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Virus-specific IgM and IgG Antibody Production by B Cells during Herpes Simplex Virus Type 2-induced Immunosuppression as Analysed by an Immunospot Assay

By S. NICK, B. METZGER, S. MULLER AND D. FALKE*

Division of Experimental Virology, Institute of Medical Microbiology, Johannes-Gutenberg-Universität, Hochhaus am Augustusplatz, D-6500 Mainz, F.R.G.

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SUMMARY

The mechanism of herpes simplex virus (HSV)-2-induced immunosuppression was analysed by determination of the number of IgM and IgG antibody-secreting B cells in female BALB/c mice using an immunospot assay. Primary HSV-1 or -2 as well as homologous or heterologous booster infections at different times were performed. In accordance with earlier results on humoral antibody generation, in contrast to HSV-1, HSV-2 induced only very low numbers of antibody-producing B cells in dose–response experiments. They appeared late after infection compared to HSV-1. Despite a homologous humoral booster reaction against HSV-1 at day 8 no IgM- or IgG-secreting cells in the spleen could be detected. This non-reactivity of the spleen had vanished 10 days later, when secondary reactions of B cells could be observed. Secondary infections with a high homologous dose of HSV-2 after a low primary dose produced only a low booster response of IgG-secreting B cells. Suppression of humoral antibody production induced by HSV-2 (high dose) waned after more than 50 days, indicating that the HSV-2-induced suppression did not impair antigen presentation or memory cell generation.

INTRODUCTION

Far reaching conclusions have been drawn from the observation that females with cancer of the cervix have higher antibody titres against herpes simplex virus (HSV)-2 than HSV-1 (Rawls *et al.*, 1968). This correlation was recently questioned (Vonka *et al.*, 1984*a*, *b*). Recent results suggest that HSV-2 may influence the humoral antibody levels by immunosuppression (Kampe *et al.*, 1985; Nick *et al.*, 1986) and consequently the local microepidemiology of HSV-2 may be affected.

We were studying the kinetics and genetics of HSV-1-induced antibody formation in mice (Knoblich *et al.*, 1983) and tested the possibility of enhancement of antibody formation (Knoblich *et al.*, 1984). Surprisingly, only very low levels of antibodies were obtained after infection with four different strains of HSV-2 (Kampe *et al.*, 1985). Some intertypic recombinants also induced only low levels of antibodies. Finally, infectious HSV-2 but not HSV-1 was able to induce suppression of humoral antibody formation against HSV-1 and -2 (Kampe *et al.*, 1985; Nick *et al.*, 1986). It was demonstrated by injection of silica that macrophages were the first target, since silica could release the suppression (Nick *et al.*, 1986).

The HSV-2-induced suppression phenomenon of antibody formation was therefore studied at the level of B cells producing HSV-specific IgM or IgG antibodies. The main purpose of the present communication was to elucidate more exactly the mechanism by which HSV-2 might interrupt antibody generation.

METHODS

Mice. Female inbred BALB/c mice were raised in our own breeding colony. Female outbred NMRI mice were purchased from Savo-Ivanovas (Kisslegg, Allgäu, F.R.G.). They were injected intraperitoneally at 6 to 8 weeks of age.

Cells and viruses. HSV-1 strain Len and HSV-2 strains HG-52 and Bry were used. The properties of these virus strains were described previously (Kampe *et al.*, 1985; Nick *et al.*, 1986). For infection of mice, virus was propagated on confluent monolayers of Vero cells grown in Medium 199 (Gibco) supplemented with 10% heat-inactivated newborn calf serum. Titres of the virus stocks were determined by plaque assays on Vero cells (Kampe *et al.*, 1985).

For preparation of antigen, virus was grown on BHK-21 cells in GMEM (Gibco) with 10% newborn calf serum (Gibco). For most immunospot assays HSV-1 was used as antigen; essentially identical results were obtained using HSV-2.

Preparation of antigen. The procedure used was described recently (Nick *et al.*, 1986). In brief, 48 h after the infection of BHK cells, the culture supernatants were harvested and centrifuged for 20 min (2500 *g*). Infected BHK cells were disrupted by repeated freezing and thawing. Comparable supernatants of non-infected BHK cells served as control antigen.

The virus particles of the supernatant were pelleted in an SW27 rotor (100000 *g*, 1 h). The pellet was carefully resuspended in phosphate-buffered saline (PBS) and layered onto 15 ml of 30% sucrose (w/w) in PBS. After a second centrifugation, the protein sediment was resuspended in PBS containing 0·1% NP40 by sonication. In the case of control antigen the 100000 *g* pellet was adjusted to the same protein concentration as the virus antigen.

Enzyme-linked immunospot assay. Antibody-secreting cells were determined according to the protocol of Sedgwick & Holt (1983). In brief, 5 cm Petri dishes (Nunc) were coated with HSV antigen or control antigen for 12 to 18 h at 5 °C. In general, 50 μg of protein per dish was sufficient. The dishes were washed twice with PBS to remove unadsorbed material and exposed to PBS containing 1·5% bovine serum albumin (BSA) (w/v) for 2 h at room temperature.

Cell suspensions were prepared by using the pooled spleens of three mice per assay. After mincing, the material was passed through a steel sieve. Cells of this suspension were pelleted (400 *g*, 5 min) and the erythrocytes lysed with 0·83% ammonium chloride. After two washes with Hanks' balanced salt solution, viable cells were counted by the trypan blue exclusion method. Spleen cells were diluted in RPMI 1640 medium with 10% calf serum and 330 μl of each dilution was added to the dishes. The dishes were placed for 5 h at 37 °C in a vibration-free incubator. To remove lymphocytes the dishes were washed carefully three times with PBS and three times with PBS containing 0·05% Tween 20. For visualization of secreted and bound antibodies, the dishes were incubated with alkaline phosphatase-conjugated goat anti-mouse IgM or IgG (μ- or γ-chain-specific; Zymed Laboratories, San Francisco, Ca., U.S.A.) for 2 h at 37 °C. Dishes were rinsed again and blue spots developed after addition of 1 ml of 2·3 mM-5-bromo-4-chloro-3-indolylphosphate (Sigma) in aminomethylpropanol buffer mixed with agarose (Sedgwick & Holt, 1983). Blue spots could be counted after 1 h at 37 °C under low magnification.

Most important for the immunospot test was the concentration of the antigen used for coating, the quality of the alkaline phosphatase-conjugated anti-mouse sera and the time of incubation.

The optimal concentration of antigen was determined by a chequerboard test. Spleen cells were obtained from animals immunized with 1×10^3 p.f.u. HSV-1 (Len) 12 days before. The optimal time for detecting immunospots was found to be after 5 to 6 h of incubation. The same procedure proved to be useful for HSV-2 antigen.

Specificity of the immunospot assay. In order to test the specificity of the assay system, spleen cells from HSV-1-immunized mice were tested for antibody formation against BHK control antigen.

Spleen cells from mice immunized 4 days before with sheep red blood cells were tested on HSV-1 antigen and control antigen. The assay proved to be specific for HSV. Comparing the number of immunospots from BALB/c or NMRI mice, we noted a greater homogeneity of the spot number in the inbred BALB/c females. NMRI mice produced somewhat higher numbers of antibody-secreting cells. There was no detectable difference in the size of the spots if HSV-1 or HSV-2 was used as catching antigen.

Neutralization-test. The method was used as described before (Kampe *et al.*, 1985; Knoblich *et al.*, 1983, 1984; Nick *et al.*, 1986). Approximately 20 to 30 p.f.u. was used for the neutralization reaction. Five mice were used per group. The values are given ± S.D.

RESULTS

Primary infections by HSV-1 and -2

We first studied the number and time of appearance of IgM- and IgG-secreting cells after primary infection of BALB/c mice with 1×10^3 p.f.u. of HSV-1 (strain Len). Fig. 1 shows the increase in the number of IgM-producing B cells from days 5 to 12. IgG-producing cells were detected until day 20. In contrast, the same dose of HSV-2 did not induce antibody-forming B cells (Fig. 2). Moreover, no increase in the total number of spleen cells was observed if compared to HSV-1.

We next studied the number of B cells in dose–response experiments as described earlier for humoral antibody formation. Fig. 3 demonstrates IgG-forming B cells after infection of NMRI

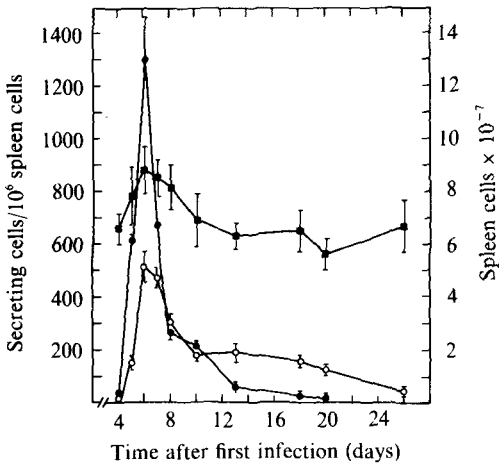

Fig. 1

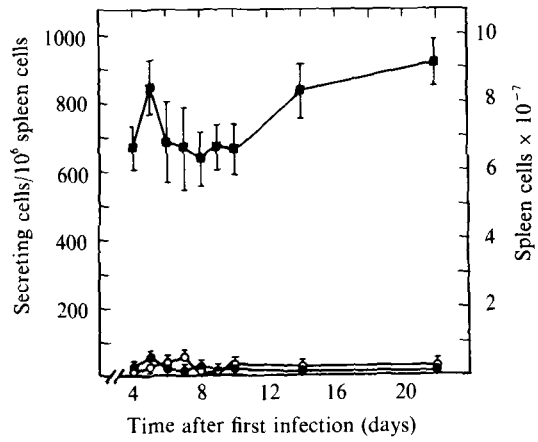

Fig. 2

Fig. 1. Number of IgM (●) and IgG (○) antibody-secreting B cells per 10^6 spleen cells after intraperitoneal primary infection with HSV-1 (strain Len, 1×10^3 p.f.u.). Total number of spleen cells (■) of BALB/c mice.

Fig. 2. Number of IgM (●) and IgG (○) antibody-secreting B cells per 10^6 spleen cells after primary infection with HSV-2 (strain HG-52, 1×10^3 p.f.u.). Total number of spleen cells (■) of BALB/c mice.

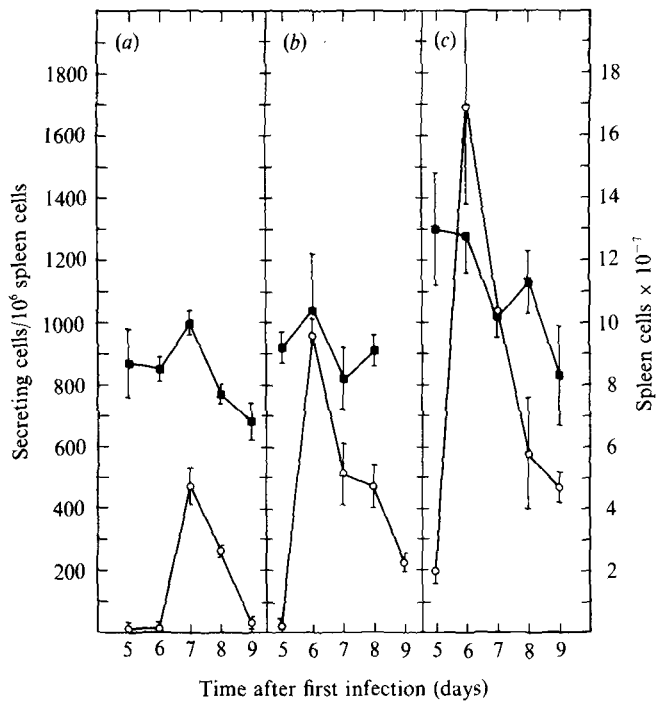

Fig. 3. Dose–response experiment of HSV-1 (strain Len) with (*a*) 1×10^2 (*b*) 5×10^3 and (*c*) 1×10^5 p.f.u. IgG-secreting B cells (○) were determined at different times after infection. Total number of spleen cells (■) of NMRI mice.

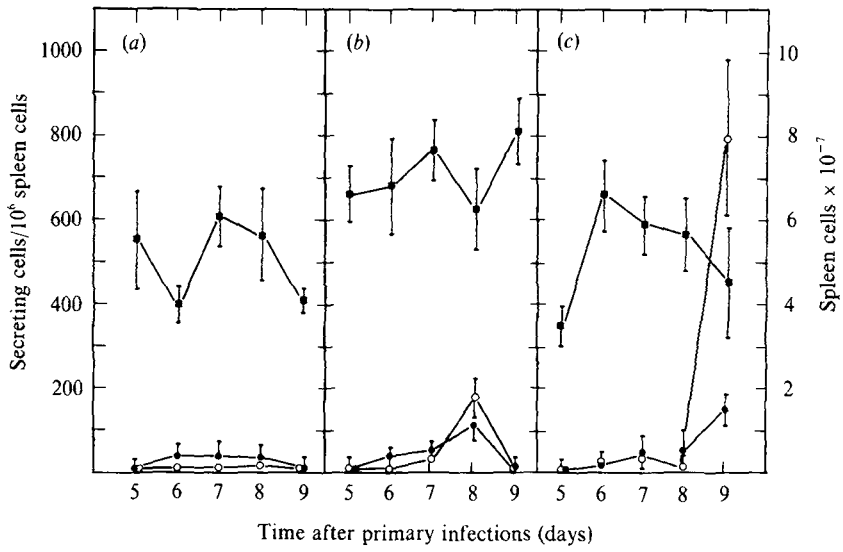

Fig. 4. Dose–response experiment with HSV-2 (strain HG-52) for IgM- (●) or IgG- (○) producing cells at different times after primary infection with (*a*) 3×10^2, (*b*) 5×10^3 and (*c*) 1×10^5 p.f.u. Total number of spleen cells (■) of NMRI mice.

mice with 1×10^2, 5×10^3 or 1×10^5 p.f.u. of HSV-1 (strain Len). It could be seen that if the dose of HSV was increased, an increase of the number of IgG-producing B cells could be detected. In BALB/c mice (data not shown) a comparable increase of antibody-generating cells was observed, however at a lower level compared to NMRI mice.

Fig. 4 shows low levels of IgM- and IgG-producing B cells of BALB/c mice after infection with increasing doses of HSV-2 (strain HG-52). Additionally, it was observed that the IgM and IgG cell responses were delayed compared to antibody-secreting B cells after HSV-1 infection. It should be noted that a high dose of this strain of HSV-2 (1×10^5 p.f.u.) produced disease in many mice. In control experiments (data not shown) all surviving mice exhibited HSV strain-typical humoral antibody formation after infection with HSV-1 or -2.

Duration of the HSV-2-induced suppression

Suppression of antibody generation was first detectable on day 5 and was most pronounced 8 days after infection with HSV-2 (Nick *et al.*, 1986). We therefore determined the duration of this suppression by using HSV-2 (strain Bry) which, because of its low pathogenicity, did not kill mice at doses of 10^4 and 10^6 p.f.u. and could therefore be used for long term testing of antibody production.

Table 1 shows the course of antibody formation after long term testing of mice Antibody formation was detectable 51 and 61 days after infection (1×10^6 p.f.u.) but not earlier. These results indicated that the suppression induced by HSV-2 disappeared later than 40 days after infection.

Secondary infection at day 8

The next question was whether secondary infections with different doses of HSV-1 or HSV-2 on day 8 after primary infections induced secondary B cell reactions. A high dose of a HSV-1 homologous re-infection in BALB/c mice (1×10^6 p.f.u.) induced only a minor booster cell response (Fig. 5*a*). No response was observed after re-infection with 5×10^3 p.f.u. (Fig. 5*b*). However, there was a typical booster reaction if humoral antibody formation was tested in NMRI mice after a second injection followed a primary dose of 1×10^3 p.f.u. (Table 2, group A).

Pre-infection with HSV-2 (1×10^3 p.f.u., strain HG-52) and second infections with either

Table 1. *Antibody formation after prolonged incubation with HSV-2 (strain Bry) after primary infection*

Day of bleeding after infection	Neutralization ± S.D.	
	1×10^4 p.f.u.	1×10^6 p.f.u.
21	1:3	<1:40
31	1:6	<1:40
41	1:2	<1:40
51	1:1	1:100 ± 26
61	1:5	1:269 ± 67

Table 2. *Antibody formation after re-infection with HSV-1 or -2 at days 8 or 21**

Group	Primary infecting virus at day zero	Dose of 1st virus (p.f.u.)	Secondary infecting virus at day 8 or 21	Dose of 2nd virus (p.f.u.)	Neutralization ± S.D.
A	HSV-1 (Len)	1×10^3	–	–	1:280 ± 114
	HSV-1 (Len)	1×10^3	HSV-1 (Len) 8	1×10^3	1:491 ± 173
B	HSV-2 (Bry)	1×10^4	–	–	<1:10
	HSV-2 (Bry)	1×10^4	HSV-2 (Bry) 8	1×10^4	<1:10
C	HSV-2 (Bry)	1×10^4	–	–	1:8 ± 7
	HSV-2 (Bry)	1×10^4	HSV-1 (Len) 8	3×10^3	1:59 ± 19
	–	–	HSV-1 (Len) 8	3×10^3	1:874 ± 341
D	HSV-2 (HG-52)	1×10^3	–	–	<1:10
	HSV-2 (HG-52)	1×10^4	–	–	1:21 ± 11
	HSV-2 (HG-52)	1×10^5	–	–	1:106 ± 53
E	HSV-2 (HG-52)	1×10^3	HSV-2 (HG-52) 8	1×10^3	<1:10
	HSV-2 (HG-52)	1×10^3	HSV-2 (HG-52) 8	1×10^4	1:32 ± 18
	HSV-2 (HG-52)	1×10^3	HSV-2 (HG-52) 8	1×10^5†	1:22 ± 26
F	HSV-2 (HG-52)	1×10^3	HSV-2 (HG-52) 21	1×10^3	<1:10
	HSV-2 (HG-52)	1×10^3	HSV-2 (HG-52) 21	1×10^4	1:110 ± 23
	HSV-2 (HG-52)	1×10^3	HSV-2 (HG-52) 21	1×10^5‡	1:169 ± 45

* The tests were done in NMRI mice, five per group. Bleeding was done 21 days after the last infection. Groups A to C and D to F were each treated in one experiment.

† Eight mice were infected, three died.

‡ Seven mice were infected, one died.

5×10^3 p.f.u. or 1×10^6 of HSV-1 showed only a cellular reaction with 1×10^6 p.f.u. of HSV-1 (Fig. 5*c* and *d*). This B cell response, however, was low compared to a primary HSV-1 infection and was characterized by only some IgM- and IgG-producing cells. A second injection with HSV-2 (1×10^3 or 1×10^6 p.f.u.) irrespective of the dose resulted in no B cell response (data not shown). A second experiment of this type (as Fig. 5*a* to *d*) gave identical results, with the exception that in the experiment corresponding to Fig. 5(*c*) the response was still lower. The results of Fig. 5(*c*) may be compared to the levels of humoral antibodies of group C in Table 2.

Booster infections at 21, 28 and 35 days after infection

Homologous booster infections by HSV-1 at day 8 produced only a weak B cell response in the spleen (see above), whereas the level of humoral antibodies was increased independently of the time of the second injection (day 8, 21, 28 or 35) (Kampe *et al.*, 1985; Nick *et al.*, 1986; Table 2). Moreover, at day 21 (group F) the degree of HSV-2 (1×10^4 or 1×10^5 p.f.u. of booster)-induced suppression was lower than at day 8 (Table 2, group E). A single injection with 1×10^5 p.f.u. of strain HG-52 induced limited antibody formation (Table 2, group D), as described earlier (Kampe *et al.*, 1985). We therefore compared the number of antibody-secreting B cells after secondary infections (low or high dose of HSV-1 or -2) at 21, 28 and 35 days after infection to the humoral antibody levels (Table 2 and Fig. 5).

A low dose homologous booster of HSV-1 administered to BALB/c mice (Table 3, group A) gave no specific B-cell response; however the high dose booster with HSV-1 at day 21 and 28

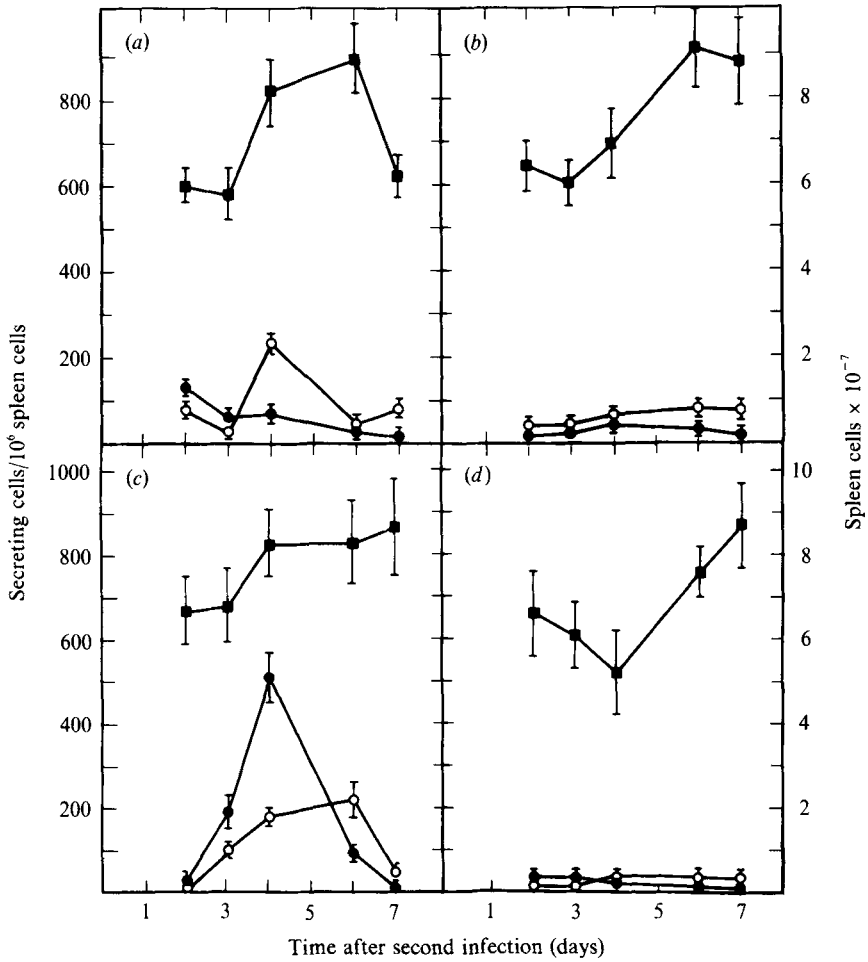

Fig. 5. IgM (●) and IgG (○) antibody-secreting B cells after a secondary infection with (*a*) 1×10^6 p.f.u. and (*b*) 5×10^3 p.f.u. of HSV-1 (strain Len) after a primary infection with HSV-1 (strain Len, 1×10^3). In (*c*) and (*d*) 1×10^3 p.f.u. of HSV-2 (strain HG-52) were used as a primary infection. As secondary infections, (*c*) 1×10^6 or (*d*) 5×10^3 p.f.u. of HSV-1 (strain Len) were used at day 8. Total spleen cells were determined (■) for BALB/c mice.

resulted in a high number of antibody-secreting cells (Table 3, groups B and D). IgM-producing cells were also detected in group B, indicating no suppression. This result contrasted with the almost complete lack of IgM- and IgG-producing cells at day 8 in the spleens of mice inoculated with 10^6 p.f.u. of HSV-1 after a primary dose of 10^3 p.f.u. of HSV-1 (Fig. 5*a*, homologous high dose booster). A high dose booster with HSV-2 at day 28 after HSV-1 priming (group E) gave a considerable cell IgG response as expected. Group F gave the data of a high dose HSV-1 booster at day 28 after HSV-2 priming. Only a low IgG reaction was induced by the suppressing HSV-2 primary infection. This result may be compared to Fig. 2 of Nick *et al.* (1986). Group C of Table 3 gives the values for a high dose booster (HSV-2). In this case no IgM-secreting cells but considerable numbers of IgG-secreting cells could be observed. It should be added that a high dose booster with HSV-1 at day 35 (compare Table 3, groups B and D) always gave strong HSV-specific cellular reactions in the spleen, whereas a low dose booster gave no reaction. Similar reactions in the spleen could be observed after a double homologous booster (at 21 and 42 days after infection) with either low or high doses of HSV-1 (data not shown). Generally it could be observed that the B cell booster response after HSV-2 priming was delayed compared to HSV-1 priming.

Table 3. *The number of IgM- and IgG-producing B cells after secondary infection with high or low doses of HSV-1 or -2 at days 21 and 28**

Group	Day of first infection	Type of first infecting virus and dose†	Day of re-infection	Type of re-infecting virus and dose†	Day of test	IgM-AsC/ 10^6 spleen cells‡	IgG-AsC/ 10^6 spleen cells	Total number of spleen cells × 10^7
A	Zero	HSV-1 (Len, 1×10^3)	21	HSV-1 (Len, 5×10^3)	2	7 ± 1	17 ± 3	$7{\cdot}0 \pm 0{\cdot}7$
					3	6 ± 4	49 ± 12	$13{\cdot}0 \pm 1{\cdot}0$
					4	7 ± 1	39 ± 4	$9{\cdot}6 \pm 1{\cdot}1$
					5	14 ± 4	32 ± 8	$11{\cdot}8 \pm 1{\cdot}0$
					8	0 ± 0	30 ± 2	$7{\cdot}8 \pm 1{\cdot}2$
B	Zero	HSV-1 (Len, 1×10^3)	21	HSV-1 (Len, 1×10^6)	1	NT§	8 ± 1	$5{\cdot}4 \pm 0{\cdot}7$
					2	14 ± 4	106 ± 7	$9{\cdot}6 \pm 1{\cdot}2$
					3	143 ± 2	1708 ± 236	$11{\cdot}6 \pm 0{\cdot}4$
					4	128 ± 23	1056 ± 108	$9{\cdot}5 \pm 0{\cdot}8$
					5	57 ± 1	877 ± 85	$9{\cdot}2 \pm 0{\cdot}8$
					8	0 ± 0	67 ± 7	$6{\cdot}7 \pm 0{\cdot}5$
C	Zero	HSV-2 (HG-52, 1×10^3)	21	HSV-2 (HG-52, 1×10^6	2	1 ± 1	4 ± 1	$6{\cdot}5 \pm 0{\cdot}3$
					3	13 ± 5	52 ± 5	$14{\cdot}3 \pm 0{\cdot}8$
					4	0 ± 0	385 ± 31	$11{\cdot}2 \pm 0{\cdot}8$
					5	0 ± 0	1080 ± 53	$11{\cdot}6 \pm 0{\cdot}8$
					6	NT	412 ± 29	$9{\cdot}8 \pm 0{\cdot}4$
					8	NT	87 ± 9	$10{\cdot}3 \pm 0{\cdot}9$
D	Zero	HSV-1 (Len, 1×10^3)	28	HSV-1 (Len, 1×10^6)	2	NT	30 ± 1	$8{\cdot}3 \pm 0{\cdot}2$
					3	NT	477 ± 78	$10{\cdot}5 \pm 1{\cdot}1$
					4	NT	2077 ± 327	$10{\cdot}2 \pm 1{\cdot}1$
					5	NT	812 ± 154	$10{\cdot}9 \pm 0{\cdot}9$
					6	NT	627 ± 121	$9{\cdot}3 \pm 1{\cdot}2$
					7	NT	292 ± 23	$8{\cdot}8 \pm 1{\cdot}5$
E	Zero	HSV-1 (Len, 1×10^3)	28	HSV-2 (HG-52, 1×10^6)	2	NT	36 ± 5	$9{\cdot}2 \pm 0{\cdot}7$
					3	NT	1003 ± 21	$8{\cdot}5 \pm 0{\cdot}3$
					4	NT	3578 ± 318	$9{\cdot}5 \pm 1{\cdot}3$
					5	NT	1549 ± 221	$10{\cdot}0 \pm 0{\cdot}4$
					6	NT	892 ± 60	$7{\cdot}1 \pm 1{\cdot}5$
					7	NT	288 ± 62	$7{\cdot}8 \pm 1{\cdot}0$
F	Zero	HSV-2 (HG-52, 1×10^3)	28	HSV-1 (Len, 1×10^6)	2	NT	17 ± 4	$5{\cdot}9 \pm 0{\cdot}9$
					3	NT	130 ± 26	$5{\cdot}3 \pm 0{\cdot}7$
					4	NT	156 ± 22	$6{\cdot}2 \pm 0{\cdot}8$
					5	NT	374 ± 50	$7{\cdot}2 \pm 0{\cdot}9$
					6	NT	233 ± 31	$7{\cdot}5 \pm 0{\cdot}9$
					7	NT	30 ± 1	$8{\cdot}5 \pm 1{\cdot}1$

* The groups A to C and D to F were each treated in one experiment. Values are averages from three BALB/c mice.

† Dose given in p.f.u.

‡ AsC, Antibody-secreting cells.

§ NT, Not tested.

DISCUSSION

The object of this study was to determine the kinetics of IgM- and IgG-producing B cells after HSV-1 and -2 infections in the spleen using an immunospot test supplemented by results on formation of neutralizing antibodies. By this experimental design we intended to obtain more insight into the mechanism of the HSV-2-induced suppression of humoral antibody formation (Kampe *et al.*, 1985; Nick *et al.*, 1986).

Dose response experiments of primary infections with HSV-1 resulted in typical responses of IgM- and IgG-producing cells 5 to 20 days after infection. In contrast, HSV-2 induced only very low numbers of HSV-specific IgM- or IgG-producing B cells. This observation coincided clearly with earlier results on humoral antibody formation (Kampe *et al.*, 1985; Nick *et al.*, 1986) and Table 2. Compared to the lymphocytic choriomeningitis virus system (Moskophidis & Lehmann-Grube, 1984) the number of HSV-specific antibody producing cells was rather low in BALB/c mice. However, they produced lower HSV-specific antibody serum titres than NMRI mice.

Concerning the total number of spleen cells, HSV-2 induced no or a lower response than HSV-1 (Nick, 1986; Nick *et al.*, 1986). This observation is supported by the experiments presented here and may be explained by a lack of certain lymphokines for cellular proliferation after HSV-2 infections. Interestingly, silica given separately induced an increase in the number of spleen cells at days 6 to 10 (data not shown) and, moreover, released the HSV-2-induced suppression probably by influencing macrophages (Nick *et al.*, 1986).

Primary infections (low dose) and homologous re-infections (high dose) with HSV-1 at day 8 produced only a weak B cell response in the spleen. However, typical HSV-1 booster reactions (low dose) of humoral antibodies could be elicited if a low dose of HSV-1 was given 8 days after primary low dose infections. We therefore assume an extra-splenic localization of antibody-forming cells under these conditions. It is known that after priming, B cells migrate from the spleen to other locations (Van Rooijen *et al.*, 1986). In contrast, re-infections at days 21, 28 and 35 (only high doses of virus) resulted in strong, specific cellular secondary responses in the spleen. This indicates that 8 days after HSV-1 infection in the spleen, but not in the remainder of the organism, a state of HSV-induced non-reactivity exists, which disappears within 10 days.

Suppression of humoral antibody formation is most effective 8 days after HSV-2 infection (Nick *et al.*, 1986). No booster cell response or antibody formation after HSV-2 booster infections was detectable early after challenge. The HSV-2-induced suppression of humoral antibodies lasts for about 40 days after infection with a high dose of strain Bry (1×10^6 p.f.u.). After this time humoral antibodies against HSV-2 appear spontaneously. Therefore, priming and memory should be intact. Even at day 21 a reduction of the suppression was observed (Table 2, group E and F). In contrast, strain HG-52 induced some humoral antibodies at a dose of 1×10^5 (Kampe *et al.*, 1985); consequently, the degree of suppression seems to be different from strain to strain of HSV-2.

At days 21, 28 and 35 a limited cellular booster could be seen (Table 3). The response was of the IgG cell type, no IgM-producing cells were detected. These results point to the stability of the priming events. Perhaps memory cells are also generated after induction of non-antibody-producing primary infections. Additionally, there is a delay in the appearance of IgG antibody-producing cells. The HSV-2-induced suppression may thus be caused by a block at different stages of antibody formation including macrophages and perhaps connected to a lack of proliferation of T helper cells or overproduction of suppressor cells. The existence of suppressor cells for HSV-1 humoral antibody generation was indicated by use of a low dose of cyclophosphamide (Knoblich *et al.*, 1984). Such cells were also observed for HSV-induced delayed type hypersensitivity (Nash *et al.*, 1985).

We hope that further analysis of the HSV-2-induced suppression phenomenon will enable us to elucidate the cellular and/or lymphokine defects as well as the HSV-2-induced peculiarities of the host–parasite relationship.

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REFERENCES

KAMPE, P., KNOBLICH, A., DIETRICH, M. & FALKE, D. (1985). Differences in humoral immunogenicity between herpes simplex virus types 1 and 2. *Journal of General Virology* **66**, 2215–2223.

KNOBLICH, A., GÖRTZ, J., HÄRLE-GRUPP, V. & FALKE, D. (1983). Kinetics and genetics of herpes simplex virus-induced antibody formation in mice. *Infection and Immunity* **39**, 15–23.

KNOBLICH, A., MÜLLER, W. E. G., HÄRLE-GRUPP, V. & FALKE, D. (1984). Enhancement of antibody formation against herpes simplex virus in mice by the T-cell mitogen bestatin. *Journal of General Virology* **65**, 1675–1686.

MOSKOPHIDIS, D. & LEHMANN-GRUBE, F. (1984). The immune response of the mouse to the LCM-Virus. IV. Enumeration of antibody producing cells in spleens during acute and persistent infection. *Journal of Immunology* **133**, 3366–3370.

NASH, A. A., LEUNG, K. N. & WILDY, P. (1985). The T-cell mediated immune response of mice to herpes simplex virus. In *The Herpesviruses*, vol. 4, pp. 87–102. Edited by B. Roizman & C. Lopez. New York: Plenum Press.

NICK, S. (1986). *Die B-Zellantwort der Milz nach der Infektion von Mäusen mit den Herpes simplex Viren vom Typ 1 und 2*. Dissertation, Johannes-Gutenberg Universität.

NICK, S., KAMPE, P., KNOBLICH, A., METZGER, B. & FALKE, D. (1986). Suppression and enhancement of humoral antibody formation by herpes simplex virus types 1 and 2. *Journal of General Virology* **67**, 1015–1024.

RAWLS, W. E., TOMPKINS, W. A. F., FIGUEROA, M. F. & MELNICK, J. L. (1968). Herpes virus type 2: association with carcinoma of the cervix. *Science* **161**, 1255–1256.

SEDGWICK, J. D. & HOLT, P. G. (1983). A solid phase immunoenzymatic technique for enumeration of specific antibody secreting cells. *Journal of Immunological Methods* **57**, 301–309.

VAN ROOIJEN, N., CLAASEN, E. & EIKELENBOOM, P. (1986). Is there a single differentiation pathway for all antibody-forming spleen cells? *Immunology Today* **7**, 193–196.

VONKA, V., KANKA, J., JELINEK, J., SUBRT, I., SUCHANEK, A., HAVRANKOVA, A., VACHAL, M., HIRSCH, I., DOMOVASZKOVA, E., ZAVADOVA, H., RICHTEROVA, V., NAPRSTKOVA, J., DVORAKOVA, V. & SVOBODA, B. (1984*a*). Prospective study on the relationship between cervical neoplasia and herpes simplex type-2 virus. I. Epidemiological characteristics. *International Journal of Cancer* **33**, 49–60.

VONKA, V., KANKA, J., JELINEK, J., SUBRT, I., SUCHANEK, A., HAVRANKOVA, A., VACHAL, M., HIRSCH, I., DOMOVASZKOVA, E., ZAVADOVA, H., RICHTEROVA, V., NAPRSTKOVA, J., DVORAKOVA, V. & SVOBODA, B. (1984*b*). Prospective study on the relationship between cervical neoplasia and herpes simplex type-2 virus. II. Herpes simplex type-2 antibody presence in sera taken at enrolment. *International Journal of Cancer* **33**, 41–66.

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