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Th1/Th2-like immunity and resistance to herpes simplex labialis ☆

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Abstract

To investigate potential immunologic mechanisms of resistance to recurrent herpes simplex labialis, we assayed serum antibody titers and cultured peripheral blood mononuclear cell (PBMC) cytokine production among patients with a history of frequent episodes (H^+S^+), herpes simplex virus (HSV)-seropositive individuals without a history of herpes labialis (H^-S^+) and HSV-seronegative persons (H^-S^-). In addition, H^+S^+ patients were exposed to experimental ultraviolet radiation (UVR) on the lips and the immunologic assay results compared among those who developed experimental lesions and those who did not. H^+S^+ patients were found to have higher median serum titers of HSV antibody and trends to lower levels of HSV-specific PBMC IFN-γ and IL-2 than H^-S^+ control patients (123 vs 66, $P = 0.04$; 424 vs 548 pg/ml, $P = 0.08$; 14 vs 26 pg/ml, $P = 0.14$, respectively). Correlation of the results with the occurrence of experimental lesions showed the inverse: the subgroup of H^+S^+ patients with UVR-induced lesions had lower titers of antibody and trends to higher levels of IFN-γ and IL-2 than H^+S^+ patients who could not be induced (93 vs 149, $P = 0.02$; 501 vs 347 pg/ml, $P =$ NS; 26 vs 11 pg/ml, $P =$ NS, respectively). The size and duration of UVR-induced lesions showed positive correlations with IFN-γ and IL-2 levels ($r = 0.60$–0.67, $P = 0.02$–0.04). Although the small number of patients

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limited the power of this study, the overall pattern of the findings suggests that a Th1-like cytokine response (IFN-γ and IL-2 production) may be associated with resistance to naturally occurring episodes of herpes labialis. The development and severity of experimental UVR-induced herpes labialis appears to be regulated differently and may involve an immunopathologic mechanism.

Keywords: Herpes simplex labialis; Herpes simplex virus; Immunity; Cytokine; Ultraviolet radiation; Immunopathology

1. Introduction

Herpes simplex virus type 1 (HSV-1) or HSV-2 infection occurs at some point in the life time of 80 and 30%, respectively, of healthy persons in the United States. Only one-third or less of these infected persons, however, experience symptomatic ocular, oral–facial or genital recurrences of the disease (Nahmias et al., 1989). The biologic basis for the difference between infected persons who do and those who do not suffer recurrences has long been a subject of clinical and laboratory investigation, including studies of genetic markers (Whitley, 1990), epidermal cell susceptibility (Spruance and Chow, 1980), relative potency of different HSV strains (Kaufman et al., 1983; Klieman et al., 1985) and multiple assays of immunologic function (Kohl, 1985; Rawls, 1985; Wildy and Gell, 1985). While these studies have generated many interesting findings, the most provocative are data suggesting that quantitative and/or qualitative alterations in cytokine production may correlate with the development of recurrent infection (Wilton et al., 1972; O'Reilly et al., 1977; Shillitoe et al., 1977; Rattray et al., 1980; Cunningham and Merigan, 1983; Green et al., 1985; Sheridan et al., 1987; Kuo and Lin, 1990).

Several laboratories have been interested in the potential role of interferon-γ (IFN-γ) in resistance to HSV infection. Green and co-workers demonstrated HSV-specific IFN-γ production by cultured peripheral blood mononuclear cells (PBMC) and showed that HSV-specific IFN-γ was absent from the PBMC cultures of 30% of persons with frequent episodes of herpes labialis, but was uniformly detectable in seropositive, disease-free controls (Green et al., 1981, 1985). Cunningham and Merigan (Cunningham and Merigan, 1983) obtained PBMC from patients at various times after the onset of herpes labialis, incubated the cells without viral antigen stimulation and detected IFN-γ in the cell culture supernatants ('spontaneous secretion'). They then found a positive correlation between the peak supernatant IFN-γ level and the time to the next recurrence of herpes labialis. An attempt to confirm the phenomenon of spontaneous IFN production was unsuccessful (Green et al., 1983). However, Torseth and Merigan (Torseth and Merigan, 1986) subsequently reported that both IFN-γ in the supernatant of HSV antigen-stimulated PBMC and IFN-γ in lesion vesicle fluid correlated with the duration of the interval between recurrences of herpes labialis.

A Th1-like immune response refers to the predilection of cultured, activated PBMC or T-cells to produce IFN-γ and interleukin-2 (IL-2) and a Th2-like response to the production by PBMC or T-cells of IL-4, IL-5, IL-6 and IL-10 (Mosmann and Coffman, 1989). Th1 cell clones are believed to represent the predominant population of T-cells

involved in delayed-type hypersensitivity and other types of cellular immune responses ('pro-inflammatory'). Th2 cell clones can down-regulate IFN-γ and IL-2 production, 'anti-inflammatory' activity, and appear to function as the main population of $CD4^+$ T-cells involved in the stimulation of antibody production (Bottomly, 1988; Hayakawa and Hardy, 1988; Paul, 1989; Cogan et al., 1994; Cohen, 1994). To see if cytokine profiles (Th1- or Th2-like) might help our understanding of resistance to recurrent herpes labialis, we studied serum antibody and the cytokine production of cultured PBMC from patients with frequent herpes labialis and from control subjects. In addition, we correlated these immunologic parameters to the concurrent susceptibility of the patients to recurrent herpes labialis by exposing their lips to experimental ultraviolet radiation (UVR).

2. Materials and methods

2.1. Study design

To determine the immune functions that might be important in the control of HSV reactivation, we studied patients with a history of recurrent herpes labialis, infected (seropositive) patients without a history of disease, and seronegative subjects. Immune parameters assessed included serum HSV neutralizing antibody and the levels of IFN-γ, IL-2, IL-4, and IL-10 produced in the supernatant of PBMC cultures exposed to HSV antigen. In addition, the concurrent susceptibility to HSV reactivation of the herpes labialis history-positive patient group was assessed by exposing their lips to experimental UVR. The immunologic data derived from these studies were then correlated with two forms of clinical evidence: (1) the patients' history of natural herpes labialis; and (2) the development of herpes labialis following experimental UVR exposure.

2.2. Patients

Healthy, immunocompetent patients with a history of herpes labialis following sun exposure were recruited (H^+S^+, H, history of herpes labialis; S, HSV serology). Patients without a history of herpes labialis were evaluated for the presence of serum HSV neutralizing antibodies and divided into a group with antibodies to HSV (H^-S^+) and a group of individuals without antibodies to HSV (H^-S^-). Western blot assay of serum was performed on all sera to confirm the results of the neutralizing antibody assay and to exclude patients who were HSV neutralizing antibody-seropositive solely on the basis of antibodies to HSV-2. All patients signed an Institutional Review Board-approved document of informed consent.

2.3. Serum HSV-neutralizing antibody assay

Serial 1 : 2 dilutions of heat-inactivated sera were incubated at 4°C for 60 min with 250 plaque-forming units of HSV-1 strain E115 (Rawls et al., 1970). After incubation, aliquots of the serum/virus mixture were used to inoculate cultures of Vero cells in

6-well plates. The plates were incubated at 37°C for 60 min and then each well was overlaid with minimal essential medium (MEM) containing 5% heat-inactivated fetal bovine serum (FBS) and 0.5% agarose. After incubation for 5 days, the plates were stained with neutral red to determine plaque number and to calculate the dilution of serum effecting a 50% reduction in plaque number compared to control wells. Titers greater than or equal to 1 : 10 were considered indicative of HSV infection.

2.4. Serum HSV antibody assay by Western blot

Coded sera was frozen at −20°C and shipped in batch to the Children's Orthopedic Hospital, Seattle, WA. Assays for HSV-1- and HSV-2-specific antibodies were kindly performed by Dr. Rhoda Ashley using standard methods (Ashley et al., 1988).

2.5. Harvest and storage of PBMC

One hundred milliliters of fresh, heparinized blood was mixed with an equal volume of tissue culture medium (RPMI 1640). Two volumes of blood in medium were layered onto one volume of Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) in 50 ml conical tubes and centrifuged at 400 *g* for 30 min at room temperature. The opaque interface containing mononuclear cells was then transferred to a sterile centrifuge tube and washed 3 × with phosphate-buffered saline (PBS). The mononuclear cell concentration was adjusted to 2×10^6 cells/ml in MEM, 20% FBS, 10% dimethyl sulfoxide on ice and 1.5 ml aliquots were transferred to screw-cap vials (Cooke Pro-Vials, Cooke Laboratory Products, Alexandria, VA). The vials were frozen to −110°C at a rate of 1°C/min in a pre-programmed controller (CyroMed Model 700A, Cryomed, New Baltimore, MI) and then stored in liquid nitrogen.

2.6. Determination of susceptibility to HSV reactivation by experimental exposure of the lips to UVR

After blood samples were obtained for isolation of PBMC, herpes labialis history-positive patients were exposed to UVR in order to induce a reactivation of the infection. The interval between blood sampling and UVR exposure was 24 h for 21 of the subjects and 2–14 days for the other 7 participants. All patients exposed to UVR had been free of herpes labialis for at least 30 days prior to the procedure.

Sensitivity to UVR (minimal erythematous dose, MED) was assessed by exposure of small areas of the inner forearm to increasing doses. The quadrant of the lips which was the usual site of recurrent herpes labialis was outlined with a black marking pen. The remainder of the lips and perioral skin outside of this zone were covered with a para-aminobenzoic acid sun screen having a sun protection factor of 15. The patient was then given 4 MED of UVR from two fluorescent tubes (FS20 T12 UVB Sunlamp, National Biological Corp., Twinsberg, OH). Patients were followed every other day for 4 visits for evidence of herpes labialis. Assessment of lesion severity was made by observation of lesion stage, size, and pain and by virus isolation from lesion specimens. Additional details of the procedure are available elsewhere (Spruance et al., 1991).

2.7. Isolation of HSV

Papular lesions were scraped with a scalpel blade and the blade with the shavings placed in 2 ml of tissue culture medium. Vesicles were opened with a needle and the fluid absorbed with a swab, and the bases of ulcers were rubbed with a medium-moistened swab. The tips of these swabs were broken off into a medium-containing tube. Specimens thus obtained were plated immediately onto susceptible mink lung cells or kept at +4°C for not longer than 72 h prior to assay (Salmon et al., 1984).

2.8. PBMC cultures

Frozen cells were rapidly thawed, examined for viability by Trypan blue exclusion, washed twice in PBS and suspended in tissue culture medium (RPMI 1640) with 10% heat-inactivated FBS at a cell concentration of 2×10^6 viable cells/ml. One ml of the cell suspension was seeded into 4 snap-cap culture tubes (6 ml) which were immediately stimulated with heat-inactivated HSV-1 strain E115-infected mink lung cell sonicate (10 μg/ml) (Spruance et al., 1984), uninfected cell sonicate (10 μg/ml), PHA (1 μg/ml) or medium. After 2 days of incubation, the culture supernatants were removed and frozen at −20°C for subsequent cytokine assays (IL-2, IL-4, IL-10). The volume of the culture was restored to 1 ml with fresh medium without antigens, and 6 days after initiation, the culture was terminated and the supernatant frozen for additional assays (IFN-γ, IL-4, IL-10).

2.9. IL-2 assay

The murine IL-2-dependent T-lymphocyte cell line, CTLL, was used to assay for human IL-2 (Daynes et al., 1990). Triplicate tubes of cells were incubated overnight with 2-fold serial dilutions of test culture supernatants, 0.02–10 U of control recombinant human IL-2 (Genzyme, Cambridge, MA), or medium. IL-2 was quantitated by the ability of the test supernatant to support growth of the cells equal to 50% of maximal viability sustained by the recombinant cytokine (defined as 1 U). The viability of the indicator cells was evaluated by the addition of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl-tetrazolium bromide (MTT) during the final 4 h of the 24-h bioassay. The formation of insoluble blue crystals due to the cleavage of MTT by living cells was measured with an ELISA reader after the addition of 20% sodium dodecyl sulfate/50% *N*,*N*-dimethyl-formamide to dissolve the MTT crystals. The ELISA readings from the test samples were analyzed by applying a second degree polynomial curve fit and determining the dilution equivalent to half-maximal stimulation by the recombinant IL-2 control.

2.10. IL-4 assay

ELISA plates for the colorimetric assay of IL-4 were prepared as follows. Mouse monoclonal anti-human IL-4 (0.5 μg, Genzyme, Cambridge, MA) in 50 μl of 50 mM Tris HCL was placed in each well of a 96-well plate (Corning ELISA Plate, Corning

Glass Works, Corning, NY) and incubated at 4°C overnight. The plate was washed 5 × with PBS, 0.05% Tween 20 (PBST), incubated at room temperature for 2 h with 200 μl/well of PBST, 1% BSA, and washed 5 × again with PBST.

To assay IL-4, the standard (recombinant human IL-4, Genzyme, Cambridge, MA) in concentrations from 39 to 2500 pg/ml or dilutions of the test samples, 50 μl/well, were added in duplicate to ELISA plate wells. After overnight incubation at 4°C, the plates were washed 5 × with PBST. Polyclonal rabbit anti-human IL-4 (Genzyme, Cambridge, MA), 10 μg/ml in PBST, 1% bovine serum albumin (BSA), was added to each well. The plates were then incubated at room temperature for 1 h and washed 5 × with PBST. Anti-rabbit IgG biotin conjugate (Sigma Immuno Chemicals, St. Louis, MO), diluted 1 : 5000 in PBST, 1% BSA, was added to each well. The plates were incubated at room temperature for 1 h and washed 5 × with PBST. Horseradish peroxidase avidin D (Vector Labs, Burlingame, CA), diluted 1 : 2000 in PBST, 1% BSA, was added to each well. The plates were incubated at 37°C for 30 min and washed 5 × with PBST. Chromogen substrate (Sigma Chemical Co., St. Louis, MO, 1.5 g/50 ml) and ice cold H_2O_2, 30% solution, were diluted in sodium citrate buffer, pH 4.2, 1 : 100 and 1 : 1000, respectively, and 50 μl of the solution was added to each well. Color was allowed to develop for 5 min and then quantitated at 405 nm wavelength in an automated ELISA plate reader. The concentration of IL-4 in the test samples was determined with an optical density plot prepared from values of the IL-4 standards.

2.11. IL-10 assay

IL-10 was assayed using commercially available ELISA plates, standards and reagents (Pharmingen, San Diego, CA). The procedures were similar to those described above for the assay of IL-4. The standard curve ranged from 32 to 4000 pg/ml.

2.12. IFN-γ assay

IFN-γ was assayed using commercially available ELISA plates, standards and reagents (Intertest Gamma, Genzyme, Cambridge, MA). The procedures were similar to those described above for the assay of IL-4. The standard curve ranged from 100 to 6400 pg/ml.

2.13. Cell proliferation

Cell proliferation was measured by the incorporation of tritiated thymidine into nascent DNA as previously described (Green et al., 1981).

2.14. Statistical procedures

Continuous variables were compared by the Mann–Whitney rank sum test. Where one of two groups was discontinuous, the Kruskal–Wallis test for two groups was employed. Correlations were examined by calculating Pearson's correlation coefficient,

and the significance of correlations was determined by ANOVA. All comparisons were two-sided and $P < 0.05$ was considered significant.

3. Results

3.1. Characteristics of the study patients

A total of 57 patients participated in the study, of whom 28 were individuals with frequent herpes labialis (H^+S^+), 19 were patients without a history of herpes labialis, but with a serologic evidence of HSV-1 infection (H^-S^+), and 10 patients had neither historical nor serological evidence for HSV infection (H^-S^-). As shown in Table 1, the 3 groups were comparable with regards to standard demographic features. Among the patients with herpes labialis, 4% had a history of genital herpes and 14% had serum antibody to both HSV-1 and HSV-2.

3.2. Correlation of immune parameters with patients' histories of herpes labialis

Immune parameters studied included HSV-neutralizing antibody titers and the level of different cytokines produced by cultured PBMC in response to HSV antigen. The results of these assays and correlation with the patients' histories of herpes labialis are shown in Table 2.

HSV antigen-specific IFN-γ, IL-2 and IL-10 were found in stimulated cell cultures from both seropositive patient groups (H^+S^+ and H^-S^+, Table 2 and Fig. 1) and were absent or detected at significantly lower levels in the cultures from the seronegative subjects ($P < 0.001$–0.002). HSV antigen-induced cell proliferation was significantly increased in the seropositive compared to the seronegative persons (data not shown).

Table 1
Characteristics of the study population

Features	History of herpes and serologic status		
	H^+S^+ ($n = 28$)	H^-S^+ ($n = 19$)	H^-S^- ($n = 10$)
Years of age, median (range)	37 (25–67)	32 (23–58)	33 (19–41)
Gender (% female)	87	70	50
Race (% Caucasian)	100	95	100
Hx of herpes labialis (%)	100	0	0
Frequency of herpes labialis, no. of episodes in the past year, median (range)	4 (2–14)	0	0
Develop herpes labialis from sun exposure (%)	100	0	0
Hx of herpes genitalis (%)	4	0	0
HSV-1 serum antibody (%) [a]	100	100	0
HSV-2 serum antibody (%) [a]	14	10	0

[a] Western blot.

Table 2
HSV antigen-specific immune function among patients with and without a natural history of herpes simplex labialis

Assay	Patient group				
	H^+S^+ ($n=28$) [a]	P [b]	H^-S^+ ($n=19$)	H^-S- ($n=10$)	P [c]
Serum antibody, (median titer)	123	0.04	66	0 (0)	< 0.001
PBMC IFN-γ, pg/ml (median)	424 (430) [d]	0.08	548 (612)	91 (92)	< 0.001
PBMC IL-2, pg/ml (median)	14 (50)	0.14	26 (62)	0 (19)	< 0.001
PBMC IL-4, pg/ml (median)					
Day 2	0 (0)	NS [e]	0 (0)	0 (0)	NS
Day 6	0 (0)	NS	0 (0)	0 (0)	
PBMC IL-10, pg/ml (median)					
Day 2	565 (1097)	NS	600 (986)	110 (502)	0.001
Day 6	624 (689)	NS	721 (849)	150 (333)	0.002

[a] The number of specimens tested is less than the total number for some of the IL-4 and IL-10 assays because of limitations in the amount of supernatant available.
[b] Mann–Whitney rank sum test comparing the H^+S^+ and H^-S^+ groups.
[c] Mann–Whitney rank sum test comparing the H^-S^- group to all seropositive patients.
[d] The number shown is the corrected, antigen-specific value, or the value for the cultures stimulated with HSV antigen minus the value for cultures stimulated with uninfected control cell antigen alone. The uncorrected values are shown in parentheses.
[e] NS, not significant.

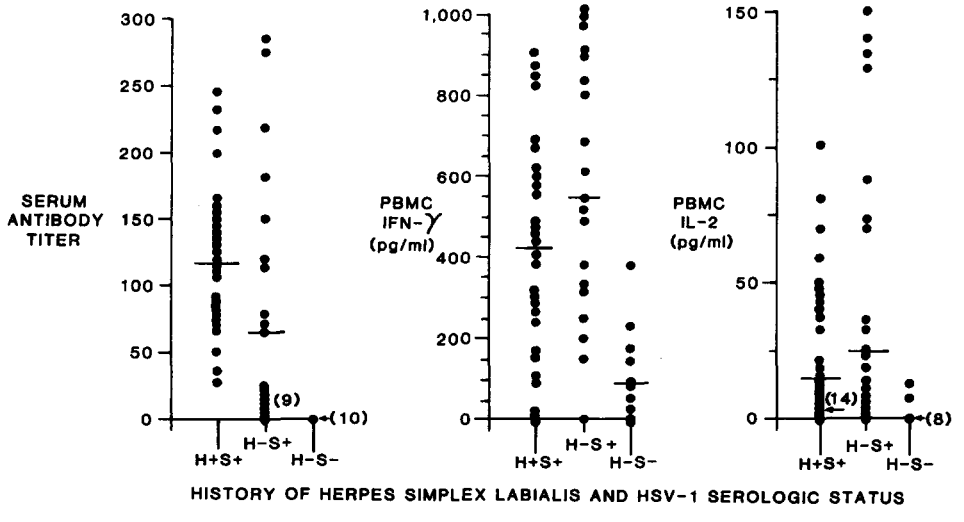

Fig. 1. Serum HSV antibody titers and in vitro cytokine production of HSV-stimulated PBMC by history of herpes labialis and HSV serologic status.

To determine if any of our immune parameters were HSV disease-specific, we compared the values among the patients with a history of herpes labialis (H^+S^+) and those who were clinically disease-free, but had evidence of prior infection with the virus (H^-S^+). As shown in Table 2 and Fig. 1, levels of HSV-neutralizing antibody were significantly higher in the history-positive than the history-negative subjects. Conversely, there were trends to lower levels of PBMC IFN-γ and IL-2 among the history-positive patients compared to the history-negative, seropositive persons. Stimulation of cells from these two groups of subjects with PHA led to similar levels of IFN-γ and IL-2, and stimulation with HSV antigen and measurement of cell proliferation by [^{3}H]thymidine incorporation was also non-discriminating (data not shown). There was no apparent difference between the two groups in culture supernatant levels of HSV antigen-induced IL-10 (Table 2).

IL-4 was only sporadically detectable in HSV antigen-stimulated PBMC culture supernatants on either day 2 or day 6 from any of the 3 groups of patients (Table 2), from unstimulated control cultures and from cultures stimulated with PHA (data not shown). Occasional cultures had low levels of IL-4 (< 50 pg/ml), but the results were negative for the majority of supernatants tested within each group. There was no apparent significance to the distribution of positive values.

Among the history-positive patients, we did not find a significant correlation between the number of reported episodes in the past year or the time since the last natural episode of herpes labialis and HSV-neutralizing antibody titers, PBMC IFN-γ or PBMC IL-2 ($r = -0.07$ to 0.31, $P =$ NS, data not shown).

3.3. Induction of herpes labialis with experimental UVR among the H^+S^+ patient subgroup

After the blood samples were taken for the immunologic studies described above, the 28 patients with a history of herpes labialis were exposed to UVR in an attempt to experimentally induce herpes simplex labialis. Twelve of the 28 patients (43%) developed a lesion within 7 days after the procedure. In 8 of the 12 cases (67%), the disease was confirmed by virus isolation. The distribution of these cases by time after UVR exposure is shown in Fig. 2. Four lesions developed within 48 h ('immediate' lesions) and the remainder 2–7 days after UVR exposure ('delayed' lesions) (Spruance et al., 1991).

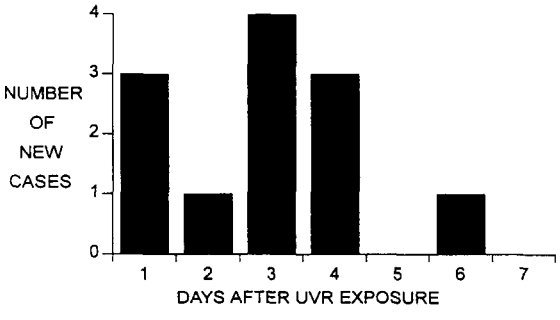

Fig. 2. Number of new cases of UVR-induced herpes labialis by time after UVR exposure.

Table 3
HSV antigen-specific immune function among patients with and without experimental UVR-induced herpes simplex labialis

Assay	Patient group		
	Lesion ($n = 12$) [a]	P [b]	No lesion ($n = 16$)
Serum antibody, (median titer)	93	0.02	149
PBMC IFN-γ, pg/ml (median)	501 (501) [c]	NS [d]	347 (352)
PBMC IL-2, pg/ml (median)	26 (59)	NS	11 (41)
PBMC IL-4, pg/ml (median)			
Day 2	0 (0)	NS	0 (0)
Day 6	0 (0)	NS	0 (0)
PBMC IL-10, pg/ml (median)			
Day 2	565 (1068)	NS	577 (1097)
Day 6	678 (812)	NS	443 (672)

[a] The number of specimens tested is less than the total number for some of the IL-4 and IL-10 assays because of limitations in the amount of supernatant available.
[b] Mann–Whitney rank sum test comparing the lesion and no lesion groups.
[c] The number shown is the corrected, antigen-specific value, or the value for the cultures stimulated with HSV antigen minus the value for cultures stimulated with uninfected control cell antigen alone. The uncorrected values are shown in parentheses.
[d] NS, not significant.

3.4. Correlation of immune parameters with susceptibility to experimental UVR-induced herpes labialis

The relationship between immune measures and concurrent susceptibility to UVR-induced herpes labialis was studied by comparing serum HSV-neutralizing antibody titers and HSV antigen-stimulated PBMC culture supernatant cytokine concentrations among the 12 patients who were successfully induced with UVR and developed a lesion compared with the 16 who did not. The correlations were opposite from those found when patients with and without a history of natural disease were compared. The patient subgroup that developed experimental UVR-induced lesions, compared to those who did not, had significantly lower median serum HSV antibody titers and a tendency to higher supernatant IFN-γ and IL-2 levels (Table 3 and Fig. 3). There was no difference between the two groups in the quantities of HSV antigen-specific IL-10.

While these differences were small, correlation of historical susceptibility to sun exposure with the immunologic data provided support for the experimental observations. History-positive patients were grouped according to whether they experienced an episode of herpes labialis less than 50% of the time after sun exposure (occasionally susceptible), or more than 50% of the time (highly susceptible). Compared to the occasionally susceptible patients, the highly susceptible patients had values of immune

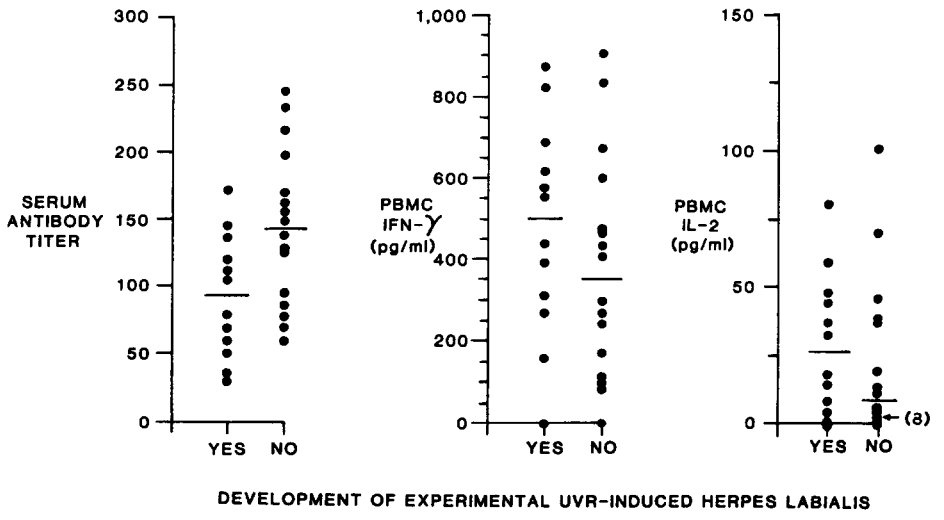

Fig. 3. Serum HSV antibody titers and in vitro cytokine production of HSV-stimulated PBMC among herpes labialis history-positive patients according to whether or not they were induced to an episode of herpes labialis by exposure to UVR.

parameters similar to those who were experimentally inducible with UVR: lower median neutralizing antibody titers (85 vs 149, P = NS), increased median PBMC IFN-γ (474 vs 269, $P = 0.08$) and increased median PBMC IL-2 (20 vs 4, $P = 0.09$).

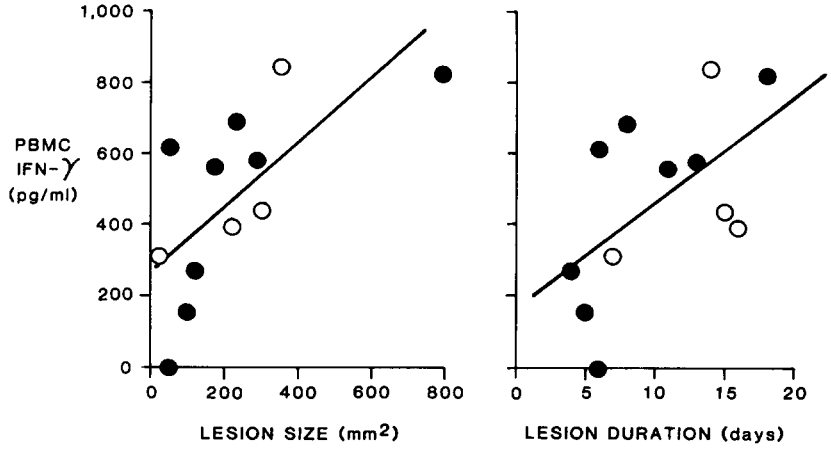

Fig. 4. Correlation between HSV-specific IFN-γ production by PBMC and the severity of UVR-induced herpes labialis. ●, delayed lesions; ○, immediate lesions. For definitions, see text. Correlations were examined by calculating Pearson's correlation coefficient, and the significance of correlations was determined by ANOVA. $r = 0.67$ for lesion size, and 0.60 for lesion duration. For delayed lesions only, $r = 0.66$ and 0.74, respectively. $P < 0.05$ for all correlations.

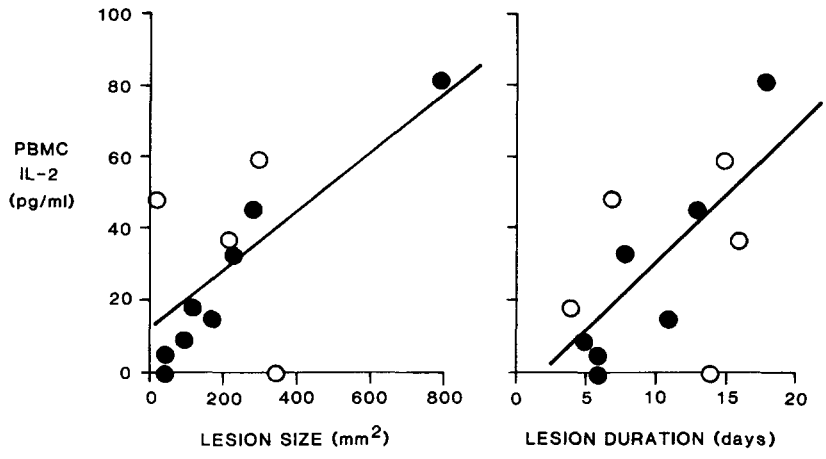

Fig. 5. Correlation between HSV-specific IL-2 production by PBMC and the severity of UVR-induced herpes labialis. ●, delayed lesions; ○, immediate lesions. For definitions, see text. Correlations were examined by calculating Pearson's correlation coefficient, and the significance of correlations was determined by ANOVA. $r = 0.63$ for lesion size, and 0.66 for lesion duration. For delayed lesions only, $r = 0.89$ and 0.96, respectively. $P < 0.05$ for all correlations.

Because these observations suggested that the development of UVR-induced lesions might be positively correlated with the magnitude of HSV antigen-specific IFN-γ and IL-2 levels, we correlated the supernatant levels of these two cytokines with two measures of lesion severity, lesion size and lesion duration (Figs. 4 and 5). Among the 12 patients who were successfully induced to have lesions by UVR exposure, the correlation coefficient (r) for the 4 comparisons ranged from 0.60 to 0.67 and the significance of the correlation from $P = 0.02$ to 0.04. We performed these correlations separately for the 'immediate' and 'delayed' onset lesion groups (Spruance et al., 1991). The immediate lesions tended to be 'outliers', and the correlation between cytokine levels and lesion severity was greater (0.66–0.96) for the delayed lesions alone.

4. Discussion

We found that titers of HSV-neutralizing antibody were higher among patients with frequent herpes labialis compared to the history-negative, HSV-seropositive control patients (Fig. 1). This difference has been noted by others and attributed to either an association between humoral responsiveness and an increased risk of recurrences or a booster effect from frequent re-exposure to viral antigens (Rawls, 1985). We believe the former explanation is correct, and hypothesize that the higher antibody levels could be evidence for a greater Th2 anti-inflammatory immune response among the subjects with frequent herpes labialis. If this antibody increase was due to a booster effect, one might also expect to find increased T-cell responses. To the contrary, we found that herpes labialis patients tended to produce less PBMC IFN-γ and IL-2, evidence of decreased

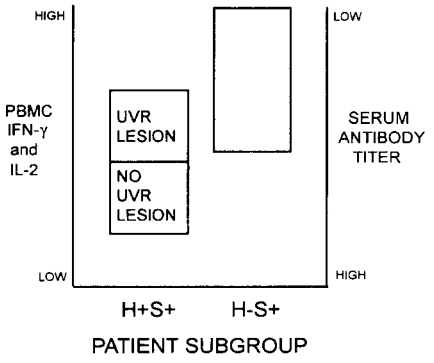

Fig. 6. Schematic representation of correlations suggested by the results. Patient subgroups differed by serum HSV-neutralizing antibody levels and HSV antigen-induced PBMC production of IFN-γ and IL-2. The two vertical scales run in opposite directions.

Th1 activity, and consistent with experimental findings that Th1 and Th2 immune responses are antithetical (Heinzel et al., 1989; Daynes et al., 1990).

In contrast to the evaluation of the patients by their history of herpes labialis, we made the novel observation that antibody titers were significantly lower among those who were induced to have herpes labialis by experimental exposure to UVR compared to those who were exposed, but did not develop lesions (Fig. 3). Of further interest, the values for PBMC IFN-γ and IL-2 tended to be greater among those who developed lesions compared to those who did not, again falling in the opposite direction from the antibody results. While the cytokine differences were not statistically significant, we found a strong positive correlation between experimental lesion severity and PBMC IFN-γ and IL-2 production (Figs. 4 and 5). These data are summarized schematically in Fig. 6.

We conclude from our UVR-induced herpes labialis study that a pro-inflammatory immune response may help to facilitate experimental disease. Since this conclusion is opposite from our correlations of immune measures with natural herpes disease, a necessary hypothesis is that UVR is a unique stimulus which can induce HSV reactivation independent of host cellular immune function. Following this event, we propose that the introduction of virus into the epidermis of an HSV-immune individual leads to a cutaneous inflammation of variable severity proportional to the intensity of the host immune response, much like a tuberculin skin test. The potential implication of this interpretation is that immunopathology may play a significant role in the pathogenesis of herpes labialis, similar to what has been demonstrated for experimental herpetic keratitis (Niemialtowski and Rouse, 1992; Hendricks et al., 1992). The dual role of immune responses (protection/pathology) is well-recognized in a variety of other infectious diseases, including bacterial meningitis, tuberculosis, and *Pneumocystis carinii* pneumonia (The National Institutes of Health-University of California Expert Panel, 1990). Alternatively, more vigorous T-cell responses and susceptibility to UVR-induced herpes labialis could be correlated because the cutaneous immune response in this subset of patients is more susceptible to impairment by UVR exposure. Temporary impairment of

Langerhans' cell function by UVR has been well-documented (Otani and Mori, 1987) and is one of the possible explanations for the relationship between UVR exposure and herpes labialis (Spruance, 1988).

Prior investigations of PBMC from patients with herpes labialis (Cunningham and Merigan, 1983; Green et al., 1985; Torseth and Merigan, 1986; Kuo and Lin, 1990) and the results of the present study provide evidence that persons susceptible to natural recurrent disease have PBMC that produce less HSV-specific IFN-γ and IL-2. What remains entirely unclear is how susceptibility to HSV recurrences originates, and whether it is genetically based or acquired; the exact mechanism(s) which creates susceptibility; and why enhanced susceptibility to disease is limited to HSV infection. As suggested by experiments in a murine model (Norval et al., 1987), this difference in responsiveness may have originated from the time of primary oral–facial HSV-1 infection, involving transient circumstances which promoted a Th2-like immune response to this specific infection (Cohen et al., 1991; Mason, 1991; Daynes and Araneo, 1992; Bonneau et al., 1993), and predisposed these individuals to a higher frequency of recurrent disease. Green (Green et al., 1985) showed that cell cultures from 2 of 8 subjects undergoing primary herpes gingivostomatitis failed to produce IFN-γ. Reeves and coworkers found that the probability of recurrences of HSV-2 infection was directly related to the presence and titer of neutralizing antibody (suggestive of a Th2-like response) following the primary infection (Reeves et al., 1981). The HSV-specific memory T-cells of these persons might bear the Th2-like cytokine phenotype of the primary immune response, leading to an inadequate inflammatory response at the time of virus reactivation.

An evaluation of IL-12 was not conducted in the present study, but would be of interest in future investigations. Patients with resistance to recurrent HSV infections could have a predisposition to increased IL-12 production by stimulated macrophages, which, in turn, could direct the immune response toward protective, Th1-like cytokine responses (Romagnani, 1992).

We had difficulty detecting IL-4 in PBMC culture supernatants and we did not find a correlation of IL-10 with clinical disease. These outcomes weaken our supposition that the Th1/Th2 paradigm explains resistance to herpes labialis. We have been able to identify IL-4 in culture supernatants of fresh PBMC using the same techniques (B.A. Araneo, unpublished data). The role of frozen, as opposed to fresh PBMC, and other laboratory parameters in these outcomes needs to be determined. Questions about the strict applicability of the Th1/Th2 paradigm in HIV infection have been raised by recent reports (Graziosi et al., 1994; Maggi et al., 1994; Mosmann, 1994). However, regardless of the outcome of this debate, the potential role of the Th1/Th2 paradigm in resistance to virus infections may be more appropriately evaluated in an illness other than HIV infection that does not cause major abnormalities in the immune system.

In summary, despite the small number of patients and the wide variation in antibody and cytokine results, several interesting hypotheses have emerged from this study for future testing. The data has suggested that patients with a history of frequent herpes labialis (H^+S^+) have a stronger Th2-like immune response and a weaker Th1 response relative to values for the HSV-infected control subjects who are free from recurrent disease (H^-S^+). Accordingly, HSV-specific Th1-like cellular immunity may be an

important factor in down-regulating the frequency of naturally occurring herpes labialis. Experimental UVR-induced herpes labialis appears to be regulated differently. Correlation of cytokine assay results with the development and severity of experimental lesions within the history-positive patient subgroup provides evidence that there is an immunopathologic component to recurrent HSV disease.

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