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Twin Studies Demonstrate a Host Cell Genetic Effect on Productive Human Immunodeficiency Virus Infection of Human Monocytes and Macrophages In Vitro

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Biological and genetic variability is a prominent feature of human immunodeficiency virus (HIV) strains, especially in tropism, syncytium formation, and replicative capacity. To determine whether there were variable host cell effects on HIV replication in monocytes, three different strains of low-passage-number monocytotropic blood isolates of HIV and the laboratory-adapted strain Ba-L were inoculated into panels of adherent monocytes drawn from 44 different donors, and peak extracellular HIV p24 antigen titers were compared. The clinical HIV strains showed patterns of either moderate or low-level replication in most donor monocytes (20 to 4,000 pg/ml). However, within this range there was marked variation in peak titers in most donors. HIV type 1 Ba-L replicated in all donor monocytes to much higher levels with less variability (30 to 40 ng/ml). Furthermore, replication of 21 clinical blood-derived strains of HIV in blood monocytes and monocyte-derived macrophages (MDM) from pairs of identical twins and age-matched unrelated donors (URD) of the same sex were compared. In all of the seven pairs of identical twins, the kinetics of replication (measured by extracellular HIV p24 antigen) of panels of four clinical HIV type 1 isolates in monocytes were similar within pairs. However, marked and significant differences in kinetics of HIV production occurred within 10 of the 12 unrelated donor pairs ($P = 0.0007$). The remaining two URD pairs showed similar kinetic patterns, but only one pair had the same HLA-DR genotype. Similar results were observed with monocytes/MDMs obtained from a second bleed of the same donor. Hence, discordant patterns of HIV replication kinetics between URD monocyte pairs contrasted with concordant patterns in identical twin monocytes. These data strongly suggest a host cell genetic effect on productive viral replication in monocytes and MDMs. So far, no consistent genetic linkage of HIV replication pattern with HLA-DR genotype has been observed.

There is marked variation among humans in susceptibility to human immunodeficiency virus (HIV) infection during the subsequent course of infection, taking from less than 1 to more than 15 years for blood $CD4^+$ cell decline or AIDS to develop, or in the degree of organ involvement, especially when infection directly results in symptoms as in HIV encephalopathy (5, 10, 38). HIV load and route of exposure are the major factors influencing susceptibility to infection (53). Viral factors, including the syncytium-inducing phenotype (28) and viral load (11, 19, 32, 40), also partially determine progression of immunodeficiency and development of clinical disease. In contrast, the role of host genetic factors in susceptibility to infection is less well established, although HLA B51 and HLA B52/44 have been associated with susceptibility and resistance, respectively (14). However, the HLA B8 DR3 haplotype has consistently shown strong associations with more rapid progression to low CD4 counts or disease (6, 20, 31, 49). T-cell receptor genes have also been linked with HIV infection, although a consistent pattern has not yet emerged (12).

In inbred animal models, both viral and host genetic factors have been shown to influence the outcome of viral infection (18). Host genetic factors have been shown to operate through immune restriction of viral replication and to influence the magnitude of viral replication in cells cultured from these animals in vitro (55).

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However, there have been few studies of the influence of the host cell genetics on HIV replication in vitro or in vivo. Using HIV passaged in T-cell lines, Williams and Cloyd (54) showed up to a 1,000-fold variation in susceptibility of mitogen-stimulated peripheral blood mononuclear cells (PBMC) to HIV infection, as determined from the maximum multiplicity of infection (MOI) needed to establish a productive infection. Some donor PBMC were completely resistant to infection at low to moderate MOI. The segregation of the HIV susceptibility pattern within a family suggested a host cell genetic effect. In contrast, Spira and Ho (48) showed only a maximum of 40-fold variation in HIV production from different donor PBMC, using low-passage-number primary HIV type 1 (HIV-1) isolates. Levels of HIV replication in different donor macrophages were not compared. The design of these studies and the differences in donor cell variability between them have created doubts about the importance of host cell genetic effects on HIV replication, which may differ between CD4 lymphocytes and macrophages. Hence, we studied the replication of a number of freshly isolated HIV-1 isolates in blood monocytes from a variety of unrelated donors (URDs) and from identical (ID) twins. The use of ID twins allows complete genetic matching of cells even when host cell genetic effects on the multiple stages of HIV replication may be polygenic. We have previously demonstrated that culture conditions can be standardized to obtain reproducible titers of extracellular (EC) virus produced by monocytes and macrophages isolated from patients on separate occasions (8).

a) HLA DRB1 genotypes in identical twin pairs and unrelated donors.

Pair*	Twin 1	Twin 2
1.	13, -	13, -
2.	4, 15	4, 15
3.	1, 15	1, 15
4.	14, 17	14, 17
5.	1, 7	1, 7
6.	9, 15	9, 15
7.	12, 15	12, 15
	URD 1	URD 2
a.	7, 15	7,15
b.	13,15	7,17

b) Blood groups in identical twin pairs

Pair	ABO	Rh	P	Duffy	Kidd	MNSs	Kell	Lewis
1	O pos	R_1r(CcDe)	P_1(+)	Fy(a neg b pos)	JK(a pos b pos)	SsMN	K1 neg K2 pos	NT
2	AB neg	r''r(cEe)	P_1(++++)	Fy(a neg b pos)	JK(a pos b neg)	sN	K2 pos	Le(a neg b neg)
3	O pos	R_2R(cDEe)	P_1(++++)	Fy(a neg b pos)	JK(a pos b pos)	sN	K2 pos	Le(a pos b neg)
4	O pos	R_1r(CcDe)	p_1(+++)	Fy(a neg b pos)	JK(a pos b pos)	sMN	K2 pos	Le(a neg b neg)
5	AB neg	NT	P_1(-)	Fy(a pos b neg)	JK(a pos b pos)	NT	K1 neg K2 pos	NT
6	O neg	NT	P_1(+)	Fy(a pos b pos)	JK(a pos b pos)	NT	K1 neg K2 pos	NT
7	NT	NT	NT	NT	NT	NT	NT	NT

FIG. 1. Characterization of subjects. pos, positive; neg, negative; NT, not tested.

MATERIALS AND METHODS

Subjects. Seven pairs of ID (33 ± 9-years-old) and two pairs of nonidentical (NID) (40- and 41-year-old) twins obtained from the Australian NHMRC Twin Registry and 12 pairs of age-matched (34 ± 6-year-old) URDs of the same sex were chosen for the study. These subjects donated 150 ml of blood on two or three different occasions.

The identity of twins was confirmed by phenotype (maternal interview), by HLA-DR genotyping, and by erythrocyte phenotyping (Fig. 1). All subjects were HIV, hepatitis B virus surface antigen and anti-hepative C virus negative and free from chronic metabolic, autoimmune, and chronic or intercurrent infectious diseases.

Isolation of monocytes. Blood mononuclear cells from chosen donors were separated over Ficoll-Hypaque (Pharmacia) and resuspended in 5 ml of elutriation medium (Hank's basal salt solution without Ca^{2+} or Mg^{2+}, 0.38% sodium citrate, and 0.4% bovine serum albumin). The cell suspension was separated into eight fractions by countercurrent elutriation in a Beckman J-6M/E centrifuge fitted with a JE-5.0 (with a 4.0-ml/chamber) elutriation rotor (Beckman Instruments) as reported previously (23). Fractions 7 and 8 were harvested, and the purity of monocytes was checked by nonspecific esterase staining. The pooled fraction contained approximately 90% monocytes. Remaining T lymphocytes were depleted by complement-dependent cytotoxicity, using a monoclonal anti-CD3 antibody (anti-OKT3; Ortho Diagnostics) and baby rabbit complement lysis. Resulting population were tested for residual T cells by flow cytometry (Epics Profile II; Coulter Electronics) and anti-CD3 monoclonal antibodies to a different epitope. Ninety-eight to 99% of purified cells were positive for nonspecific esterase, and <1% were positive for T cells (Sigma kit no. 180).

Viruses. Twenty-one first-passage HIV isolates from the blood of Australian patients (three groups: HIV+ immunodeficiency; HIV− immunodeficiency; and AIDS-related complex/AIDS) with asymptomatic HIV infection with (n = 5) or without (n = 10) immunodeficiency (CD4, <500), AIDS-related complex, or AIDS (n = 6) and laboratory-adapted macrophage tropic Ba-L were tested for the ability to replicate in human monocytes from several donors. HIV isolates were passaged once or twice in phytohemagglutinin-stimulated donor PBMC for expansion. These isolates were concentrated by ultracentrifugation (45,000 × g for 18 h) to remove residual contaminating mitogen or cytokines present in the supernatants. The resulting pellets were resuspended to a 1:50 ratio in growth medium.

Multiplicity of infection (MOI). Virus titer was determined by serial dilution in pooled phytohemagglutinin-activated PBMC, and then virus stocks were aliquoted and stored at −70°C.

HIV infection of monocytes and macrophages. A total of 2×10^6 adherent monocytes or macrophages were cultured in 24-well tissue cluster plates (Costar) or in Labtek dual-chamber slides in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 10% heat-inactivated pooled human AB serum. In preliminary experiments, low-passage-number clinical HIV-1 isolates were used to infect purified monocytes adherent to plastic substrate for less than 24 h (monocytes) or for 3 to 5 days (monocyte-derived macrophages [MDMs]). Approximately 10 to 15% (7 of 56) of clinical isolates recently tested in our

laboratory were able to productively infect monocytes, as measured by the production of significant EC HIV (detected by p24 antigen and reverse transcriptase assay) and, in some, the appearance of intracellular HIV DNA by PCR. Considerable variability in replication among these isolates was observed (reference 8 and unpublished observations).

Further experiments were designed to eliminate technical factors as possible causes of this variability. As a result, care was taken to treat all cultures identically, including aliquoting and storage of ultracentrifuged virus (separating from possible cytokine contamination) at −70°C, infection with the same MOI (0.03 50% tissue culture infective dose [$TCID_{50}$] per cell), aliquoting and storage of all culture supernatants at −70°C for standardization, and batching of HIV antigen assays to achieve consistency. In definitive experiments with twins and URDs, replicate wells were inoculated with four different cell-free HIV isolates and were allowed to adsorb to cells for 2 h. The medium was aspirated, the monocytes were washed three times with RPMI 1640, and fresh medium was added (1.5 ml per well).

Sample collections. For kinetic studies of these infected cells, half of the cultured supernatant was collected and replenished with fresh medium every 3 to 4 days. Supernatant samples were stored and batched at −70°C freezer for later assay for HIV-1 p24 antigen.

Detection and quantitation of HIV infection. Supernatant samples were assayed for HIV-1 p24 antigen and quantified by using the manufacturer's standards (HIV antigen assay; Abbott Laboratories, North Chicago, Ill.). The limit of detection of HIV p24 antigen was 9 pg/ml. When the upper limit of 300 pg/ml was exceeded, the samples were diluted with the kit diluent and reassayed.

Comparison of kinetics of HIV (p24 antigen) production in monocyte pairs. Two sets of criteria for concordance or discordance of the kinetic curves of EC HIV-1 p24 antigen were applied prospectively. If the mean p24 antigen levels at the two peaks (or the two latest time points) were insignificantly different (by *t* test) or within 50% of each other, the HIV kinetic curves within a pair of donors were regarded as concordant. If kinetics curves for all four strains tested on the pair were concordant, the kinetic curves of HIV replication for the pair were then classified as concordant. Population means and standard deviations were compared, and significance was determined with the Student *t* test, adjusted for unequal variances.

Cell surface labeling for flow cytometry. Membrane CD4 expression was quantified by immunofluorescence and flow cytometry. Cells were washed and resuspended at 10^6/ml in phosphate-buffered saline (PBS) with 0.1% azide and 1% fetal calf serum (PBSAz). Surface labeling of monocytes was performed by blocking nonspecific binding with a 5-min preincubation of the cell pellet with 50 μl of heat-inactivated human serum; 20 μl of anti-Leu3a (Becton Dickinson, San Jose, Calif.) or immunoglobulin G1 isotype control antibody was then added, and the mixture was incubated at 4°C for 30 min. After two washes in PBSAz, the cell pellet was again resuspended in human serum for 5 min, and 20 μl of rat anti-mouse kappa-phycoerythrin conjugate (Becton Dickinson) was added for a second 30-min incubation at 4°C. The cells were washed in PBSAz twice and resuspended in PBS with 1% paraformaldehyde for flow cytometry.

Flow cytometry analysis. Samples were analyzed on a FACScan flow cytometer (Becton Dickinson) equipped with a 15-mW air-cooled 488-nm argon ion laser. The green fluorescence was collected after passage through a 530 ± 30-nm band pass filter. The red fluorescence was collected after passage through a 585 ± 20-nm band pass filter. Electronic compensation was used among the fluorescence channels to remove residual spectoral overlap. Samples were gated on low-angle (forward scatter) versus 90° angle (side scatter) to exclude debris and clumps and select cell populations of interest. A minimum of 10,000 events were collected for each sample. Immunofluorescence data were displayed on a four-decade log scale (24).

HLD-DR genotyping. All twin pairs and 6 of 11 URDs were HLA typed at the DR locus by PCR-sequence-specific primers (SSP). Briefly, genomic DNA was prepared by the salting-out method, and PCR amplifications were carried out in a Perkin-Elmer 9600 thermal cycler. Amplified products were separated on a 2% (wt/vol) agarose gel, and allele assignments were as described by Olerup and Zetterquist (37).

Erythrocyte phenotyping. Phenotyping was performed by using standard immunohematology techniques with commercial antisera according to the manufacturers' instructions (4).

RESULTS

HIV infection of monocytes from two URDs. Previous kinetic experiments showed extracellular HIV antigen titers to peak between 5 and 10 days postinfection (8). Hence, the culture supernatants were aspirated at 7 days postinfection for EC HIV p24 antigen assays in these preliminary experiments. As shown in Fig. 2A, monocytes from two URDs, D1 and D2, were infected with four different HIV-1 clinical isolates, WM379, WM355, WM373, and WM380. Supernatants were harvested at day 7 postinfection and assayed for EC HIV p24 antigen. Isolate WM373 produced significantly higher levels of EC p24 in monocytes from donor D2 than in donor D1 in all six replicate wells. The other three isolates in donor D2 produced low levels of EC p24. All four isolates in D1 produced low levels of EC p24. To exclude one obvious source of variability, we measured the levels of CD4 antigen by flow cytometry on the monocytes from donors D1 and D2 labeled with a Leu3a monoclonal antibody. There were no significant difference in the CD4 levels between the monocytes from two unrelated donors (data not shown). The whole HIV-monocyte experiment was repeated a month later under identical conditions (Fig. 2B), and the results obtained were very similar to our first set of results. Supernatants were also assayed for reverse transcriptase activity, and results similar to those with HIV antigen were obtained (data not shown).

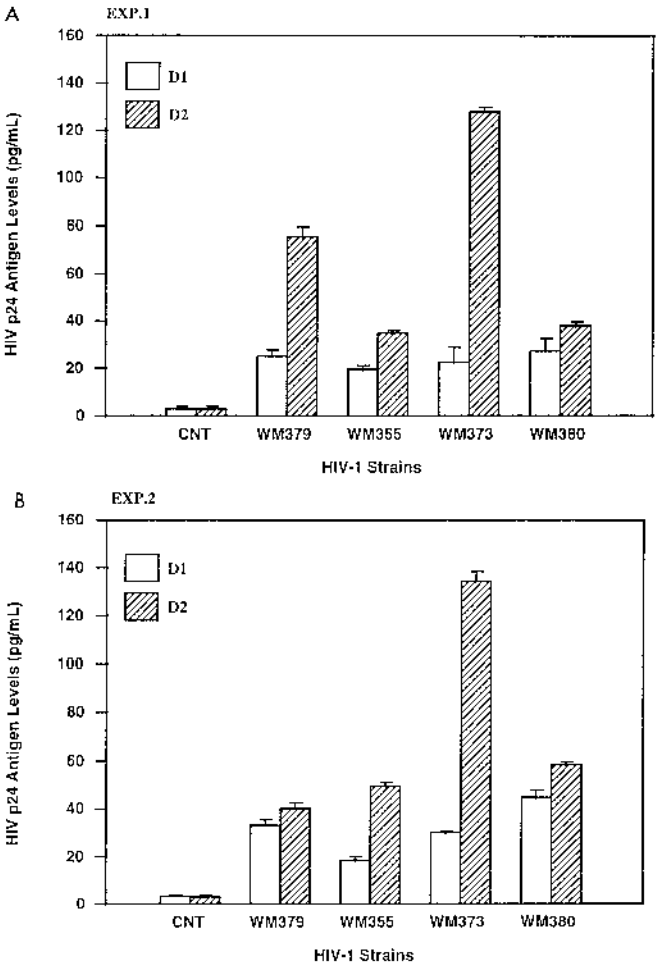

FIG. 2. HIV infection of monocytes from two URDs: effects on HIV production. Monocytes from two URDs, D1 and D2, were infected with four different HIV clinical isolates, WM379, WM355, WM373, and WM380 (MOI of 0.03 $TCID_{50}$ per cell). Culture supernatants were harvested and assayed for HIV p24 antigen 7 days postinfection. Isolate WM373 produced higher levels of EC p24 in monocytes from donor D2 than in those from D1 (A). The experiment was repeated one month later (B), and very similar results were obtained.

Differences in HIV replication in panels of human monocytes. Further confirmation of the foregoing results, which suggested that different host cells have different effects on HIV replication in monocytes, was sought by using a larger panel of donor monocytes and HIV-1 isolates. Monocytes from 11 URDs were infected with a single HIV-1 isolate, WM333;

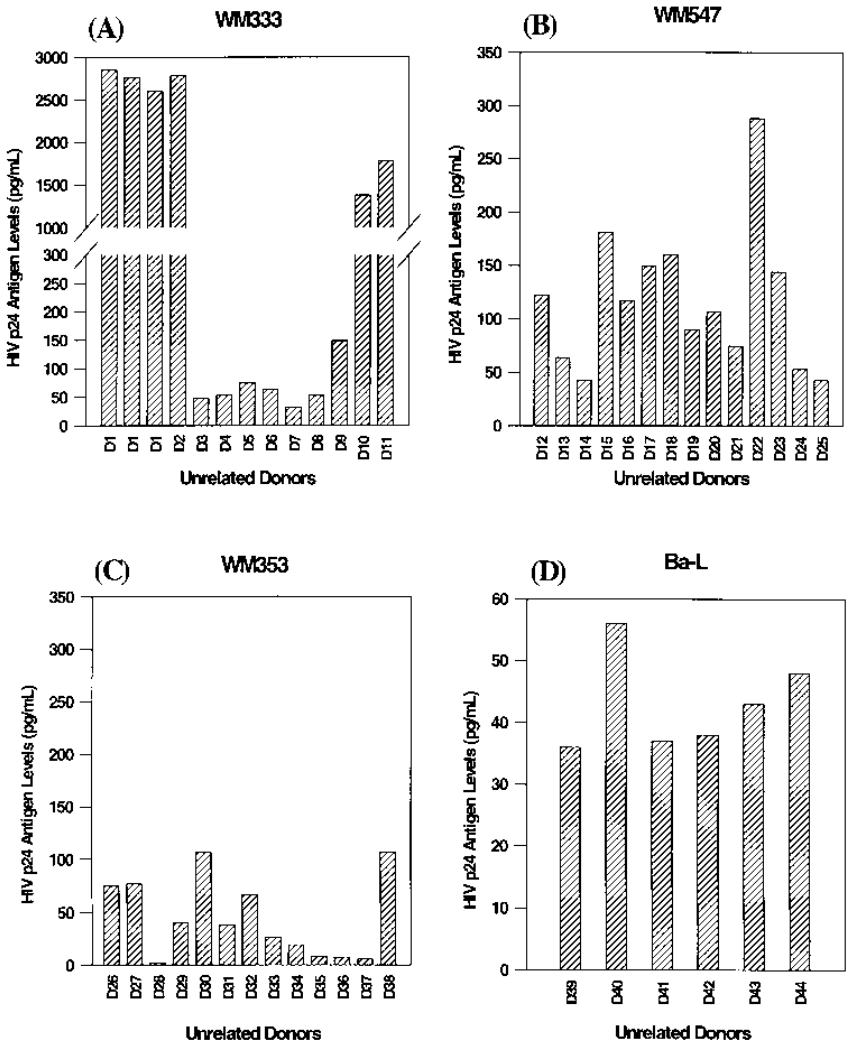

FIG. 3. Monocytes from different donors determine productivity of HIV infection. Forty-four URDs were infected with three different low-passage-number clinical isolates and one laboratory-adapted strain, Ba-L. HIV isolates and their culture supernatants assayed for EC p24 HIV antigen 7 days postinfection. Note the viral strain and donor cell variability. Isolate WM353 always replicated poorly, whereas WM333 and WM547 varied in replicative ability from moderate to high, depending on the host cell, as indicated by EC p24 antigen levels. Standard deviations were all <15% for each p24 antigen concentration shown. Laboratory-adapted strain Ba-L replicated to higher levels in all donors and with less variability. WM333 was isolated from the blood of a patient with AIDS and isolates WM353 and WM547 from HIV-positive patients with a low blood CD4 count.

14 URD monocytes were infected with WM547, 13 URD monocytes were infected with isolate WM353, and an additional 6 URD monocytes were infected with the laboratory-adapted macrophage-tropic isolate Ba-L for comparison (Fig. 3).

In these experiments, the highest levels of WM333 were produced by monocytes from donors 1, 2, 10, and 11. The same isolate replicated to a moderate level in donor 9 and to relatively low levels in the rest of the donors (Fig. 3A). Three separate experiments with isolate WM333 in donor 1 monocytes are shown to demonstrate the reproducibility of these results.

Isolate WM547 replicated to moderate levels in most donors except donor 22, for whom the highest level of EC p24 production was recorded (Fig. 2B). Isolate WM353 replicated to low or negligible levels in all of the URD monocytes tested (Fig. 2C). Hence, isolate WM353 was considered a low-replicating strain compared with isolates WM333 and WM547.

The laboratory-adapted macrophage-tropic strain HIV-1 Ba-L replicated to much higher levels in all patient monocytes and displayed much less variability than the clinical isolates (Fig. 2D).

These results indicate that some clinical isolates (WM353) usually replicate poorly in monocytes, whereas the others vary in productive replication from moderate to high, depending on the host cell.

Kinetics of HIV production by infected monocytes from twins and URDs. In view of the variable host cell effect on HIV replication shown above, we began a formal study of replication kinetics of HIV strains in 7 pairs of ID twins, 12 pairs of URDs, and 2 pairs of NID twins. The hypothesis to be tested was that HIV replication in monocytes is influenced by host cell genetic factors.

Genetics of the twin and URDs. All seven pairs of putative ID twins (classified by maternal interview) were genotyped by PCR-SSP for HLA-DR alleles, and six of seven pairs were serologically typed with a panel of erythrocyte blood groups. (Patient compliance for three large venesections prevented

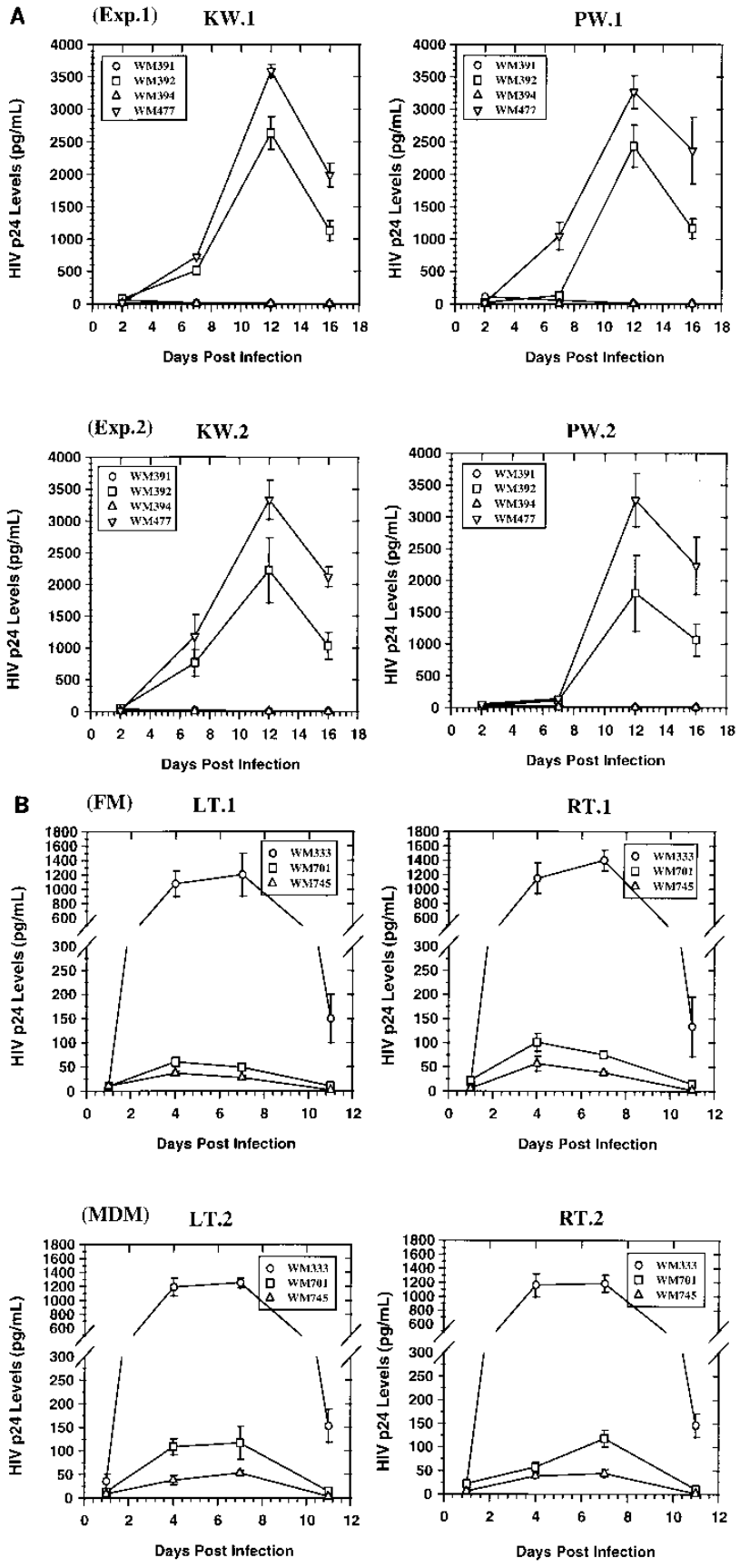
A
(Exp.1)
KW.1
PW.1
(Exp.2)
KW.2
PW.2
B
(FM)
LT.1
RT.1
(MDM)
LT.2
RT.2
WM391
WM392
WM394
WM477
WM333
WM701
WM745
HIV p24 Levels (pg/mL)
Days Post Infection

FIG. 4. (A) Kinetics of HIV production in an ID twin monocyte pair (KW1 and PW1). Monocytes from the ID twin pair were inoculated with four HIV clinical strains (MOI of 0.03 $TCID_{50}$ per cell). Supernatants were assayed for EC p24 antigen in sextuplicate at the time points shown. Two HIV strains (WM392 and WM477) grew to high titers, whereas the other two (WM391 and WM394) failed to replicate in these donors. The viral replication kinetic patterns within the ID twin monocyte pairs were similar to each other (experiment 1). Similar congruent kinetics of HIV production within the ID twin pair was observed when repeated a month later (experiment 2). (B) Kinetics of HIV production in monocytes from ID twins LT and RT. Monocytes were infected with three HIV-1 isolates 3 days after their initial adherence as for panel A. A congruent kinetic pattern of viral replication was repeatedly obtained with MDMs from the twin pair.

complete typing of the last twin pair by both methods.) All seven ID twin pairs were identical for HLA-DR alleles, and of the six tested panels of blood group antigens, all were identical (Fig. 1). Six of twelve URD pairs, including the two shown in Fig. 1, were HLA-DR typed.

HIV infection of fresh monocytes and MDMs from ID twins. (i) Monocytes adherent for <24 h. In Fig. 4A, the kinetics of HIV production by an ID twin monocyte pair (KW1 and PW1) is shown. Of the four HIV-1 strains used in this experiment, two strains (WM392 and WM477) grew to high titers and the other two (WM391 and WM394) failed to produce significant levels of EC p24 antigen in both donors. When we compared the kinetic patterns of all four isolates within the ID twin pair monocytes, the patterns were remarkably similar to each other. This experiment was repeated a month later (Fig. 4A, experiment 2), and results similar to those of the first experiment were obtained in similar experimental conditions. Congruent HIV replication kinetic patterns within the ID twin pairs were observed in all of seven ID twin pairs (Fig. 4A and Tables 1 and 2). In each of these experiments, at least one and usually two or three of the infecting strains produced significant levels of HIV-1 EC p24 antigen.

(ii) Comparison of MDMs adherent for 3 days. To determine whether maturation of blood monocytes to macrophages alters the comparative kinetic patterns of HIV production in twin monocytes, the following experiment was performed. Freshly elutriated monocytes from an ID twin pair were cultured for 3 days (allowing these cells to mature into macrophages) prior to infection with HIV. All three strains exhibited congruent HIV replication kinetic patterns in MDMs within the twin pair on two separate occasions (Fig. 4B).

HIV-1 infection of monocytes from URD pairs. Monocytes from 12 URD pairs were infected with four HIV isolates under the same experimental conditions as used for the ID twins. Figure 5A shows monocytes from URDs BK and JS infected with four different HIV-1 isolates (WM373, WM379, WM477, and WM506). The viral replication patterns in monocytes from these two URDs were dissimilar. The monocytes from donor BK supported isolate WM477 replication strongly and isolate WM379 to a moderate level. The other two isolates, WM373 and WM506, showed no evidence of productive infection in BK donor monocytes within 16 days postinfection. In contrast, JS donor monocytes did not support a productive HIV replication with any of the four isolates used within 16 days. Similar results were obtained when the experiment was repeated a month later (Fig. 5B). The monocytes of donor JS which were inoculated with all four HIV strains showed the normal increase in size and maturation over a week and were comparable with uninfected controls. Staining with trypan blue at the termination of the experiment showed 99% viability of all cultures. In further similar experiments, another nine URD pairs showed significant differences in HIV replication kinetics (Tables 2 and 3).

The remaining two URD monocyte pairs, RW and BB (Fig. 6A) and AK and SH (Fig. 6B), unexpectedly showed remarkably similar HIV kinetic patterns within each URD monocyte pair. Furthermore, both AK and SH (URD pair) had the same HLA-DR type (DR2,7). However, the other URD pair (RW and BB) had dissimilar HLA-DR genotypes yet displayed concordant viral kinetic patterns (Fig. 1).

HIV infection of monocytes from NID twin pairs. Monocytes from NID twins (RA and MA) showed a dissimilar viral kinetic patterns within the pair (Fig. 7A), although isolate WM477 was the best-replicating isolate in both donor monocytes. Repeatedly, donor RA supported only the replication of isolate WM477 (to moderate levels). Infection with the other three HIV strains was nonproductive over 14 days. In contrast, isolate WM477 replicated to high titers and isolate WM453 replicated moderately in monocytes from MA, both peaking at day 7 postinfection. The other two isolates (WM391 and WM392) failed to establish a productive replication in donor MA monocytes. Once again, the experiment was repeated a month later (Fig. 7B) and similar results were obtained.

Supernatants from selected experiments in the ID twin,

TABLE 1. Comparison of peak titers of HIV antigen achieved in HIV-infected monocytes from ID twins

Pair	HIV-1 strain	Peak titer of HIV antigen (pg/ml)[a]	
		Twin 1	Twin 2
1	WM379	2,380	2,170
	WM547	70	105
	WM391	40	60
	WM506	10	10
2	WM391	170	160
	WM453	20	30
	WM392	15	15
	WM353	26	30
3	WM370	3,450	3,530
	WM477	185	200
	WM373	70	86
	WM506	60	70
4	WM701	51	55
	WM745	37	32
	WM484	22	20
	WM353	8	8
5	WM379	160	155
	WM477	135	140
	WM353	132	110
	WM506	64	60

[a] There were no significant differences between the pairs according to the criteria described in Materials and Methods.

TABLE 2. Similarity of kinetics of HIV production from infected monocytes of twins and URDs

Source of monocytes	Kinetics of HIV production from infected monocytes (comparisons between pairs)[a]		
	No. concordant	No. discordant	Total
ID twins	7	0	7
URDs	2	10	12
NID twins	0	2	2

[a] Comparison of ID twins and URDs, $P = 0.0007$ (Fisher's exact test).

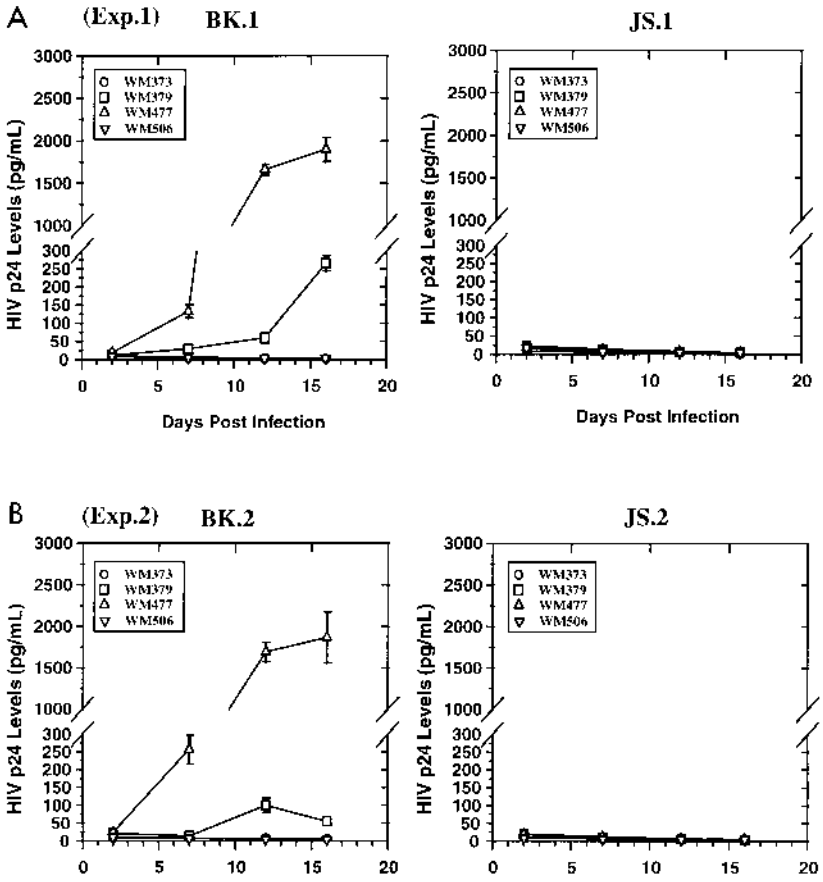

FIG. 5. Kinetics of HIV production in monocytes from an URD pair (BK and JS). (A) Monocytes from donors BK and JS were inoculated with four HIV clinical strains (MOI of 0.03 $TCID_{50}$ per cell), and their cultured supernatants were assayed for EC p24 antigen at the time points shown. The viral replication kinetic pattern with this URD monocyte pair differed considerably. Isolates WM379 and WM477 replicated to moderate or relatively high levels in donor BK monocytes. In contrast, all four isolates failed to grow to significant levels in monocytes from donor JS, although the monocyte cultures were healthy. Similar results were obtained when the experiment was repeated a month later (B).

URD, and NID twin groups were reassayed for reverse transcriptase activity, and similar comparative levels were observed (data not shown). As shown in Table 2, 7 of 7 pairs of ID twins showed concordant kinetic patterns of HIV production, compared with 2 of 12 URDs and none of two NID twins. The differences between ID twins and URDs were highly significant ($P = 0.0007$).

DISCUSSION

Low-passage-number blood isolates from asymptomatic patients or those with AIDS were used to infect blood monocytes within the first day after initial adherence. In some experiments, MDMs adherent for 3 days were used. There has been debate about the susceptibility of monocytes at various stages after initial adherence (47). In our laboratory as in others (8, 22, 50–52), it has been found that a small proportion (10 to 15%) of low-passage-number blood HIV isolates will productively infect blood monocytes in vitro within a day of adherence, although the productivity of infection progressively increases when cells are infected 1 and 2 days after plating (8, 44, 47). In addition, more blood- or tissue-derived low-passage-number HIV strains replicate in 5 day adherent macrophages than in fresh monocytes from the same patient, as detected by PCR for intracellular HIV cDNA and p24 antigen and by reverse transcriptase assays for productive infection (15, 34a). Hence, although selective tropism for both macrophages and monocytes occurs, the latter appears to be even more selective (8, 17). The matching of blood isolates and fresh monocytes in these experiments is also supported by recent reports demonstrating that HIV-infected circulating blood monocytes can be detected in vivo (33, 35, 45) and contain a relatively homogenous population of HIV cDNAs (with non-syncytium-inducing patterns of V3 loop sequences). These HIV-infected monocytes also appear to contribute to the plasma pool of HIV, although this level was variable and less than from blood CD4 T lymphocytes (56).

In this study, we showed that after HIV infection of panels of 44 donor monocytes, different HIV strains differ in overall productivity of infection, with some isolates producing relatively low levels of HIV in monocytes of every donor tested and others producing much higher levels of HIV in most donor monocytes. Nevertheless, there was quite marked variability of the EC HIV levels produced from monocytes of different donors (up to 90-fold [Fig. 3A]). These differences were reproducible, with similar levels of HIV produced by monocytes drawn from the same donor at different times. Hence, in most donor monocytes, individual HIV isolates (e.g., WM333) will replicate moderately well whereas others (e.g., WM353) will grow relatively poorly in all donor monocytes. In contrast, the laboratory-adapted macrophage-tropic strain Ba-L replicated to higher levels in all donor monocytes (and MDMs in unpub-

TABLE 3. Comparison of peak titers of HIV antigen achieved in HIV-infected monocytes from URDs

Pair	HIV-1 strain	Peak titer of HIV antigen (pg/ml)	
		URD1	URD2
1	WM547	112	53[a]
	WM391	70	38[a]
	WM353	43	30
	WM506	20	38[a]
2	WM477	176	870[a]
	WM353	30	60[a]
	WM506	20	8
	WM484	15	8
3	WM547	55	160[a]
	WM391	27	107[a]
	WM353	86	260[a]
	WM484	29	92[a]
4	WM333	1,230	3,870[a]
	WM547	198	65[a]
	WM391	26	15
	WM608	10	12
5	WM477	2,200	3,587[a]
	WM379	30	178[a]
	WM506	30	8[a]
	WM353	8	8
6	WM477	215	1,870[a]
	WM379	60	120[a]
	WM506	52	20[a]
	WM484	8	23
7	WM477	550	120[a]
	WM379	65	48[a]
	WM373	18	25
	WM506	17	18
8	WM333	3,408	110[a]
	WM379	61	90[a]
	WM353	18	36[a]
	WM608	10	20
9	WM547	80	131[a]
	WM562	37	72[a]
	WM624	45	10[a]
	WM661	77	21[a]

[a] Significant differences between pairs: $P < 0.05$, Student t test corrected for unequal variances.

lished experiments) with much less variability than found for clinical isolates. This finding provides further evidence for the greater specificity of the host cell effect for clinical strains in monocyte cultures.

Comparisons of the kinetics of production of four isolates of HIV from infected monocytes of ID twins and URD pairs over a period of 3 weeks allowed these differences to be quantified and tested for significance. The use of ID twins provided donor monocytes which were genetically homogeneous within the pair. This provided the most stringent test for an effect of host cell genetics, as such effects may be polygenic and influence multiple stages of HIV replication. In these experiments, there was also marked variability in the ability of each viral strain to replicate in monocytes of any URD or between (not within) ID twin pairs (up to 400-fold). However, there was remarkable similarity in the kinetics and peak concentrations of HIV EC p24 antigen produced from infected monocytes of ID twins (<2-fold).

Furthermore, this similarity was reproducible at repeat venesection 2 to 4 weeks later. Conversely, in most (10 of 12) of the URDs, the kinetics and concentrations of EC HIV produced from infected monocytes between donor pairs being compared were markedly and significantly different. Similar differences were observed in HIV production between NID twins. Two sets of criteria were applied prospectively to define concordance or discordance of the kinetic curves of the four virus isolates used in each twin or URD pair. In all 7 ID twin pairs, the kinetic curves for each HIV isolate were classified as concordant by two methods, whereas in 10 of 12 URD pairs (or the two NID twin pairs), the kinetic curves were classified as discordant. In 2 of the 12 URD pairs, the kinetic curves were classified as concordant. Interestingly, one of the latter URD pairs had the same HLA-DR genotype (DR7). However, when the HLA-DR genotypes of all the other URDs were considered, no consistent association of any HLA-DR genotype with high or low viral productivity was observed, although surprisingly HLA-DR3 was poorly represented in this mostly caucasian population. The use of different panels of clinical HIV isolates for each pair was necessary because the supply of each isolate was exhausted by the need for a large number of replicates of low passage isolated. Nevertheless, one advantage of this approach was to demonstrate consistent results with 23 different strains of HIV. These results strongly suggest a host cell genetic influence on productive HIV infection in blood monocytes and macrophages in addition to the well-described tropism of a proportion of HIV of strains for monocytes and macrophages.

Some of the twin and URD experiments were repeated with MDMs which had been adherent for 3 days (Fig. 4B) or differentiated macrophages adherent for 5 days (data not shown). In these experiments, we observed to patterns similar those in recently adherent monocyte pairs. The kinetics and peak levels of HIV were concordant for ID twins. Hence, the same host cell effect was observed regardless of the state of maturation of monocytes/macrophages.

Although we and others have reported data suggesting that host cell characteristics and culture conditions may influence productive HIV infection in monocytes and macrophages (8, 17), this evidence in twins is the first to demonstrate a clear effect of host cell genetics. However, in HIV infection of T lymphocytes or PBMC, evidence that the predominant infected cell is the CD4 T lymphocyte has been found. A marked difference in host cell specificity for HIV infection of PBMC from different donors was reported as early as 1987 (13). More recently, William and Cloyd (54) reported that marked variability (up to 1,000-fold) in susceptibility of PBMC to HIV strains in vitro segregated within a family and appeared to be related to HLA haplotypes (54). However, Spira and Ho (48), using low-passage-number primary HIV isolates rather than strains passaged in T-cell lines, demonstrated less variability (40-fold) in productivity between donor PBMC and could not initially demonstrate relative resistance to infection of any donor PBMC (at low MOI). In a recently published report, however, Paxton et al. showed relative resistance of some donor CD4 lymphocytes to HIV strains (39a). Furthermore, coculture of a single donor PBMC with three separate donor PBMC produced very similar DNA quasispecies. In our system, variation of EC HIV titers with donor monocytes/macrophages reached a maximum of 400-fold but HIV production was much lower than in mitogen-stimulated lymphocytes. Furthermore, some URD pairs showed high-level HIV production in one donor's monocytes and no production in the other donor's monocytes, which were both viable and healthy cultures. The replication patterns in ID twins provided a control for natural variation in cells of identical genotype.

Although twins who were discordantly infected with HIV have been reported (57), there have been no in vitro studies of HIV infection of cells from twins. In contrast, the role of host

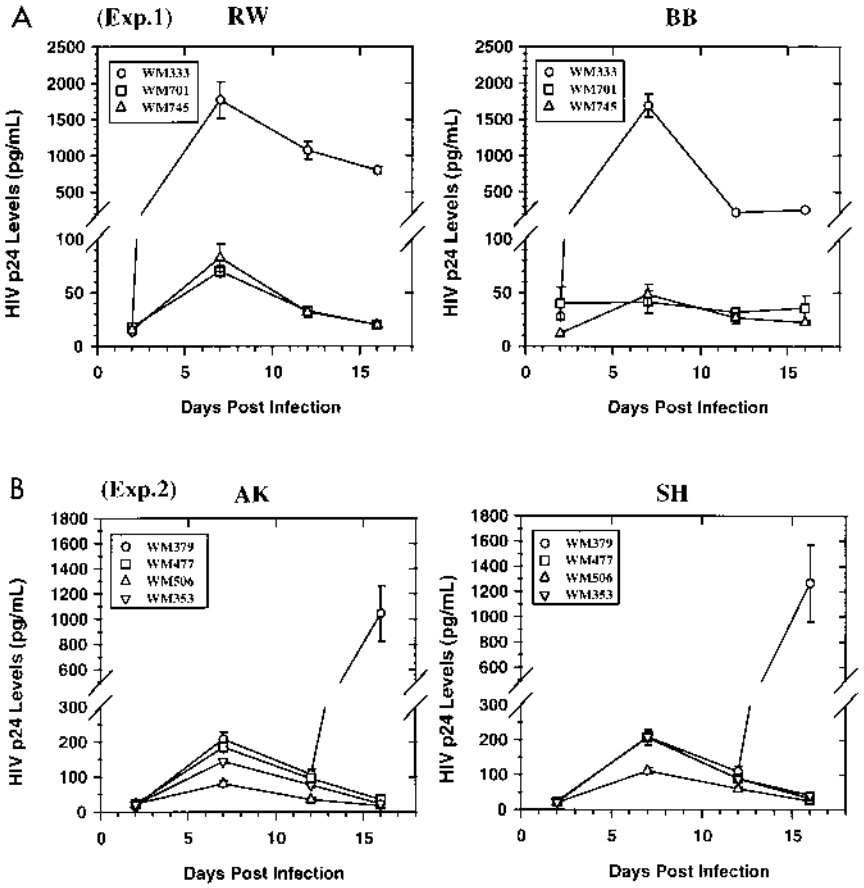

FIG. 6. Two URD monocyte pair supported similar HIV kinetic patterns. Monocytes from two URD pairs (RW and BB [A] and AK and SH [B]) were inoculated with four different HIV clinical strains (MOI of 0.03 $TCID_{50}$ per cell). Cultured supernatants were harvested for EC p24 antigen at the time points shown. Both pairs displayed similar HIV replication kinetic patterns within each URD monocyte pair.

cell genetics in influencing viral replication and productive infection is well documented for a number of viruses both in inbred animals and in the cells derived from these animals. Individual genes such as those in the H-2 complex, which influence, for example, the replication of murine cytomegalovirus in inbred strains of mice (CBA and C57BL) and their explanted cells in culture, have been described (16, 18). Other animal virus systems in which host cell genetics influence viral replication include flaviviruses in mice (41) and herpes simplex virus in the peripheral nervous system and epidermal cells of mice (46). The mechanisms of these genetic effects appear to be diverse. They may be mediated through constitutive differences in viral replication in the host cell, through interferon production, or through immune mechanisms including the competence of natural killer and cytotoxic T cells in the whole animal.

The mechanisms for these differences in productive infection of host cell monocytes (in twin pairs and URDs) are currently being examined in our laboratory. The multiple stages of HIV replication within macrophages could be influenced by cellular processes. However, many reports have suggested that the strongest determinant of HIV tropism for monocytes and macrophages appears to be at virus entry, because of differences in gp120 (and especially the V3 loop) between lymphocyte- and monocyte/macrophage-tropic viruses (9, 36, 40). Most are nonsyncytium inducing with a neutrally charged V3 loop, in contrast to the syncytium-inducing strains tropic for CD4 lymphocytes. Regions of envelope (other than the V3 loop) have also been shown to be important determinants of tropism with some HIV strains (25). However, there is some evidence that other stages of replication may also influence the productivity (not tropism) of HIV infection in monocytes and macrophages, both in vitro and in vivo. Mikovits et al. (33) have recently reported evidence for latent HIV infection of monocytes in vivo, demonstrating the presence of HIV DNA but not RNA in monocytes freshly isolated from asymptomatic seropositive patients. Coculture of these latently infected monocytes with activated T cells resulted in the production of infectious virus. The original studies of Cheng-Mayer et al. (9), defining the V3 loop as the principal determinant for HIV tropism for macrophages, also suggested that other genes may influence the degree of HIV replication in vitro. In addition, Fouchier et al. (17) showed evidence for a restriction on HIV replication in infected monocytes subsequent to the stage of reverse transcription. Mori et al. (34) have also described the presence of mutations in simian immunodeficiency virus outside the envelope region which appeared to influence macrophage tropism of simian immunodeficiency virus. *vpr*, *vif*, and *nef* have recently been shown to be essential for replication in macrophages, although natural variation in these genes affecting such replication has not been documented (2, 3, 21, 30). Hence, ID twins were used to define a host cell genetic effect, as there was potential for a polygenic influence of cellular gene product on HIV replication at vari-

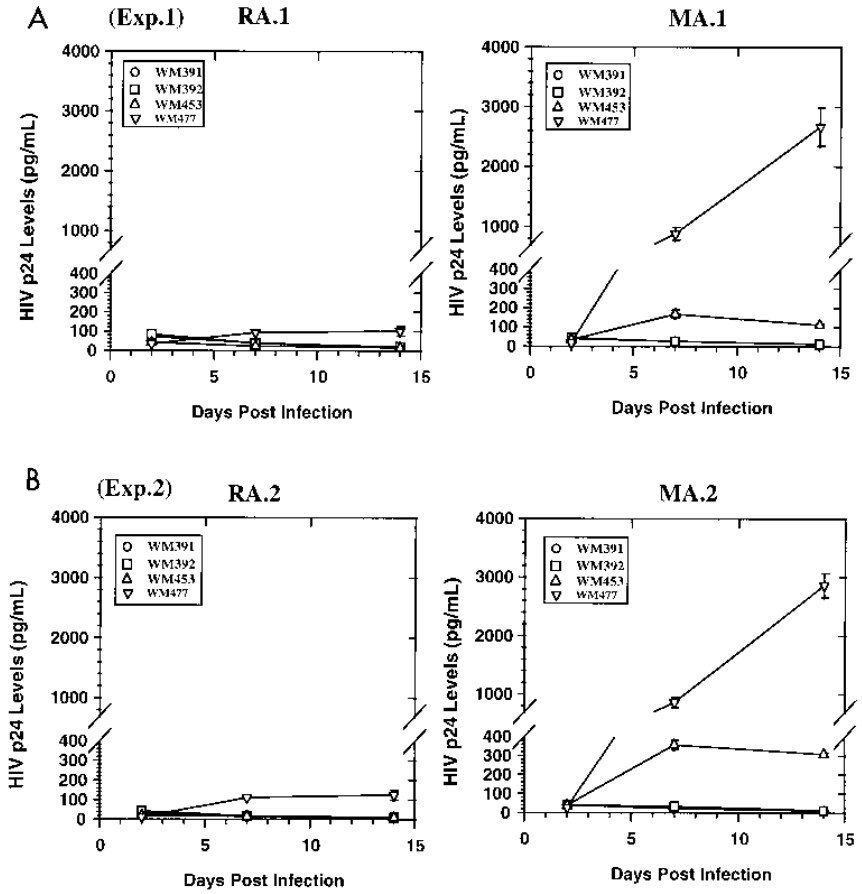

FIG. 7. Kinetics of HIV production within an NID twin monocyte pair. (A) Isolates WM453 and WM477 grew to higher titers in donor MA monocytes than in donor RA monocytes, although the WM477 isolate was the best-replicating isolate in both donors' monocytes. Nevertheless, the kinetic patterns of production of the four strains within NID twin monocyte pair were (significantly) dissimilar. Similar results were obtained when the experiment was repeated a month later under identical conditions (B).

ous stages within the cell. Currently we are examining the relative importance of virus entry and the later stages of HIV replication in determining these genetic effects in monocytes. The influence of host cell genetics on replication of primary strains of HIV in T lymphocytes may also be better resolved in twin studies.

The influence of host cell genetics on HIV replication in monocytes/macrophages in vivo is likely to be important. Macrophages are the predominant infected cells in the brain (27), a reservoir for HIV infection in other tissues (e.g., lung and lymph node) (29), and a major source of virus in genital secretions which is transmitted during sexual intercourse (1). Monocytes are also a minor source of HIV in blood (42, 56). Non-syncytium-inducing macrophage-tropic HIV predominates in the early stages of infection (43). HIV load has recently been shown to be a risk factor for the progression of HIV disease independent of CD4 lymphocyte concentration (32). Hence, individual genetic differences in the ability of tissue reservoir macrophages to support HIV production could strongly influence the progression of HIV disease locally or generally. In recently published studies of long-term nonprogressors, characteristic changes in HIV sequence suggestive of attenuated viruses were identified in only a few (12a, 26, 39). However, the others also had persistently low blood viral loads (7, 39). These patients may be genetically resistant to the late increase in HIV replication, either through constitutive differences in the ability of host cell to support HIV replication or through a more competent immune system (e.g., T-lymphocyte cytotoxicity). Hence, investigation of the mechanism by which host cell genetic effects influence HIV replication could assist in the development of future prognostic tests for patients with a high risk of progression or even define new therapeutic strategies.

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