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Reactivation from Latency and Recurrence of HSV Infections Due to Impaired Th-1 Cytokines

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ABSTRACT

HSV infections are ubiquitous and cause significant morbidity worldwide. After a productive primary HSV infection, the virus gets cleared off spontaneously in the majority of the individuals. However, in a proportion of patients, the virus establishes a chronic state known as latent infections in the neurons. In general HSV-1 causes lesions above the waist in contrast HSV-2 below waist although vice versa infections are not uncommon. During latency, the HSV infects the dorsal root ganglion but it remains almost silent in terms of replication. Upon a poorly defined trigger or insult, HSV gets reactivated i.e the virus replicate enormously (prodrome phase) and migrate towards the same neuro dermatome through which it entered and cause agonizing painful necrotizing lesions of genitals or mouth. It has been a myth about what is such trigger or insult that provokes recrudescence. It has been postulated that conditions such as stress, Catamenia (menstrual cycle), fever, exposure to sunlight, radiation and trauma either alone or in the combination of multiple factors are believed to be the cause of recurrence. In this paper, we report a novel mechanism that a decrease of normal Th-1 cytokine milieu may trigger the HSV reactivation and recurrent infection.

INTRODUCTION

Herpes Simplex Viruses (HSV) infections are ubiquitous and they cause infections ranging from mild gingivostomatitis to life threatening encephalitis in immunocompromised individuals[1]. HSV consists of two types namely HSV-1 and HSV-2 and these viruses upon primary infection they reach the nervous system via the nerve ending. In the nervous system, they migrate towards the dorsal root ganglion and HSV-1 normally encroach trigeminal ganglion and HSV-2 the lumbosacral ganglion. At the periphery, HSV causes lytic infections and in the ganglion, they undergo some metamorphosis and acquire a special chronic and silent type of infection known as latent infection[2]. Once the organism causes latent infection in the neurons the virus stays in the body for the rest of the life of the patient[3]. During latent infection, the virus shuts down almost all its genes and up-regulates a new gene cluster called latency associated transcripts (LATs) which are the only transcripts found at this time. Lytic infections are prone to the immune attack of the virus and elimination. In contrast, latent infection provides a survival advantage to the virus since it remains asymptomatic and evade immune surveillance. The exact genes responsible for latency is complex. The elements surrounding the ICP-0 gene bind with a host protein called neuronal restrictive silencing factor (NRSF). This binding leads to histone deacetylation upstream of ICP-0 gene which prevents the transcription for a lytic infection[4]. In general, HSV-1 causes infections above waist and HSV-2 below waist though vice versa is also common. Though HSV-1 and HSV-2 are antigenically distinct and variable tissue tropism their trigger mechanism and reactivation appears to similar. Once triggered they undergo a lytic replication and migrate centrifugally from the CNS to the exactly same neuro dermatome through which they entered and cause devastating psychosomatic herpes disease.

While spatial regulation of viral proteins involved in the disease development cellular factors such as cytokines predominantly determines the outcome of the diseases. During HSV infections, Th-1cytokines namely IFN- γ, IL-2, and TNF-α are beneficial to host while Th-2 cytokines namely IL-10 and IL-4 are not. This is because HSV infections are intracellular and only CMI can identify and destroy them[5]. Th-1 cells, in general, promote CMI mounted by T cells. Th-2 cells generally govern the humoral immunity (HI) i.e. antibody mediated immunity. Antibodies are better to eliminate all the circulating viruses in the body fluids but their intracellular action is minimal and hence not beneficial to the host[5]. This up/down regulation of either Th-1 or Th-2 cytokine determines the outcome of HSV mediated disease.

Since T helper cytokines are such pivotal in determining the disease outcome we made an attempt to study its role of cytokines in HSV latency. For this, we collected serum samples from patients suffering from active HSV lesion and subjected for Th-1 and Th-2 cytokine estimation.

MATERIALS AND METHODS

Study population:

In this study, we enrolled 323 individuals of which 183 were HSV (1 and 2) active infection positive and 140 were HSV negative. Human ethical clearance was obtained from Institutional Human Ethical Committee (UM/IHEC/02-2017-I). A proforma was made and informed consent from each participant was obtained.

Blood Collection:

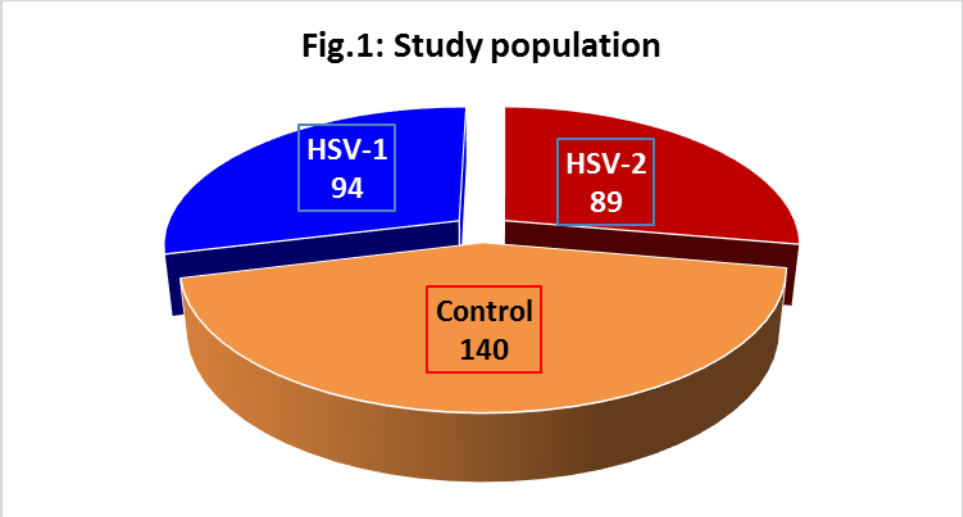
3-5 ml of whole blood was collected from the out-patient (OP) units (Voluntary Health Services, Chennai) who had lesions on genital area or oral cavity. A similar volume of blood was collected from healthy controls also. Serum was separated and stored at -20°C until usage.

Determination of HSV positivity and Cytokines profile:

The active HSV infection was confirmed by serum Anti IgM and the past HSV infection by Anti-IgG ELISA (CALBIOTECH Cat. No. H1M4908, H2M4989, H1G5010, AND H2G4881). Then the serum collected from all the patients and healthy controls were subjected to the presence of both Th-1 cytokines (IFN-γ (Cat. No. KHIFNG and IL-2 (Cat. No.900-M12) and Th-2 cytokines IL-10 (Cat. No.900-K21) and IL-4 (Cat. No900-M14) by EIA kits were procured from Thermo Fisher Scientific, Inc. USA (BD-Pharmagen). A similar study was done for serum collected from healthy individuals to confirm HSV negativity. Then the cytokine EIA results were scrutinized for any predilection towards any gender or age. EIA was performed on serum samples according to the manufacturer's instructions. Then the plates were read at 450 nm in an ELISA plate reader. A standard curve was plotted and the concentration of the unknown sample was calculated by from the standard curve.

RESULTS

Confirmation of HSV positivity of patients by ELISA:

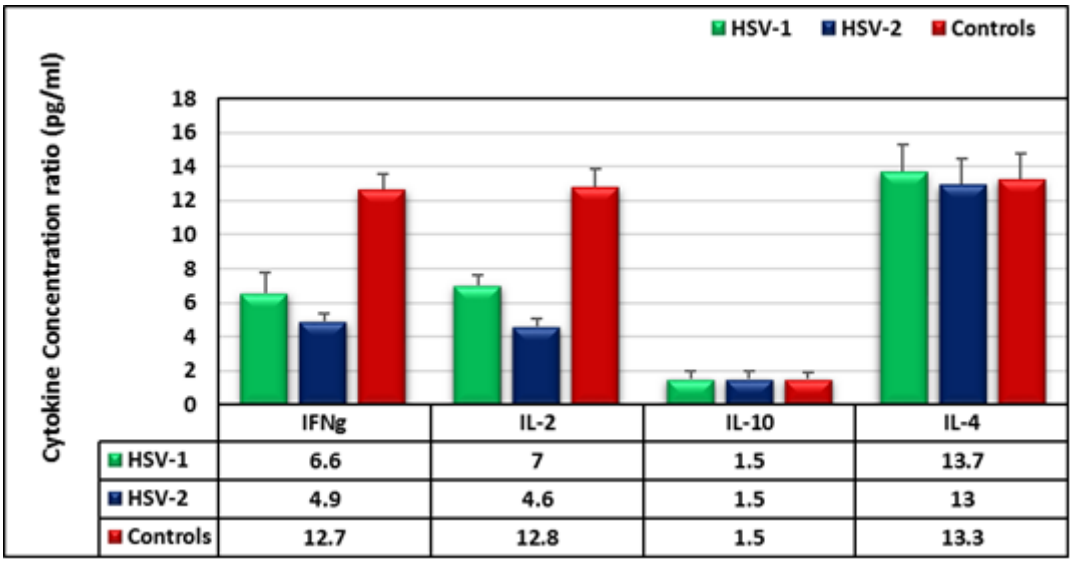


Serum samples collected from HSV patients (n=183) and controls (n=140) (Fig.1) were subjected for anti-HSV IgM and IgG and the data is provided in the table-1. Anti HSV IgM study of serum samples showed that all the patients with oral lesions were positive for anti-HSV-1 IgM and all the patients with genital lesion were positive for anti-HSV-2 IgM. (Table-1).Anti HSV IgM was not detected in the control. Taken together the data suggested that indeed patients with lesion have significantly higher IgM accentuating the active current infection. In contrast, all the HSV samples and controls had detectable levels of both anti HSV-1 and anti-HSV-2 IgG. This data suggest that all the patients (both HSV) and control had prior exposure (past infection) of both HSV infections.

Table 1: Prevalence of anti-HSV antibodies among the study group. Serum samples collected from HSV-1 and HSV-2 patients (clinically suspected) were subjected for anti-HSV IgM and IgG antibodies by ELISA. Each sample from the patients with active HSV infection and healthy controls were subjected to this evaluation.

Antibody	Specificity	HSV-1 Patients (Oral lesion) (n=94)	HSV-2 Patients (Genital lesion) (n=89)	Control (n=140)
IgM	HSV-1	94 (100%)	-	-
	HSV-2	-	89 (100%)	-
IgG	HSV-1	94 (100%)	94 (100%)	140 (100%)
	HSV-2	89 (100%)	89 (100%)	140 (100%)

Serum Cytokine levels among HSV positive and control individuals:



	IFNg	IL-2	IL-10	IL-4
HSV-1	6.6	7	1.5	13.7
HSV-2	4.9	4.6	1.5	13
Controls	12.7	12.8	1.5	13.3

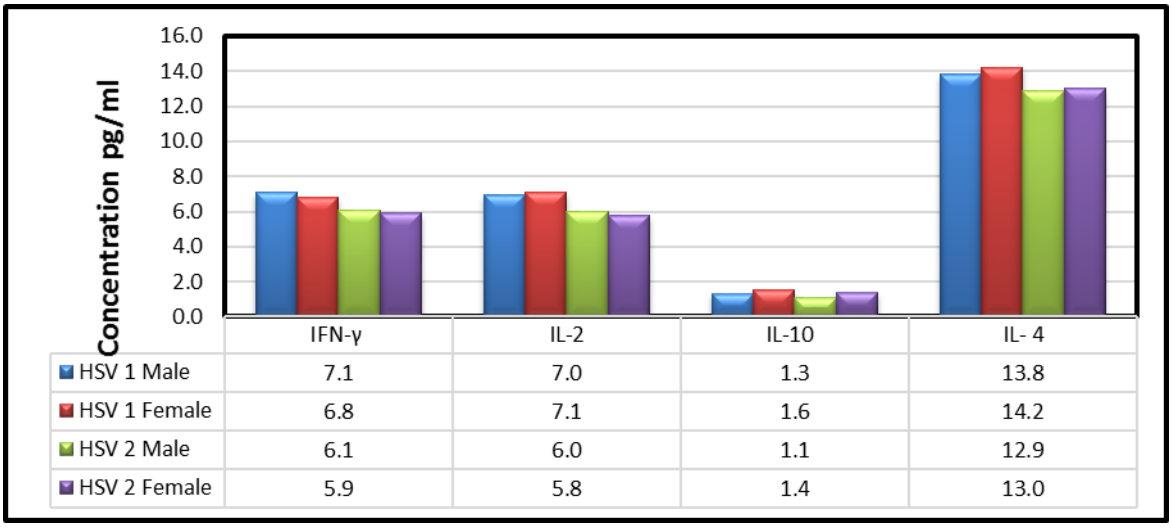
Fig 2: Shows the overall serum cytokine levels of patients with active HSV infections and healthy controls. IFNg and IL-2 are Th-1 cytokines and IL-10 and IL-4 are Th-2 cytokines, which were estimated by ELISA. The lowest detection limit of cytokine by these ELISAs was 0.05 pg/ml. The figure shows the mean ± SD of three independent experiments.

Serum Th-1 cytokine estimation of healthy control subjects showed 12.8 and 12.7 pg/ml of IL-2 and IFN-γ, respectively. Same samples when subjected for Th-2 cytokines showed 1.5 pg/ml and 13.3 pg/ml of IL-10 and IL-4, respectively. One of the key observations with HSV-1 positive individuals was that serum Th-1 cytokine concentrations were dipped almost by half (Fig-2). These individuals had IL-2 and IFN-γ concentrations at 7.1 pg/ml and 6.6 pg/ml, respectively. This reduction was statistically significant ($p<0.001$; Students *t* test) when compared with healthy controls. The decrease in Th-1 cytokines was further augmented among HSV-2 positive individuals. They showed a reduction by one third i.e. IL-2 was 4.6 pg/ml and IFN-γ was 4.9 pg/ml (Fig.2) which was again statistically significant ($p<0.001$; Students *t* test). Neither HSV-1 nor HSV-2 positive individuals showed any such alterations in Th-2 cytokines as they had comparable levels ($p>0.5$; Students *t* test). Another important observation was that there was a positive correlation with respective IgM antibody positivity and declination of Th-1 cytokine productivity (data not shown). Such an association was not observed with IgG antibody positivity. From this data, we propose that Th-1 cytokines significantly diminish among the HSV infected individuals and such an insult or trigger may provoke reactivation, recrudescence, and recurrent herpetic infections. Taken together it

could be assumed that Th-1 cytokines prevent HSV reactivation. Reduction or failure of Th-1 cytokine secretion may be a trigger for HSV reactivation and recurrent infection.

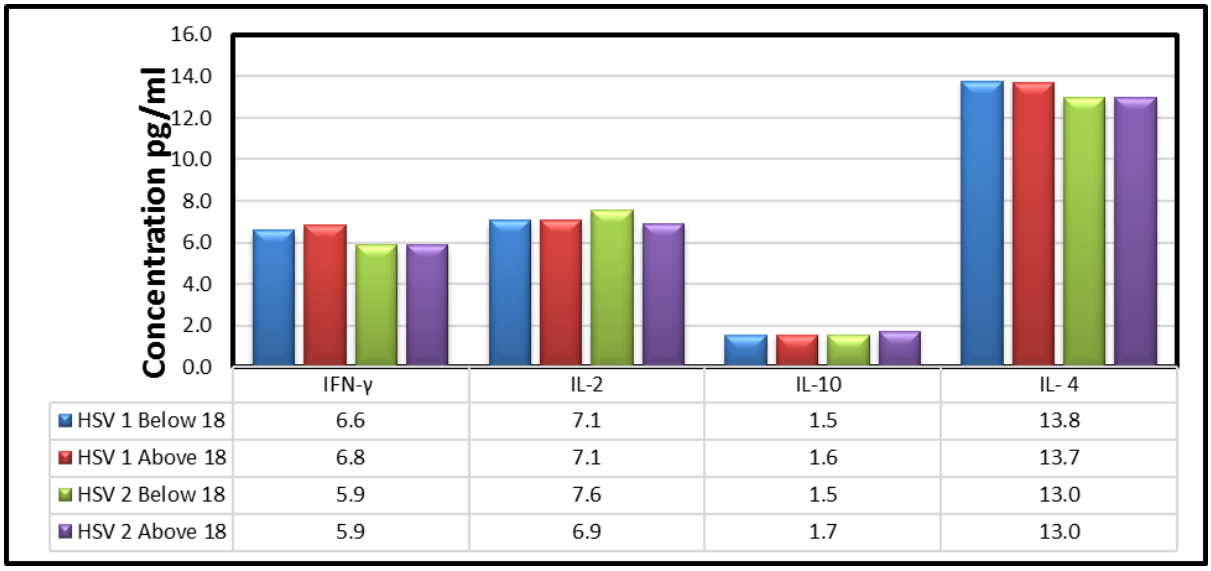
Correlation of Gender or Age and impairment of Th-1 cytokine secretion among HSV Patients:

As discussed above there was a correlation observed between impairment of Th-1 cytokines and recurrence of HSV infections. A similar correlation was again noticed between anti-HSV IgM antibody positivity and diminished Th-1 cytokine production and recurrent HSV infections. Next, we wanted to elucidate whether such correlation existed between gender or age of the patients with the change in cytokine secretion or HSV recurrence. As shown in the Fig. 3 and Fig.4 there were comparable levels of Th-1 and Th-2 cytokines were produced irrespective of the gender or age and there was no significant difference within HSV groups. This suggests that there was no such correlations or bias existed between gender or age and cytokines produced or HSV recurrence.



	IFN-γ	IL-2	IL-10	IL- 4
HSV 1 Male	7.1	7.0	1.3	13.8
HSV 1 Female	6.8	7.1	1.6	14.2
HSV 2 Male	6.1	6.0	1.1	12.9
HSV 2 Female	5.9	5.8	1.4	13.0

Fig 3: Shows the gender-wise classification of HSV positive subjects. The study population was segregated based on their sex i.e. male versus female and the level of each Th-1 and Th-2 cytokines are plotted. Among the 94 HSV-1 positive individuals 43 were males and 51 were females. Similarly, among 89 HSV-2 positive individuals, 42 were males and 47 were females.



	IFN-γ	IL-2	IL-10	IL- 4
HSV 1 Below 18	6.6	7.1	1.5	13.8
HSV 1 Above 18	6.8	7.1	1.6	13.7
HSV 2 Below 18	5.9	7.6	1.5	13.0
HSV 2 Above 18	5.9	6.9	1.7	13.0

Fig 4: Shows the age wise classification of HSV positive subjects. The study population was segregated based on their age i.e. <18 years versus >18 years and the level of each Th-1 and Th-2 cytokines are plotted. Among the 94 HSV-1 positive individuals 45 were <18 years and 49 were >18 years old. Similarly, among 89 HSV-2 positive individuals, 43 were <18 years and 46 were >18 years old.

DISCUSSION

As per 2015 WHO estimates there are 140 million of HSV-1 infections and 417 million of HSV-2 infections threaten humans globally. In India 33.3% are seropositive for HSV-1 and 16.6% are seropositive for HSV-2[6]. Though herpes was known for more than 2000 years and it is embarrassing that there are no vaccines or drugs to effectively control it. HSV genome is about 152.3 kb and it encodes for more than 70 known proteins. While its ability to orchestrate the expression of its genes effectively is important in establishing an infection the cytokine milieu that is the virus is equally important to successfully establishment of such infection by HSV.Waves of such cytokines are prognostic to viral infections i.e. some combination of cytokines are enhancers of the disease and some combination of cytokines are detrimental to the virus. During human HSV infections Th-1 cytokines (IFN-γ and IL-2) are protective and Th-2 cytokines (IL-10 and IL-4) are detrimental to the virus (4). HSV can cause either lytic infections or latent infections and for causing either one is primarily depends on the competency of host immune system especially the driven cytokine niche. In this study, we included 183 actively infected (94 with HSV-1 and 89 with HSV-2) patients and 140 healthy controls and subjected them to serum cytokines levels namely IFN-γand IL-2

(Th-1 cytokine) and IL-10 and IL-4 (Th-2 cytokine). Results showed that patients suffering from HSV had significantly lower Th-1 cytokines while Th-2 cytokines were unaffected. In addition, such disparity was not found to be associated with their gender or age. From this observation, we speculate that a decrease in Th-1 cytokine milieu led to lowering of resistance which provokes HSV prodrome and recurrence.

IFN-γ was first reported by Issacs and Lindenmann (1957) and was confirmed by Nagano and Kojima (1958) [7]. IFN-γ is important to both innate immunity and acquired immunity [8]. IFN-γ is produced by the innate immune system by NK cells and adaptive immune system by T cells (both CD4 and CD8 T cells).IFN-γ, also called Type II interferon, is a homodimeric glycoprotein containing approximately 21 to 24 KD subunits. The human IFN-γ gene, situated on chromosome 12, contains three introns; the four exons code for a polypeptide of 166 amino acids, 20 of which constitute the signal peptide [9]. Upon secretion, it binds specifically to IFN-γ receptor (IFNGR) and up regulate JAK which further induce STAT1 which further stimulate several IFN regulatory Factors (IRF) which translocate to nucleus and bind to gamma activated sequence (GAS). This leads to activation of cells with IFN-γ receptor[7]. Besides that binding of IFN-γ to its receptor down regulate many events through suppressors of cytokine signaling (SOCS) of which SOCS-1and SOCS-3 play primary role [10]. IFN-γ is a prominent Th-1 cytokine which governs the cell mediated immunity (CMI). Besides, it promotes CMI it suppresses Th-2 cells and humoral immunity [11]. We and others have shown that IFN knockout mice failed to clear HSV infections[12-14].

During lytic infection which normally occurs in the periphery such as epithelial cells and others the virus replication occurs in a productive way and at the end of the event the host cell will be lysed and the progeny virus will be released into the circulation which seeks new cells to infect. During latent infection, the virus migrates via sensory neurons and reach the dorsal root ganglia where it causes latent infection. At the time of latent infection, the only activity seen would be the latency associated transcript (LAT)[15]. The next step is critical. When the latent virus is insulted or triggered with some stimuli it initiates the prodromal stage. This stage is followed by the bout of reactivation that results in formation of cold sores or lesions in the oral cavity if the infected virus is HSV-1 or genital tract if the virus is HSV-2. The reactivation is initiated by triggers produced by stress, menstrual cycle, fever, exposure to UV light for sunlight and trauma. Lawrence J. Mathers et al 2006 suggested that harsh breast

feeding child induced reactivation [16]. In this paper, we propose that a decrease of IFN-γ could be one another trigger that can cause HSV reactivation.

Previous studies using animal models had shown that IFN-γ is indeed a primary cytokine that suppresses reactivation. Ting Liu et al 2001 cultured HSV-1 in trigeminal ganglion (TG) cultures and have proved that IFN-γ prevented reactivation[17]. E.M. Cantin et al 1995 again showed that TG cultures of IFN knockout mice there was enhanced replication which could be completely ablated by monoclonal antibody to IFN-γ[18]. In an another study they found that IFN-γ acts on latently infected neurons to inhibit (i) HSV-1 reactivation, (ii) ICP0 promoter activity, (iii) gC promoter activity, and (iv) reactivation in neurons in which the ICP0 or gC promoter is active[19]. Thomas Cherpes et al. 2008 showed that ex vivo TG cultures injected with medroxyprogesterone acetate (MPA), the compound most commonly used for injectable hormonal contraception dramatically reduced IFN-γ production among infiltrating CD8 cells which eventually promoted HSV reactivation[19]. Similarly in vivo MPA treatment of latently infected ovariectomized mice inhibited IFN-γ production and lytic granule increase resulted in increased activation of HSV. All these above studies and others suggested that presence of IFN-γindeed prevents HSV recrudescence. In our study, we found that a decrease in IFN-γ leads to reactivation which goes in agreement with the above studies.

Recently Mohamed Motamedifar et al 2015 showed that during HSV-1 infection, the human serum level of TNF-α drastically decreased (from 11.59 pg/ml to 6.61 pg/ml) while serum IL-10 level was unaffected. Authors of this paper claimed that a decrease of TNF-α appears to be one of the predisposing factors for the HSV reactivation [20]. TNF-α is a Th-1 cytokine [21]. In our study, we tested two other important Th-1 cytokines namely IFN-γ and IL-2. Our study revealed that there was a drastic reduction from healthy controls (i.e. from12.7 pg/ml (Healthy controls) to 6.6 pg/ml for HSV-1 patients and 4.9 pg/ml to HSV-2) and IL-2 (from 12.8 pg/ml to 7.0 pg/ml for HSV-1 and 4.6 pg/ml for HSV-2). Our study clearly showed that a decrease in Th-1 cytokines making the patients susceptible to HSV recurrence and thus our results corroborated the results of Mohamed Motamedifar's work. In their study, they tested only HSV-1 only while we tested both HSV-1 and HSV-2. Taken together Th-1 cytokines protect humans from prodrome and reactivation and any decrease in it make the patients vulnerable to the flurry of reactivation which can affect the patients both physically and psychologically.

CONCLUSION

HSV causes both lytic and latent diseases to humans. While productive infection leads to lytic HSV disease latency is a fascinating completely different ball game. Host pressure fights the virus to give-up all its strengths and forcing them to hide inside the neural ganglion until the development of a congenial environment for the virus. Once the triggering signals are sensed by the virus it undergoes a prodromal phase followed by waves of reactivation which cause lesions and blisters that are so painful. While stress, trauma, menstrual period and radiation has been described as predisposing factors for HSV recrudescence in this paper we propose that a decrease in Th-1 cytokines could provoke the virus and cause recurrent herpetic lesions.

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