Efficacy of transfer factor in treating patients with recurrent ocular herpes infections

Renato Meduri¹, Emilio Campos¹, Lucia Scorolli¹, Caterina De Vinci², Giancarlo Pizza² & Dimitri Viza³
¹Eye Physiopathology Clinical Service, University of Bologna, Italy; ²Immunotherapy Unit, 1st Division of Urology, S. Orsola-Malpighi Hospital, Bologna, Italy; ³Laboratoire d’Immunobiologie, URA 1294 CNRS, Faculté de Médecine des Saints-Pères, Paris, France

Key words: Cell-mediated immunity, herpes simplex virus, keratitis, ocular herpes, transfer factor, uveitis

Abstract

Recurrent ocular herpes is an insoluble problem for the clinician. As cellular immunity plays an important role in controlling herpes relapses, and other studies have shown the efficacy of HSV-specific transfer factor (TF) for the treatment of herpes patients, an open clinical trial was undertaken in 134 patients (71 keratitis, 29 kerato-uveitis, 34 uveitis) suffering from recurrent ocular herpetic infections. The mean duration of the treatment was 358 days, and the entire follow-up period 189121 before, and 64062 days after TF treatment. The cell-mediated immune response to the viral antigens, evaluated by the lymphocyte stimulation test (LST) and the leucocyte migration test (LMT) (P<0.001), was significantly increased by the TF treatment. The total number of relapses was decreased significantly during/after TF treatment, dropping from 832 before, to 89 after treatment, whereas the cumulative relapse index (RI) dropped, during the same period, from 13.2 to 4.17 (P<0.0001). No side effects were observed. It is concluded that patients with relapsing ocular herpes can benefit from treatment with HSV-specific TF.

Abbreviations: C.equ.: cell equivalent; CMI: cell-mediated immunity; CMV: cytomegalovirus; EBV: Epstein-Barr virus; HIV: human immunodeficiency virus; HK: herpes keratitis; HSV: herpes simplex virus; KU: kerato-uveitis; LMT: leucocyte migration test; LST: lymphocyte stimulation test; MIF: migration inhibition factor; RHK: relapsing herpes keratitis; RI: relapse index; TF: transfer factor; VZV: varicella zoster virus

Introduction

Current therapy for relapsing herpes keratitis (RHK) is unsatisfactory and restricted to the acute phase of the illness with topical applications and/or systemic administration of interferons and other antivirals, e.g. acyclovir. However, no treatment to eradicate the persistent viral infection causing the relapses is available.

It is known that healthy adults with humoral immunity to various ubiquitous viruses, e.g. cytomegalovirus (CMV), herpes simplex (HSV) and varicella-zoster virus (VZV), also present an adequate cellular immune response, as assessed by the lymphocyte stimulation test (LST) in presence of the corresponding viral antigens [1–3]. The work of Centifanto et al. [4] and our own studies [5–7] have shown that during remission, patients suffering from genital or labial herpes display, probably transiently, insufficient cell-mediated immunity (CMI) to HSV as assessed by the leucocyte migration test (LMT) [8–10]. The CMI response to HSV antigens in these patients increases 6–8 days after a relapse, with a subsequent decrease over time [5,6]. Similarly, in a preliminary study we observed that patients suffering from RHK have deficient CMI responses to HSV-1, and also to CMV [11].

HSV-specific transfer factor (TF) has previously been successfully used for treating patients with genital and labial herpes infections [5,6,12–14]. Furthermore, encouraging results using TF have been reported by Abramson et al. [15] in treating uveitis patients while in a limited number of RHK patients, we have confirmed that HSV-specific TF reduces the number of relapses and also significantly decreases HSV-1-caused opacities in rabbits’ eyes [6,16]. These results
were recently confirmed in a pilot study concerning a limited number of patients [11]. The data reported here were gathered on 134 patients and continue to support the initial contention: HSV-specific TF is efficacious in treating patients with relapsing ocular herpes.

**Material and Methods**

**Patients.** 134 patients (67 male, 67 female), 72 suffering from herpes keratitis (HK), 29 from kerato-uveitis (KU) and 33 from uveitis (U), aged 4–81 years were assessed for the presence of antibodies against HSV-1 and CMV, and were treated with HSV-1/2-specific TF. The cellular immune response to CMV and HSV-1 was assessed using the LMT in 70 and the LST in 63 patients. Prior to TF administration, all uveitis patients received topical applications of corticosteroids during their relapses, whereas KU and HK patients were treated topically with α-interferon and/or acyclovir. Criteria for relapse in uveitis were: moderate to severe ocular pain, circumcorneal ciliary injection, photophobia, turbidity of the aqueous humour with appearance of inflammatory cells and diminished visual acuity. For keratitis, relapse criteria were: recurrence of dendritic keratitis with characteristic branched lesions of the cornea, foreign-body sensation, lacrimation, photophobia, conjunctival injection, corneal ulceration. Disappearance of the reported symptoms was correlated with remission.

**Transfer factor.** Bovine transfer factor was produced and its activity assessed as previously described [5, 6, 13]. Briefly, calves were injected with HSV-1 and HSV-2 live viruses, and sacrificed 3–4 weeks later after skin testing had produced evidence for reactivity to HSV. The activity of the dialysate obtained from the calves’ lymphocytes was assessed in vitro using the LMT, and in vivo using a protection-to-lethal-HSV-challenge mouse model [7]. CMV/EBV-specific TF was obtained from a patient with nasopharyngeal carcinoma in remission and strongly reactive against both viruses [17, 18]. Active dialysates thus obtained were replicated in vitro using standard methods established in our laboratory [19, 20]. The in-vitro-replicated TF was encapsulated and orally administered at an average dose of 4x10⁸ cell equivalent (c.equ.) per week in the first 2 weeks of treatment (induction phase), and then at 10⁸ c.equ. per week for the following 6–12 months.

**Immunological assays.** ELISA was used for the evaluation of anti-HSV antibody titers in the patients’ serum (Behring-Germany). The LMT was carried out as described by Centifanto et al. [4] and by Søberg et al. [9], while the LST has been described elsewhere [21]. HSV antigens used for other then ELISA studies were obtained from Ismunit (Pomezia, Italy).

**HLA soluble antigens.** Quantification of serum soluble HLA class I were performed following the double determinant immunoassay as described by Inostroza et al. [22] with minor modifications. Briefly, ELISA Pro-Bind 3915 assay plates (Becton Dickinson, Lincoln Park, NJ, USA) were coated overnight at room temperature with 100μl of mouse monoclonal antibody W6/32 (25 μg/ml) diluted with carbonate-bicarbonate buffer. Subsequently, each well was washed 5 times with phosphate buffer saline (PBS) containing 0.05% Tween-20 (PBS-Tween), and blocked with 300μl of PBS-Tween containing 3% bovine serum albumin, at 35°C for 1 hour, followed by 5 additional washings, with PBS-Tween. Serum was diluted at 1:10, 1:25, 1:50 with PBS-Tween and the diluted samples were added to the wells in triplicate and incubated for 60 minutes at 37°C. The quantity of bound sHLA was detected by adding 100μl of a 1:1000 dilution of a polyclonal β-2-microglobulin-specific antiserum conjugated with peroxidase (Glostrup, Denmark). After incubation for 60 minutes at 37°C, the wells were washed 5 times and incubated at room temperature in the dark. The colour development was read 30 minutes later, at 492 nm, in a ELISA Microplate Reader 400 (Packard, Meriden, CT, USA). Values of sHLA were standardized using standard dilutions of lyophilized soluble HLA antigens (Sang Sta, Melo Park, CA, USA).

**Results**

**Immunological studies.** With regard to viral serology, 88.8% of the patients had antibodies to CMV and 87.31% to HSV-1; 4.4% were reactive only to CMV, 3.73% only to HSV-1 and 1.5% only to VZV, whereas 61.9% were positive for CMV, HSV-1 and VZV; 15.6% for CMV and HSV-1; 6.72% for HSV and VZV. LMT results are shown in Table 1. Before treatment, 65/299 tests (21.74%) performed in keratitis patients were positive at the lowest antigen dilutions (10 and 25); during/after TF treatment a significant increase was observed: 39.47% (P<0.001). For KU patients the percentages were, 17.11% before and 42.62% after TF therapy (P<0.001), and for U patients respectively, 16.67% and 42.67% (P<0.001). No sig-