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Cell-Mediated Immunity Against Herpes Simplex Virus Envelope, Capsid, Excreted, and Crude Antigens

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Cell-mediated immunity to herpes simplex virus envelope, capsid, excreted, and crude antigens was studied by in vitro lymphocyte stimulation tests during 198 recurrent attacks in 69 patients. Excreted antigen caused no blast transformation. Envelope and capsid antigen-induced lymphocyte stimulation was at the maximum 7 to 14 days postinfection, declining thereafter to a rather constant level in 1 to 2 months. The lowest levels were measured just a few days before a new attack. In persons with frequent relapses, the fluctuation was more rapid and stimulation index levels stayed higher, although no protective level seemed to exist. Cultures stimulated with the crude antigen in autologous serum showed rapid increases and declines in the stimulation index values, contrary to those grown in agamma serum, in which the stimulation level stayed rather constant up to 1 year postinfection.

Herpes simplex virus (HSV) infections are an annoying medical problem. About 80 to 90% of the population gets the infection, and a recurrent infection occurs in 50 to 60% of these persons.

Different causes have been suggested for the recurrent attacks. Interest has been focused on both humoral and cell-mediated immune responses. A slightly elevated level of specific anti-HSV antibodies has been detected in persons who develop several recurrences compared with those who have no repeat infections (3–5). In cell-mediated immunity, increased (2), decreased (8, 21), and fluctuating values in blast transformation tests have been reported (8, 18), suggesting that a possible defect lies in the cell-mediated immune system. Differences in the mediators released from the lymphocytes have also been found (11, 15, 18, 20).

When virus-specific antibodies are measured against different structural components of HSV, a time sequence is found in their appearance. Antibodies against envelope antigen are detected first, followed by those against capsid and excreted antigens (5). A similar time sequence in the response to different HSV antigens might exist in cell-mediated immunity as well. Animal models of HSV infections suggest that the envelope antigen is the major target for immunoresponsive lymphocytes and mediators released by lymphocytes (9, 13). So far no human study exists in which cell-mediated immunity against these structural subunits has been measured during HSV infections. In this report we have followed persons with relapsing and nonrelapsing HSV infections and measured blast transformation induced by HSV envelope, capsid, excreted, and crude antigens and the different immunoglobulin class antibodies (immunoglobulin A [IgA], IgG, IgM) to the HSV envelope antigen during the course of infection and then after recovery up to 18 months postinfection.

MATERIALS AND METHODS

Patients. The study group consisted of 71 patients selected from the outpatient clinic of the Department of Dermatology, University of Turku; 69 had a history of frequently relapsing HSV infection. Of these patients, 38 had labial infections, 8 had genital infections, and 23 had local infections in various areas of the body. The clinical diagnosis of an HSV infection was confirmed by a virus isolation in each case. The mean age of the patients was 35 years, and the majority (80%) were women. The patients were followed from 2 to 18 months in the Department of Dermatology. Two patients who had no history of HSV infections and no circulating anti-HSV antibodies served as negative controls.

Blood samples. The blood samples used to follow both the cell-mediated and humoral immune responses were taken immediately at the time of a recurrent attack and later, about 1, 2, 4, and 8 weeks and 6 to 18 months after infection. A 40-ml sample of heparinized blood was used for the lymphocyte transformation tests and T- and B-cell determinations. Separate samples were taken for leukocyte counting and for antibody determinations. The serum specimens were stored at −20°C until used in the antibody assay.

Antigens. The various viral antigens were produced from Vero cells infected with the VR strain of HSV type 1. Crude antigen was prepared from infected cell lysates by ultracentrifugation through a linear sucrose gradient as described previously (6). Capsid and envelope antigens were prepared according to Martin et al.

(10). For capsid antigen preparation, infected cells were harvested into Nonidet P-40 solution and disrupted by sonication. The suspension was purified by filtration and ultracentrifugation. For envelope antigen preparation, the infected cells were pelleted, disrupted and treated with diethyl ether, and ultracentrifuged. Excreted antigen was produced according to Kaplan et al. (7). Corresponding control antigens were prepared from noninfected cells in a manner similar to that for viral antigens.

For blast transformation assays, the HSV antigens were inactivated by β-propionlactone treatment according to the method of Neff and Enders (12).

In lymphocyte transformation tests, different antigen concentrations were used, as follows: crude antigen, 30 and 6 μg/ml; capsid antigen, 2, 0.4, and 0.08 μg/ml; envelope antigen, 3, 0.6, and 0.12 μg/ml; and excreted antigen, 15, 3, and 0.6 μg/ml.

Blast transformation was also induced by phytohemagglutinin (375 and 75 μg/ml; Difco Laboratories, Detroit, Mich.) and purified protein derivative (4 and 0.04 μg/ml; CVL, London, England).

Lymphocyte stimulation tests. Lymphocytes were isolated by a Ficoll-Hypaque gradient (Pharmacia, Uppsala, Sweden) according to the method described by Böyum (1), with slight modifications.

Samples of 1.5×10^5 cells per 100 μl were placed in the wells of round-bottom microtiter plates (A/S Nunc, Roskilde, Denmark) in medium 199 containing 10 μg of gentamicin per ml. A 50-μl portion of either inactivated HSV negative human serum (AB, Rh−) from which the immunoglobulins were removed by sodium sulfate precipitation (agamma serum) or autologous serum and 50 μl of the respective antigen dilution were added to the wells. The plates were thereafter incubated at 37°C in 100% humidity and a 5% CO_2 atmosphere for 7 days. For the last 20 h, 1 μCi of [^{3}H]thymidine (The Radiochemical Centre Ltd., Amersham, England) was added to each well. The cells were then harvested with a semiautomatic harvester (MASH II, Microbiological Associates, Bethesda, Md.). Cell-bound radioactivity was measured in a liquid scintillation counter (LKB-Wallac 81000, Turku, Finland). All experiments were done in triplicate.

Data are presented as stimulation index (SI) values: SI = (counts per minute of stimulated cultures − background)/(counts per minute of unstimulated cultures − background).

T- and B-cell determinations. T-cell determinations were made according to Wybran et al. (22) and B-cell determinations were made according to Pettersson et al. (17).

Antibody determinations. A solid-phase radioimmunoassay used to detect class-specific HSV antibodies has been described in detail earlier (5, 6). In this study, IgA, IgG, and IgM class antibodies against HSV envelope antigen were determined.

RESULTS

During the follow-up, 198 recurrent HSV infections were registered. One attack was seen in 16 patients; 2, in 19 patients; 3, in 4 patients; and 4 or more, in 15 patients. Fifteen persons were completely symptom-free during the period followed, although they had a history of several recurrent HSV infections before the follow-up started. However, blood samples were taken regularly, and thus these patients served as HSV-positive, nonrecurrent controls.

Both the relative number of leukocytes and the amount of T- and B-cells showed some variation. However, generally the proportions were within normal limits. The number of T-cells represented 50 to 75% of the lymphocyte population and that of B-cells represented 5 to 20%. The total leukocyte and B- or T-cell counts showed no evident connection with the recurrent infections or changes in SI levels to various HSV antigens in individual patients.

Cell-mediated immunity. HSV excreted antigen and the control antigens caused no blast transformation. The persons who had no history of previous HSV infections were also negative in lymphocyte stimulation studies with HSV antigens.

For the envelope, capsid, and crude antigens the SI values were at their lowest just before the onset of infection, staying at the low level 1 to 5 days after the viral vesicles had appeared. The mean SI values for all patients during recurrent attacks are presented in Fig. 1 for the different HSV antigens, when the strongest antigen concentrations were used for stimulation. Different curves are established for those patients who had frequent, rather frequent, and nonrelapsing attacks. The SI values started to increase in a few days and were highest 7 to 14 days after the beginning of the eruption. The level started to decline soon after the maximum was gained, and a definite decrease was seen by 30 days after the beginning of the eruption. In patients who had frequent recurrent infections the SI values started to decline more rapidly than in those who had only occasional relapses. Capsid antigen-induced blast transformation was exceptional since the mean SI value was rather constant; however, in individual patients the tendency to fluctuate was evident (Fig. 2). When lower concentrations of viral antigens were used for stimulation, a similar fluctuation was noticed, although the SI values stayed at a lower level.

The blast transformation induced by the crude antigen was studied by incubating the cells with both agamma and patients' own autologous sera. When autologous serum was used the SI level started to decrease 30 days after the attack. In contrast, the blast transformation index with agamma serum stayed at a higher level up to 1 year after infection (Fig. 1D).

Differences were found in SI values in individual patients. No clear correlation was found between these and the frequency of the HSV infections or the duration or extent and healing of the lesions. Some patients had very low and some had rather high, but still fluctuating, SI

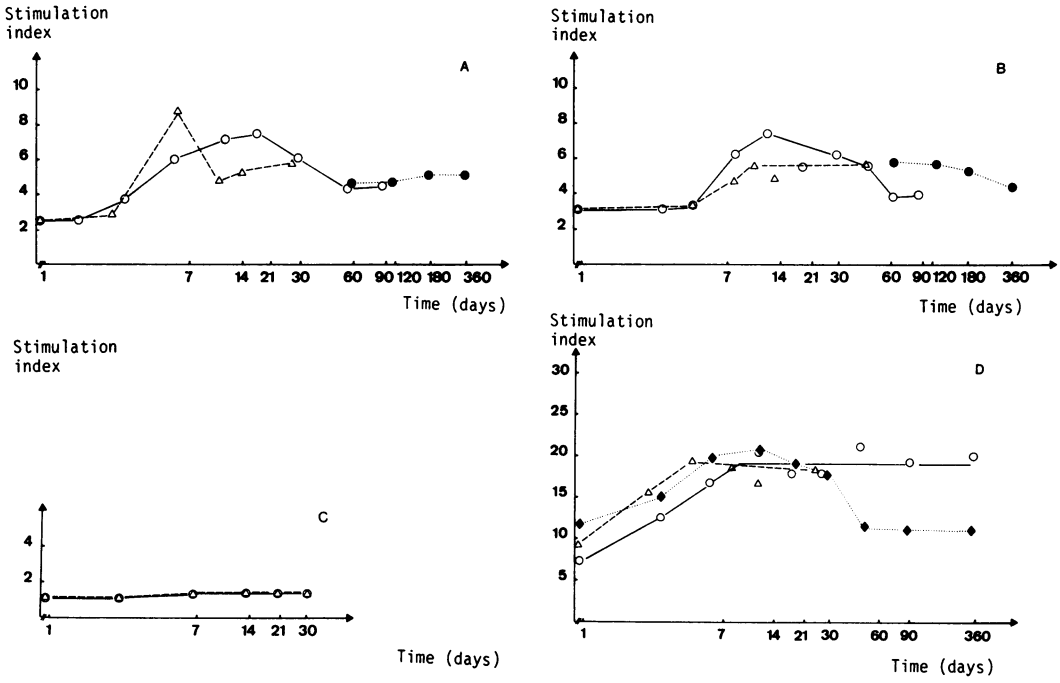

FIG. 1. Lymphocyte SI values after a recurrent HSV infection: (A) for envelope antigen in all patients (○), in those with frequent recurrences (△), and in a group with no recurrence for 2 months to 1 year (●); (B) for capsid antigen in all patients (○), in those with frequent recurrences (△), and in a group with no recurrence for 2 months to 1 year (●); (C) for excreted antigen in all patients (○) and in those who had frequent recurrences (△); (D) for crude antigen in all patients in agamma serum (○) or autologous serum (◆) and in patients with frequent recurrences in agamma serum (△).

values always. As an example, two patients with rather frequent labial HSV attacks, one with a relatively low SI and another with a high SI, are illustrated in Fig. 2. The maximal values reached in a new attack were dependent on the average SI level of the patient. If the person had many attacks in a short time, the SI values had a tendency to increase. However, no protective level of SI could be found, since a new attack developed while the values, though decreasing, were still rather high (Fig. 2).

The HSV antigens used induced lymphocyte stimulation in each patient studied. Patients with HSV infection in the genital area or other locations did not differ from those with labial infections.

The SIs for phytohemagglutinin and purified protein derivative fluctuated somewhat after infection. For purified protein derivative the mean SI value rose from 22 to 41, declining to 29 about 1 month after the recurrent attack.

Humoral immunity. A relatively high antibody level to HSV type 1 envelope antigen was found in each patient with a recurrent HSV infection. The radioimmunoassay titer varied from 1:10,000 to 1:20,000 for IgG and from 1:500 to 1:2,000 for IgA. No evident fluctuations could be noted in the course of the disease, nor did the antibody levels follow the variations in blast transformation induced by the specific mitogens.

IgA antibodies to HSV were detected in 63 of the patients, in 34 patients with labial and in 6 with genital recurrent infections. IgM class antibodies were found in three of these patients, the most severe cases.

DISCUSSION

Recently, animal experiments have shown that cell surface antigens participate in antibody-dependent cell-mediated cytotoxicity during HSV infection. The viral antigens on infected cell surfaces could be recognized in cell culture as early as 3 to 4 h postinfection (13, 19). The humoral antibody response after primary HSV infections was also directed first against the envelope antigen and then against capsid and excreted antigens (5). However, the specific antibodies as such will evidently not prevent recurrent eruptions.

In the present work the role of HSV envelope, capsid, and excreted antigens in cell-mediated immunity was studied during 198 recurrent HSV attacks in 69 patients. Excreted antigen caused

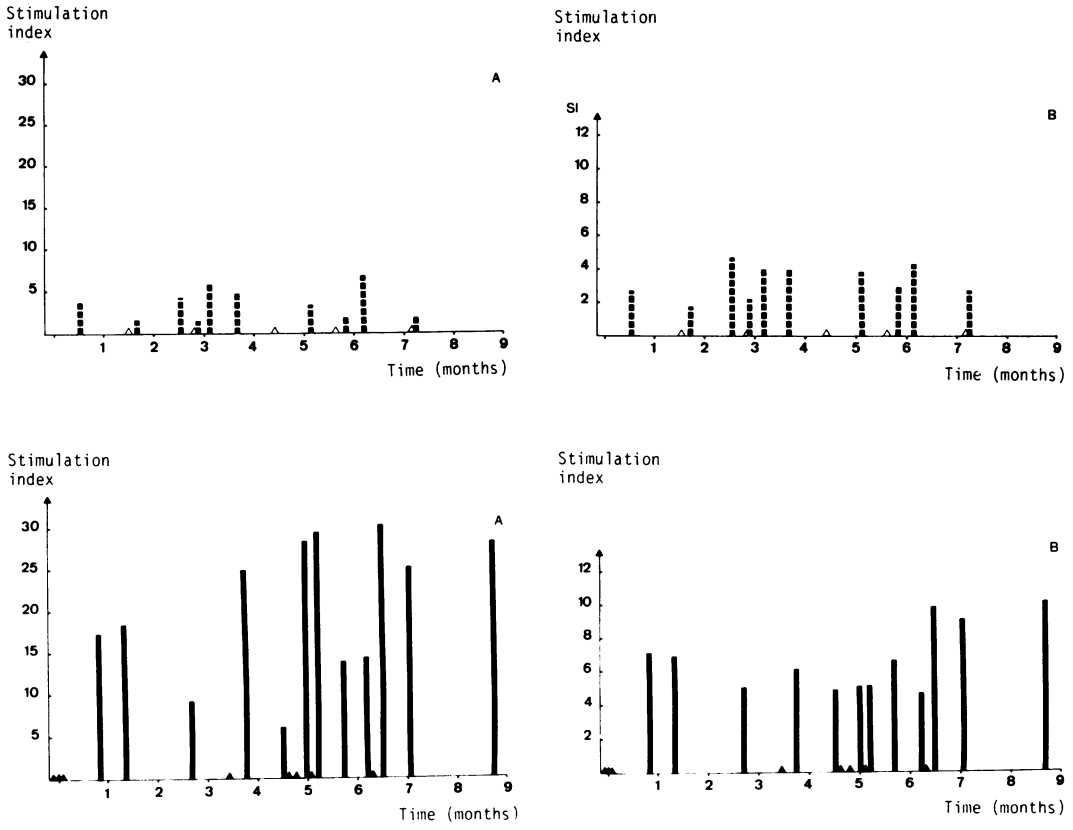

FIG. 2. Lymphocyte SI values for envelope (A) and capsid (B) antigens in two patients (broken and solid columns) during a 9-month follow-up. Symbols: △, ▲, recurrent infections.

no blast transformation, as expected since it is possibly not a structural viral antigen (5). Envelope, capsid, and crude antigens did induce a blast transformation, which reached the maximum 1 to 2 weeks after the eruption and was at the lowest just before the beginning of the next eruption. The increase and decrease in SI level induced by envelope antigen preceded that induced by capsid antigen. The SI levels evoked by the crude antigen were highest, as expected since it represents a combination of various HSV antigens. The structural antigens used were not type specific since no differences were seen in the patients with various clinical pictures. This is in accordance with previous findings (5).

In patients who had frequent recurrences, the SI values induced by HSV antigens declined faster than in the others. This supports the idea of a possible immunological defect in these patients, noticed previously by Kirchner et al. (8).

The effect of autologous serum in the incubation solution was compared with agamma serum in crude antigen-induced cultures. An evident difference was seen in that cultures stimulated with autologous serum showed decreasing stimulation values 14 days after eruption whereas the SI levels in agamma serum-treated cultures stayed at a high level up to 1 year after infection. It has been suggested that antibodies could bind to the specific antigens, and depending on the antigen-antibody ratios in the system, the antibodies may increase or impair the antigen-induced stimulation of lymphocytes (14). On the other hand, antibody-mediated cytotoxicity may affect the circulating cell population, recognizing and killing HSV-sensitized cells (16). However, the blast transformation indices in autologous serum fell rather rapidly after the acute phase of the infection, although the HSV antibody levels did not show fluctuations.

Although a constant fluctuation was seen in the structural HSV antigen-induced lymphocyte SI values, the clinical course of the attacks did not follow these parameters strictly. High SI levels did not prevent further attacks. It thus appears evident that immunotherapy, e.g., vaccination with HSV antigen, will not prevent future relapses.

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