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Surveillance Network for Herpes Simplex Virus Resistance to Antiviral Drugs: 3-Year Follow-Up

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Herpes simplex virus (HSV) infections are very common in the general population and among immunocompromised patients. Acyclovir (ACV) is an effective treatment which is widely used. We deemed it essential to conduct a wide and coordinated survey of the emergence of ACV-resistant HSV strains . We have formed a network of 15 virology laboratories which have isolated and identified, between May 1999 and April 2002, HSV type 1 (HSV-1) and HSV-2 strains among hospitalized subjects. The sensitivity of each isolate to ACV was evaluated by a colorimetric test (C. Danve, F. Morfin, D. Thouvenot, and M. Aymard, J. Virol. Methods 105:207-217, 2002). During this study, 3,900 isolated strains among 3,357 patients were collected; 55% of the patients were immunocompetent. Only six immunocompetent patients excreted ACV-resistant HSV strains (0.32%), including one female patient not treated with ACV who was infected primary by an ACV-resistant strain. Among the 54 immunocompromised patients from whom ACV-resistant HSV strains were isolated (3.5%), the bone marrow transplantation patients showed the highest prevalence of resistance (10.9%), whereas among patients infected by human immunodeficiency virus, the prevalence was 4.2%. In 38% of the cases, the patients who excreted the ACV-resistant strains were treated with foscarnet (PFA), and 61% of them developed resistance to PFA. The collection of a large number of isolates enabled an evaluation of the prevalence of resistance of HSV strains to antiviral drugs to be made. This prevalence has remained stable over the last 10 years, as much among immunocompetent patients as among immunocompromised patients.

Herpes simplex virus (HSV) infections are very common; they are localized on the face and torso in the case of HSV type 1 (HSV-1) and in the genital region in the case of HSV-2. HSV-1 infections in the genital region are on the increase (40). Ocular herpes is less frequent, and neonatal herpes and herpetic meningoencephalitis are very rare but have a severe functional and vital prognosis (37).

Since acyclovir (ACV) {9-[(2-hydroxyethoxy)methyl)guanine]} was introduced to the market in 1983, it has been used primarily in the prevention and treatment of HSV infections. ACV-resistant HSV strains have been observed in vivo since the first large therapeutic trials (5, 10, 36). These resistant strains are detected in vitro by phenotypic tests which determine the antiviral concentration inhibiting viral replication by 50%. Several methods have been used to evaluate the sensitivity of the HSV strains to ACV, including techniques to detect the intensity of the cytopathic effect, such as the plaque reduction (17, 31) and colorimetric (11, 22, 26) techniques, but also the detection of DNA replication by hybridization (39) or antigen production by flow cytometry (30).

Previous surveys among immunocompetent patients have shown a prevalence of resistance to ACV varying between 0 and 0.6%, whereas among immunocompromised patients, the prevalence varied between 3 and 6% (9, 16, 29). The use of ACV is constantly growing, particularly in the general population, where it is used mainly in self-medication (since 1996) for the treatment of labial herpes. Among immunocompromised patients, the increase in the use of ACV is linked to the growth in the number and survival of patients among whom it is used for prophylactic or curative treatment. Furthermore, the development of new antiviral molecules derived from ACV (thymidine kinase [TK] dependent), such as valacyclovir, penciclovir, and ganciclovir, increases the risk of selection pressure of resistant strains. We could not get accurate data about the consumption of various antiherpes drugs, hospitalized patients did not receive any over-the-counter drug.

Since the last French survey goes back to the beginning of the 1990s (29), we deemed it essential to conduct a prospective

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TABLE 1. Prevalence of resistance to ACV according to the immune status of the patients

Virus excreted	Immunocompetent patients		Immunocompromised patients		Total patients	
	No. of patients[a]	No. of strains	No. of patients	No. of strains	No. of patients	No. of strains
HSV-1	1,237 (3 [0.2%])	1,326	1,168 (36 [3%])	1,523	2,405 (39 [1.6%])	2,848
HSV-2	618 (3 [0.5%])	664	334 (18 [5.4%])	411	952 (21 [2.2%])	1,075
Total	1,855 (6 [0.3%])	1,990	1,502 (54 [3.6%])	1,933	3,357 (60 [1.8%])	3,923

[a] The number and percentage of patients who excreted ACV-resistant HSV strains are given in parentheses.

and coordinated multicenter national study of the emergence of ACV-resistant viruses. This would allow us to establish a correlation between in vitro resistance and clinical resistance and to show a possible cross-resistance among different antiviral drugs, by distinguishing a large number of isolated HSV strains among immunocompetent and immunocompromised patients. For this purpose, a surveillance network was maintained from May 1999 to April 2002 at the Laboratory of Virology in Lyon, France, with the participation of 14 other French virology laboratories, two-thirds of which had already participated in the previous surveillance network (29). For this study we developed a fast colorimetric test (11), a standardized form for clinical information concerning the patient, and a database for processing information.

MATERIALS AND METHODS

Cells. An African green monkey kidney cell line (Vero; American Type Culture Collection) was cultivated in 199 medium containing a penicillin-streptomycin mix (25,000 U and 25,000 μg, respectively, per ml) supplemented with 7% fetal calf serum and was maintained in Eagle minimum essential medium with the addition of 1% L-glutamine, 1% amino acids (Eagle minimum essential medium nonessential amino acid solution), penicillin-streptomycin mix (25,000 U and 25,000 μg, respectively, per ml), and 2% fetal calf serum. All of these the products were obtained from BioWhittakerEurope, Verviers, Belgium.

Virus. (i) Reference strains. The ACV-sensitive (SC16) and ACV-resistant (SC16-DM21) HSV-1 strains were from the Wellcome Laboratory (Paris, France) (15). The ACV-sensitive HSV-2 strain (G-HSV-2) was supplied by B. Roizman (University of Chicago, Chicago, Ill.). The ACV-resistant (average 50% inhibitory concentration, 100 μM) HSV-2 strain (91-411) was isolated at the Laboratory of Virology in Lyon (France) in 1991 from a patient who had received a bone marrow transplant. The TK gene of this strain shows a frameshift mutation due to the deletion of a thymine (T) at the level of the T homopolymer (nucleotide 482 to 485).

(ii) Clinical isolates. Between May 1999 and April 2002, HSV strains were isolated in 14 virology laboratories from fibroblast embryonic cells from human lung (MRC-5 and Hel cells) and in 1 laboratory from African green monkey kidney cells (BGM cells). The isolates were sent in dry ice to the Lyon Laboratory of Virology, where they were cultivated in Vero cells. The cytopathic effect of the viruses was checked every day, and when more than 70% of the cells were lysed, the cells and the medium were collected, aliquoted, and stored at −80°C.

Clinical record. Each isolate was accompanied by a standardized form of clinical information that included the following.

(i) Identification. The patient and the hospital department were identified.

(ii) Date and location of the sample. The date and the location of the sample were classified according to the following categories: (a) upper region, including eye, skin (face, hand, neck, and back), mouth, upper respiratory tract (URT), and lower respiratory tract (LRT); (b) lower region, including anus, genital apparatus, and skin (buttocks and legs); and (c) systematic follow-up, including samples taken mainly from the URT and bronchoalveolar lavage but also from urine of patients who were regularly monitored and not showing any clinical signs.

(iii) Clinical definition. Primary infection corresponds to the first infecting contact with HSV-1 or HSV-2. Primary manifestation corresponds to the first manifestation symptomatic of a herpetic infection, whether the subject was or was not previously infected by the other viral type. Recurrence corresponds to the clinical expression of a viral reactivation in a patient previously infected by the same viral type.

(iv) Antiviral treatments. Antiviral treatments administered before, during, or after isolation of HSV were noted.

(v) Immune status. Immune status (immunocompetent or immunocompromised) was as reported by the physicians. Immunocompromised patients were grouped into nine categories according to the type of pathology that brought about the immunosuppression: (a) patients with hemopathies, including leukemias, lymphomas, and myelomas; (b) patients with human immunodeficiency virus (HIV) infections; (c) patients who had received bone marrow transplantation (auto or heterologous); (d) patients who had received organ transplantations, (e) patients with cancers, (f) patients who had received corticotherapy, (g) patients with burns, (h) miscellaneous, which groups together patients who had received chemotherapy for a nonspecified cause and patients with immunodeficiency, Hodgkin's disease, Crohn's disease, Sezary's syndrome, Still's disease, Wegener's granuloma and Waldenstrom's macroglobulinemia, pemphigus, pneumopathy, and rectocolitis; and (i) patients with immunosuppression due to an unknown cause. Immune status had to be filled in for the patient to be included in the study.

The clinical record and the viral strain corresponding to the same patient were given the same identification number, and then the clinical information was entered into an ACCESS database (Microsoft Windows).

Typing of HSV strains. Typing of HSV strains was carried out in each of the 15 participating laboratories by indirect immunofluorescence with three monoclonal antibodies (anti-HSV-1, anti-HSV-2, and anti-HSV-1 and -2) (Argène-Biosoft, Varhiles, France), developed with fluorescein-conjugated goat anti-mouse antibodies (Argène-Biosoft).

Colorimetric test for detecting sensitivity to ACV. The colorimetric test for detecting sensitivity to ACV (11) was based on the chessboard technique developed by Langlois et al. (22) but with only two virus dilutions (10^{-1} and 10^{-2}) tested in relation to two antiviral doses (5 and 10 μM). The results were expressed as the percent reduction of viral replication in the presence of the antiviral drug, for each virus dose and each antiviral concentration. For all of the ACV-resistant strains, the 50% inhibitory concentrations of ACV, foscarnet (PFA), ganciclovir (GCV), and penciclovir (PCV) were measured by the chessboard technique (22). The resistance thresholds had been defined by Langlois et al. (22) for ACV (6.5 μM for HSV-1 and 13.5 μM for HSV-2), for PFA (>400 μM for HSV-1 and HSV-2), and for GCV (>5 μM for HSV-1 and HSV-2) and by Edert et al. (14) for PCV (25 μM for HSV-1 and 60 μM for HSV-2).

Information from the study was published in a monthly bulletin.

RESULTS

General characteristics of the population studied. Between May 1999 and April 2002, 4,098 isolates, corresponding to 3521 patients, were collected. In 95% of the cases the patients had been hospitalized, and in 5% of cases they were outpatients, patients of doctors in private practice, or patients from state-run clinics.

As some isolate cultures were negative (1.6%) or came from patients whose immune status could not be revealed (2.9%), the study was carried out with 3,923 strains excreted by 3,357 patients. The population studied was 55% immunocompetent patients (n = 1,855) and 45% immunocompromised patients (n = 1,502) (Table 1). The 2,848 strains of HSV-1 and the 1,075 strains of HSV-2 were excreted by 1,781 women and 1,512 men; the genders of 64 patients (2%) were not specified. In total, of the 3,357 patients, 60 excreted ACV-resistant strains.

TABLE 2. Distribution of herpetic infections according to sample sites

Sites	No. of patients[a]			
	Immunocompetent		Immunocompromised	
	With HSV-1	With HSV-2	With HSV-1	With HSV-2
Upper (eye, skin, orolabial, LRT, URT, and upper region[b])	822 (3 [0.4%])	42	958 (35 [3.6%])	18 (1 [5.6%])
Lower (anal, skin, genital, and lower region[c])	301	550 (3 [0.5%])	58 (1 [1.7%])	287 (15 [5.2%])
Upper + lower[d]	25	12	23	17 (1 [6%])
Systematic follow-up	50	2	70	3 (1 [33%])
Miscellaneous[e]	23	4	47	8
Nonspecified	16	8	12	1
Total	1,237	618	1,168	334

[a] The number and percentage of patients excreting ACV-resistant strains are given in parentheses.
[b] Upper region indicates that there were several strains for the same patient but at different localizations above the waist.
[c] Lower region indicates that there were several strains for the same patient but at different localizations below the waist.
[d] The strains for the same patient were localized in both the upper and lower regions.
[e] Isolated strains from urine or biopsies (kidney or esophagus) are classified under miscellaneous.

Immunocompetent patients. Of the 1,855 immunocompetent patients, 1,237 excreted HSV-1 strains and 618 excreted HSV-2 strains (Table 1). The HSV-1 infections represented 95% of infections of the upper region (822 of 864), and 35% of infections of the lower region (301 of 851) (Table 2). Infections of both the upper and lower regions were observed in 2% of cases (two-thirds due to HSV-1 and one-third due to HSV-2). Primary infections represented 22% of cases, primary manifestations represented 20.5%, and herpetic recurrences represented 39%. More than 72% of the patients had not received antiviral treatment when the samples were taken (Table 3).

Six patients excreted ACV-resistant strains (0.3%), including three infected by HSV-1 (0.2%) and three by HSV-2 (0.5%) ($0.3 < P < 0.5$; no significant difference) (Table 1). These patients consisted of five females and one male, who were hospitalized for dermatology (four patients), infectious diseases (one patient), and geriatrics (one patient). The HSV-1 strains were isolated from lesions on the face, neck, hand, and mouth and the HSV-2 strains were isolated from the vulva and the buttocks. One female patient excreted an ACV-resistant strain while suffering from a primary infection, and another female patient did so while suffering from a primary manifestation. For four patients, there was a herpetic recurrence. The ACV-resistant strains occurred in two patients treated with this antiviral drug, in one untreated female patient, and in three patients for whom the treatment was not recorded (Table 4).

TABLE 3. Division of patients according to antiviral treatments

Treatment	No. (%) of patients	
	Immunocompetent	Immunocompromised
None	1,343 (72.4)	826 (55)
Before sampling		
ACV[a]	104 (5.6)	255 (17)
Other[b]	5 (0.2)	30 (2)
During sampling		
ACV	235 (12.7)	300 (20)
Other	20 (1)	15 (1)
Nonspecified treatment	148 (8)	75 (5)
Total	1,855 (100)	1,502 (100)

[a] ACV and Val-ACV
[b] GCV, Vidarabine, and PFA.

We monitored the case of a woman who since 1984 had secreted HSV-2 strains resistant to ACV (27). This 73-year-old patient, who was diagnosed as having monoclonal gammapathy, was not considered immunocompromised. She suffered chronic erosive vulvar lesions between 1984 and 1993, which received long-term treatment with ACV and PFA. Since 1995, we have tested the sensitivity of 14 HSV-2 isolates to ACV and to PFA; 3 were found to be sensitive to both antiviral drugs, and 11 were resistant to ACV only. In the cases with which the research was carried out, the ACV-resistant strains showed cross-resistance to GCV and penciclovir PCV.

The patient infected primarily by HSV-1 had been hospitalized for Kaposi Juliusberg syndrome. She was pregnant and had suffered from an atopic condition with eczema since childhood. This woman was probably infected by her young daughter (age unknown), who was undergoing treatment for herpetic stomatitis. This hypothesis could not be confirmed, because no samples were taken from the child.

Immunocompromised patients. The population studied consisted of 1,502 immunocompromised patients, 1,168 having excreted HSV-1 strains and 334 having excreted HSV-2 strains (Table 1). The main causes of immunosuppression were hemopathy (32% of the cases), HIV infections (21% of the cases), organ transplantations (13.5% of the cases), and bone marrow transplantations (13.5% of the cases) (Table 5). Eleven patients had leukemia and received a bone marrow transplant during the study; they have been included in the bone marrow transplantation category. HSV-1 strains were isolated to the upper region in 98% of the cases (958 of 976) and to the lower region in 17% of the cases (58 of 287) (Table 2). Infections of both the upper and lower regions were observed in 2.7% of the cases. There was a primary infection in 3.5% of the cases, a primary manifestation in 15.5% of the cases, and a recurrence in 54% of the cases. More than 55% of patients had not received antiviral treatment when the samples were taken (Table 3).

The ACV-resistant strains were excreted by 54 immunocompromised patients (3.6%), including 36 infected by HSV-1 and

TABLE 4. Characteristics of six immunocompetent patients who excreted ACV resistant strains

Patient no.	HSV type	No. of strains	Sex[a]	Age (yr)	Type of infection	Antiviral treatment	Sample mo/yr	IC_{50}[b] (μM)			
								ACV	PFA	GCV	PCV
00-40[c]	1	1	F	28	Primary infection	None	12/1999	43.5	131	11.3	97.6
01-1081	1	1	M	22	Primary manifestation	Unknown	09/2001	31.2	213	7.15	63
01-1288	1	1	F	25	Recurrence	Unknown	11/2001	76.5	87	ND[d]	ND
01-473	2	1	F	63	Recurrence	Zovirax ointment for 2 days	11/2000	39.1	125	148	263
00-362	2	1	F	83	Recurrence	Unknown	02/2000	41.3	175	158	300
99-174[e]	2	14	F	73	Recurrence	Intermittent Val-ACV, PFA, and Cidofovir	12/1995	100	143	ND	ND
							12/1995	100	143	ND	ND
							04/1997	4.5	145	ND	ND
							03/1998	100	182	ND	ND
							04/1998	100	163	ND	ND
							06/1998	100	173	ND	ND
							01/1999	5.7	201	ND	ND
							07/1999	83	173	132	268
							08/1999	100	134	ND	ND
							10/1999	6.5	125	1.8	10.5
							02/2000	25	125	ND	ND
							03/2000	100	154	190	321
							06/2001	100	161	ND	ND
							01/2002	116	125	ND	ND

[a] F, female; M, male.
[b] IC_{50}, 50% inhibitory concentration.
[c] Pregnant patient with Kaposi Juliusberg syndrome and eczema since childhood.
[d] ND, not determined.
[e] Monoclonal gammopathy described by Mouly et al. (27).

18 infected by HSV-2 ($0.1 < P < 0.2$; negligible difference) (Table 1). They consisted of 20 females and 34 males, age 3 to 78 years. HSV-1 strains were excreted by patients who had had a bone marrow transplant ($n = 22$) or an organ transplant ($n = 4$), who had shown a hemopathy ($n = 9$), or who had had corticotherapy for pemphigus ($n = 1$) (Table 5). HSV-2 strains were excreted by patients with HIV infection ($n = 13$), hemopathy ($n = 2$), or common variable immune deficiency ($n = 2$) or who had had an organ transplant ($n = 1$) (Table 5).

(i) Patients who received a bone marrow transplant. Of the 201 patients who received bone marrow transplants, 22 excreted ACV-resistant HSV-1 strains (10.9%). This value was 18.4% for allotransplantations (16 of 87) and 1.2% for autotransplantations (1 of 81) ($P < 0.001$). In all the cases there were herpetic recurrences in the upper region, with 11 of these patients having stomatitis. Nineteen patients had used ACV before, and 2 had used GCV for a cytomegalovirus infection (prophylaxis not specified). Of the 12 ACV-resistant patients treated with PFA, 7 excreted PFA-resistant strains (Table 6).

(ii) Patients with hemopathy. Of the 512 patients with hemopathy, 11 excreted ACV-resistant strains (2.1%). Among the nine patients excreting HSV-1 strains, four had lymphoma, three had leukemia (two acute leukemia and one lymphatic leukemia), and two had bone marrow aplasia. Among the two patients excreting HSV-2 strains, one had lymphoma and the other had myelodysplasia. Ten of these suffered a recurrent infection localized in the upper region, of whom six had stomatitis. One female patient had a recurrent infection in both the upper and lower regions (Table 6). Previous treatment with ACV was reported for 10 patients. Treatment with PFA was specified for only one of the two patients with PFA-resistant strains; the second was treated with ACV, but we had no information concerning any PFA treatment.

TABLE 5. Frequency of ACV-resistant HSV infections according to cause of immunosuppression and HSV type

Cause of immunosuppression	No. (%) of patients excreting:				Total no. of patients	% ACV resistant
	HSV-1		HSV-2			
	ACV sensitive	ACV resistant	ACV sensitive	ACV resistant		
Bone marrow transplant	160	22 (12.1)	19		201	10.9
HIV infection	133		164	13 (7.5)	310	4.2
Hemopathies	464	9 (1.9)	37	2 (5.1)	512	2.1
Organ transplant	164	4 (2.4)	32	1 (3.1)	201	2.5
Miscellaneous[a]	90	1 (1.1)	26	2 (7.1)	119	2.5
Cancer	62		22		84	
Corticotherapy	47		11		58	
Burns	24		0		24	
Nonspecified	24		5		29	
Total	1,132	36	316	18	1,502	3.6

[a] See Materials and Methods.

TABLE 6. Characteristics of immunocompromised patients who excreted ACV-resistant strains

Cause of immunosuppression	No. of patients	Type of infection	Site	ACV and GCV treatment	No. receiving PFA treatment	No. with PFA-resistant strains
Bone marrow transplant	22	HSV-1, recurrent	Upper	19 ACV	10	6
				2 GCV	2	1
				1 not known	0	0
HIV infection	13	HSV-2, recurrent	Lower	12 ACV	6	3
				1 not known	0	0
Hemopathy	9	HSV-1, recurrent	Upper	8 ACV	Not known	1
				1 not known	0	0
	2	HSV-2, recurrent	Upper Upper and lower	2 ACV	2	1
Organ transplant	4	3 HSV-1, recurrent; 1 HSV-1, nonrecurrent	3 Upper, 1 lower	2 ACV	1	1
				1 GCV	0	0
				1 not known	0	0
	1	HSV-2, recurrent	Systematic follow-up (urine)	1 ACV	0	0
Common variable immune deficiency	2	HSV-2, recurrent	Lower	2 ACV	1	1
Pemphigus	1	HSV-1, recurrent	Upper	1 ACV	0	0

(iii) Patients with HIV infection. Of the 310 patients with HIV infection, 13 excreted ACV-resistant HSV-2 strains (4.2%). Among these, eight had a CD4 level of less than 200/mm^3 (between 18 and 200/mm^3), and four had a higher level (up to 800/mm^3). These recurrent infections were localized to the anogenital region (Table 6). Treatment with ACV had been administered to 10 of the 11 patients, and among the 5 patients who had been treated with PFA, 2 excreted PFA-resistant strains.

(iv) Patients who received an organ transplant. Of the 201 patients who received an organ transplant, 5 excreted ACV-resistant strains (2.5%). These transplants consisted of two heart transplants, two heart-lung transplants, and one liver transplant. The HSV-1 strains were isolated to the upper region ($n = 3$) and to the lower region ($n = 1$), and there were three cases of recurrent infection (Table 6). A type 2 strain was detected in the urine during systematic monitoring of a patient who had already had herpetic infections (Table 6). For the four patients for whom we received information regarding treatment, three had been treated with ACV and one had been treated only with GCV for a cytomegalovirus infection (prophylaxis not specified). PFA treatment had been administered to the patient who excreted a PFA-resistant strain.

(v) Patients in the miscellaneous category. Three of the 119 patients belonging to the miscellaneous category excreted ACV-resistant strains. Two patients with common variable immune deficiency excreted ACV-resistant HSV-2 strains during recurrent genital infections (Table 6); they had previously been treated with ACV. It should be noted that the first patient excreted an ACV-resistant strain (sacrum) and a ACV-sensitive strain (vulva) on the same day at two different sites and that the second patient excreted four ACV-resistant strains, two of which were resistant to PFA under treatment with PFA, and then a strain sensitive to ACV and to PFA. The third patient had pemphigus, for which he was treated with corticotherapy, and he excreted the herpesvirus during a recurrent infection of the upper region. This patient had previously been treated with ACV.

(vi) Other immunocompromised patients who did not excrete ACV-resistant HSV strains. The other immunocompromised patients included in the study did not excrete ACV-resistant strains; 84 of these patients had solid cancer, 58 were treated with corticotherapy for causes such as asthma or lupus or while waiting for a transplantation, and 24 who had serious burns excreted HSV-1 strains. The type of cancer and the cause of the immunosuppression were not specified for 13 and for 29 patients, respectively.

During the course of the 6 half-years of surveillance, the prevalence of resistance to ACV of immunocompetent patients varied between 0.5 and 0.2%, and that of immunocompromised patients varied between 5 and 3.6%. These overall half-yearly variations are negligible for both the immunocompetent patients and for each category of immunocompromised patients.

TABLE 7. Clinical resistance among patients who excreted ACV-resistant strains

Immune status	No. of patients							
	Total	Clinical resistance			Excretion			
		Yes	No	Not known	Chronic	Recurrent	Short term	One strain
Immunocompetent	6	1	5	0	1	0	0	5
Immunocompromised	54	36	8	10	24	5	10	15[a]

[a] Including the 10 patients whose clinical resistance was not known.

In conclusion, 0.3% of immunocompetent patients excreted ACV-resistant strains, in contrast to 3.6% of immunocompromised patients ($P < 0.001$). The percentage of ACV-resistant HSV-2 strains is not significantly higher than the percentage of HSV-1 strains. The prevalence of resistance is highest (10.9%) in patients who have had a bone marrow transplant, particularly for those who have had allotransplants (18.4%), with a significant difference in relation to the other categories of immunocompromised patients ($P < 0.001$).

Relationship between excretion of ACV-resistant strains and clinical resistance. Clinical resistance, reported by the doctor, can be seen through persistent lesions with chronic excretion and multiple clinical recurrences. The viral excretions have therefore been put into four categories according to their duration: chronic if the excretion was continuous for longer than 1 month, recurrent if the time between the excretion of two strains was longer than 1 month, short term when the period of excretion was less than 1 month, and "one strain only" when we had only one sample available.

Of the six immunocompetent patients, the female patient with monoclonal gammopathy had persistent genital lesions with chronic excretion of resistant strains. Only one strain was isolated from each one of five other patients, and clinical resistance was not reported.

Of the 54 immunocompromised patients, 36 had clinical resistance, 24 of whom excreted chronic strains and 5 of whom had multiple recurrences. Ten excreted short-term strains, and 15 excreted only a single strain; for 4 of these patients, a resistance to treatment was noted (Table 7).

Chronic excretion of ACV-resistant strains is frequent in patients with HIV infection (61%) and in patients who have received a bone marrow transplant (41%). With regard to the five recurrent excretions, in one case there were successive excretions of ACV-resistant and ACV-sensitive strains, and in four cases there were excretions of only ACV-resistant strains.

DISCUSSION

The network for surveillance of the resistance of HSV strains to ACV consisted of 15 French virology laboratories. Patients were recruited between May 1999 and April 2002. The colorimetric test for sensitivity to ACV (11), which is fast and reasonably inexpensive, appears to be well adapted to evaluating a large number of isolates, as only 5% have been taken up by the conventional checkerboard technique (22).

In total, 3,357 patients, corresponding to 3,923 strains, have been included in this study. Of the population studied, the vast majority (95%) of whom were recruited from hospitals, 55% were immunocompetent and 45% were immunocompromised. The immunocompetent patients had never received antiviral treatment in 72% of the cases, in comparison to 55% of the immunocompromised patients ($P < 0.001$).

For immunocompetent patients, the prevalence of resistance to ACV of 0.3% is not significantly different from the prevalences observed by Nugier et al. (29) in France (0.33%) in 601 patients and by Christophers et al. (9) in England (0.28%) in 1,775 patients. Acquisition of resistance to ACV remains rare, and the cases described are mostly cases of genital herpes (21, 27, 38). Besides the female patient described by Mouly et al. (27), we report two new cases of recurrent genital herpes caused by ACV-resistant strains, or a prevalence of 0.55%, which is close to that observed by Christophers et al. (9) (0.47%). Two recent studies have reported cases of resistance to ACV during recurrent orolabial herpetic infections in immunocompetent patients. The prevalence observed by Boon et al. (2) was 0.11%, and that observed by Bacon et al. (1) was 0.2%. In this study, we report two cases of orolabial ACV-resistant HSV strains: one stemming from a recurrent infection, with a prevalence of 0.4%, and one stemming from a primary infection in an untreated female patient, with a prevalence of 0.24%. Some patients with genital herpes excreted ACV-resistant HSV strains without having received antiviral treatment (9) (3 patients out of 708 [0.42%]).

Selection of ACV-resistant strains can be fast; indeed, in the case of patient 01-473, an ACV-resistant strain was observed 2 days after treatment began. Sarisky et al. (35) described the case of a resistant strain isolated 17 h after beginning treatment with PCV. The heterogeneity of HSV populations, i.e., the coexistence of viruses sensitive and resistant to antiviral drugs, facilitates this rapid selection of ACV-resistant strains.

In immunocompromised patients, the prevalence of resistance of 3.6% is lower but not significantly different from those observed in the different studies carried out during the last 10 years: 4.7% of 148 patients (16), 5.4% of 184 patients (29), 6.3% of 95 patients (9), and 4.2% of 971 patients (result of a study carried out at the Laboratory of Virology in Lyon between 1986 and 1996 [M. Aymard et al., unpublished data]). The prevalence of resistance to ACV is very strongly linked to the type of immunosuppression, and when serious immunosuppression is combined with lengthy exposure to ACV, the risk of appearance of strains resistant to antiviral drugs increases considerably. Thus, the study of Englund et al. (16) shows a prevalence of resistance to ACV of 13.7% in bone marrow transplant patients. In our investigation, the prevalence of resistance in these same patients was 10.9% overall and reached 18.4% in patients receiving allotransplants. A recent study showed an incidence of 30% while monitoring allotransplant patients for 4 years in the same hospital depart-

ment (F. Morfin, K. Bilger, F. Najioullah, A. Boucher, A. Thiebaut, N. Raus, S. Bosshard, M. Aymard, M. Michallet, and D. Thouvenot, submitted for publication).

The prevalence of resistance in HIV-infected patients decreased from 7 to 3.4% during the last 10 years (9, 16, 20). The overall prevalence of 4.2% that we report confirms this decrease, which is probably linked to a reduction in the number of opportunistic infections since the implementation of tritherapy (24).

For 51 of the 54 immunocompromised patients who excreted ACV-resistant strains, treatment with ACV was reported; in 10 cases this was prophylactic treatment. In the cases of a bone marrow transplantation (two cases) and a heart transplantation (one case), there was only one treatment with GCV reported, and we do not know if it was preventive for cytomegalovirus infection. The risk of cross-resistance during administration of antiviral drugs in which the mode of action is TK dependent was previously reported in the literature (16, 29, 32). However, during a study carried out at the Laboratory of Virology in Lyon between 1986 and 1996, prophylactic treatment with ACV was found to carry less risk of a resistant virus emerging in comparison to curative treatments (0.6 versus 2.7%). Prophylactic treatment with GCV in cytomegalovirus-seropositive patients is also accompanied by a decrease in the risk of ACV-resistant HSV strains emerging (6).

Antiviral drugs with a non-TK-dependent mode of action, such as PFA, have been used since the emergence of ACV-resistant strains (7, 28), and PFA-resistant strains have appeared (8, 13, 33, 34). In our study, among the 22 ACV-resistant patients who received PFA, 14 excreted PFA-resistant strains (64%). Likewise, a prevalence of 50% of strains resistant to ACV and to PFA was reported by Chen et al. (8) for 14 patients who received bone marrow transplants. Among the 11 patients who received PFA alternating with ACV following excretion of ACV-resistant strains, 8 excreted PFA-resistant strains (72%) in an average of 13 days after the treatment was administered (between 5 and 43 days). This speed of development in PFA-resistant strains was first reported by Chen et al. (8). They describe three patients who excreted PFA-resistant strains when they received PFA for the first time and two patients, having previously received treatment with PFA, who developed PFA-resistant strains after only 5 and 7 days of treatment. Langston et al. (23) described three bone marrow transplantation patients who excreted ACV-resistant HSV strains and who rapidly developed PFA-resistant strains while under treatment with PFA (after 3, 6, and 10 weeks). Alternation of antiviral treatments remains, however, the best strategy for overcoming the emergence of resistant strains, because when antiviral treatment stops, the strains again become sensitive to the antiviral drugs. In this study, for three of the six patients who received PFA alternating with ACV (50%), strains sensitive to ACV and to PFA were isolated.

Resistance to ACV is demonstrated by the presence of mutations in the TK gene (12, 18) and, more rarely, in the DNA polymerase gene (25). Resistance to PFA is explained by the presence of a mutation in the DNA polymerase gene (19). The combination of phenotypic tests for cross-resistance and genetic tests would allow us to improve our knowledge about the molecular mechanism of resistance to antiviral drugs, to develop fast methods to detect resistant mutants (for example, by direct sequencing of the pathological product), and to implement a rational therapeutic strategy in the choice of secondary antiviral drugs, such as PFA and cidofovir (the latter is still reserved for patients excreting strains resistant to both ACV and PFA) (3, 4).

It was necessary to collect a large number of isolates in order to evaluate the prevalence of resistance to antiviral drugs and to study changes in resistance. It is interesting to observe the stability of frequency of resistance in the immunocompetent and immunocompromised populations, as well as the tendency to a reduction in resistance of the HSV in subjects infected by HIV. Indeed, despite self-medication by immunocompetent patients and curative and prophylactic treatments for immunocompromised patients, resistance to ACV has not increased during the course of the last 10 years. In bone marrow transplantation patients receiving multiple antiviral treatments as a prophylactic or therapeutic measure, the risk of selecting ACV-resistant HSV strains is very high and justifies virological surveillance as well as a phenotypic study of cross-sensitivity to other antiviral drugs. With more than 3,300 patients, this study has demonstrated the benefit of regularly monitoring patients at risk to detect in vitro resistance in order to adapt therapeutic care to the needs of immunocompromised patients.

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