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The Extent of Early Viral Replication Is a Critical Determinant of the Natural History of Simian Immunodeficiency Virus Infection

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Different patterns of viral replication correlate with the natural history of disease progression in humans and macaques infected with human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV), respectively. However, the viral and host factors influencing these patterns of viral replication in vivo are poorly understood. We intensively studied viral replication in macaques receiving identical inocula of SIV. Marked differences in viral replication patterns were apparent within the first week following inoculation, a time prior to the development of measurable specific immune effector responses to viral antigens. Plasma viral RNA levels measured on day 7 postinoculation correlated with levels measured in the postacute phase of infection. Differences in the susceptibility of host cells from different animals to in vitro SIV infection correlated with the permissiveness of the animals for early in vivo viral replication and hence with the postacute set point level of plasma viremia. These results suggest that host factors that exert their effects prior to full development of specific immune responses are critical in establishing the in vivo viral replication pattern and associated clinical course in subjects infected with SIV and, by extension, with HIV-1.

In primary human immunodeficiency virus type 1 (HIV-1) infection, a peak of plasma viremia is typically followed by a decrease in levels of circulating virus of up to 2 to 3 orders of magnitude over the succeeding few weeks (9, 13, 48, 49). While both cytolytic and noncytolytic cellular and humoral immune responses have all been suggested as potential mechanisms involved in the curtailment of viral replication (5, 28–31, 35, 46, 50, 54, 58, 62), establishment of a direct causal relationship between the onset of any particular immune response to HIV infection and the decrease in levels of circulating virus associated with resolution of primary infection has proved elusive. Indeed, it has been suggested that the empirically observed patterns of changing levels of circulating virus may be obtained in mathematical models that a priori exclude any role for the immune response (47).

While the mechanisms underlying downregulation of levels of circulating virus with resolution of primary infection remain uncertain, several studies have clearly demonstrated that in most infected subjects, following resolution of acute primary infection, plasma virus levels equilibrate and remain relatively stable over the short term. The level at which plasma viremia stabilizes in the postacute infection period is believed to be a reflection of the overall quasi-steady-state levels of ongoing in vivo viral replication and clearance (12, 26, 48, 60) and has been shown to be an important prognostic determinant. Higher levels of plasma viremia at 6 to 12 months following primary HIV-1 infection are associated with a significantly increased relative risk for more rapid disease progression (32, 33, 39, 56). Although the prognostic significance of this viral load set point is broadly acknowledged, the mechanisms responsible for establishing the level observed in a given individual and the significance of different viral and host factors in determining this level are poorly understood. However, follow-up observations involving individuals infected with essentially the same viral quasispecies demonstrate widely differing courses and outcomes, clearly indicating the potential for host factors to exert a major influence on clinical course (34, 40).

Studies with simian immunodeficiency virus (SIV)-infected macaques have also documented extensive and continuous viral replication throughout the course of infection, as well as correlations between different patterns of viral replication and clinical course (2, 7, 24, 25, 59). In contrast to the study of acute HIV infection in human subjects, in experimental SIV infection, the investigator can precisely control the inoculum used and the timing, amount, and route of inoculation. Multiple specimens can be obtained early in infection at defined times following inoculation, making the SIV-infected macaque model especially well suited for studies aimed at understanding the viral and host determinants that contribute to establishment of different, prognostically significant patterns of viral replication. We used a sensitive quantitative competitive (QC)-reverse transcription (RT)-PCR-based assay for monitoring SIV RNA in longitudinal plasma specimens to characterize the viral dynamics of early primary SIV infection, in an effort to identify patterns that might correlate with and predict the

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postacute level of viral replication, which is itself correlated with disease course.

MATERIALS AND METHODS

Virus stocks. Cell-free stocks of SIVsmE660 (19) and SIVsmE543-3 (23) were prepared in phytohemagglutinin A (PHA)-activated peripheral blood mononuclear cells (PBMC) from a *Macaca nemestrina* donor.

Inoculation of macaques. Four juvenile *Macaca mulatta* (body weight, 4 to 6 kg at the time of inoculation) and 12 *M. nemestrina* (body weight, 3 to 4 kg at the time of inoculation) macaques were inoculated intravenously (i.v.) with 50 50% monkey infective doses of a cell-free stock of SIVsmE660, the titer of which was determined in *M. nemestrina* macaques (19). Four additional juvenile *M. mulatta* (4 to 6 kg at the time of inoculation) macaques were inoculated with 1,000 50% tissue culture infective doses ($TCID_{50}$) of a cell-free stock of SIVsmE543-3 (23) (the titer of which was determined on PHA-activated PBMC from *M. nemestrina*). All animal care was in accordance with the institutional guidelines of the National Institute of Allergy and Infectious Diseases.

QC-RT-PCR analysis of plasma viral RNA levels. QC-RT-PCR analysis of plasma virion-associated RNA was performed essentially as described in detail elsewhere (24), with serial dilutions containing known amounts of in vitro transcripts derived from the internally deleted SIV *gag* construct pSGΔ83 as an internal control. Acid citrate dextrose-A-anticoagulated plasma specimens were extracted with commercial reagents (Purescript; Gentra Systems, Minneapolis, Minn.). Randomly primed reverse transcription was performed (42°C, 30 min) as described previously (24), and a highly conserved region of the SIV *gag* region was amplified from the resulting cDNA (45 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 1 min), with the primers S-GAG03 (5′-CAGGGAAiiiAAGC AGATGAATTAG-3′, where i indicates inosine) and S-GAG04 (5′-GTTTCAC TTTCTCTTCTGCGTG-3′). The products were quantified by computer-assisted video image analysis of ethidium bromide-stained gels, and the calculated input template copy number for the test sample was determined by interpolation on a log/log plot of input internal control template copy number versus the ratio of amplified product for internal control/test sample, as described elsewhere (24, 48). Interassay variation was <25% (coefficient of variation [CV]).

In situ hybridization (ISH) analysis. ISH was performed on formalin-fixed, paraffin-embedded tissues essentially as described elsewhere (25), with digoxigenin-labeled antisense RNA probes generated by SP6 or T7 polymerase transcription from 1- to 2-kb subclones spanning the entire SIVmac239 genome. Lack of signal with sense probes indicated the specificity of hybridization. Tissues were hybridized with 1.75 ng of riboprobe/ml at 52°C overnight, washed sequentially in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–50% formamide and 2× SSC, and treated for 30 min at 37°C in an RNase solution (RNase T_1 and RNase A in 2× SSC). Slides were blocked in Tris-NaCl buffer (pH 7.4) containing 2% (vol/vol) horse serum, then incubated for 1 h with sheep antidigoxigenin-alkaline phosphatase conjugate, and incubated overnight with nitroblue tetrazolium–5-bromo-4-chloro-3-indoylphosphate toluidinium substrate at room temperature. Slides were counterstained with nuclear fast red, dehydrated, and coverslipped. Sections from inguinal and axillary lymph nodes were examined by light microscopy by an observer unaware of plasma viral load measurements for the animals evaluated. The number of SIV-expressing cells per high-power field (40× objective) was determined for 12 independent fields and averaged to obtain a semiquantitative estimate of the relative frequency of productively infected cells. Due to the relative lack of sensitivity of the technique, ISH underestimates the total level of viral replication.

Flow cytometric analysis. Cells were stained for analysis on a Becton Dickinson (San Jose, Calif.) FACScan flow cytometer by standard methods and with the following fluorochrome-conjugated monoclonal antibodies: anti-CD4 (OKT4a-fluorescein isothiocyanate [FITC]; Ortho), anti-CD8 (anti-Leu2–FITC; Becton Dickinson), anti-CD2 (T11-phycoerythrin; Ortho), and anti-HLA-DR (I3-FITC), all shown to cross-react in preliminary studies with macaque cells (51a).

Measurements of cytokines. MIP-1α, MIP-1β, and RANTES were measured in plasma samples and in supernatants of 24-h unstimulated and 48-h PHA-stimulated PBMC cultures by antigen capture sandwich enzyme-linked immunosorbent assay (ELISA), with antibody pairs and standards obtained from R&D Systems (Minneapolis, Minn.). The 24-h supernatants from the unstimulated PBMC cultures were intended to reflect secretion triggered by in vivo stimuli, while the 48-h supernatants from the PHA-stimulated PBMC cultures were intended to provide an index of the capacity for secretion under conditions of maximal stimulation. Plasma measurements were performed on substantially platelet-free plasma specimens prepared by sequential centrifugation steps. Interleukin 2 (IL-2) and IL-10 were measured in plasma samples and in supernatants of 24-h unstimulated and 48-h PHA-stimulated PBMC cultures by antigen capture sandwich ELISA, with antibody pairs and standards obtained from R&D Systems; gamma interferon was measured with Duoset antibodies from Genzyme (Cambridge, Mass.). For each ELISA, the intra-assay CV was less than 5%; the interassay CVs were less than 12%, with threshold sensitivities of approximately 25 pg/ml for each analyte.

Limiting dilution in vitro SIV susceptibility assay. PBMC isolated from each animal by Ficoll density centrifugation just prior to the in vivo inoculation (animals shown in Fig. 3A) or 2 weeks prior to in vivo inoculation (animals shown in Fig. 3B) were tested either fresh or cryopreserved for in vitro susceptibility to SIV infection. PBMC from each animal were activated with PHA in separate bulk cultures (RPMI 1640, 10% heat-inactivated fetal calf serum, antibiotics, and PHA) for 48 h, then analyzed by flow cytometry for T-lymphocyte subsets ($CD4^+$ $CD8^+$ T cells) and HLA-DR as a marker for cellular activation, and plated out in replicate cultures of 10^6 cells/1.0 ml each. These replicate cultures were then inoculated with serial 10-fold dilutions of the SIV stock used for in vivo inoculation (SIVsmE660, 1.6×10^3 $TCID_{50}$/ml; SIVsmE543-3, 1.0×10^4 $TCID_{50}$/ml). Following a 2-h incubation at 37°C, the cells were washed and then cultured in human recombinant IL-2-containing medium (5 half-maximal units/ml; Advanced Biotechnologies, Inc.). Supernatants were harvested twice weekly and replaced with fresh medium. Supernatants were analyzed for p27 antigen content by using a commercial kit with a threshold sensitivity of 50 pg/ml (Coulter, Hialeah, Fla.) as an index of productive infection.

RESULTS

Early SIV replication and postacute viral replication set point. In a previous study of a candidate SIV vaccine in rhesus macaques (*M. mulatta*), we noted markedly different patterns of viral replication among the control group of four SIV-naive animals that received identical inoculations with the challenge virus SIVsmE660 (24). Viral replication patterns, as reflected by plasma viral RNA levels measured by QC-RT-PCR (24), correlated with clinical course and outcome. In two animals, uncontrolled viral replication was associated with rapidly progressive disease and death 14 and 22 weeks postinoculation, without evidence of seroconversion (Fig. 1A; RH168 and RH147). In a third animal, high levels of plasma viremia at 1 and 2 weeks postinoculation were followed by a subsequent substantial drop in plasma virus levels, but this gave way to eventual progressive increases in viral replication and associated clinical progression to AIDS and death 54 weeks postinoculation (Fig. 1A; RH187). The fourth animal showed sustained, substantial control of viral replication (Fig. 1A; RH174) and remains clinically well, with normal numbers of $CD4^+$ T cells nearly 4 years postchallenge, despite the demonstrable continued presence of low levels of infectious virus. Plasma viral RNA levels obtained through the postacute period of infection (8 weeks) were related to clinical course and survival (Fig. 1A) (24).

To evaluate the possibility that viral replication patterns very early in the course of infection might contribute to determining the postacute viral replication set point, we measured plasma SIV RNA levels twice weekly, beginning on day 4 postinoculation, in 12 SIV-naive macaques (*M. nemestrina*) that received identical i.v. inocula of SIVsmE660 (19). Different patterns of viral replication were observed, with the levels of viral RNA in plasma observed in the postacute phase of infection (week 6) varying by nearly 3 orders of magnitude among animals (Fig. 1B and C). On day 4 postinoculation, 8 of 12 animals had plasma viral RNA levels below the threshold for quantification (400 copy eq/ml; Fig. 1B). The other four animals had plasma viral RNA levels between 760 and 2,400 copy eq/ml (Fig. 1C). By day 7 postinoculation, all animals had quantifiable plasma viral RNA levels (mean, 584,000; standard deviation, 436,000; range, 58,400 to 1,280,000 copy eq/ml). Plasma viral RNA levels continued to increase after day 7, reaching a peak value at day 10 ($n = 2$), day 14 ($n = 6$), or day 17 ($n = 4$), with some animals showing double peaks. As an index of viral replication in the postacute phase of infection, we calculated the average of plasma viral RNA levels on days 36, 38, and 42 postinoculation (mean, 910,000 copy eq/ml; standard deviation, 820,000 copy eq/ml; range, 3,900 to 2,610,000 copy eq/ml).

To determine whether the extent of early viral replication was related to the level established in the postacute phase of infection, we performed analysis of covariance (ANCOVA) and linear regression analysis on plasma viral RNA levels measured on day 7 and in the postacute period of infection. The

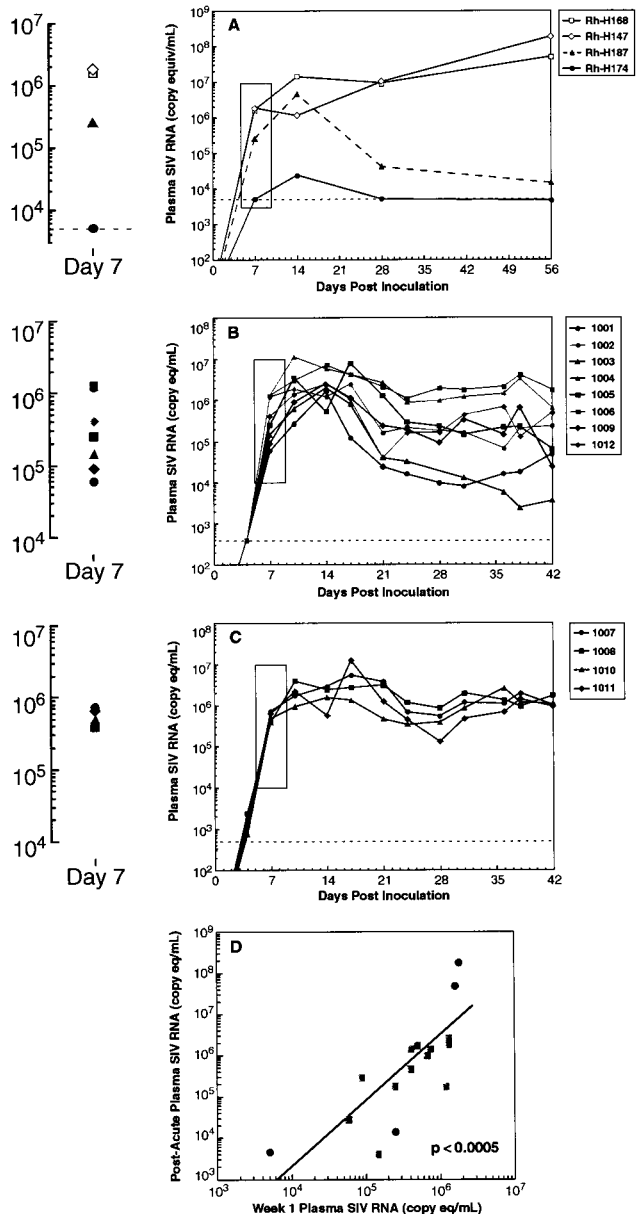

FIG. 1. Plasma viral load levels through primary infection in SIV-naive macaques inoculated with SIVsmE660. (A) Rhesus macaques (*M. mulatta*): RH168 and RH147 (open symbols, solid lines) died with rapidly progressive SIV disease 14 and 22 weeks postinoculation, respectively; RH187 (solid triangles, dashed line) died with progressive disease 54 weeks postinoculation; RH174 (solid circles, solid line) remains healthy nearly 4 years postinoculation. The lighter dashed line indicates threshold sensitivity of the QC-RT-PCR assay employed, 4,500 copy eq/ml; detail shows plasma viral RNA levels on day 7 postinoculation. (B) Pigtail macaques (*M. nemestrina*) with plasma viral RNA levels below the threshold for quantification in the QC-RT-PCR assay employed (dashed line, 400 copy eq/ml) on day 4 postinoculation. (C) Pigtail macaques (*M. nemestrina*) with plasma viral RNA levels above the threshold for quantification in the QC-RT-PCR assay employed (dashed line, 400 copy eq/ml) on day 4 postinoculation. (D) Correlation and regression between day 7 plasma viral RNA levels and postacute plasma viral RNA levels. Day 7 and postacute plasma viral RNA data points for results shown in panels A (circles) and B and C (squares) were pooled after ANCOVA showed no significant differences in the regression patterns for the two data sets. The linear least-squares regression of postacute plasma viral RNA on day 7 viral RNA levels is highly significant ($P < 0.0005$); the r^2 value of 0.59 indicates that approximately 60% of the variation in postacute viremia levels can be explained in terms of variation in the day 7 viremia. Separate analysis for data shown only in panels B and C also showed a significant correlation between day 7 and postacute plasma viral RNA levels ($P < 0.013$; $r^2 = 0.48$). (A detailed analysis of the kinetics of viral replication and mathematical modeling of the dynamics of primary SIV infection is presented separately [37a].)

ANCOVA showed that the data presented in Fig. 1A, B, and C could be pooled, as they did not differ in their regression patterns. As shown in Fig. 1D, regression analysis demonstrated that day 7 plasma viral RNA level significantly predicts postacute plasma viral RNA level ($P < 0.0005$). We interpret the level of plasma viremia on day 7 as an indicator of the relative extent of viral replication and spread through the first week of infection. The r^2 value of 0.59 for the regression of postacute plasma viremia levels on day 7 plasma RNA levels indicates that approximately 60% of the variation in postacute RNA levels can be explained in terms of variation in plasma RNA levels already existing by day 7 (Fig. 1D). This observation strongly suggests that factors exerting their effects within the first week of SIV infection play an important role in influencing the overall pattern of viral replication established in an infected animal and in determining the associated long-term clinical course. The observed differences in viral replication patterns seen among animals receiving identical inocula implicate host factors in determining these differences. This finding is particularly noteworthy, as it has been well documented that measurable specific antiviral immune responses, such as the effector phase of cytotoxic T-lymphocyte (CTL) activity (28, 50, 62) or production of antibodies to viral antigens, are not optimally developed during this initial phase of infection.

Detailed evaluation of viral replication in early primary SIV infection. To study viral replication during the early phase of primary SIV infection in greater detail, and in a manner that would further minimize the potential contribution of viral factors, four rhesus macaques (*M. mulatta*) were inoculated with a well-characterized, molecularly cloned SIV isolate (23). The virus used, SIVsmE543-3, is dual tropic (T cells and macrophages) and difficult to neutralize in vitro, properties it shares with primary HIV-1 isolates. Following analysis of baseline samples and i.v. inoculation with 1,000 $TCID_{50}$ of virus, comprehensive analysis of viral replication was performed, including daily measurements of plasma viral RNA levels through the first 2 weeks postinoculation, with continued plasma viral RNA measurements through the course of follow-up, along with ISH analysis (25) of serial lymph node biopsy specimens obtained 1, 2, and 4 weeks postinoculation. Marked differences in the rate and extent of early viral replication were seen between animals receiving identical inocula of molecularly cloned virus (Fig. 2A). Analysis of serial lymph node biopsy specimens taken at weeks 1, 2, and 4 postinoculation indicated that monitoring the plasma compartment provided a good reflection of the status of viral replication in lymphoid tissues (Fig. 2B).

Candidate host correlates of viral replication patterns. We evaluated several candidate host factors other than antigen-specific immune responses with potential for contributing to determining the observed different patterns of early viral replication in different animals. Differences before or at the pre-inoculation baseline or over the first week of infection in secretion by PBMC of either chemokines (RANTES, MIP-1α, and MIP-1β [11, 45]) or representative T_h1-like or T_h2-like cytokines (3, 10, 65) did not correlate in any consistent fashion with differences in patterns of in vivo viral replication (data not shown). Levels of chemokines in plasma also did not correlate, while cytokines were not present in plasma at measurable levels (data not shown).

To evaluate potential differences in the inherent susceptibility of target cells from different animals to SIV infection, we performed limiting dilution in vitro susceptibility culture assays on PBMC from each animal. Culture supernatants were harvested twice weekly and analyzed for p27 antigen as an index of productive infection. Marked differences were noted in the

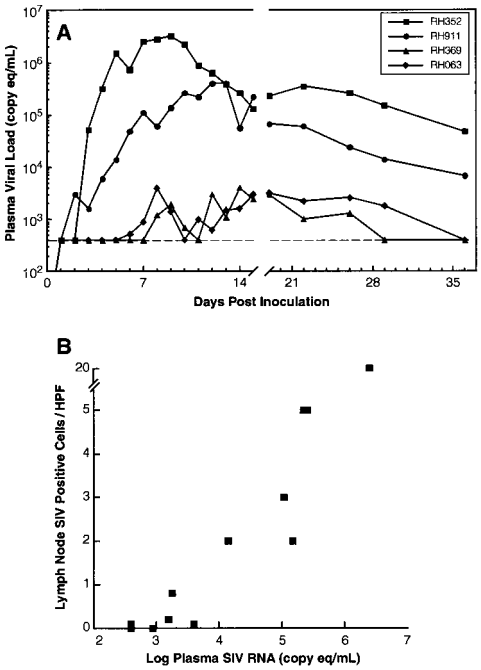

FIG. 2. (A) Plasma viremia levels during primary infection in four macaques (*M. mulatta*) inoculated with SIVsmE543-3 (23). (B) Agreement between levels of viral replication as determined by ISH in lymph nodes (biopsy specimens obtained on days 7, 14, and 28) and simultaneous plasma levels ($P = 0.0034$; $r^2 = 0.59$). Levels of plasma viral load also paralleled ISH results for lymph node biopsy specimens obtained on day 36 postinoculation for animals shown in Fig. 1B and C (data not shown). HPF, high-power field (40× objective).

susceptibility of mitogen-activated PBMC from different animals to in vitro infection (Fig. 3). The susceptibility to in vitro infection was significantly correlated with in vivo permissiveness for early viral replication (as reflected by plasma viral load at day 7 postinoculation) (Fig. 3; $P = 0.0015$ by Spearman rank correlation).

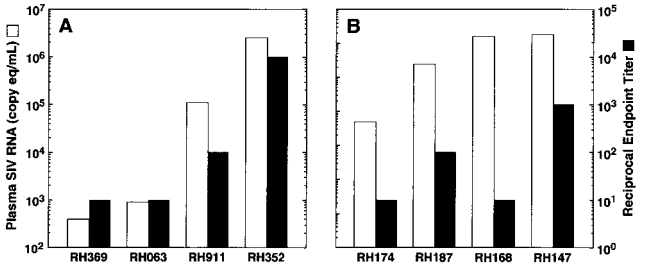

FIG. 3. Correlation of in vitro susceptibility of PBMC cultures and early in vivo viral replication for animals inoculated with SIVsmE543-3 (A) or SIVsmE660 (B). Figure shows in vitro reciprocal limiting dilution endpoint (black; 1/last dilution of input virus giving above-threshold levels of p27 antigen [>50 pg/ml] in culture supernatants on day 10 [A] or day 11 [B]) and in vivo plasma SIV RNA copy equivalents per milliliter on day 7 postinoculation (white). In vitro susceptibility was significantly correlated with in vivo permissiveness ($P = 0.0015$ by Spearman rank correlation for combined results shown in panels A and B). Rank order of in vitro susceptibility of PBMC from the animals shown in panel A to two additional SIV isolates (SIVsmH4 and SIVsm62d) was the same as shown for SIVsmE543-3 (data not shown). Limiting dilution endpoint rank order of susceptibility described above was comparable to the rank order based on absolute p27 antigen levels in day 3 supernatants of cultures inoculated with undiluted virus, the earliest available time point with measurable levels of p27 antigen in all culture supernatants. In ongoing separate prospective studies of a cohort of four additional rhesus macaques, limiting dilution endpoint rank order was maintained in studies with three sequential blood samples spread over 12 weeks, and rank order was comparable for fresh and cryopreserved specimens (51a).

These susceptibility studies were deliberately performed with unfractionated PBMC in order to allow any interactions between different cellular subpopulations, including noncytolytic SIV suppression mediated by $CD8^+$ cells (30, 31, 58), that might modulate viral infection. However, flow cytometric analysis performed on the activated cells just prior to inoculation of the cultures indicated that the observed differences in susceptibility were not trivially explainable based on differing percentages of $CD4^+$ cells or activated $CD4^+$ T cells present at the initiation of the cultures (data not shown). These results suggest that intrinsic differences in susceptibility to SIV infection of target cells from different animals may help determine differences in the rate and extent of early viral replication and spread in vivo, with important ramifications for clinical course.

DISCUSSION

We took advantage of unique aspects of the SIV-infected macaque model to study factors leading to the establishment of the postacute viral replication set point, with its associated prognostic significance (24, 32, 33, 56, 59). Longitudinal measurements of plasma viral RNA levels paralleled viral replication in lymph nodes, as assessed by ISH in serial lymph node biopsy specimens, and by inference provide a good index of viral replication in the presumed major site of viral replication—the lymphoreticular tissues (21, 43, 44).

Strikingly, marked differences in viral replication patterns between animals receiving identical inocula were apparent even within the first week following inoculation. Viral factors are unlikely to be the cause of these differences. The differential emergence of viral variants with different replicative capacity within the first week of infection seems unlikely among animals that received identical i.v. inocula of the same molecularly cloned virus (SIVsmE543-3), while heteroduplex mobility analysis of the V1/V2 region, the most variable portion of the SIV envelope, indicates that little or no viral diversity emerges within the first week of infection in animals infected with the nonclonal virus isolate SIVsmE660 (21a).

Levels of plasma viral RNA measured by day 7 postinoculation differed among animals and were significantly related to the eventual plasma viremia set point in the postacute phase of infection. This is noteworthy since the set point level is highly predictive of subsequent clinical course and outcome in both SIV-infected macaques and HIV-1-infected humans (24, 32, 33, 39, 56, 59). Specific immune responses have been postulated to be the dominant mechanisms through which viral replication is curtailed in primary infection, leading to resolution of the acute phase of infection and equilibration of viral replication (5, 9, 13, 28, 29, 48, 49, 56, 62). However, our observations suggest that factors that exert their effects during the very early stages of infection, before the effector phase of specific antiviral immune responses is optimally developed (29, 50, 62), also play a critical role. This represents a departure from previous models of AIDS pathogenesis.

The data suggest two alternative scenarios. In the first, host factors other than specific immunity may play the dominant role in establishing both the initial and postacute levels of viral replication. Thus, the rate and extent of early viral replication may simply reflect the intrinsic susceptibility of cells of a given host or may be affected by the size, location, and nature of the cell populations initially infected in vivo (seeding), influencing pathogenesis in ways that are largely independent of effects of specific immune responses. The correlation that we have demonstrated between the extent of early in vivo viral replication

and susceptibility of PBMC from different animals to SIV infection in vitro implies that intrinsic susceptibility of target cells contributes importantly to this process. This relative susceptibility likely influences the level of early viral replication, which is predictive of the postacute set point plasma viremia level, which in turn is correlated with clinical course and prognosis. In this model, the decrease from peak viremia may be associated in part with infection and destruction of the initially available pool of target cells that are most highly susceptible to infection (38, 47).

However, the extent and pattern of variation in plasma viremia at day 7 postinoculation do not fully account for the greater degree of variation demonstrable in the postacute phase of infection, suggesting the importance of factors in addition to those such as intrinsic susceptibility to infection that are operative at the outset of infection. Regression analysis suggests that approximately 60% of the variation observed in postacute plasma SIV RNA levels is explainable in terms of the variation observed in day 7 levels. This is consistent with specific immune responses playing a critical role in the modulation of viral replication and disease progression.

Antigen-specific immune responses may exert much of their activity in the context of other host factors such as differential susceptibility of target cells influencing levels of early viral replication. In this view, the effectiveness of the immune response in curtailing viral replication may be directly impacted by factors influencing the level of viral replication present during the initial stages of the development of the immune response. The quality of specific immune responses may be critically determined by the level and kinetics of antigen exposure, as demonstrated in a variety of animal models (18, 36, 37, 51, 55, 63, 64). Indeed, in a murine leukemia virus model, inoculation of neonatal mice with low doses of virus induced a protective CTL response while inoculation with higher levels of the same virus was associated with development of a nonprotective T_h2 cytokine profile, failure to develop effective CTL, and disease manifestations (55).

Compromise of the ability of the immune system to curtail viral replication following an initial period of high levels of viremia may reflect deletion of virus-specific clones (20, 36, 37, 41, 42, 63, 64). It has recently been demonstrated that differences in the qualitative nature of the primary immune response to HIV-1 were associated with prognosis, independent of the level of plasma viremia measured at the time of presentation with symptomatic primary HIV-1 infection, and it was hypothesized that poor prognosis was related to the deletion of HIV-specific $CD8^+$ CTL clonotypes (20, 41). However, it is possible that these differences in immune responses were in turn influenced by differences in viral replication rates earlier in infection, obscured by sampling limitations inherent in the analysis of clinical specimens in studies of primary HIV-1 infection.

The importance of host factors and early events affecting SIV dissemination is reinforced by studies in which early postinoculation treatment of infected animals with the antiretroviral agent *R*-9-(2-phosphonylmethoxypropyl)adenine (57) was capable of profoundly modulating viral replication and subsequent clinical course, even leading to the apparent clearance of infection following withdrawal of treatment. Consistent with our data underscoring the importance of early events, optimal results were seen only when treatment was initiated within 24 h of inoculation (57). Similar data have been reported for postinoculation zidovudine treatment in murine retroviral models (52, 53), while in the SIV-infected macaque model, transient early intervention with anti-SIV immune globulin preparations has been reported to result in sustained downmodulation of viral replication in treated animals, with improved clinical course and survival, compared to animals treated with a control immune globulin preparation or untreated controls (22).

We observed a clear relationship between the rank order susceptibility of PBMC from different animals to in vitro SIV infection and the rank order of permissiveness for early in vivo viral replication in the same cohort. This relationship suggests that target cells from different animals differ in their intrinsic susceptibility to SIV infection, with important in vivo consequences. While the underlying basis of this differential susceptibility to infection remains to be determined, differences in susceptibility to infection related to cellular expression of chemokine receptors (1, 4, 14–17, 27, 45) and effects of noncytolytic $CD8^+$ T-cell suppressive activity (30, 31, 58) on early viral replication are interesting parameters for further evaluation. In preliminary cell fractionation studies, the differential susceptibility to in vitro SIV infection among animals appears to be maintained, even when enriched $CD4^+$ T lymphocytes are used as target cells, suggesting that properties of the target cells themselves, rather than differences in interactions between PBMC subpopulations, likely account for the differential susceptibility. Analysis of such in vitro susceptibility may facilitate studies in the SIV-infected macaque model either by allowing assignment of animals of defined expected in vivo permissiveness (based on measurement of in vitro susceptibility) to appropriate experimental groups or by allowing interpretation of results obtained with randomly assigned animals in the light of the in vitro susceptibility data.

In summary, animals receiving identical SIV inocula showed markedly different levels of early viral replication, which correlated with the postacute viral replication set point that was established. The extent of early in vivo viral replication in different animals was in turn correlated with the susceptibility of their PBMC to infection with SIV in vitro. These findings implicate host factors in addition to specific immune responses to viral antigens as important determinants of levels of viral replication and associated disease course, while other observations suggest their relevance to HIV-1-infected humans. Extended follow-up of subjects infected with HIV-1 via common transfusion sources has demonstrated wide differences in clinical course, despite inoculation with the same viral quasispecies, implicating host factors as the basis for the differences (40). In recent studies of early specimens from plasma donors undergoing seroconversion for HIV-1, the earliest measurable levels of plasma viral RNA were related to the virus levels in plasma established in the postacute set point period (6). In addition, prior studies with human PBMC have demonstrated longitudinally stable differences in the in vitro susceptibility to HIV-1 infection of PBMC and enriched $CD4^+$ T cells from different HIV-1-seronegative donors, consistent with the observations reported here (61). Elucidation of the mechanisms that account for these observations will provide insight into the pathogenesis of SIV–HIV-1 disease and may have important practical consequences for the development of an effective vaccine for prevention of HIV-1 infection.

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