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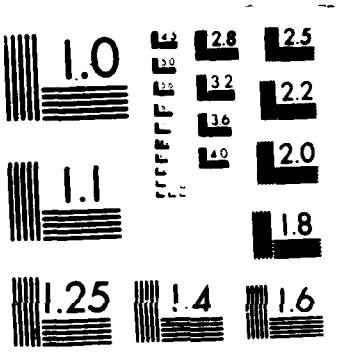

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IMMUNOLOGIC AND GENETIC SELECTION OF ADENOVIRUS VACCINE STRAINS: Synthesis and Characterization of Adenovirus Antigens

Annual Report

by

Harold S. Ginsberg, M.D.

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(For the period January 1981-January 1982)

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

H7ts88 replicates in 293 cells at 39.5°C although it physically maps in the L1 region of the genome, which encodes the IIIa protein. However, it expresses E1a and E1b gene products normally. At 39.5°C it produces large amounts of the hexon protein, which is responsible for neuralizing antibodies, as well as all other functional late proteins except IIIa, but it does not produce infectious virus at the non-permissive temperature.

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The penton base (protein III) and the fiber (protein II) were characterized, which had not been previously done. The penton base had a molecular weight of 68,000 and the fiber had a molecular weight of 30,000 to 33,000. Previously, the fiber had been identified incorrectly.

Fifty conditionally lethal, temperature-sensitive (ts) mutants of the type 7 adenovirus vaccine strain have been isolated and characterized. Genetic analyses indicate that forty-nine of the mutants could be divided into 10 complementation groups. Selected mutants from each group were localized on the adenovirus genome by recombination and marker rescue analyses. The mutations were predominantly located in the late transcription regions L1, L2, and L3 which code for the 55-58K and IIIa proteins (L1), the penton base and pV1 (L2), and hexon protein (L3). It was striking that only one mutation involved an early gene product, the DNA-binding protein. The IIIa protein mutants are potentially excellent candidates for use in an attenuated, live virus vaccine since it produces large amounts of hexons which is the primary antigen responsible for neutralizing antibodies. Indeed, these mutants produce as much immunologically reactive hexons, fiber, and penton base as does wild-type 7 adenovirus. This contract period was devoted to characterization of these mutants in greater detail, particularly H7ts88.

19., con't.

Penton base protein
Fiber protein
293 cells

ANNUAL REPORT
1981-1982

ABSTRACT

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Growth of H7ts88 on 293 cells at 39.5oC

The mutation in H7ts88 appeared to be suppressed at the non-permissive temperature when the mutant was grown in 293 cells (human embryonic kidney cells transformed by Ad5 DNA, which constitutively express early genes 1a and 1b): the yield at 39.5oC was only 20- to 50-fold lower than that at 32oC. In addition, similar but less marked effect was observed in GPT+ KB cell line 16 (KB cells expressing the left-most 15% of Ad5 genome) and GPT+ KB cell line 8 (KB cells expressing the left-most 4.5% of Ad5 genome), but not in GPT+ KB cell line 18 (KB cells which contain and express only Ad5 genes located between map co-ordinates 4.5 and 15.5 on the viral genome), suggesting that the suppression of the H7ts88 mutation was due to the constitutive expression of adenovirus early gene 1a (E1a) products. It should be noted that H7ts88 appears to make functional E1a and E1b products, since it complements type 5 adenovirus mutants known to have large deletions in this region of the genome (Ad5 d1312, d1313, d1314 and sub 315). Moreover, the mutation in H7ts88 has been mapped to lie between map co-ordinates 34 and 36.7 on the viral genome. In contrast, other mutants in the same complementation group, and which map in the same area of the genome, fail to grow on 293 cells at 39.5oC. Thus, H7ts88 maps in the gene encoding the IIIa protein and appears to be an ideal candidate to be employed in a vaccine. Infection results in high yields of hexons, the critical antigen for production of protective antibodies; but infectious virus is not made in large amounts, and thus viral spread is unlikely. Unfortunately transformation studies could not be completed owing to cancellation of the contract.

Identification of the type 7 adenovirus penton base and fiber polypeptides.

The structural proteins of Ad7 had not been fully characterized when this study began, and their electrophoretic pattern in SDS-polyacrylamide was an essential prerequisite to phenotypic characterization of Ad7 mutants. Determination of the size of the fiber polypeptide became a particular problem early in these studies, when it was discovered that it was much smaller than the fiber polypeptide of type 2 and type 5 adenoviruses. The prototype strain of type 7 adenovirus, the Gomen strain, was included in these studies for comparison. The following data indicated that the molecular weight of the fiber polypeptide of the vaccine strain is approximately 30,000, and that of Gomen strain is 33,000, while the molecular weight of the penton base of the two strains is approximately 68,000:

1. Pentons released from purified Ad7 virions by dialysis against 0.005M tris-maleate buffer, pH 6.2, contained two polypeptides. The larger of these had a molecular weight of 68,000 and was trypsin-sensitive, suggesting that it was the penton base. The smaller polypeptide had a molecular weight of 30,000 or 33,000, in the case of vaccine strain and the Gomen strain, respectively. Moreover, the same two polypeptides were present in the particles which banded at a buoyant density of 1.26gms/ml in equilibrium CsCl gradients and were considered to be aggregates of 12 pentons (so-called "dodecons").

2. Cytoplasmic RNA was obtained from vaccine strain- and from Gomen strain-infected KB cells late in infection. Specific RNA, selected by hybridization to DNA fragments containing the fiber gene (Ad7 vaccine strain DNA restriction fragment EcoRI-B, 87-100 map units, or Gomen strain DNA fragment BamHI-D, 83.1 - 92.9, map units) was translated *in vitro* using the rabbit reticulocyte reaction mixture. The resulting product was a single polypeptide of 30,000 or 33,000 daltons when the RNA was selected from vaccine strain- or Gomen strain-infected cells, respectively, regardless of which of the two DNA fragments was used in the selection procedure.

1. Praszkier, J., and Ginsberg, H.S. Isolation and characterization of conditionally lethal, temperature-sensitive mutants of type 7 adenovirus. J. Virology. In Press.

2. Praszkier, J., and Ginsberg, H.S. Characterization of a L1 temperature-sensitive mutant of type 7 adenovirus. (Submitted for publication).

3. Praszkier, J., and Ginsberg, H.S. Characterization of the type 7 adenovirus fiber protein. (Submitted for publication).

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