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Effects of the Homeopathic Preparation Engystol on Interferon-γ Production by Human T-Lymphocytes

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There is a growing interest in complementary medical practices, but few studies have investigated mechanisms behind the possible benefits. The effects of the homeopathic preparation Engystol on interferon-γ producing T-lymphocytes were studied in vitro. Lymphocytes were isolated from 30 healthy human volunteers and the percentage of interferon-γ producing cells was analysed by fluorescence activated cell sorting. Cells were treated with NaCl (control) or Engystol at concentrations from undiluted to 2%. All concentrations of Engystol increased the percentage of interferon-γ producing lymphocytes significantly, from a mean of 20.9% ± 10.5% to over 24%. There was no dose-dependence of the effect at the concentrations tested.

Keywords Prophylaxis, Homeopathy, Inflammation, Fluorescence activated cell sorting.

BACKGROUND

Although there is a continual increase in the worldwide interest and use of complementary and alternative medical practices (Barnes et al., 2004; Schneider et al., 2004) there are few studies available to furnish a scientific rationale for the benefits commonly reported in clinical practice. The present study addressed a possible mechanism of action for the homeopathic preparation Engystol (Heel GmbH, Baden-Baden, Germany), which is frequently used in complementary medicine for the prophylactic treatment of infectious diseases.

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Engystol is a complex homeopathic preparation of *Vincetoxicum hirundinaria* (swallowort) and sulphur in several different dilutions (*V. hirundinaria* at 10^{-6}, 10^{-10} and 10^{-30}; sulphur at 10^{-4} and 10^{-10}). Engystol is used in the form of tablets or in solution for injections. The ingredients are listed in the Homeopathic Pharmacopoeia of the United States (HPUS) (1979). *Vincetoxicum hirundinaria* is used in complementary medicine as a stimulant of host defence mechanisms and sulphur is a common component of homeopathic remedies often used to treat acute and chronic disease (Reckeweg, 1991).

Prophylactic benefits of Engystol have been reported from clinical practice with this preparation in respiratory infections as well as in patients with influenza and common cold (Herzberger and Weiser, 1997; Tiraspolski et al., 1998; Torbicka et al., 1998). The remedy has been shown to be an effective ancillary treatment of viral infections in infants (Wagner et al., 1986). Reports have indicated immunostimulatory effects specifically on phagocytic activity, granulocyte function and improved humoral response (Denys and Siewierrska, 1999; Heilmann, 1994; Matusiewicz, 1997; Siewierrska and Denys, 1999). Further, a recent in vitro analysis suggested that Engystol may interact directly with virus particles and reduce infectivity independently of the possible effects on the immune system (Oberbaum et al, personal communication).

On the basis of the results described in the preceding studies it was postulated that part of the effects of Engystol might be achieved through an increase in the production of interferon-γ by immune cells. Interferon-γ is produced by several varieties of cells such as helper T-cells, cytotoxic T-cells natural killer cells and others. Production is induced by specific contact with antigens or through unspecific stimulation by substances that may be of biological or chemical origin (Cohen and Cohen, 1996; Lunney, 1998). Thus it was investigated whether Engystol treatment might increase the number of interferon-γ producing T-lymphocytes in vitro.

METHODS

Blood samples were obtained from 30 randomly selected healthy human volunteers aged 20–56 years, 24 men and 6 women. All subjects were blood donors at the Institute of Experimental Haematology and Transfusion medicine at the University Clinic Bonn, Germany. The anonymity of each donor was guaranteed and all subjects gave their informed consent. Lymphocytes were isolated from 9 ml blood samples. Total lymphocyte counts and differential cell counts were carried out on 1.2 ml of the samples. Autologous serum was prepared from 4.5 ml blood. All preparations were performed within 2 hours of drawing blood.

Lymphocytes were isolated from blood samples diluted 1:1 with Roswell Park Memorial Institute (RPMI)-1640 medium. Seven ml HISTOPAQUE –1077 solution (Sigma-Aldrich) were overlayed with 7 ml blood samples in sterile centrifugation tubes and centrifuged for 20 minutes at 300 × *g*. The band of lymphocytes formed by this procedure was removed with a sterile pipette, and the cells were washed twice by mixing with RPMI-1640 medium and sedimentation by centrifugation for 10 minutes at 200 × *g*. The washed lymphocytes were taken up in 1 ml RPMI-1640 medium.

Cell viability was assessed by staining 10 µl freshly isolated lymphocytes, diluted with 40 µl RPMI-1640 medium, with 50 µl 0.4% Trypan Blue solution for 5 minutes at 30°C. Living (unstained) and dead (blue) cells were counted in a Neubauer cell counter. Cell preparations were only used for assays if they contained >95% living cells.

The lymphocyte preparations were diluted with medium to a mean cell density of 0.526×10^6 cells/ml. The dilution medium consisted of (per 10 ml): 1ml autologous serum (10%), 103 µl glutamine (200 mmol), 5 µl streptomycin, 50 µl gentamycin, 8.842 ml RPMI-1640 medium. Engystol solutions were obtained from Heel GmbH, Baden-Baden Germany. The dilution medium in Engystol ampoules is sterile physiological NaCl solution, which was also used to dilute the Engystol preparations further.

For each assay, 5 µl diluted Engystol solution was mixed with 95 µl lymphocyte suspension in microtitre plates. After 19 hours incubation at 37°C, cells were activated by the addition of phorbol 12-myristat 13-acetate, ionomycin and monensin. After a further 5 hour incubation at 37°C, cells were washed in phosphate-buffered saline solution (PBS) and fixated with 5% formaldehyde solution for 10 minutes. After washing twice in PBS, samples were resuspended in 1 ml PBS and stored in the dark at 4°C.

Production of interferon-γ was measured by fluorescence activated cell sorting (FACS; Becton Dickinson). Harvested cells from 1 ml preparations were taken up in 50 µl buffer and mixed with 10 µl anti-human-interferon-γ-FITC-antibodies or anti-human CD3-phycoerythrin-antibodies (Hölzel Diagnostika, Köln, Germany). Samples were incubated at 4°C for 20 minutes, washed and resuspended in 150 µl PBS. Expression was measured on the same day by FACS. The percentage of FACS-counted interferon-γ expressing cells (labelled by anti-human-interferon-γ-FITC-antibodies) of the total number of lymphocytes (labelled by anti-human CD3-phycoerythrin-antibodies) were calculated for each sample. Each experiment was carried out in triplicate. Analysis of normally distributed data was performed by ANOVA; for data showing different distributions Kruskal-Wallis one-way analysis of variance on ranks was done (SigmaStat 2.0; Jandel Scientific Software). Data from Engystol-treated cells were compared with those from controls using multiple pairwise analysis (log-rank testing). The limit for statistical significance was set to 0.05.

RESULTS AND DISCUSSION

The effects on interferon-γ production of incubation with Engystol at different concentrations are shown in (Figure 1). First, 20.9% (20.9% ± 10.5%) of untreated lymphocytes expressed interferon-γ. Treatment with Engystol increased this number to >24%. Increases were seen with at all dilutions of Engystol and with no apparent dose-dependent effect. All increases in percentage of interferon-γ producing cells compared with control were statistically significant ($p < 0.001$; Table 1). Although the standard deviations were relatively high both for measurements from control cells and from Engystol-treated cells, there was no increase in scatter of the data with Engystol treatment (Table 1).

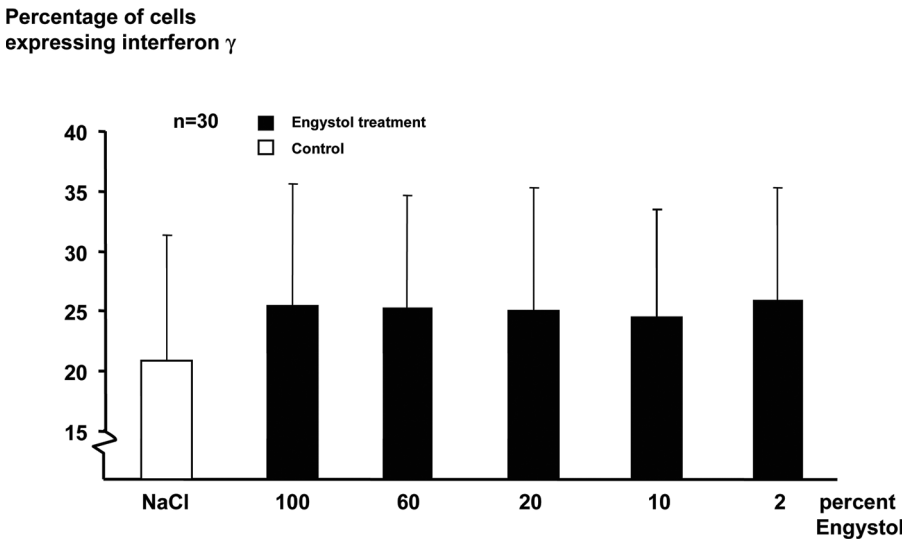

Figure 1: Percentages of cells producing interferon-γ after stimulation with NaCl control (open bar) or Engystol (filled bars). The lines indicate standard deviations.

Table 1: Percentages of T-lymphocytes expressing interferon-γ after treatment with different dilutions of Engystol or with control (NaCl solution). All subjects; n = 30.

		Engystol				
	Control	**100%**	**60%**	**20%**	**10%**	**2%**
Mean % of cells expressing IFN-γ	20.9 ± 10.5	25.5 ± 10.1	25.2 ± 9.4	25.0 ± 10.2	24.5 ± 9.0	25.9 ± 9.4
P for comparison with control		0.001	0.001	0.001	0.001	0.001

The large variability between individuals in interferon-γ production in response to stimulation is in accordance with what has been reported in other experiments with healthy human volunteers (Klein et al., 1997). Several factors influence this response, such as the nature of the stimulant and the relative number of naive and memory cells (Schauer et al., 1996). Despite the variance, the increases in interferon-γ producing cells were highly significant, indicating that the analysis was adequately powered to detect a relevant change from baseline in this population. That the effects were of similar magnitude with Engystol at all concentrations, with no apparent dose response, indicates that the active ingredients in the agent have a quite high stimulating activity. We did not attempt to establish the highest Engystol dilution needed to elicit a response.

The group of patients who reported with allergies at baseline showed generally lower levels of interferon-γ producing cells, both at baseline and after stimulation with Engystol (Figure 2). The effects of Engystol on percentages of interferon-γ producing cells was not significant in this group of patients (p = 0.07; Table 2). It should be noted that the number of patients in this group was small (n = 8) and the 95% confidence interval correspondingly larger than for the overall patient group. Thus, the analysis was not adequately powered to show a statistically significant increase in interferon-γ producing cells in this patient group, although the trend in this group was similar to that in patients without allergies. The latter patient group showed significant increases in the percentages of interferon-γ producing cells of a magnitude and

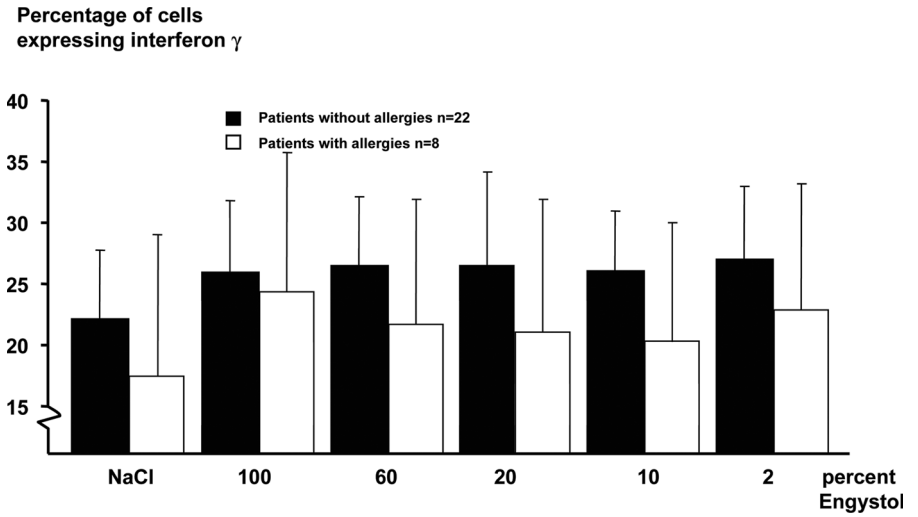

Figure 2: Percentages of cells producing interferon-γ after stimulation with Engystol or NaCl control in patients without allergies (filled bars) and patients with allergies (open bars). The lines indicate standard deviations.

Table 2: Percentage of T-lymphocytes producing interferon-γ after Engystol treatment in patients with (+; n = 8) and without (—; n = 22) allergies.

			Engystol									
	NaCl		100%		60%		20%		10%		2%	
	+	—	+	—	+	—	+	—	+	—	+	—
Mean % of cells expressing IFN-γ	22.1 ± 11.6	17.5 ± 5.7	25.9 ± 11.4	24.4 ± 5.9	26.5 ± 10.2	21.7 ± 5.6	26.5 ± 10.8	21.1 ± 7.6	26.0 ± 9.7	20.3 ± 5.0	27.0 ± 10.2	22.9 ± 5.9
P for comparison with control			0.001	0.07	0.001	0.07	0.001	0.07	0.001	0.07	0.001	0.07

statistical significance similar to that of the overall population (Figure 2; Table 2). As with the overall population, there was no apparent dose-response effect of Engystol (Figure 2). This may, in part, be an effect of the assay used, since it only looked at percentages of cells producing interferon-γ but not at the levels of production by single cells. Thus, the response measured in the assay represents more an on/off switch than an increased stimulation of interferon-γ production by individual cells. A similar explanation might be brought forward for the lack of apparent dose-response observed.

A comparison of effects of Engystol on interferon-γ production by T lymphocytes from smokers (n = 9) and non-smokers (n = 13) did not show any significant differences in effects between these groups (data not shown). Patients with allergies were excluded from this analysis. Baseline interferon levels were not significantly lower in smokers than in non-smokers (22.4 ± 9.2 in non-smokers vs. 21.7 ± 15.1 in smokers). Engystol treatment increased the percentage of interferon-γ producing cells significantly ($p < 0.001$ vs baseline) and to the same extent in both groups. Thus, the response of lymphocytes to the homeopathic agent did not appear to be compromised in these smokers. The higher baseline values in this set of patients are a reflection of the exclusion of patients with allergies, who had low baseline levels of interferon-γ producing cells.

The mechanism behind the observed effects still needs to be elucidated. Engystol contains two main active ingredients, *V. hirundinaria* and sulphur. Both constituents are frequently used in homeopathic therapies to stimulate host defences and work on Engystol has shown effects on phagocytic activity, granulocyte function and improved humoral response. (Denys and Siewierrska, 1999; Fimiani et al., 2000; Heilmann, 1994; Matusiewicz, 1997; Siewierrska and Denys, 1999). Recent data (R. Glatthaar, personal communication, August 2005) indicate that the preparation may influence virus-specific components necessary for viral replication, but without a direct interaction with viral surface proteins.

An increase in interferon-γ production indicates an activation of the immune system. Since such activation can be specific or unspecific and the activating substances may be of biological or chemical origin (Cohen and Cohen, 1996; Lunney, 1998), it is unclear whether one or both of the components of the remedy are responsible for the effects. The practice of homotoxicology is based on a postulated stimulation of the immune system by small amounts of activators (Schmid and Hamalcik, 1962), which is in line with what was observed in these in vitro experiments.

The relevance if from these in vitro results to clinical reality needs to be established. However, a therapy that improves interferon-γ production in response to stimuli might be expected to confer benefits to patients at risk of infection or exposed to infectious agents. As has been shown previously, induction of cytokines is often not restricted to one single type of cytokine and

although it was not measured in the assay, one would expect Engystol stimulation to be accompanied by increased production of IL-2 and TNF-α as well (Mascher et al., 1999). Such wider implications of the current limited experiments would warrant further research.

In summary, this study observed that peripheral human lymphocytes respond to treatment with the homeopathic preparation Engystol in vitro by increased interferon-γ production. Such effects indicate a possible mechanism for the claimed benefits of this preparation in the treatment of patients with infections. This limited study might stimulate further research into this and other alternative medical remedies.

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REFERENCES

Barnes, P. M., Powell-Griner, E., McFann, K., Nahin, R. L. (2004). Complementary and alternative medicine use among adults: United States, 2002. *Adv. Data.* 343:1–19.

Cohen, M. C., Cohen, S. (1996). Cytokine function. A study in biologic diversity. *Am. J. Clin. Pathol.* 105:589–598.

Denys, A., Siewierrska, K. (1999). Efficacy of preoperative immunoprophylaxis in patients with neoplastic diseases – II. Estimation of antihaemagglutinin and antineuraminidase antibody titre or influenza viruses A and B. *Int. Rev. Allergol. Clin. Immunol.* 5:46–50.

Fimiani, V., Cavallaro, A., Ainis, O., Bottari, C. (2000). Immunomodulatory effect of the homoeopathic drug Engystol-N on some activities of isolated human leukocytes and in whole blood. *Immunopharmacol. Immunotoxicol.* 22:103–115.

Heilmann, A. (1994). A combination injection preparation as a prophylactic for flu and common colds. *Biomed. Ther.* 12:249–253.

Herzberger, G., Weiser, M. (1997). Homeopathic treatment of infections of various origins: a prospective study. *Biomed. Ther.* 15:123–127.

(1979). *The Homeopathic Pharmacopoeia of the United States* (HPUS). 8th ed. Falls Church, VA, USA.

Klein, S. A., Dobmeyer, J. M., Dobmeyer, T. S., Pape, M., Ottmann, O. G., Helm, E. B., Hoelzer, D., Rossol, R. (1997). Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. *AIDS* 11:1111–1118.

Lunney, J. K. (1998). Cytokines orchestrating the immune response. *Res. Sci. Tech. Off. Int. Epiz.* 17:84–94.

Mascher, B., Schlenke, P., Seyfarth, M. (1999). Expression and kinetics of cytokines determined by intracellular staining using flow cytometry. *J. Immunol. Meth.* 223:115–120.

Matusiewicz, R. (1997). The effect of a homeopathic preparation on the clinical condition of patients with corticosteroid-dependent bronchial asthma. *Biomed. Ther.* 15:70–74.

Reckeweg, H. (1991). *Materia Medica, Homoeopathia Antihomotoxica.* Second English ed., Vol. 1. Baden-Baden, Germany: Aurelia-Verlag.

Schauer, U., Jung, T., Krug, N., Frew, A. (1996). Measurement of intracellular cytokines. *Immunol. Today* 17:305–306.

Schmid, F., Hamalcik, P. (1962). [Vincetoxicum: Therapeutisches Prinzip]. *Antihomotoxische Medizin.* Vol. 2. Baden-Baden, Germany: Aurelia Verlag.

Schneider, B., Hanisch, J., Weiser, M. (2004). Complementary Medicine Prescription Patterns in Germany. *Ann. Pharmacother.* 38:502–507.

Siewierrska, K., Denys, A. (1999). Efficacy of preoperative immunoprophylaxis in patients with neoplastic diseases – I. Phagocyte activity and bactericidal properties of peripheral blood granulocytes. *Int. Rev. Allergol. Clin. Immunol.* 5:39–45.

Tiraspolski, I. V., Kuzmin, V. N., Kashirina, T. N., Busorgina, O. V. (1998). [Antihomotoxic therapy for cytomegalus primary infections in pregnant women]. *Biol. Med.* 27:206–211.

Torbicka, E., Brzozowska-Binda, A., Wilczynski, J., Uzerowicz, A. (1998). RSV infections in infants: therapy with a homeopathic preparation. *Biomed. Ther.* 16:256–260.

Wagner, H., Jurcic, K., Doenicke, A., Rosenhuber, E., Behrens, N. (1986). The effect of homoepathic preparations on the phagocygotic activity of granulocytes. *Drug. Res.* 36:1421–1426.