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TITLE: GENETICALLY-ENGINEERED POXVIRUSES AND THE
CONSTRUCTION OF LIVE RECOMBINANT VACCINES

PRINCIPAL INVESTIGATOR: Enzo Paoletti, Ph.D.

CONTRACTING ORGANIZATION: Albany State Health Laboratory
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) A survey of the transcript levels associated with vaccinia virus was performed. Specific and unique vaccinia promoters were identified by standard techniques and manipulated to drive the expression of foreign genes such as Beta lactosidase and glycoproteins. Promoters of all three classes (early, intermediate, late) were identified and studied. No single promoter was found to be exceptional. Most strong promoters C10LW, H6, 11K, IL3 gave good expression which fell within several fold of each other. No promoter was found that gave magnitude increased levels of expression. Generally, irrelevant proteins such as Beta galactosidase could be expressed at high levels sufficient to visualize on Coomassie stained gels. Unfortunately, this was not the case when relevant proteins such as viral glycoprotein were expressed. The additional factors that need to be involved for high level expression by vaccinia virus vectors are still unknown.					
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FOREWORD

In conducting research using animals, 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 (NIH Publication No. 86-23, Revised 1985).

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The Research Objectives of the Research Contract #DAMD17-85-C-5232 BASIC were twofold:

- i) To genetically engineer poxvirus vectors to optimize the expression of foreign gene(s) pertinent to the construction of live recombinant vaccines.
- ii) To genetically engineer poxvirus vectors to further attenuate the virus resulting in a vaccine vector that is safer than any currently available vaccine strain.

SUMMARY

- A) A survey of the transcript levels associated with vaccinia virus was performed. Specific and unique vaccinia promoters were identified by standard techniques and manipulated to drive the expression of foreign genes such as Beta lactosidase and glycoproteins. Promoters of all three classes (early, intermediate, late) were identified and studied. No single promoter was found to be exceptional. Most strong promoters C10LW, H6, 11K, IL3 gave good expression which fell within several fold of each other. No promoter was found that gave magnitude increased levels of expression. Generally, irrelevant proteins such as Beta galactosidase could be expressed at high levels sufficient to visualize on Coomassie stained gels. Unfortunately, this was not the case when relevant proteins such as viral glycoproteins were expressed. The "additional" factors that need to be involved for high level expression by vaccinia virus vectors are still unknown.

Significantly, however, with regard to vaccine formulation the selection of the proper temporally regulated promoter element may be more critical than the actual level of protein expression.

- B) The genetic engineering of the virus to construct an attenuated vector has been carried out in several ways with overall significant success.
- i) In order to genetically stabilize, i.e. to free the genome thus rendering it less susceptible to mutations, we looked for and investigated the genetic functions responsible for recombination. A genetic analysis of temperature sensitive mutants was performed to determine the genes involved in recombination. An unexpected finding was that the vaccinia virus DNA polymerase was involved in recombination at a level other than DNA synthesis and replication. This fact obviously complicates manipulation of the vaccinia virus recombination functions since the DNA polymerase is an essential viral gene.
 - ii) A DNA ligase function with obvious implications in recombination was identified. It was shown to be an early protein. Genetic manipulation revealed that it was not an essential gene for the virus. This unexpected result reveals an unprecedented complexity in the recombinational machinery of poxviruses.
 - iii) A number of regulatory genes responsible for host cell restriction of growth in tissue culture were identified and manipulated. These genes specifying viral replication in tissue culture cells of human, porcine and rabbit cells were described. Manipulation

of these genes, for example differential deletion analysis, revealed that attenuation of the virus could be affected without significantly diminishing the ability of the virus to act as a vector for vaccination purposes.

Manipulation of the host range genes also provided for the cloning and expression of foreign genes in vaccinia virus vectors that greatly simplified and accelerated the construction and analysis of vaccinia recombinants.

iv) In order to set a baseline and to have available the organizational information of the genetic basis of the vaccinia virus the entire DNA genome was sequenced. This analysis revealed that vaccinia virus has 198 open reading frames and 191,636 base pairs in the entire genome. This information will be invaluable for understanding the biology of the virus.

v) Partly due to the availability of the DNA sequence, a number of genes have been deleted from the virus. Many of these genes have been implicated in undue virulence. Hence such manipulations have resulted in greatly attenuated vaccinia virus strains that have been shown in preclinical studies to retain their efficacy for vaccination purposes. To date, more than sixty (60) genes have been deleted from the virus. Significantly, such an analysis has allowed us to manipulate (genetically) vaccinia virus such that it is now susceptible to antiviral intervention via conventional, well defined and therapeutically acceptable methods.

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PATENT APPLICATION

The contract support was noted in a filed patent application,

"Recombinant Poxvirus Host Range Selection System."

PERSONNEL

Personnel receiving support from the contract were:

Dr. Elizabeth Norton
Dr. Gerard Johnson