig. 1E).

Finally, review of the microarray data revealed that 109 panels, representing 96 unique transcripts, comprised a viral footprint—that is, were increased twofold or more by MOI 1.0 vs. MOI 0.0 (mock-infected) at 16 hours (data not shown). More than half (55, 57%) represent adenovirus upregulation of host proliferation systems. Twenty-five (26%) of these tran-

scripts respond to host transcription factor E2F, which is itself a target for adenovirus E1A protein.⁶⁰ An example of this group is *RRM2* (ribonucleotide reductase subunit 2) which is E2F-responsive and fulfills adenovirus' dependence on host ribonucleotide reductase.⁶¹ As expected, both techniques show that *RRM2* is substantially increased by viral infection at 16 hours (Fig. 1F).

Direct Incubation Antiviral Assays

The defensin-like chemokines IP-10 and I-TAC, identified as antiviral host genes (Table 1), were assayed for antiviral activity in vitro (Fig. 2) against Ad serotypes with different preferred targets (tissue tropisms) but all capable of infecting the eye. Representative serotypes of group B (Ad3) and group C (Ad5) are known to infect the oropharynx and respiratory system preferentially, but can produce a mild, self-limiting conjunctivitis. In contrast, group D serotypes, Ad8 and Ad19, are highly specific for the eye and generally cause a more serious keratoconjunctivitis with possible long-term visual sequelae.⁶² Figure 2 shows that the viral titers representing the respiratory serotypes, Ad3 and Ad5, are both significantly inhibited by IP-10, the peptide product of *CXCL10*; only Ad3 is significantly inhibited by I-TAC, the peptide product of *CXCL11*. In contrast, neither IP-10 nor I-TAC demonstrated any significant inhibitory activity against the ocular adenoviral serotypes Ad8 and Ad19, or against HSV-1, another ocular viral pathogen.

Figure 3 summarizes the antiviral activity of selected defensins, in the same assay. Human α defensin-1 (*HDEF1A*) significantly decreased viral titers for the respiratory serotypes Ad3 and Ad5, but had no inhibitory effect on the ocular serotypes, Ad8 and Ad19. The β -defensins *bDEFB1* and *bDEFB2*, which are present in human conjunctival and corneal epithelial cells were ineffective against either the respiratory or ocular serotypes.

DISCUSSION

The study of viral pathogenesis has advanced rapidly in recent years after the application of gene array technology. The co-evolution of the host cell and virus has been investigated for many important human pathogens HSV-1,¹² hCMV,¹³ HIV,¹⁴ KSHV,¹⁵ human papilloma virus,¹⁶ hepatitis C virus,^{17,18} and dengue virus.¹⁹ Of particular interest has been the elucidation of details of the life cycle of the virus, host cell antiviral defenses, and successful viral immune evasion strategies. Adenovirus pathogenesis has been studied in vitro¹¹ and in respiratory⁶³ and ocular animal models.^{64,65} The principal immune evasion strategies have been categorized as follows: (1) inhibition of the interferon response, (2) inhibition of multiple apoptosis pathways, and (3) inhibition of cellular immune responses by blocking MHC 1 cell surface molecules, CTL-mediated cell lysis, and NK cell activity.^{21,66,67} In the present study, inhibition of the IFN response attenuated the innate immune response, decreasing expression of antimicrobial peptides (defensin-like chemokines and defensins). In addition, unlike previous studies, we chose conditions for our gene array studies that more closely mimicked conditions of naturally occurring ocular infections: (1) a differential MOI strategy to identify host antiviral genes, (2) use of a pathogenic clinical ocular isolate, (3) use of conjunctival epithelium as the target cell, (4) use of primary cell cultures from fresh whole eyes, and (5) appropriate times of analysis after infection.

At 1.5 hours after infection the virus has penetrated the cell, achieved nuclear localization, and commenced early transcription (viral genes E1A, E2A, E4). By 6 hours, late viral transcription is in progress, with a viral blockade in place, both of host mRNA transport and host translation. Host apoptosis is also

TABLE 1. Host Antiviral Genes Upregulated at 16 Hours after Infection

Affymetrix ID	Gene Symbol	Entrez Gene	Ratio MOI (0.1)/ MOI (1.0)	Class	Comment
Secreted products					
204533_at	CXCL10	3627	2.9	A*	IFN γ inducible ³²
210163_at	CXCL11	6373	2.1	C*	IFN γ inducible ³³
211122_s_at	CXCL11	6373	3.0	A*	
209774_x_at	CXCL2	2920	2.0	B	
215101_s_at	CXCL5	6374	2.1	B	
205916_at	S100A7	6278	2.2	C*	Psoriasin: chemotactic for CD4+ T lymphocytes and neutrophils, also newborn innate defense ³⁴⁻³⁶
Induced by IFN and/or virus					
218943_s_at	DDX58	23586	2.2	C	RIG-I: essential to double-stranded RNA-induction of innate antiviral response ³⁷
213797_at	RSAD2	91543	5.0	A*	Viperin/cig5: induced by IFN and HCMV ³⁸
205483_s_at	GIP2	9636	2.7	A*	Aka ISG15, IFI15. Induced by HSV-1 infection ³⁹
219211_at	USP18	11274	2.4	C*	Aka ISG43: IFN induced protease specifically removes ISG15 from conjugated proteins ⁴⁰
202270_at	GBP1	2633	2.6	A	
209417_s_at	IFI35	3430	2.3	A	Translocated to the nucleus on IFN treatment ⁴¹
214453_s_at	IFI44	10561	2.2	A	Induced by IFN α /IFN β and hepatitis C virus ⁴²
204439_at	IFI44L	10964	3.2	A*	
219209_at	IFIH1	64135	3.2	A	IFN-inducible RNA helicase, which causes activation of IFN β promoter ⁴³
203153_at	IFIT1	3434	3.0	A*	Aka IFI-56. Induced by HSV-1 infection ³⁹
204747_at	IFIT3	3437	3.1	A*	Interferon-and virus-inducible gene ISG-60 ⁴⁴
229450_at	IFIT3	3437	4.4	B	
201601_x_at	IFITM1	8519	2.2	B*	Induced by HSV-1 infection ³⁹
214022_s_at	IFITM1	8519	2.2	B*	
212203_x_at	IFITM3	10410	2.1	B	
202086_at	MX1	4599	2.7	A*	Induced by HSV-1 infection ³⁹
204994_at	MX2	4600	2.5	A*	
205552_s_at	OAS1	4938	2.7	B	IFN induced ⁴⁵
204972_at	OAS2	4939	2.8	C*	
206553_at	OAS2	4939	2.6	C	
205660_at	OASL	8638	2.7	A	TRIP14: Induced by dengue virus ¹⁹ ; no oligoadenylate synthetase activity; interacts with thyroid receptor and with transcription repressor MBD1 ⁴⁶
210797_s_at	OASL	8638	2.0	C	
IFN signaling					
208436_s_at	IRF7	3665	2.4	A*	
AFFX-HUM-ISGF3A/M97935_MA_at	STAT1	6772	2.0	B	IFN-induced antiviral state requires Stat 1 ⁴⁷ ; adenoviral infection suppresses Stat1 function ⁴⁸
209969_s_at	STAT1	6772	2.4	C	
Transcriptional control					
209211_at	KLF5	688	2.0	C	Transcription regulator; positive regulator of cell proliferation, normally expressed in cornea but not conjunctiva ⁴⁹
Membrane proteins					
207907_at	TNFSF14	8740	2.6	B	LIGHT: Herpesvirus entry mediator; role in IFN- γ mucosal inflammatory responses ⁵⁰
209498_at	CEACAM1	634	2.2	B	Receptor for <i>Haemophilus influenzae</i> , <i>Neisseria gonorrhoeae</i> , and <i>Neisseria meningitidis</i> in humans ⁵¹
Apoptosis					
210538_s_at	BIRC3	330	3.3	A	BIRC3/CLAP2 is a ubiquitin ligase for the apoptosis inducer Smac/DIABLO. ⁵²
203140_at	BCL6	604	2.4	B	
202037_s_at	SFRP1	6422	2.4	C*	Cells transfected with SFRP1/SARP2 have increased sensitivity to proapoptotic stimuli. ⁵³
Enzymes					
204733_at	KLK6	5653	3.4	A*	Kallikrein peptidase
221750_at	HMGCS1	3157	2.0	A	3-Hydroxy-3-methylglutaryl-coenzyme A synthase
Unassigned					
213796_at	SPRR1A	6698	2.2	B	Cross-linking molecules within keratinocyte cornified cell envelope ⁵⁴
208539_x_at	SPRR2A	6700	2.5	B	Modulated by human papillomavirus infection ⁵⁵
228285_at	TDRD9	122402	2.2	A	Also known as hypoxia-inducible gene 1 (HIG1), upregulated during nickel-induced carcinogenesis ⁵⁶
205569_at	LAMP3	27074	2.6	A*	
31799_at	MRPS22	56945	2.5	A	
204885_s_at	MSLN	10232	2.2	B	
219691_at	SAMD9	54809	2.1	C	
205241_at	SCO2	9997	2.0	B	
Unknown					
53720_at	FLJ11286	55337	2.0	C	Unknown function
218712_at	FLJ20508	54955	2.1	B	Unknown function

The data are sorted into functional groups and are also assigned to one of three different classes (A-C). In class A (21 transcripts) the differences among all three MOIs were significant. In class B (16 transcripts), the MOI (0.1) was not significantly different from MOI (0), so a decrease in MOI (1.0) was predominantly responsible for the change in MOI (0.1)/MOI (1.0). In class C (12 transcripts), the MOI (1.0) was not significantly different from MOI (0), so an increase in MOI (0.1) was predominantly responsible for the change in MOI (0.1)/MOI (1.0). For a further 21 transcripts, MOI (0.1) vs. MOI (1.0) was the only significant difference. These are omitted for brevity. Ten uncharacterized Affymetrix panels were altered but are omitted from the table: 214329_x_at, 224646_x_at, 233722_at, 233890_at, 234136_at, 234330_at, 235183_at, 241245_at, 241569_at, and 241859_at. Affymetrix, Santa Clara, CA.

* Also suppressed twofold by an MOI of 1.0 at the earliest time studied.

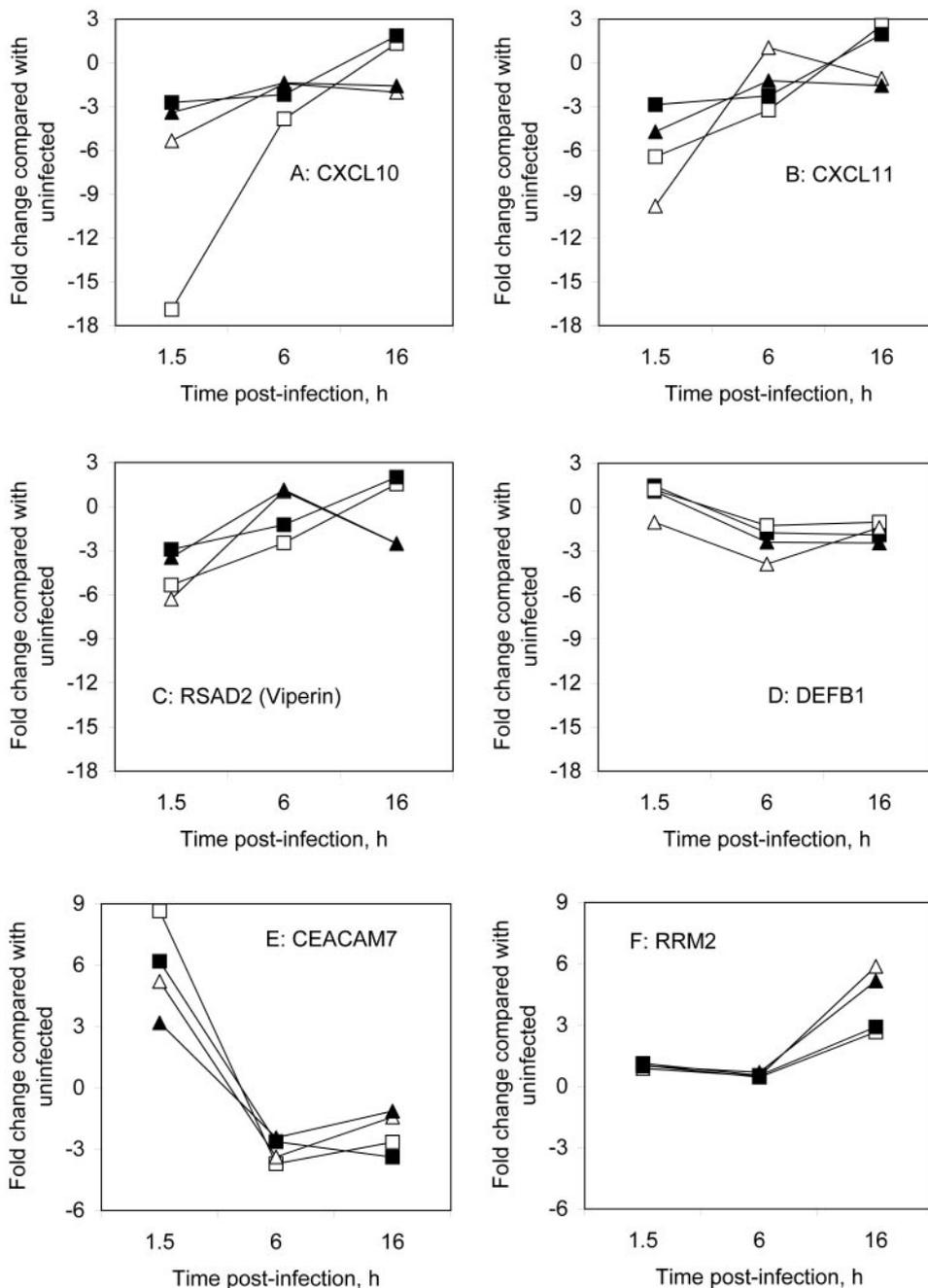


FIGURE 1. (A-F) Verification of microarray data by QRT-PCR for selected transcripts. Selected transcripts that had been identified by microarray as significantly altered were verified by QRT-PCR. (A) *CXCL10*, (B) *CXCL11*, and (C) *RSAD2* (upregulated at T16, MOI 0.1) are from Table 1. In contrast (D) *DEFB1* is downregulated by both MOIs at T16. (E) *CEACAM7* is an early-responding innate immune gene, whereas the increase in (F) *RRM2* is virus-directed. For each time point, expression in infected cells is relative to mock-infected (MOI 0.0) expression. *Filled symbols*: microarray data; *open symbols*: RT-PCR. *Triangles*: MOI 1.0; *squares*: MOI 0.1. Negative values on the y-axes indicate x-fold decrease (e.g., -3 is a threefold decrease).

blocked, and S-phase is induced in the host cell to facilitate viral DNA replication. Finally, by 16 hours viral assembly is in full swing, the host cytoskeleton is disrupted, and host interferon and MHC defenses are blocked. At this time host cell lysis and release of mature virus is 2 to 4 hours away.

We compared the present data with the only other microarray analysis of adenovirus effects in ocular cells, Natarajan et al.¹¹ At 2 hours after infection in human corneal fibroblasts, they surveyed 1176 transcripts and found 58 upregulated and 14 downregulated in infected versus uninfected cells, a discovery rate of (72/1176 = 6.1%). At a comparable time point (T1.5), we found that 310 unique transcripts were changed at least twofold in at least one of the three comparisons between different MOIs. There are ~13,300 nonredundant characterized transcripts on the U133A chip, giving a discovery rate of (310/13,313 = 2.4%). Because the transcripts surveyed by Natarajan et al. are all likely to be on the microarray chip that

we used (U133A GeneChip; Affymetrix) random chance dictates that our data set contains 2.4% of 72 (i.e., ~2) of their transcripts. Exact matches between the data sets are *BRC2*, *RAB2*, and *F2R*. Three matches are not substantially greater than random chance. Natarajan et al. also reported the “transcription-initiation factor TFIIID”. The TFIIID is a complex of the TATA box-binding protein (TBP) with multiple TBP-associated factors (TAFs). Our data contain TAF9L, which we score as an inexact match. The low level of concordance between the present report and that of Natarajan et al. is probably due to their use of a different cell type (corneal fibroblasts versus conjunctival epithelial cells) and an oculotropic viral serotype (Ad19). We are presently conducting experiments using conjunctival epithelial cells and Ad19. Our recent microarray analysis of cultured human corneal fibroblasts (Harvey SAK, et al. *IOVS* 2005;46:ARVO E-Abstract 1207) yielded 31 immune or defensive genes with expression that is altered relative to their

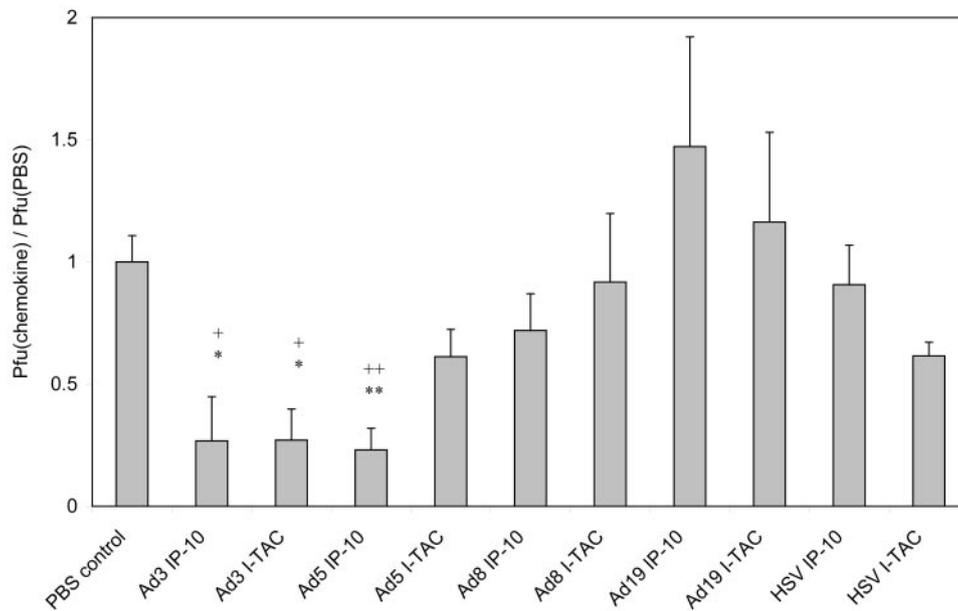


FIGURE 2. Direct antiviral effects of IP-10 and I-TAC, the products of *CXCL10* and *CXCL11*. Mixtures containing 10^2 to 10^5 pfu/mL of the virus and 0 or 100 $\mu\text{g/mL}$ peptide stocks were incubated at 37°C for 75 minutes, and then used to infect A549 cell monolayers. Data are the mean \pm SEM of determinations in two to four independent experiments, except PBS (0 $\mu\text{g/mL}$) control, which includes data from 18 determinations. Symbols: * $P < 0.05$, *t*-test; ** $P < 0.01$, *t*-test; + $P < 0.05$, Dunnett's test; ++ $P < 0.01$, Dunnett's test.

originating cultured keratocytes. Eighteen of these genes were interferon associated, and all 18 were upregulated, suggesting that the corneal fibroblasts used by Natarajan et al. may be intrinsically more resistant to viral infection than the originating keratocytes. Among these 18 transcripts are 14 that appear in Table 1: *DDX58*, *RSAD2*, *G1P2*, *GBP1*, *IFI44*, *IFI44L*, *IFIT1*, *IFIT2*, *IFITM1*, *MX1*, *MX2*, *OAS1*, *OAS2*, and *OASL*.

It is well established that binding and/or internalization of virus particles can stimulate changes in host gene expression that are independent of those directed by the viral proteome. For example, binding of UV-inactivated human cytomegalovirus to human monocytes induces immunoregulatory gene expression.⁶⁸ Furthermore, whereas IFN- γ upregulates IP-10 expression in murine renal epithelial cells, UV-inactivated Ad5 also upregulates IP-10 by a parallel independent NF- κ B-signaling pathway, without eliciting a host IFN response.⁶⁹ However, the important role of the viral proteome in further modulating host gene expression is clearly demonstrated at 24 hours after infection in microarray experiments in which an intact adeno-

virus altered expression of 476 transcripts in embryonic lung fibroblasts compared with only 147 (30%) transcripts altered by an adenovirus depleted of genomic DNA.⁷⁰ The chemotactic functions of *CXCL10* and *CXCL11* genes and their peptide products IP-10 and I-TAC, respectively, are well described for their recruitment function of leukocytes (lymphocytes, macrophages, and neutrophils) during inflammation.^{71,72} Although some expression is reported in bacterial,⁷³ fungal,⁷⁴ and protozoan⁷⁵ infections, they are expressed principally in response to a wide range of DNA and RNA viruses: recombinant Ad vectors,^{44,76-78} HSV-1,^{79,80} hCMV,⁸¹ influenza A,⁸² hepatitis C virus,⁸³ RSV,⁸⁴ and HIV.⁸⁵ Their dual function of chemoattraction and direct defensin-like killing has only been recently described in bacteria.^{86,87} Yount and Yeaman⁸⁸ correctly predicted that *CXCL9*, *CXCL10*, and *CXCL11* would have antibacterial defensin-like properties based on their discovery of a universal multidimensional signature motif that characterized the structure of all cationic peptides. In the present study, we report for the first time that the antimicrobial activity of

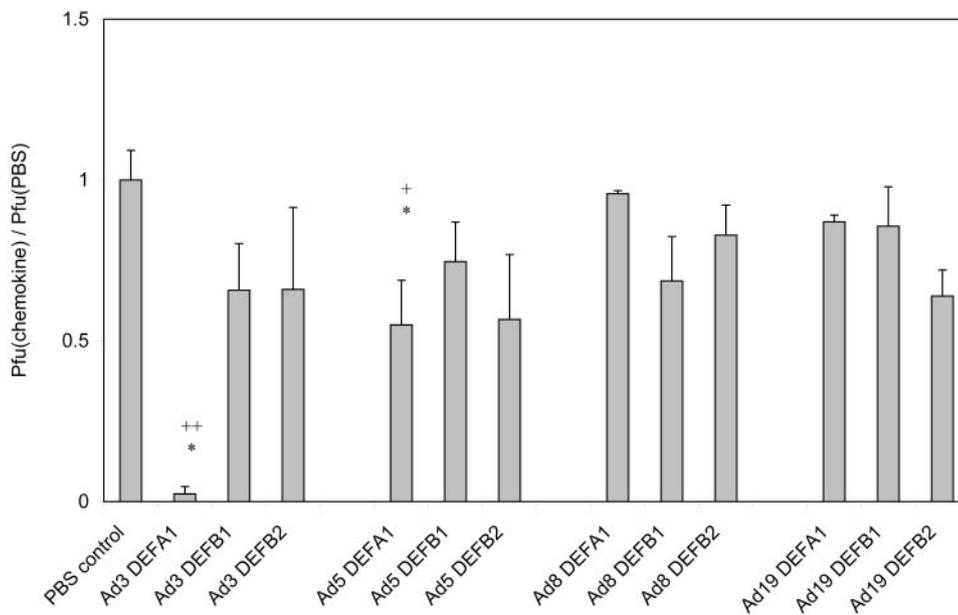


FIGURE 3. Direct antiviral effects of selected human defensins. As for Figure 2, except that virus-peptide mixtures were incubated at 37°C for 60 minutes before use. The PBS (0 $\mu\text{g/mL}$) control includes data from 33 determinations.

these defensin-like chemokines can be expanded to include the common respiratory adenoviral serotypes, Ad3 and Ad5, but not the ocular serotypes Ad8 or Ad19 or HSV-1, another ocular viral pathogen. The mechanism of the killing action is unknown, but it is unlikely to be the same as for bacteria and enveloped viruses (i.e., permeabilizing lipid membranes,⁸⁹) as adenoviridae are nonenveloped viruses. Carr et al.⁷⁹ have recently shown that CXCL10/IP-10 has no direct protective effect against HSV-1, although the chemokine is absolutely essential in mounting an ocular inflammatory response against the virus.

Recently, there has been a burgeoning interest in the cationic antimicrobial proteins and peptides (α - and β -defensins, cathelicidin, CAP-37) and their role in the innate immune defense of all mucosal surfaces including the eye.^{8,90,91} Of the defensins tested in the current antiviral study, only hDEFA1, demonstrated statistically significant activity against the respiratory serotypes, Ad3 and Ad5. Other studies have demonstrated significant antiviral inhibitory activity of α -defensins against recombinant Ad5 vectors,^{25,92} HSV-1 and 2,⁹³⁻⁹⁵ and HIV.⁹⁶⁻⁹⁹ For example, human α -defensin 1 at 50 μ g/mL inhibits Ad5 infection by 97%, whereas human β -defensin 2 at the same concentration provides <10% inhibition.⁹² In our study, α -defensin 1 also was effective against Ad3 but not against Ad8 or Ad19.

In the present study, the human defensin- β 1 and - β 2 genes were expressed by primary conjunctival epithelial cells but were suppressed after Ad5 infection. Their peptides were ineffective against any adenovirus serotype or against HSV-1. However, both α -defensin-5 and human β -defensin-1 were effective (two- to threefold decrease) against the Ad5-derived vector Av1CF2s when the defensins were transfected into host cells.²⁵ To our knowledge, this result is consistent with the literature in which no antiviral activity has yet to be shown by β -defensins against a replicating wild-type adenovirus.²⁵ In contrast, β -defensins have been reported to inhibit HIV *in vitro*.²⁷

The β -defensins hDEF-1, hDEF-2, and hDEF-3 show increased expression by conjunctiva and corneal epithelia in response to bacterial infection, proinflammatory cytokines, and injury,^{8,91} and are downregulated in *Pseudomonas aeruginosa* murine keratitis.¹⁰⁰ The specificity of their antibacterial function on the ocular surface is supported by the present data that they have no substantial antiviral function.

The present data show that IP-10, ITAC, and hDEFA1 can inhibit the common respiratory adenoviral serotypes, Ad3 and Ad5, but have no effect on the ocular serotypes, Ad8 and Ad19. A preliminary report (Gordon YJ, et al. *IOVS* 2005;46:ARVO E-Abstract 2797) shows that similarly, the antimicrobial protein CAP37 is effective against Ad3 and Ad5, but not against Ad8 or Ad19. Overall, these and other data support the notion of ocular tissue tropism wherein the viral pathogens Ad8 and Ad19 have evolved effective immune evasion strategies to promote replication and transmission in a specific ecological niche—for example, the ocular surface. Preliminary data suggest that the cells' antiviral response (measured by the secretion of IP-10 protein) in response to Ad19 occurs later and is less pronounced than their response to Ad5, which is exactly what one would expect if Ad19 practices immune evasion in the ocular niche. Coevolution generally favors a dynamic equilibrium that is achieved when host-mediated virus clearance is modulated, in part, by successful evasion strategies. Additional gene arrays studies are planned with ocular adenoviral serotypes that infect ocular conjunctival epithelia, to explore these issues.

Acknowledgments

The authors acknowledge the invaluable assistance of Michelle Rigatti in culturing conjunctival epithelial cells.

References

1. Kaufman SHE, Medzhitov R, Gordon S. *The Innate Immune Response to Infection*. Washington, DC: ASM Press; 2004.
2. Beutler B. Innate immunity: an overview. *Mol Immunol*. 2004; 40:845-859.
3. Levy O. Antimicrobial proteins and peptides: anti-infective molecules of mammalian leukocytes. *J Leukoc Biol* 2004;76:909-925.
4. Gallo RL, Nizet V. Endogenous production of antimicrobial peptides in innate immunity and human disease. *Curr Allergy Asthma Rep*. 2003;3:402-449.
5. Scott MG, Hancock RE. Cationic antimicrobial peptides and their multifunctional role in the immune system. *Crit Rev Immunol*. 2000;20:407-431.
6. Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. *Annu Rev Immunol*. 2004;22:181-215.
7. Haynes RJ, Tighe PJ, Dua HS. Antimicrobial defensin peptides of the human ocular surface. *Br J Ophthalmol*. 1999;83:737-741.
8. McDermott AM, Redfern RL, Zhang B, Pei Y, Huang L, Prose RJ. Defensin expression by the cornea: multiple signalling pathways mediate IL-1 β stimulation of hBD-2 expression by human corneal epithelial cells. *Invest Ophthalmol Vis Sci*. 2003;44:1859-1865.
9. Kocuzalla AR, Bals R. Antimicrobial peptides: current status and therapeutic potential. *Drugs*. 2003;63:389-406.
10. Gordon YJ, Romanowski EG, McDermott AM. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr Eye Res*. In press.
11. Natarajan K, Shepard LA, Chodosh J. The use of DNA array technology in studies of ocular viral pathogenesis. *DNA Cell Biol*. 2002;21:483-490.
12. Higaki S, Deai T, Fukuda M, Shimomura Y. Microarray analysis in the HSV-1 latently infected mouse trigeminal ganglion. *Cornea*. 2004;23:S42-S47.
13. Challacombe JF, Rechtsteiner A, Gottardo R, et al. Evaluation of the host transcriptional response to human cytomegalovirus infection. *Physiol Genomics*. 2004;18:51-62.
14. Wang Z, Trillo-Pazos G, Kim SY, et al. Effects of human immunodeficiency virus type 1 on astrocyte gene expression and function: potential role in neuropathogenesis. *J Neurovirol*. 2004;10:25-32.
15. Poole IJ, Yu Y, Kim PS, Zheng QZ, Pevsner J, Hayward GS. Altered patterns of cellular gene expression in dermal microvascular endothelial cells infected with Kaposi's sarcoma-associated herpesvirus. *J Virol*. 2002;76:3395-3420.
16. Chang YE, Laimins LA. Microarray analysis identifies interferon-inducible genes and Stat-1 as major transcriptional targets of human papillomavirus type 31. *J Virol*. 2000;74:4174-4182.
17. Li K, Prow T, Lemon SM, Beard MR. Cellular response to conditional expression of hepatitis C virus core protein in Huh7 cultured human hepatoma cells. *Hepatology*. 2002;35:1237-1246.
18. Smith MW, Yue ZN, Korth MJ, et al. Hepatitis C virus and liver disease: global transcriptional profiling and identification of potential markers. *Hepatology*. 2003;38:1458-1467.
19. Warke RV, Khaja K, Martin KJ, et al. Dengue virus induces novel changes in gene expression of human umbilical vein endothelial cells. *J Virol*. 2003;77:11822-11832.
20. Brodsky FM, Lem L, Solache A, Bennett EM. Human pathogen subversion of antigen presentation. *Immunol Rev*. 1999;168: 199-215.
21. Burgert HG, Ruzsics Z, Obermeier S, Hilgendorf A, Windheim M, Elsing A. Subversion of host defense mechanisms by adenoviruses. *Curr Top Microbiol Immunol*. 2002;269:273-318.
22. Seet BT, Johnston JB, Brunetti CR, et al. Poxviruses and immune evasion. *Annu Rev Immunol* 2003;21:377-423.

23. Zhao H, Granberg F, Elfineh L, Pettersson U, Svensson C. Strategic attack on host cell gene expression during adenovirus infection. *J Virol.* 2003;77:11006-11015.
24. Worgall S, Wolff G, Falck-Pedersen E, Crystal RG. Innate immune mechanisms dominate elimination of adenoviral vectors following in vivo administration. *Hum Gene Ther.* 1997;8:37-44.
25. Gropp R, Frye M, Wagner TO, Bargon J. Epithelial defensins impair adenoviral infection: implication for adenovirus-mediated gene therapy. *Hum Gene Ther.* 1999;10:957-964.
26. Stilwell JL, Samulski RJ. Role of viral vectors and virion shells in cellular gene expression. *Mol Ther.* 2004;9:337-346.
27. Quinones-Mateu ME, Lederman MM, Feng Z, et al. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. *AIDS.* 2003;17:F39-F48.
28. Braida L, Boniotto M, Pontillo A, Tovo PA, Amoroso A, Crovella SA. single-nucleotide polymorphism in the human beta-defensin 1 gene is associated with HIV-1 infection in Italian children. *AIDS.* 2004;18:1598-1600.
29. Alp S, Skrygan M, Schlottmann R, et al. Expression of beta-defensin 1 and 2 in nasal epithelial cells and alveolar macrophages from HIV-infected patients. *Eur J Med Res.* 2005;10:1-6.
30. Risse Marsh BC, Massaro-Giordano M, Marshall CM, Lavker RM, Jensen PJ. Initiation and characterization of keratinocyte cultures from biopsies of normal human conjunctiva. *Exp Eye Res.* 2002;74:61-69.
31. Pellegrini G, Golisano O, Paterna P, et al. Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. *J Cell Biol.* 1999;145:769-782.
32. Luster AD, Ravetch JV. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). *J Exp Med.* 1987;166:1084-1097.
33. Cole KE, Strick CA, Paradis TJ, et al. Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. *J Exp Med.* 1998;187:2009-2021.
34. Jinquan T, Vorum H, Larsen CG, et al. Psoriasin: a novel chemotactic protein. *J Invest Dermatol.* 1996;107:5-10.
35. Gläser R, Harder J, Bartels J, Christophers E, Schröder J-M. Psoriasin (S 100a7) is a major and potent E. coli-selective antimicrobial protein of healthy human skin (Abstract). *J Invest Dermatol.* 2001;117:768.
36. Yoshio H, Tollin M, Gudmundsson GH, et al. Antimicrobial polypeptides of human vernix caseosa and amniotic fluid: implications for newborn innate defense. *Pediatr Res.* 2003;53:211-216.
37. Yoneyama M, Kikuchi M, Natsukawa T, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol.* 2004;5:730-737.
38. Chin KC, Cresswell P. Viperin (cig5), an IFN-inducible antiviral protein directly induced by human cytomegalovirus. *Proc Natl Acad Sci USA.* 2001;98:15125-15130.
39. Nicholl MJ, Robinson LH, Preston CM. Activation of cellular interferon-responsive genes after infection of human cells with herpes simplex virus type 1. *J Gen Virol.* 2000;81:2215-2218.
40. Malakhov MP, Malakhova OA, Kim KI, Ritchie KJ, Zhang DE. UBP43 (USP18) specifically removes ISG15 from conjugated proteins. *J Biol Chem.* 2002;277:9976-9981.
41. Wang X, Johansen LM, Tae HJ, Tapparowsky EJ. IFP 35 forms complexes with B-ATF, a member of the AP1 family of transcription factors. *Biochem Biophys Res Commun.* 1996;229:316-322.
42. Kitamura A, Takahashi K, Okajima A, Kitamura N. Induction of the human gene for p44, a hepatitis-C-associated microtubular aggregate protein, by interferon-alpha/beta. *Eur J Biochem.* 1994;224:877-883.
43. Andrejeva J, Childs KS, Young DF, et al. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc Natl Acad Sci USA.* 2004;101:17264-17269.
44. de Veer MJ, Sim H, Whisstock JC, Devenish RJ, Ralph SJ. IFI60/ISG60/IFIT4, a new member of the human IFI54/IFIT2 family of interferon-stimulated genes. *Genomics.* 1998;54:267-277.
45. Zilberstein A, Kimchi A, Schmidt A, Revel M. Isolation of two interferon-induced translational inhibitors: a protein kinase and an oligo-isoadenylyl synthetase. *Proc Natl Acad Sci USA.* 1978;75:4734-4738.
46. Andersen JB, Strandbygard DJ, Hartmann R, Justesen J. Interaction between the 2'-5' oligoadenylyl synthetase-like protein p59 OASL and the transcriptional repressor methyl CpG-binding protein 1. *Eur J Biochem.* 2004;271:628-636.
47. Horvath CM, Darnell JE Jr. The antiviral state induced by alpha interferon and gamma interferon requires transcriptionally active Stat1 protein. *J Virol.* 1996;70:647-650.
48. Look DC, Roswit WT, Frick AG, et al. Direct suppression of Stat1 function during adenoviral infection. *Immunity.* 1998;9:871-880.
49. Chiambaretta F, De Graeve F, Turet G, et al. Cell and tissue specific expression of human Kruppel-like transcription factors in human ocular surface. *Mol Vis.* 2004;10:901-909.
50. Cohavy O, Zhou J, Granger SW, Ware CF, Targan SR. LIGHT expression by mucosal T cells may regulate IFN-gamma expression in the intestine. *J Immunol.* 2004;173:251-258.
51. Blau DM, Turbide C, Tremblay M, et al. Targeted disruption of the Ceacam1 (MHVR) gene leads to reduced susceptibility of mice to mouse hepatitis virus infection. *J Virol.* 2001;75:8173-8186.
52. Hu S, Yang X. Cellular inhibitor of apoptosis 1 and 2 are ubiquitin ligases for the apoptosis inducer Smac/DIABLO. *J Biol Chem.* 2003;278:10055-10060.
53. Melkonyan HS, Chang WC, Shapiro JP, et al. SARPs: a family of secreted apoptosis-related proteins. *Proc Natl Acad Sci USA.* 1997;94:13636-13641.
54. Hohl D, de Viragh PA, Amiguet-Barras F, Gibbs S, Backendorf C, Huber M. The small proline-rich proteins constitute a multigene family of differentially regulated cornified cell envelope precursor proteins. *J Invest Dermatol.* 1995;104:902-909.
55. Lehr E, Brown DR. Infection with the oncogenic human papillomavirus type 59 alters protein components of the cornified cell envelope. *Virology.* 2003;309:53-60.
56. Salnikow K, Davidson T, Zhang Q, Chen LC, Su W, Costa M. The involvement of hypoxia-inducible transcription factor-1-dependent pathway in nickel carcinogenesis. *Cancer Res.* 2003;63:3524-3530.
57. Gordon YJ, Huang LC, Romanowski EG, Yates KA, Proske RJ, McDermott AM. Human cathelicidin (LL-37), a multi-functional peptide, is expressed by ocular surface epithelia and has potent antibacterial and antiviral activity. *Curr Eye Res.* 2005;30:385-394.
58. Ruan X, Chodosh J, Callegan MC, et al. Corneal expression of the inflammatory mediator CAP37. *Invest Ophthalmol Vis Sci.* 2002;43:1414-1421.
59. Virji M. CEA and innate immunity. *Trends Microbiol.* 2001;9:258-259.
60. Reichel R, Kovsdi I, Nevins JR. Activation of a preexisting cellular factor as a basis for adenovirus E1A-mediated transcription control. *Proc Natl Acad Sci USA.* 1988;85:387-390.
61. Wojcik BE, Dermody JJ, Ozer HL, Mun B, Mathews CK. Temperature-sensitive DNA mutant of Chinese hamster ovary cells with a thermolabile ribonucleotide reductase activity. *Mol Cell Biol.* 1990;10:5688-5699.
62. Gordon YJ, Aoki K, Kinchington PR. Adenovirus keratoconjunctivitis. In: Pepose JS, Holland GN, Wilhelmus KR, eds. *Ocular Infection and Immunity.* St. Louis: Mosby; 1996:877-894.
63. Pacini DL, Dubovi EJ, Clyde WA Jr. A new animal model for human respiratory tract disease due to adenovirus. *J Infect Dis.* 1984;150:92-97.
64. Gordon YJ, Romanowski E, Aralullo-Cruz T. An ocular model of adenovirus type 5 infection in the NZ rabbit. *Invest Ophthalmol Vis Sci.* 1992;33:574-580.
65. Tsai JC, Garlinghouse G, McDonnell PJ, Trousdale MD. An experimental animal model of adenovirus-induced ocular disease: the cotton rat. *Arch Ophthalmol.* 1992;110:1167-1170.
66. Mahr JA, Gooding LR. Immune evasion by adenoviruses. *Immunol Rev.* 1999;168:121-130.
67. Fessler SP, Delgado-Lopez F, Horwitz MS. Mechanisms of E3 modulation of immune and inflammatory responses. *Curr Top Microbiol Immunol.* 2004;273:113-135.

68. Yurochko AD, Huang ES. Human cytomegalovirus binding to human monocytes induces immunoregulatory gene expression. *J Immunol.* 1999;162:4806-4816.
69. Borgland SL, Bowen GP, Wong NC, Libermann TA, Muruve DA. Adenovirus vector-induced expression of the C-X-C chemokine IP-10 is mediated through capsid-dependent activation of NF-kappaB. *J Virol.* 2000;74:3941-3947.
70. Stilwell JL, McCarty DM, Negishi A, Superfine R, Samulski RJ. Development and characterization of novel empty adenovirus capsids and their impact on cellular gene expression. *J Virol.* 2003;77:12881-12885.
71. Farber JM. Mig and IP-10: CXC chemokines that target lymphocytes. *J Leukoc Biol.* 1997;61:246-257.
72. Spandau UH, Toksoy A, Verhaart S, Gillitzer R, Kruse FE. High expression of chemokines Gro-alpha (CXCL-1), IL-8 (CXCL-8), and MCP-1 (CCL-2) in inflamed human corneas in vivo. *Arch Ophthalmol.* 2003;121:825-831.
73. Nau GJ, Schlesinger A, Richmond JF, Young RA. Cumulative Toll-like receptor activation in human macrophages treated with whole bacteria. *J Immunol.* 2003;170:5203-5209.
74. Schuh JM, Blease K, Hogaboam CM. CXCR2 is necessary for the development and persistence of chronic fungal asthma in mice. *J Immunol.* 2002;168:1447-1456.
75. Ritter U, Korner H. Divergent expression of inflammatory dermal chemokines in cutaneous leishmaniasis. *Parasite Immunol.* 2002;24:295-301.
76. Tibbles LA, Spurrell JC, Bowen GP, et al. Activation of p38 and ERK signaling during adenovirus vector cell entry lead to expression of the C-X-C chemokine IP-10. *J Virol.* 2002;76:1559-1568.
77. Zhang N, Schroppel B, Chen D, et al. Adenovirus transduction induces expression of multiple chemokines and chemokine receptors in murine beta cells and pancreatic islets. *Am J Transplant.* 2003;3:1230-1241.
78. Zaiss AK, Liu Q, Bowen GP, Wong NC, Bartlett JS, Muruve DA. Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors. *J Virol.* 2002;76:4580-4590.
79. Carr DJ, Chodosh J, Ash J, Lane TE. Effect of anti-CXCL10 monoclonal antibody on herpes simplex virus type 1 keratitis and retinal infection. *J Virol.* 2003;77:10037-10046.
80. Lundberg P, Cantin E. A potential role for CXCR3 chemokines in the response to ocular HSV infection. *Curr Eye Res.* 2003;26:137-150.
81. Cheeran MC, Hu S, Sheng WS, Peterson PK, Lokensgard JR. CXCL10 production from cytomegalovirus-stimulated microglia is regulated by both human and viral interleukin-10. *J Virol.* 2003;77:4502-4515.
82. Julkunen I, Sarenava T, Pirhonen J, Ronni T, Melen K, Matikainen S. Molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression. *Cytokine Growth Factor Rev.* 2001;12:171-180.
83. Harvey CE, Post JJ, Palladinetti P, et al. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. *J Leukoc Biol.* 2003;74:360-369.
84. Bitko V, Garmon NE, Cao T, et al. Activation of cytokines and NF-kappa B in corneal epithelial cells infected by respiratory syncytial virus: potential relevance in ocular inflammation and respiratory infection. *BMC Microbiol.* 2004;4:28.
85. Poluektova L, Moran T, Zelivyanskaya M, Swindells S, Gendelman HE, Persidsky Y. The regulation of alpha chemokines during HIV-1 infection and leukocyte activation: relevance for HIV-1-associated dementia. *J Neuroimmunol.* 2001;120:112-128.
86. Cole AM, Ganz T, Liese AM, Burdick MD, Liu L, Strieter RM. Cutting edge: IFN-inducible ELR+ CXC chemokines display defensin-like antimicrobial activity. *J Immunol.* 2001;167:623-627.
87. Durr M, Peschel A. Chemokines meet defensins: the merging concepts of chemoattractants and antimicrobial peptides in host defense. *Infect Immun.* 2002;70:6515-6517.
88. Yount NY, Yeaman MR. Multidimensional signatures in antimicrobial peptides. *Proc Natl Acad Sci USA* 2004;101:7363-7368.
89. Yeaman MR, Yount NY. Mechanisms of antimicrobial peptide action and resistance. *Pharmacol Rev.* 2003;55:27-55.
90. Haynes RJ, McElveen JE, Dua HS, Tighe PJ, Liversidge J. Expression of human beta-defensins in intraocular tissues. *Invest Ophthalmol Vis Sci.* 2000;41:3026-3031.
91. McDermott AM. Defensins and other antimicrobial peptides at the ocular surface. *Ocul Surface.* 2004;2:229-247.
92. Bastian A, Schafer H. Human alpha-defensin 1 (HNP-1) inhibits adenoviral infection in vitro. *Regul Pept.* 2001;101:157-161.
93. Daher KA, Selsted ME, Lehrer RI. Direct inactivation of viruses by human granulocyte defensins. *J Virol.* 1986;60:1068-1074.
94. Jenssen H, Andersen JH, Mantzilas D, Gutteberg TJ. A wide range of medium-sized, highly cationic, alpha-helical peptides show antiviral activity against herpes simplex virus. *Antiviral Res.* 2004;64:119-126.
95. Sinha S, Cheshenko N, Lehrer RI, Herold BC. NP-1, a rabbit alpha-defensin, prevents the entry and intercellular spread of herpes simplex virus type 2. *Antimicrob Agents Chemother.* 2003;47:494-500.
96. Mackewicz CE, Yuan J, Tran P, et al. alpha-Defensins can have anti-HIV activity but are not CD8 cell anti-HIV factors. *AIDS.* 2003;17:F23-F32.
97. Guo CJ, Tan N, Song L, Douglas SD, Ho WZ. Alpha-defensins inhibit HIV infection of macrophages through upregulation of CC-chemokines. *AIDS.* 2004;18:1217-1218.
98. Zhang L, Yu W, He T, et al. Contribution of human alpha-defensin 1, 2, and 3 to the anti-HIV-1 activity of CD8 antiviral factor. *Science.* 2002;298:995-1000.
99. Zhang L, Lopez P, He T, Yu W, Ho DD. Retraction of an inter-pretation. *Science.* 2004;303:467.
100. Huang X, Hazlett LD. Analysis of *Pseudomonas aeruginosa* corneal infection using an oligonucleotide microarray. *Invest Ophthalmol Vis Sci.* 2003;44:3409-416.