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Effects of a *Sarcocystis gigantea* Extract (SGE) on the Replication of Human Immunodeficiency Virus (HIV)

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ABSTRACT

As recently reported, a strong stimulation of noninfected $CD4^+$-positive $H9^-$ cells by *Sarcocystis gigantea* (Syn. *S. ovifelis*) extract (SGE) was observed using the lymphocyte proliferation assay. After SGE-prestimulation, HIV-infected $H9^+$ cells showed an exacerbation of the virus replication. In the present study, we investigated the reactivity of HIV-infected human monocytes by SGE. The highly sensitive p24 core profile ELISA was used to examine directly the amount of HIV produced. Experiments were performed using the permanent monocytic cells U937. Permanent incubation as well as preincubation with SGE before virus infection were able to stimulate HIV expression in all the cells. In U937 cells, the virus release per cell was 64 times higher in permanent stimulation with 320µg SGE compared to controls and 9 times higher in case of prestimulation.

INTRODUCTION

Investigations on the interaction of microorganisms with the immune system (especially interference with defence mechanisms) have recently attracted increased attention. Sarcocystiosis is a protozoan infection which is widespread throughout the world. Via the food chain, the *Sarcocystis* spp. could infect human, as shown by the antibody titer in large parts of the population (Aryeety and Piekarski 1976; Hiepe 1977). It was the aim of the model presented here to characterize the immunomodulating effect of a protozoan parasite, *Sarcocystis gigantea* extract (SGE), on the immune system of man (several human

mononuclear cell lines) concomitantly with another pathogen, Human Immunodeficiency Virus (HIV-1) , in vitro.

The basis of this study were the previous findings regarding isolation and characterization of a *Sarcocystis gigantea* lectin (SGL) which has the capacity to activate human mononuclear cells ($CD3^+$, $CD4^+$, $CD8^+$, Mp, B lymphocytes) (Montag et al. 1987; Tietz et al. 1986a, 1986b, 1989, 1990, 1991).

As recently reported, HIV-susceptible, immortalized lymphocytes ($H9^-$, MT-4) were investigated to establish their reactivity to SGE containing the mitogen above mentioned (Drössigk et al. 1995; Pötzsch 1994). Using the lymphocyte proliferation assay, a strong stimulation of noninfected $CD4^+$-positive $H9^-$ cells by SGE was observed. After SGE stimulation (20-160μg), HIV-infected $H9^+$ cells showed an exacerbation with an optimum at day 4. The virus replication in the latently HIV-infected $H9^+$ cells could be detected indirectly because of the higher cytotoxicity in the MT-4 cell system measured with the MTT cell vitality assay. In parallel, the highly sensitive p24 core profile ELISA was used to examine directly the amount of produced HIV.

In this study, experiments were performed using monocytes (U937) prestimulated as well as permanently stimulated with SGE.

MATERIALS AND METHODS

Preparation of SGE

The extract was prepared from *Sarcocystis macrocysts* as described by Tietz (1986a) and contained 6.5 mg/ml protein. The values of agglutination titers obtained in suspensions of erythrocytes and GDA erythrocytes amounted to 729 and 59,049, respectively and could inhibited by N-acetyl-(D)-galactosamine. Addition of SGE in a concentration of 160μg/ml to human peripheral lymphocytes increased the lymphocyte proliferation 85 fold over the background measured by ^3H thymidine incorporation into the cell DNA.

Cell cultures

The permanent cells U937 (monocytes) were preincubated in 96-well microtiter plates containing several concentration of SGE for 3 days. Cell concentrations amounted to $4x10^5$ mononuclear cells/ml. Cultures were washed and infected with HIV (exactly HIV-1) for 3 hrs and washed again. The further incubation were performed without and with substitution of SGE in a concentration corresponding to the preincubation. Cultures were generally performed in RPMI 1640 supplemented 10% with heat-inactivated fetal calf serum.

Virus identification

On days 2, 4 and 7 after HIV infection samples were investigated to determine the amount of virus produced in picograms (pg). The number of viable culture cells was determined by means of trypanblue staining. In order to detect HIV the HIV-1 p24 core profile ELISA (Du Pont de Nemours, Bad Homburg, Germany) was used. This highly sensitive and highly specific diagnostic test is commercially available and contains a monoclonal mouse antibody against the nuclear protein p24 of HIV.

RESULTS

As shown in Fig. 1 and Fig. 2, a virus exacerbation in monocytes was effected by SGE. The virus replication in the U937 cells was nearly 4 times stronger than the control. No significant differences were observed on day 2 and 4 in preincubated and permanently stimulated cell cultures. On day 7, the permanently stimulated cell culture shows a distinct depletion. The number of cells in the course of the incubation in the two systems are presented in Fig. 3 and Fig. 4. A decrease in number of cells depending on the concentration of SGE and time was noted for permanent SGE incubation.

In order to compare HIV production per cell with permanent SGE addition, preincubation and control (Fig. 5 and Fig. 6) the quotient of pg p24 SGE and pg24 control was considered in relation to the viable number of U937 cells. The virus release per cell was 10 times higher in permanent stimulation with 320µg SGE compared with preincubation of SGE.

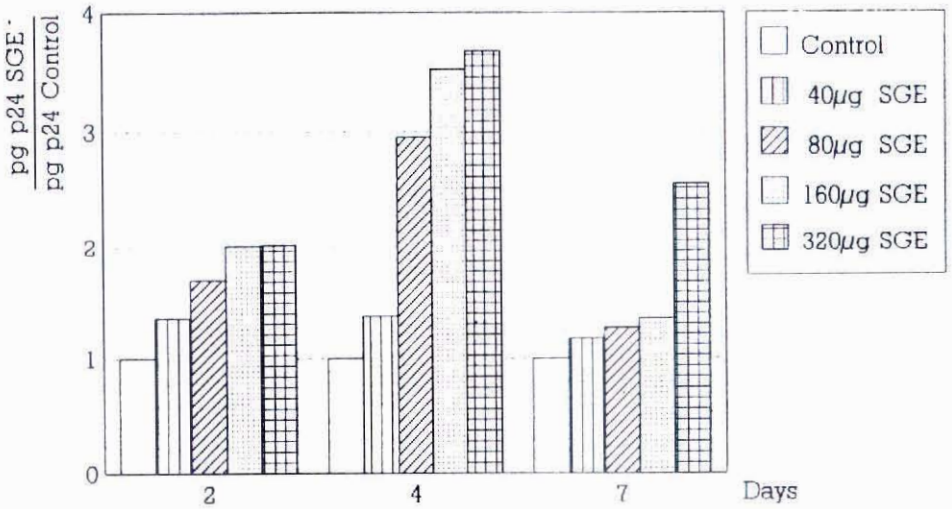

Fig. 1. Expression of HIV in U937 cells after preincubation with SGE.

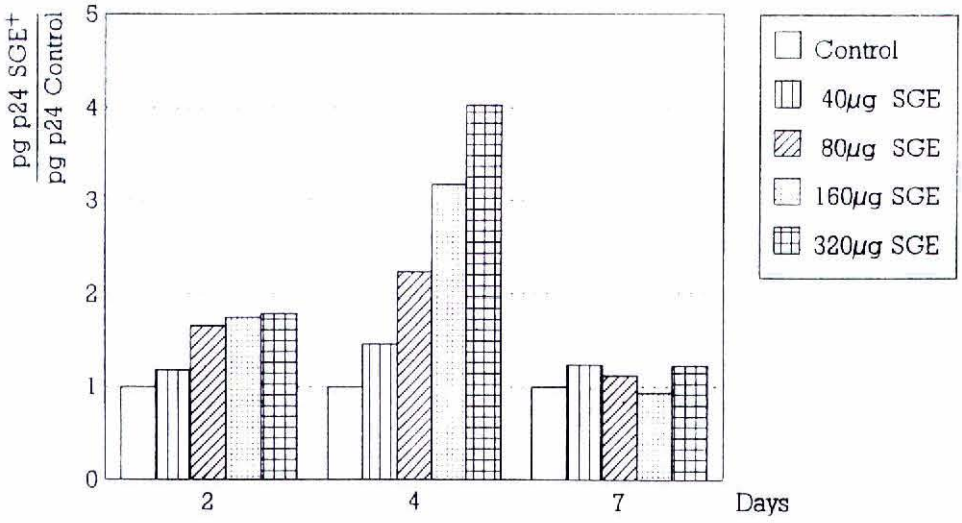

Fig. 2. Expression of HIV in U937 cells after permanent incubation with SGE.

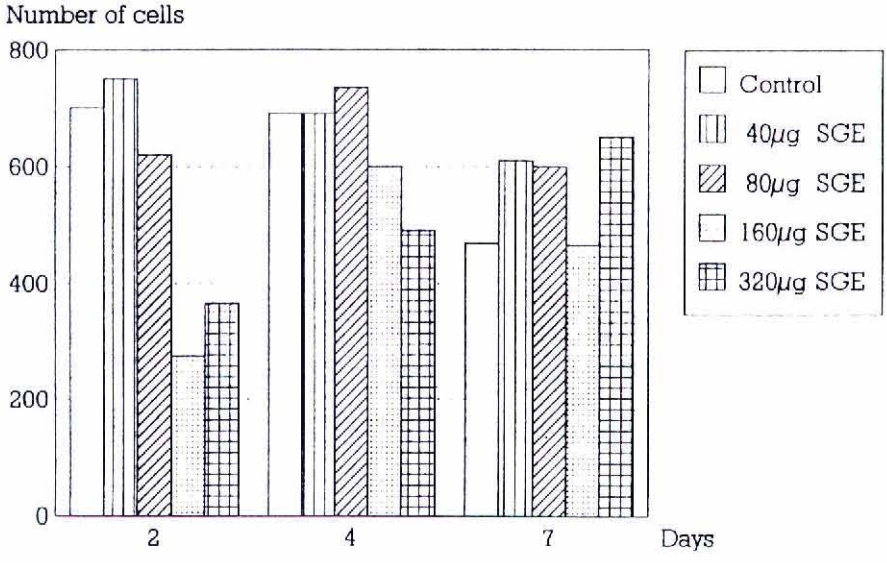

Fig. 3. Number of U937 cells after preincubation with SGE.

To summarize, it appeared that the virus expression increased in HIV-infected monocytes incubated by SGE. Permanent stimulation by SGE caused a strong decrease in the number of U937 cells. This small number of cells was able to produce the same amount of HIV as the preincubated group with a large number of cells.

EFFECT OF *S. GIGANTEA* EXTRACT ON HIV REPLICATION

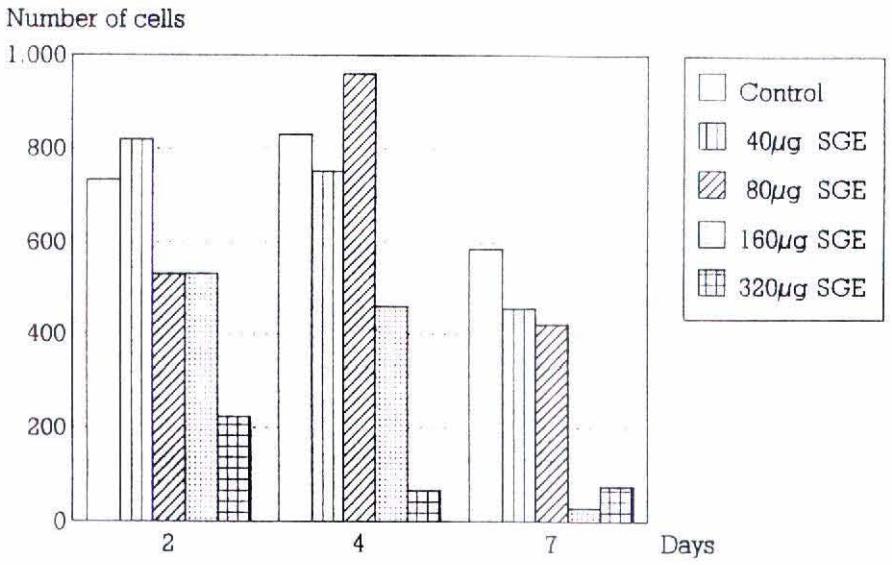

Fig. 4. Number of U937 cells after permanent incubation with SGE.

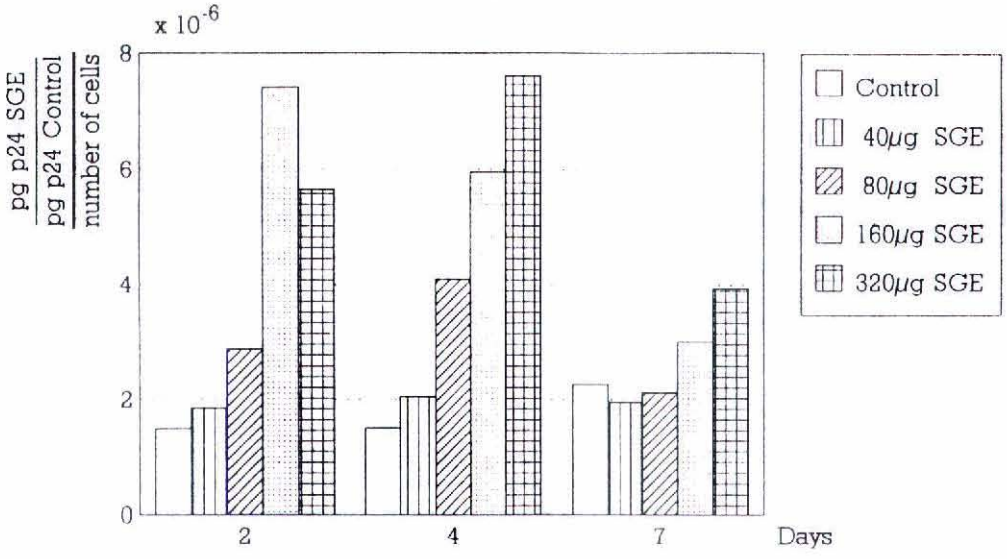

Fig. 5. Expression of HIV per U937 cell after preincubation with SGE.

DISCUSSION

As has been shown for the $CD4^+$-positive $H9^+$ and MOLT-4 cells, the addition of SGE to cultures of human MNCs caused lymphocyte proliferation and expression of virus in HIV-infected cells (Dörsigk et al. 1995; Pözsch 1994). It was found by means of fluorescence-activated cell sorter (FACS) that SGL was linked by 68% of lymphocytes but 93% of monocytes (Tietz et al. 1991). Besides

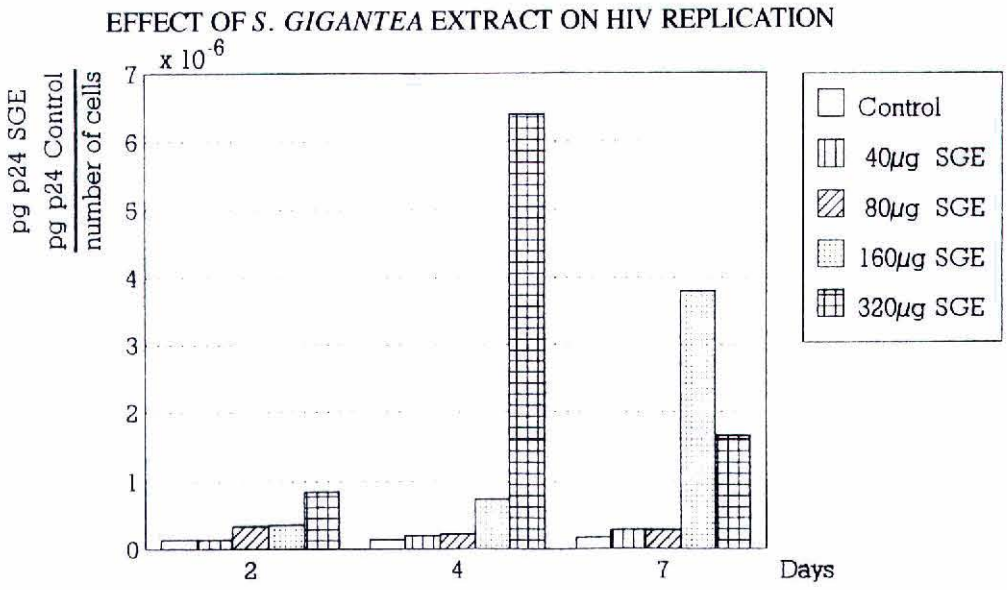

Fig. 6. Expression of HIV per U937 cell after permanent incubation with SGE.

$CD4^+$-positive lymphocytes, monocytes may also be infected by HIV. Monocytes play an important role especially in the early phase of HIV pathogenesis. For this reason, it was of interest for us to investigate the effects of SGE on monocytes in the established model, using U937 cells. Fig. 1 and Fig. 2 present the mean values in pg of HIV obtained by p24 profile ELISA under the conditions mentioned above. The monocytes were also stimulated to virus exacerbation in relation to time and amount of SGE and reached the highest expression of HIV on day 4 with 320µg. This result is in accordance with the literature concerning virus expression by different lectins (Alouf et al. 1986; Masihi et al. 1990; Pineau et al. 1989). There is no significant difference in the two trials (permanent and prestimulation). On day 7, the U937 cells seem to be more exhausted in permanent stimulation. Therefore, we investigated virus exacerbation in relation to the number of U937 cells counted. Surprisingly, the data presented in Fig. 3 and Fig. 4 show considerable differences in the number of cells. In case of the prestimulated cells, the number of cells reached the number of the control after a slow decrease whereas the permanently stimulated cells decreased in number depending on time and SGE amount. Considering virus release per cell, it was approximately 9 times higher in permanent stimulation with 320µg SGE compared with permanent stimulation and 64 times compared with control. Phillips and McMichael (1993) reported HIV exacerbation induced by mitogens in latently infected cells. Beside this, an increased apoptosis of HIV infected cells influenced by lectins was shown by Groux et al. (1991).

To summarize the results obtained, the increased virus release after SGE

stimulation could be induced in the established model. A comparison of the results obtained with conditions in vivo is not possible yet, because immortalized cells are only partially suitable for this. Furthermore, it is unknown whether SGE represents other developmental stages and species of the genus *Sarcocystis*. Nevertheless, it is possible to draw the conclusion that some Apicomplexa might be able to influence the defence system of man and animals by immunomodulating effects. It could not be ruled out that such protozoans or microorganisms might be cofactors for the activation of pathogens, related closely to the defence system, such as HIV, FIV and FeLV.

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