Drug-Resistant HIV Infection among Drug-Naive Patients in Israel

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Background. In Israel, <0.06% of the general population is infected with human immunodeficiency virus (HIV), with a much higher prevalence among specific groups. These groups are distinguished demographically by risk behavior category and by virus subtype. We investigated transmission of drug resistance within groups to assess the impact of these factors.

Methods. Plasma samples from >15% of all patients with new diagnoses of HIV infection were randomly collected between June 1999 and June 2003. Sequences from 176 drug-naive patients included 20 of subtype A, 20 of subtype AE, 2 of subtype AC, 29 of subtype B, 100 of subtype C, and 5 of subtype F.

Results. Major drug resistance mutations (protease: L90M; reverse transcriptase: M41L, K103N, V106M, M184V, Y181S, G190A, L210W, T215Y/F, and K219R) were detected in 1 subject with A subtype, 3 with subtype B, and 9 with subtype C. In addition, 1 subject with A subtypes, 2 with subtype B, and 10 with subtype C had secondary mutations (protease: M46I; reverse transcriptase: A98G, K101Q, and V108I). Only 1 patient had mutations associated with >1 class of drugs. Among subjects who contracted HIV infection in Israel, 16 of 56 (1 of 7 with subtypes A or AE, 4 of 17 with subtype B, and 11 of 32 with subtype C; $P = .7$–1.0) carried resistant virus—a significantly higher proportion ($P < .001$) than in subjects infected in other countries (10 of 120 infected).

Conclusions. Drug-resistant virus was detected in 14.8% of patients with new diagnoses of HIV infection but in 28.6% of patients known to have been infected in Israel. The implications include a need for pretreatment resistance testing and for better programs aimed at prevention of transmission, directed particularly at patients. We did not find significant differences in transmission of resistant virus between those infected with subtypes B and C, despite the different demographic background. A conclusive analysis and interpretation should await a more extensive study.

Transmission of drug resistance from treatment-experienced patients to newly infected persons has been observed repeatedly in countries with access to antiretroviral therapy [1–11]. Reports in the literature suggest that 8%–30% of people who contract HIV infection in Europe or the United States acquire a virus with drug resistance–conferring mutations [12–14] (reviewed in [15]). The transmission of drug-resistant strains increases despite all prevention efforts, raising major public health concerns [16]. The clinical management of patients who have never been exposed to therapy may be unfavorably affected [17–20].

Although the incidence of HIV infection in Israel is relatively low (47 cases per million persons in 2000) [21], the prevalence is much higher among particular groups. Most of the infected patients or their parents contracted the virus in other countries. Infected immigrants had come from many parts of the world. Consequently, most of the circulating subtypes and recombinants can be found in various proportions. The major groups are the following: Israeli citizens of Ethiopian origin, who account for only 1.7% of the total population [22] but contribute 45.4% of all new HIV/AIDS cases diagnosed [21]; injection drug users who recently

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immigrated from the former Soviet Union, where a large epidemic among injection drug users broke out in the late 1990s [23]; and men who have sex with men, usually infected in Israel or in other Western countries. These 3 groups can be clearly distinguished demographically, by risk behavior category and by virus subtype. Cross-infection between groups is very rare. The general availability of antiretroviral treatment in Israel leads to transmission of resistant virus among members of each group. We investigated the frequency of drug-resistant HIV infection among drug-naive patients within the different groups.

METHODS

Clinical specimens and database. Samples obtained from drug-naive patients selected at random by the treating physicians between June 1999 and June 2003 were sent for genotypic analysis to the National HIV Reference Laboratory (NHRL; Tel-Hashomer, Israel). Participating physicians from the different national AIDS health care centers sent all patients who arrived for follow-up visits during the relevant period without further selection. The treatment center laboratories determined plasma HIV RNA levels and $CD4^+$T cell counts. Demographic information (including date and place of birth, date of immigration to Israel, and presumed locale where infection was acquired), together with the current $CD4^+$ cell counts and HIV RNA levels, were provided by the participating physicians on standardized forms along with the samples and were stored in an anonymous database at the NHRL. Subtype identification was based on comparing the polymerase sequence with consensus sequences from the Stanford public database (http://hivdb.stanford.edu) [24–26]. Several sequences documented in the present study are shared with SPREAD, a scientific surveillance program supported by the European Commission [27] (authors' unpublished data).

HIV-1 RNA extraction and sequencing. Extraction and genotyping of viral RNA were performed as described elsewhere [28].

Statistical analysis. Clinical data and individual mutation frequencies were compared across patient groups by the χ^2 test and Fisher's 2-tailed exact test. A mutation summary score was computed for each of the 3 groups of drugs by adding the number of mutations present. The mutation summary scores were compared across patient groups with the Wilcoxon rank sum test.

Phylogenetic analysis. Phylogenetic relationships of newly derived viral sequences were estimated from comparisons with previously reported representative HIV-1 isolates. These are listed in figure 1. The comparison was made by use of the CLUSTAL W profile alignment option. The sequences were aligned according to their nucleotides (nucleotides 2262–2549 and 2659–3290 of HXB2 [GenBank accession number K03455] of the protease amino acids 4–99 and reverse transcriptase amino acids 38–248, respectively). A tree was drawn by the TreeView program.

RESULTS

Screening of HIV-infected patients. HIV/AIDS case management is done in 7 regional AIDS centers in Israel. Testing for HIV by ELISA is available on request, free of charge. In addition, all new immigrants from Ethiopia aged >9 years are screened for HIV infection on arrival. Systematic screening is done in jails and for some military recruits [21]. Regional laboratories and the blood services perform ELISA testing for HIV antibodies. All samples positive by ELISA are forwarded to the NHRL for confirmation by Western blot analysis. Confirmed cases are reported back to the medical source of specimen and to the AIDS Department in the Ministry of Health, where the data are stored in a National Register. About 300 new patients were given a diagnosis of HIV infection each year during the study period.

Because no systematic screening for resistance is done in Israel, samples from patients with new diagnoses were collected at random by participating physicians at the HIV treatment centers and sent to the NHRL. Plasma samples were collected from patients coming for a second consultation, together with plasma used to determine baseline $CD4^+$ cell counts and viral RNA. All samples received at NHRL by July 2003 were genotyped and were included in the study. The participating physicians sent all patients who arrived for follow-up during the relevant period without further selection. These constituted between 12% (large centers) and 60% (small centers) of all patients who were given diagnoses at the participating health care centers (5 of 7) during the study period. The donors made up >15% of all patients with new diagnoses between June 1999 and June 2003. Distribution of risk groups in the collected samples was in accordance with the prevalence of these groups in the population of infected patients during these years (58%, 24%, and 10% heterosexuals, injection drug users, and men who have sex with men, respectively, in the study samples, and 62%, 25%, and 13% in the total population; $P = 1$); 51% of the study patients were of Ethiopian origin, compared with 50% in the total population ($P = 1$).

Patients and sample classification. Sequences were obtained from a total of 178 drug-naive patients. These included 42 with A subtypes (20 with subtype A, 20 with AE, and 2 with AC), 29 with subtype B, 100 with subtype C, and 5 with subtype F (table 1). Samples from 2 patients did not amplify and were excluded from this study.

Most of the patients had recently been given a diagnosis of HIV infection (median, 3 days since confirmation by positive result of a Western blot assay). All 5 patients with subtype F were born and infected in Argentina. Most of the patients with A subtypes (79%) came from the former Soviet Union—in

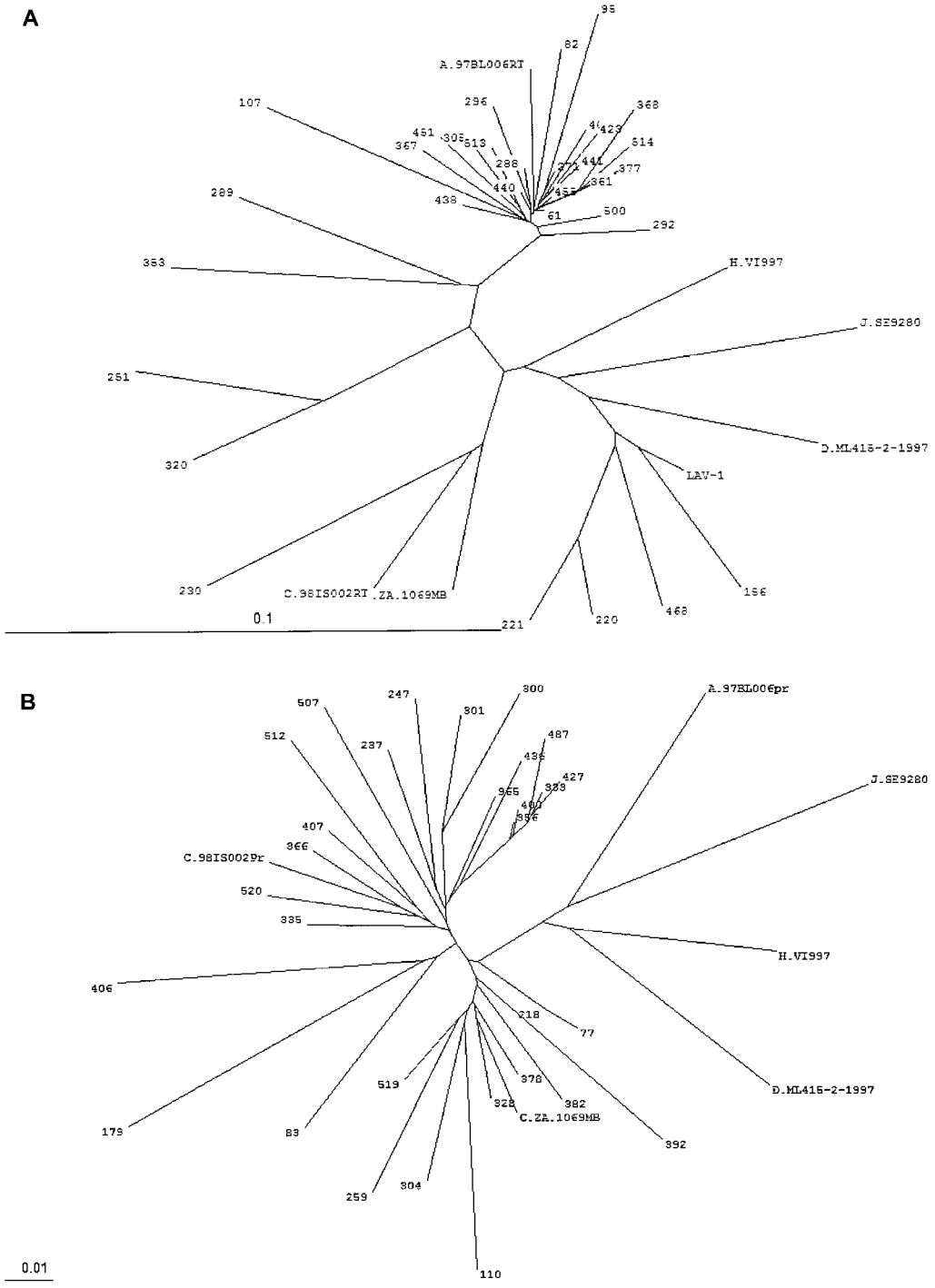

Figure 1. Phylogenetic trees of protease and reverse-transcriptase genes of drug-naive patients given diagnoses of HIV infection in Israel. Sequences of Israeli patients were compared with reference sequences from the Los Alamos database: A subtypes from Belarus (A/97BL006; GenBank accession number AF193275), B (HIV-1_{Lav-1}; GenBank accession number K02013), C from Israel and South Africa (C 98IS002 and C.ZA.1069MB; GenBank accession numbers AF286233 and AY585268, respectively), D from Kenya (D.ML415-2-1997; GenBank accession number AY322189), H isolated in Belgium (H.V1997; GenBank accession number AF190128), and J isolated in Sweden (J.SE9280; GenBank accession number AF082394). The sequences were aligned according to their nucleotides (protease: amino-acids 4–99; reverse transcriptase: amino acids 38–248). A tree was drawn by the TreeView program. The figure shows a phylogenetic analysis of the reverse transcriptase sequences of patients with HIV subtypes A *(A)* and of protease sequences of 32 patients with subtype C known to have been infected in Israel *(B).* The multiple infections involving families of epidemiologically linked viruses is depicted in the cluster of injection drug users who immigrated from the former Soviet Union infected with HIV subtype A and CRF01_AE *(A);* a chain of transmissions originating from a common source is evident in *B:* patients 333, 386, 400, 427, and 487. The latter patients received their diagnoses at 4 different centers during the last year of the study, 2 of them were diagnosed before seroconversion; no independent information regarding a common source or any other connection between these patients exists.

Table 1. Demographic characteristics of newly HIV-infected drug-naive patients in Israel.

Factor	Subtype			*P*		
	A (n = 42)[a]	B (n = 29)	C (n = 100)	A vs. B	B vs. C	A vs. C
Sex				>.001	>.001	.1
Male	64	76	43			
Female	36	24	57			
Risk group				>.001	>.001	>.001
Injection drug users	76	21	2			
Heterosexuals	14	21	87			
Men who have sex with men	7	48	…			
Mother-to-child transfer	…	…	9			
Other	3	…	2			
Not available	…	10	…			
Locale where infection was acquired				<.001	.001	.216
Israel	14	55	30			
Abroad	43	31	51			
Not available	43	14	19			
Age, years				NS	NS	NS
Mean ± SEM	33.35 ± 1.38	30.13 ± 3.06	31.85 ± 1.40			
Median	31.3	32.4	31.0			
Time since first Western blot, months				NS	NS	NS
Mean ± SEM	0.60 ± 0.17	1.21 ± 0.56	1.07 ± 0.24			
Median	0.1	0.0	0.1			
HIV RNA, copies/mL				0.7	0.5	0.2
Mean ± SEM	185,668 ± 34,850	299,123 ± 87,947	496,291 ± 198,387			
Median	36,700	89,000	93,500			
Log_{10} HIV load				NS	NS	NS
Mean ± SEM	5.27 ± 4.52	5.48 ± 4.94	5.70 ± 5.30			
Median	4.54	4.95	4.97			
$CD4^+$ cell count, cells/μL				NS	NS	NS
Mean ± SEM	454 ± 64	310 ± 52	424 ± 90			
Median	483	289	264			

NOTE. Data are percentage of patients, unless otherwise indicated. The χ^2 test was used to compare clinical data. NS, not significant.

[a] Twenty patients with subtype A, 20 patients with subtype AE, and 2 patients with subtype AC.

particular, the Ukraine; 43% of patients with A subtypes are known to have been infected in the former Soviet Union, for 43% the country of infection was not available, and 14% were infected in Israel. Most patients with subtype B were born and infected in Israel (59% and 55%, respectively) and in the former Soviet Union (34% and 21%, respectively). For 14% of patients with subtype B, the country of infection was not known, and the rest (10%) were infected in various Western countries. Most patients with subtype C, or their parents, were born in Ethiopia (92%), and a few were born in the former Soviet Union (3%), Israel (2%), India, and Africa; 51% of patients with subtype C were infected in Ethiopia, and 30% were infected in Israel; for 19%, the locale where infection was acquired was unknown.

Demographic data varied between the 3 groups in a number of additional parameters: sex, age, and mode of infection (table 1). HIV loads and $CD4^+$ cell counts at diagnosis were similar for the 3 groups, and although the average HIV load was lower and $CD4^+$ cell count higher among patients with A subtypes, the differences did not reach statistical significance (table 1).

Resistance-associated mutations and polymorphisms. Twelve patients—1 with an A subtype (2% of all with A subtypes), 3 with subtype B (10% of all with B), and 9 with subtype C (9% of all with C), or 7% of the total—had major (primary) mutations, which included the protease N88D (2 patients with subtype C) and L90M (1 with subtype C) or different combinations of reverse-transcriptase mutations, including M41L (1 with subtype B), K103N/T (1 with an A subtype, 1 with subtype B, 2 with subtype C), V106M (1 with subtype C), M184V (1 with subtype B), G190A (1 with subtype C), L210W (1 with subtype B), T215Y/F (1 each with subtype B and subtype C) or K219R (1 with subtype B). In addition, 14 patients, 1 with an A subtype (2%), 2 with subtype B (7%), and 11 with

Table 2. Frequencies of drug resistance–conferring mutations in samples obtained from drug-naive patients in Israel.

Gene, mutation	Patients infected with subtype			Patients with subtype C infected in Israel vs. other patients with subtype C		
	A (n = 42)[a]	B (n = 29)	C (n = 100)	Israel (n = 32)	Others (n = 68)	P
Protease						
Major mutations						
M46I	…	7	4	3	4	NS
N88D	…	3	3	3	1	NS
L90M	…	…	1	3	…	NS
Minor mutations						
L10V	7	3	8	13	7	NS
K20R	12	7	12	16	10	NS
L33F	…	10	1	…	1	NS
L63P	34	59	44	56	39	NS
A71T	2	21	1	…	1	NS
V77I	10	21	2	…	3	NS
V82I	2	…	4	13	1	.007
Other[b]						
M36I	90	10	98	73	97	.552
I93L	76	3	89	59	90	.749
PR score,[c] no including 36 and 93, average no. of mutations per patient	0.67	1.31	0.8	1.1	0.7	.01
Reverse transcriptase						
Thymidine-associated mutations						
M41L	…	…	2	3	…	NS
D67N	…	…	…	…	…	…
K70E/R	…	…	2	…	3	NS
L210W	2	3	…	…	…	NS
T215F/Y	…	3	1	…	1	NS
K219R	…	3	…	…	…	NS
NRTI-associated mutations and polymorphisms						
E44D	…	…	6	16	3	.02
A62V	15	…	2	2	3	NS
K70E/R	…	…	2	…	3	NS
F77L	2	…	…	…	…	NS
F116I	…	3	2	…	3	NS
V118I	…	…	2	…	3	NS
Q161K	…	…	1	3	…	NS
M184V	…	3	…	…	…	NS
NRTI,[c] average no. of mutations per patient	0.02	0.07	0.11	0.16	0.09	0.5
NNRTI-associated mutations						
A98G	…	…	6	19	…	>.001
K101R	…	…	7	16	3	.02
K103N	2	3	2	6	…	.09
V106M	…	…	1	3	…	NS
V108I	2	…	1	…	1	NS
V179I	17	…	4	6	4	NS
Y181S	…	…	1	…	1	NS

(continued)

Table 2. ***(Continued.)***

Gene, mutation	Patients infected with subtype			Patients with subtype C infected in Israel vs. other patients with subtype C		
	A (n = 42)[a]	B (n = 29)	C (n = 100)	Israel (n = 32)	Others (n = 68)	P
G190A	…	…	1	3	1	NS
F227Y	2	…	…	…	…	NS
Other[b]						
A98S	2	10	26	25	29	.5
NNRTI,[c] including A98S, average no. of mutations per patient	0.22	0.03	0.22	0.35	0.1	.005

NOTE. Data are percentage of patients with mutation. For clarity, only mutation frequencies of ⩾1% are shown (empty spaces in the table represent 0 occurrence of the mutation in the tested samples). In addition to significant difference in the frequency of M36I and I93L between patients infected with B and non-B subtypes, significant differences were found in the frequency of protease K20R in patients infected with subtype B vs. C (P < .04), A71T in patients infected with B vs. non-B subtypes (P < .001), V77I in patients infected with B vs. A subtypes (P = .04) or C subtypes (P = .002); and reverse transcriptase A62V in patients infected with subtypes A vs. B (P = .02) or C (P < .009), A98S of patients infected with subtypes A vs. C (P = .001) and V179I in patients infected with A vs. non-A subtypes (P = .01). Clinical data and individual mutation frequencies were compared across patient groups by χ^2 test and Fisher's 2-tailed exact test. NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NS, not significant.

[a] Twenty patients with subtype A, 20 patients with subtype AE, and 2 patients with subtype AC.

[b] Protease mutations M36I and I93L are present in almost all non-B variants as part of the structure of the wild type non-B virus; reverse transcriptase mutation A98S is prevalent in patients infected with subtype C.

[c] A mutation summary score was computed for each group of drugs by adding the number of mutations present, not including 36 and 93. The mutation summary scores were compared across patient groups with the Wilcoxon rank sum test.

subtype C (11%), 8.2% of the total, had different secondary (minor) mutations, including the protease M46I (2 with subtype B and 4 with subtype C) or the reverse transcriptase A98G (4 with subtype C), K101Q (1 with subtype C), V108I (1 with an A subtypes and 1 with subtype C), and Y181S (1 with subtype C). Other minor mutations and polymorphisms, which have been documented previously to exist in subtypes other than B [28–32], irrespective of treatment, are shown in table 2. One patient with subtype C had protease L90M plus reverse transcriptase A98G and K101R); 1 patient with subtype B had M184V plus L210W plus T215Y (table 3).

Resistance in drug-naive patients infected in Israel. The frequency of resistance mutations varied between patients who were infected in Israel and those infected in countries where treatment is not generally available.

Because immigrants from Ethiopia are screened for HIV infection on arrival, the locale where they were infected is well documented. Place of infection is not as completely documented for the others. Fifty-six patients are known to have been infected in Israel (7 with HIV A subtypes, 17 with subtype B, and 32 with subtype C), and 74 are known to have been infected outside of Israel; for 46, information is not available. Patients with subtype C infected in Israel received the diagnosis on average 10.7 years (median, 11 years) after their arrival, 9 of them during the acute stage of infection. Genotyping was done either immediately after diagnosis or within 13 months of their positive result of Western blot testing (median, 1.3 months). The mean HIV RNA level (±SEM) was 904,182 ± 710,791 copies/mL (median, 197,500 copies/mL; range, 3980–18,000,000 copies/mL), and the mean $CD4^+$ cell count (±SEM) was 480 ± 226 cells/μL (median, 280 cells/μL). The mean time after immigration to Israel that patients with subtype C had HIV infection diagnosed 0.7 years (median, 0.5 years); their mean HIV RNA level (±SEM) was 318,189 ± 84,439 copies/mL (median, 197,500 copies/mL), and the mean $CD4^+$ cell count (±SEM) was 411 ± 97 cells/μL (median, 251 cells/μL). No significant differences in HIV RNA levels and $CD4^+$ cell counts were found between the patients with subtype C infected in Israel and those infected in the country of origin (mainly Ethiopia).

During the study period (June 1999 to June 2003), an increase in the spread of resistance was not observed. In the 4 study years, 10, 3, 7, and 6 patients, respectively, had viruses with resistance-conferring mutations. Overall, the differences were not significant.

Frequencies of resistance-conferring mutations correlated with the place where infection was acquired: higher frequencies were seen in patients infected in Israel, particularly in patients with subtype C, who acquired nonnucleoside reverse-transcriptase inhibitor–associated mutations. Among those who contracted HIV in Israel, 10 (17.9%) of 56 (1 of 7 with A subtypes, 3 of 17 with subtype B, and 6 of 32 with subtype C; P =

Table 3. Major and strong minor resistance-conferring mutations in patients HIV-infected in Israel versus others.

Type of mutation, subtype	Patient no.	Place of acquisition of infection	PI-associated mutation	NRTI-associated mutation(s)	NNRTI-associated mutation(s)	Other mutations and polymorphisms	
						Protease	Reverse transcriptase
Major							
A	457	NA	…	…	K103T	M36I, L63P, I93L	R211S
B	706	Israel	…	F116I, K219R	…	L63P	R211K
	76	Israel	…	M184V, L210W, T215Y/F	…	L10V, L63P	…
	38	Israel	…	…	K103N	M36I	…
C	83	Israel	N88D	…	…	M36I, L63P, I93L	A98S
	297	Abroad	N88D	…	…	M36I, I93L	A98S
	406	Israel	L90M	…	A98G, K101R	L10V, M36I, L63P, I93L	…
	237	Israel	…	…	G190A	M36I, I93L	E44G, A98S, V179I
	300	Israel	…	M41L, E44D	…	M36I, L63P, I93L	…
	373	Abroad	…	T215Y	…	M36I, A71T, I93L	…
	400	Israel	…	E44D	A98G, K103N, V106M	M36I, L63P, I93L	E44A
	328	Israel	…	…	K103N	L10I, M36I, I93L	A98S
Minor							
A	451	Abroad	…	…	V108I	M36I	R211S
B	84	NA	M46I	…	…	L63P	…
	312	Israel	M46I	…	…	K20R, M36I	…
C	77	Israel	M46I	…	…	K20I, M36I, I93L	…
	111	Abroad	M46I	…	…	L33F, M36I	…
	337	Abroad	M46I	…	…	M36I, I93L	…
	416	Abroad	M46I	…	K101R	M36I, L63P, I93L	R211K
	333	Israel	…	…	A98G, K101R	M36I, L63P, I93L	…
	356	Israel	…	…	A98G, K101R	M36I, L63P, I93L	…
	427	NA	…	…	A98G, K101R	M36I, D60E, L63P, I93L	…
	487	Israel	…	…	A98G	M36I, D60E, L63P, I93L	R211K
	507	Israel	…	…	K101Q	M36I, L63V, I93L	…
	281	Abroad	…	…	V108I	K20R, M36I, L63T, I93L	A62V, A98S
	472	Abroad	…	…	Y181S	M36I, L63T, I93L	…

NOTE. NA, not available; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

0.7–1) carried virus with major resistance mutations, a significantly higher proportion ($P < .001$) than the proportion infected in other countries (3 [2.5%] of 120). Six patients infected in Israel (10.7%) carried strong minor mutations, compared with 7 (5.8%) of 120 infected elsewhere or with an unknown place of acquisition of infection (table 3).

Phylogenetic analyses of the patients' sequences was done in comparison with 7 reference strains from HIV-1 group M from the Los Alamos sequences database [33] by use of the CLUSTAL W [34] profile alignment option. As seen in figure 1, 2 kinds of transmission chains are apparent: multiple infections involving families of epidemiologically linked viruses (e.g., the cluster of injection drug users emigrating from the former Soviet Union infected with HIV subtype A and CRF01_AE; figure 1*A*); and a chain of transmissions originating from a common source (e.g., subtype C patients no. 333, 386, 400, 427, and 487; figure 1*B*). The latter patients were given diagnoses at 4 different health care centers during the last year of the study, 2 of them before seroconversion; no independent information regarding a common source or any other connection between these patients exists.

DISCUSSION

We did not find significant differences in transmission of resistant virus between the groups infected with subtype B and subtype C. The rate of transmission of resistant virus within a group reflects the proportion of HIV carriers under antiretroviral treatment, the proportion of treated patients engaged in risky behavior, and factors such as adherence to treatment.

Differences among the 3 groups could have been expected, reflecting differences in behavior and adherence to treatment associated with the diverse demographic characteristics and cultural backgrounds of these patients, but these factors act in a complex manner. For example, depending on the degree of nonadherence to treatment, these patients may or may not carry more resistant viruses than do adherent patients. Therefore, the similarity in transmission of resistance between groups may result from mutual cancellation of opposite effects. Alternatively, it may indicate that cultural and socioeconomic differences may be less confounding than presently thought when analyzing heterogeneous populations. A conclusive interpretation should await a more extensive investigation.

Our estimates of resistance in drug-naive patients in Israel are conservative, incorporating only major and strong minor resistance-conferring mutations. In the Stanford database [24], these mutations are found among drug-naive patients in frequencies of 0 to 0.6% only. Three of the 4 patients with subtype C harboring M46I as the only strong minor resistance mutation were infected abroad, suggesting that this mutation in patients with subtype C might have arisen without drug selection pressure [28]. However, in the Stanford database, the frequency of this mutation in drug-naive patients is <0.2% for patients with either subtype B or subtype C.

We found drug-resistant virus in 26 (14.8%) of 176 recently infected patients. Of those who did not carry resistance-conferring mutations, at least 55% were infected in countries where drug treatment was not available (Ethiopia and the former Soviet Union). Of the 56 patients known to have been infected in Israel, 16 (28.6%) had virus harboring resistance-conferring mutations (major for 10 patients and strong minor for 6; table 3). This is 2.6-fold higher ($P < .001$) than the frequency (11.1%) recently reported in Europe (the CATCH Study) [27, 35]. To what extent the difference is the result of more complete treatment coverage in Israel, a higher rate of risky behavior, and/or partial adherence remains to be determined.

Our finding of drug-resistant viruses in a significant proportion of newly infected patients implies that many HIV-infected persons receiving HAART continue to engage in risk-related behavior. The public health implications of this include the need to intensify programs directed at such patients that address prevention of transmission.

Our results regarding the spread of drug-resistant virus in Israel support the rationale behind the guidelines recommendation for testing of all recently infected patients for drug resistance [20]. Indeed, testing for genotypic resistance before initiation of therapy has been found in a US study to be cost-effective when the rate of primary resistance exceeds 4% [36]. Our data show considerable baseline drug resistance in patients with newly diagnosed HIV infection who were infected in Israel. Pretreatment resistance testing should thus be the standard of care for all persons who were recently infected either in Israel or in other countries where HAART is available to the general public. The primary purposes of such early resistance testing would be to help select appropriate treatment regimens for the individual patient and to keep track of the spread of drug-resistant viruses.

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References

1. Yerly S, Rakik A, Kinloch-de-Loes S, et al. Prevalence of transmission of zidovudine-resistant viruses in Switzerland. l'Etude suisse de cohorte VIH [in French]. Schweiz Med Wochenschr **1996**; 126:1845–8.
2. Brodine SK, Shaffer RA, Starkey MJ, et al. Drug resistance patterns, genetic subtypes, clinical features, and risk factors in military personnel with HIV-1 seroconversion. Ann Intern Med **1999**; 131:502–6.
3. Sugiura W, Matsuda M, Abumi H, et al. Prevalence of drug resistance-related mutations among HIV-1s in Japan. Jpn J Infect Dis **1999**; 52: 21–2.
4. Yerly S, Kaiser L, Race E, Bru JP, Clavel F, Perrin L. Transmission of antiretroviral-drug-resistant HIV-1 variants. Lancet **1999**; 354:729–33.
5. Little SJ, Daar ES, D'Aquila RT, et al. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. JAMA **1999**; 282:1142–9.
6. Puig T, Perez-Olmeda M, Rubio A, et al. Prevalence of genotypic resistance to nucleoside analogues and protease inhibitors in Spain. The ERASE-2 Study Group. AIDS **2000**; 14:727–32.
7. Salomon H, Wainberg MA, Brenner B, et al. Prevalence of HIV-1 resistant to antiretroviral drugs in 81 individuals newly infected by sexual contact or injecting drug use. Investigators of the Quebec Primary Infection Study. AIDS **2000**; 14:F17–23.
8. Weinstock H, Respess R, Heneine W, et al. Prevalence of mutations associated with reduced antiretroviral drug susceptibility among human immunodeficiency virus type 1 seroconverters in the United States, 1993–1998. J Infect Dis **2000**; 182:330–3.
9. Wegner SA, Brodine SK, Mascola JR, et al. Prevalence of genotypic and phenotypic resistance to anti-retroviral drugs in a cohort of therapy-naive HIV-1 infected US military personnel. AIDS **2000**; 14: 1009–15.
10. UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance. Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. BMJ **2001**; 322:1087–8.
11. Tanuri A, Caridea E, Dantas MC, et al. Prevalence of mutations related to HIV-1 antiretroviral resistance in Brazilian patients failing HAART. J Clin Virol **2002**; 25:39–46.
12. Yerly S, Vora S, Rizzardi P, et al. Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. AIDS **2001**; 15: 2287–92.
13. Little SJ, Holte S, Routy JP, et al. Antiretroviral-drug resistance among patients recently infected with HIV. N Engl J Med **2002**; 347:385–94.
14. Hanna GJ, Balaguera HU, Freedberg KA, et al. Drug-selected resistance mutations and non-B subtypes in antiretroviral-naive adults with es-

tablished human immunodeficiency virus infection. J Infect Dis **2003**; 188:986–91.
15. Wensing AM, Boucher CA. Worldwide transmission of drug-resistant HIV. AIDS Rev **2003**; 5:140–55.
16. Wainberg MA, Friedland G. Public health implications of antiretroviral therapy and HIV drug resistance. JAMA **1998**; 279:1977–83.
17. Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. Lancet **1999**; 353:2195–9.
18. Yeni PG, Hammer SM, Carpenter CC, et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. JAMA **2002**; 288:222–35.
19. US Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Rockville, MD: US Department of Health and Human Services, **2003**. Available at: http://aidsinfo.nih.gov/guidelines. Accessed 23 March 2004.
20. Hirsch MS, Brun-Vezinet F, Clotet B, et al. Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society–USA Panel. Clin Infect Dis **2003**; 37:113–28.
21. Chemtob D, Grossman Z. Epidemiology of adult and adolescent HIV infection in Israel—a country of immigration. Int J STD AIDS **2004**;15: 691–6.
22. Statistical abstract of Israel. Jerusalem: The State of Israel, Central Bureau of Statistics, **2001**. Available at: http://www1.cbs.gov.il/.
23. Adler MW. ABC of AIDS—development of the epidemic. BMJ **2001**; 322:1226–9.
24. Kantor R, Machekano R, Gonzales MJ, Dupnik K, Schapiro JM, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database: an expanded data model integrating natural language text and sequence analysis programs. Nucleic Acids Res **2001**; 29:296–9.
25. Kuiken C, Korber B, Shafer RW. HIV sequence databases. AIDS Rev **2003**; 5:52–61.
26. Wu TD, Schiffer CA, Gonzales MJ, et al. Mutation patterns and structural correlates in human immunodeficiency virus type 1 protease following different protease inhibitor treatments. J Virol **2003**; 77:4836–47.
27. Wensing AMJ, van de Vijver DAMC, Asjo B, et al. Prevalence of transmitted drug resistance in Europe is largely influenced by the presence of non-B sequences: analysis of 1400 patients from 16 countries: the CATCH-Study [abstract 117]. Antiviral Ther **2003**; 8:S131.
28. Grossman Z, Vardinon N, Chemtob D, et al. Genotypic variation of HIV-1 reverse transcriptase and protease: comparative analysis of clade C and clade B. AIDS **2001**; 15:1453–60.
29. Pieniazek D, Rayfield M, Hu DJ, et al. Protease sequences from HIV-1 group M subtypes A-H reveal distinct amino acid mutation patterns associated with protease resistance in protease inhibitor–naive individuals worldwide. HIV Variant Working Group. AIDS **2000**; 14:1489–95.
30. Cane PA, de Ruiter A, Rice P, Wiselka M, Fox R, Pillay D. Resistance-associated mutations in the human immunodeficiency virus type 1 subtype C protease gene from treated and untreated patients in the United Kingdom. J Clin Microbiol **2001**; 39:2652–4.
31. Kantor R, Carvalho AP, Wynhoven B, et al. Nucleic acid differences between HIV-1 non-B and B reverse transcriptase and protease sequences at drug resistance positions [abstract 53]. Antiviral Ther **2003**; 8(Suppl 58):S58.
32. Kantor R, Zijenah LS, Shafer RW, et al. HIV-1 subtype C reverse transcriptase and protease genotypes in Zimbabwean patients failing antiretroviral therapy. AIDS Res Hum Retroviruses **2002**; 18:1407–13.
33. Los Alamos HIV sequence database: drug resistance alignments. Los Alamos, NM: Los Alamos National Laboratory, **2004**.
34. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **1994**; 22:4673–80.
35. Wensing AMJ, van de Vijver DAMC, Asjo B, et al. Analysis from more than 1600 newly diagnosed patients with HIV from 17 European countries shows that 10% of the patients carry primary drug resistance: the CATCH-study [abstract LB 1]. In: Proceedings of the 2nd International AIDS Society Conference on HIV Pathogenesis and Treatment (Paris), **2003**.
36. Weinstein MC, Goldie SJ, Losina E, et al. Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. Ann Intern Med **2001**; 134:440–50.