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Project Summary

Investigation on the Potential Environmental Hazards of Pesticidal Viruses

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Due to the environmental and ecological effects of toxic chemical pesticides, the usage of insect viruses have been considered as one of the alternatives for the control of agriculture insect pests. In fact in the past 3 decades, several baculoviruses have been used as viral pesticides for pest control. These viruses have not been demonstrated to be hazardous to nontarget organisms using the classical infectivity and morphological alteration as measuring factors. In this research project, molecular biological approaches were used to characterize the molecular structure of one of the insect viruses in order to investigate and elucidate the possible pathogenicity and oncogenicity of pesticidal viruses to human and other mammals at *in vitro* level. The study suggests that the pesticidal virus Spodoptera Frugiperda (SF) can not productively infect human fibroblast or HEP-2 cell lines and cannot induce morphological transformation of human fibroblast.

Besides the study on the biopathology of a pesticidal virus, *Spodoptera fragiperda* nuclear polyhedrosis virus (SfNPV), the molecular structure of the genome of this virus was also extensively studied in developing nonhazardous universal pesticidal viruses. The complete set of virus DNA fragments have been cloned in pBR322 plasmid. This set of the recombinant plasmid is now available for further gene function study.

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This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

In recent years, there has been great interest in industry and government in searching for the possible usage of insect viruses as an alternative to chemical pesticides to control agricultural insect pests. The impetus to use viral pesticides is based on the environmental and ecological effects of toxic chemical pesticides. For example, the three most commonly used chemical insecticides, methylparathion, malathion, and carbaryl, are highly toxic and teratogenic to mammals. Others such as DDT, are extremely stable in nature. The accumulation of residual stable chemicals pose a great problem on environmental health.

The nuclear polyhedrosis viruses (NPVs) are known to be pathogenic to invertebrates. This group of viruses causes lethal disease in their insect hosts. The virus particles of this group usually consist of enveloped nucleocapsids which frequent-

ly are included in a large protein lattice or polyhedron. The nucleocapsids are rod-shaped with dimensions of about 250 x 50 mμ, and are usually singly enveloped; but in some instances, more than one nucleocapsid can occur within one virus envelope. Viral genomes of this group were found to contain covalently closed supercoiled double-stranded DNA with a molecular weight of approximately 75 to 100 x 10^6 daltons. These viruses comprise the best known insect viruses. All together, the NPVs have been found in more than 200 species of Lepidoptera, in 20 species of Hymenoptera, and in 9 species of Diptera. They are all in the genus Baculovirus.

These bacilliform viruses replicate in the nuclei of the infected cells. During the process of infection, a substantial portion of virions is enveloped and subsequently occluded in the protein matrix of polyhedra. The intact polyhedra are not infectious in *in vitro* insect cell cultures, but they are the key "vector" by which virus infections are transmitted in nature. When insect larvae ingest the polyhedra, the infectious virions are released from polyhedra through the solubilization of the protein matrix of polyhedra in the alkaline environment and by enzymatic digestion in larvae gut. The purified NPV DNA was proved to be infectious in the insect cell cultures.

Viral Pesticides

Several baculoviruses have been used as viral pesticides for pest control during the last 3 decades; e.g., the NPV of the Alfalfa caterpillar (*Colias eurytheme*); the NPV of cabbage looper (*Trichoplasia ni*); the NPV of the beet army worm (*Spodoptera exigua*) and NPVs isolated from sawflies for forest protection in the USA and Canada. *T. ni* was introduced to Columbia, South America from California and has been used with great success in recent years.

Four NPV viral insecticides have been registered in the USA, and are commercially available for field application. The first, "Elcar", containing the NPV of the boll worm (*Heliothis zea*) is registered by the pharmaceutical firm Sandoz, Inc., for the control of cotton bollworm. The second available product named "TM Bioctrol 1" is registered by the US Forest Service, and contains the NPV of the Douglas fir tussock moth (*Orgyia pseudotsugata*). The NPV of the Gypsy Moth (*Porthetria dispar*) and NPV of pine sawfly (*Neodiprion sertifer*) are two other NPVs which have been registered.

With the hazardous environmental deterioration by chemical pesticides, and with urgent need for promoting the world food production in mind, the use of viral pesticides might be conceptually a practical and useful approach. But before any great revolutionary events happen, a precise evaluation of the benefit as well as the potential environmental health problem exhibited by this approach should be made. It is estimated that in the western hemisphere, 30% of the current pest problems in agricultural crop production can be treated with viral pesticides. In California among the pest species group causing major crop losses, 46% are susceptible to baculoviruses. Theoretically, viral pesticides can effectively solve certain problems such as toxic chemical pollution and inefficiencies of certain chemical pesticides in crop production. As far as safety and environmental health is concerned, relative amounts of *in vivo* and *in vitro* tests have been performed. But most of the tests applied used acute infectivity, antigenicity, and morphological alteration as measuring factors. The fate of viral DNA, possibilities of genetic recombination and viral gene integration, viral oncogenicity as well as low level of persistent infection have never been extensively examined.

Potential Hazards

There are several important considerations and noteworthy facts to be carefully examined and evaluated. First, the candidate pesticidal virus may infect insect hosts other than the target pest. Second, insect virus may be able to induce infection in other invertebrate or even vertebrate via either permissive or abortive infection. Third, as the consequence of persistent infection or non-fetal infection, the insects are known to be carriers of a variety of animal aborviruses. Pesticidal virus might follow the same pattern, and introduce itself into human beings or other vertebrate through its vector host by an unnatural route. Fourth, the so-called host specificity in virology is neither a fundamental nor a stable characteristic; the condition of the host and the nature of infectious agent (intact virion or naked DNA) will affect the entire susceptibility to infection. Although numerous *in vitro* and *in vivo* experiments have been done to prove the species specificity and the safety of pesticide virus, the striking report of transfection of Fogh-Lund human amnion cell with the silk worm NPV-DNA and the demonstration of viral DNA and antigens in vertebrate cells have raised the question of species specific and real meaning of safety as monitor solely by the infectivity and cytopath effect. Furthermore, various cocarcin gens and tumor promoting agents, su as phorbol ester, which probably ex widely in nature, might induce an une pected virus and host interaction whi might lead to the oncogenic transform tion of cells infected by pesticidal viruse

Detection and Molecular Interaction

In the application of pesticidal viruse two important issues require immedia attention. First of all, it is essential improve the methodology and sensitiv in detecting virus and host cell (includi vertebrate cell and human cell) intera tion at the molecular level and effects cocarcinogen on virus and host cell inte action; the alternative way of virus infe tion, the fate of viral DNA, possible vir gene integration and recombination, vir oncogenicity and persistent infectic require a molecular biological method detection and observation other tha infectivity assay. Secondly, the structur function, and genetic relatedness baculovirus have to be carefully studie and examined; a universal pesticid virus or a multifunctional pesticidal viru may be constructed.

Other than the classic methods detection and analysis, there are sever recent major technical approaches whic can be applied to insect virus systems ar will add a great impact to the understan ing of viral genome status, gene stru ture, gene function, and pathogenesi Such as:

(a) Nucleic acid hybridization (inclu ing DNA-DNA reassociation kine ics analysis, *in situ* (RNA-DN cytohybridization, Southern's bl hybridization, etc.

Detection of viral DNA, defective c non-defective, can be achieved by DNA DNA reassociation kinetics analysis. Us ing highly specific radioactive viral DN probes, it has been possible to dete small numbers of copies or portions c viral genomes in the DNA isolated fror cells suspected of carrying viral informa tion. It does not matter whether viral DN is replicating or defective, integrated c plasmid, biologically active or latent. Thi technique is able to tell the degree c homology and relatedness between tw viruses or two individuals. The degree c viral gene expression, in regard to trans

criptional mRNA, can also be detected by this technique.

As far as localization of viral nucleic acid and detection of susceptible cell types is concerned, the technique of *in situ* RNA-DNA cytohybridization will fulfill the goal. The great advantage of this technique is its ability to localize virus-specific DNA or RNA according to cell type and intracellular location by autoradiography. In combination with these nucleic acid hybridization techniques, a more advanced study of the interaction of insecticidal virus with the mammalian cell, especially human cells, can be achieved.

(b)Restriction endonuclease and specific DNA fragmentation.

The DNA fragmentation by restriction endonuclease has become a very powerful tool for analyzing not only small viral genomes but also genomes of increasing complexity and molecular size. Cleavage of DNA into specific terminal fragments and construction of a DNA fragment map will provide elements needed for the detailed characterization of viral genome, and also for the regulation of gene transcription and gene interaction. The restriction enzyme cleavage pattern will also provide a detailed comparison of strain variation and strain relatedness.

In adenovirus system, by DNA fragment transfection and DNA-DNA reassociation kinetics analysis (using restriction endonuclease fragments as probe), it was found that only the extreme left-hand 7% of the adenovirus type 2 DNA is sufficient for transformation of rat kidney cell *in vitro*. The EcoR1-C fragment, the left 16% of the viral genome, of adenovirus type 12 DNA has been proved to carry a transforming gene, and was used as a powerful probe for the study of the association of adenovirus type 2 with various types of human cancer.

The structure and function of several viral genomes such as ΦX174, SV40, adenovirus, etc., have been elucidated by the application of restriction endonucleases. Using DNA fragments generated by various restriction enzymes and nucleic acid hybridization techniques, the virus gene expression and gene regulation in SV40 and adenovirus-infected permissive and non-permissive cells have been defined. The utilization of restriction endonuclease and nucleic acid hybridization in the human cytomegalovirus system has been very successfully performed in our laboratory. We feel that these techniques can be effectively applied to study gene interaction and gene expression in pesticidal virus-infected permissive and non-permissive cells

(c) Transfection of viral DNA using calcium phosphate and dimethylsulfoxide (DMSO).

Viral infection can be initiated in an alternate route in an *in vitro* system. By infection of cells treated with calcium phosphate and DMSO, adenovirus DNA and herpes simplex DNA have been proved to be infectious. It is not necessary to have intact virus particles to initiate the infection process. Transformation of rat cells by DNA of adenovirus type 5 was also achieved by this method. As mentioned above, the specific DNA fragment carrying the transforming gene has also been detected by calcium phosphate method. Using this technique to advantage, there is an urgent need for the examination of the biological activity of pesticidal viral DNA. Mass application of pesticidal virus will generate numerous defective or naked DNA and on some occasions these particles might become a potential environmental hazard and dangerous to human health.

(d) Gene cloning and recombinant DNA technology.

Gene cloning and recombinant DNA technology has become a revolutionary tool not only for the study of molecular biology but also for industrial application. Numerous genes of biochemical and genetic interest have been isolated and studied due to the achievements of recombinant DNA research. Virus genomes can be constructed and amplified *in vitro* without the natural hosts, and a wide host range, non-hazardous pesticidal virus might therefore be constructed with a minimum risk to health and environment.

Summary

This Summary contains three main elements which reflect the work performed with the support of a grant from EPA: the interaction of SfNPV with various mammalian cells *in vitro*, the genomic structure of SfNPV, and cloning of SfNPV DNA (Hind III fragments) in plasmid pBR322. The details are described in the full report.

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The complete report, entitled "Investigation on the Potential Environmental Hazards of Pesticidal Viruses," (Order No. PB 85-242 527/AS; Cost: $11.95, subject to change) will be available only from:
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