Graefe's Arch Clin Exp Ophthalmol (1996)
234: S 133–S 138 © Springer-Verlag 1996

Justus Garweg
Matthias Böhnke

Slow viral replication of HSV-1 is responsible for early recurrence of herpetic keratitis after corneal grafting

Received: 18 April 1995
Revised version received:
13 November 1995
Accepted: 13 November 1995

J. Garweg (✉) · M. Böhnke
Department of Ophthalmology,
University Hospital Bern, CH-3010 Bern,
Switzerland
Tel. +41-31-632 85 38;
Fax +41-31-381 70 66

Abstract ● Background: The presence of herpetic DNA has been shown in diseased and healthy corneal tissue. A clinical correlation with the activity of the disease has not yet been demonstrated. This study was done to evaluate the use of DNA amplification for HSV-1 from different sites for the clinical prognosis after corneal grafting. ● Patients and methods: Eighteen patients with herpetic keratitis, 8 patients with other forms of keratitis, and 15 patients with corneal disease unrelated to herpes undergoing penetrating keratoplasty were investigated. From these, aqueous humor was obtained at the time of surgery. The excised cornea was divided into three parts for paraffin embedding, 24 h tissue culture and preparation of minced tissue. All samples were processed for HSV-1 glycoprotein D PCR followed by Southern blot and DNA hybridization. ● Results: In the herpes group, target DNA was detected in 4/18 aqueous humor samples, 7/16 minced tissue preparations, 6/18 explant culture fluid samples and 4/15 paraffin sections. In the control groups of other keratitis and non-herpetic eye disease, respectively, target DNA was found in 0/5 and 2/12 aqueous humor samples, 1/6 and 0/12 minced tissue preparations, 0/8 and 0/15 explant culture fluid samples and in 1/6 and 1/14 paraffin sections. Five of six patients in whom herpes DNA was detected in the short-term tissue culture experienced an episode of herpes reactivation within 4 months after transplantation, whereas only one of the remaining patients in all three groups did so ($p=0.0005$). ● Conclusion: A slow viral replication may be responsible for early recurrence of herpetic keratitis after corneal grafting. Detection of herpetic DNA in short-term tissue cultures from explant tissues may help to define the patients at risk.

Introduction

Herpetic keratitis is thought to be the most frequent cause of corneal blindness in developed countries [11], with a prevalence of 1.5 per 1000 persons [23].

According to current concepts, recurrent herpetic stromal disease results from reactivation of the latent virus within the trigeminal ganglion followed by transneuronal spread into the cornea [12, 25]. Ganglionic latency as a source for viral reactivation was first suspected by Goodpasture [16]. Clinical evidence for this was found by Stevens and Cook, who recovered replicative virus from trigeminal ganglia 3–4 months after footpad inoculation in mice [39]. Silent viral shedding from the infected ganglia into the tear film has been shown [18], as has the presence of viral DNA in the cornea [34] without evidence of clinical disease.

Replication of the virus within the cornea has been suspected in cases of active stromal keratitis because of the electron-microscopic demonstration of viral parti-

cles in the corneal stroma [12]. The successful isolation of virus from cultured explant corneae in cases of stromal keratitis supported this view [9, 13, 38, 41]. However, the long time of 5–11 days until isolation of living virus from cultured corneae led to the idea of viral latency in the cornea and in vitro reactivation [13, 29]. The application of different molecular biological techniques has recently reinforced this concept [3, 6, 7, 15, 36]. The clinical significance of corneal latency for the recurrence of disease, however, is unknown.

The aim of this study was to define patients at risk for herpes reactivation after corneal grafting by means of the polymerase chain reaction (PCR) technique. We combined short-term corneal explant culture with DNA amplification using the PCR, followed by Southern blot analysis of aqueous humor, minced fresh explant tissue, paraffin sections and culture fluid after 24 h.

Patients and methods

Eighteen consecutive patients with the clinical diagnosis of herpetic keratitis, 8 patients with interstitial keratitis and 15 controls with nonherpetic corneal disease were included in the study (Table 1).

The diagnosis of herpetic keratitis was established when (1) a history of dendritic keratitis or recurrent stromal keratitis within the last few years was reported and/or (2) recurrent stromal disciform keratitis with endothelial precipitates, recurrent diffuse interstitial keratitis with deep stromal vascularization or acute stromal necrosis was documented which had responded to combined systemic Acyclovir and local steroid treatment [22, 43]. The disease activity and treatment at the time of surgery in the herpetic keratitis group are detailed in Table 1.

All eyes were rinsed with 0.6% polividone-iodine solution and 10 ml of balanced salt solution, pH 7.2, at the beginning of surgery. Aqueous humor was obtained at the beginning of surgery with a 30-gauge needle and a tuberculin syringe. The aqueous humor was pelleted (10000 *g*, 10 min) and the supernatant removed. The pellet was resuspended in 50 µl K buffer (Tris-Cl 10 mM, KCl 50 mM, $MgCl_2$ 2 mM, Tween 20 0.5%, proteinase K 0.1 mg/ml, pH 8.3), covered with two drops of light mineral oil, heated to 56° C for 1 h and to 95° C for 10 min, then stored at −20° C until used for DNA amplification [19].

The excised corneal tissue was divided into two parts immediately after surgery. One half was fixed in a buffered 4% formaldehyde solution and embedded in paraffin. From this tissue, three sections with a thickness of 10 µm each were processed in 50 µl K buffer as described above.

From the remaining corneal tissue, a small sample (thickness approximately 0.5–1 mm) was dissected, minced and lysed in 100 µl of K buffer, sedimented and the supernatant used for DNA amplification. The remaining tissue was placed in organ culture [3 ml Eagle's MEM, supplemented with 10% fetal calf serum and penicillin (100 µg/ml), streptomycin (100 µg/ml), and amphotericin B (0.3 µg/ml)] and incubated for 24 h at 32° C in a humidified atmosphere containing 5% CO_2. After 24 h of culture, a sample of the culture fluid was used for viral culture. The remaining medium was stored frozen until processed for DNA amplification.

DNA amplification and hybridization

DNA amplification was run under standard conditions [37]. The primers were located in the glycoprotein D gene of herpes simplex virus type 1 (HSV-1; primer sequences and positions see Table 2 [1]). The amplification mix consisted of 10 mM Tris (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 0.02% gelatine (w/v), 200 µM of desoxinucleoside triphosphates, 1.0 U *Thermus brockianus* DNA polymerase (Finnzymes, Espoo, Finland), 50 pmol of each primer, and a sample volume of 5 µl to a final volume of 50 µl. The amplification protocol included DNA melting at 95° C for 5 min, then 35 cycles each consisting of denaturation at 95° C for 1 min, primer annealing at 60° C for 0.5 min and fragment elongation at 72° C for 1 min, followed by a final fragment elongation step at 72° C for 7 min. All buffers and media used were routinely run as internal negative controls to exclude viral contamination. Controls for specificity included culture supernatants from cells infected with HSV-1, HSV-2, VZV or CMV. In each amplification experiment, a positive control containing 10 ng HSV-1 DNA was also included. The amplified DNA fragment had a length of 221 bp. It was visualized on a 3% NuSieve agarose gel (FMC Bioproducts, Rockland, Me., USA) after staining with ethidium bromide 0.01% and compared to a routinely run molecular weight marker (pBR322, *Hae*III Digest; Sigma Chemicals, Louisville, Mo., USA), then transferred onto nylon membranes (Hybond N^+, Amersham, Arlington Heights, Ill., USA) by capillary blotting [40] and alkali fixed with 0.4 N NaOH [33]. For DNA hybridization, we used a buffer containing 50% formamide, 5×SSC, 0.02% SDS, 0.1% *N*-lauroyl-sarcosine (w/v), 0.1% BSA and 20 µg/ml salmon sperm DNA. After prehybridization for 1 h, 20 pmol of digoxigenin-labeled probe was added. This probe was generated by introduction of digoxigenin-11-dUTP (Boehringer Mannheim,

Table 1 Patient data (*ACV*= systemic antiviral treatment with Acyclovis)

Group	*n*	No antiviral therapy	ACV at surgery
Herpetic keratitis	18	11	7
Inactive scar		10	1
Acute keratitis/keratouveitis		1	3
Chronic stromal keratitis		0	3
Undetermined keratitis	8	7	1
Acute corneal ulceration in elderly		1	
Stromal keratitis of unknown origin		3	
State after multiple rejections		2	
Corneal degeneration	15	15	0
Keratoconus		7	
Secondary endothelial atrophy		5	
Chemical burn		1	
Chronic keratitis		2	

Table 2 Primer sequences and location in the glycoprotein D gene of HSV-1 (primer sequences according to [1])

Primer	Sequence (5'–3')	Location
Outer		
BJHSV1.1	ATCACGGTAGCCCGGCCGTGTGACA	19–43
BJHSV1.2	CATACCGGAACGCACCACACAA	218–239
Inner[a]		
BJHSV1.3	CCATACCGACCACACCGACGA	51–71
BJHSV1.4	GGTAGTTGGTCGTTCGCGCTGAA	166–188

[a] The inner primers were used only to generate the random labeled DNA probes

Table 3 Results of HSV-1 glycoprotein D gene ampliciation (number of positive samples)

	Nonherpetic corneal disease	Undefined keratitis	HSV-1 keratitis
Aqueous humor	0/12	0/5	4/18
Minced tissue	0/12	1/6	7/16
Explant culture	0/15	0/8	6/18
Paraffin sections	1/14	1/6	4/15
Total	3/53	2/25	21/67*

* $P=0.0002$ (chi-squared test)

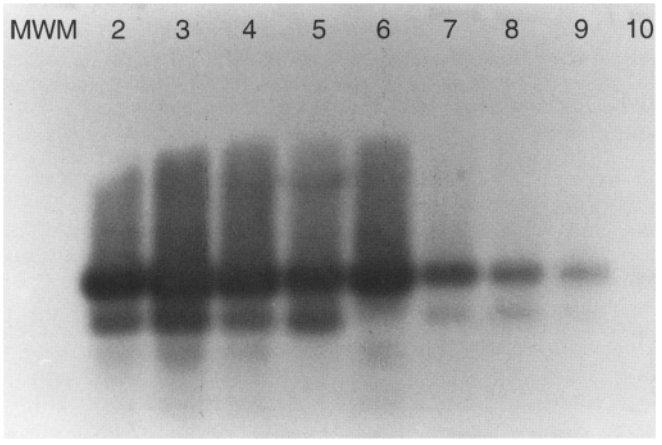

Fig. 1 Southern blot detection of glycoprotein D gene DNA after PCR amplification using a digoxigenin random-labelled probe. *Lane 1* Molecular weight marker (*MWM*) pBR322·*Hae*III digest (Sigma Chemicals, St. Louis, Mo., USA); *lanes 2–10* 5, 5×10^{-1}, 5×10^{-2}, 5×10^{-3}, 2×10^{-3}, 1×10^{-3}, 5×10^{-4}, 2×10^{-4}, 1×10^{-4} pfu of HSV-1. 2×10^{-4} pfu of HSV-1 are detected

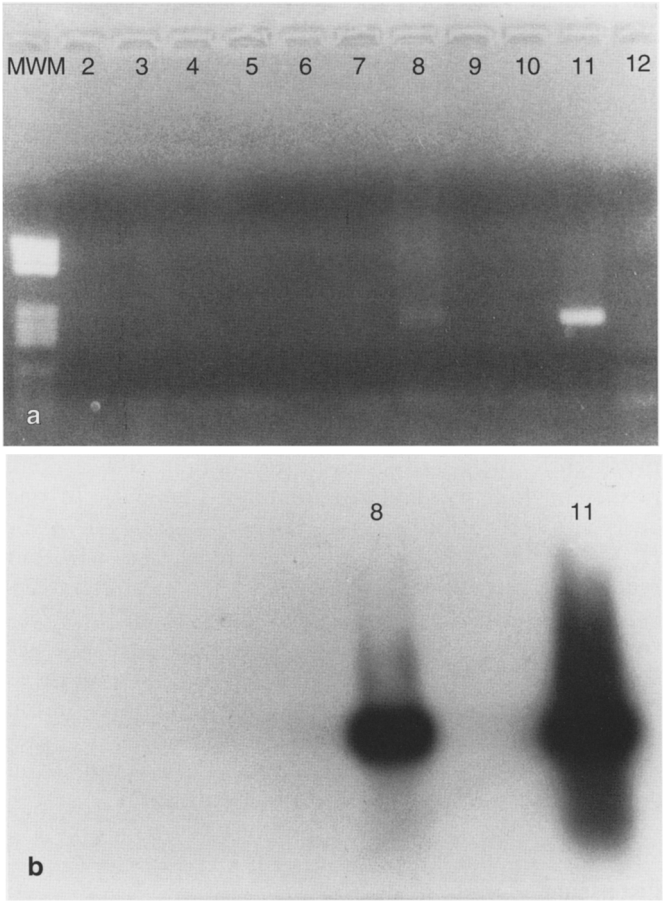

Fig. 2 **a** DNA amplification from herpes keratitis samples. 3% NuSieve agarose gel, staining with 0.01% ethidium bromide. *Lane 1* molecular weight marker pBR322·*Hae*III digest, *lane 2* aqueous humor, *lane 3* culture medium, *lanes 4, 7, 9, 10* paraffin sections, *lanes 5, 6, 8* minced tissue samples, *lane 11* positive control containing 5×10^{-2} pfu of HSV-1, *lane 12* negative control (PCR buffer). **b** Southern blot and DNA hybridization with a nonradioactive probe to **a**

Germany) in a nested PCR setting using internal primers (location 51–71 and 166–188 [1]). The conditions and protocol for this nested reaction were as described above, except for a sample volume of 1 µl, an annealing temperature of 62° C and 30 amplification cycles. Bound probe was detected according to the manufacturer's instructions (Boehringer Mannheim, Germany). In short, the membrane was incubated with an alkaline phosphatase-conjugated antibody to digoxigenin. AMPPD (Lumigen-PPD; Boehringer Mannheim, Germny) served as substrate for the alkaline phosphatase. Finally, the membrane was exposed to an X-ray film (Fuji RX) for 30 min at room temperature. Each result was confirmed by two independent control amplifications. Additionally, DNA amplification of a fragment of the thymidine kinase gene (amplificate length of 110 base pairs) was performed [24].

Results

Testing the specificity of the amplification system using clinical isolates of different viruses of the herpes family, we found an amplification only for HSV-1. In the ethidium bromide-stained agarose gel, we detected DNA from 10^{-3} plaque-forming units (pfu; data not shown). With Southern blot and DNA hybridization, 2×10^{-4} pfu were detectable (Fig. 1).

The results of DNA amplification and hybridization from patient samples are summarized in Table 3. The

Table 4 Correlation of results in HSV-1 patients (/ sample not available, *i* inactive scar, *c* chronically active keratitis, *a* acute stromal keratitis/keratouveitis)

	Patient number																	
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18
DNA amplified from																		
Aqueous humor	+	−	+	+	−	−	−[a]	−	−	−	−	−	−	−	−	+[b]	−	−
Explant culture	+	+	+	−	−	−	−	−	−	(+)	−	−	−	−	−	+	+	−
Minced tissue	/	−	+	−	/	−	(+)	−	−	−	+	+	+	+	−	−	+	+
Paraffin sections	−	−	−	+	+	−	+	−	−	−	−	−	/	−	−	/	/	+
Viral activity at surgery	i	a	a	a	i	i	i	i	i	c	i	c	i	i	i	a	c	i
Clinical reactivation	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	+	+	−

[a] In a vitreous sample taken at the same time as HSV-1 DNA was detected
[b] Same patient as 04, re-keratoplasty after 19 months due to graft failure

amplification from aqueous humor, paraffin sections, fresh tissue and explant cultures did not correlate with the clinical activity of the keratitis. Additionally, herpetic DNA was detected in fresh and paraffin sections from some patients with interstitial keratitis and nonherpetic corneal disease (Fig. 2).

However, we observed a recurrence of herpetic keratitis in six patients within 4 months after transplantation: once without Acyclovir therapy, twice a few weeks after stopping Acyclovir therapy and three times despite oral therapy with Acyclovir 2–3×400 mg daily. In four of these cases, HSV-1 DNA had been detected in the overnight explant culture fluid. In another case, a weak positive signal was not reproducible (Table 4). In one case of reactivation, explant culture was negative for HSV-1 DNA, and in another, culture was positive but recurrence was not observed within 1 year postoperatively under systemic Acyclovir prophylaxis. In three patients of the herpetic keratitis group, no viral DNA was detected in any of the materials (Table 4). None of these patients experienced a recurrence of the keratitis in the graft (χ^2 test: $P=0.0005$).

Living virus was not detected in viral cultures from aqueous humor (50 µl) or in explant cultures.

Discussion

Corneal infection with herpes simplex may present in various forms, ranging from mild epithelial infection to severe stromal necrosis. The reactivation of viral disease as well as the immune response in the cornea may contribute to clinical disease [22, 43]. Recurrence of herpes in the corneal transplant impairs the prognosis of corneal grafting [5, 14]. The transplantation trauma itself was only recently established to be a potent stimulus for a recurrence of the disease after transplantation [26].

With the application of DNA amplification [37], a viral etiology of keratitis may be confirmed [10, 17, 27]. However, a high incidence of HSV-1 DNA has been observed in healthy corneal transplant tissue [3, 4, 30], sometimes leading to total endothelial necrosis in culture without evidence of preexisting clinical disease [2, 4, 42]. Vice versa, viral DNA has sometimes not been detected in cases of active clinical disease [17]. Thus, the method needs further evaluation before its application to clinical samples.

This study was done to define the incidence of viral DNA from different ocular sites after DNA amplification. Furthermore, we wanted to establish whether release of HSV-1 DNA into the culture fluid was possible. To exclude an in vitro reactivation of latent virus as a source for this, a culture interval of only 24 h was chosen. Thus, we wanted to overcome the drawbacks of the single methods. Glycoprotein D gene amplification was chosen, because this region of the viral genome is highly preserved and thus not strain dependent [1]. Moreover, the clinical use of glycoprotein D gene amplification is well established. Finally, the sensitivity of glycoprotein D gene PCR was, in our hands, superior to that of the often used thymidine kinase gene [24], in accordance with the results from other laboratories [20]. In their article about glycoprotein D PCR, Aurelius et al. emphasized the value of nested PCR in improving the sensitivity of the test system [1]. Cantin et al. clearly demonstrated the great difference between non-nested and nested PCR on paraffin sections of herpetic corneae [3]. One has to expect a high proportion of false-positive results with nested PCR. The relevance of the low copy number that requires nested PCR for its detection is not known [21]. Thus, we decided not to use a nested setting, but to increase the sensitivity and specificity by DNA hybridization to increase its clinical relevance.

Our results from paraffin sections and from fresh corneal tissue are in accordance with published data [3, 17, 30, 35]. A positive amplification signal from any single source investigated does not necessarily establish a clinical diagnosis. The detection of viral DNA in the aqueous humor, however, showed some association with the clinical activity of disease at the time of surgery, but

also with early postoperative viral reactivation. The origin of this viral DNA in the aqueous humor is subject to speculation. Most likely it comes from the iris and ciliary body and not from the cornea. Thus, reactivation or chronic subclinical viral activity not only in the cornea, but also in the uvea has to be assumed. In one patient with an inactive corneal scar and no signs of viral activity, HSV-1 DNA was amplified from the aqueous humor. This patient developed an early postoperative recurrence of herpetic keratitis in the graft (patient 1, Table 4).

Moreover, we were not able to detect HSV-1 DNA in any of the materials from some patients with herpetic keratitis (patients 6, 8, 9 and 15, Table 4). Detection of viral DNA in multiple samples from a single patient may predict increased risk for HSV reactivation in the graft. This is currently under investigation.

The important finding of our study is the demonstration of HSV-1 DNA in short-term corneal explant cultures. A simple wash-out of herpetic DNA from the excised cornea is not likely, because no correlation between the presence of HSV-1 DNA in corneal tissue and in the culture fluid was found. In two samples, no HSV DNA was detected in minced tissue, whereas it was demonstrated in the explant cultures (Table 4). For in vitro reactivation of herpes in the cornea, a time of 24 h is obviously too short [13]. Though viral cultures of tear film have not been done, contamination from this site is not likely after application of 0.6% polividone-iodine and washing of the conjunctival sac with saline prior to surgery [32].

Several molecular biological studies have addressed the molecular site of viral latency in corneal disease [6–9, 24, 28, 31]. The clinical relevance of these studies is an improved understanding of the mechanism of viral reactivation and the identification of patients at risk [15, 44].

In contrast to this, our results provide evidence of a slow viral replication in the cornea in distinct cases. The replicative activity at the time of surgery was too low to be detected by viral culture, but the short-term tissue culture may have increased the viral DNA to detectable levels in five cases and to a nearly detectable level in a sixth case. Clinically, five of the six positive patients experienced an episode of herpes reactivation in the transplant within 4 months after corneal grafting. In conclusion, we suggest that slow viral replication, rather than reactivation of the latent virus in the cornea, contributes at least partially to early reinfection of the corneal graft and an increased rate of recurrence after corneal transplantation.

Acknowledgement This study was supported in part by a grant of the Swiss Science Foundation No. 32-39730.93.

References

1. Aurelius E, Johannson B, Sköldenberg B, Staland A, Forsgren M (1991) Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. Lancet 337: 189–192
2. Böhnke M, Garweg J, Germann D (1992) Spontaneous endothelial necrosis in human donor cornea. International Conference on Herpetic Eye Disease, New Orleans, La, Abstract 23: 4
3. Cantin EM, Chance J, McNeal J, Willey DE, Openshaw H (1991) Detection of herpes simplex virus DNA sequences in corneal transplant recipients by polymerase chain reaction assays. Curr Eye Res 10 [Suppl]:15–21
4. Cleator GM, Klapper PE, Dennett C, Sullivan AL, Bonshek RE, Marcyniuk B, Tullo AB (1994) Corneal donor infection by herpes simplex virus: herpes simplex virus DNA in donor corneas. Cornea 13: 294–304
5. Cobo LM, Coster DJ, Rice NS, Jones BR (1980) Prognosis and management of corneal transplantation for herpetic keratitis. Arch Ophthalmol 98: 1755–1759
6. Cook SD, Brown SM (1986) Herpes simplex virus type I persistence and latency in cultured epithelial cells, keratocytes, and endothelial cells. Br J Ophthalmol 70: 642–650
7. Cook SD, Hill JH (1991) Herpes simplex virus: molecular biology and the possibility of corneal latency. Surv Ophthalmol 36: 140–148
8. Cook SD, Aitken DA, Loeffler KH, Brown SM (1986) Herpes simplex virus in the cornea; an ultrastructural study on viral reactivation. Trans Ophthalmol Soc UK 105: 634–641
9. Coupes D, Klapper PE, Cleator PM, Bailey AS, Tullo AB (1986) Herpes virus simplex in chronic human stromal keratitis. Curr Eye Res 5: 735–738
10. Crouse CA, Pflugfelder SC, Pereira I, Cleary T, Rabinowitz S, Atherton SS (1990) Detection of herpes virus genomes in normal and disease corneal epithelium. Curr Eye Res 9: 569–581
11. Dawson CR, Togni D (1976) Herpes simplex eye infections: clinical manifestations, pathogenesis and management. Surv Ophthalmol 21: 121–135
12. Dawson C, Togni B, Moore TE (1968) Structural changes in chronic herpetic keratitis. Arch Ophthalmol 79: 740–747
13. Easty DL, Shimeld C, Claque CPM, Menage M (1987) Herpes simplex isolation in chronic stromal keratitis: human and laboratory studies. Curr Eye Res 6: 69–74
14. Ficker LA, Kirkness CM, Rice NS, Steele AD (1988) Longterm prognosis for corneal grafting in herpes simplex keratitis. Eye 2: 400–408
15. Gamus D, Romano A (1988) Herpetic imprint and privileged areas of its target organ: local latency and reactivation in herpetic keratitis. Ped Syst Ophthalmol 11: 37–40
16. Goodpasture EW (1929) Herpetic infections with special reference to involvement of the nervous system. Medicine (Baltimore) 8: 223–2430
17. Holbach LM, Font RL, Bear W, Tittler SJ (1991) HSV antigens and HSV-DNA in avascular and vascularized lesions of human herpes simplex keratitis. Curr Eye Res 10 [Suppl]:63–68
18. Kaufman HE, Brown DC, Ellison EM (1967) Recurrent herpes in the rabbit and man. Science 156: 1628–1629

19. Kawasaki I (1990) Sample preparation from blood, cells, and other fluids. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols. Academic Press, San Diego, Calif, pp 146–152
20. Klapper PE, Cleator, Tan SV, Guiloff RJ, Scaravilli F, Ciardi M, Aurelius E, Forsgren M (1993) Diagnosis of herpes simplex encephalitis with PCR. Lancet 341:691
21. Kwok S, Higushi R (1989) Avoiding false positives with PCR. Nature 339:237–238
22. Liesegang TJ (1988) Ocular herpes simplex infection: pathogenesis and current therapy. Mayo Clin Proc 63:1092–1105
23. Liesegang TJ, Melton LJ III, Daly PJ, Ilstrup DM (1989) Epidemiology of ocular herpes simplex: incidence in Rochester. Minnesota, 1950 through 1982. Arch Ophthalmol 197:1155–1159
24. Lynas, C, Cook SD, Laycock KA, Bradfield JW, Maitland NJ (1989) Detection of latent virus mRNA in tissues using the polymerase chain reaction. J Pathol 157:285–289
25. Nesburn A, Cook M, Stevens JG (1972) Latent herpes simplex infection from trigeminal ganglia of rabbits with recurrent eye infections. Arch Ophthalmol 88:412–417
26. Nichols SM, Benylles A, Shimeld C, Easty DL, Hill TJ (1994) Recurrent herpes simplex after corneal transplantation in rats. Invest Ophthalmol Vis Sci 35 [Suppl]:1681
27. Ohashi Y, Yamamoto S, Kohj N, Okamoto S, Kinoshita S, Hayashi K, Manabe R (1991) Demonstration of herpes simplex virus DNA in idiopathic corneal endotheliopathy. Am J Ophthalmol 112:419–423
28. O'Neal FJ, Goldberg RJ, Rapp F (1972) Herpes simplex virus latency in cultured human cells following treatment with cytosine arabnoside. J Gen Virol 14:189–197
29. Openshaw H (1983) Latency of herpes simplex virus in ocular tissue of mice. Infect Immun 39:960–962
30. Openshaw H, McNeill JI, Lin XH, Chen J, Cantin EM (1992) HSV-1 DNA sequences in human and rabbit corneas. International Conference on Herpetic Eye Diseases, New Orleans, La, Abstract 21:9
31. Pavan-Langston D, Rong BL, Dunkel EC (1989) Extraneuronal herpetic latency: Animal and human corneal studies. Acta Ophthalmol 67 [Suppl]:135–141
32. Rackur FM (1985) New aspects of mechanism of action of povidone-iodine. J Hosp Infect 15:13–23
33. Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acid Res 13:7207–7221
34. Rong BL, Kenyon KR, Bean KM, Langston DP, Dunkel EC (1988) Detection of the HSV genome in human corneal buttons. Invest Ophthalmol Vis Sci 29 [Suppl]:158
35. Rong BL, Pavan-Langston D, Weng QP, Martinez R, Cherry JM, Dunkel EC (1991) Detection of herpes simplex thymidine kinase and latency-associated transcript gene sequences in human herpetic corneas by polymerase chain reaction amplification. Invest Ophthalmol Vis Sci 32:1808–1815
36. Sabbaga EM, Pavan-Langston D, Bean KM, Dunkel EC (1988) Detection of HSV nucleic acid sequences in the cornea during acute and latent ocular disease. Exp Eye Res 47:545–553
37. Saiki RK, Gelfand DH, Stoffel S, Sharf SJ, Higuchi R, Horn GT, Mullis KB, Ehrlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA-polymerase. Science 239:487–490
38. Shimeld C, Tullo AB, Easty DL, Thomsitt J (1982) Isolation of herpes simplex virus from the cornea in chronic stromal keratitis. Br J Ophthalmol 66:643–647
39. Stevens JG, Cook ML (1971) Latent herpes simplex virus in spinal ganglia of mice. Science 173:843–845
40. Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517
41. Tullo AB, Easty DL, Shimeld C, Stirling PE, Darville JM (1985) Isolation of herpes simplex virus from corneal discs of patients with chronic stromal keratitis. Trans Ophthalmol Sco UK 104:159–165
42. Tullo AB, Marcyniuk B, Bonshek R, Dennett C, Cleator GM, Lewis AG, Klapper PE (1990) Herpes virus in a corneal donor. Eye 4:766–767
43. Wilhelmus KR (1987) Diagnosis and management of herpes simplex stromal keratitis. Cornea 6:286–291
44. Wilhelmus KR, Coster DJ, Donovan HC, Falcon MG, Jones BR (1981) Prognostic indicators of herpetic keratitis. Analysis of a 5 year observation period after corneal alteration. Arch Ophthalmol 99:1578–1582