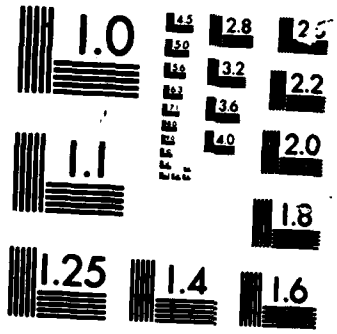
AD-A166 436 TRYPANOSOME SURFACE ANTIGEN GENES: ANALYSIS USING RECOMBINANT DNA(U) ISSAQUAH HEALTH RESEARCH INST WA K D STUART 27 MAR 85 DAMD17-82-C-2016 1/1

UNCLASSIFIED F/G 6/13 NL



AD _____

TRYPANOSOME SURFACE ANTIGEN GENES:

ANALYSIS USING RECOMBINANT DNA

ANNUAL REPORT

12/2/83 TO 8/31/84

KENNETH D. STUART, Ph.D.

March 27, 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland, 21701-5012

Contract No. DAMD17-82-C-2016

Issaquah Health Research Institute
1595 N.W. Gilman Blvd.
Issaquah, Washington 98027

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Issaquah Health Research Institute 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 1595 N.W. Gilman Blvd. Issaquah, Washington 98027	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-82-C-2016
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M162770; TASK NO. 870 AH; WORK UNIT ACCESSION NO. 008

11. TITLE (Include Security Classification)

Trypanosome Surface Antigen Genes; Analysis Using Recombinant DNA.

12. PERSONAL AUTHOR(S)
Kenneth D. Stuart, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 83/2/12 TO 84/8/31	1985 March 27	10

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	
06	13	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
African Trypanosomes, Surface Antigenes, Sleeping Sickness, Gene Cloning.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The objective of this project is to identify and isolate early (frequently expressed) variant antigenic types (VATs), and clone and characterize their expressed variant surface flycoprotein (VSG) genes. Numerous syringe passaged and cyclically transmitted, frequently expressed VATs have been isolated, monoclonal antibodies prepared to their VSGs, and the expressed VSG genes have been cloned. We have shown that many diverse stocks express VSG epitopes related to the early IsTat epitopes. The VSG gene organization in the genome and sequence organization has been characterized. We have confirmed sequence homology at the 3' terminus of the VSG genes and have discovered additional homology near the 5' terminus of unrelated VSG genes.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code) / 22c. OFFICE SYMBOL

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

SUMMARY

PURPOSE

The purpose of this study is to identify, isolate and characterize variant surface glycoprotein (VSG) genes expressed by frequently occurring variant antigenic types (VATs) of African trypanosomes.

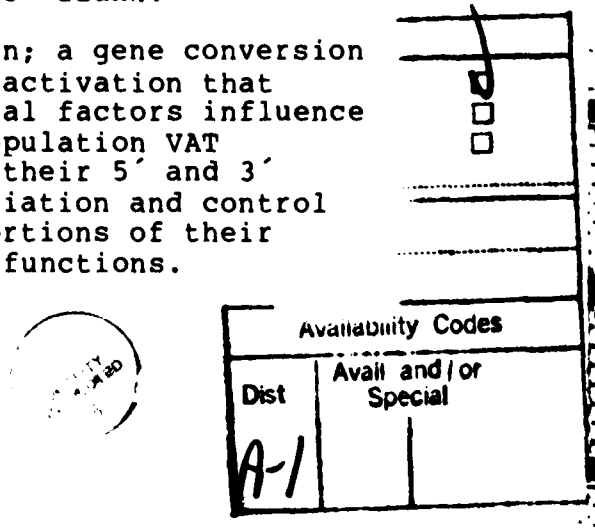
METHODS

VAT occurrence in relapses and other stocks was examined using VSG specific monoclonal antibodies which we prepared. The genomic organization of VSG genes was examined by molecular hybridization analyses combined with nuclease digestion and physical characterization experiments. The characteristics of VSG genes were examined by nucleotide sequence and molecular hybridization analyses.

RESULTS AND CONCLUSIONS

Early IsTaR 1 VATs occur frequently in IsTaR 1 relapses and initial and relapse infections of other stocks but not in initial or relapse populations of cyclically transmitted IsTaR 1. The early IsTar 1 VAT VSG genes have a telomeric location. Thus telomeric VSG genes appear to be preferentially expressed but telomeric VSG genes can be replaced by others by gene conversion altering the early VAT repertoire. Different VATs differ in their growth rates and switching frequencies. Thus, several factors influence the VAT composition of relapse populations. Most VSG genes appear to be intrachromosomal but many are telomeric and VSG genes are always telomeric when expressed. Many telomeric VSG genes are located on the 50-150 kb minichromosomes. All telomeric VSG genes have a characteristic sequence on their 5′ flank which we call the V sequence. This sequence may function in gene conversion since it occurs at the 5′ boundary of the gene conversion. We cannot yet exclude the possibility that the V sequence may have a role associated with control of VSG gene expression. Many, but not all, intrachromosomal VSG genes have the V sequence in their 5′ flank. All expressed VSG genes and most, but not all, other VSG genes conserve a characteristic sequence at their 3′ end. All the IsTaR 1 VSG genes also have conserved portions of their coding sequence. The telomeric VSG genes conserve additional sequences in their 3′ flank.

Two processes mediate antigenic variation; a gene conversion process and a process that we call telomeric activation that activates existing telomeric VSG genes. Several factors influence the sequence of VST occurrence and relapse population VAT composition. VSG genes conserve sequences in their 5′ and 3′ flanks which may have a role in antigenic variation and control of VSG gene expression. They also conserve portions of their coding sequences which may reflect conserved functions.



FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

AD _____

TRYPANOSOME SURFACE ANTIGEN GENES:

ANALYSIS USING RECOMBINANT DNA

ANNUAL REPORT

12/2/83 TO 8/31/84

KENNETH D. STUART, Ph.D.

March 27, 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland, 21701-5012

Contract No. DAMD17-82-C-2016

Issaquah Health Research Institute
1595 N.W. Gilman Blvd.
Issaquah, Washington 98027

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

(1.) OBJECTIVES

The overall objective of this project was to investigate the characteristics of variant surface antigen (VSG) genes in African trypanosomes in order to determine if they had common characteristics that might be of value for immunoprophylaxis. This integrated biological/immunological/molecular genetic project had the following specific objectives for the period of support:

1.1 To identify and characterize frequently occurring and cyclically transmitted variant antigenic types (VATs). These experiments were designed to identify factors that are important to the frequent occurrence of VATs.

1.2 Examine the VSG genes of frequently occurring VATs to seek common characteristics within these genes to determine if they contain common sequences.

1.3 To characterize the flanking sequences of early and later VAT VSG genes. These experiments are designed to determine the molecular basis for the frequent expression of some VSG genes.

1.4 Determine the occurrence of IsTar 1 VATs among various stocks of African trypanosomes. These studies are designed to determine if particular epitopes are conserved among various stocks of trypanosomes.

(2.) PROGRESS REPORT

We have found that the rate at which VATs switch to other VATs and the relative growth rates of the VATs are important factors in the order in which VATs are expressed. While we have no direct evidence for the basis of the growth rate differences, our studies suggest that the characteristics of sequences flanking VSG genes and the chromosomal location of VSG genes influences the switching frequencies. Using monoclonal antibodies we studied the relative growth rates and switching frequencies of VATs A, 1, 3, 5, 7, and 11 of the IsTaR 1 serodeme. We found that the A VAT was the predominant VAT since all the other VATs spontaneously switched to this VAT. The rate at which the VATs switched to A was characteristic to each VAT clone. The rate at which A VATs replaced the other VATs in these populations reflected the relative growth rates of the VATs and their switching frequency.

We have confirmed that the A, 1, 3, and 5 IsTat 1 VATs are early VATs in infections begun with the original A or 1 clones by the criterion of repeated occurrence in first relapse populations. VAT 7 occurs rarely in such first relapse populations and VAT 11 not at all. We have found that the frequency of expression of any VAT is dependent on the genomic organization and that both the genomic organization and frequency of expression can be altered. Whether or not a gene is located on a telomere (chromosome end) and which telomere appears to be one of the most important factors responsible for the frequent

expression of a VATs. Non telomeric VSG genes also differ with respect to their frequency of expression. This may reflect the characteristics of their flanking sequences.

Original IsTaR 1 clones have no telomeric 7 or 11 VSG genes but 7 VATs occur more frequently than 11 VATs illustrating that intrachromosomal VSG genes are expressed at differing frequencies. A telomeric 11 VSG gene is produced by gene conversion in VAT 11. This gene is inactivated but retained in a subsequent VAT (A^{11}). This clone now expresses 11 frequently in first relapse populations illustrating that the probability and order of VAT expression can be altered and that telomeric VSG genes are expressed more frequently than intrachromosomal VSG genes. All the VATs that we have examined contain a telomeric 5 VSG gene. As with VAT 11 a VAT 5 was isolated that contained a second 5 VSG genes that was created by gene conversion. This second gene was retained in an inactive state in a subsequent VAT (A^{5}). The additional 5 VSG gene did not increase the frequency of VAT 5 occurrence which was lower than that for VAT 11 from clone A^{11}. This illustrates that different telomeric VSG genes are expressed at different frequencies. VAT A is the most predominant VAT of the IsTaR 1 serodeme; it invariably represents 100% of early first relapse populations in lethally irradiated mice. We have isolated a VAT which has lost the telomeric A gene that is expressed in the A VATs. Although this clone retains an intrachromosomal A gene A VATs do not occur in first relapse populations from this VAT. This illustrates that the most predominant VATs can be changed. These results taken together suggest that the sequence of VAT expression is not fixed for a serodeme but that the sequence of expression reflects the genomic location of the VSG genes (which is in flux) and characteristics of the VSG genes.

We found that none of the VATs that were early VATs occurred in any relapses of cyclically transmitted clones of the IsTaR 1 serodeme. Genomic analyses have shown that while many of the VSG genes present in the clone before cyclic transmission are present after cyclic transmission, many genes are absent. This result is so surprising that we have sent several clones to Dr. Jenni (STIB-Switzerland) and to Dr. Esser (WRAIR) to repeat this experiment. The cyclically transmitted cells have not yet been returned to us. These results suggest that substantial genomic alteration may occur during cyclic transmission.

Molecular hybridization studies have revealed a sequence that is conserved within the coding sequence among all the expressed VSG genes that we cloned. This sequence is located between 30 and 600 nt from the 5′ end of the coding sequence. Our sequence analyses have not yet located this sequence. Our immunological studies using both monoclonal and polyclonal antibodies have not detected cross reaction among these VSGs suggesting that while nucleotide sequence is sufficiently conserved to detect molecular hybridization, this is not reflected in epitope conservation. Complementary studies have found sequences flanking VSG genes that are conserved. We have found

that a 5′ flanking sequence from a genomic clone containing a 5 VSG gene hybridizes to the 5′ flank of several but not all VSG genes. We have localized the sequence responsible for this hybridization and determined its nucleotide sequence. This sequence is an unusual sequence that contains a tandem (TAA)n repeat, followed by an alternating CG motif followed by an alternating AT motif. This sequence occurs in one or two tandem copies and the (TAA)n repeat occurs only once within each complex. The n can be as large as 109. This sequence occurs in the vicinity of the 5′ boundary of gene conversion and may represent a recognition sequence for this process. The variation among these sequences may contribute to the differences observed in the switching (gene conversion) frequencies among VSG genes. The VSG genes also have conserved sequences in their 3′ flanking sequences. These sequences have CCTC, AT rich, and 13 mer segments. These may represent the 3′ equivalent of the 5′ conserved sequence. Their lower degree of variation suggests that they may not influence switching frequency.

We have found that there are two processes that mediate antigenic switching (variation). One process is gene conversion which results in the duplication of a VSG gene and insertion of the gene copy near a telomere where it may be expressed. The second process that we call telomeric activation unlike gene conversion transcriptionally activates an alternate telomere and inactivates the previously expressed telomere. We have shown that these two processes are independent and that gene conversions that do not activate VSG genes can occur. These results confirm that there are two distinct mechanisms of antigenic variation; one requires recombination like processes while the other requires processes that activate a telomeric VSG gene while concomitantly inactivating another. These results also indicate that rearrangements can accumulate in the genome of African trypanosomes suggesting that the potential for VSG gene expression is subject to constant drift.

We have found that the 5′ flanks of VSG genes vary substantially both in their nucleotide sequence and the length of the sequence between the coding sequence and the barren region. For example, the 1 VSG gene has about 1.5 kb between the coding sequence and the barren region while the 11 VSG gene has over 5 kb. The restriction maps of these region are distinct and they do not cross hybridize. We have found that the 1 VSG gene is located on a 69 kb minichromosome and can be expressed on that chromosome. We have probed minichromosomes for the presence of sequences that occur in the 5′ flanks of VSG genes to determine what sequences are retained among VSG gene flanks and are essential for expression. We have found that minichromosomes lack the 35 nt spliced leader sequence and extensive TAA repeats but that they contain the 76 bp repeat in which the TAA repeat is often embedded. Thus, many ,if not all, expressed VSG genes retain 76 bp sequences but are not associated with 35 nt SL sequences or extensive TAA repeats. We have also probed minichromosomal DNA with a conserved telomeric sequence which contains within it the conserved repeated sequence CCCTAA. This

sequence hybridized with all size classes of chromosomes indicating that many, if not all, telomeres retain this sequence motif.

As reported in the 12/2/81-12/1/82 and 12/2/82-12/2/83 reports, we have observed the IsTaR 1 VSG gene sequences and the expression of VSGs that react with IsTaR 1 VSG specific monoclonal antibodies in other stocks. Thus, the genes that we are studying are not only expressed frequently in this IsTaR 1 serodeme but also by other strains from various regions of Africa.

DISTRIBUTION LIST

12 copies	Director Walter Reed Army Institute of Research Walter Reed Army Medical Center ATTN: SGRD-UWZ-C Washington, DC 20307-5100
4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, Maryland 21701-5012
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100

END

FILMED

5-86

DTIC