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(30) 1996/10/11 (60/028,169) US
(54) **PROCEDE PERMETTANT DE DETERMINER L'EFFICACITE D'UN TRAITEMENT MEDICAMENTEUX CHEZ DES SUJETS INFECTES PAR LE VIH**
(54) **PROCESS OF DETERMINING THE EFFICACY OF DRUG TREATMENT IN HIV INFECTED SUBJECTS**

(57) Procédé permettant de déterminer l'efficacité d'une thérapie antivirale chez un sujet infecté par le VIH et recevant ladite thérapie. Ce procédé consiste (a) à détecter le taux de VIH à activité de transcription dans des monocytes du sujet à plusieurs moments différents, (b) à comparer les taux de VIH détectés et (c) à mettre en corrélation les taux de VIH détectés sur la période concernée avec la thérapie. Ledit procédé peut être utilisé pour surveiller l'efficacité d'un traitement à base d'agents anti-VIH tels que AZT, 3TC, DDc, Indivar ou Saquinavir. Des baisses des taux de VIH avec le temps indiquent un traitement efficace. Des augmentations des taux de VIH détectés indiquent une résistance au traitement.

(57) The present invention provides a process for determining the efficacy of anti-viral therapy in an HIV-infected subject receiving such therapy. The process includes the steps of a) detecting the level of transcriptionally active HIV in monocytes of the subject at a plurality of different times, b) comparing the detected HIV levels, and c) correlating changes in the detected HIV levels over time with the therapy. The process can be used to monitor the efficacy of treatment with any anti-HIV agent such as AZT, 3TC, DDc, Indivar, or Saquinavir. Decreases in HIV levels over time indicate an efficacious treatment. Increases in detected HIV levels over time indicate resistance to treatment.

**PCT**

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(54) Title: PROCESS OF DETERMINING THE EFFICACY OF DRUG TREATMENT IN HIV INFECTED SUBJECTS

(57) Abstract

The present invention provides a process for determining the efficacy of anti-viral therapy in an HIV-infected subject receiving such therapy. The process includes the steps of a) detecting the level of transcriptionally active HIV in monocytes of the subject at a plurality of different times, b) comparing the detected HIV levels, and c) correlating changes in the detected HIV levels over time with the therapy. The process can be used to monitor the efficacy of treatment with any anti-HIV agent such as AZT, 3TC, DDc, Indivar, or Saquinavir. Decreases in HIV levels over time indicate an efficacious treatment. Increases in detected HIV levels over time indicate resistance to treatment.

# PROCESS OF DETERMINING THE EFFICACY OF DRUG TREATMENT IN HIV INFECTED SUBJECTS

## Cross-Reference to Related Application

5      The present application is a continuation in part of United States Provisional Patent Application No. 60/024,404, filed August 22, 1996 and a continuation-in-part of United States Provisional Patent Application No. 60/028,169, filed October 11, 1996.

## Technical Field of the Invention

10     The field of the present invention is HIV therapy.  More particularly, the present invention pertains to a process of determining the efficacy or efficiency of drug treatment in HIV infected subjects by determining the level of HIV in  monocytes of the subject.

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## Background of the Invention

HIV is known to productively infect a variety of different cell types *in vitro* and *in vivo*.  The extent to which HIV infects and replicates in these cells has important implications concerning dissemination from
20     portals of entry, cell function, and disease progression given the finite number of target cells.  End products of viral replication including expression of unspliced HIV mRNA and plasma free virus has led to virologic determinants as a measure of disease state and therapeutic efficacy.  A marked increase in the ratio of unspliced to spliced HIV
25     mRNA as might occur during the shift from latent to productive infection precedes precipitous drops in CD4 count.  Plasma viral load has been shown to correlate with disease progression and has been used to determine HIV kinetics *in vivo*.  These measurements, however, fail to provide information on the cell type of origin—a weakness considering
30     the effects of HIV expression on cell function, the role of infected cells in transmission and dissemination, and the therapeutic potential of blocking cell type specific co-receptors.

Plasma viral burden analysis have allowed researchers to
35     estimate kinetic parameters of HIV-1 life cycle *in vivo*. The life span of productively infected T-lymphocytes was estimated to be 2.2 days.  The

detection and quantification of productively infected lymphocytes, therefore, is technically difficult requiring very sensitive techniques. Further, the turnover of these cells may be too rapid to measure these cells on a continuous basis. In addition, the contribution of free virus to the infective pool by cell types not destroyed by viral replication has not been experimentally addressed and has only been included as an aside in most kinetic models.

The transmission of HIV characteristically involves the early appearance of NSI (non-syncitial inducing), macrophage tropic viral isolates despite the presence or absence of SI (syncitial inducing) and NSI variants in the donor. Effective anti-HIV immune responses have been suggested to temper the replication of SI variants after transmission. Following seroconversion, SI variants start to appear as a result of immune dysfunction. Some studies, however, suggest that NSI, macrophage tropic isolates persist throughout the disease course in spite of the abundance of SI variants.

Recent data lends support to the model that selective transmission may occur as the virus penetrates mucosal surfaces and encounters cells of monocyte/ macrophage lineage. The virus can then be distributed throughout the lymphoid system and tissue compartments via these cells. Once in the lymphoid compartment, the virus encounters the overwhelming majority of the body's lymphocytes. The virus accumulates in and eventually destroys the T-cell reservoir leaving only macrophages in T-cell depleted lymph nodes. Although free virus determinations have helped define HIV-1 kinetics *in vivo*, resent elucidation of macrophage and T-cell tropic coreceptors demands further characterizations of the cells producing virus at various times during disease progression and during antiretroviral therapy.

Brief Summary of the Invention

The present invention provides a process of determining or monitoring the efficacy of drug treatment in HIV infected subjects. In accordance with this process, the levels of HIV RNA in monocytes of

2

the subject are measured over the course of treatment with one or more drugs (e.g., AZT, 3TC, DDC). As drug treatment efficacy increases, the levels of HIV RNA in monocytes decreases. Conversely, where a subject develops resistance to a drug, that resistance is evident from an

5        increase or lack of decrease in monocyte HIV RNA levels. In other words there is a direct correlation between the effectiveness of treatment and monocyte HIV RNA levels. Preferred monocytes for use in this invention are CD14$^+$ monocytes.

10        In accordance with the present invention, dual immunophenotyping PCR *in situ* hybridization (DIPDISH) is used to detect cells containing HIV-1 DNA, dual immunophenotyping fluorescence *in situ* hybridization (DIPFISH) is used to detect and quantify gag-pol mRNA in cells and quantitative RNA analysis is used

15        to quantify plasma viral load. The present invention discloses that monocytes, and particularly CD14$^+$ monocytes, are persistently productive of HIV message. Furthermore, the levels of HIV mRNA in those monocytes respond in parallel with plasma viral load to drug therapy. As viral message production is an earlier event in virion

20        production a process of the present invention is more a more sensitive indicator of drug efficacy and drug resistance than prior art methods.

Productively infected cell types in patients infected by HIV have been identified and quantified. As shown previously, very few CD4

25        positive lymphocytes were productively infected by HIV although many contain proviral DNA. The present invention discloses that monocytes are the major productively infected cell type in HIV seropositive individuals and viral production in these cells is altered by antiretroviral therapy. The percentage of productively infected monocytes

30        corresponded with viral burden analysis in patients on no, single, combination, and triple drug therapy.

Brief Description of the Drawings

In the drawings, which form a portion of the specification:

5          FIG. 1 shows a time course experiment of ACH-2 cells stimulated
with TNF-α and detected using FISH.  Less than 5 percent of the cells
express any HIV-1 mRNA at time point 0 (A), expression of HIV-1
multiply spliced mRNA is produced within 1 hour (B) and unspliced
transcripts start to be produced at the 12 hour timepoint (C), and
expression of all mRNA is seen in over 90% of the stimulated cells at
10        24 hours (D).  The ACH-2 cell line (AIDS Research and Reagent
Program, NIAID, NIH Bethesda, MD), containing a single copy of
integrated HIV-1 proviral DNA per cell, was harvested at an early
passage number and used as the HIV-1 infected cell copy number
control cells were stimulated with μg/ml TNF-α.

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FIG. 2 shows dot plots of patient PBMCs following DIPFISH.  To
determine the cell types containing HIV RNA using DIPFISH cells are
labeled with optimized concentrations of phycoerythrin-conjugated
antibodies specific for the cell types of interest (CD4, CD14) and fixed
20        and permeabilized by the addition of 50 μl of Permeafix (Ortho
Diagnostics, Inc.) per $10^6$ cells at ambient incubation temperature for at
least 60 minutes.  The cells are then washed twice in PBS, pH 7.4 at
ambient temperature and once in 2x SSC at ambient temperature.  The
cells are then resuspended with 50 μl hybridization buffer containing a
25        cocktail of 5- or 6- carboxyfluorescein-labeled oligonucleotides specific
for HIV RNA- Positive (ribosomal RNA) and negative control cocktails
are used in replicate samples.  Probe was hybridized to target for 30
minutes at 43°C in a water bath.  The cells were washed for 5 minutes
with buffer A at 42°C, 30 minutes with buffer B at 42°C.
30        Autofluorescence was quenched using quenching reagent.
Multiparameter analysis of cell surface molecules and HIV RNA was
performed on a Coulter XL flow cytometer (Coulter, Hialeah, FL).

FIG. 3 shows a scattergram illustrating the relationship of
35        productively infected monocytes (abscissa) and plasma viral load
(ordinate) in patients on no ◆, single ■ , combination ▲, and triple x

4

therapy. Forty HIV seropositive patients from the VA Lakeside Hospital
followed routinely were evaluated using CD4 count, DIPDISH,
DIPFISH, and quantitative RNA. Five HIV seronegative patients were
evaluated using the same assays. Patients were either on no therapy,
single drug therapy with AZT (200 mg three times/day) or DDC (0.75
mg three times/day) , combination therapy with AZT and 3TC (150 mg
two times/day), or triple therapy with AZT 3TC and either Indinavir (800
mg three times/day) or Saquinavir (600 mg three times/day). All
patients were on therapy for at least 30 days prior to blood donation.
PBMCs were isolated from fresh heparinized blood layered on a
Histópaque 1077 (Sigma, St. Louis, MO) discontinuous density
gradient and centrifuged at 600xg for 30 minutes at ambient
temperature. The turbid layer was removed, washed twice with 3
volumes of RPMI and once with phosphate buffered saline (PBS).

Detailed Description of the Invention

The present invention provides a process for determining the
efficacy of anti-viral therapy in an HIV-infected subject receiving such
therapy. The process includes the steps of a) detecting the level of
transcriptionally active HIV in monocytes of the subject at a plurality of
different times, b) comparing the detected HIV levels, and c) correlating
changes in the detected HIV levels over time with the therapy. The
process can be used to monitor the efficacy of treatment with any anti-
HIV agent such as AZT, 3TC, DDc, Indivar, or Saquinavir. Decreases
in HIV levels over time indicate an efficacious treatment. Increases in
detected HIV levels over time indicate resistance to treatment.

The level of transcriptionally active HIV is detected by measuring
the level of HIV mRNA and, preferably gag-pol HIV mRNA. The HIV
mRNA is detected using *in situ* hybridization. *In situ* hybridization is
accomplished by exposing monocytes *in situ* to an oligonucleotide
probe that specifically hybridizes to at least of a portion of the HIV
mRNA. The probe is labeled with a detectable marker, the detection of
which indicates the presence of HIV mRNA.

A process of this invention is particularly useful when the
monocytes harboring the transcriptionally active HIV are CD14$^+$
monocytes. CD14$^+$ monocytes are identified in blood samples of the
subject using immunophenotyping. In accordance with such

5      immunophenotyping, a blood sample is exposed to an antibody
directed against CD14$^+$, which antibody is labeled with a detectable
marker. In a preferred embodiment, the immunophenotyping and *in
situ* hybridization are carried out simultaneously. In accordance with
that preferred embodiment, CD14$^+$ is labeled with an anti-CD14$^+$

10     antibody having a first detectable label attached thereto, the labeled
monocytes are then fixed and permeabilized in the presence of an
oligonucleotide probe that hydridizes to the HIV mRNA, which probe is
labeled with a second detectable marker, and the amount of both
detectable labels measured. Preferably, the first and second

15     detectable labels are fluorescent labels. A preferred first detectable
label is phycoerythrin. A preferred second detectable label is 5- or 6-
carboxyfluorescein.

The level of HIV RNA in monocytes is determined in blood

20     samples from the subject using a combination of cellular antigen (e.g.,
CD14) detection (using labeled antibodies) and *in situ* hybridization
using a tagged probe directed against a region of HIV RNA. Details of
such methods are set forth below. A detailed description of
simultaneous *in situ* cellular antigen detection and nucleic acid

25     amplification can be found in United States Patent Application Serial
Number 08/521,467, filed August 30, 1995, the disclosure of which is
incorporated herein by reference.

**Study subjects.** Thirty-seven HIV seropositive patients from the VA

30     Lakeside Hospital followed routinely were evaluated using CD4 count,
DIPDISH, DIPFISH, and quantitative RNA analysis. Ten HIV
seronegative patients were evaluated using the same assays. Patients
were either on no therapy, single drug therapy with AZT (200 mg, three
times a day) or DDC (0.75 mg, three times a day), combination therapy

35     with AZT and 3TC (150 mg, two times a day), or triple therapy with AZT
3TC and either Indinavir (800 mg, three times a day) or Saquinavir

6

(600 mg, three times a day). All patients were on therapy for at least 30 days prior to blood donation.

5       Histogram gates were set based on a negative control cocktail directed against cytomegalovirus and on a positive control cocktail directed against 28 ribosomal RNA. Fine tuning of the gates on each patient sample were set based on internal HIV-negative and CD4 or CD14 negative populations. CD4^{+} T-lymphocytes containing HIV-1 DNA ranged from 4% to 43%. The percentage of cells containing HIV-

10      1 DNA did not correlate with CD4 count or drug therapy suggesting that this population may be a reservoir for defective viral genomes, non-productive infection of resting T-cells, or provirus capable of subsequent activation.

15      **Cells and cell lines.** PBMCs were isolated from fresh heparinized blood layered on a Histopaque 1077 (Sigma, St. Louis, MO) discontinuous density gradient and centrifuged at 600 x g for 30 minutes at ambient temperature. The turbid layer was removed, washed twice with 3 volumes of RPMI and once with phosphate

20      buffered saline (PBS). The ACH-2 cell line (AIDS Research and Reagent Program, NIAID, NIH, Bethesda, MD), containing a single copy of integrated HIV-1 proviral DNA per cell, was harvested at an early passage number and used as the HIV-1-infected cell copy number control.

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**Dual Immunophenotyping/Fluorescence in situ Hybridization (DIPFISH).** To determine the cell types containing HIV RNA using DIPFISH, cells were labeled with optimized concentrations of phycoerythrin (PE)-conjugated antibodies specific for the cell types of

30      interest (CD4, CD14) and fixed and permeabilized by the addition of 50 $\mu$l of a water-soluble, non-aldehyde fixative, Permeafix (Ortho Diagnostics, Inc.) per $10^6$ cells at ambient incubation temperature for at least 60 min. The cells were then washed twice in PBS, pH 7.4 at ambient temperature and once in 2 x SSC at ambient temperature.

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The cells were then resuspended with 50 μl hybridization buffer containing a cocktail of 5- or 6-carboxyfluorescein-labeled oligonucleotides specific for HIV RNA. Positive (ribosomal RNA) and negative control cocktails were used in replicate samples. Probe was

5     hybridized to target for 30 minutes at 43°C in a water bath. The cells were washed with buffer at 42°C. Autofluorescence was quenched using quenching reagent. Multiparameter analysis of cell surface molecules and HIV RNA was performed on a Coulter XL flow cytometer (Coulter , Hialeah, FL).

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**Dual Immunophenotyping/PCR-driven in situ hybridization (DIPDISH).** Cell samples were adjusted to a final concentration of 1 x $10^6$ cells/ml. A 400 μl aliquot of each sample was centrifuged at 600 x g for 2 minutes at ambient temperature and the cell pellet was washed

15    twice in PBS. The cells were then fixed and permeabilized by the addition of 50 μl of Permeafix (Ortho Diagnostics, Inc.) at ambient temperature for 60 minutes. Cells were then pelleted as above, washed with PBS and resuspended in 190 μl of PCR reaction mixture consisting of 10 mM Tris HCL (pH 8.3); 50 mM KCL; 1.5 mM $MgC_4$;

20    0.25 mM each dATP, dCTP, dGTP; 0.14 mM dTTP; 4.3 μM dUTP-11-digoxigenin; 100 pmole each forward and reverse primer; 1.0 μl (5 Units) *Taq* polymerase (Amplitaq, Perkin-Ehner, Norwalk CT); and gelatin 0.001% w/v.

25    The DNA in the reaction mixture was amplified in 500 μl tubes inserted into the wells of a 48 well thermocycler (Perkin-Elmer Cetus) programmed for 25 cycles of thermal denaturation (94°C, 1 minute), primer annealing (58°C, 2 minutes), and primer extension (74°C, 1.5 minutes), with 5 seconds added for each of 25 cycles. Appropriate

30    positive and negative controls amplified with or without the addition of *Taq* polymerase were simultaneously run with each sample. After amplification, cells were pelleted and resuspended in 25 μl of 10 mM Tris HCL (pH 8.3), 50 mM KCL, and 1.5 mM $MgC_4$. A 100 mg aliquot of the appropriately labeled target specific oligonucleotide probe in 10

35    μg/ml sonicated herring sperm DNA (Sigma) was added to the reaction tube. The product DNA was denatured at 95°C for 3 minutes then

8

allowed to hybridize with the respective oligonucleotide probe at 56°C
for 2 hours.

5        After hybridization, the cells were washed for 30 minutes with 2x
SSC/50% formamide/500 μg/ml bovine serum albumin (BSA) at 42°C,
30 minutes with 1x SSC/50% formamide/500 μg/ml BSA at 42°C, 30
minutes with 1x SSC/500 μg/ml BSA at ambient temperature and then
briefly with PBS at ambient temperature. Following the last wash, the
cells were resuspended in 80μl of PBS and 20μl strepavidin-
10       phycoerythrin (PE) and incubated for 30 minutes at ambient
temperature. The cells were then washed in PBS as described above.

**Flow Cytometry.** The cell suspension was filtered through a 37 mm
nylon mesh and analyzed by flow cytometry using an EPICS XL flow
15       cytometer. Laser excitation was 15 mW at 488 nm, and the FITC and
PE florescence was detected with standard optical filter set-up (550
dichroic, 525 bandpass (FITC) and 585 bandpass (PE)). Instrument
sensitivity was standardized before each experiment employing
Immuno-Bright calibration beads (Coulter Source, Marriette, GA). The
20       percent fluorescence-positive cells was determined by integration over
a range of 0.2% positive counts on the identically treated negative
sample (100% uninfected PBMCs).

**Viral Burden.** Quantitative RNA determinations were performed on
25       plasma using the Amplicor RNA kit (Roche Molecular Systems,
Alameda, CA) as per manufacturer instructions.

In peripheral blood, a significant proportion of PBMCs contain
HIV-1 DNA while very few contain transcriptionally active virus.
30       Simultaneous immunophenotyping not only allowed unequivocal
identification of infected cell subtypes but also enriched cell subtypes
for the various infected cell types present in the blood. To determine
the cell types with productive or latent HIV infection,
immunophenotyping was combined with a novel *in situ* hybridization
35       strategy using 220 5'- and 3', 6-carboxyfluorescein-labeled
oligonucleotides complementary to gag-pol targets or with *in situ* PCR

for proviral DNA. The *in situ* hybridization experiments were calibrated using the ACH-2 cell line with and without TNF-α stimulation. Time course experiments (FIG. 1) reveal that >98% of unstimulated ACH-2 cells express high levels of HIV-1 mRNA which agrees with previous

5       estimates. Twenty four hours following stimulation, >90% of ACH-2 cells express high levels of HIV-1 mRNA which corresponds to between 300-400 genome copies per cell.

        Using simultaneous immunophenotyping with CD4 and CD14

10      and *in situ* hybridization, the data show that a significant proportion of monocytes harbor transcriptionally active HIV whereas very few CD4 T-lymphocytes harbor transcriptionally active virus at any point in time (FIG. 2). The percentage of productively infected monocytes ranged from <1% to 93% percent and the percentage of productively infected

15      CD4 lymphocytes ranged from <1 to 6% which, most likely, represents the more rapid turnover of productively infected CD4 cells and the persistence of productively infected monocytes. It was previously suggested that productive infection of monocytes occurred following differentiation to macrophages and indeed a significant proportion of

20      cells were found in peripheral blood with the CD14 low, CD16 high phenotype indicative of macrophage differentiation. Backgating on the cells expressing HIV RNA, however, did not reveal a specific, infected subset of CD14 positive cells.

25      To determine the relationship between the percentage of infected cells and plasma viral load, plasma was obtained from the same sample used to isolate PBMCs and quantitative competitive RNA PCR was performed on each sample (FIG. 3). Six groups were identified: those patients with high viral load, high percentage of

30      infected monocytes; high viral load, low percentage of infected monocytes; low viral load, high percentage of infected monocytes; low viral load, low percentage of infected monocytes; and intermediate or low viral load, intermediate percentage of infected monocytes. A low viral load in the presence of a high percentage of monocytes

35      expressing HIV-1 mRNA may represent early indication of drug resistance. A high viral load in the presence of a low percentage of

monocytes expressing HIV-1 mRNA may represent production of free virus predominantly in other cell types as may occur in the phenotypic switch preceding symptomatic infection. Even in the extreme cases of a patient with a high viral titer in spite of triple therapy or a patient with a

5      low viral titer in the absence of therapy, the percentage of infected monocytes paralleled the viral load.

Nine out of fifteen patient (60.0%) with a high percentage of monocytes expressing HIV-1 mRNA had viral loads over 50,000

10      copies/ml while four out of twenty-three patient (17%) with less than 50% monocytes expressing HIV-1 mRNA had viral loads over 50,000 copies/ml. As viral message production is an earlier event in the viral lifecycle, this measurement is likely a more sensitive indicator of drug efficacy and drug resistance.

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Productively infected cell types in blood from patients infected by HIV were identified and quantified. CD14 positive monocytes were the major persistently productive cells infected with HIV. A specific subtype of infected monocytes was not identified even though CD14 low, CD16

20      high cells phenotypically resembling macrophages have been identified in the peripheral blood of HIV seropositive individuals. Interestingly, productively infected monocytes in several patients exhibited an increased CD14 mean peak fluorescence relative to the HIV negative monocytes in the same sample. Increases in CD14

25      expression in monocytes has been shown in the peripheral blood of HIV-infected patients although the presence of HIV in these cells was not determined in this previous study. CD14 is a glycosyl-phosphatidynositol -anchored molecule on the surface of monocytes and to a lesser extent granulocytes. Monocyte activation by

30      lipopolysaccharide requires CD14, therefore alterations of CD14 could conceivably explain the functional defect of monocytes at the cellular level in HIV-infected individuals. As shown previously, very few CD4 positive lymphocytes were productively infected by HIV although many contain proviral DNA.

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The transmission of HIV involves the early infection of antigen presenting cells such as Langerhans cells and monocytes following breach of mucosal barriers. Virus is shuttled in these cells via peripheral blood to lymphoid and other tissue. Within lymphoid tissue

5    free virus produced by productively infected cells can infect other permissive cells or become trapped within the follicular dendritic cell (FDC) network.

Following infection, non-syncytium-inducing (NSI), macrophage

10   tropic viral isolates predominate despite the presence or absence of syncytium-inducing (SI) and NSI variants in the donor. Effective anti-HIV immune responses have been suggested to temper the replication of SI variants after transmission. Following progression to symptomatic disease, SI variants start to appear as a result of immune dysfunction.

15   The studies herein show that cells of the monocyte/macrophage lineage are likely a major reservoir of virus capable of producing virions. Yet this reservoir has been omitted from mathematical models proposed to examine the relationship between virion production and host cell destruction.

20

Much debate has centered on the question of whether monocytes are infected, productively or latently, by HIV *in vivo*. The absolute monocyte count and the absolute CD4 count were calculated in all of the patients. Based on the percentage of productively infected

25   CD4 T-lymphocytes and productively infected monocytes, and given that monocytes release anywhere from 10% to 50% of the number of virions as T-cells, the amount of free virus contributed by these cell types should be roughly equivalent. The exception to this would be patients with high viral loads and a low percentage of infected

30   monocytes (FIG. 3). These patients were all symptomatic with CD4 counts less than 200 suggesting the switch to the T-cell tropic, SI phenotype had occurred in these patients. This may explain why other studies in patients with AIDS indicate that monocytes are rarely a source of virus.

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Quantification of infected monocytes/macrophages is not only critical for a complete kinetic model of HIV infection but also for therapeutic monitoring. Since these cells are not destroyed by viral production and persistently infected cells such as macrophages, may

5        have a lifespan five to six times longer than infected T-lymphocytes, these cells are ideal for monitoring HIV activity at the cellular level including lymphoid tissue such as tonsil and lymph nodes.

Clearly, the contribution of productively-infected monocytes to

10       the free virus pool must be distinctly defined. With the discovery of new tropism dependent co-receptors for HIV and inevitable therapy directed at blocking these co-receptors, determination of productively infected cell types and their contribution to the free virus pool is critical. The ease of the technologies used to define the viral lifecycle in this study

15       most likely portends the end of surrogate markers as we know them and reaffirms the trend toward viral lifecycle monitoring.

WHAT IS CLAIMED IS:

1.      A process for determining the efficacy of anti-viral therapy
in an HIV-infected subject receiving such therapy, the process
comprising the steps of:
        a)      detecting the level of transcriptionally active HIV in
monocytes of the subject at a plurality of different times;
        b)      comparing the detected HIV levels; and
        c)      correlating changes in HIV levels over time with the
therapy.

2.      The process of claim 1 wherein the monocytes are CD14$^+$
monocytes.

3.      The process of claim 1 wherein the anti-viral therapy is
treatment with AZT, 3TC, Ddc, Indivar, or Saquinavir.

4.      The process of claim 1 wherein decreases in HIV-RNA
levels over time indicate an efficacious treatment.

5.      The process of claim 1 wherein increases in detected
HIV-RNA levels over time indicate resistance to treatment.

6.      The process of claim 1 wherein detecting is accomplished
by detecting the level of HIV mRNA using *in situ* hybridization.

7.      The process of claim 6 wherein *in situ* hybridization is
accomplished by exposing the monocyte to an oligonucleotide probe
that specifically binds to at least a portion of the mRNA, which probe is
labeled with a detectable marker.

8.      The process of claim 7 wherein the portion of HIV mRNA
is the gag-pol portion.

9.      The process of claim 6 wherein the detectable label on
the probe is a fluorescent label.

14

10.　　The process of claim 9 wherein the fluorescent label is a 5- or 6- carboxyfluorescein.

5　　　　11.　　The process of claim 2 wherein the CD14+ monocytes are labeled with an antibody against CD14, which antibody is labeled with a detectable marker.

12.　　The process of claim 11 wherein the detectable label on 10　　the antibody is a fluorescent label.

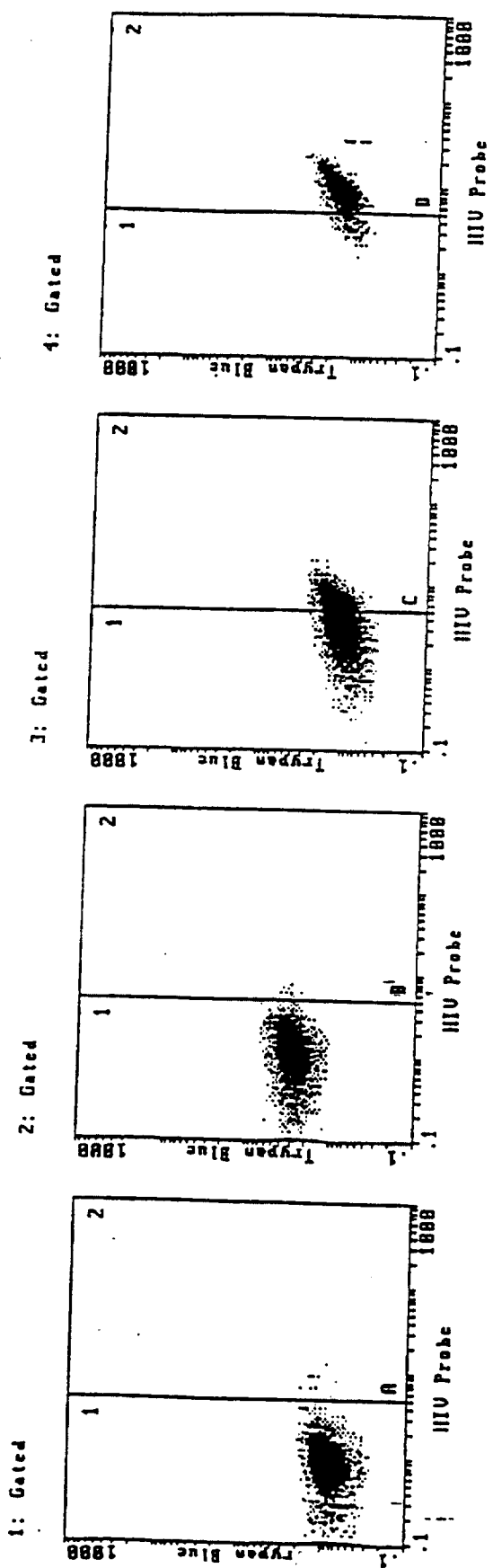13.　　The process of claim 12 wherein the detectable fluorescent label is phycoerythrin.
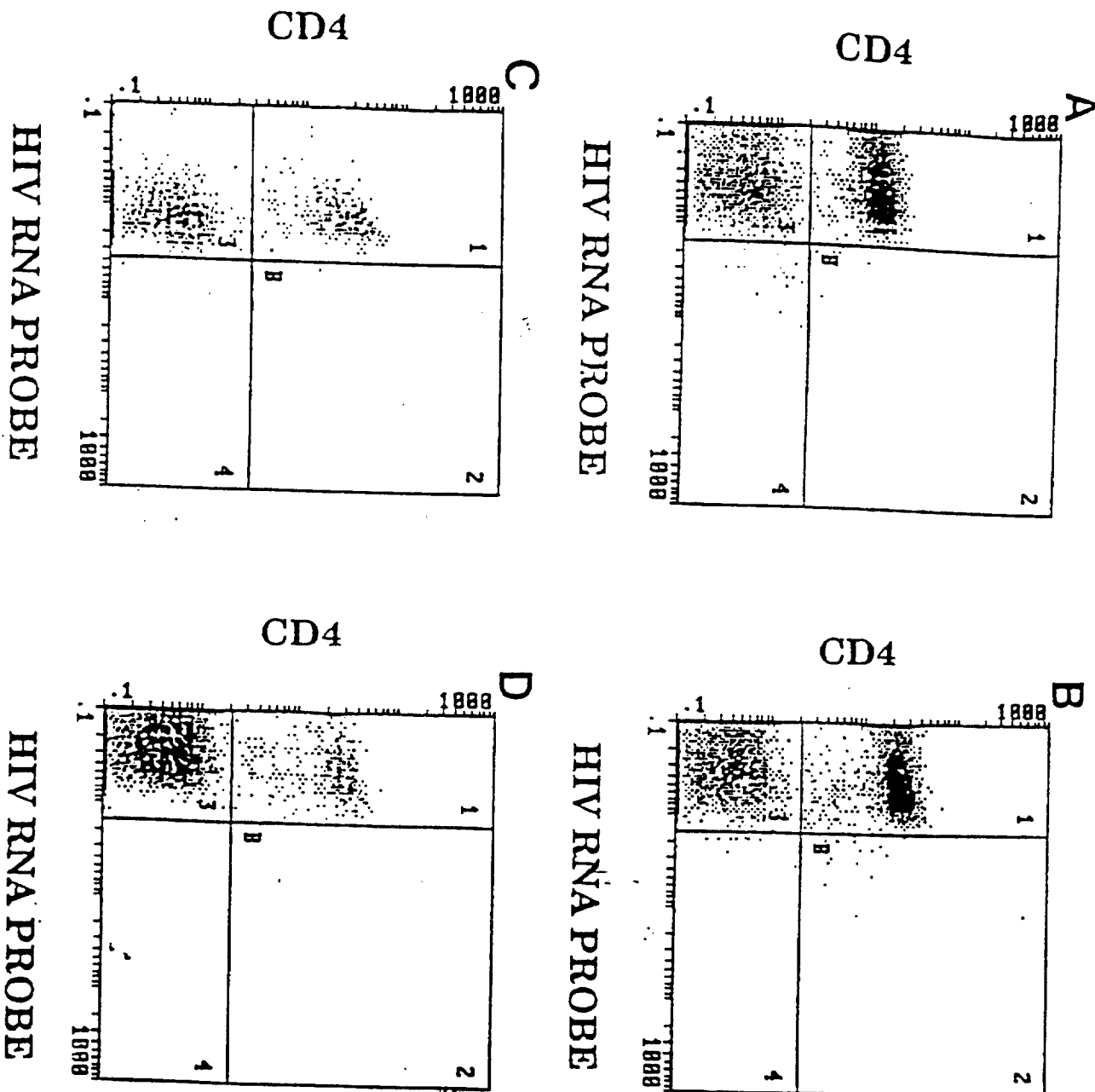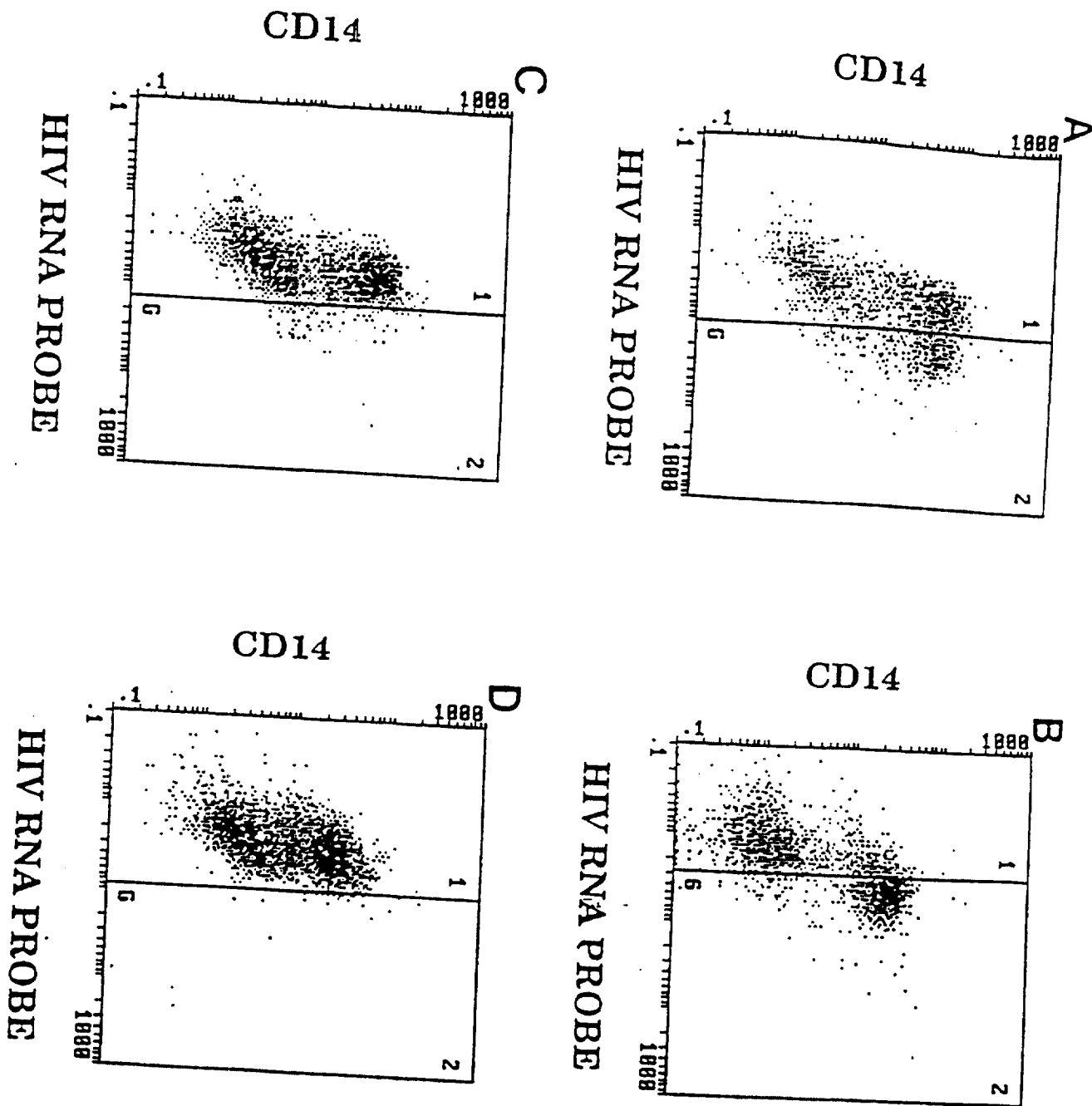
FIG. 1

FIG. 2a

FIG. 2b