TABLE 1. (*continued*). Significantly Upregulated or Downregulated Genes of Human Corneal Endothelial Cells after HSV-1 Infection

GenBank ID*	Gene Symbol	Change	Regulation
XR_019109	*LOC650517*	4.3	Up
NM_001823	*CKB*	4.3	Up
NM_001451	*FOXF1*	4.3	Up
NM_000882	*IL12A*	4.3	Up
NM_194284	*CLDN23*	4.3	Up
NM_004405	*DLX2*	4.3	Up
NM_130436	*DYRK1A*	4.3	Up
XR_015273	*LOC728371*	4.2	Up
BU681302	*PPP2R2D*	4.2	Up
NM_031458	*PARP9*	4.2	Up
NM_003335	*UBE1L*	4.2	Up
NM_003649	*DIO*	4.2	Up
NM_004184	*WARS*	4.2	Up
CD556746	*CD556746*	4.2	Up
NM_145343	*APOL1*	4.2	Up
NM_001050	*SSTR2*	4.2	Up
BX110908	*BX110908*	4.1	Up
AK128592	*DNHD2*	4.1	Up
BC024745	*BC024745*	4.1	Up
ENST00000382591	*FAM90A10*	4.1	Up
AK226060	*BUB3*	4.1	Up
NM_001017534	*COPI*	4.1	Up
NM_014059	*C13orf15*	4.1	Up
AL049782	*CG012*	4.1	Up
NR_002139	*HCG4*	4.1	Up
NM_001003845	*SP5*	4.1	Up
NM_015564	*TRRTM2*	4.1	Up
NM_053001	*OSR2*	4.1	Up
BQ130147	*BQ130147*	4.0	Up
ENST00000294663	*GBP2*	4.0	Up
NM_004024	*ATF3*	4.0	Up
AK092450	*AK092450*	4.0	Up
BC000772	*BC000772*	4.0	Up
NM_016585	*THEG*	4.0	Up
NM_024522	*FAM77C*	4.0	Up
NM_173649	*FLJ40172*	4.0	Up
NM_024989	*PGAP1*	4.0	Up
NM_152542	*PPM1K*	4.0	Up
AL833749	*LOC146439*	4.0	Up
NM_014177	*C18orf55*	5.2	Down
NM_025184	*EFHC2*	4.9	Down
NM_001629	*ALOX5AP*	4.5	Down
NM_022469	*GREM2*	4.4	Down
AK126014	*KIAA1211*	4.3	Down
NM_000812	*GABRB1*	4.2	Down
ENST00000379426	*ENST00000379426*	4.2	Down
NM_014905	*GLS*	4.0	Down

$P < 0.01$.

* http://www.ncbi.nlm.nih.gov/Genbank/, National Center for Biotechnology Information, Bethesda, MD.

transcriptional regulation-related genes: cAMP-response element binding protein (*Cbp*)/p300, CCAAT/enhancer binding protein alpha (*CEBPA*), CREB binding protein (*CREBBP*), activating transcription factor 3 (*ATF3*), histone, and retinoic acid receptor-related genes (cytochrome P450, family 26, subfamily A, polypeptide 1; *CYP26A1*), nuclear receptor co-repressor 1 (*N-cor*), promyelocytic leukemia (*PML*), retinoid acid receptor (*Rar*), retinoid X receptor (*Rxr*), and SWI/SNF complex. The retinoid acid receptor family genes are nuclear receptors and act as transcriptional repressors, which are involved in antiproliferative effects of retinoic acid.

Corneal Endothelial Responses to HSV-1 in Common with Corneal Epithelial Cells

To understand the specific responses of the HCEn cells, we then compared the transcriptome of HCEn cells and the reported transcriptome of HCEp cells after HSV infection (12 hours PI).[8] Of the 10 highest induced genes in HCEn cells, *RASD1*, *DLL1*, *SOX3*, *ARC*, *DIO3*, *FLJ00049*, and tripartite motif-containing 43 (*TRIM43*) were also observed in the transcriptome of HCEp cells. In contrast, *IDO1* and *IP-10* were observed only in the HCEn transcriptome. Therefore, we reasoned that the networks of the HCEn cells represent general antiviral responses to HSV and corneal endothelium–specific responses.

TABLE 2. Molecules Significantly Associated with Antigen Presentation, as Revealed by Functional Analysis

Molecules in Network	*P*
IL15RA, AIM2, IL6, TBX21, IFIH1, APOL3, CXCL10, SOD2, IFI44L, TNFSF9, CCL8, FGR, TNFSF13B, GZMA, DLL1, CXCL9, TRIM21, HSH2D, ZC3HAV1, LAG3, CSF3, IRF1, APOBEC3G, IL18BP, IRF7, DUOX2, PLCG2, DDX58, RARRES3, IDO1, PNOC, PIK3AP1, STAT2, IL29, TRIM22, CX3CL1, IL12A, IFNB1, CIITA, TNFSF10, TNFAIP3, CCL5, CCL3, LGALS9, SECTM1, TAP1, NGFR, CASP1, CD38, CCRL1, GBP2, TLR3, TNFRSF1B, STAT1, PLSCR1, CXCL11, DHX58, MX2, OAS1, IRF4, MYD88, CXCR4, ALOX5AP, MX1, UNC93B1, IFI44, APOL1, CEACAM1, FOS, ZBTB32, NMI, CXCL2, CSF2, ISG20, BCL2L11, and *TNFSF14*	5.05×10^{-16}–5.34×10^{-4}

To delineate the general responses of HCEn cells to HSV, we constructed shared networks using genes detected in the transcriptomes of both HCEn and HCEp. IPA generated two major biological networks with high significance scores ($P < 10^{-30}$; Table 5). Shared network 1 was annotated as embryonic development, tissue development, and skeletal and muscular system development and function. Shared network 1 was characterized by interferon response, MAPK, and NFκB cascades. *IL-12*, chemokine (C-X-C motif) ligand 2 (*CXCL2*), and fibroblast growth factor 9 (*FGF9*) were identified as shared mediators (Table 5). As a co-stimulatory molecule, *TNFSF9* (*4-1BB-L*) was also observed in this network. Shared network 2 was annotated as cellular development, hematologic system development and function, and hematopoiesis and was characterized by retinoic acid metabolism.

Corneal Endothelial Responses to HSV-1 Distinct from Corneal Epithelial Cells

Next, we analyzed how HCEn cells respond to HSV-1 infection. Genes in the transcriptome of HCEn cells that were shared with HCEp with more than fourfold difference compared to the mock-infection control were eliminated. After complementing with statistically significant connecting nodes, the IPA

TABLE 3. Canonical Pathway Analysis of HSV-1–Induced Transcriptome of Corneal Endothelial Cells

Canonical Pathway	*P*	Ratio
Interferon signaling	1.11×10^{-11}	11/29 (0.379)
Role of pattern recognition receptors in recognition of bacteria and viruses	1.34×10^{-10}	15/88 (0.17)
Activation of IRF by cytosolic pattern recognition receptors	1.2×10^{-7}	11/74 (0.149)
IL-15 Production	2.18×10^{-4}	5/30 (0.167)
Role of RIG1-like receptors in antiviral innate immunity	4.12×10^{-4}	6/52 (0.115)

TABLE 4. Transcriptional Networks of HSV-1–Infected Corneal Endothelial Cells

Network	Molecules in Network	Score (−log *P*)	Function
1	*AIM2, BATF, DDX58(RIG-1), DHX58, FOXF1, IFI35, IFI44, IFIH1 (MDA5), IFIT1, IFIT2, IFIT3, IFN&α;/β, IL-29, IRF, IRF1, IRF7, ISG15, ISGF3(IRF9), NFkB (complex), Oas, OAS1, OAS2, OAS3, PARP9, RARRES3, REL/RELA/RELB, RNF19B, RSAD2, S100P, SP110, STAT2, Stat1-Stat2, TLR3, TNFSF9 (4-1BB-L),* and *TRIM69*	44	Antigen presentation, antimicrobial response, cell-mediated immune response
2	*ADRA2C, ALK, ANGPTL1, ASCL2, BCL2L14, BCR, BTC, CLDN5, CSF3, CX3CL1, CXCR4, DIO3, DUSP4, EPHA4, ERK, Fcer1, Fgf, FGF9, Ige, INSM1, MAP2K1/2, OVGP1, PLC gamma, PLCG2, PPP2R2D, Rap1, RASD1, RASGRP3, RET, Stat1 dimer, SYK/ZAP, SYNJ1, TBX21, TRIB1,* and *TXK*	40	Cellular development, hematological system development and function, hematopoiesis
3	*BCL2L11, Caspase, CCL3, CCL5, CCL8, CCRL1, Cytochrome c, FZD4, GLS, GZMA, HBA1, HBA2, HSH2D, IFITM1, IgG, Igm, Ikb, IKK (complex), IL1, IL12 (complex), IL12 (family), IL12A, IRF4, ISG20, Jnk, KRT16, MYD88, NGFR, RRAD, SP100, STAT1, Tnf receptor, TNFRSF1B, TNFSF10,* and *TNFSF13B*	38	Cell-to-cell signaling and interaction, hematological system development and function immune cell trafficking
4	*BUB3, CD38, CXCL2, CXCL9, CXCL10, CXCL11, GBP2, HLA-DR, IDO1, IFN Beta, Ifn gamma, IFNB1, IL6, IL15RA, IL18BP, Interferon alpha, IRF3 dimer, MHC CLASS I (family), MX1, MX2, NF-kappaB (family), NfkB-RelA, Nucleotidyltransferase, PMAIP1, PNPT1, Sod, TAP1, Tlr, TNFAIP3, TNFRSF8, TNFSF14(HVEML), TRAF, USP18, WARS,* and *XAF1*	36	Antigen presentation, cell-mediated immune response, humoral immune response
5	*ALOX5AP, ATF3, Cbp/p300, CEBPA, CIITA, CREBBP, CYP26A1, FOXA1, FOXA2, GBP1, GCH1, Growth hormone, HISTONE, Histone h3, Histone h4, HOXA5, KLF4, MHC Class I (complex), N-cor, NIACR2, NMI, NR3C2, P38 MAPK, PEPCK, PML, Rar, Rxr, SECTM1, Sox, SOX3, SOX8, SOX17, SWI-SNF, TRIM21,* and *Vitamin D3-VDR-retinoid X receptor, gamma*	30	Cellular growth and proliferation, embryonic development, gene expression

generated four major biological networks with high significance scores ($P < 10^{-30}$; Table 6).

The HCEn-preferred network 1 of highest significance was annotated as antimicrobial responses, inflammatory responses, and infection mechanisms. This network was characterized mainly by interferon responses. In network 2, antigen-presentation–related genes, *TNFSF10* (TRAIL), *TNFRSF1B* (TNFR-2), and *CIITA*, and granulocyte-macrophage colony stimulating factor (*CSF2, GMCSF*), were identified. Network 3 was annotated as infection mechanism, infectious disease, embryonic development, and was characterized by antiviral mediators including *CCL3, CCL5* (*RANTES*), *IL-12*, and interferon α. HCEn-preferred network 4 was annotated as cell-to-cell signaling and interaction, hematologic system development and function, and cellular movement. This network was characterized by antigen presentation and lymphocyte function-determinant–related genes, including *IDO1, HLA-DR, TNFSF14* (*HVEML*), *CXCL9, CXCL10* (*IP-10*), *CXCL11*, interferons, *IL-6*, and *IL-12*. Thus, all four HCEn-preferred networks were found to share or to be involved in the antigen-presentation–related function.

Production of Inflammatory Cytokines by HSV-1–Infected Corneal Endothelial Cells

We examined whether the observed transcriptional responses may indeed translate into a special profile of secreted proteins. The supernatant collected from HSV-1–infected HCEn cells at 12 hours PI was analyzed for a cytokine secretion profile by using protein array analysis. Significant increases in the secretion of IL-6, IL-8, monocyte chemotactic protein 1 (MCP-1, CCL2), tissue inhibitor of metalloproteinase 1 (TIMP-1), RANTES (CCL5), IP-10, I-309, macrophage migration inhibitory factor (MIF), monocyte chemoattractant protein 2 (MCP-2, CCL8), TNFSF14 (HVEML), IL-10, stromal cell-derived factor 1 (SDF-1), and interferon-γ were found in a descending order (Fig. 2). Antigen-presentation–related genes, including *IL-6, IP-10, CCL8, HVEML*, and interferon-γ, were confirmed for induction by HSV-1 infection.

Priming of Allogeneic T Lymphocytes by HSV-1–Infected Corneal Endothelial Cells

Finally, we tested whether HCEns may indeed function as APCs. HCEn cells infected with HSV-1 were treated with MMC to suppress DNA synthesis and proliferation, and then cocultured with allogeneic T cells from donors previously infected with HSV-1. The proliferation of $CD4^+$ T cells measured by BrdU uptake was significantly stimulated by HSV-1–primed HCEn cells at an MOI of 5 (Fig. 3A). Allogeneic T cells from healthy donors without a history of HSV-1 infection did not show an appreciable stimulatory effect (data not shown). For the control of allogeneic responses, Vero cells (kidney epithelial cells derived from the African Green monkey) were used as a stimulator. As expected, no significant T-cell proliferation was observed (Fig. 3B) To confirm HSV-1–stimulated allogeneic responses provoke a Th1-type response, we assessed interferon-γ secretion. HSV-1–primed HCEn cells stimulate allogeneic T cells to produce significant amounts of interferon-γ (Fig. 3C). No interferon-γ was produced by T cells cocultured with HSV-1 primed Vero cells.

DISCUSSION

Our results showed that HSV infection affected the expression of numerous genes, and the majority of the mRNAs were transcriptionally activated. Importantly, our bioinformatics analysis of HSV-induced transcriptome of HCEn cells showed that the molecular signature profile of these genes is strongly directed to initiate the acquired immune system as APCs.

Generally, HSV infection induces global silencing of host-derived transcripts.[12–14] This is mediated by viral proteins or by the immediate early genes including *ICP0, ICP27*, or *ICP34.5*.[12,15,16] Thus, global transcriptional activation after HSV infection, which was also observed in HCEp cells,[8,17] appears to be an uncommon event. Epithelial cells, including HCEps, are part of the primary defense system that initiates an

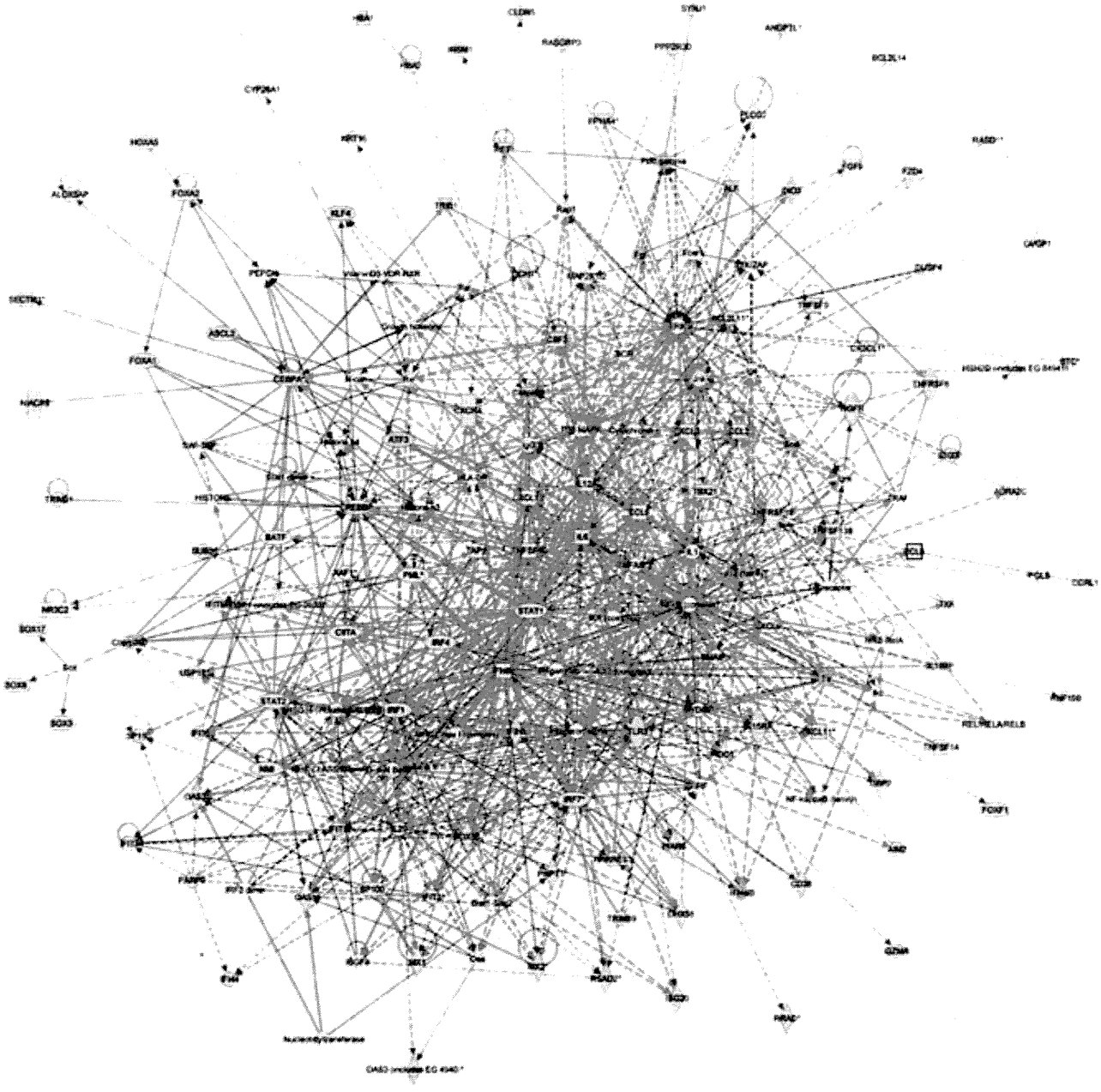

FIGURE 1. Network analysis of the biological processes underlying the HSV-1 infection–induced responses of HCEn cells. Networks 1 to 5 are summarized as the merged networks. Interactions between the networks are shown as *yellow lines*.

arsenal of proinflammatory mediators on infection. This may explain the presumed evolutionary requirement to resist transcriptional silencing exploited by HSV. In contrast, corneal endothelial cells are located inside the eye and are not easily accessible to HSV, which is different from corneal epithelial cells. Thus, the resistance of HCEn cells to transcriptional silencing appears to reflect a specific property.

On infection, HSV hijacks the transcriptional machinery of the host and diverts the canonical NFκB inflammatory signaling cascade for its own replication.[18] To resist viral replication, HCEn cells initiate an antiviral program with global transcriptional activation, which results in the release of inflammatory cytokines (Fig. 2). These cytokines subsequently prime the acquired cellular immunity to protect the corneal endothelial cells.

In the HSV-induced transcriptome of HCEn cells, we detected several antigen-presentation–related genes. For example, MHC class II is used for the presentation of exogenous proteins. The expression of MHC class II is regulated by the master transcriptional regulator CIITA (Fig. 1, Tables 4, 6), which is a signature molecule of professional APCs. Moreover, HCEn cells express the co-stimulatory molecules CD80 and CD86 on the cell surface, and they are stimulated to express CD40 after interferon-γ stimulation.[6] All these molecules are essential for APCs to provide the appropriate strength of antigen stimuli to recognize T-cell receptors.

Another important signal for APC function is a maturation stimulus, which is typically mediated by GMCSF (CSF2; Tables 1, 6; Fig. 1). Thus, these observations further support the functional capability of HCEn cells as APCs after HSV-1 infection.

TABLE 5. Transcriptional Networks Shared with Corneal Endothelial and Epithelial Cells after HSV-1 Infection

Network	Molecules	Score (−log *P*)	Functions
1	*ALP, CaMKII, CBX4, CDK5R1, CDKN1C, Ck2, CRABP1, Creb, CREBBP, CXCL2, Cyclin A, DLL1, DLX2, FGF9, FOS, FOXF1, GADD45G, Gsk3, IGF2, IL12 (complex), Interferon alpha, KCNC1, LDL, MSX1, MUC5AC, NFkB (complex), P38 MAPK, PDGF BB, RRAD, STAT5a/b, TBX21, Tgf beta, TNFSF9, TRIB1,* and *WNT1*	43	Embryonic development, tissue development, skeletal and muscular system development and function
2	*ARC, BST2, CCDC116, CLDN5, CLDN10, ELAVL3, EWSR1, GBX2, GLI1, HTT, IRX4, KCNQ2, LOC387763, MDFI (includes EG:4188), MED9, MIR18A, NEFL, NEFM, NPM1 (includes EG:4869), PAX3, PCDH8, POU5F1, PPARγ ligand-PPARγ-Retinoic acid-RARα, PTCHD2, RARA, RASL11B, retinoic acid, Retinoic acid-RAR, RPS17 (includes EG:20068), SIK3, SOX3, SYNJ1, TBX15, YWHAZ,* and *ZNF133*	33	Cellular development, hematological system development and function, hematopoiesis

Recently, an analysis of the transcriptional signature of the genome of dendritic cell (DC) responding to pathogen stimuli has been conducted, and crucial regulatory circuits were found. These circuits comprised 125 transcription factors, chromatin modifiers, and RNA-binding proteins.[19] The study showed that the responses of dendritic cells to pathogens consisted essentially of inflammatory and antiviral programs. In the inflammatory program, IL-6, IL-12, CXCL2, and IL-1β were representative effector molecules,[19] and, in our study, these molecules were found in major networks 3 and 4 of HCEn cells (Table 4). In contrast, IP-10 (CXCL10), interferon-stimulated protein, 15 kDa (ISG15), and interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) are other representatives of antiviral programs.[19] They were identified in networks 1 and 4 of the HCEn cells (Table 4). Interestingly, IP-10 was the eighth highest induced gene in the HCEn transcriptome (Table 1). IP-10 directly inhibits HSV-1 replication.[20]

In dendritic cells, antiviral programs are initiated by viral sensors, including TLRs, melanoma differentiation associated protein-5 (MDA5, IFIH1), and DDX58 (RIG-1), which again are found in network 1 of HCEn cells (Table 4). In contrast, HCEp networks were clearly distinctive in their transcriptional profile, and their identified nodes did not match those of dendritic cells. Thus, the representative transcriptional network nodes of HCEn cells are essentially matched to those for representative effector molecules in dendritic cells.

In the antiviral program of dendritic cells, signal transducer and activator of transcription 1 (STAT1) and STAT2 regulate components of the antiviral effector molecules. Consistent with this, STAT1 was positioned centrally in the transcriptional network of HCEn cells (Fig. 1). Other representative transcriptional regulators of the antiviral program in dendritic cells were IRF8, IRF9, activating transcription factor 3 (ATF3), ets variant 6 (ETV6), JUN, STAT4, and retinoblastoma-like 1 (RBL1). Of these, the IRFs (network 1) and ATF3 (network 5) were also found in HCEn cell networks. This result is consistent with the functional capability of HCEn cells as APCs, and may also reflect the HCEn cell–specific responses to pathogens.

Our results showed that HCEns can function as APCs. Generally, HSV-1 is known to block antigen presentation of infected cells.[15,21] The observed allopriming effect of HCEn cells would be beneficial for the effective eradication of HSV-infected cells. On the other hand, such elimination may lead to endothelial cell loss, which could lead to potentially blinding bullous keratopathy. So, how does the host avoid such a deleterious phenomenon? It has been shown that HCEns can serve as immune regulatory cells that dampen the cytotoxic effects induced by activated T cells. This action may protect the

TABLE 6. Transcriptional Networks Preferred by Corneal Endothelial Cells after HSV-1 Infection

Network	Molecules in network	Score (−log *P*)	Functions
1	*AIM2, BATF, DDX58, DHX58, IFI35, IFI44, IFIH1, IFIT1, IFIT2, IFIT3, Ifn, IFN TYPE 1, IL29, IL18BP, Interferon-α Induced, IRF, IRF7, ISG15, ISGF3, MX1, MX2, NFkB (complex), OAS1, OAS3, PARP9, RARRES3, REL/RELA/RELB, RNF19B, RSAD2, S100P, STAT2, Stat1-Stat2, TNFRSF8, TRIM69,* and *UBA7*	41	Antimicrobial response, inflammatory response, infection mechanism
2	*Akt, ALOX5AP, ANGPTL1, C13ORF15, CD38, CIITA, Collagen Alpha1, CSF2, CYP2J2, FAM65B, GBP1, GLS, GPR109B, Growth hormone, HSH2D, IFITM1, Ikb, IKK (complex), Ikk (family), IL1, Interferon Regulatory Factor, IRF1, IRF4, LDL, NfkB1-RelA, NR4A3, PARP, PARP12, PAR P14, Tnf receptor, TNFAIP3, TNFRSF1B, TNFSF10, TRAF,* and *WISP1*	33	Cell death, cellular growth and proliferation, connective tissue development and function
3	*APOBEC3G, APOL2, APOL3 (includes EG:80833), ATF3, CCL3, CCL5, CEBPA, CSF3, CYP26A1, FOXA1, FOXA2, GCH1, HOXA5, IgG, Igm, IL12 (complex), Interferon alpha, IRF3 dimer, ISG20, KLF4, KRT16, Nfat (family), NR4A2, P38 MAPK, PEPCK, PMAIP1, Rxr, SECTM 1, Sod, SOD2, TRIL, TRIM21, UNC93B1, VitaminD3-VDR-RXR,* and *ZC3HAV1*	31	Infection mechanism, infectious disease, embryonic development
4	*ACE2, BUB3, CCL8, CCRL1, CHEMOKINE, CXCL9, CXCL10, CXCL11, GBP2, HLA-DR, IDO1, IFN Beta, Ifn gamma, Ifnar, IFNB1, IFNα/β, Iga, IL6, IL23, IL12 (family), IL12A, IRAK, MYD88, NfkB-RelA, Oas, OAS2, Pro-inflammatory Cytokine, SMOX, SP110, Tir, TLR3, TNFSF14, TNFSF13B, WARS,* and *XAF1*	30	Cell-to-cell signaling and interaction, hematological system development and function, cellular movement

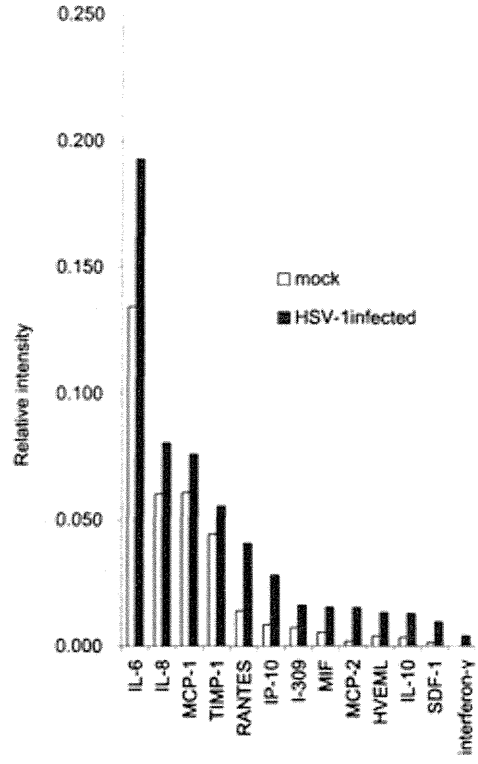

FIGURE 2. Induction profile of inflammatory cytokines by HSV-1–infected HCEn cells. HCEn cells were adsorbed with HSV-1 at an MOI of 0.1 for 1 hour and refed with DMEM. After 12 hours' incubation, the supernatant of HSV-1–infected HCEn cells was assayed with a cytokine array. A panel of inflammatory cytokines was significantly induced by HSV-1 infection. $n = 4$ per group. $P < 0.05$.

endothelial cells from death while maintaining their priming of immunologic memory responses. For example, HCEn cells impair Th1 $CD4^+$ cells by PD-L1, which is strongly expressed on its surface.[6] $CD8^+$ T cells can also be converted to regulatory T cells by HCEn cells via TGF-β.[7] In the HSV-1–induced transcriptome of HCEn cells, the sixth highest induced gene, *IDO1*, produces an immune regulatory enzyme that induces anergy or regulatory T-cell differentiation. In the HCEn networks, nuclear receptor transrepression pathways appear also to regulate inflammation by N-cor or Rxr, which are representative repressors of inflammatory responsive promoters (Table 4, network 5).[22]

The most striking difference between the HCEn and HCEp transcriptional networks was the interferon-related response. This result is consistent with their functional ability of antigen presentation. Interferons induce representative antiviral responses and modulate the immune system, and they render neighboring cells resistant to viral infection. In general, interferon responses are commonly observed after viral infection, including human cytomegalovirus.[23,24] In contrast, interferon responses are generally silenced in cases of HSV infection.[11,20] This silencing does not occur when viral replication is impaired.[11] After HSV infection of HCEn cells, we observed an induction of known interferon-inducible antiviral genes, including *OAS1/2/3*, and myxovirus resistance 1 (*MX1*)/2 in networks 1 and 4, respectively. OAS activates latent RNase L to induce viral RNA degradation.[25] The MX proteins are dynamin superfamily GTPases that interfere with viral replication.[26] Thus, HCEn cells have a strong propensity for interferon-related antiviral or inflammatory programs to resist HSV-1 infection, despite their susceptibility to infection.

The HSV-induced host genes of HCEn cells determined by network analysis showed significant association with the Jun N-terminal kinase (JNK), p38, extracellular signal-regulated kinase (ERK), and NF-κB signaling pathways. An association of these cascades with the HCEp network was also observed,[8] indicating that they are common signaling cascades after HSV-1 infection.

After HSV infection, the HCEn cells produce large amounts of IL-6, similar to HCEp cells.[8] Network analysis indicated that IL-6 was the most significantly shared effector molecule. It was centrally located in the inflammatory program of the transcriptional network, and activations of NF-κB and JNK were shown to be related to IL-6 induction.[27] In addition, IL-6 is a representative effector molecule downstream of TLR2, TLR3, and TLR9, which sense HSV entry. On infection, IL-6 mediates an acute phase reaction that influences antigen-specific immune responses.[28,29] Importantly, IL-6 converts T cells into cytotoxic T cells or the Th17 lineage and stimulates B cell differentiation.[30] In herpetic keratitis, IL-6 contributes to the massive neutrophil attraction to the corneal stroma[31–33] and stimulates bystander populations and HCEp cells to induce vascular endothelial growth factor (VEGF).[8,32–34]

Collectively, our data provide strong evidence that HCEn cells can serve as APCs after HSV-1 infection. Understanding the immune-modulating properties of the corneal endothelium would help develop efficacious strategies to block

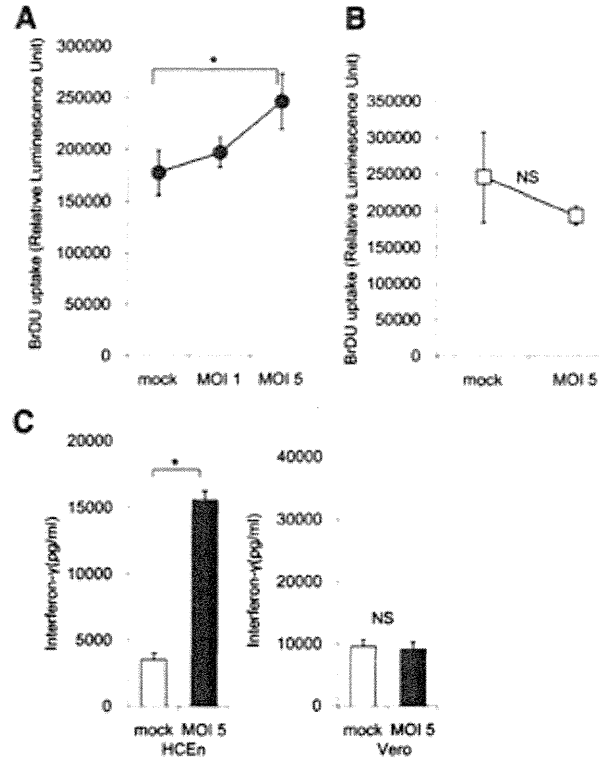

FIGURE 3. HSV-1–specific T-cell proliferation and interferon-γ secretion stimulated by HSV-1–treated HCEn cells. HCEn cells were adsorbed with HSV-1 for 1 hour at the indicated MOI and treated with mitomycin C. HSV-1–specific $CD4^+$ T cells were isolated from HSV-1 infected-allogeneic donors, and co-cultured with the HSV-1–adsorbed HCEn cells (responder: stimulator ratio, 4:1) (**A**) or xenogeneic Vero cells as the control (**B**). HSV-1–specific T-cell proliferation was examined by BrdU uptake, which was assessed with a chemiluminescence-based, BrdU-specific ELISA. HSV-1–specific interferon secretion from the T cells co-cultured with the HSV-1–primed HCEns or Vero cells was measured with ELISA (**C**). The HSV-1–primed HCEn cells, but not the Vero cells, significantly stimulated interferon-γ secretion. *$P < 0.05$.

HSV-1–induced inflammatory responses and endothelial cell loss.

Acknowledgments

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Roles Played by Toll-like Receptor-9 in Corneal Endothelial Cells after Herpes Simplex Virus Type 1 Infection

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Purpose. To determine the roles played by toll-like receptor 9 (TLR9) in cultured human corneal endothelial (HCEn) cells after herpes simplex virus type 1 (HSV-1) infection and to characterize the TLR9-mediated antiviral responses.

Method. Immortalized HCEn cells were examined for TLR expression. The upregulation of inflammatory cytokines after HSV-1 infection was determined by real-time RT-PCR or protein array analyses. The TLR9-mediated HSV-1 replication was determined by real-time PCR and plaque assay. To determine whether there was an activation of the signal transduction pathway, HCEn cells that were transfected with pathway-focused transcription factor reporters were examined for promoter activity.

Results. TLR9 was abundantly expressed intracellularly in HCEn cells. The CpG oligonucleotide, a TLR9 ligand, stimulated the NF-κB activity in HCEn cells. HSV-1 infection also stimulated NF-κB and induced NF-κB -related inflammatory cytokines, including RANTES, IP-10, MCP-2, MIF, MCP-4, MDC, MIP-3α, IL-5, TARC, MCP-1, and IL-6. The induction of these cytokines was significantly reduced by blocking the activity of TLR9. In addition, viral replication in HCEn cells was significantly reduced by the inhibition of TLR9, but was preserved by a concomitant activation of the NF-κB cascade. Of the different HSV-1–induced inflammatory cascade–related transcription factors, TLR9 was found to activate NF-κB, cyclic AMP response element (CRE), and the CCAAT-enhancer-binding proteins (C/EBP) the most.

Conclusions. Corneal endothelial cells transcriptionally initiate inflammatory programs in response to HSV-1 infection related to NF-κB, CRE, and C/EBP and express arrays of inflammatory cytokine induction by TLR9. On the other hand, HSV-1 exploits TLR9-mediated NF-κB activation for its own replication. (*Invest Ophthalmol Vis Sci.* 2011;52:6729–6736) DOI:10.1167/iovs.11-7805

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The tissues of the ocular surface help maintain the clarity of the cornea and protect the eye from numerous environmental pathogens or dead cell constituents. The endothelial cells lining the inner surface of the cornea are also responsible for maintaining the optical clarity of the cornea. Because endothelial cells do not replicate in vivo, a decrease in their density can lead to blinding bullous keratopathy.[1,2]

Generally, mucosal surfaces are armed with pattern-recognition receptors (PRRs) for sensing foreign materials. The PRRs recognize various types of ligands, such as bacterial and fungal cell wall components, bacterial lipoproteins, and nucleic acids derived from bacteria, virus, and self.[3] An invasion by viruses is recognized by the toll-like receptor (TLR) family, and the recently recognized categories of intracellular PRRs that detect nucleic acids in the cytoplasm.[4] The retinoic acid-inducible gene I (*RIG-I*) and melanoma differentiation-associated gene 5 (*MDA5*) detect the RNA of pathogens. DNA-dependent activator of interferon-regulatory factors (*DAI*), absent in melanoma 2 (*AIM2*), RNA polymerase III, leucine-rich repeat flightless-interacting protein 1 (*LRRFIP1*), and interferon γ-inducible protein 16 (*IFI16*) detect intracellular DNA.

The TLRs were the first discovered and major category of PRRs. The recognition of pathogens by TLRs leads to the induction of innate immunity, inflammation, and adaptive immunity. The TLRs on the mucosal body surface function not only to keep bacteria from invading the body but also to form mutually beneficial relationships.[5] TLRs recognize commensal or pathogen-associated molecular patterns to control the function of the mucosal surface cells. For example, the TLRs regulate the proliferation of epithelial cells after intestinal injury. An absence of TLRs significantly impairs the repair of the epithelial barrier.[5] Signaling by the TLRs leads to increased inflammation and promotes the development of inflammation-associated neoplasia.[6] Thus, intricate interactions operate for the host and microbes by the many functions of the TLRs.

Corneal endothelial cells have been recently found to act as immune modulators that suppress T cell receptor-mediated $CD4^+$ T cell proliferation. They also stimulate the conversion of $CD8^+$ T cells into regulatory T cells.[7,8] These functions may contribute to the immune privilege of the eye. TLRs are especially recognized as important modulators of innate and acquired immunity. Thus, understanding how the endothelial cells behave after TLR stimulation may provide important clues on how to control immune-mediated diseases.

TLR9 is a well-known sensor of the nucleic acids of viruses and microbes. HSV-1 is the most common viral pathogen permissive to the corneal endothelial cells, and an infection by HSV-1 is manifested as herpetic keratitis. To recognize herpesvirus, the host uses a distinct repertoire of TLRs. First, the surface glycoproteins ligate to TLR2.[9,10] Second, the DNAs of herpesvirus which are rich in CpG sequences, stimulate TLR9.[11,12] And third, double-strand RNAs, generated through

self-hybridization of viral genes, activate TLR3.[13,14] TLR9 has been reported to be a crucial component in corneal epithelial cells that recognize the HSV-1 infection.[15,16] However, the roles played by TLRs in corneal endothelial cells have not been determined.

The activation of TLR9 can also cause collateral damage or exacerbation of immune-mediated diseases. For example, when self nucleic acids activate TLR9 chaperoned by anti-DNA autoantibody,[3] the TLR9 activation initiates or exacerbates autoimmune diseases.[17–19] Thus, understanding the roles played by TLR9 may help develop effective strategies to prevent unwanted inflammatory responses in the anterior chamber or corneal endothelium.

The purpose of this study was to determine the response of the TLR9 in cultured human corneal endothelial (HCEn) cells after herpes simplex virus type 1 (HSV-1) infection and to characterize the TLR9-mediated anti-viral responses. We shall show that HCEn cells express TLR9 intracellularly, and HSV-1 infection leads to the upregulation of arrays of inflammatory cytokines mediated by TLR9. Especially important was that the NF-κB cascade downstream of TLR9 can be hijacked by HSV-1 and diverted for its own replication.

Materials and Methods

Cells

An HCEn cell line was established as described in detail.[7] The HCEn cells were propagated to confluence on 6- or 96-well plates in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum. Primary corneal endothelial cells were obtained from the corneoscleral rims of donor eyes after the central cornea was used for keratoplasty.

The procedures used conformed to the tenets of the Declaration of Helsinki.

Viruses

Confluent monolayers of Vero cells were infected with the KOS strain of HSV-1 (generous gift from Kozaburo Hayashi, National Institutes of Health [NIH], Bethesda, MD) After 1 hour of adsorption, the medium containing the virus was aspirated, and the monolayers of cells were refed with fresh HSV-1-free medium. After attaining the maximum cytopathic effect (48–72 hours after infection), the medium was discarded, and cells with the small amount of remaining medium were frozen, thawed, and sonicated. The supernatant was collected after centrifugation at 3000 rpm for 10 minutes and overlaid onto a sucrose density gradient (10–60% wt/vol). The solution was centrifuged with a swing rotor (SW28; Beckman Instruments, Fullerton, CA) for 1 hour at 11,500 rpm. The resultant visible band at the lower part of the gradient containing the HSV-1 was washed using centrifugation at 14,000 rpm for 90 minutes and resuspended in a small volume of serum-free DMEM. The virus was then divided into aliquots and stored at −80°C until use. To infect the HCEn cells with HSV-1, the HCEn cells were adsorbed with the sucrose-density gradient purified virus stock for 1 hour at a multiplicity of infection (MOI) of 0.01 to 1, and refed with fresh medium.

Flow Cytometry

Flow cytometry was used to determine the degree of TLR expression using the following monoclonal antibodies (mAbs): TLR2 (Alexis, Plymouth Meeting, PA), TLR4 (Monosan, Uden, Netherlands), TLR3 (abCam, Cambridge, UK), and TLR9 (Oncogene, San Diego, CA). Mouse isotype IgG was used as the control. FITC-conjugated anti-mouse IgG_1 or IgG_2 (BD Pharmingen, Franklin Lakes, NJ) was used as the secondary antibody.

For flow cytometric analysis of the surface expression of TLRs on the HCEn cells, a suspension of subconfluent cells was obtained by adding 0.5% trypsin/EDTA to the HCEn cells and incubated with anti-TLR antibodies. This was followed by incubation with FITC-conjugated anti-mouse IgG (BD PharMingen). For intracellular staining of the TLRs, HCEn cell suspensions were permeabilized (Cytofix/Cytoperm; BD Biosciences) before staining. After they were washed twice in PBS, the stained cells (live-gated on the basis of the forward and side scatter profile and propidium iodide exclusion) were analyzed by flow cytometry.

Luciferase Reporter Assays

HCEn cells were transfected with luciferase reporter plasmids for AP-1, C/EBP, CRE, Elk-1, ISRE, NFAT, or NF-κB (Agilent, Santa Clara, CA). For the , internal control, HCEn cells were co-transfected with pRL-CMV (Promega, Madison, WI; using Geneporter 3000 transfection reagent; Genlantis, San Diego, CA).

For inhibition of TLR-9, TLR-9 inhibitory oligonucleotide (forward 5′-TCCTGGCGGGGAAGT-3′) (Alexis, San Diego, CA) or TLR-9 siRNA (Qiagen, Hilden, Germany) was used. For activation of the NF-κB cascade, the IκBα on the HCEn cells was inhibited by IκBα siRNA (Invitrogen, Carlsbad, CA). For transfection of siRNA, HCEn cells were transfected (RNAifect; Qiagen) 2 days after transfection of the reporter plasmids, according to the manufacturer's protocol. HCEn cells were infected with HSV-1 48 hours after siRNA transfection. The luciferase activity was measured using the dual-luciferase reporter assay system (Promega).

The target sequences of the siRNA were TLR-9 siRNA: forward 5′-CGGCAACTGTTATTACAAGAA-3′, and IκBα siRNA: forward 5′-GAGCTCCGAGACTTTCGAGGAAATA-3′.

Pharmacologic Inhibition of NF-κB Cascade

An IKK inhibitor peptide or control peptide (Merck, Darmstadt, Germany) was used to block the IκB kinase activity. The IKK inhibitor peptide contained a sequence corresponding to the active IκB phosphorylation recognition sequence. For inhibition of NF-κB p65, NF-κB p65 (Ser276) inhibitor peptide or control peptide (Imgenex, San Diego, CA) was used.

Real-Time RT-PCR

Total RNA was isolated from HSV-1-infected HCEn cells and reverse transcribed (QuantiTect Reverse Transcription Kit; Qiagen). The cDNAs were amplified and quantified on a thermal cycler (LightCycler; Roche, Mannheim, Germany) using a PCR kit (QuantiTect SYBR Green; Qiagen). The sequences of the real-time PCR primer pairs were VEGF: forward 5′-GCAGCTTGAGTTAAACGAACG-3′, reverse 5′-GGTTCCCGAAACCCTGAG-3′; IL-6: forward 5′-GATGAGTACAAAAGTCCTGATCCA-3′, reverse 5′-CTGCAGCCACTGGTTCTGT-3′; HSV-1 DNA polymerase: forward 5′-CATCACCGACCCGGAGAGGGAC-3′, reverse 5′-GGGCCAGGCGCTTGTTGGTGTA-3′; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward 5′-AGCCACATCGCTCAGACAC-3′, reverse 5′-GCCCAATACGACCAAATCC-3′.

To ensure equivalent loading and amplification, all products were normalized to GAPDH transcript as an internal control.

Enzyme-Linked Immunosorbent Assay

To determine the levels of secreted IL-6, supernatants collected from HSV-1-infected HCEn cells were assayed with a commercial ELISA kit (Peprotech, Rocky Hill, NJ).

For inflammatory cytokine and chemokine profiling after HSV-1 infection, supernatants were collected from HCEn cells 12 hours post infection (pi) and assayed with human cytokine antibody arrays (RayBiotech, Norcross, GA). This analysis determined the level of expression of 80 cytokines and chemokines. The intensity of the chemiluminescence signals was digitized (LAS-1000plus; Fujifilm, Tokyo, Japan, and MultiGauge software ver.2.0; Fujifilm) and normalized by using the positive control signals in each membrane.

Pathways Analysis

The set of extracted genes was analyzed for transcriptional networks of molecular events using computerized pathway analysis (Pathways Analysis 7.0; Ingenuity Systems, Redwood, CA; based on the Ingenuity Pathways Knowledge Base). The resulting networks were evaluated by the significance scores, which were expressed as the negative logarithm of the P value. The obtained score indicate the likelihood that the assembly of a set of focus genes in a network could be explained by random chance alone.

Statistical Analyses

Data are presented as the mean ± SEM. Statistical analyses were performed using t-tests or ANOVA, as appropriate.

Results

TLR9 Expression in HCEn Cells

We used flow cytometry to determine whether TLRs are expressed on HCEn cells grown in culture or primary HCEn cells, because TLRs can be expressed on the cell surface in nonhematopoietic lineage cells. No significant cell surface expression was observed (data not shown).

Next, we assessed the intracellular expression of TLRs by staining permeabilized HCEn cells and primary corneal endothelial cells. TLR9 was found to be significantly expressed intracellularly, whereas expression of TLR2, TLR3, and TLR4 was barely detectable (Fig. 1).

TLR9-Mediated NF-κB Promoter Activation in HCEn Cells after HSV-1 Infection

To determine whether the input from TLR9 is functional, we examined whether TLR9 ligand activates the NF-κB cascade, since NF-κB is the representative signaling cascade of TLR-mediated signaling. When TLR9 was stimulated with B class CpG oligonucleotide, a TLR9 ligand, there was a significant upregulation of NF-κB promoter activity, indicating that TLR9 is functional in HCEn cells (Fig. 2).

We next evaluated whether HSV-1 infection would activate the NF-κB cascade in HCEn cells and whether TLR9 plays a role in this activation. We found that HSV-1 infection significantly stimulated the promoter of NF-κB as early as 6 hours pi, and the level of expression continued to increase up to 12 hours pi (Fig. 3) The elevated NF-κB promoter activity was significantly reduced by an inhibition of TLR9. Thus, the TLR9 cascade that stimulates NF-κB is activated after HSV-1 infection.

TLR9-Mediated Inflammatory Cytokine and Chemokine Induction in HCEn Cells after HSV-1 Infection

Next, we examined whether TLR9 is involved in the induction of cytokines and chemokines in HCEn cells after HSV-1 infection. After HCEn cells were infected with HSV-1, the level of IL-6 transcript was significantly increased at 12 hours pi (i.e., the IL-6 expression relative to GAPDH was $1.4 \times 10^{-4} \pm 1.0 \times 10^{-5}$ at an HSV-1 MOI of 0.1 and $2.0 \times 10^{-6} \pm 3.1 \times 10^{-7}$ for mock infection ($P < 0.01$). The level was lower at 24 hours pi: $1.1 \times 10^{-4} \pm 4.0 \times 10^{-6}$ IL-6/GAPDH at an HSV-1 MOI of 0.1 and $2.3 \times 10^{-6} \pm 3.3 \times 10^{-7}$ for mock infection.

To examine the contribution of TLR9 to the IL-6 induction, HCEn cells treated with TLR9 inhibitory oligonucleotide were infected with HSV-1 and assessed for IL-6 induction by real-time PCR. The HSV-1 infection significantly elevated IL-6 induction at 12 hours pi (Figs. 4A, 4B). The level of IL-6 after HSV-1 infection was significantly reduced by blocking TLR9 with a TLR9 inhibitory oligonucleotide (Fig. 4A). Inhibition of TLR9 by siRNA transfection also had a similar inhibitory effect on the IL-6 induction (Fig. 4B).

We then determined whether HSV-1 infection can stimulate IL-6 secretion through TLR9. When supernatants of HSV-1–infected HCEn cells were assessed for IL-6 by ELISA, we found that HSV-1 infection significantly stimulated IL-6 secretion (Figs. 4C, 4D). This HSV-1 infection–induced IL-6 secretion was significantly suppressed by a TLR9 inhibitory oligonucleotide in a dose-dependent manner (Figs. 4C, 4D).

Next, we assessed how HSV-1 infection modulated the cytokine and chemokine milieu of HCEn cells through TLR9. Supernatants from HSV-1–infected HCEn cells were assayed for 80 cytokines and chemokines using protein array analysis and were tested for their sensitivity to TLR9 inhibition. Twenty cytokines and chemokines were significantly upregulated after HSV-1 infection, and of them,TLR9 inhibition significantly reduced the upregulation of RANTES (CCL5), IP-10 (CXCL10), MCP-2 (CCL8), MIF, MCP-4 (CCL13), MDC (CCL22), MIP-3α (CCL20), IL-5, TARC (CCL17), and MCP-1 (CCL2) (Fig. 5).

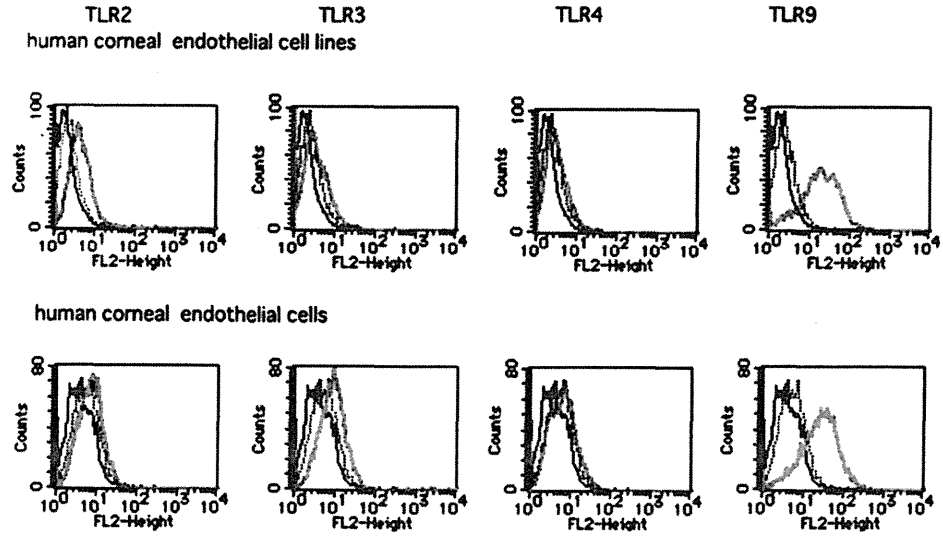

FIGURE 1. Intracellular expression of TLRs in HCEn cells. TLR 9 was significantly expressed in HCEn cells and primary human corneal endothelial cells. Expression of TLR2, -3, and -4 was barely detectable. *Solid line*: unstained; *dotted line*: control IgG stained, *gray line*: anti-TLR antibody stained.

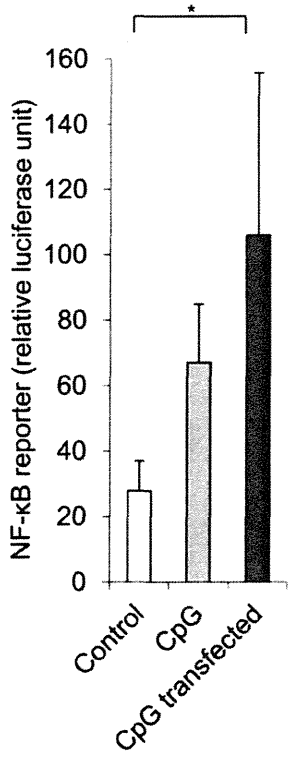

FIGURE 2. NF-κB promoter activation in HCEn cells by TLR9. HCEn cells transfected with NF-κB reporter plasmids were stimulated with CpG oligonucleotide for 24 hours and measured for luciferase activity. CpG transfection significantly elevates the NF-κB promoter activity ($n = 6$; $^*P < 0.05$).

Role of TLR9-Mediated NF-κB Activation and HSV-1 Replication

TLR9 participates in the primary defense systems against viral infection and functions to induce inflammatory cytokines after infection of HCEn cells by HSV-1. We examined whether TLR9 affects the entry and replication of HSV-1 into HCEn cells. To accomplish this, HSV-1 was adsorbed on HCEn cells, and the number of HSV-1 copies was determined by real-time PCR of HSV-1 DNA polymerase. After TLR9 was inhibited by pretreatment with TLR9 inhibitory oligonucleotide, a significant reduction in the copy number was not observed (Fig. 6A).

The contribution of TLR9 to viral replication was determined by real-time RT-PCR, and the results showed a significant reduction in the expression of the mRNA of HSV-1 DNA polymerase in HCEn cells after exposure to TLR9 inhibitory oligonucleotide (Fig. 6B). This reduction was also confirmed by titration (control oligo-treated at an MOI of 0.1: $9.3 \times 10^8 \pm 0.3 \times 10^8$; TLR9 inhibitory oligonucleotide-treated at an MOI of 0.1: $9.8 \times 10^7 \pm 0.9 \times 10^7$, $P < 0.01$).

We then assessed whether the TLR9-mediated viral replication in HCEn cells was related to NF-κB activation. The classic NF-κB cascade is regulated by IκB kinase (IKK), leading to the nuclear translocation of p65, a component of the NF-κB pathway. When HCEn cells were treated with IKK inhibitory peptide, which contained sequences corresponding to the active IκBα phosphorylation recognition sequence, the induction of HSV-1 DNA polymerase was significantly inhibited in HSV-1–infected HCEn cells (Fig. 6C). Treatment with a p65 inhibitor also significantly reduced the number HSV-1 copies (data not shown). These findings indicate that the classic NF-κB cascade is involved in HSV-1 replication in HCEn cells.

We next examined whether TLR9-inhibition–mediated suppression of HSV-1 replication can be restored by NF-κB activation. Because the activation of the classic NF-κB cascade is regulated by the degradation of IκBα, the NF-κB cascade is activated by siRNA-mediated inhibition of IκBα. Exposure to TLR9 inhibitory oligonucleotide reduced the copy number of HSV-1 DNA polymerase mRNA, and the transfection of IκBα siRNA reduced the effect of TLR9 inhibition (Fig. 6D). Collectively, these findings indicate that HSV-1 used the TLR9-mediated NF-κB activation for its own replication in HCEn cells.

Alternative Transcription Factor Activation by TLR9 in HSV-1–Infected HCEn Cells

HSV-1 infection induces an array of inflammatory cascades. This can be summarized by the transcriptional induction profiles of representative transcriptional factors. To characterize the profiles of the signaling cascades activated by HSV-1 infection and show the possible involvement of TLR9, we determined whether HSV-1 infection can activate representative transcriptional factors related to the TLR9 cascades by using transfection of reporter plasmids. The activities of transcriptional factors of cascades of NF-κB, MAPK/ERK, cAMP/PKA, MAPK/JNK, C/EBP, interferon response, and PKC/calcium were measured using reporter plasmids for NF-κB, Elk-1, cyclic AMP response element (CRE), AP-1, C/EBP, ISRE, and NFAT, respectively. HSV-1 infection significantly stimulated the transcription factors of NF-κB, Elk-1, CRE, AP-1, C/EBP, and NFAT at 24 hours pi (Fig. 7).

We then tested whether TLR9 contributes to the induction of transcription factor activities of the inflammatory cascades. When TLR9 was inhibited by siRNA transfection, the HSV-1–induced activation of CRE and C/EBP reporters was significantly reduced (Fig. 8). The other transcription factor activities, including Elk-1, AP-1, and NFAT were not appreciably affected (data not shown). Thus, HCEn cells used TLR9 leading to various types of promoter activation, including NF-κB, after HSV-1 infection.

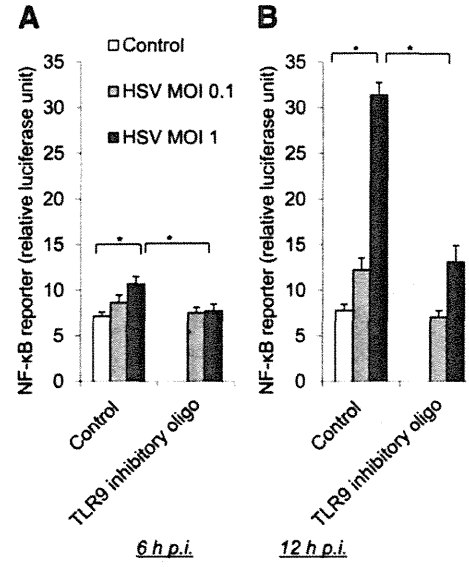

FIGURE 3. Inhibition of HSV-1 infection–induced NF-κB promoter by TLR9 inhibition. HCEn cells transfected with reporter plasmids were stimulated with HSV-1 infection for 6 (A) and 12 (B) hours, and measured for luciferase activity. Treatment by TLR9 inhibitory oligonucleotide significantly inhibited NF-κB promoter activation ($n = 6$; $^*P < 0.05$).

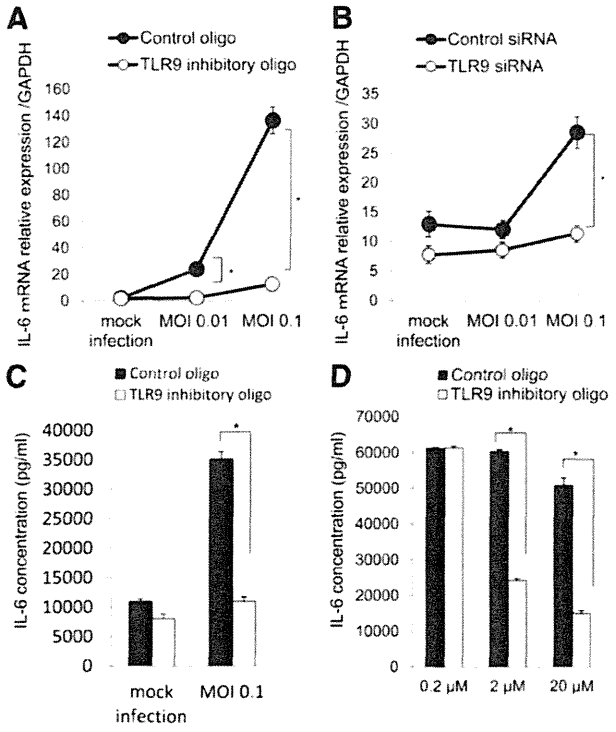

FIGURE 4. Inhibition of HSV-1 infection–induced IL-6 activation. Effect of inhibiting HSV-1 infection–induced IL-6 mRNA induction by TLR9 inhibitory oligonucleotide treatment (**A**) and by transfection of siRNA of TLR9 (**B**) at 12 hours. TLR9 blockade by inhibitory oligonucleotide or siRNA significantly reduced the HSV-1 infection–induced IL-6 mRNA activation. $n = 4$; $^{*}P < 0.01$. (**C**, **D**) Reduction of HSV-1 infection–induced IL-6 secretion by TLR9 blockade. TLR9 inhibitory oligonucleotide significantly reduced IL-6 secretion at 12 (**C**) and 24 (**D**) hours pi in a dose-dependent manner ($n = 6$; $^{*}P < 0.01$).

TLR9-Mediated Inflammatory Network after HSV-1 Infection

To summarize how HCEn cells used TLR9-mediated signals after HSV-1 infection, the TLR9-dependent cytokines induced after HSV-1 infection were analyzed for signaling interactions using a systems biological approach. Using a database of known signaling networks (Ingenuity Pathways Knowledge Base; Ingenuity Systems), we successfully generated two major biological networks with high significance scores (network 1, $P < 10^{-51}$; network 2, $P < 10^{-15}$). The most significant network was network 1, which was annotated as cell-to-cell signaling and interaction, hematologic system development and function, and immune cell trafficking, where NF-κB was centrally positioned (data not shown).

Discussion

Our results showed that TLR9 was abundantly expressed in HCEn cells and was used to initiate inflammatory responses after HSV-1 infection. HSV-1 exploited the TLR9-mediated NF-κB activation for its own replication. To resist the assault, HCEn cells transcriptionally initiate an array of inflammatory programs related to the cascades of NF-κB, ERK, MAPK (P38), JNK, cAMP/PKA, PKC, and interferon responses. Of these, TLR9 activation was especially used for the signal transduction cascades of NF-κB, CRE, C/EBP, and arrays of inflammatory cytokines, including IL-6.

In sensing microbial pathogens, conserved structural moieties are recognized by germline encoded PRRs, including the TLRs, NOD-like receptors, and C-type lectin receptors.[20] Apoptotic or necrotic cells or degradation products of the extracellular matrix, damage-associated molecules or cytokines, such as dsDNA, RNA, high-mobility group box 1 (HMGB1), ATP, hyaluronan, versican, heparin sulfate, and heat shock proteins, are abundantly present. These damage-associated molecular patterns (DAMPs) are also recognized by PRRs. Of the different PRRs, the TLRs are the most important class of receptors that are able to sense pathogen-associated molecular patterns (PAMPs). Nucleic acids, especially DNAs, are a major class of molecules that stimulate TLRs. Previously, the DNAs derived from bacteria had been considered the exclusive ligand of TLR9. However, viral genomes and self DNAs derived from necrotic or apoptotic cells have also been shown to activate TLR9. Physiologically, ligands of TLRs, including TLR9, are ubiquitous, and the corneal endothelium is continuously exposed to various components of PRR ligands. Thus, the cornea and the host are exposed to and sense the environment using combinations of PRRs. In this setting, cascades initiated from such PRRs generally converge to NF-κB or inflammasomes, where the converged signal inputs can elicit robust immune responses in synergy.[21]

For entry of HSV-1 into the host, glycoproteins, gB, gD, gH, and gL are required. For example, gB binds to paired immunoglobulin-like type 2 receptor α (PILRα) on the host,[22] gD binds to herpesvirus entry mediator (HVEM), nectin-1, or 3-O sulfated heparan sulfate, after which the host recognizes the viral invasion by the PRRs. In the TLR-mediated recognition cascade, three major molecular components—TLR2, TLR9, and TLR3—are engaged to activate innate immune responses.[23] However, the TLR-mediated interaction does not appear to affect viral entry (Fig. 6A). The sequential recognition of TLR2 and -9 that occurs after HSV-1 infection leads to a robust NF-κB activation which then induces a wide array of cytokines, chemokines, and interferons, where NF-κB plays a central role in regulating numerous cellular metabolic events. Concomitantly, HSV-1 redirects the host transcriptional machinery to

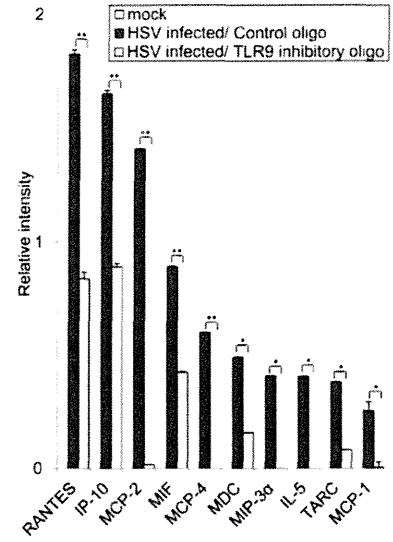

FIGURE 5. TLR9-mediated inflammatory cytokine and chemokine induction by HSV-1–infected HCEn cells. HCEn cells were adsorbed with HSV-1 at an MOI of 0.1 for 1 hour and refed with the DMEM. After 12 hours of incubation, the supernatant of HSV-1–infected HCEn cells was assayed for cytokines. TLR9-induced inflammatory cytokines and chemokines were significantly reduced by exposure to TLR9 inhibitory oligonucleotide ($n = 4$/group; $^{*}P < 0.05$, $^{**}P < 0.01$).

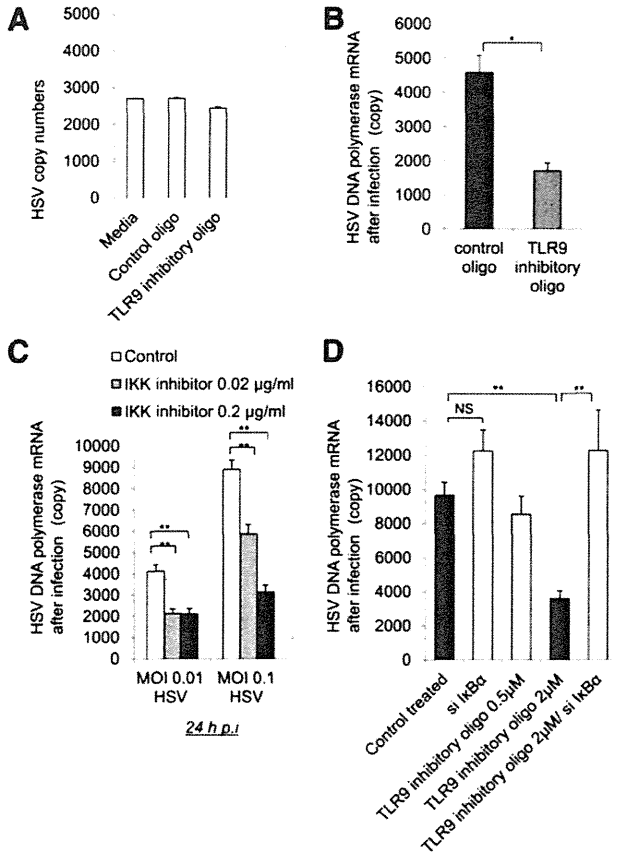

FIGURE 6. Inhibition of HSV-1 proliferation by blockade of TLR9 and NF-κB signaling cascade. (**A**) Unperturbed entry of HSV-1 into the HCEn cells by TLR9 inhibition. HSV-1 was adsorbed on HCEn cells for 1 hour. HCEn cells were washed and assessed for HSV-1 DNA polymerase copy number by using real-time PCR ($n = 8$). TLR9 inhibitory oligonucleotide did not appreciably affect HSV-1 absorption. (**B**) TLR9 inhibitory oligonucleotide impaired HSV-1 replication, shown by the reduction in copy number of HSV-1 DNA polymerase mRNA ($n = 4$, 24 hours pi). (**C**) Reduced proliferation of HSV-1 by IKK inhibitor. HCEn cells were infected with HSV-1 at the indicated MOI and assessed at 24 hours pi for copy number of HSV-1 DNA polymerase mRNA, with reverse transcription real-time PCR ($n = 4$; $P < 0.01$). (**D**) Restoration of TLR9 inhibition–mediated reduction of HSV-1 proliferation by IκBα inhibition. Treatment of TLR9 inhibitory oligonucleotide significantly reduced the copy number of HSV-1 DNA polymerase mRNA at 24 hours pi. This reduction was restored by NF-κB activation using transfection of siRNA of IκBα ($n = 4$; $^*P < 0.05$, $^{**}P < 0.01$).

express its own genes in a tightly regulated temporal cascade.[24] The three classes of genes, the immediate-early (IE) genes, including ICP-0, -4, -22, -27, and -47, followed by the early and the late genes are sequentially expressed.

To initiate productive replication of HSV-1, ICP0 plays a crucial role as a strong activator of all classes of HSV-1 genes and a propagator of lytic infections. ICP0 possesses NF-κB-binding elements on its promoter. The transcription of ICP0 is dependent on activation of NF-κB of the host, which is triggered by the recruitment of p65/RelA.[24] Inhibition of the NF-κB cascade, including the inhibition of IKK or dominant negative IκBα, significantly suppresses viral replication (Fig. 6).[24–26] Very recently, the UL31 of HSV-1 was also shown to be necessary for optimal NF-κB activation and expression of ICP4, ICP8, and glycoprotein C.[27]

The use of host NF-κB for viral replication is not limited to HSV-1 because NF-κB-binding sites are also located in the genome of different members of the herpes virus family.[28,29] Moreover, HSV-1 is equipped with the ability to effectively block multiple innate signaling for its survival. For example, virion host shutoff protein (VHS) degrades the host mRNA by its RNase function. US11 or γ34.5 inhibits PKR (RNA-activated protein kinase), and ICP47 inhibits MHC class I loading.[30–37] After the viral replication is completed, ICP-0 directs the inhibition of inflammatory responses by ubiquitin-specific peptidase 7 (USP7) translocation, which leads to the inhibition of NF-κB and JNK.[30] Thus, HSV-1 hijacks and exploits the crucial components of the host immune system, TLR9 and NF-κB, for its own use.

There are two major signaling pathways for TLR: NF-κB and MAPKs. In the MAPK cascade, the JNK, p38, and ERK pathways are conventionally activated, leading to the activation of AP-1, CRE, Elk-1, and C/EBP elements in the promoters. In addition, the C/EBP family of transcription factors is involved in many biological functions, including regulating cytokine expression, proliferation, and tumor progression.[38–40] We found that the reporter activity of NF-κB, CRE, and C/EBP are activated by TLR9 after HSV-1 infection. Analysis of the HSV-1 infection–induced transcriptome of HCEn cells showed strong inductions of CREBBP and C/EBPα, which are representative transcription factors related to CRE and C/EBP.

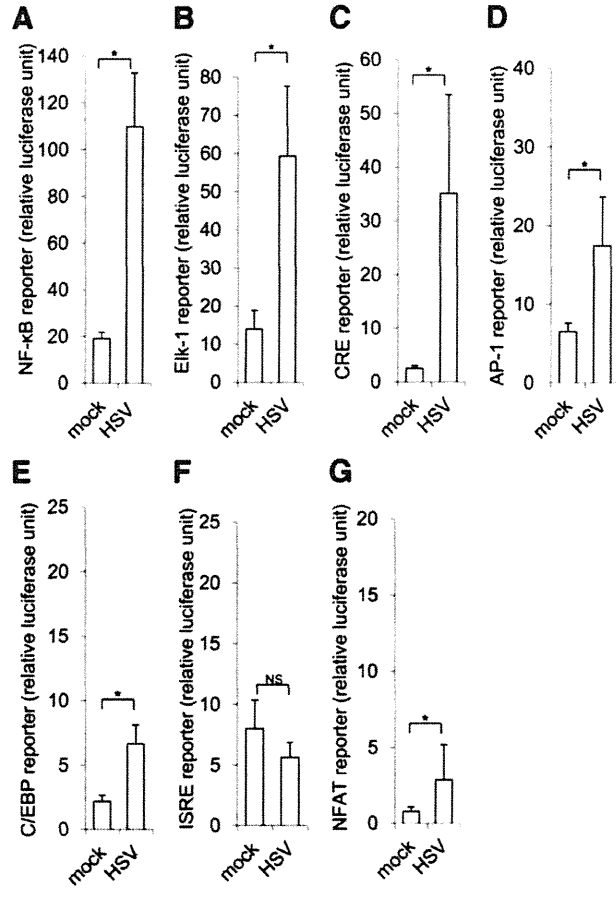

FIGURE 7. Signaling cascade–focused promoter activation in HCEn cells by HSV-1 infection. HCEn cells transfected with reporter plasmids were stimulated with HSV-1 infection for 24 hours at an MOI of 0.1 and measured for luciferase activity for (**A**) NF-κB, (**B**) Elk-1, (**C**) CRE, (**D**) AP-1, (**E**) C/EBP, (**F**) ISRE, and (**G**) NFAT. HSV-1 infection significantly elevated promoter activities of NF-κB, ELK-1, CRE, AP-1, C/EBP, and NFAT ($n = 6$; $^*P < 0.05$).

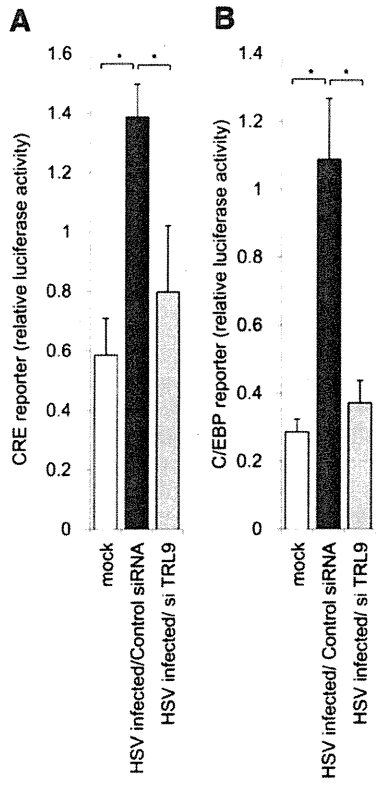

FIGURE 8. Inhibition of HSV-1 infection-induced CRE and C/EBP promoter activation by TLR9 inhibition. HCEn cells transfected with reporter plasmids were infected with HSV-1 at an MOI of 0.1 and measured for luciferase activity at 12 hours pi. Transfection of siRNA of TLR9 significantly inhibited the reporter activities of CRE (**A**) and C/EBP (**B**). ($n = 6$; $^*P < 0.05$).

Generally, transcriptional activation is regulated by different levels of transcriptional factor activation and interactions. On infection by *Helicobacter pylori*, the AP-1 and CRE elements in the cyclooxygenase promoter are activated by TLR2 and -9.[41] In TLR-mediated activation of IL-6 and TNF-α, both NF-κB and C/EBP binding elements in the promoter are critical for their transcriptional activation.[42–44] In HSV-1–infected HCEn cells, TLR9 input activated the NF-κB signal transduction cascade (Fig. 3), and our bioinformatic analysis of the induced cytokines and chemokines which are sensitive to TLR9 inhibition, were summarized as NF-κB-dependent inflammatory cascade. However, the NF-κB cascade may not be sufficient to fully explain the transcriptional activation of inflammatory cytokines. In HCEn cells, the activations of CRE, C/EBP, and NF-κB were involved in the TLR9-mediated signaling cascade (Fig. 8) and presumably in the TLR9-mediated induction of inflammatory cytokines and chemokines. At least two of the recognition sequences of these transcription factors exist in the promoters of TLR responsive cytokines and chemokines (data not shown). This may explain the unexpectedly wide array of inflammatory cytokines that was inhibited by TLR9 suppression.

We used immortalized HCEn cells as models of corneal endothelial cells in situ. The HCEn cells have similar capabilities as primary corneal endothelial cells and organ cultured corneal endothelial cell in inducing representative cytokines including MCP-1, IL-6, IL-8, CXCL2, TGFβ2, and thrombospondin 1.[8,45] However, there is still some question of whether immortalized HCEn cells can truly reflect the in vivo properties of corneal endothelial cells such as HSV-1 infection-induced endotheliitis. For this, in vivo analysis may help in gaining a better understanding of the physiological roles of the endothelial cells during a viral infection.

At present, a murine model of HSV-1–induced corneal endotheliitis is not available. We used the KOS strain for this study because our initial hypothesis was based on the findings of our earlier studies.[46,47] Very recently, the KOS strain has been reported to have a mutation of the *US8A* gene and defective *US9* gene.[48] *US9* is especially involved in neuronal virulence. However, a defective *US9* does not appear to affect the cell-to-cell spread in permissive epithelial cells.[49] In addition, no apparent dysfunction was reported for the elongated US8A by mutation.

To summarize, corneal endothelial cells express TLR9 intracellularly to recognize dsDNAs and HSV-1 infection. HSV-1 usurps this TLR-mediated NF-κB activation for its own replication.

Acknowledgments

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（総　　説）

全身疾患に関連したヒトヘルペスウイルス眼感染症

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鳥取大学医学部感覚運動医学講座　視覚病態学

Human herpes virus ocular infection related with systemic diseases

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要旨　ヒトヘルペスウイルス（human herpes virus：HHV）は全身のさまざまな臓器に多岐にわたる感染症を引き起こしてくる．眼においてもヘルペス性角膜炎をはじめとした多くの疾患を起こしてくる．眼感染症単独で発症するものが一般的には多いが，全身的な疾患に関連したヘルペスウイルス眼感染症も多く，アトピー性皮膚炎に伴うヘルペス性角膜炎，帯状疱疹，水痘角膜炎，サイトメガロウイルス網膜炎など多彩である．全身の免疫状態との関連で起こってくることも多く，免疫の状態でその病態が変化する．全身の免疫の状態を示す一つの指標になるともいえる．最近はPCR法によりヘルペスウイルスDNAを容易に検出できるようになり，従来診断できなかったものが診断可能となってきている．

Key words：アトピー性皮膚炎，帯状疱疹，水痘角膜炎，サイトメガロウイルス網膜炎

はじめに

ヒトヘルペスウイルス（human herpes virus；HHV）は現在8種が知られており，α，β，γの3つの亜科に分けられている（表）．ヘルペスウイルスは人体に潜伏感染をする性質やヒトの免疫を巧みに回避するさまざまなしくみを持っている．そのことによって，ヒトにうまく寄生することによって人類とともに歩んできたウイルスであり，全身のさまざまな臓器に多岐にわたる感染症を引き起こしてくる．一方，眼においてもαヘルペスは種々の眼感染症の原因ウイルスとして古くから知られており，βヘルペスも重要な眼感染症の原因ウイルスとして最近話題になっている．また，γヘルペスも眼との関連が言われている．

本総説ではさまざまなヒトヘルペスウイルス眼感染症のうち全身疾患との関連で生じてくるものについてまと

表　ヒトヘルペスウイルスの名称

一　般　名	和　　名	系　統　名	亜科
herpes simplex virus type 1	単純ヘルペスウイルス1型	human herpes virus-1	α
herpes simplex virus type 2	単純ヘルペスウイルス2型	human herpes virus-2	α
varicella-zoster virus	水痘帯状疱疹ウイルス	human herpes virus-3	α
Epstein-Barr virus	エプスタイン-バー・ウイルス	human herpes virus-4	γ
human cytomegalovirus	ヒトサイトメガロウイルス	human herpes virus-5	β
human herpes virus-6	ヒトヘルペスウイルス6	human herpes virus-6	β
human herpes virus-7	ヒトヘルペスウイルス7	human herpes virus-7	β
Kaposi's sarcoma-associated herpes virus	ヒトヘルペスウイルス8（カポジ肉腫関連ヘルペスウイルス）	human herpes virus-8	γ

めた.

単純ヘルペスウイルス (herpes simplex virus：HSV)

1　HSVによる通常の眼感染症

HSVは神経向性があり，神経節に潜伏感染する特徴がある．年齢が進むとともに潜伏感染率は上昇していくが，最近わが国では若年者に未感染者が増加しており，逆に重症の感染を起こす機会が増加している点は他のウイルスと同様である．HSVは多彩な感染症を引き起こしてくるが，1型（HSV-1）と2型があり（HSV-2），顔面の皮疹や口唇ヘルペスはHSV-1によるものが多く，特にヘルペス性角膜炎はほとんどHSV-1による．一方，性器ヘルペスはHSV-2によるものが多い．HSVは潜伏と再発を繰り返して宿主を悩ますが，宿主を殺すことは滅多にない．しかし，まれに重篤なヘルペス脳炎で死亡するケースもある．

HSVによる眼感染症（眼瞼単純疱疹，ヘルペス性結膜炎，ヘルペス性角膜炎，急性網膜壊死）を起こす患者の多くは他に全身疾患をもたない免疫正常者である．HSVによる眼感染症と性器ヘルペスを同時に起こすようなことはなく，ヘルペス脳炎を発症するようなこともない．そういう点でHSVはヘルペスウイルスの中でもっとも眼感染症の頻度が高いにもかかわらず，全身疾患との関連が逆に少ないといえる．ただ，例外として，アトピー性皮膚炎（atopic dermatitis）と関連したHSV眼感染症が問題となり，最近のアトピー性皮膚炎患者の増加に伴い，よく見られるようになってきている．

2　アトピー性皮膚炎とHSV感染症

1）アトピー性皮膚炎におけるHSV皮膚感染

アトピー性皮膚炎はアレルギー反応によって，特徴的な慢性皮膚炎症の寛解と増悪を繰り返す疾患であり，血清総IgEの上昇をともなっている．患者は皮膚の掻痒感が強く，その閾値が低くなっている．そのため患者はつねに皮膚をこすったり掻いたりするため皮膚炎症は悪化し，更に強い掻痒感を起こして，悪循環となる．遺伝的素因と環境因子の両者がこの疾患の発症に関与している．わが国では最近アトピー性皮膚炎患者が非常に増加し，世界でも有数の罹病率となっている．

アトピー性皮膚炎ではアトピー性角結膜炎・アトピー白内障・円錐角膜・網膜剥離など多くの眼合併症を併発してくることが知られているが，感染症をおこしやすいことも問題である．特に黄色ブドウ球菌とHSVが問題となる[1]．

アトピー性皮膚炎には重症のヘルペス皮膚感染を生じることがあり，カポジ水痘様発疹（Kaposi's varicelliform eruption：KVE）といわれている．カポジ水痘様発疹はEczema herpeticum（EH）ともいわれており，1887年にKaposiによって最初に報告された．湿疹様皮膚にHSVが感染することによって生じる広範な水疱性疾患であり，基礎疾患としてはアトピー性皮膚炎に限定されないが，アトピー性皮膚炎がもっとも頻度が高く重要である．皮膚症状のみならず，発熱，倦怠感，所属リンパ節腫脹を伴うこともある．最近アトピー性皮膚炎患者の増加と若年者の未感染者の増加にともない，このカポジ水痘様発疹が珍しい疾患とはいえなくなってきている．

アトピー性皮膚炎に重症のヘルペス感染がなぜ伴うか，その機序については不明な点も多いが，もっとも大きな要因としては，HSVに対する細胞性免疫の不全が考えられている[2]．アトピー性皮膚炎は狭義の免疫不全疾患には入らないが，免疫反応に異常があることは間違いなく，免疫変異疾患であるといえる．免疫の要因以外にも，正常皮膚に比較してアトピー性皮膚炎患者の皮膚ではHSVが増殖しやすいこと[3]，手で掻くことが皮膚でのHSV感染を拡げる要因となっていること[4]なども報告されている．

カポジ水痘様発疹では眼表面にもHSVの感染がおよぶ可能性が高く，ヘルペス性角膜炎がカポジ水痘様発疹に合併してくることがある．

また，カポジ水痘様発疹が顔面片側に限局している場合にあたかも帯状疱疹のように見えることがあり，帯状単純疱疹（zosteriform simplex）といわれている．皮疹の性状だけでは皮膚科の専門医でも帯状疱疹との鑑別が難しい．この際にpolymerase chain reaction（PCR）法によって，HSVやVZVのDNAを検出すれば，的確に鑑別をおこなうことができる．また，このためのサンプルとして皮膚ではなく涙液でも可能である．

2）アトピー性皮膚炎患者でのヘルペス性角膜炎の特徴

アトピー性皮膚炎患者は上記のように皮膚にHSV感染を生じている場合だけでなく，一般に角膜においてもヘルペスを起こしやすいことが知られている．

ヘルペス性角膜炎は一般的には片眼性の疾患であり，角膜の表面の上皮細胞でウイルスが活発に増殖する上皮型（典型例では木の枝別れ様に上皮が欠損する樹枝状角膜炎という特徴的な形態を呈する）と，角膜実質に蓄積したウイルス抗原に対する免疫反応が起こる実質型，病態不明ながら角膜内皮に感染を生じ角膜浮腫を起こす内皮型に大別されるが，いずれにおいても三叉神経節に潜伏したHSVによって生じるため，再発を繰り返すのが特

徴であり，再発を繰り返すことによってしだいに角膜が混濁し，角膜移植が必要となる例も多い．

アトピー性皮膚炎患者におけるヘルペス性角膜炎は一般的なヘルペス性角膜炎と異なり，両眼性が多く（ただし同時発症はまれ），主として上皮型であり，再発が多いのが特徴であり，また上皮の修復が遅いために表層実質に瘢痕が残りやすいことが報告されている[6]．逆に両眼性のヘルペス性角膜炎患者の40％にアトピー性皮膚炎を合併していたとの報告もある[6]．

また，アトピー性皮膚炎患者ではアシクロビル耐性株によるヘルペス性角膜炎の発症が報告されている[7]（図1）．もともとアシクロビル耐性株は増殖力が弱いことが多いが，アトピー性皮膚炎患者ではこのように増殖しにくいウイルスでもヘルペス性角膜炎が発症してしまうことになる．増殖力が弱いため，典型的な樹枝状角膜炎の形をとらないため，診断が難しい．このようなケースでもPCRによるHSV DNAの証明は有用である．

水痘帯状疱疹ウイルス（varicella-zoster virus：VZV）

1　VZVによる水痘と帯状疱疹

名前の通り，VZVの初感染は水痘の形で生じる．VZVは最初上気道に感染し，ウイルス血症を生じた後に，全身に水疱を生じ，やがて終息する．しかしHSV同様に神経向性があり，各神経節に潜伏感染が成立する．これが年余を経て再活性化したものが帯状疱疹である．そして，三叉神経第1枝領域に生じると眼部帯状疱疹としてさまざまな眼合併症を生じてくる．

VZVはHSVと異なり再発病変としての帯状疱疹を生じるのは生涯に一度のことがほとんどである．しかし，単純疱疹と異なりその範囲は広く，神経痛を含めて合併症も多彩で重症となる．

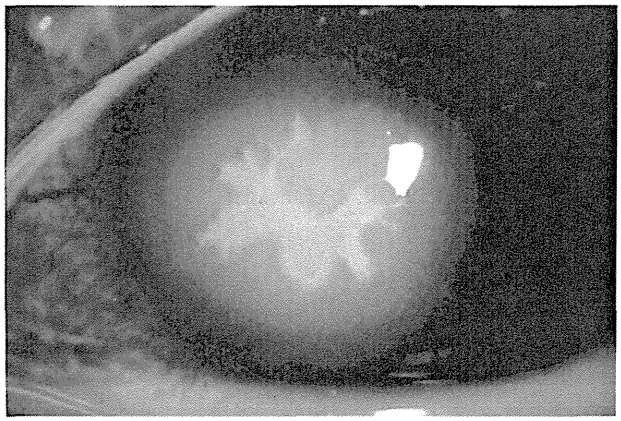

図1　アトピー性皮膚炎患者に生じた耐性ヘルペスによる樹枝状角膜炎
フルオレセイン染色してブルーフィルターで観察しているため，ウイルスが増殖して上皮が欠損した部位が緑色に染色されている

2　眼部帯状疱疹（herpes zoster ophthalmicus）

眼部帯状疱疹は三叉神経節1枝領域に神経痛をともなった皮疹として発症するため，その診断は比較的容易だが，時に神経痛が先行することがある．この段階で眼科を受診した患者に対して，眼や皮膚に所見がなくても，痛みの性状をよく聞いてピリピリした痛みを訴える場合は，後に皮疹が発症したらすぐ皮膚科・眼科を受診するようにアドバイスをすることが重要である．

眼合併症は皮疹のピークよりも遅れて生じてくることが多いので，最初は眼所見がなくても引き続き眼科でも経過観察が必要である．特に，皮疹の範囲をよく確認し，皮疹がたとえ全体に軽度でも，もし鼻尖・鼻翼に皮疹があれば眼合併症を起こしてくる頻度が高い（Hutchinsonの法則）ので，特に注意を要する．これは鼻と眼がどちらも三叉神経第1枝の枝である鼻毛様体神経の支配を受けていることによる．

眼合併症としては結膜炎，上皮型角膜炎（HSVの場合と比較して細く小さい偽樹枝状角膜炎を呈する），実質型角膜炎（小浸潤から銭型，円板状など種々の大きさと形態の角膜混濁を呈する）（図2），強膜炎（図2），上強膜炎，虹彩炎，虹彩萎縮，眼筋麻痺などきわめて多彩である．近年，涙腺炎をともなう症例など過去に知られていなかった眼合併症も報告されるようになってきている[8]．眼部帯状疱疹の眼合併症は一旦終息すればHSVの場合と異なり再発することはきわめてまれだが，遷延例はかなり認められ，また，ステロイド点眼で消炎した際に早期にやめると，再燃することがある．

帯状疱疹の中には，皮疹を伴わず帯状疱疹に特有の神経痛を認める無疹性帯状疱疹（zoster sine herpete）もあるが，眼科領域では皮疹を伴わず，帯状疱疹に特徴的な眼合併症を認めるものもzoster sine herpeteとよんでいる．帯状疱疹の眼合併症はもともと多彩であり，多くの眼炎

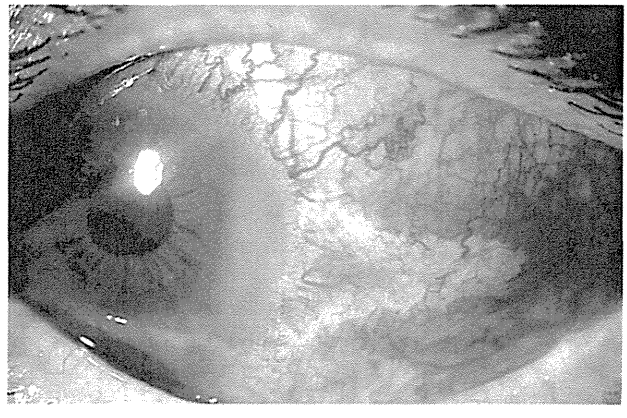

図2　帯状疱疹後の強膜炎と角膜炎
強い充血を認める強膜炎と周辺部の深層角膜の浸潤を伴っている

症性疾患の中に実はVZVによるものが含まれている可能性がある．たとえば虹彩炎の患者で前房水を採取してPCRをおこなうことによって，その原因としてVZVがみつかることがある．VZVはHSVに比べてもともと臨床サンプルから培養で検出することが難しいウイルスであったため，それだけにPCRの恩恵は非常に大きい．

3　水痘角膜炎（varicella keratitis）

水痘罹患後数か月を経て，片眼に円板状角膜炎の形で，角膜中央の浮腫と混濁を生じてくることがまれにあり，水痘角膜炎といわれている[9]．小児例が多いため，発症に気づくのが遅れるので，実際の発症時期は不明だが，水痘罹患後まもなくその続発症として生じている可能性が高いと思われる．しかし，一度潜伏したVSVの再活性化による病態である可能性も否定はできない．

以前は既往や血清抗体価によってしか診断できなかったが，最近は涙液をPCRにかけて診断できる．小児でも涙液は採取できるので，その点非常に診断しやすくなったといえるが，まず水痘を原因として疑うことが重要である．成人に比べて角膜の混濁や浮腫が強く，ステロイド点眼とアシクロビル眼軟膏で軽快するが，早期にやめると再燃することが多い．おそらく成人に比べて強い免疫反応が生じているのではないかと思われる．加えて，弱視の発症もともなうため，健眼と同等の良好な視力を得ることが難しい．

4　進行性網膜外層壊死（progressive outer retinal necrosis：PORN）

VZVは免疫正常者の網膜で急性網膜壊死を起こしてくる．これはVZVによって眼底周辺部に網膜血管炎が広範に生じ，網膜壊死から，網膜裂孔，網膜剥離と進展して失明する疾患である．この急性網膜壊死は珍しい疾患であるが，世界ではじめての報告が日本の報告である[10]ことから，桐沢型ぶどう膜炎（Kirisawa uveitis）の呼称が日本では広く使用されている．ただ，病態の首座からいうと急性網膜壊死の方が名称として合致している．急性網膜壊死は免疫正常者の網膜内層に発症し，病態にウイルス増殖と免疫反応による炎症の両方が関与しており，炎症が強く，前眼部炎症や硝子体混濁も伴う．

進行性網膜外層壊死はこの急性網膜壊死よりも更に重症であり，重度の免疫不全の患者の網膜外層に急激に発症・拡大する．抗ヘルペス薬を使用しても視力予後はきわめて不良である．次のサイトメガロウイルス網膜炎と同様，AIDSのコントロールが最近よくなったため，もともと珍しい疾患であったが，最近はさらにまれとなっている．ただ，免疫の状態によって同じウイルスが異なる病態を示す点から大変興味深い疾患である．

ヒトサイトメガロウイルス
(human cytomegalovirus：HCMV)

1　HCMVによる眼感染症

HCMVによる眼感染症としては網膜炎が有名である．サイトメガロウイルス網膜炎は免疫不全の患者におこるのが大きな特徴であり，眼球以外の臓器で生じてくる多くのHCMV感染とその点は共通している．ところが最近，サイトメガロウイルスによって虹彩炎，角膜内皮炎[11]が発症することがトピックとなっているが，これらは免疫正常者で生じてくる点が他のサイトメガロウイルス感染と大きく異なっている．ここでは全身の免疫不全に関連して生じる網膜炎について述べる．

2　サイトメガロウイルス網膜炎

1）臨床的特徴

免疫不全の患者に多くは両眼性に起こる．AIDSの重要な症状の一つであったが，それに関しては近年，抗HIV治療において，非核酸系逆転写酵素阻害薬，プロテアーゼ阻害薬のいずれかと核酸系逆転写酵素阻害薬2剤を含む3剤以上の抗ウイルス薬を組み合わせる強力なhighly active anti-retroviral therapy（HAART）療法が導入され，AIDSは死亡する病気から生存可能な病気となるとともに，サイトメガロウイルス網膜炎の頻度は減少し，重症例も減っている．臨床所見としては後極部の血管周囲の出血・滲出斑を特徴としている（図3）．免疫不全で生じるため，硝子体混濁や前眼部炎症は認められないか，あってもごく軽度である．

2）検査

眼局所においてはPCRによる眼内液からのウイルスDNA検出が重要である．眼球の奥の網膜の炎症であるが，硝子体液だけでなく前房からでもPCRでCMV の

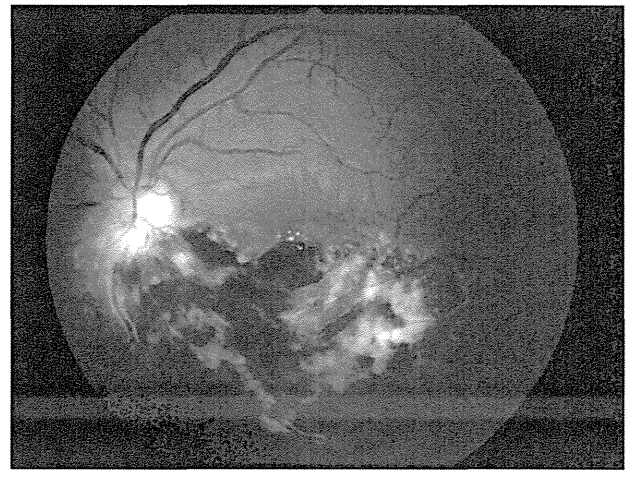

図3　サイトメガロウイルス網膜炎
網膜血管を中心として出血・滲出斑を認める．乳頭・黄斑にもかかっており，視機能に大きな影響を与えている

DNAが検出される．CMVで網膜炎を起こしている患者の場合，免疫不全を背景として全身の他の臓器の感染の可能性もあり，血清の抗CMV抗体価の上昇，血清のCMV抗原血症（antigenemia）の証明などの全身的なウイルス検索も重要となる．

3）治療

抗CMV薬（ガンシクロビル，バラガンシクロビル）の投与とともに，免疫不全の改善が必要だが，改善に伴い炎症が生じることもあるので注意が必要であり，immune recovery uveitisと言われている．これはHAART療法などの導入により，CMV網膜炎を有する患者で臨床的な免疫能の回復に伴って眼内炎症を生じるケースが認められるようになり，こう呼ばれているもので，強い前房内や硝子体の炎症を生じてくれば逆に少し免疫をおさえる必要が生じてくるため，ステロイドによる治療が必要となる．

その他のヘルペスウイルス

その他のヘルペスウイルス属についても眼感染症の原因ウイルスとしての報告があるが，確立されたものは少ない．Epstein-Barr virus（HHV-4）やHHV-6はHCMVとともに造血幹細胞移植後に再活性化をすることが報告されており，免疫不全患者でのさまざまな合併症に関連している可能性が指摘されているが，そのような場合において眼感染症に関与したという報告はまだない．

HHV-8はAIDS患者のカポジ肉腫から発見されたウイルスであり，当然眼瞼のカポジ肉腫の原因ウイルスである．これはAIDSなどの免疫不全患者で生じるので，その他のヘルペスウイルスの中で全身疾患と関連した唯一確かなものであるといえる．

おわりに

このように，全身的な疾患に関連したヘルペスウイルス眼感染症は多岐にわたっており，アトピー性皮膚炎に伴うヘルペス性角膜炎，帯状疱疹，水痘角膜炎，サイトメガロウイルス網膜炎など多彩である．全身の免疫状態との関連で起こってくることが多く，また，免疫状態とのバランスでその病態が変化する．全身の免疫の状態を示す一つの指標になるともいえるので，その発症には注意を払う必要がある．最近はPCR法によりヘルペスウイルスDNAを容易に検出できるようになり，従来診断できなかったものが診断可能となってきている．ヘルペス属ウイルスは診断ができれば，抗ヘルペス薬，抗サイトメガロウイルス薬によって対応することができるので，多彩な疾患の原因となりうることを十分理解し，原因として少しでも疑われる疾患があれば検査をしてみることが重要である．

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30. サイトメガロウイルス角膜内皮炎

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近年，日和見感染症の原因として知られるサイトメガロウイルスが，免疫機能正常者における難治性の角膜内皮炎の原因となることが報告されている．本疾患では，コインリージョンとよばれる特徴的な角膜後面沈着物を認め，虹彩炎や高眼圧を伴うことが多い．水疱性角膜症に至る重症疾患であり，前房水PCR (polymerase chain reaction) を用いた早期診断と，抗ウイルス薬による治療が必要である．

角膜内皮炎は角膜内皮細胞に特異的な炎症を生じる疾患で，1982年にKhodadoustらによって報告された．当時は自己免疫疾患と考えられていたが，その後の研究により，単純ヘルペスウイルス (HSV) や水痘帯状疱疹ウイルス (VZV) などのウイルス感染が関与していることが知られるようになった．最近になって，抗ヘルペス薬による治療が奏効しない難治性の角膜内皮炎のなかに，サイトメガロウイルス (CMV) によるものが少なからずあることが報告され，注目されている[1~3]．

●CMV角膜内皮炎の臨床的特徴

角膜内皮炎では，細胞浸潤や血管侵入を伴わない限局性の角膜浮腫と，浮腫の範囲に一致した角膜後面沈着物 (keratic precipitates：KPs) が認められる (図1，2) が，特にCMV角膜内皮炎では円形に配列したKPsからなる衛星病巣 (コインリージョン) を伴うことが特徴である (図1)．ヒト角膜内皮細胞は生体内における増殖能が乏しいため，角膜内皮細胞の障害によって角膜内皮細胞密度の低下を生じ，進行すると水疱性角膜症に至る．片眼性の症例が多いが両眼性症例も報告されている．CMV角膜内皮炎の症例では，虹彩毛様体炎や続発緑内障を合併していることが多い．CMV角膜内皮炎は免疫機能不全のない患者にも発症することが特徴である．

●CMV角膜内皮炎の診断と治療

診断には，PCR (polymerase chain reaction) を用いた前房水中のCMV DNAの検索が有用である．PCRは非常に感度が高いため，病態と無関係のウイルスDNAを検出する可能性があるため注意が必要で，PCRの結果と臨床所見，抗ウイルス治療に対する反応などを総合的に判断してCMV角膜内皮炎と診断する必要がある．平成22年度厚生労働省難治性疾患克服研究事業「特発性角膜内皮炎研究班」が提唱したCMV角膜内皮炎の診断基準を**表1**に示す．

CMV角膜内皮炎に対する治療は，CMV網膜炎に準じた抗CMV療法と，消炎を目的としたステロイド療法を併用する．初期治療としてガンシクロビル全身投与

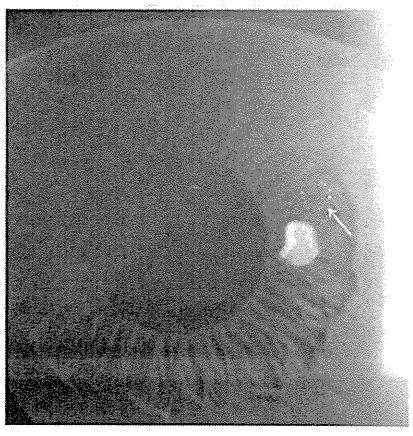

図1　CMV角膜内皮炎 (51歳，男性)
上方周辺部から中央へと進行する角膜浮腫を認め，透明角膜側にはコインリージョンが存在する (矢印)．前房水PCRでCMV DNAを検出し，ガンシクロビル治療が有効であった．(文献1より改変)

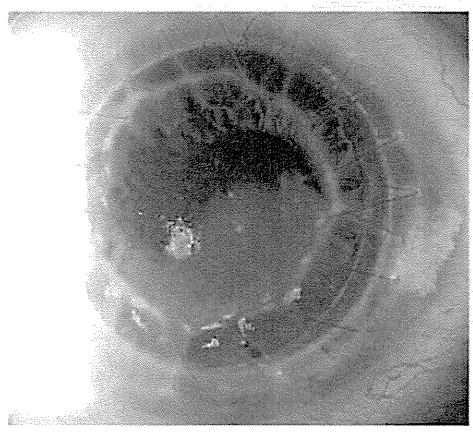

図2　角膜移植後に発症したCMV角膜内皮炎 (78歳，女性)
角膜混濁に対する角膜移植の5カ月後に，下方から中央へ進行する角膜浮腫と浮腫に一致した範囲の角膜後面沈着物を認めた．ステロイド治療には反応せず，前房水からCMV DNAを検出した．(文献5より)

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