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The report summarizes the first year of contract of the research aimed to define newly recognized high risk population for HTLV-I infection. To define the extent of HTLV-I infection among groups of Jewish immigrants to Israel with an increased frequency of Adult T-cell Leukemia (ATL). Various serological and molecular screening of methods were used, including ELISA and PCR use for molecular survey by amplification of HTLV-I proviral DNA from preipheral blood mononuclear cells DNA. Overall rate of infection if 12% for Jews arriving from Khurusan-North-Eastern Iran. No positive HTLV-I carriers were found from other parts of Iran, Ethiopia, and other Mid-Eastern countries.

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MOLECULAR STUDIES OF HTLV-I INFECTION IN NEWLY RECOGNIZED HIGH RISK POPULATION - MIDTERM REPORT

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INTRODUCTION:

Human retroviruses have been recognized and well characterized during the last decade after the detection of human T-cell leukemia virus type I (HTLV-I) by Poizes and Galo (1) and Yoshida (2) endemic areas were described in southern Japan and parts of the Caribbean and South America (3-7), as well as high risk groups of IVDA (Intravenous Drug Abusers) (8).

The following the discovery of human immunodeficiency virus types 1 (HIV-1) and 2 (HIV-2) and their implication in AIDS accelerated the intensity of research for possible oncogenic viruses.

It was found that leukemic cells in malignancies associated with HTLV-I contained clonically integrated provirus, leading to the direct viral involvement in the oncogenic process, while in contrast, neoplasms frequently seen in the setting of HIV infection - (Kaposis Sarcoma and high grade B-cell Lymphoma) did not appear to arise as a direct consequence of viral transformation of HIV-1 infected cells.

The epidemiological link between HTLV-I and ATLL resulted from two complementary events. The first, an appreciation of Uchiyame and Takatsuki, that ATLL represents a unique clinical entity (9), allowed the geographic localization of the disease to the southern islands of Japan. Epidemiological studies showed that exposure shortly after birth is a major risk factor for subsequent development of HTLV-I associated malignancy (10), while a very small proportion (<5%) of contacts, after a period of twenty or more latent years, develop malignancies, suggesting a multiple step process that may be involved in leukemogenesis, while a viral infection may be a prerequisite, it alone may be insufficient to produce the leukemic phenotype.

These general epidemiologic features of ATLL suggest that systematic reevaluation of appropriate candidate malignancies and viruses may reveal other oncogenic viruses. Careful cataloguing and description of clinical syndromes is essential to derive epidemiological clues that may lead to virus identification. As example: the differentiation of ATLL from mycosis-fungoides is a case in point. While some

of the special features of ATLL, as the enhanced expression of interleukin-2 (IL-2) receptor alpha (IL-2Rα) chain (Tac antigen) on the cell surface, was initially ignored while recognition of the subtler clinical aspects of the syndrome allowed for subsequent epidemiologic observations (9). The HTLV-I model for malignancy as a consequence of viral infection suggests that careful molecular reexamination of the role of already recognized viruses in suspect malignancies is in order. In the case of HTLV-I over one million of infected individuals in Japan give rise to about 500 cases of ATLL per year. Hodgkin's disease may provide another case in point. The bimodal age distribution, the age prevalence, anectodal description of geographic clustering and outbreaks of HD suggest possible viral ethiology (11-13).

The increasingly frequent reports of Epstein Bar Virus (EBV) genome detection in some cases of HD suggest that EBV can be important in the pathogenesis of subsets of HD patients (14), as well as in some ENT malignancies (15).

The example of the last five cases of ATLL-like T-cell Lymphoma in Israel, occurring in Iranian immigrants from the area of north eastern Iran (16), raised the possibility of a new population at risk for HTLV-I infection. This is an example of geographic, familial and ethnic clustering of HTLV-I infection that may yield important clues to viral etiology, in view of conflicting reports about clustering in children less than six years of age (17-19) or reports about geographic clustering associated with high socioeconomic status (20-21), while others question those reports (22-23).

In view of the clustering of ATLL in Jewish newcomers from north eastern Iran (16,24) we have designed this study being a systematic survey of HTLV-I serology in newcoming communities to Israel, with an emphasis on middle eastern communities.

The methodology of the research was based on a general survey of Israeli blood donors. Annually about 250.000 units of blood are donated in Israel, big part of them through the Israel Defence Forces. All data of blood donors is computerized and part of the National Medical Database. This national database is based on the fact that virtually all non-Arab citizens of the country are drafted (25).

Conditions in Israel are favorable for epidemiologic research. The country is small, densely populated, with a special ethnic mixture of the Jewish Community and quite society stable population.

Reliable population registers are available and any individual may be traced by his personal, seven digit, identification number, used ago for administrative and research purposes. Data is available from records of hospital admissions and discharges, as well as from the National Compensatory Health Draft Registry, enabling total population studies on Jewish young adults. Numerous epidemiologic studies, done by our group, have used this database (26-34). In addition to conducting an epidemiologic survey, using serologic methods, two additional aimes of this study are to disparate additional disease entities related to HLTV-I and to conduct a molecular survey of HTLV-I.

Approximately five years following the discovery of HTLV-I it was found that the pathology caused by HTLV-I in one setting may not predict other forms of pathology related to the virus.

A specific illustration is afforded by the two major non-overlapping syndromes associated with HTLV-I. While HTLV-I may cause ATLL, another subset of infected individuals that some of them will develop a slow neurologic disease characterized by a gradual development of spastic paresis of the lower extremities with minimal sensory loss. This illness of tropical spastic paresis or HTLV-I-associated myelopathy (HAM), differentially diagnosed from multiple sclerosis, is connected to the virus. The association between the illness – HAM and HTLV-I - was discovered by Gessian et al (35), while screening for neurologic diseases in Martinique. Regardless of underlying mechanism involvement of HTLV-I, in a slow neurologic disease, is quite surprising. So far, up to the present research, no HAM cases were described in Israel in view of the description of ATLL, an additional aim of our work, as part of the epidemiologic work to screen for possible HTLV-I-associated neurologic disease in Israel.

Serologic survey is backed by western blotting. The demonstration of Seiki and colleagues of the unique coding regions located in the 3' end of the genome provided a first clue as to potential oncologic mechanisms of HTLV-I (36). The 3' ends of the genome of HTLV-I, -II

and the leukemogenic bovine leukemia virus (BLV) all contain coding sequences for at least two overlapping genes (37-40). These genes, known as tax and rex, are now known to encode a transcriptional and post-transcriptional regulator of viral expression. The initial discovery of the tax gene was surprising, in that such transacting transcriptional regulators had only been previously identified in DNA viruses such as herpes simplex virus, varicella-zoster virus, and adenovirus.

The HTLV-I tax gene encodes a 40-kilodalton (kDa) protein, and the HTLV-II tax gene encodes a 37-kDa protein (36-40). These nuclear phosphoproteins are highly homologous, and have similar effects on transcription. Tax expression is necessary for transcriptional activation of virus replication and efficient RNA production from the viral long terminal repeat (LTR); however, an additional property noted early was the ability of Tax to trans-activate other viral and cellular promoters, including those involved in regulation of T-cell growth. Examples of such promoters include those for IL-2, IL-2Rα, and the lymphokine, granulocyte-macrophage colony-stimulating factor (GM-CSF) (41-44). In the case of HTLV-I and -II, Tax appears to interact with three 21-base pair (bp) repeats located in the U3 portion of the viral LTR. Within the sequence of the 21-bp repeats are core sequences known to bind cellular transcription factors (45-46). A variety of these proteins have now been identified and partially characterized.

The aim of this research is also to back the serologic and epidemiological work with molecular screening in addition to western blotting, measuring of antibodies to HTLV-I p40tax, with recombinant p40tax as an antigen.

MIDTERM REPORT:

Blood samples were drawn from Israeli blood donors, through the central blood services of Magen David Adom. Additional sources of samples was the collection of blood through the Association of Iranian immigrants to Israel and through the community services of Iranian and Mashadi Jews in Israel: clinics, elderly homes, schools and synagogues in Bney Barak, Holon, Bat-Yam, Herzlia and Tel-Aviv,

all towns and communities in the central part of Israel.
Additional parts of this survey were samples from patients on long-term hemodialysis, peritoneal dialysis, Adult T-cell Leukemia (ATLL), T-cell malignant disorders, other than Adult T-cell Leukemia, Mycosis fungoides, Sezary's Syndrome Complex, Parapsoriasis, and the collection of blood samples from immigrants from Ethiopia.

During the report period, up to the end of May 1992, we have studied altogether 9658 blood samples including 8960 blood donors (all of them negative for HTLV-I), 480 Iranian Jews of them 212 Mashadi Jews, 102 Ethiopian immigrants, 24 T-cell malignancies, 20 hemodialysis patients, 40 ICPD patients and 32 Mycosis Fungoides/Parapsoriasis patients.

METHODS:

Serologic screening for HTLV-I antibodies in serum or plasma samples was done by means of an enzyme-linked immunosorbent assay-ELISA (Abbott Laboratories). Confirmatory was done by western blotting and/or radioimmunoprecipitation assay - RIPA with Sulphur S^{35}-labeled methionine. HTLV-I-infected HUT 102B lysates were also done (47). Samples, positive in the ELISA, were tested by both confirmatory methods. Antibodies to HTLV-I p40tax were measured by means of an ELISA with recombinant p40 tax as an antigen on solid phase of polystrene beads (Abbott Laboratories Diagnostic Division North Chicago Illinois, USA).

HTLV-I seropositive-infected samples, with a known reactivity against p40tax on RIPA, were used as positive controls (Dr. Rosenblatt's Laboratory Division of Hematology-Oncology Department of Medicine UCLA School of Medicine, Los Angeles California USA) and samples negative for HTLV-I on ELISA and western blot as negative controls. Samples were scored as a positive for p40tax, if the optimal density exceeded 4-5 times the mean negative control value. The polymerase chain reaction - PCR - was used to amplify HTLV-I sequence of DNA from peripheral blood mononuclear cells with primers to a 159bp segment contained with the tax/rex gene as described before (48).

RESULTS:

Out of 9658 blood samples tested for HTLV-I we have found 25 positive for HTLV-I in ELISA and western blot and 12 positive in anti p40tax ELISA. The positive results are detailed in the following tables.

TABLE 1: Prevalence of HTLV-I infection

Patients	HTLV-I & western blot	
	# tested	# Positive (%)
Iranian Jews	480	5.20% (25)*
Mashadi Jews	210	12 % (25)
Ethiopian Jews	107	0 %
T-cell malignancy	24	0%
Haemodialysis	20	0%
CIPD	40	0%

*All positive patients were of Mashadi origin

TABLE 2: Anti p40tax ELISA results

Patients	Anti p40tax ELISA	
	# tested	# Positive (%)
Mashadi Jews	128	12 (9%)
Other Iranian Jews	40	0
Ethiopian Jews	25	0
Haemodialysis	20	0
CIPD	40	0

The extensive social and ethnic ties between Jews of Mashadi origin allowed selective sampling of this population within Israel. On repeated assys, 25 of 210 (11.9%) Mashadi serum samples were positive by ELISA for antibodies to HTLV-I and were confirmed by western

blotting. In contrast, none of the control samples was positive by ELISA (Tables).

In an effort to survey the oldest members of the Mashadi population, we tested 32 unrelated elderly Mashadi women living in an old people's home in Tel-Aviv, 14 (44%) were seropositive, a rate three times higher than that in the general Mashadi population. At our first sample collection, 12 (52%) of 23 long-standing residents of the home were seropositive, 2 of 9 new residents surveyed a year later were seropositive. Of 26 Mashadi women, older than 60 years, who lived elsewhere in Israel only 3 (12%) were seropositive.

To determine whether serologocal assays had detected all infected subjects, we carried out PRC amplification of HTLV-I DNA from 29 of the residents of the old people's home. 19 were positive for HTLV-I sequences: 14 of these were seropositive, 4 were seronegative, and 1 was seronegative by standard ELISA but reactive in the anti-p40tax ELISA. A second PCR on repeat samples from 10 of these women showed concordant results in 6 of 6 PCR-positive and 2 of 3 PCR-negative cases; 1 woman originally positive on PCR was negative on the second sample, and 1 woman originally PCR-negative was faintly positivie on her second sample and had antibodies to p40tax by ELISA. This unusual clustering of HTLV-I infection within the old people's home suggested acquisition of infection within the institution.

In addition to antibodies to virion components, we assayed for antibodies to the non-structural p40tax protein. Of 128 Mashadi samples tested 12 had absorbance levels 4-5 or more times those of the negative control and were judged positive (Tables). 103 samples were negative by both assays, 8 samples were seropositive for both anti-HTLV-I and anti-p40tax; 13 samples were positive for anti-HTLV-I and negative for anti-p40tax; and 4 were positive for anti-p40tax but repeatedly negative for anti-HTLV-I by ELISA and western blot. 3 of the latter group of samples were negative on PCR and the other, from a resident of the old people's home, was positive. All 4 were negative by western blotting and by RIPA. Therefore, the reason for the high titres in three samples in the anti-p40tax ELISA is unclear.

Direct comparison of anti-p40tax ELISA results and those of the RIPA was done for 28 subjects. 8 samples were positive for anti-p40tax by

both RIPA and ELISA; 1 with traces of antibody by RIPA was negative by ELISA; the 3 samples mentioned previously were negative by RIPA but repeatedly positive by ELISA and 16 were negative by both tests. The usefulness of the anti-p40tax ELISA in detecting true additional HTLV-I-infected seronegative individuals remains unclear, since seronegative infection was corroborated by other means of detection in only 1 of the 4 questionable cases.

The finding of a higher rate of seropositivity among elderly Mashadi women, than in the general Mashadi population, suggested a potential for gradual development of seropositivity with age, as has been reported by others. Because of the possibility that serological screening may not detect all positive subjects we carried out HTLV-I-specific PCR on DN from 68 randomly selected Mashadi blood samples. 5 of 7 known seropositive samples were positive in the PCR assay, and 61 sero-negative samples were all negative by PCR. Hence, PCR did not increase sensitivity of detection within this sample above that was achieved with serological assays alone. All PCR-positive samples contained HTLV-I and not HTLV-II, as shown by discriminatory PCR.

We have characterized the Iranian HTLV-I isolate using a combination of Southern blotting, PCR and sequencing. We have developed a primary T-cell line from a Mashadi HTLV-I carrier containing integrated HTLV-I provirus.

DISCUSSION:

We have identified a high risk of HTLV-I infection in Iranian Jews originating from the city of Mashad in Khurusan, northeastern Iran. This group seems to have an overall infection rate of about 12%, though the rate was much higher among residents of a Mashadi home for old people. The reason for this clustering is unclear, although the findings suggest acquisition of infection within the institution. The overall level of infection among other Iranian Jews seems to be substantially lower than that among Mashadis. The explanation may be geographic, and Mashad may be within a previously unrecognised endemic region. Since we did not test non-Jewish Mashadis, this possibility cannot be excluded. In the present situation we will not be able to test this population unless USAMRD, or any other federal agency, may

help us with a proper connection with the Mashad University or any other scientific group in Iran.

The unique history of the Mashadi community may help to explain their high rate of HTLV-I infection. In 1839, the Jewish community in Mashad was subjected to a series of violent attacks and forced to convert to the Islam, though the majority of the community continued to practise Judaism covertly (49). To safeguard the community secret necessitated a very high rate of intermarriage among community members. Members of this community continued to marry close relatives for over the next 150 years. Markers of consanguinity are high among Mashadi Jews - for example, over 20% have a deficiency of glucose-6-phosphate dehydrogenase (50). The chance introduction of HTLV-I infection into the community could have resulted in rapid transmission of the virus by sexual means and by breastfeeding.

It was recently shown by Krivine (51) that there is an retrovirus replication in the first weeks of life. In a prospective longitudinal study of 50 infants, born to HIV-seropositive women, blood samples were obtained at birth at 4-9 weeks, and 5-9 month of age and were tested for HIV RNA by the polymerase chain reaction (PCR), viral culture and p24 antigen measurement. 16 were diagnosed as HIV infected by the age of 4-9 weeks by PCR and culture, while only 10% of the newly born - 5 were detected as HIV-positive at birth.

No changes in HIV infection were detected on both ages of testing for HIV. We intend to perform a similar study in HTLV-I-seropostive women, assuming that perinatal HTLV-I infection could also be demonstrated by PCR or culture , after the first two months of life. Such a work may also give some clues about the ways of transmission of HTLV-I and possibility of HTLV-I-like HIV during the first weeks of life.

An estimated 5000-6000 Mashadis now live in Israel. This is the first Israeli ethnic group identified as having a high rate of HTLV-I infection. Members of the Mashadi Jewish community also migrated to the USA and parts of Europe. We estimate that similar prevalence of HTLV-I infection could be found in other Jewish Mashadi communities. We intend to continue this work during the next year.

In addition, it is estimated that Mashadi and Iranian Jews have moved to the East, to the regions of Uzbekistan, Afganistan, Kazahstan, Armenia and Pakistan (supplement map). We intend to study samples of those newly coming immigrants from Eastern Soviet republics (parts of former USSR). We estimate that at least 20.000 immigrants have arrived to israel from those areas. There was also a settlement of Iranian Jews in China, in the area of Kaifen. But at present we have no possibility to explore this community, that partly assimilated in the general Chinese population.

In this study we use several methods to detect HTLV-I infection. Our rate of anti-p40tax seropositivity in carriers was somewhat lower than that found by others among healthy HTLV-I carriers (50-53), independent evidence for infection using PCR was obtained only in 1 out of 4 samples, thus detection of anti-p40tax antibodies did not appreciably add to the estimate of the rate of infection, and there is no point of its further use in our study.

Our findings on the use of PCR suggest that in a high risk population such as the Mashadi population in the old people's home we have studied, or in families with HTLV-I carriers, PCR would increase the number of detected infected individuals above those that are detected by serological survey.

The results in general population survey, in quite big sample, suggests that there is no point to continue such screening in high risk populations only. The usefulness of PCR as a screening assay in approriate setting requires further study.

The limited number of Ethiopian Jews studied does not confirm previously published results (54) about HTLV-I infection in the Falashi community and further work is needed in this group too. We have not summarized, yet, the data of Mycosis Fungoides and Parapsoriasis patients. Further work is also needed with the study of Mashadi isolates and their sequence as compared to Japanese and other isolates. The successful identification of HTLV-II by vitro of limited nucleic acid homology and antigenic similarity to HTLV-I suggests that variants and new retroviruses can be identified. We have published our initial result in a paper attached to this report (55).

Over a decade following initial identification of the role of the retrovirus in human disease, HTLV-I remains as the only example for human retroviral leukemogenesis. Several viruses can be derived from the HTLV model. First, leukemia may be the rare consequence of infection with a relatively common virus. Second, a long latency period may be involved following acquisition of infection, and the leukemogenic process may involve multiple steps beyond initial infection. Third, the mechanism by which the virus may predispose to development of leukemia may be different from that seen with other retroviruses, and may involve transacting viral genes, either regulators of DNA transcription or regulators of viral mRNA expression. Finally, the role of HTLV-I as an etiologic agent in both HAM and ATLL suggests that viruses, not thought to have oncogenic properties, may on occasion predispose to malignancy. The overall lesson from the HTLV experience is that when suitable or suggestive epidemiologic factors are found, such as ethnic, geographic and/or familial clustering in the absence of a known inheritance pattern, a search for underlying viruses should be initiated. The search would involve removal of malignant tissue, when possible, for culture in vitro and a search using available molecular and serologic probes.

CONCLUSIONS:

During the first year of the project, summarized in this midterm report, we have studied 9658 blood samples for HTLV-I serology and molecular detection of the virus.

We have been successful in identifying a new high risk population for HTLV-I in the Middle East, a population of immigrant Jews from the region of Khurusan - northeastern Iran - and mainly from the town of Mashad.

This special population with isolates of ATLL and HAM gives a special and new insight on HTLV-I epidemiology and clinically, with special concentration on HTLV-I positives in senior population.

Various serological and molecular screening methods, including enzyme-linked immunosorbent assay (ELISA) for anti-HTLV-I, ELISA for an antibody for recombinant HTLV-I p40tax protein, and molecular detec-

tion of infection by a polymerase chain reaction (PCR) amplification of HTLV-I proviral DNA, from peripheral mononuclear cells DNA, were used.

By HTLV-I ELISA the overall rate of infection was 12% among immigrants from Khurusan (northeastern Iran), non HTLV-I carriers were detected in a general survey of the population and other high risk groups, including other Iranian and Ethiopian Jews as well as some clinical condition such as ATLL, othe T-cell malignancies and haemodialysis patients.

We have found an unexplained clustering of HTLV-I infection in a cohort of 32 elderly women of similar geographic origin (Mashad) in a home of senior citizens, 14 were seropositive in ELISA and 19 of 28 were positive by PCR.

These findings and this newly identified high risk population suggests that, in addition to ELISA, other screening techniques may be required to detect all carriers in high risk population.

The study continues in screening of high risk populations and molecular identifications of the new isolates.

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Serological and molecular survey for HTLV-I infection in a high-risk Middle Eastern group

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To define the extent of human T-cell leukaemia virus (HTLV-I) infection among a group of Jewish immigrants to Israel with an increased frequency of adult T-cell leukaemia, various serological and molecular screening methods, including enzyme-linked immunosorbent assay (ELISA) for anti-HTLV-I, ELISA for antibody to recombinant HTLV-I p40*tax* protein, and molecular detection of infection by polymerase chain reaction (PCR) amplification of HTLV-I proviral DNA from peripheral blood mononuclear cell DNA, were used. By HTLV-I ELISA the overall rate of infection was 12% (24 of 208) among immigrants from Khurusan, northeastern Iran; no HTLV-I carriers were detected among 111 unselected Jewish immigrants from other parts of Iran. There was unexplained clustering of HTLV-I infection within a cohort of 32 elderly women of similar geographic origin in a home for old people—14 were seropositive by ELISA and 19 of 29 were positive by PCR. The findings in this newly identified high-risk population suggest that in addition to ELISA, other screening techniques may be required to detect all carriers in high-risk populations.

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Introduction

Human T-cell leukaemia virus type I (HTLV-I) infection has been described in southern Japan, the Caribbean basin, and the northern parts of South America, and in certain high-risk groups, such as intravenous drug abusers in the United States.[1,2] Previous reports of HTLV-I infection among Ethiopian Jews in Israel were not confirmed.[3,4] During the past 4 years, sporadic cases of adult T-cell leukaemia linked to HTLV-I have been reported in Israel[5–11] and 4 of the 5 latest cases were among immigrants to Israel who originated from the city of Mashad in northeastern Iran.[11] Because of these findings, we undertook a systematic survey of Iranian Jews in Israel, focusing on immigrants with links to Mashad.

Subjects and methods

Blood samples from Israeli blood donors of Iranian origin were obtained from the Israeli Magen David Adom Blood Services Center, Tel Aviv. The criterion for classification as an Iranian control was that the country of birth of the blood donor or at least one of his or her parents was Iran. Blood samples were collected on three occasions from residents of a Mashadi home for elderly women in the Tel Aviv area and from three Mashadi community synagogues in the cities of Bnei Brak and Tel Aviv. Samples were classified as Mashadi if the donor or at least one of his or her parents originated from Mashad, Iran. 20 samples from patients on long-term haemodialysis, 8 from patients with T-cell malignant disorders other than adult T-cell leukaemia, and 12 from Ethiopian Jewish immigrants were also included.

Serological screening was done for HTLV-I antibodies on serum or plasma samples by means of an enzyme-linked immunosorbent assay (ELISA; Abbott Laboratories). Confirmatory western blotting and/or radioimmunoprecipitation assay (RIPA) with sulphur-35-labelled methionine HTLV-I-infected HUT 102B

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PREVALENCE OF HTLV-I INFECTION

	HTLV-I ELISA and western blot		Anti-p40*tax* ELISA	
	No tested	No (%) positive	No tested	No (%) positive
Mashadi Jews	208	24 (12%)	127	12 (9%)
Other Iranian Jews	111	0	20	0
Ethiopian Jews	12	0	12	0
Haemodialysis patients	20	0	20	0
Patients with T-cell malignant disorders	8	0	ND	..

lysate were also done.[12] Samples positive in the ELISA were tested by both confirmatory methods. Antibodies to HTLV-I p40*tax* were measured by means of an ELISA with recombinant p40*tax* as antigen on the solid phase (polystyrene beads) (Abbott). HTLV-I seropositive infected samples with known reactivity against p40*tax* on RIPA were used as positive controls, and 4 samples negative for HTLV-I on ELISA and western blot as negative controls. Samples were scored as positive for p40*tax* if the optical density exceeded 4·5 times the mean negative control value. The polymerase chain reaction (PCR) was used to amplify HTLV-I sequences of DNA from peripheral blood mononuclear cells with primers to a 159 bp segment contained within the *tax rex* gene as previously described.[12]

Results

The extensive social and ethnic ties between Jews of Mashadi origin allowed selective sampling of this population within Israel. On repeated assays, 24 of 208 (11·5%) Mashadi serum samples were positive by ELISA for antibodies to HTLV-I and were confirmed by western blotting. In contrast, none of the 151 control samples was positive by ELISA (table).

In an effort to survey the oldest members of the Mashadi population, we tested 32 unrelated elderly Mashadi women living in an old people's home in Tel Aviv. 14 (44%) were seropositive, a rate three times higher than that in the general Mashadi population. At our first sample collection in 1988, 12 (52%) of 23 long-standing residents of the home were seropositive; 2 of 9 new residents surveyed a year later were seropositive. Of 26 Mashadi women older than 60 years who lived elsewhere in Israel only 3 (12%) were seropositive.

To determine whether serological assays had detected all infected subjects, we carried out PCR amplification of HTLV-I DNA from 29 of the residents of the old people's home. 19 were positive for HTLV-I sequences: 14 of these were seropositive, 4 were seronegative, and 1 was seronegative by standard ELISA but reactive in the anti-p40*tax* ELISA. A second PCR on repeat samples from 10 of these women showed concordant results in 6 of 6 PCR-positive and 2 of 3 PCR-negative cases; 1 woman originally positive on PCR was negative on the second sample, and 1 woman originally PCR-negative was faintly positive on her second sample and had antibodies to p40*tax* by ELISA. This unusual clustering of HTLV-I infection within the old people's home suggested acquisition of infection within the institution.

In addition to antibodies to virion components, we assayed for antibodies to the non-structural p40*tax* protein. Of 128 Mashadi samples tested 12 had absorbance levels 4·5 or more times those of the negative control and were judged positive (table). 103 samples were negative by both assays. 8 samples were seropositive for both anti-HTLV-I and anti-p40*tax*; 13 samples were positive for anti-HTLV-I and negative for anti-p40*tax*; and 4 were positive for anti-p40*tax* but repeatedly negative for anti-HTLV-I by ELISA and western blot. 3 of the latter group of samples were negative on PCR, and the other, from a resident of the old people's home, was positive. All 4 were negative by western blotting and by RIPA. Therefore, the reason for the high titres in three samples in the anti-p40*tax* ELISA is unclear.

Direct comparison of anti-p40*tax* ELISA results and those of the RIPA was done for 28 subjects. 8 samples were positive for anti-p40*tax* by both RIPA and ELISA; 1 with traces of antibody by RIPA was negative by ELISA; the 3 samples mentioned previously were negative by RIPA but repeatedly positive by ELISA; and 16 were negative by both tests. The usefulness of the anti-p40*tax* ELISA in detecting true additional HTLV-I-infected seronegative individuals remains unclear, since seronegative infection was corroborated by other means of detection in only 1 of the 4 questionable cases.

The finding of a higher rate of seropositivity among elderly Mashadi women than in the general Mashadi population suggested a potential for gradual development of seropositivity with age, as has been reported by others. Because of the possibility that serological screening may not detect all positive subjects' we carried out HTLV-I-specific PCR on DNA from 68 randomly selected Mashadi blood samples. 5 of 7 known seropositive samples were positive in the PCR assay, and 61 seronegative samples were all negative by PCR. Hence, PCR did not increase sensitivity of detection within this sample above that achieved with serological assays alone. All PCR-positive samples contained HTLV-I and not HTLV-II, as shown by discriminatory PCR (data not shown).

Discussion

We have identified a high risk of HTLV-I infection in Iranian Jews originating from the city of Mashad in Khurusan, northeastern Iran. This group seems to have an overall infection rate of about 12%, though the rate was much higher among residents of a Mashadi home for old people. The reason for this clustering is unclear, although the findings suggest acquisition of infection within the institution. The overall level of infection among other Iranian Jews seems to be substantially lower than that among Mashadis. The explanation may be geographic, and Mashad may be within a previously unrecognised endemic region. Since we did not test non-Jewish Mashadis, this possibility cannot be excluded.

The unique history of the Mashadi community may help to explain their high rate of HTLV-I infection. In 1839, the Jewish community in Mashad was subjected to a series of violent attacks and forced to convert to Islam, though the majority of the community continued to practise Judaism covertly.[13] To safeguard the community secret necessitated a very high rate of intermarriage among community members. Members of this community continued to marry close relatives over the next 150 years. Markers of consanguinity are high among Mashadi Jews—for example, over 20% have a deficiency of glucose-6-phosphate dehydrogenase (D. M., unpublished). The chance introduction of HTLV-I infection into the community could have resulted in rapid transmission of the virus by sexual means and by breastfeeding.

An estimated 5000–6000 Mashadis now live in Israel. This is the first Israeli ethnic group identified as having a high rate of HTLV-I infection. Members of the Mashadi Jewish community also migrated to the USA and parts of

Europe. We predict that the rate of infection among these migrants would be similar to that of the Israeli cohort.

In this study, we used several methods to detect HTLV-I infection. Our rate of anti-p40*tax* seropositivity in carriers was somewhat lower than that found by others among healthy HTLV-I carriers.[7,14,16] Serum samples from 4 subjects were positive only for anti-p40*tax* antibodies. Independent evidence of infection was obtained by PCR in only 1 of the 4. Thus, detection of anti-p40*tax* antibodies did not appreciably add to the estimate of the rate of infection. Our findings on the use of PCR suggested that in a high-risk population, such as the old people's home we studied or in families of HTLV-I carriers, PCR would increase the number of infected individuals above that detected by serological means. The usefulness of PCR as a screening assay in appropriate settings requires further study.

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Quinine-induced disseminated intravascular coagulation

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Recurrent disseminated intravascular coagulation occurred in 3 women after ingestion of quinine tablets for cramp. All had circulating quinine-dependent antibodies to platelets and in 2 there was initial evidence of antibody consumption, with low titres that rose steeply over the next few days and remained high for many months.

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Introduction

Recognised haematological problems associated with ingestion of quinine include thrombocytopenia, erythrocyte haemolysis, and neutropenia. Quinine was first implicated as a cause of purpura in the late 19th century,[1] and there have been several reports of associated thrombocytopenia.[2–5] However, we are aware of only two published cases of disseminated intravascular coagulation induced by quinine,[6,7] and report three further cases.

Patients and methods

Case histories

A 71-year old woman was admitted 5 times over 3 years with various symptoms, which included acute shortness of breath, wheeze, generalised abdominal pain, fever, lower back and chest pain, melaena, haematemesis or haemoptysis, and bruising and petechiae. Most episodes occurred shortly after going to bed. Investigations on each occasion (table) showed evidence of disseminated intravascular coagulation (DIC). On the first 2 admissions she was treated with antibiotics, although blood cultures were always negative. On the third admission she was treated for asthma, and on the last 2 occasions no specific treatment was given. On each occasion, fever and other symptoms resolved within 24 h with a subsequent resolution of coagulation abnormalities. At least 2 similar, but milder, episodes also occurred for which she did not attend hospital. On 3 occasions recent quinine ingestion was clearly remembered by the patient or documented in the admission notes. A check on the number of tablets left in the bottle after her initial prescription indicated that 10 tablets had been taken over 7 years. Retrospective quinine-dependent platelet antibody analysis on samples stored from the last 3 admissions and from intervening periods showed low or undetectable antibody concentrations during the first 2 days of each acute episode, which then rose sharply

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EPIDEMIOLOGIC AND MOLECULAR CHARACTERIZATION OF HTLV-I INFECTION IN ISRAEL

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Human T-cell leukemia viruses type I (HTLV-I) and type II (HTLV-II) are the two human retroviruses directly implicated in pathogenesis of human leukemia. HTLV-I has been linked to adult T-cell leukemia/ lymphoma (ATLL), and HTLV-II to rare cases of chronic T-cell leukemia. Epidemiologic and molecular studies of both viruses have identified several themes underlying the leukemogenic process. Leukemia is a rare consequence of infection, and both viral infection and secondary transforming events appear to be involved. Due to the absence of a virally encoded oncogene, or preferred viral integration sites, novel mechanisms of leukemogenesis must be invoked. We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran (Lancet 336:1533-1535,1990). We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax-rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. An immortalized T-cell line was derived from a Mashadi HTLV-I carrier. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 differrent restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Further characterization of Iranian HTLV-I isolates is underway. A potential role for the HTLV-I/II transregulatory proteins Tax and Rex in leukemogenesis is discussed. Insights derived from the study of HTLV-I/II can be applied to the search for other oncogenic viruses.

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MOLECULAR CHARACTERIZATION OF IRANIAN HTLV-I ISOLATES

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We recently reported a high rate of HTLV-I seropositivity among immigrants to Israel from Mashad in Northeastern Iran (Lancet 336:1533-1535, 1990). We have now characterized the Iranian HTLV-I isolate using a combination of Southern blotting, polymerase chain reaction (PCR) and sequencing. DNA from 10 HTLV-I seropositive Mashadi carriers was isolated from peripheral blood mononuclear cells and amplified by PCR using primers to the tax/rex region. All 10 samples were found to be HTLV-I and not HTLV-II using discriminatory PCR. An immortalized T-cell line was derived from a Mashadi HTLV-I carrier. The cell line contained integrated HTLV-I provirus. Southern blotting and restriction mapping of the Iranian isolate demonstrated marked similarity to published Japanese isolates using 10 different restriction enzymes. Amplification and preliminary sequencing of a 900 bp segment of env from two Mashadi isolates disclosed approximately 95% nucleic acid sequence homology with the published sequence of Japanese isolates, and >98% homology between two Mashadi isolates. These data indicate substantial conservation of sequence between Iranian and Japanese HTLV-I isolates. Further characterization of Iranian HTLV-I isolates is underway. (Supported by the Rashi Foundation, Doron Foundation, ICRF, and a grant from the USAMRDC).

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