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ANTIBODY TO THE RNA-DEPENDENT DNA POLYMERASE OF HTLV-III: CHARACTERIZATION AND CLINICAL ASSOCIATIONS

ANNUAL REPORT

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OCTOBER 1, 1991

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-87-C-7020

Cornell University Medical College
1300 York Avenue
New York, New York 10021

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1, 1991	Annual Report 15 Nov 88 - 14 Nov 89

4. TITLE AND SUBTITLE

Antibody to the RNA-Dependent DNA Polymerase of HTLV-III: Characterization and Clinical Associations

5. FUNDING NUMBERS

DAMD17-87-C-7020

603105A ✓
3M263105DH29 AB
DA311658

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS

RA 1; AIDS; Antibody; Clinical; HTLV-III; Infectious Diseases

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

1989 ANNUAL REPORT: DAMD 17-87-C-7020
"Antibody to the RNA dependent DNA polymerase of HIV"

Problem under study: The identification and characterization of anti-HIV reverse transcriptase antibodies in the sera of HIV seropositive individuals, and correlation of their levels with clinical status and defects in immune function.

In my Annual Report of 1987 we described the identification of a series of purified IgGs from HIV seropositive individuals capable of blocking the catalytic activity of HIV-associated reverse transcriptase. The specificity of these antibodies, lack of cross-reactivity with mammalian and prokaryotic DNA polymerases, and correlation with clinical health was described. In 1988 we had greatly expanded these studies. We prepared bulk quantities of these anti-polymerase IgGs, and these are on deposit with the NIH as:

AIDS Research and Reference Reagents Program
AIDS Program
NIAID, NIH
"Antibody to HIV reverse transcriptase"
Catalog number: 187.

We also received 78 (38 HIV seropositive) serum samples from Dr. Zvi Bentwich, Kaplan Hospital, Rehovot, Israel together with clinical and immunologic data, and correlated serum p24 antigen levels with anti-RT activity and clinical course.

Summary of progress in 1989

1. At the beginning of 1989 we received 150 coded serum samples from Dr. Robert Redfield of WRAIR, all of which are from HIV seropositive individuals at various clinical stages of infection. During this year, and hopefully continuing into a follow-up period, we have used some of these samples, as well as others derived from our previous cohorts, to design a synthetic peptide-based ELISA system for detection of anti-RT antibodies. This would enable us to avoid the laborious procedures needed for characterization of anti-RT activity by enzyme purification and enzyme inhibition.

2. As is apparent from our list of WRAIR-assisted publications, while our work with anti-polymerase antibodies was in progress we came to realize the importance of several biologic properties of HIV also relevant to immune intervention. We thus began investigating the biologic activity of consensus sequences for hormone responsive elements in the HIV-LTR, as a model for the investigation of certain steroid-based compounds which may have immunomodulatory activity as well as inhibit the induction of chronic viral infection. We noted one such substance, tamoxifen, explained in Goal 2, below.

1989-1990 PUBLICATIONS, ALL ACKNOWLEDGING USAMRDC SUPPORT AND RELEVANT TO THIS PROPOSAL:

1. Laurence J, Freidman SM, Chartash EK, Crow MK, Posnett DN. Human immunodeficiency virus infection of helper T cell clones Early proliferative defects despite intact antigen-specific recognition and interleukin 4 secretion. J Clin Invest 83:1843-48, 1989.

2. Laurence J, Sellers MB, Sikder SK. Effect of glucocorticoids on chronic human immunodeficiency virus (HIV) infection and HIV promoter-mediated transcription. Blood 74:291-297, 1989.

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Goal 1. Define linear pol epitopes recognized by sera from HIV infected individuals at early clinical stages.

In an attempt to define a linear sequence of HIV-1 pol which could be used in a serologic assay to quickly detect that subset of IgG antibodies capable of inhibiting the catalytic activity of RT, synthetic peptides were prepared from nucleotide sequences of HIV pol regions homologous to the catalytic sites of other mammalian polymerases. The initial peptides, ranging in length from 8 to 15 amino acids, were synthesized at The New York Blood Center by the solid-phase method using an automated peptide synthesizer (Applied Biosystems, Foster City, CA). Peptide resins were cleaved by hydrogen fluoride, extracted, and analyzed for purity by HPLC.

The five sequences we initially employed were:

Code	Sequence	aa Position	Activity	Reference
I	LDVGDAYF	109-116	NTP binding site	1
II	VLPQGWKGSP	158-157	conserved region	1
III	IQKLVGKLNW	257-266	conserved region	1
IV	PENPYNTPVFAIKK	219-232	conserved region	2
V	DSRNPLWKGPAKLLW	496-509	COOH terminus	2

ELISA. Peptide solutions of 10mg/ml in PBS were air drived overnight at 37^0C in polyvinyl, 96-well microtiter plates to yield 1-5 ug/well. Nonspecific binding sites were blocked with BSA. After a 6 h incubation at 25^0C and a 16h incubation at 4^0C, serum samples were added at 1:100, 1:200 and 1:400 dilutions. After 2 h at 25^0C the wells were washed 10-20 times with 0.2% Tween 20 in PBS, after which affinity-purified, alkaline phosphatase conjugated goat antibody to human IgG was added. After 2 h the plates were washed, and substrate solution was added. After a 30 in. incubation the reaction was stopped by adding 1N H2S04, and the optical densities were analyzed on an automated ELISA scanner. Seropositivity was defined as any value greater than three standard deviations obtained with negative controls.

SAMPLES. The samples analyzed included 100 sera obtained from our original longitudinal survey (1979-1985). An additional 150 samples have been obtained from Dr. Robert Redfield at WRAIR and are in the process of being analyzed.

A statistically significant but imperfect correlation was found between the presence of antibodies capable of blocking the catalytic activity of RT, as reported by our functional assay (3) and anti-peptide I, II or IV reactivity. The latter two residues correspond to the putative HIV catalytic site as determined by insertional mutagenesis (4,5). Interestingly, they are outside the regions of the pol gene thought to be immunogenic by hydrophobicity plots (6) or B cell eiptope computer analysis (7), yet are within the few discrete regions of pol predicted to be strong T cell epitopes (7).

The data, gathered on 100 HIV seropositive individuals at various clinical stages of infection and 15 seronegative controls were as follows:

0/15 HIV seropositive individuals were positive by ELISA with any of the 5 synthetic peptides.

38/100 HIV seropositive individuals were positive for one or more pol peptides. Of these 38, only 3 had WR stage 5-6 disease, with all others in the WR 1-2 category.

Of the 62 seropositive individuals who were non-reactive, all were more advanced than WR 2, and only 6 had anti-RT catalytic activity.

The raw data for the 38 reactive patient samples were:

Peptide No.	I	II	III	IV	V
No. sera +	31	26	3	8	3
% positive	81.6	68.4	7.9	21.1	7.9

Serologic Reactivity vs. RT Inhibitory Activity

	Serologic Activity to peptides II and/or IV	No serologic activity to peptides II and/or IV
No anti-RT activity	2	7
Anti-RT activity	20	9

Interestingly, Peptides III and V gave low frequencies of positive reactions similar to those for pol synthetic peptides selected on the basis of hydrophobicity plots (6).

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Goal 2. Investigation of certain steroids and steroid antagonists with anti-HIV and immunomodulatory activity.

A variety of nuclear proteins acting as positive or negative transcriptional regulators bind to specific sequences within the long terminal repeat (LTR) of the two subtypes of the human immunodeficiency virus, HIV-1 and HIV-2. Proceeding upstream from the start site, there are consensus sequences for the TATA binding factor, SP1, NF-kB, and other less well-studied factors (1). Downstream of the start site, overlapping the tat target, TAR, there are binding regions for at least two other molecules, nuclear factor-1 and leader binding protein (1). A change in the amount or activity of these cellular transcription factors is thought to underlie the sensitivity of HIV mRNA accumulation to a myriad of stimuli. These include signals as disparate as cytomegalovirus and herpes simplex virus co-infection, monokine and lymphokine treatment, antigenic stimulation, and phorbol ester exposure. All are capable of converting a latent or chronic HIV infection to a productive one (1), and many appear to involve protein kinase C (PKC) activation (2). These studies are particularly pertinent with regard to the use of immune modulators or vaccines, as cytokines and antigen may in some instances stimulate viral replication.

DNA sequences responsive to steroid hormone regulation have also been located near mammalian retroviral promoters (3). For example, the rate of transcription of mouse mammary tumor virus (MMTV) DNA is augmented by glucocorticoids via a mechanism that increases, rapidly and selectively, the utilization of a transcription initiation site within the MMTV-LTR (4). Progesterone also elevates MMTV RNA expression in vivo and in vitro, an effect that is synergistically enhanced by estradiol (5). Consenus sequences for such hormone responsive elements have similarly been identified in HIV (6, 7).

In preliminary reports, we had utilized model systems for induction of HIV-1 from chronically infected cells. Particularly, we have worked with non-transformed clonal CD4+ T cells (2) and subclones of two cell lines, the U937-derived promonocytic line U1.1 and the CD4+ T-cell derived line 8E5 (8, 9). The cell lines contain integrated provirus inducible by immunologic or chemical (PKC activators, demethylators) stimulation. We had established the PKC dependence of these systems, and defined their susceptibility to relatively toxic PKC inhibitors such as the isoquinolone H7, 2-aminopurine and staurosporine, and immunosuppressive adrenocortical steroids (7). Recent data with gonadal steroid antagonists such as tamoxifen, which share anti-PKC activity and immune enhancing properties, suggest that regulation of chronic infection with an immunotherapeutic may be a feasible goal in HIV disease.

Briefly, we have recently shown that induction of HIV from chronically infected T cells or monocytes could be blocked by the triphenylethylene anti-estrogen tamoxifen (TMX), at concentrations which did not affect cellular DNA synthetic

responses. This effect correlated with tamoxifen's ability to completely inhibit PMA enhancement of HIV-promoter mediated trans-activation in both monocytic and CD4+ T cells (Fig. 1). No interference with a primary infection was noted. Tamoxifen's mechanism of action may relate both to its capacity to inhibit PKC, as well as to consensus sequences for gonadal steroid responsive elelments in the HIV LTR. Thus, TMX inhibited HIV-LTR-directed tat activity with an IC_{50} of 2uM, and its IC_{50} for inhibition of PKC is 6.1 uM (10); typical serum levels of this drug in man are on the order of 2uM (11).

Manipulation of gonadal HREs in relationship both to viral regulation and immune modulation may be especially pertinent in HIV infection for several reasons. First, TMX has a variety of immunologic effects. In vitro modulation of human lymphocyte reactivity includes increases in pokeweed mitogen-driven immunoglobulin production, inhibition of CD8+ T suppressor cell but not CTL phenomena, and augmentation of natural killer cell activity (12). Second, a prominent manifestation of HIV infection, Kaposi's sarcoma, is most prevalent among men. A model for this disease, produced when tat under the control of the HIV LTR was introduced into the germline of mice, was elicited only in male transgenic animals, despite equivalent levels of tat mRNA expression their female counterparts (13). The possibility that this sexual difference in phenotype is hormonally based is under investigation. A further indication that hormonal therapy might have a therapeutic role in HIV infection was the preliminary observation that the synthetic progesterone megestrol acetate (Megace) led to significant improvements in appetite, lethargy, and body weight in some HIV infected patients (14), and is under clinical evaluation at Walter Reed.

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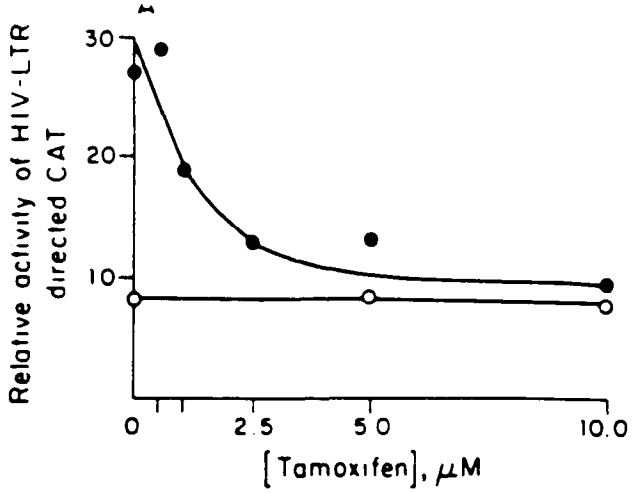

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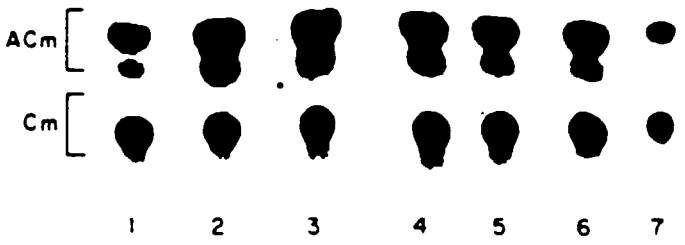

	1	2	3	4	5	6	7
Conversion (%)	16.9	57.8	62.0	39.0	26.0	28.1	19.7
PMA (50ng/ml)	-	+	+	+	+	+	+
TMX (μM)	0	0	0.5	1.0	2.5	5.0	10

Fig. 1. Effect of tamoxifen on PMA-mediated enhancement of HIV-LTR-CAT. The CAT plasmid (1μg DNA) was co-transfected into U1.1A cells (2×10^6) with a plasmid containing tat (1μg DNA). PMA (50 ng/ml) and/or TMX (1-10 uM) was present throughout the 48h culture period. The percent conversion of [^{14}C]chloramphenicol (Cm) to its acetylated forms (ACm) was determined.

A. Dose-response curve for inhibition of PMA-driven upregulation of HIV-LTR-CAT activity. This activity was measured in the presence (closed circles) and absence (open circles) of PMA.

B. Chromatographic data for conversion of Cm to ACm.

FUTURE PLANS

Goal 3: To strengthen our work in refining these epitopes, we sought to use recombinant peptides which extended the sequences derived from our preliminary data. We established a collaboration with Dr. Sean Nowlan, currently with Baxter-Travenol, Inc., who had described three highly immunogenic regions of pol:

Epitope designation	Amino acid residues	HIV-1 nucleotides	Provisional epitope map (amino acids)
rI	9-214	2101-2774	144-191
rII	191-335	2700-3134	214-335
rIII	335-567	3134-3832	511-536

These pol sequences, and 5 additional non-reactive recombinant peptides from pol which we have also received, will be valuable in refining the exact specificity of pol targets for both antibody and CTL.