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MT-2 Cell Tropism as Prognostic Marker for Disease Progression in Human Immunodeficiency Virus Type 1 Infection

ANDERS KARLSSON,[1,2]* KATARINA PARSMYR,[3] ERIC SANDSTRÖM,[2] EVA MARIA FENYÖ,[1] AND JAN ALBERT[3]

Department of Virology, Karolinska Institute,[1] Department of Clinical Virology, Swedish Institute for Infectious Disease Control,[3] and Department of Dermatovenereology (Venhälsan), Södersjukhuset,[2] Stockholm, Sweden

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The ability of human immunodeficiency virus type 1 (HIV-1) isolates to replicate in MT-2 cells was investigated as a prognostic marker for disease progression and CD4$^+$ lymphocyte depletion in 53 HIV-1-infected, asymptomatic individuals. MT-2-negative viruses were isolated from 49% of the patients both early and late during the follow-up period; 38% converted from being MT-2 negative to MT-2 positive, while 11% were MT-2 positive throughout the study. One individual showed a fluctuating virus phenotype. The loss of CD4$^+$ lymphocytes was significantly more rapid in MT-2-positive patients. We found a broad spectrum of CD4$^+$ lymphocyte changes in patients whose virus changed its MT-2 tropism. Our data suggest that the changes could be divided into three general patterns. A stable or slowly decreasing CD4$^+$ lymphocyte count changed into a more rapid fall in 44% of the patients, no significant change in rate of decline could be noted in 44% of the patients, while a stable CD4$^+$ lymphocyte level after a change in MT-2 tropism was noted in 12% of the patients. A correlation between MT-2 tropism and clinical symptoms was also noted. Half of the patients with MT-2-negative virus throughout the study were still asymptomatic after a mean follow-up time of 80 months, while only 15% of those who converted remained asymptomatic. All patients with MT-2-positive viruses at the time of inclusion in the study developed HIV-1-related symptoms, and half of them died during the study. The MT-2 status of 16 patients could be determined at the time of AIDS diagnosis; 50% were MT-2 positive, while 50% were MT-2 negative. No difference in AIDS-defining diagnoses or CD4$^+$ lymphocyte counts at the time of diagnosis was noted. Knowledge of the HIV-1 phenotype may improve the early recognition of progressive disease.

AIDS was described more than 10 years ago, and the human immunodeficiency virus (HIV) has since been recognized as the etiological agent underlying the immunodeficiency. The immunodeficiency is, at least in part, a result of depletion of the CD4 antigen-positive helper subset of T lymphocytes, but the precise mechanisms by which the CD4$^+$ lymphocytes are destroyed are not well understood.

Biological differences between different HIV type 1 (HIV-1) isolates have been proposed as one important factor in pathogenesis (1, 2, 7, 8, 10, 25, 29). Several studies have demonstrated that in vitro differences in virus replication rate, syncytium-inducing capacity, and ability to infect tumor cell lines correlate with the severity of the immunodeficiency (2, 25, 29).

Because of the lack of simple assays to study these biological properties, different researchers have used slightly different classification systems as well as different terminologies. The replicative capacity of HIV-1 isolates has been the basis of one of the classification systems. Accordingly, viruses which have been called rapid/high (R/H) (2) replicate rapidly in peripheral blood mononuclear cells (PBMCs), produce syncytia in PBMCs, and replicate in CD4$^+$ tumor cell lines. Such viruses are usually found only in patients with advanced immunodeficiency. In contrast, slow/low (S/L) viruses replicate slowly and inefficiently in PBMCs, show a variety of cytopathic effects (cell killing and/or small syncytia), or lack cytopathogenicity in PBMCs altogether. An important characteristic of S/L viruses is that they do not replicate in cell lines or replicate only transiently (11). Such virus variants are found primarily in patients with mild disease but also in a substantial proportion of patients with AIDS (2, 29).

Another classification system is based on the capacity of primary isolates to induce syncytia in PBMC cultures. Accordingly, HIV-1 isolates may be classified as syncytium inducing (SI) or non-syncytium inducing (NSI) (25). Subjects harboring SI viruses have a more rapid course of disease progression than those harboring NSI viruses, and, using these correlates, the biological phenotype has been shown to be an independent predictor of progression to AIDS (14). The presence of SI variants correlates with a rapid CD4$^+$ lymphocyte decline (14, 21, 27). In untreated asymptomatic individuals, conversion from NSI to SI viruses has been reported to be followed usually by a more rapid decline in the number of CD4$^+$ lymphocytes, whereas prior to the conversion, the number of CD4$^+$ lymphocytes and the rate of decline are similar to those in individuals persistently harboring NSI isolates (14, 21, 26).

Recently, a simple assay for biological characterization of HIV-1 isolates, the MT-2 cell assay, has been described (4, 16). In two independent studies, most R/H and SI isolates have been shown to replicate in MT-2 cells, whereas most S/L and NSI isolates lacked this capacity (16, 18). We

* Corresponding author. Mailing address: Department of Dermatovenereology, Södersjukhuset, S-118 83 Stockholm, Sweden. Phone: +46 8 616 25 00. Fax: +46 8 616 25 09.

therefore used the MT-2 cell assay to assess the biological phenotypes of sequentially obtained HIV-1 isolates from 53 individuals and investigated whether the MT-2 cell assay can be used to monitor and predict disease progression and $CD4^+$ lymphocyte depletion in HIV-1-infected individuals.

MATERIALS AND METHODS

Subjects. The subjects were HIV-1-seropositive (initially or subsequently) adult homo- or bisexual men who were part of a cohort to which men have been recruited and prospectively followed since November 1982 at the Gay Men's Health Clinic at South Hospital, Stockholm, Sweden (13). In 1987, a group of 53 asymptomatic men were selected because they were considered likely to remain asymptomatic or show progressive disease. These men have been monitored since then, and blood samples have been collected at regular intervals.

The mean age of the patients at the start of the follow-up was 34 years (range, 23 to 56 years). For 24 (45.3%) of the 53 patients, the dates of the last negative and the first positive HIV-1 antibody tests were known. The median interval between these dates was 13 months. The date of seroconversion was taken as the midpoint between the two dates. The median length of follow-up from the first positive anti-HIV-1 test or the midpoint between the last negative and the first positive test to the end of the study or death was 80 months (range, 35 to 139 months). Thirty-seven (69.8%) patients developed symptoms included in the definition for CDC group IV, of whom 19 (51.4%) developed AIDS. Fifteen (28.3%) patients died during the follow-up period, 13 from complications of AIDS and 2 from unrelated causes (1 from suicide and 1 from lung cancer).

Antiretroviral treatment was given according to the "state of the art" at any given time. No patients were on antiretroviral treatment at the start of the study. Altogether, 38 patients received zidovudine during the study period. The mean time of zidovudine was 25 months. Thirteen of these patients switched to didanosine and were treated for a mean time of 6 months. Ten patients were given intermittent foscarnet treatment for a mean time of 8 months, (seven for cytomegalovirus retinitis and the rest for other serious cytomegalovirus complications). Fifteen (28.3%) patients did not receive any antiretroviral treatment.

Clinical evaluation. Patients were seen at least every third month, and their symptoms were classified according to the system developed by the Centers for Disease Control and Prevention (5). AIDS was defined in accordance with the revised surveillance definition (6).

$CD4^+$ lymphocyte count. A series of T-cell subset determinations was carried out for each patient. The median number of samples collected from an individual and analyzed for the number and percentage of $CD4^+$ lymphocytes was 23 (range, 3 to 42). The T-cell subsets in blood were determined by direct or indirect immunofluorescence by using monoclonal antibodies (Ortho Diagnostics) on an Ortho Spectrum III flow cytometer according to the manufacturer's recommendations.

MT-2 cell assay. HIV-1 was isolated from PBMCs by cocultivation with phytohemagglutinin-stimulated PBMCs from healthy blood donors as described previously (19). Supernatants were harvested and assayed by an in-house HIV-1 antigen enzyme-linked immunosorbent assay (23) every 3 to 4 days. Negative cultures were continued for at least 4 weeks. The time between the start of the culture and the peak of antigen production was calculated.

The virus isolates were then tested for their ability to replicate on MT-2 cells (kindly provided by C. Boucher). Fresh or cryopreserved cell-free supernatants from the PBMC cocultures were inoculated in parallel on 10×10^6 phytohemagglutinin-stimulated PBMCs from two or three different HIV-1-antibody negative blood donors and 5×10^6 MT-2 cells. The cells were incubated at 37°C for 2 h, after which the supernatants were replaced with fresh medium (RPMI 1640; GIBCO, Paisley, Scotland) supplemented with 10% heat-inactivated fetal calf serum (Flow, Irvine, Scotland), 2 mg of polybrene (Sigma, St. Louis, Mo.) per ml, antibiotics, and, for the PBMC cultures, 10 U of recombinant interleukin-2 (Amersham, Buckinghamshire, United Kingdom) per ml.

The cultures were observed for cytopathic changes and syncytium formation twice a week. The virus isolates were classified as MT-2 positive if the MT-2 cultures showed both cytopathic changes and increasing levels of HIV-1 antigen in two consecutive samples. Isolates which did not fulfill the criteria given above but which replicated on PBMCs were classified as MT-2 negative.

The ability to replicate on MT-2 cells was tested for virus from each individual on samples collected on at least two occasions, that is, as early and as late as possible during the study period. The mean time between the two virus isolations was 42 months (range, 9 to 67 months). If the phenotype of the virus isolates showed a change from MT-2 negative to MT-2 positive, additional isolates were characterized in order to pinpoint the time of the change.

Statistical analysis. The rate of change in the $CD4^+$ lymphocyte population was fitted by linear regression analysis. In order to avoid any effects associated with the primary infection, only results obtained at least 6 months after the first HIV-1-seropositive test were included. Repeated counts obtained after the patient's $CD4^+$ lymphocyte count had fallen to less than 20×10^6/liter were not analyzed. In all analyses, both the absolute number of $CD4^+$ lymphocytes and the percentage of $CD4^+$ lymphocytes were used. The correlation between the two was very good (data not shown), and only slight differences in the results were found. Only results obtained by using the absolute number of $CD4^+$ lymphocytes are presented in this report.

A person was considered to carry MT-2-negative virus until the last culture with an MT-2-negative phenotype was observed and to carry MT-2-positive virus from the time the first culture with an MT-2-positive phenotype was observed. When a switch in HIV-1 phenotype was observed, no $CD4^+$ lymphocyte counts between the last culture date with the MT-2-negative phenotype and the first culture date with the MT-2-positive phenotype were included. In this way, linear trends related to the different HIV-1 phenotypes could be calculated for each individual. Differences between the regression coefficients were analyzed by the Student t test. All P values are two-tailed.

RESULTS

Sequential virus isolates from 53 HIV-1-infected homo- or bisexual men were tested for their ability to replicate in MT-2 cells. Virus isolates from 26 patients (49.1%) were MT-2 negative throughout the study, while 6 (11.3%) patients carried MT-2-positive isolates throughout the study. In 20 patients (37.7%), a conversion from MT-2-negative to MT-2-positive isolates was detected. The virus isolates from one individual showed a fluctuating virus phenotype, from MT-2 negative to MT-2 positive (68 months), to MT-2

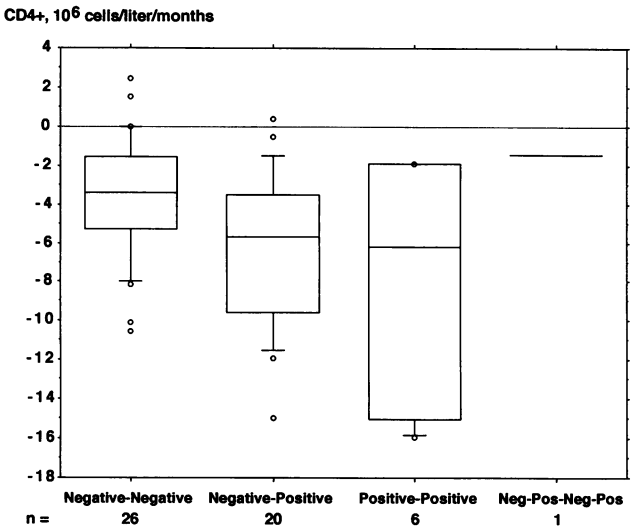


FIG. 1. Box plot showing mean regression coefficients of linear trends fitted through individual CD4+ lymphocyte counts. The MT-2 cell tropisms of viruses from 53 individuals were classified early and late during the follow-up period. The mean time between the two isolations was 42 months. The 10th, 25th, 75th, and 90th percentiles are indicated.

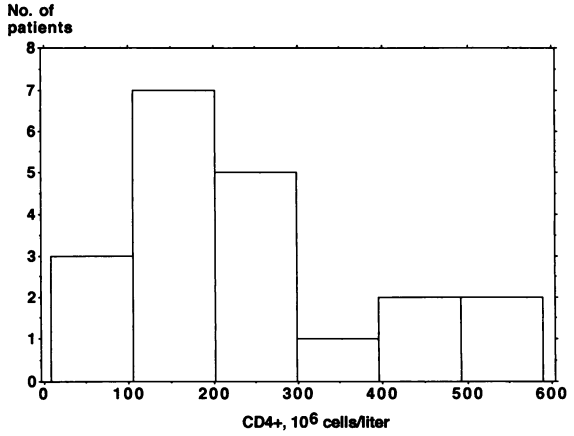


FIG. 2. CD4+ lymphocyte counts at the time of the first MT-2-positive test for the 20 men whose viruses converted from MT-2 negative to MT-2 positive.

negative (100 months), and back to MT-2 positive again (107 months).

The results from the MT-2 cell assay were correlated to changes in CD4+ lymphocyte counts. The rates of these changes were evenly distributed between a decline of 15.9 and an increase of 2.4 × 10^6 cells per liter/month, with a mean decrease of 5.1 × 10^6 cells per liter/month (Fig. 1). Patients with MT-2-negative virus isolates both early and late had a mean decrease of 3.5 × 10^6 cells per liter/month compared with 6.4 cells per liter/month for patients who showed a change in virus phenotype ($P = 0.01$) and 7.9 cells per liter/month for patients with MT-2-positive isolates throughout the study ($P < 0.02$). However, there were large individual differences, especially among the patients who were MT-2 positive at the time of inclusion in the study.

At the end of the study, the mean CD4+ lymphocyte counts of patients who were or became MT-2 positive differed significantly from those who remained MT-2 negative throughout the study. At the baseline, the differences between the groups were less pronounced and all three groups had normal or only moderately depressed CD4+ lymphocyte counts (Table 1). Patients in the group who remained MT-2 negative received less antiretroviral treatment than the patients in the other two groups (Table 1). Thus, the differences in the rate of CD4+ lymphocyte decline might have been even larger if no antiviral treatment had been given.

The events that occurred during the transition from MT-2-negative to MT-2-positive isolates were carefully studied in the 20 patients whose isolates underwent a phenotypic switch. There were considerable differences in the absolute CD4+ lymphocyte counts when conversion occurred, the lowest being 8 × 10^6 cells per liter and the highest being 580 × 10^6 cells per liter, with a median of 200 × 10^6 cells per liter (Fig. 2).

Interestingly, 9 of the 20 patients (45%) whose virus changed from an MT-2-negative to an MT-2-positive phenotype had a transient but significant increase in CD4+ lymphocyte counts between the last negative and the first positive MT-2 isolation. This change is illustrated in Fig. 3 for one representative patient. The mean increase for this period was 95 × 10^6 cells per liter. There were no significant differences between the patients with and without this transient increase in CD4+ lymphocyte counts at the time of the

TABLE 1. Mean CD4+ lymphocyte count at baseline and the end of the study and proportion of patients receiving antiretroviral treatment during the study[a]

MT-2 status	No. of patients	No. of CD4+ lymphocytes (10^6 cells/liter) at:		Antiretroviral treatment (% of patients)		
		Baseline	End of study	Zidovudine	Didanosine	Foscarnet
Neg/Neg	26	489	327	46	15	15
Neg/Pos	20	397	104[b]	100[b]	40	25
Pos/Pos	6	308[c]	57[d]	83[e]	17	17
N/P/N/P	1	460	330	100	0	0

[a] The following four patient groups were studied: patients whose virus was MT-2 negative at the time of entry into the study and that remained so (Neg/Neg), patients whose virus converted from MT-2 negative to MT-2 positive (Neg/Pos), patients whose virus was MT-2 positive at the time of entry into the study and that remained so (Pos/Pos), and the patient with fluctuating MT-2 results (N/P/N/P).

[b] $P \leq 0.001$ compared with Neg/Neg.

[c] $P < 0.05$ compared with Neg/Neg.

[d] $P < 0.01$ compared with Neg/Neg.

[e] One patient, who did not receive any antiretroviral treatment, had MT-2 positive virus isolates for 59 months without developing AIDS.

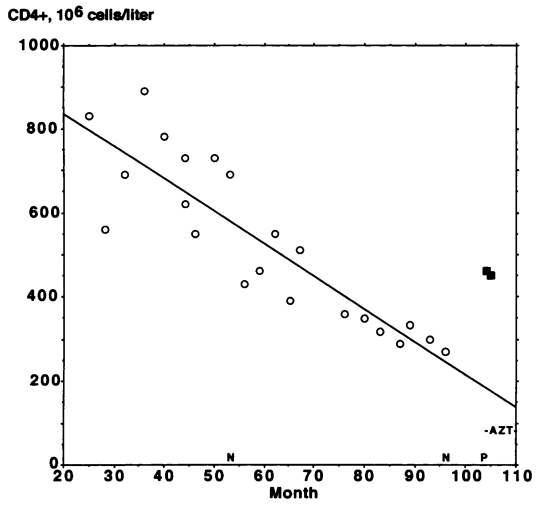


FIG. 3. Increase in the CD4+ lymphocyte count between the last MT-2-negative and first MT-2-positive virus isolations in patient 7. N, MT-2-negative isolation; P, MT-2-positive isolation; ○, CD4+ lymphocyte count while MT-2 negative; ■, CD4+ lymphocyte count while MT-2 positive. The patient did not receive any zidovudine (AZT) treatment before the MT-2 positive isolation and the increase in CD4+ lymphocyte count.

switch regarding the time between the MT-2-negative and the MT-2-positive isolations or the time to the peak of antigen production in PBMC culture. Furthermore, there was no significant difference in the antiretroviral drug treatment histories between the groups. One-third of the patients with increasing CD4+ lymphocyte counts were treated with zidovudine, one-third started zidovudine treatment, and one patient was treated with didanosine during this period. Among the patients with decreasing CD4+ lymphocyte counts, 55% were being treated with zidovudine, while 27% started zidovudine treatment. One patient treated with zidovudine also started treatment with foscarnet during this period.

For the patients whose virus changed its MT-2 tropism, we found a broad spectrum of CD4+ lymphocyte changes. Three types of patterns are exemplified in Fig. 4. (i) A stable or slowly decreasing CD4+ lymphocyte count changed into a more rapid fall in connection with the change from MT-2-negative to MT-2-positive status (Fig. 4a). (ii) No significant change in the rate of CD4+ lymphocyte decrease was found (Fig. 4b). (iii) Stable or even slowly increasing CD4+ lymphocyte levels were observed after MT-2 conversion (Fig. 4c). Of the 16 patients with enough CD4+ lymphocytes for an accurate analysis, 7 patients (44%) showed a doubling in the rate of CD4+ lymphocyte decline (pattern a), 7 patients (44%) with relatively high rates of CD4+ lymphocyte decline showed little or no increase in the rate of decline (pattern b), while 2 patients (12%) showed a stable or increasing CD4+ lymphocyte level (pattern c).

A correlation between the MT-2 cell tropism of the virus and clinical symptoms was also noted. Half of the patients with MT-2-negative virus throughout the study were still asymptomatic after a mean follow-up time of 80 months, while only 15% of those with MT-2 conversion remained asymptomatic. Conversely, all patients with an MT-2-positive virus phenotype at the time of inclusion in the study developed HIV-1-related symptoms, and half died during the study (Table 2). A total of 19 patients (35.8%) developed AIDS during the study. The MT-2 status of three patients could not be determined at the time of the AIDS diagnosis. Of the viruses from the remaining 16 patients, half were MT-2 positive at the time of AIDS diagnosis, while half were MT-2 negative. No difference in AIDS-defining diagnoses or CD4+ lymphocyte counts at the time of diagnosis could be noted between patients with MT-2-negative and MT-2-positive isolates.

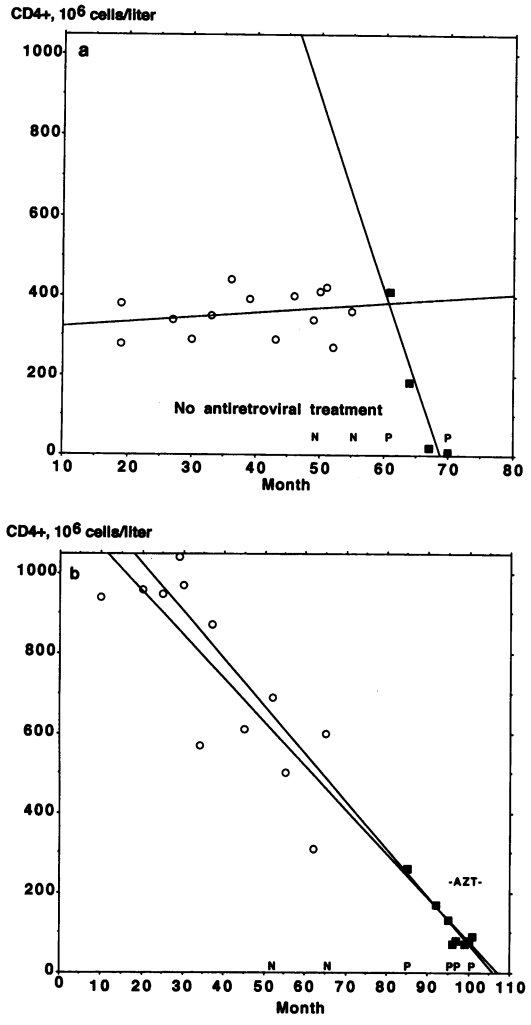
A comparison between the ability of the HIV-1 isolates to replicate in MT-2 cells and the kinetics of replication in the original PBMC isolations was made. The mean time to the peak of antigen production was 11.3 ± 4.6 days for the MT-2-positive virus isolates, while it was 15.1 ± 5.8 days for the MT-2-negative isolates ($P < 0.0001$).

DISCUSSION

In the present study, we investigated whether patients carrying MT-2-positive and MT-2-negative HIV-1 strains differ in terms of clinical stage, rate of disease progression, and rate of CD4+ lymphocyte depletion. The MT-2 cell assay was described by Koot et al. (16) and Boucher et al. (4) as a simple and convenient method of determining the biological phenotype of HIV-1 isolates. Like those investigators, we found a correlation between MT-2 tropism and disease progression. However, clinical deterioration in individuals with MT-2-negative virus is not uncommon. Koot et al. (16) found that 36% of the patients who developed AIDS had MT-2-negative virus, while Boucher et al. (4) found that proportion to be only 11%. In our study, 50% of the patients who developed AIDS carried MT-2-negative isolates. Similar results have been reported for SI isolates (25). On the other hand, there are also important differences between our study and other studies investigating the relationship between biological phenotype and disease progression. Tersmette et al. (26) reported that conversion to an SI virus phenotype is always followed by a rapid decline in CD4+ lymphocytes, and Koot et al. (14) reported that a phenotype conversion was followed by a threefold increase in the rate of CD4+ lymphocyte decline. In contrast, we found a much broader spectrum of events, with large individual differences. Some individuals experienced an immunological disaster in connection with the change from MT-2-negative to MT-2-positive status, while others showed little or no increase in the rate of CD4+ lymphocyte decline. In a few individuals, there were even slight improvements in CD4+ lymphocyte counts following the change in MT-2 tropism.

At variance with earlier studies, our findings demonstrate that the phenotypic switch can occur at all stages of immune depletion. The lowest CD4+ lymphocyte count at which the change occurred was 8×10^6 cells per liter and the highest was 580×10^6 cells per liter. In a study by Boucher et al. (4), all eight individuals who converted to isolates with the SI phenotype had relatively intact immune systems and the lowest CD4+ lymphocyte count at which the switch occurred was 270×10^6 cells per liter. In the study by Koot et al. (14), the mean CD4+ lymphocyte count at the time of phenotypic conversion was 480×10^6 cells/liter.

Koot et al. (14) found that patients who develop AIDS caused by SI variants had lower CD4+ lymphocyte counts at the time of diagnosis than persons who progressed to AIDS caused by NSI variants. In contrast, we found no significant difference ($P = 0.10$) in the CD4+ lymphocyte counts between the two groups. One possible explanation for the difference could be the limited size of the two groups in our study.



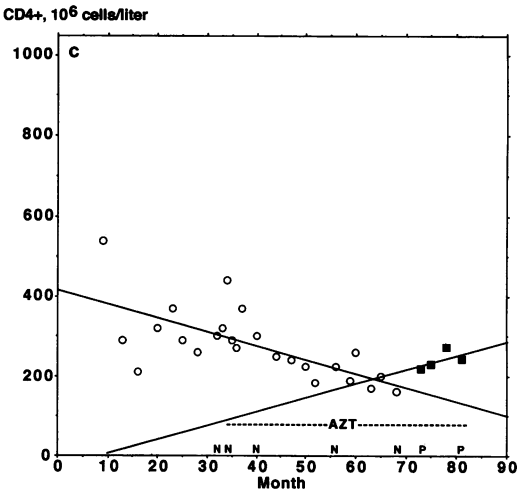


FIG. 4. Three examples of individual patients representing different patterns of MT-2 tropism and $CD4^+$ lymphocyte count change. The duration and type of antiretroviral treatment are indicated. N, MT-2-negative isolation; P, MT-2-positive isolation; O, $CD4^+$ lymphocyte count while MT-2 negative; ■, $CD4^+$ lymphocyte count while MT-2 positive. (a) Pattern 1, a stable or slowly decreasing $CD4^+$ lymphocyte count changes into a more rapid fall in connection with the change in MT-2 tropism (patient 28). (b) Pattern 2, no significant change in the rate of $CD4^+$ lymphocyte decrease (patient 3). (c) Pattern 3, a stable, or even slowly increasing $CD4^+$ lymphocyte level after MT-2 conversion (patient 37).

It has been clearly shown in this and earlier studies that changes in the biological phenotype of the infecting HIV-1 isolates correlate with disease stage. It is not clear, however, whether the changes in replicative capacity, cytopathogenicity, and cell tropism are the cause or the consequence of the deterioration of the immune system. The strong correlation between virus phenotype and disease progression suggests a direct role for the virus in the pathogenic process. However, we still need to explain why some patients deteriorate and develop AIDS without having MT-2 tropic virus, why some patients appear to show immunological improvement after the phenotypic switch, and why the phenotypic switch occurs over such a broad range of $CD4^+$ lymphocyte levels. One explanation could be that the HIV-1 isolates from blood are not always representative of the viruses present and acting in other tissue compartments (9, 17).

In our study, 45% of the patients had an increase in their $CD4^+$ lymphocyte counts during the transition of their infecting virus from an MT-2-negative to an MT-2-positive phenotype. This finding is intriguing and suggests that there may be an immunological activation at the time of conversion. Again, it is difficult to determine whether the increase in $CD4^+$ lymphocytes is a cause or a consequence of the phenotypic switch.

There is evidence that the biological properties of different clones within a bulk isolate differ and that the phenotype of an isolate obtained by PBMC coculture is determined by the phenotype of the most replication-competent clone in that bulk isolate (12, 28). Coexisting clones may interact and thereby influence the biological phenotype of a viral population (24). In our study, the virus isolates from one individual showed a fluctuating phenotype. This fluctuation indicates that the expression of phenotype is gradual rather than absolute and may be influenced by factors in the immune system.

In the present study, there was a strong correlation between the ability of an isolate to replicate in MT-2 cells and the time to peak antigen production in the primary PBMC coculture. This is in line with the findings of Koot et al. (16), who reported that MT-2 cell tropism is very closely related to other methods that discriminate NSI and SI isolates. Similarly, we have observed (18) that almost all

TABLE 2. Clinical status at the end of the study in relation to the ability of virus isolates to grow on MT-2 cells[a]

Patient status	No. (%) of patients whose virus had the following MT-2 tropism[b]:		
	Neg/Neg	Neg/Pos	Pos/Pos
Asymptomatic	13 (50)	3 (15)	0
CDC group IVa, b, c-2	8 (31)	7 (35)	2
AIDS	1 (4)	4 (20)	1
Dead (of AIDS)	4 (15)	6 (30)	3
Total	26 (100)	20 (100)	6

[a] One patient whose virus had fluctuating MT-2 tropism developed CDC group IV c-2 symptoms, but data for that patient are not included here.

[b] See footnote *a* of Table 1 for definitions of abbreviations defining the virus phenotypes.

isolates classified as R/H replicate in MT-2 cells, which is in contrast to the case for S/L isolates. This indicates that the MT-2 assay is indeed a sensitive and convenient method of distinguishing R/H, SI isolates from S/L, NSI isolates.

The use of the MT-2 cell assay for the biological phenotyping of HIV-1 isolates has many advantages over the cumbersome characterization performed in PBMCs. It is easy to perform, it is reliable with a high degree of sensitivity and specificity, and the correlation to the replication rate in PBMC culture is very good. It can be used for routine testing of large numbers of infected individuals. Knowledge of the HIV-1 phenotype may, together with other virological and immunological markers, improve the early recognition of progressive disease. The occurrence of an MT-2-positive virus isolate in an HIV-1-infected individual should always be an indication for closer surveillance of the patient and should raise the question of antiretroviral treatment.

It has been suggested that the HIV-1 phenotype may influence the effect of antiretroviral treatment. Recent data suggest that MT-2-positive HIV-1 isolates respond less well to zidovudine treatment than MT-2-negative isolates (3, 15, 20). This may be due in part to a faster development of high-level zidovudine resistance in patients infected with MT-2-positive virus (4, 17). Indeed, the biological phenotype of HIV-1 appears to be a stronger risk factor for disease progression than does the development of drug resistance (22). Furthermore, the conversion from MT-2-negative to MT-2-positive phenotype appears not to be influenced by zidovudine treatment (4, 15, 20). Together, these data suggest that there is an intricate relationship between the biological phenotype of HIV-1, as determined by the MT-2 assay, and the response to antiretroviral treatment. This must be taken into account when clinical trials of antiretroviral treatment are designed and the usefulness of the MT-2 cocultivation assay as a marker for efficacy must be investigated.

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REFERENCES

1. **Anand, R., F. Siegal, C. Reed, T. Cheung, S. Forlenza, and J. Moore.** 1987. Non-cytocidal natural variants of human immunodeficiency virus isolated from AIDS patients with neurological disorders. Lancet **ii**:234–238.
2. **Åsjö, B., L. Morfeldt-Månsson, J. Albert, G. Biberfeld, A. Karlsson, K. Lidman, and E. M. Fenyö.** 1986. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet **ii**:660–662.
3. **Boucher, C., J. Lange, F. Miedema, G. Weverling, B. Larder, and M. Tersmette.** 1992. HIV-1 phenotype rather than high level AZT resistance is associated with rapid clinical progression of asymptomatic treated patients, abstr. PoB 3570, p. B182. VIII Int. Conf. AIDS, Amsterdam, The Netherlands.
4. **Boucher, C. A. B., J. M. A. Lange, F. F. Miedema, G. J. Weverling, M. Koot, J. W. Mulder, J. Goudsmit, P. Kellam, B. A. Larder, and M. Tersmette.** 1992. HIV-1 biological phenotype and the development of zidovudine resistance in relation to disease progression in asymptomatic individuals during treatment. AIDS **6**:1259–1264.
5. **Centers for Disease Control.** 1986. Classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infections. Morbid. Mortal. Weekly Rep. **35**:334–339.
6. **Centers for Disease Control.** 1987. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. Morbid. Mortal. Weekly Rep. **36**(Suppl. 1S):1–15.
7. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biologic features of HIV-1 that correlate with virulence in the host. Science **240**:80–82.
8. **Dahl, K., K. Martin, and G. Miller.** 1987. Differences among human immunodeficiency virus strains in their capacities to induce cytolysis or persistent infection of a lymphoblastoid cell line immortalized by Epstein-Barr virus. J. Virol. **61**:1602–1609.
9. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature (London) **362**:359–362.
10. **Evans, L. A., T. M. McHugh, D. P. Stites, and J. A. Levy.** 1987. Differential ability of human immunodeficiency virus isolates to productively infect human cells. J. Immunol. **138**:3415–3418.
11. **Fenyö, E. M., L. Morfeldt-Månson, F. Chiodi, B. Lind, A. Von Gegerfelt, J. Albert, E. Olausson, and B. Åsjö.** 1988. Distinct replicative and cytopathic characteristics of human immunodeficiency virus isolates. J. Virol. **62**:4414–4419.
12. **Groenink, M., R. A. M. Fouchier, R. E. Y. De Goede, F. De Wolf, R. A. Gruters, H. T. M. Cuypers, H. G. Huisman, and M. Tersmette.** 1991. Phenotypic heterogeneity in a panel of infectious molecular human immunodeficiency virus type 1 clones derived from a single individual. J. Virol. **65**:1968–1975.
13. **Karlsson, A., G. Bratt, G. von Krogh, L. Morfeldt-Månsson, B. Böttiger, and E. Sandström.** 1991. A prospective study of 115 initially asymptomatic HIV positive gay men in Stockholm, Sweden. Scand. J. Infect. Dis. **23**:431–441.
14. **Koot, M., I. P. M. Keet, A. H. V. Vos, R. E. Y. De Goede, M. T. L. Roos, R. A. Coutinho, F. Miedema, P. T. A. Schellekens, and M. Tersmette.** 1993. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of $CD4^+$ cell depletion and progression to AIDS. Ann. Intern. Med. **118**:681–688.
15. **Koot, M., P. T. A. Schellekens, J. W. Mulder, J. M. A. Lange, M. T. L. Roos, R. A. Coutinho, M. Tersmette, and F. Miedema.** 1993. Viral phenotype and T cell reactivity in human immunodeficiency virus type 1-infected asymptomatic men treated with zidovudine. J. Infect. Dis. **168**:733–736.
16. **Koot, M., A. H. V. Vos, R. P. M. Keet, R. E. Y. De Goede, M. W. Dercksen, F. G. Terpstra, R. A. Coutinho, F. Miedema, and M. Tersmette.** 1992. HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay. AIDS **6**:49–54.
17. **Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci.** 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature (London) **362**:355–358.
18. **Scarlatti, G., V. Hodara, P. Rossi, L. Muggiasca, A. Bucceri, J. Albert, and E. M. Fenyö.** 1993. Transmission of human immunodeficiency virus type 1 (HIV-1) from mother to child correlates with viral phenotype. Virology **197**:624–629.
19. **Scarlatti, G., V. Lombardi, A. Plebani, N. Principi, C. Vegni, G. Ferraris, A. Bucceri, E. M. Fenyö, H. Wigzell, P. Rossi, and J. Albert.** 1991. Polymerase chain reaction, virus isolation and antigen assay in HIV-1-antibody-positive mothers and their children. AIDS **5**:1173–1178.
20. **Schellekens, P., M. Roos, M. Koot, J. Lange, J. W. Mulder, M. Tersmette, and F. Miedema.** 1992. Immunological and virological parameters in a double-blind placebo-controlled study of zidovudine (ZDV) treatment in HIV-1 infected asymptomatic homosexual men, abstr. PoB 3478, p. B166. VIII Int. Conf. AIDS, Amsterdam, The Netherlands.
21. **Schellekens, P. T. A., M. Tersmette, M. T. L. Roos, R. P. Keet, F. De Wolf, R. A. Coutinho, and F. Miedema.** 1992. Biphasic rate of $CD4^+$ cell count decline during progression to AIDS correlates with HIV phenotype. AIDS **6**:665–669.
22. **St. Clair, M. H., P. M. Hartigan, J. C. Andrews, M. S. Simberkoff, and J. D. Hamilton.** 1992. Zidovudine resistance,

syncytium-inducing phenotype, and disease progression in matched progressors and nonprogressors, abstr. p. 24. HIV Drug-resistance Workshop, Noordwijk, The Netherlands.

23. **Sundqvist, V. A., J. Albert, E. Ohlsson, J. Hinkula, E. M. Fenyö, and B. Wahren.** 1989. Human immunodeficiency virus type 1 p24 production and antigenic variation in tissue culture of isolates with various growth characteristics. J. Med. Virol. **29**:170–175.
24. **Tan, W., R. Fredriksson, Å. Björndal, P. Balfe, and E. M. Fenyö.** 1993. Cotransfection of HIV-1 molecular clones with restricted cell tropism may yield progeny virus with altered phenotype. AIDS Res. Hum. Retroviruses **9**:321–329.
25. **Tersmette, M., R. E. Y. De Goede, B. J. M. Al, I. N. Winkel, R. A. Gruters, H. T. Cuypers, H. G. Huisman, and F. Miedema.** 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. J. Virol. **62**:2026–2032.
26. **Tersmette, M., R. A. Gruters, F. De Wolf, R. E. Y. De Goede, J. M. A. Lange, P. T. A. Schellekens, J. Goudsmit, H. G. Huisman, and F. Miedema.** 1989. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. J. Virol. **63**:2118–2125.
27. **Tersmette, M., J. M. A. Lange, R. E. Y. De Goede, F. De Wolf, J. K. M. Eeftink-Schattenkerk, P. T. A. Schellekens, R. A. Coutinho, J. G. Huisman, J. Goudsmit, and F. Miedema.** 1989. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet **i**:983–985.
28. **Tersmette, M., and F. Miedema.** 1990. Interactions between HIV and the host immune system in the pathogenesis of AIDS. AIDS **4**(Suppl. 1):S57–S66.
29. **Von Briesen, H., W. B. Becker, K. Henco, E. B. Helm, H. R. Gelderblom, H. D. Brede, and H. Rübsamen-Waigmann.** 1987. Isolation frequency and growth properties of HIV-variants: multiple simultaneous variants in a patient demonstrated by molecular cloning. J. Med. Virol. **23**:51–66.