Immunity to Herpes Simplex Virus Type 2: Recurrent Lesions Are Associated with the Induction of Suppressor Cells and Soluble Suppressor Factors

T. IWASAKA,1 J. F. SHERIDAN,2,3 AND L. AURELIAN1,2,3∗

Division of Biophysics1 and the Johns Hopkins Sexually Transmitted Diseases Research Unit,2 The Johns Hopkins Medical Institutions 21205, and Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine,3 Baltimore, Maryland 21201

Received 17 June 1983/Accepted 27 August 1983

Cell-mediated immunity to herpes simplex virus type 2 was investigated in infected inbred strain 13/N guinea pigs with (45%), and without, a history of recurrent herpetic disease (A. D. Donnenberg, E. Chaikof, and L. Aurelian, Infect. Immun. 30:99–109, 1980). Induction of suppressor cells capable of reducing the proliferative response of herpes simplex virus type 2-stimulated immune lymphoid cells was demonstrated in spleen cells from animals with a history of recurrent disease at recrudescence and convalescence but not in spleen cells from quiescent animals or from animals without a history of recurrent herpetic disease (seropositive controls). Suppressor cells were also detected in the peripheral blood but only from three of seven studied animals, and only at recrudescence. In addition to inhibitory cell-cell interactions, the herpes simplex virus type 2-activated regulatory cells of animals with recrudescent herpetic lesions elaborated soluble suppressor factors affecting lymphocyte proliferation. Suppression mediated by suppressor factors was observed only when suppressor factors were added at an early stage of in vitro culture and was reversed by medium exchange throughout the 6 days of culture. Sephadex chromatography revealed the presence of factors capable of differentially modulating the proliferative response of herpes simplex virus-stimulated immune cells and concanavalin A-stimulated normal lymphoid cells.

The hallmark of herpetic disease in humans is the ability of the virus to cause recurrent disease in a proportion of seropositive individuals. Recovery from infection with herpes simplex virus (HSV) is associated with the development of: (i) delayed-type hypersensitivity, a persistent response reflective of the establishment of immune memory (20, 23), (ii) lymphoproliferation (LT) (15, 25), an in vitro response that correlates with HSV-specific delayed-type hypersensitivity (22), and is widely accepted as indicative of previous exposure to the antigen, and (iii) antigen-driven cell-mediated effector responses that can be measured in vitro, such as the production of lymphokines (3, 5, 26a). However, approximately one half of the infected individuals exhibit recurrent disease that occurs as a consequence of the reactivation of latent virus (10, 26a, 27). The observation that in the immunocompetent host, HSV lesions are self-limiting and of relatively short duration, whereas individuals with impaired cell-mediated immunity (CMI) suffer severe herpetic infections of endogenous origin (1, 18), has led to the hypothesis that recurrent HSV disease is associated with regulatory defect(s) in virus-specific CMI (2, 6, 27).

The following observations are pertinent with respect to the studies described in this report. The experimental design was made feasible by the development of a guinea pig model of recurrent herpetic disease (6, 26; A. D. Donnenberg, Ph.D. thesis, The Johns Hopkins University, Baltimore, Md., 1980). This model is particularly well suited for CMI studies since immune regulation in the guinea pig closely resembles that in humans (4, 5). Using this model, workers at our laboratory have previously shown that CMI responses to HSV antigens are impaired in animals with a history of recurrent disease studied during quiescence (6).

To investigate the possibility that suppressor lymphoid cells play an immune regulatory role in recurrent HSV disease, lymphoid cells obtained at various stages of the recurrent disease cycle were studied for their effect on T-cell dependent proliferation. The data indicate that spleen cells (SC) and peripheral blood lymphocytes (PBL) harvested during recrudescence (but not during
quiescence) contain suppressor cells capable of inhibiting virus-induced lymphoproliferation. Suppression appears to be mediated by soluble factors that differentially modulate HSV-induced proliferation of immune lymphoid cells, and concanavalin A (ConA)-stimulated proliferation of normal SC.

MATERIALS AND METHODS

Cells and virus. Primary guinea pig embryo cells (13/N fetuses, 15 to 25 days of gestation) were grown in Eagle minimal essential medium with 10% fetal calf serum. They were used in the propagation and plaque purification of the G strain of herpes simplex virus type 2 (HSV-2 [G]) used in these studies. The isolation and properties of this virus have been previously described (9).

Animal inoculation. Inbred strain 13/N guinea pigs were originally obtained from C. K. Hsu, University of Maryland School of Medicine, Baltimore. They were bred in a closed colony and infected with HSV-2 (G) as previously described (5, 6). Briefly, young adult animals (500 to 600 g) were injected subcutaneously in the right hind footpad with 2 10^4 PFU of a single preparation of HSV-2 (G) (50 μl). Consistent with previous findings (6), clinical symptoms were observed within 24 to 48 h and seroconversion was observed within 3 to 5 weeks postinfection. Guinea pigs were monitored three times per week for the development of recurrent disease, defined as at least moderate erythema and swelling, with virus shedding for 5 to 8 days after the onset of symptoms (6). Approximately 45% of the infected animals developed a history of recurrent disease within 1 to 4 months after infection and evidenced somewhat higher viral antibody titters than those without such history (seropositive controls) (6).

Antigens. HSV-2 (G) was passaged twice in guinea pig embryo cells at 0.2 PFU/cell. Virus pelleted by centrifugation at 100,000 × g for 1 h, was resuspended in phosphate-buffered saline (PBS) to a final concentration of 3.2 10^7 PFU/ml (250 μg of protein per ml) and inactivated by exposure to UV irradiation at 17 cm from a Sylvania G15T8 source for 30 min as described previously (5, 6). This preparation was designated UV-HSV-2 (G).

In vitro culture of lymphoid cells. SC and PBL were obtained as described previously (5, 6). They were cultured (2.0 10^6 cells per ml) at 37°C in freshly made RPMI 1640 medium with 10% decapitated normal 13/N guinea pig serum (GPS), 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, 5 × 10^-5 M 2-β-mercaptoethanol, 10 U of mycostatin and 50 μg of gentamycin per ml (RPML-10% GPS) as described previously (5, 6). Cultures (1-ml volume) were set in snap-cap tubes (12 by 75 mm; Falcon 2054) in the presence of 0.1 ml of UV-HSV-2 (G) antigen (final concentration, 6.0 μg of protein per ml), ConA (final concentration, 5 μg/ml), or PBS control. In mixed cultures, SC or PBL from seropositive control animals served as immune responder cells. They were mixed with various proportions (100, 80, 50, 20, and 0%) of lymphoid cells from animals with a history of recurrent disease (regulatory cells), or with mitomycin C-treated (50 μg/ml, 30 min, 37°C) nonimmune cells (control) and grown in presence of UV-HSV-2 (G) or PBS control.

LT assay. The LT assay was performed as described previously (5, 6). At appropriate intervals of in vitro culture, cells were suspended by blending in a Vortex mixer, and samples (100 μl) were transferred in triplicate to microtiter wells (Falcon 2040). Cells were pulsed for 4 h at 37°C with 25 μl of titrated thymidine ([3H]Tdr; 40 μCi/ml) well and harvested onto glass fiber filters (Reeve Angel grade 934 AH). Results were expressed as net cpm = (mean cpm_experimental) - (mean cpm_control), if it registered ≥200 net cpm. Responses to mock antigen in a series of control experiments were 38.3 net cpm, where upper and lower 99% confidence intervals were 81.0 and 0.0 net cpm, respectively (5).

Detection of soluble suppressor factor(s). SC or PBL grown in the presence of UV-HSV-2 (G) antigen (6.0 μg of protein per ml) were centrifuged at 300 × g for 10 min. Supernatants were collected and filtered through a 0.45-μm Millipore filter. They were assayed for suppressor factor(s) (SF) activity on cultures (2 × 10^6 cells) of UV-HSV-2 (G)-stimulated immune responder cells pulsed with [3H]Tdr on day 5 in culture (virus-specific suppression) or on ConA-stimulated (5 μg/ml) lymphoid cells obtained from normal (nonimmune) animals and pulsed with [3H]Tdr at 3 days in culture (nonspecific suppression). SF activity was expressed as the percent suppression calculated from the following formula: % suppression = 100 - % residual cpm where % residual cpm = mean cpm_conA - 100% GPS + SF/mean cpm_conA - 100% GPS × PBS × 100.

Sephadex chromatography of culture supernatants. Sephadex G-100 and G-200 (Pharmacia Fine Chemicals, Piscataway, N.J.) columns, 84 by 1.5 cm in diameter, were poured in PBS (pH 7.2) at 4°C. The columns were calibrated by eluting a solution of blue dextran and phenol red. Supernatants from day 5 in vitro cultures of SC from recrudescence animals were pooled and loaded on G-100 columns. Supernatants to be loaded on G-200 columns were first concentrated to a 1-ml volume (20 to 30 mg of protein) by ultrafiltration through an Amicon (PM10) membrane filter (Amicon, Lexington, Mass.). Columns were eluted with PBS, and 0.7-ml fractions were collected. Protein concentration was determined for each fraction by adsorption at 280 nm in a Beckman DBG spectrophotometer, and alternate fractions were assayed for virus-specific and nonspecific SF activity.

RESULTS

HSV-2-induced LT response in animals with recurrent disease. SC and PBL obtained from guinea pigs with a history of recurrent disease at recrudescence (0 to 7 days after onset of clinical symptoms), convalescence (8 to 14 days), and quiescence (>15 days) and from seropositive animals without a history of recurrent disease (seropositive controls) were grown in vitro in the presence of UV-HSV-2 (G) and assayed for [3H]Tdr incorporation at 3 and 6 days in cul-
Consistent responses were significantly higher in SC from seropositive controls than that from animals with a history of recurrent disease. In the latter group, the lowest responses were those of the SC obtained during recrudescence (Fig. 1). In PBL cultures, there were large variations in the LT responses of individual animals. Nevertheless, similar LT responses were observed in seropositive controls and in animals studied during quiescence and convalescence. However, during recrudescence, PBL from three of the seven animals studied in these series (animals 407, 318, and 303) had significantly (20- to 100-fold) lower responses (Table 1).

LT responses in mixed SC and PBL cultures. Experiments with mixed SC and PBL cultures were designed to determine whether reduced LT responses (Fig. 1 and Table 1) are related to the presence of active suppressor cells. SC and PBL from seropositive controls (immune responder cells) mixed with various proportions of lymphoid cells obtained at recrudescence, convalescence, or quiescence (regulatory cells) were cultured with UV-HSV-2 (G) and assayed for \(^3\)H-TdR incorporation at 3 and 6 days in culture. Controls consisted of immune responder cells mixed with various proportions of mitomycin C-treated (50 \(\mu\)g/ml, 30 min, 37°C), normal lymphoid cells. The magnitude of \(^3\)H-TdR incorporation in mixed cultures containing normal SC appeared to represent a virtually linear dilution of the immune responder cells. On the other hand, cultures containing as little as 20% recrudescent SC evidenced significantly reduced levels (58 to 85%) of \(^3\)H-TdR incorporation (Fig. 2). A similar reduction was not observed in cultures in which the regulators were SC obtained during quiescence, even if these were differentiated or amplified by 3 days of in vitro growth in the presence of UV-HSV-2 (G) before mixing (Table 2). In PBL cultures (Fig. 2C), reduced levels of \(^3\)H-TdR incorporation were observed only when the regulators were obtained at recrudescence, from the three animals (407, 318, and 303) with impaired LT responses shown in Table 1. PBL obtained at convalescence, quiescence, or from the seropositive control animals shown in Table 1 [including those with lower LT responses (viz. animal 300)] did not inhibit the proliferation of the immune responder PBL.

Production of soluble SF. In these experiments, SC obtained during recrudescence and grown in vitro in the presence of UV-HSV-2 (G) were centrifuged (300 \(\times\) g, 10 min.) at daily intervals, suspended in fresh RPMI-10% GPS with or without UV-HSV-2 (G) antigen, and assayed for \(^3\)H-TdR incorporation. As shown in Table 3, recrudescent SC suspended in fresh

![FIG. 1. \(^3\)H-TdR incorporation in cultures of SC from seropositive control (○), recrudescent (●), convalescent (□) and quiescent (●) guinea pigs incubated in the presence of UV-HSV-2 (G) antigen (6.0 \(\mu\)g of protein per ml). The data represent means ± standard deviation.](http://iai.asm.org/)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Net cpm*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seropositive controls</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>42,668 ± 607</td>
</tr>
<tr>
<td>157</td>
<td>61,368 ± 1,225</td>
</tr>
<tr>
<td>232</td>
<td>88,088 ± 4,346</td>
</tr>
<tr>
<td>230</td>
<td>95,787 ± 1,698</td>
</tr>
<tr>
<td>229</td>
<td>117,655 ± 1,438</td>
</tr>
<tr>
<td>435</td>
<td>130,445 ± 7,294</td>
</tr>
<tr>
<td>228</td>
<td>164,781 ± 3,455</td>
</tr>
<tr>
<td>437</td>
<td>252,520 ± 4,902</td>
</tr>
</tbody>
</table>

* PBL were obtained from animals, without a history of recurrent disease at 14 to 24 weeks after primary infection, that were seropositive for neutralizing antibody assayed as described in the text (seropositive controls) and from animals with virus-positive recrudescent lesions (0 to 7 days after the onset of symptoms), studied within 6 to 14 weeks after primary infection (recrudescent).
medium (with or without viral proteins) had five to ninefold higher levels of DNA synthesis than those observed in cultures maintained in the original medium. Conversely (Fig. 3), the original culture supernatant (diluted 1:5 in RPMI-10% GPS) produced a 30.8% ± 1.2 reduction in the LT response of UV-HSV-2 (G)-stimulated immune responder SC. Supernatants from cultures of PBL obtained from the three animals with impaired LT responses (Table 1) caused a similar reduction (40.3% ± 10) in the proliferation of immune responder cells. Supernatants from cultures of SC or PBL from seropositive control animals did not have a similar effect (7.6% ± 4.2 and 13% ± 10.9 suppression, respectively). These studies demonstrate that supernatants from cultures of lymphoid cells that are positive for active suppressor cells (Fig. 2), contain SF(s) capable of suppressing HSV-induced lymphoproliferation. The levels of SF activity in the supernatants increased as a function of time in culture, reaching maximal values on days 4 to 5 (Fig. 4).

**Effects of SF on LT response.** Cultures of UV-HSV-2 (G)-stimulated immune responder cells grown in the presence or absence of SF (20% final concentration) added at initiation (day 0) were assayed for [3H]Tdr incorporation on days 1, 3, 5, and 6. LT responses increased exponentially throughout the first 5 days (Fig. 5A).

However, the time required to generate a minimal positive response (200 net cpm) in the presence of SF could be graphically estimated as 2 days in culture as compared to 1 day in SF-free medium. The slopes of the responses were indistinguishable, indicative of a similar doubling

---

**TABLE 2.** [3H]Tdr incorporation in mixed cultures containing SC from quiescent animals

<table>
<thead>
<tr>
<th>Proportion of immune responder SC (%)</th>
<th>Quiescent SC$^a$</th>
<th>Amplified quiescent SC$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>9,604 ± 537</td>
<td>4,786 ± 146</td>
</tr>
<tr>
<td>80</td>
<td>12,869 ± 514</td>
<td>5,024 ± 177</td>
</tr>
<tr>
<td>50</td>
<td>12,008 ± 1,091</td>
<td>6,061 ± 304</td>
</tr>
<tr>
<td>20</td>
<td>7,594 ± 296</td>
<td>5,521 ± 281</td>
</tr>
<tr>
<td>0</td>
<td>7,394 ± 851</td>
<td>4,196 ± 524</td>
</tr>
</tbody>
</table>

$^a$ Immune responder SC were mixed with various proportions of SC obtained during quiescence and grown in the presence of UV-HSV-2 (G) (6.0 μg of protein per ml). [3H]Tdr incorporation was assayed on day 6.

$^b$ SC obtained during quiescence were grown for 3 days in the presence of UV-HSV-2 (G) (6.0 μg of protein per ml) before mixing with various proportion of immune responder SC. Mixed cultures were grown for 6 days with UV-HSV-2 (G) and assayed for [3H]Tdr incorporation.
TABLE 3. SF production in cultures of SC obtained at recrudescencea

<table>
<thead>
<tr>
<th>Time to medium exchange (day)</th>
<th>Net cpm (×103) on day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>No exchange</td>
<td>0.1 ± 0.5</td>
</tr>
<tr>
<td>1</td>
<td>14.5 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>9.6 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

a SC were grown in the presence of UV-HSV-2 (G) (6.0 μg of protein per ml). At daily intervals (days 1 to 5) they were centrifuged (300 × g, 10 min.), suspended in fresh RPMI-10% GPS and reincubated at 37°C. They were assayed for [3H]Tdr incorporation on days 3, 5, and 6 in culture. Identical results were obtained in cultures suspended in SF-free, RPMI-10% GPS containing UV-HSV-2 (G) (6.0 μg of protein per ml).

The dose-response relationship for SF inhibition of UV-HSV-2 (G)-stimulated proliferation is shown in Fig. 5B. Dilutions of SF >1:20 were routinely not suppressive. The effects of the delayed addition of SF on UV-HSV-2 (G)-induced proliferation is shown in Fig. 5C. The addition of SF after the initial 24 h of antigen stimulation greatly reduced its suppressive potential. The addition of SF at 3 days failed to produce suppression, suggesting that the SF-mediated inhibition of cell growth results from the interruption of a relatively early event in the lymphocyte activation sequence.

The SF-induced suppression of HSV-stimulated proliferation, is reversible (Table 4). Thus, replicate cultures of UV-HSV-2 (G)-stimulated immune responder SC grown in the presence of SF (30% final concentration) were suspended in SF-free medium and assayed for [3H]Tdr incorporation at various times in culture. Suppression was reversed in all cases, including cultures (6 days) suspended in SF-free medium immediately before the [3H]Tdr pulse. Reversal was not due to elimination of the viral proteins, since identical results were obtained in cultures suspended in SF-free medium containing UV-HSV-2 (G).

Virus-specificity of SF activity. To determine the specificity of the SF activity, UV-HSV-2 (G)-stimulated immune responder SC, and ConA-stimulated (5 μg/ml) normal (nonimmune) SC were grown in medium with or without 20% SF. HSV-stimulated cultures were assayed for [3H]Tdr incorporation on day 5, whereas ConA-induced proliferation was assayed on day 3 in culture. SF reduced the levels of [3H]Tdr incorporation in both cultures (51 and 52% suppression in HSV- and ConA-stimulated cultures, respectively). However, suppression appears to be mediated by different factor(s). Thus, factors inhibiting ConA-induced (and antigen-induced) proliferation fractionated on Sephadex columns within three fractions with respective molecular weights ranging between 5,000 and 30,000 (Fig. 6A). On the other hand, two fractions with approximate molecular weights of 50,000 to 60,000, and 110,000 to 160,000, respectively (Fig. 6B), did not suppress the ConA-induced proliferation but exhibited strong suppressive activity for UV-HSV-2 (G)-stimulated immune responder SC.

FIG. 3. SF activity in supernatants of UV-HSV-2 (G)-stimulated 5-day cultures of SC and PBL from seropositive control (□) and recrudescent (△) animals (5 in each group). For SF assay, supernatants (20% final concentration) were added to UV-HSV-2 (G)-stimulated cultures (6.0 μg of protein per ml) of immune responder SC (2 × 106 cells per ml) on day 0. [3H]Tdr incorporation was assayed on day 5 in culture. The data represent the mean ± SD.
the proliferation of HSV-2-stimulated immune lymphoid cells. Suppressor cells and soluble SFs are not detected in the spleen or peripheral blood of animals studied while quiescent, nor in those from animals without a history of recurrent HSV-2 disease (seropositive controls).

The mixed culture experiments (Fig. 2) unequivocally identify functionally active suppressor cells. Thus, we have previously shown that at a given antigen dose, the magnitude of [3H]TdR incorporation is proportional to the number of antigen-specific immune cells that are induced to proliferate (5). Consistent with this finding, a virtually linear dilution of [3H]TdR incorporation was observed in cultures containing mixtures of immune responder SC and increasing proportions (0 to 100%) of normal (non-immune) SC. Proliferation was not due to the stimulation of the normal cells by a mitogenic factor, since they were mitomycin C-treated before mixture. On the other hand, in parallel cultures containing as little as 20% lymphoid cells obtained during recrudescence, the magnitude of [3H]TdR incorporation was significantly reduced. Recrudescence was defined as 0 to 7 days after lesion onset, since lesions were virus positive for 5 to 8 days (6; data not shown). Regulatory cells obtained at quiescence did not cause a similar suppression, confirming the specificity of the experimental design for detecting functionally active suppressor cells. Consistent with previous findings (2, 5, 6, 27), immune lymphoid cells were virtually unstimulated by exposure to PBS or antigen prepared from cells mock infected with PBS instead of virus, and

FIG. 4. Kinetics of SF production. SC obtained at recrudescence were grown in presence of UV-HSV-2 (G) (6.0 µg of protein per ml), and the supernatants collected on days 1 (D1-S), 3 (D3-S), 4 (D4-S), and 5 (D5-S) in culture were assayed for SF activity at a final concentration of 50%. The assay was as described in the legend to Fig. 3. The data represent the mean ± SD.

FIG. 5A A Time course of SF-mediated suppression. Immune responder SC were grown with UV-HSV-2 (G) (6.0 µg of protein per ml) in the presence (●) or absence (○) of SF (30% final concentration) and assayed for [3H]TdR incorporation on days 1, 3, and 5. B, Dose effect of SF-mediated suppression. Immune responder SC were grown with UV-HSV-2 (G) (6.0 µg of protein per ml) and increasing concentrations of SF (0 to 50%) added on day 0. [3H]TdR incorporation was measured on day 5 in culture. C, Effect of the delayed addition of SF (50% final concentration) on UV-HSV-2 (G)-stimulated (6.0 µg of protein per ml) proliferation of immune responder SC. [3H]TdR incorporation was assayed on day 5. SF was obtained from cultures of recrudescent SC. Data represent the mean ± SD.
suppression was not observed in mixed cultures grown in the presence of antigen prepared from mock-infected cells (data not shown).

Recent studies have focused on soluble factors elaborated by activated lymphoid cells that participate in the regulation of immune responsiveness. Thus, ConA stimulation of murine and human lymphocytes has been shown to selectively produce (or amplify) active suppressor cells (8, 20). Despite some evidence to the contrary (21), it appears that suppression by these lectin-activated cells involves, at least in part, the elaboration of several short-range factors that inhibit target cell function. Indeed, ConA-activated human lymphocyte supernatants were shown to contain suppressor factors that inhibit the mixed leukocyte reaction (16, 30), mitogen- and antigen-stimulated T-cell proliferation, and B-cell immunoglobulin production (13, 29).

In the present study, experiments were designed to determine whether the suppression mediated by lymphoid cells obtained at recrudescence (Fig. 1 and 2) requires cell to cell contact or whether it could be mediated by soluble SF(s) released by these cells. Our findings indicate that SC obtained during recrudescence and grown in vitro in the presence of UV-HSV-2 (G) produce soluble SFs that are released into the culture medium. These factors dampen the UV-HSV-2 (G)-stimulated proliferation of the cells that elaborate them (feedback inhibition) (Table 3), the HSV-stimulated responses of immune responder cells (Fig. 3 and 5 and Table 4) and the ConA-stimulated LT responses of normal lymphocytes (Fig. 6A). Suppression was not mediated by the viral proteins themselves, since it was reversed by SF-free medium supplemented with 6.0 μg of UV-HSV-2 (G) per ml (Tables 3 and 4). However, Sephadex molecular sieve chromatographic profiles (Fig. 6), suggest that factors involved in the suppression of HSV-induced DNA synthesis by the immune cells differ from those involved in the suppression of ConA-induced proliferation of normal lymphoid cells.

At present, we do not know whether the various fractions containing SF activity represent structurally similar or different molecular entities, nor whether the high-molecular-weight factor(s) are also capable of inhibiting proliferation induced by antigens other than HSV. Furthermore, the mechanism of SF action is still unclear. Most of our studies were done with unfractionated supernatants, thereby precluding final conclusions pertaining to the suppressive effects of the apparently different SF moieties and their respective role in the pathogenesis of herpetic disease. However, suppression of DNA synthesis in HSV-stimulated immune lymphoid cells was only observed when SF was added at early stages (day 0 to 1) of the culture period (Fig. 5C), suggesting that SF exerts its influence on an initiation event. In the presence of SF, the onset of the proliferative response was delayed by 1 day (Fig. 5A), and the maximal levels of [3H]TdR incorporation were significantly decreased (Fig. 5B). However, as evidenced by identical doubling times in SF-treated and untreated cultures (Fig. 5A), SF did not modify the antigen sensitivity (5) of the lymphoid cells. SF-mediated suppression was reversible throughout the 6 days of in vitro culture (Tables 3 and 4), indicating that suppression is not merely a consequence of toxicity. Taken in toto, the findings are consistent with the interpretation that SF interacts with defined cell surface receptors that represent inhibitory domains within the cell membrane (24). The cell population involved in SF synthesis, the structural identity of the various SF fractions detected in these series, and their respective roles in the pathogenesis of the disease remain to be established.

It may be profitable to consider the findings within the context of our previously stated hypothesis (2, 6, 27) that posits regulatory aspects
FIG. 6. Molecular sieve chromatography of SF. (A) Supernatants from UV-HSV-2 (G)-stimulated recrudescent SC cultures (day 5) were fractionated on a Sephadex G-100 column prepared in PBS (pH 7.2). Fractions (20% final concentration) were assayed for SF activity (expressed as percent suppression) in UV-HSV-2 (G)-stimulated immune responder SC (○) as described in the legend to Fig. 3 and on ConA-stimulated normal SC (■). [3H]TdR incorporation in ConA-stimulated cultures was assayed on day 3. Protein concentration is indicated by percent absorbance at 280 nm (●). Volumes of elution of standard calibration proteins are indicated (arrows). (B) Supernatants obtained as in part A were concentrated by ultrafiltration through a PM 10 Amicon membrane (10,000-dalton exclusion) fractionated on a Sephadex G-200 column prepared in PBS (pH 7.2) and assayed as in part A. Protein concentration is indicated by percent absorbance at 280 nm (●). Volumes of elution of virus-specific SF activity is indicated by bars. Nonspecific SF activity (ConA cultures) was not observed.
the spleen and the peripheral blood, and they produce SFs that dampen the proliferative responses of the cultures that elaborate them. The proportion of suppressor cells decreases with time after lesion onset. In the spleen (but not PBL), suppressor cells are still detectable during convalescence. However, their proportion is lower, as evidenced by the finding that twice as many convalescent as compared to recrudescent SC, are required to reduce the 2-day lymphoproliferative response by one half (Fig. 2A). Suppressor cells are no longer detectable during quiescence, even when SC (or PBL) are differentiated or amplified by 3 days of in vitro growth in the presence of viral antigen. These findings suggest that at recrudescence, suppressor cells are induced by reactivated virus. Similar findings were obtained in exogenously reinfected HSV-2 seropositive animals (J. F. Sheridan et al., manuscript in preparation).

The following are implicit in these interpretations. First, during quiescence, suppressor cells or their precursors must persist at a site other than the spleen or the peripheral blood. This site may be the lymph nodes. Indeed, we find that suppressor cells differ in their distribution at different stages of the herpetic disease (viz. SC versus PBL), and different tissue distribution of various cell subpopulations has been independently demonstrated (11, 12, 15, 19). Secondly, the impaired LT responses of SC obtained during quiescence (as compared to seropositive controls [6; Fig. 1]) may reflect the presence of fewer cells that are committed to proliferate in the presence of HSV antigen, or cells with lower antigen avidity. Mathematical modeling of lymphoproliferative responses based on exponential growth as a function of time and logistic growth as a function of log antigen dose supports this interpretation (Donnenberg, Ph.D. thesis). However, final conclusions must await the results of limiting dilution studies, presently in progress in our laboratory, that are designed to define the frequency of HSV-responsive cells at different stages of the recurrent herpetic disease cycle.

ACKNOWLEDGMENTS

These studies were supported by Public Health Service grant AI16959 from the National Institute of Allergy and Infectious Diseases.

We thank Melinda Beck and Rita Fishelewich for excellent technical assistance and Jean Roberson for help with the manuscript.

LITERATURE CITED


