

SHORT REPORT

Immunological markers of frequently recurrent genital herpes simplex virus and their response to hypnotherapy: a pilot study

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Summary: Patients were recruited for hypnotherapy from a clinic for patients with frequently recurrent genital herpes simplex virus (rgHSV). Psychological and immunological parameters were measured 6 weeks prior to hypnotherapy and 6 weeks afterwards, during which time each patient kept a diary of symptoms of rgHSV. Following hypnotherapy there was a significant overall reduction in the number of reported episodes of rgHSV, accompanied by an increase in the numbers of CD3 and CD8 lymphocytes, which may represent a non specific effect of hypnosis. The improvers showed significant rises in natural killer (NK) cell counts, HSV specific lymphokine activated killer (LAK) activity, and reduced levels of anxiety when compared to non-improvers. NK cell numbers and HSV specific LAK activity may therefore be important in the reduction in rgHSV following hypnotherapy.

Keywords: HSV-2, hypnosis, immunology, anxiety

INTRODUCTION

In 1974 Lycke *et al.* found the first evidence for a link between psychological distress and immunity to HSV when increased titres of antibodies to the latent herpes viruses HSV, Epstein-Barr virus and cytomegalovirus were found in a population of depressed patients when compared with healthy controls¹. These findings were confirmed in studies of medical students at examination time, and of spouses entering into divorce proceedings, when in addition, the antibody changes were associated with a reduction in NK cell activity, CD4 lymphocyte counts, and lymphocyte proliferative responses². It has become evident that NK cells, macrophages, CD4 and CD8 lymphocytes, interferon alpha and gamma, interleukin 2 (IL-2) and leukocyte migration inhibitory factor are all significant in protection against HSV³.

There is evidence that psychological intervention⁴ such as self-hypnosis training may buffer the effects of psychological distress on the immune system: in medical students, improved mood

ratings at examination time following self hypnosis training correlated with increases in NK cell, CD4 and CD8 lymphocyte counts^{5,6}.

CD8 lymphocytes and NK cells both have the capacity to lyse HSV-infected cells. NK cell lysis of HSV-infected cells can occur in a number of ways: in the absence of interferon or accessory cells⁷; following activation by IL-2 (termed LAK cell activity); and through the intermediary of specific antibody (termed antigen dependent cellular cytotoxicity [ADCC]). Klemke *et al.* found lower numbers of NK cells, and lower HSV specific LAK and ADCC activity in those with frequent recurrences compared to those with less frequent recurrences⁸. In patients with recurrent labial HSV-1, a striking decrease in HSV-1 specific NK activity, with reduced levels of IL-2 and alpha interferon, is seen during acute recurrences^{9,10}.

We investigated the effect of hypnotherapy on a variety of immune parameters, namely CD4, CD8, CD3, CD16 (NK cells) and CD19 lymphocyte counts, cortisol levels and specific cellular cytotoxicity in response to HSV. These were then related to changes in mood ratings post-hypnosis and to changes in the frequency of rgHSV.

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METHODS

Twenty subjects were recruited from a clinic for the treatment of frequently recurrent genital HSV-2 in the Department of Genitourinary Medicine at the Chelsea & Westminster Hospital. The study group consisted of patients who were due to discontinue prophylactic antiviral medication for a trial period, or who were reluctant to take such prophylaxis. Frequent recurrences were defined as a minimum of 6 episodes a year, but because of the short study period we aimed to recruit subjects who experienced 12 or more episodes a year when not taking prophylaxis. HIV seropositive patients were excluded. Subjects were not selected for any particular hypnotic facility.

At entry to the study the following tests were performed: the Barber hypnotic suggestibility score¹¹ (a subjective and objective measure of responsiveness to a range of suggestions), the Hospital Anxiety and Depression scale (HAD)¹², the Personality Syndromes Questionnaire (Gruzelier, unpublished), and the Eysenck Personality Questionnaire¹³. Blood was taken between 10:00 and 11:00 h (to reduce the effects of diurnal variations), for the following

- (A) *Lymphocyte sub-populations*, which were monitored by flow cytometry (Ortho CytoRon Absolute) using fluorescently tagged specific cell surface markers.
- (B) *Plasma cortisol levels* were assayed by automated ELISA.
- (C) *Peripheral blood mononuclear cell (PBMC) non-specific NK cell cytotoxic activity* was measured in 18 h chromium release assays against K562 human erythroleukaemia cell lines¹⁴.
- (D) *HSV specific NK cell cytotoxic activity of PBMCs* was measured, similarly, using the human epithelial type 2 cell line (Hep-2) infected with HSV-1 24 h prior to the test. (HSV-1 was used because HSV-2 is known to be cytopathic for Hep-2 cells and because HSV-1 infected cells have been used successfully to analyse the immune responses to both HSV-1 and HSV-2¹⁵). The background responses obtained with uninfected Hep-2 cells were subtracted from those obtained with HSV infected Hep-2 cells.
- (E) *HSV specific LAK activity* was measured as for D, but following stimulation with IL-2.
- (F) *HSV specific ADCC activity* was measured as for D, following addition of autologous plasma.

A diary was provided to record any recurrent lesions over a 6-week period, before each subject was given a standardized session of hypnotherapy using suggestions designed to increase mood and self confidence. More specific suggestions concerning boosting immunity and attacking infection were also used. A recording of the hypnotherapy session was supplied with instructions to listen to the tape at least 3 times per week for 6 weeks. The symptom diary was continued throughout this period, and in it the number of tape sessions per week was also recorded. At the end of the 6-week follow-up period the blood investigations were repeated, together with the HAD scale.

Subjects were deemed not to have improved clinically if they had recorded the same number or a greater number of recurrences in the 6 weeks immediately following the session of hypnotherapy compared to the 6 weeks immediately preceding intervention.

The data were analysed using the Statistical Package for the Social Sciences¹⁶, using the paired *t*-test to compare data pre- and post-intervention and the independent samples *t*-test to compare the improvers with the non-improvers group.

RESULTS

Twenty subjects were recruited between October 1997 and February 1998. Their median age was 36, and their history of HSV-2 culture positive rgHSV was well documented in the hospital case notes, with a median duration of 10 years (Table 1). The median number of annual recurrences in the absence of prophylactic medication was 12 (Table 1). Eight (40%) of subjects discontinued maintenance prophylaxis at entry: 6 were taking acyclovir, and 2 were on the placebo arm of a study of echinacea, a herbal extract currently under investigation for its therapeutic potential in rgHSV. The standardized sessions of hypnotherapy were performed between November 1997 and April 1998.

Thirteen patients (65%) improved clinically following hypnotherapy, 7 did not, of whom 3 reported an increased number of recurrences. There was no significant difference in baseline demographics (Table 1), including the Barber hypnotic suggestibility scores, between improvers and non improvers. Of the 6 who discontinued acyclovir prophylaxis, 4 went on to improve, as did one of the 2 who discontinued echinacea.

Table 1. Demographics of study population, parameters of recurrent genital herpes simplex virus (rgHSV) and mean Barber scores

	Improvers (n=13)	Non-improvers (n=7)	Combined groups
Median age in years (range)	36 (27 to 64)	40 (23 to 51)	36 (23 to 64)
Female gender (%)	61%	71%	65%
Median duration of rgHSV in years (range)	12 (1.5 to 26)	4 (1 to 23)	10 (1 to 26)
Median number of annual recurrences (range)	12 (7 to 25)	10 (6 to 16)	12 (6 to 25)
Barber score (0 to 8)	5.1	4.3	4.8

Table 2. Changes in recurrent genital herpes simplex virus and immune parameters following hypnotherapy

	Pre	Post	Difference	95% CI	P value if significant (paired <i>t</i> -test)
Episodes in 6 weeks					
Improvers	2.50	0.84	-1.66	-0.79 to -2.51	0.001
Non improvers	1.35	2.14	+0.78	0.08 to 1.48	0.033
Whole group	2.10	1.30	-0.8	-0.006 to -1.59	0.048
Immune profile (cells/mm ³)					
CD3 cells	1252	1386	+133	22 to 244	0.021
CD8 cells	386	446	+59	11 to 102	0.019
CD4 cells	776	831	+55		
CD16 (NK) cells					
Improvers	152	254	+112	23 to 201	0.019
Non-improvers	111	93	-18		
CD19 cells	219	227	+8		
Cortisol levels (nmol/l)	327	328	+1		
Functional immune activity (% cytotoxicity)					
Non-specific NK	41.67	44.48	+2.81		
HSV specific NK	6.82	7.21	+0.38		
HSV specific ADCC	16.09	16.56	+0.47		
HSV specific LAK in improvers	11.36	29.80	+18.51	6.9 to 30.0	0.007
HSV specific LAK total	11.26	26.55	+15.2	2.8 to 27.7	0.022

CI=confidence interval, NK=natural killer, LAK=lymphokine activated killer, ADCC=antigen dependent cellular cytotoxicity, HSV=herpes simplex virus

Overall, the number of recurrences fell significantly following intervention (Table 2). The median number of recurrences in the 6 weeks prior to intervention was 2 (range 0 to 6), and following intervention was 1 (range 0 to 3). The pre-intervention number of recurrences was greater in those who subsequently improved (Table 2), but not significantly so.

Immune parameters

There was a significant increase in CD3 and CD8 lymphocytes in both improvers and non improvers following hypnotherapy (Table 2). The non-improvers group had lower lymphocyte counts than the improvers both at baseline and follow-up (Figure 1), but the difference was not statistically significant. NK cell numbers rose significantly in the improvers and fell slightly in the non improvers (Table 2). A rise in the percentage of NK cells as a proportion of the total lymphocyte count was matched by an equivalent fall in the percentage of CD4 lymphocytes (Table 3).

Herpes simplex virus specific LAK activity more than doubled following hypnotherapy, an increase which was highly statistically significant, and confined to the improvers group (Table 2). There was no increase in HSV specific NK or ADCC activity following intervention. Cortisol levels were also unchanged.

A detailed analysis of the psychological parameters will be presented elsewhere.

DISCUSSION

Hypnotherapy can be used to promote relaxation, reduce anxiety, lift mood, and impart coping

mechanisms. Regular relaxation has been shown to improve non-specific NK cell function, and to reduce antibody titres to HSV^{17,18}. The hypnotic state, however, is more than a state of relaxation. Hypnosis produces neurophysiological changes which can be measured, and which are not found with ordinary relaxation techniques¹⁹. It has been shown to have a direct effect on the left versus right hemisphere dominance of temporo-limbic influences¹⁹. In asymptomatic HIV-1 infected individuals followed over 30 months, dominant left-sided influences have been shown to correlate with significantly higher CD4 and CD8 counts compared to patients with right-sided dominance²⁰.

This is the first time that hypnotherapy has been associated with improvement in immune function

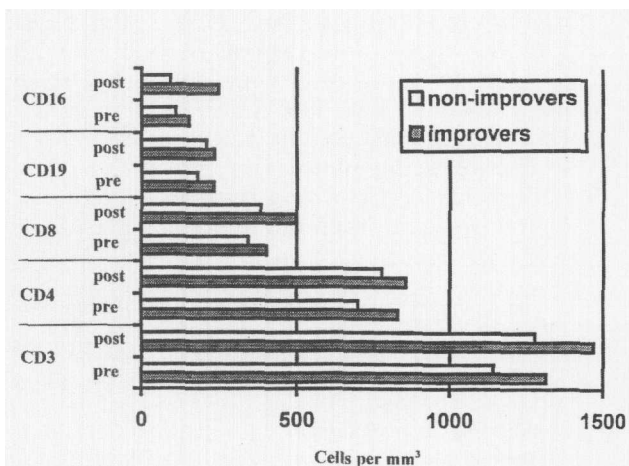


Figure 1. Pre- and post-hypnotherapy immune profiles in subjects with reported improvement in number of episodes of recurrent genital herpes simplex virus versus reported non-improvement

Table 3. Significant results of independent samples t tests comparing improvers with non-improvers

	Improvers	Non-improvers	Difference	95% CI	P value
Change in CD16 count (cells/mm ³)	+112	-18	130	20.4 to 240.4	0.023
Change in CD16 cells as a percentage of total lymphocytes	+4.3	-2.9	7.2	1.7 to 12.7	0.015
Change in CD4 cells as a percentage of total lymphocytes	-4.66	+2.33	7.0	0.89 to 13.1	0.028
Change in HAD anxiety score	-3.0	+2.0	5.0	1.5 to 8.4	0.007
Change in number of rgHSV episodes in 6 weeks	-1.66	+0.78	2.45	0.76 to 3.2	0.003
Change in number of days of symptomatic HSV in 6 weeks	-7.46	+7.42	14.89	6.47 to 23.3	0.002

HAD=Hospital Anxiety and Depression scale, rgHSV=recurrent genital herpes simplex virus, CI=confidence interval

coincident with a reduction in the severity of a chronic medical condition. It has previously been shown that hypnotherapy can increase numbers of CD4, CD8 and NK lymphocytes in healthy subjects^{4,5}. We confirmed that hypnotherapy significantly increases CD3 and CD8 lymphocyte counts. These increases may represent a non-specific effect of hypnosis, suggested by the fact that the increase in these parameters was significant in both clinical improvers and non-improvers. Increasing daylight is most unlikely to account for the differences over time, as although lymphocyte numbers are known to be subject to circadian rhythms, the variations are diurnal, not seasonal. CD8 and NK cell numbers are reported to increase during daylight hours, an increase which is impaired by sleep deprivation²¹. The absence of seasonal variation is supported by data from the 2 patients who were previously on the echinacea study, in whom monthly CD4, CD8 and NK numbers were measured in the year prior to hypnosis.

The rise in HSV antibodies at times of distress may be secondary to an impairment of specific cell mediated immunity directed against HSV. This is supported by the mouse animal model²². In humans, prospective studies have not shown any direct relationship between stressful life events and frequency of recurrence of HSV^{23,24}, although those who suffer frequent recurrences believe the two to be linked²⁵, and score more highly on stress indices than those with less frequent recurrences²⁶. There is, however, support for a link between negative mood (defined as anxiety, depression and hostility) and frequency of recurrence^{24,27}. Two studies have detected increased levels of anxiety in the 4 to 10 days preceding a recurrence^{28,29}, independent of prodromal symptoms²⁹. It is possible that stressful life events influence recurrence *indirectly* by causing negative mood. We found that a reduction in anxiety following hypnotherapy correlates with a reduction in rgHSV.

It is to be expected that clinical improvement would reduce anxiety levels in itself, while those for whom the hypnotherapy was not working would become even more anxious. Our observation of the patients suggests to us that the reduction in anxiety, at least in part, preceded clinical improvement: subjects reported feeling more confident that they would improve clinically after their

hypnotherapy session. They further reported feeling very much less anxious and stressed by everyday life events immediately following their episode of hypnotherapy.

The mechanisms by which negative mood might impair cellular immune responses to HSV-2 have not been elucidated, although an ever increasing number of interactions between the nervous system and the immune system is coming to light³⁰. In particular, the hypothalamo-pituitary-adrenal axis is known to interact with lymphokines, including IL-2 and gamma interferon; lymph nodes are known to receive innervation from the nervous system; and lymphocytes and macrophages have receptors for neurotransmitters, including endorphins³⁰.

That the NK cell plays a pivotal role in the cell mediated immunity directed against HSV-infected cells is strongly supported by the association between a clinical response to hypnotherapy and a rise in NK cell indices in the improvers group. The absence of a rise in NK numbers in the non-improvers group despite an increase in CD8 is intriguing, as self hypnosis training in medical students does produce such a rise. It is possible that this represents a specific immune defect.

Natural killer cells are activated by IL-2 to become LAK cells. Reduced NK cell numbers and reduced HSV specific LAK activity were previously shown to be linked with increased frequency of rgHSV⁷. Reduced IL-2 levels and reduced HSV specific NK activity have been described at the time of HSV-1 recurrences^{9,10}. We found that a rise in HSV specific LAK activity is associated with reduced frequency of rgHSV.

This was a small pilot study, and the results require confirmation in a controlled clinical trial using a larger cohort of patients. We continue to see all of the patients in clinic, and some who have remained off prophylaxis have experienced very little rgHSV in the year since hypnotherapy. We do not suggest that our data in any way support replacement of antiviral treatment for the average patient with genital herpes, but it may be beneficial in the small subgroup of patients with very frequent symptoms, who may have increased anxiety levels. Hypnotherapy is shown to have the potential to produce significant immune changes, which suggests to us that it could have wider application as an investigative tool. The

postulated link between negative mood and recurrent HSV needs further clarification, in particular the link between anxiety and stress.

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