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HSV-1 INFECTION AND IMMUNITY IN IMMUNOSUPPRESSED MICE

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Summary. — The effect of cyclophosphamide (CY) and hydrocortisone (HY) on the susceptibility of mice to intracerebral and intraperitoneal infection with herpes simplex virus type 1 (HSV-1) was investigated. The mean survival time, the survival ratio, the localization of HSV-1 antigen in brain, spleen and liver as well as immunity after immunization with inactivated virus were determined. In the case of primary infection, an increased susceptibility to HSV-1 was observed after administration of the immunosuppressive drug. Immunization increased the resistance of mice to virus challenge, but no such effect was observed when the virus challenge was accompanied by administration of CY or HY. The influence of CY and HY on the immunization process itself was divergent: when HY was given at the time of immunization, the resistance to virus challenge was abolished. On the other hand, CY given simultaneously with inactivated HSV-1 did not depress the immunization effect.

Key words: herpes simplex virus type 1; immunosuppression; cyclophosphamide; hydrocortisone

Introduction

Damage of cellular immunity is the substantial cause promoting the development of infections with various viruses such as HSV, cytomegalovirus, vaccinia virus, and measles (Nahmias 1970; Logan, Tindell, and Elson 1971; Merigan and Stevens 1971; Muller, Herrmann and Winklemann 1972; Burns and Allison 1975; Lopez and O'Reilly 1977; Virelizier 1977). This was found especially as result of immunosuppressive therapy in posttransplantation patients (Thomas, Mendez-Picon and Thomas 1978; Jamieson, Stinson and Shumway 1979; Salaman and Miller 1979) as well as in various inflammations and autoimmune diseases (Gerber and Steinberg 1976, 1976*a*). The application of immunosuppressive drugs may activate latent virus infections. However, immunosuppressive drugs do not decrease the level of already existing antiviral antibodies (Armstrong *et al.* 1971). It was found that acute HSV infections often occur in persons immunosuppressed after transplantations (Anderson and Spencer 1969; Betts *et al.* 1977).

In guinea pigs resistant to latent HSV-1 infection, latency was observed in the ganglion after administration of HY (Tenser and Hsiung 1977). In hamsters latently infected with measles virus, CY activated the virus (Wear and Rapp 1971). The purpose of the experiments described in this paper was to determine the effect of CY and HY on primary HSV-1 infection in mice, on the infection in mice previously immunized with inactivated HSV-1 as well as the influence of these immunosuppressors on the immunization itself.

Materials and Methods

Virus. HSV-1, McIntyre strain, obtained from the Institute of Hygiene in Freiburg, was propagated in primary rabbit kidney cells and then passaged in the primary *Cercopithecus aethiops* monkey kidney cells. The virus titre was $10^{5.5}-10^{6.6}$ $TCID_{50}$/1 ml.

Immunosuppressive drugs. Cyclophosphamide (VEB Jenapharm) and hydrocortisone (Polfa) were used. CY was given in a single dose of 300 mg per kg of body weight 24 hr prior to virus administration. HY in the dose of 2.5 mg was given on the day of and 72 hr after infection.

Experimental animals. Inbred mice, strain CFW/Pzh, weighing about 16–18 g were used.

The complement fixation (CF) test was performed by micromethod introduced by Sever (1962). Freeze-dried HSV-1 antigen fixing the complement was applied.

ELISA. The test was performed with HSV-1 antigen according to the method described by Jankowski (1980), applying peroxidase conjugated rabbit serum against mouse IgG. The highest dilution in which the extinction value was twofold as compared with mean OD value of the negative serum diluted 1 : 1000 was regarded for the serum antibody titre.

Immunofluorescence (IF) test. Fragments of various organs were quickly frozen at −80 °C. Serial sections (4 μm) were cut, fixed in cold acetone and stained with fluorescein-conjugated human anti-HSV-1 gammaglobulin. To control the specificity of the assay, the staining was blocked on some sections with nonconjugated anti-HSV-1 serum, then the sections were overlaid with the anti-HSV-1 conjugate.

The evaluation of pathogenicity of HSV-1 (in vivo). Percentage of survivors was calculated by the formula:

$$\text{survivors (\% of mouse)} = \frac{\text{number of living mice on 14 th day}}{\text{number of mice in the group}} \times 100$$

The mean survival time was calculated by the formula given by Grunert *et al.* (1965):

$$\text{mean survival time} = \frac{f\,(d-1)}{N}$$

f — number of dead mice on the particular day — d
N — number of mice in the group

The observation time was 14 days following virus administration. All survived mice on day 14 were included into the calculation.

Immunization and infection of animals. Mice were infected either intracerebrally(i.c.) with 3 $TCID_{50}$ or intraperitoneally (i.p.) with $5 \times 10^{5.5}$ $TCID_{50}$.

Statistical analysis. Student's t test with significance of $P = 0.01$ and χ^2 test with significance of $P = 0.05$ were applied.

Results

Effect of HY and CY on primary HSV-1 infection

In i.c. infected mice a linear relationship was found between survival rate and the mean survival time on one hand and the infecting dose on the other hand (Fig. 1). The dose of 3 $TCID_{50}$ was chosen for the further experiments.

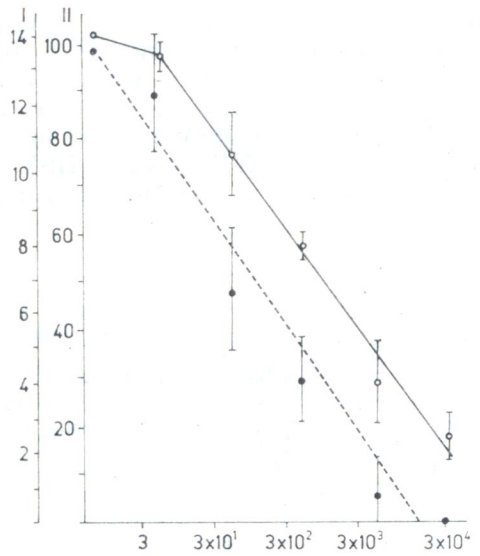

Fig. 1.
Per cent of survivors and mean survival time after i.c. infection of mice with different doses of HSV-1
○——○ % of survivors; ●– – ● mean survival time
Abscissa: Virus dose ($TCID_{50}$); ordinates: I — mean survival time (days p.i.), II — per cent of survivors.

Its defined parameters were 87 % survival and 13.4 days mean survival time at fortnight observation. Thus, it was possible to follow the effect of immunosuppression. Even after this low infecting dose, CY decreased the mice survival to 21.5 % while the mean survival time was shortened to 7.7. days. After administration of HY the values were 5.5 % and 6.3 days, respectively (Fig. 2).

Statistical analysis (χ^2) indicated significant differences both in determinations o survival-rate ($P_0 < 0.001$) and the mean survival time ($P_0 < < 0.001$) between the group of mice infected with the dose of 3 $TCID_{50}$ and groupsjo animals which were given the virus together with CY or HY.

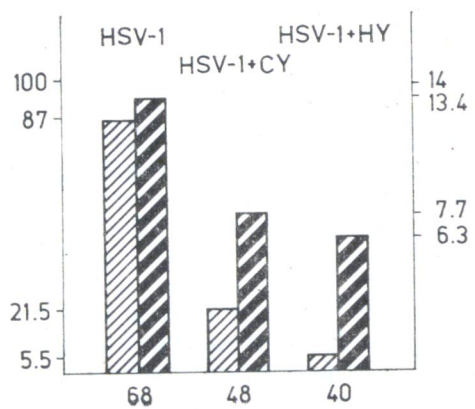

Fig. 2.
Effect of cyclophosphamide and hydrocortisone on the mortality and mean survival time of mice infected with HSV-1 by i.c. route (3 $TCID_{50}$)
Thin lines: % of survivors; thick lines: mean survival time
Abscissa: no. of mice; ordinates: in the left — per cent of survivors; in the right — mean survival time.

Tab. 1. Effect of immunosuppressive drugs on survival-rate, mean survival time and frequency of virus antigen detection in organs of i.c. infected mice.

Dose of HSV-1	Per cent of survivors	Mean survival time (days)	Per cent of positive results by IF		
			Brain	Liver	Spleen
3 $TCID_{50}$	87.0	13.4	none	none	none
3 $TCID_{50}$ + cyclophosphamide	21.5	7.7	100	75	75
3 $TCID_{50}$ + hydrocortisone	5.5	6.3	100	90	88.8

After i.p. virus administration (dose $5 \times 10^{5.5}$ $TCID_{50}$) the percentage of survivors was 46 % and the mean survival time — 12 days (Fig. 3). The CY or HY administration caused further decrease of mice survival (12.8 % and 0 % respectively). The analysis of quality variables (χ^2) revealed a significant difference between the group infected with HSV-1 in a dose of $5 \times \times 10^{5.5}$ $TCID_{50}$ and the groups of mice which were given virus together with immunosuppressive drugs. The critical values for the mean survival time and mice survival-rate were $P_o < 0.001$.

Using IF, localization of virus antigen in brain, liver and spleen and its frequency were defined in the same animals (e.g. Figs. 4, 5 and 6). In immunosuppressed animals the relationship was found between the increased presence of HSV-1 antigens in the organs and the decreased per cent of survivors and the lower mean survival time both after i.c. (Tab. 1) and i.p. (Tab. 2) infections.

Tab. 2. Effect of immunosuppressive drugs on survival rate, mean survival time and frequency of virus antigen detection in organs of i.p. infected mice.

Dose of HSV-1	Per cent of survivors	Mean survival time (days)	Per cent of positive results in IF		
			Brain	Liver	Spleen
$5 \times 10^{5.5}$ $TCID_{50}$	46	12.0	33	39	60
$5 \times 10^{5.5}$ $TCID_{50}$ + cyclophosphamide	12.8	6.6	58	95	83.3
$5 \times 10^{5.5}$ $TCID_{50}$ + hydrocortisone	0	6.5	44.4	100	94

Tab. 3. Effect of immunosuppression on survival-rate and mean survival time of i.p. infected mice after immunization with inactivated HSV-1.

No. of mice	Inactivated HSV-1		Immuno-suppres-sive drug	HSV-1 $5 \times 10^{5.5}$ $TCID_{50}$	
	Per cent of survivors	Mean survival time (days)		Per cent of survivors	Mean survival time (days)
29	100	14	—	89.7	13.5
26	100	14	CY	25.6	8.7
18	100	14	HY	5.5	7.4

CY — cyclophosphamide; HY — hydrocortisone

Influence of immunosuppressive drugs on the susceptibility to virus challenge in immunized mice

Intraperitoneal immunization with inactivated HSV-1 diminished the susceptibility of the animals to virus challenge ($5 \times 10^{5.5}$ $TCID_{50}$). The mice survival increased from 46 % to 89.7 % and the mean survival time from 12 to 13.5 days (Table 3). Such effect of immunization was not observed when immunosuppressive drugs were given together with infectious HSV-1 ($5 \times 10^{5.5}$ $TCID_{50}$). Despite of the presence of antibodies in the titre of 160 as determined in ELISA as well of CF antibodies in the titre of 16, CY decreased the percentage of survivors to 25.6 % and shortened the mean survival time to 8.7 days. After HY administration these values were 5.5 % and 7.4 days, respectively.

A significant difference was found in the survival ($P_0 < 0.001$) and in mean survival time ($P_0 < 0.001$) between mice immunized and infected with $5 \times 10^{5.5}$ $TCID_{50}$ and animals immunosuppressed at the time of their challenge.

Tab. 4. Effect of immunosuppressive drugs given at the time of immunization on i.p. HSV-1 infection.

No. of mice	Immunosupres-sive drugs	Inactivated HSV-1		HSV-1 $5 \times 10^{5.5}$ $TCID_{50}$	
		Per cent of survivors	Mean survival time (days)	Per cent of survivors	Mean survival time (days)
29	—	100	14	89.7	13.5
28	CY	100	14	71.4	12.4
21	HY	93	13.8	47.6	11.4

CY — cyclophosphamide; HY — hydrocortisone

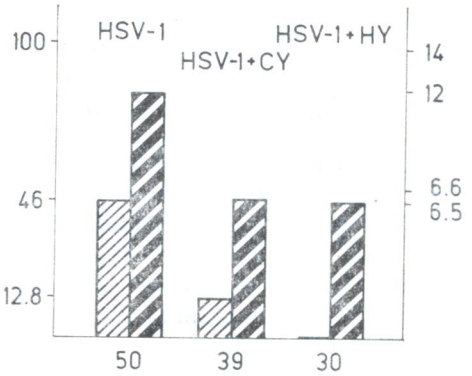

Fig. 3.
Effect of cyclophosphamide and hydrocortisone on the mortality and mean survival time of mice infected with HSV-1 by i.p. route ($5 \times 10^{5.5}$ $TCID_{50}$)
Thin lines: % of survivors; thick lines: mean survival time
Abscissa: no. of mice; ordinates: in the left — per cent of survivors; in the right — mean survival time.

Effect of immunosuppressive drugs on the immunization with inactivated herpes virus

CY or HY given to mice together with inactivated HSV-1 decreased the formation of specific antibodies. No anti-HSV-1 antibodies were detected by ELISA; the titre of CF antibodies was equal 2. In non-immunosuppressed mice, however, the antibody titres were 160 and 16, respectively.

In the group of animals which have received CY together with inactivated HSV-1,after virus challenge ($5 \times 10^{5.5}$ $TCID_{50}$) the rate of mice survival (71.4 %) and the mean survival time (12.4 days) was similar to those observed in non-immunosuppressed animals (Tab. 4). χ^2 test did not indicate any significant differences of survival ($P_0 > 0.3$) and in mean survival time ($P_0 > 0.2$). HY given during immunization diminished the resistance of animals to administration of infectious HSV-1. In comparison with the control group, the percentage of survivors decreased from 89.7 % to 47.6 % and the mean survival time from 13.4 to 11. 4 days (Tab. 4). The differences in survival of mice ($P_0 < 0.005$) and the mean survival time ($P_0 < 0.02$) were statistically significant. These data were, however, close to those obtained in non-immunized mice (46 % and 12 days, respectively).

Discussion

Herpes viruses cause numerous lethal syndroms as well infections reactivated in immunosuppressed persons (e. g. Fiala *et al.*, 1975;Galasso 1981; Nahmias *et al.*, 1981). Therefore they are the subject of intensive investigations. The aim of the presented experiments was to define the effect of immunosuppressive drugs on the progress of virus infection in mice infected with HSV. After administration of CY or HY a considerable decrease of survival time as well as of the survival rate was found both in i.c. and i.p. infected animals.

In immunosuppressed mice a lower ability to limit virus replication was observed. It was especially evident in mice infected i.c. with the dose as low as 3 $TCID_{50}$. This was demonstrated by IF showing larger number and size

of HSV-1 foci in brain, liver and spleen of immunosuppressed mice. These data are in accordance with original and well documented observations of Rajčáni *et al.* (1974) and the results published later by Rager-Zisman and Allison (1976), who found increased replication of HSV and more severe pathological changes in i.p., oraly or intravenously infected mice immunosuppressed with CY. In their interpretation of the effect of immunosuppressive drugs Kirchner *et al.* (1978) went even further suggesting that these compounds cause the loss of genetic resistance to HSV-1 infection in C57BL/6 mice.

HSV-1 is an example of virus which spreads from cell to cell. The virus penetrates the neighbouring cells prior to lysis of previously infected cells (Notkins 1974). Virus spread may occur in the infected organism in the presence of high titres of specific antibodies (Nahmias and Roizman 1973; Price *et al.*, 1982) since they are not able to neutralize sufficiently the intracellular virus. This is the reason why despite already existing anti-HSV antibodies not depressed during immunosuppression (Armstrong *et al.*, 1971; Prince *et al.*, 1971) the virus still spreads in immunosuppressed patients.

Observations in men are in agreement with experimental results presented in this paper. It was shown that previous i.p. immunization of animals with inactivated virus does not protect mice against pathogenic virus effect if CY or HY was given at the time of infection, despite the presence of specific antibodies. But in immunized mice which were not immunosuppressed after infection with the virulent strain, the percentage of survivors as well as the mean survival time were significantly increased in comparison to the group of non-vaccinated mice. It was also observed that immunosuppression did not decrease the existing level of herpes virus antibodies. That is related to observations made by Suvansirikula *et al.* (1977) in kidney transplated recipients when immunosuppressive therapy did not decrease the level of cytomegalovirus antibodies.

It was found in the present investigations that the effect of CY and HY on the immunization itself was divergent. No antibodies were detected in animals immunosuppressed with CY or HY at the time of immunization. Despite the absence of humoral response in mice received CY after challenge with wild virus, the percentage of survivors and the mean survival time were comparable with values obtained in unimmunosuppressed animals. CY depressed mainly the humoral response related to its preferential effect on B lymphocytes (Turk 1972). The dose administrated in these experiments (300 mg/kg body weight) applied even for a short time also diminishes the cellular response. While the delayed hypersensitivity returned to a normal level already within three days since the administration of drug (Turner 1979). The described results indicate that CY given during immunization decreased the progress of humoral response. It does not inhibit the cellular response which after administration of virulent HSV-1 strain contributed to the elimination of virus infection. On the other hand, however, HY decreases the number of B lymphocytes and inhibits the process of differentiation of B cell precursors in the cells producing antibodies (Bach 1976); it mainly exerts a destructive effect on the progress of cellular response.

As it appears from many investigations performed on clinical material (Nahmias 1970; Logan *et al.*, 1971; Merigan and Stevens 1971; Muller *et al.*, 1972) and in animals infected with HSV (Mori, Tasaki and Kimura 1967; Nahmias *et al.*, 1969; Zisman *et al.*, 1970; Ennis 1973*a*, 1973*b*) mainly the cellular response is responsible for protection against HSV infection. The inhibiting effect of HY on cytotoxic reactions, interferon synthesis and the population of macrophages (Weissman and Thomas 1962; Fauci *et al.*, 1976) contribute to the elimination of HSV (Mogensen 1979) and might also cause that immunization with inactivated virus (administrated together with HY) did not protect mice against virus challenge.

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Explanation to Figures (Plate XLVII).

Fig. 4. Intracellular and extracellular HSV-1 antigen in the brain.

Fig. 5. Varying amounts of HSV-1 antigen in hepatocyte nuclei, their cytoplasm and apparently in the sinusoidal spaces at the periphery of a hepatic lobule.

Fig. 6. Heavy deposit of HSV-1 antigen in the splenic capsule and in the cytoplasm of a few macrophages.