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FINAL REPORT

PROJECT NO. IS-2707-96

**Molecular Interactions between Herpes and
Retroviruses in Dually Infected Chickens and Turkeys**

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**Project Title: MOLECULAR INTERACTIONS BETWEEN HERPES AND
RETROVIRUSES IN DUALY INFECTED CHICKENS AND TURKEYS**

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**Keywords: In vivo, molecular integration, avian oncogenic viruses, Marek's disease virus,
avian retroviruses.**

Abbreviations commonly used in the report, in alphabetical order:

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Authorizing Official, Principal Institution**

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	2	6	4	12
Submitted, in review, in preparation	1		1	2
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses			1	1
Abstracts	1		4	5
Not refereed (proceedings, reports, etc.)	2		3	5

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1	1	3
Longer Visits (Sabbaticals)				

Description of Cooperation:

Continuous contacts by e-mail were kept throughout the entire project. Dr. Witter met Davidson in August 1997 at the AVMP/AAAP Annual Meeting, and in October 1997 at an Intl. Symp. at IAH UK; Drs. Kung and Davidson met in July 1998 at the MDV Intl. Workshop Smolnice, Slovak Republic. The three collaborators met in May 1999 in Sacramento, CA, USA for one week to presented the findings and discuss the project, and a special workshop was organized by Dr. H-J Kung. Dr. Kung provided Davidson primers for the MDV hotspots and Dr. Davidson sent several DNA of "chimeric molecules" to Dr. H-J Kung for cloning and sequencing. Dr. Witter provided Dr. Davidson the recombinant virus RM1 DNA and performed three in vivo experiment of dual infection with prototype MDV and ALV-J and sent the samples to Dr. Davidson for analysis. Six joint publications resulted from the cooperation.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

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Project Title:

**MOLECULAR INTERACTIONS BETWEEN HERPES AND RETROVIRUSES
IN DUALY INFECTED CHICKENS AND TURKEYS**

Abstract

Tumors in commercial poultry are caused mainly by infection with avian herpes and retroviruses, the herpesvirus Marek's disease virus (MDV) and the retroviruses, reticuloendotheliosis (REV), lymphoid leukosis, subgroups A-I and J (ALV and ALV-J) in chickens, or lymphoproliferative disease (LPDV) in turkeys. Infection with one virus aggravates the clinical outcome of birds that are already infected by another oncogenic virus. As these viruses do not interfere for infection, MDV and one or more retroviruses can infect the same flock, the same bird and the same cell. While infecting the same cell, herpes and retroviruses might interact in at least three ways: a) Integration of retrovirus genomes, or genomic fragments (mainly the LTR) into MDV; b) alteration of LTR-driven expression of retroviral genes by MDV immediate-early genes, and c) by herpesvirus induced cellular transcriptional factors.

The first type of molecular interaction have been demonstrated to happen efficiently *in vitro* by Dr. Kung, in cases multiple infection of cell cultures with MDV and REV or MDV and ALV. Moreover, Dr. Witter showed that an *in vitro*-created recombinant, RM1, had altered *in vitro* replication and *in vivo* biological properties. A more comprehensive characterization of RM1 was carried out in the present project. We sought to highlight whether events of such integrations occur also in the bird, *in vivo*. For that, we had first to determine the prevalence of dually-infected individual birds in commercial flocks, as no systematic survey has been yet reported. Surprisingly, about 25% of the commercial flocks infected with avian oncogenic viruses had a multiple virus infection and 5% of the total samples analysed had multiple virus sequences. Then, we aimed to evaluate and characterize biologically and molecularly the resulting recombinants, if formed, and to analyse the factors that affect these events (virus strains, type and age of birds and time interval between the infection with both viruses). The perception of retrovirus insertions into herpesviruses *in vivo* is not banal, as the *in vivo* and *in vitro* systems differ in the viral-target cells, lymphocytes or fibroblasts, in the MDV-replicative type, transforming or productive, and the immune system presence.

We realized that previous methods employed to study *in vitro* created recombinant viruses were not adequate for the study of samples taken directly from the bird. Therefore, the Hot Spot-combined PCR was developed based on the molecularly known RM1 virus. Also, the PFGE that was used for tissue cultured-MDV separation was inefficient for separating MDV from organs, but useful with feather tips as a source of bird original MDV. Much attention was dedicated now to feathers, because if a recombinant virus would be formed *in vivo*, its biological significance would be evident by horizontal dissemination through the feathers. Major findings were: a) not only *in vitro*, but also *in vivo* MDV and retrovirus co-infections lead to LTR integrations into MDV. That was shown by the detection of chimeric molecules. These appeared in low quantities and as quasispecies, thus interfering with sequence analysis of cloned gel-purified chimeric molecules. Mainly inserts were located in the repeat long MDV fragments. In field birds chimeric molecules were detected at a lower frequency (2.5%) than in experimentally infected birds (30-50%). These could be transmitted experimentally to another birds by inoculation with chimeric molecules containing blood. Several types of chimeric molecules were formed, and same types were detected in birds infected by a second round. To reproduce viral integrations, *in vivo* infection trials were done with field inoculae that contained both viruses, but the chimeric molecule yield was undetectable.

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Achievements

Significance of main scientific achievements or innovations

With the support of this grant, we have provided conclusive evidence that recombination between retrovirus and herpesvirus can occur at a detectable frequency. In a broader context, this has far-reaching implications in the generation of new pathogens and in the evolution of viruses. In a narrower context, RM1 promises to be an extremely interesting virus to reveal the MDV properties related to vaccine, in vivo replication and latency. The 100% protectivity of RM1 against very virulent strains of MDV provides a framework to identify genes implicated in vaccination. The fact that RM1 replicates extremely well in thymus allows probing the genes involved in the tropism of virus. The LTR insertion could transcriptionally activate a number of adjacent genes, by virtue of its strong enhancer sequences, which function in an orientation and position-independent manner to enhance transcription. Future work will involve the definition of those genes activated by LTR and elucidation their functions in in vivo replication.

The issue of retroviral sequence integration into herpesviruses *in vivo*, in cases of double virus-infection is of a wide significance in general virology and veterinary medicine and also represents a special case of gene transposition. We aimed to determine occurrence of such integrations *in vivo* by following the presence of chimeric molecules. Several conditions were analysed: a) Commercial birds that acquired naturally a mixed infection; b) Experimentally infected chickens with prototype strains of MDV and ALV-J; c) Commercial chickens infected experimentally with virus inoculae obtained from commercial cases of double infection with MDV and ALV-J, in the same flock or the same bird. In the two first categories we found that integration events happened at various rates, depending on the experimental system used. While in commercial flocks the event was limited (about 2.5% of the 2926 DNA samples), it reached a 30-50% rate in experimentally-infected birds with prototype viruses, and was undetectable in experimentally-infected birds with field inoculae. It seems that by increasing the virus adaptation to laboratory conditions, the rate of retrovirus LTR integration into MDV increases, as judged by the extent of chimeric molecules.

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Unlike *in vitro*, where recombinant viruses were separated by several rounds of plaque purifications and limiting dilutions, the *in vivo* situation differs; many different events occurred simultaneously in the same bird and normally each cell produces many herpes virions. Now various molecules were formed and detected in the same DNA preparation, therefore recombinant virus isolation is problematic. Only a biological advantage would enable a recombinant virus to dominate in an infected bird. As the feather follicles are the site of MDV productive replication and dissemination, the feather tip DNA was studied in regard to evidences of retro-MDV integration events that would be evident by the presence of chimeric molecules.

We showed that commercial poultry co-infections not only have the potential for a collective clinical influence, but also can result in the emergence of recombinant viruses, possibly with unexpected biological properties. In the course of the present study two incidence of molecular interaction that happened between two avian viruses were revealed; the emergence of ALV-J, as a consequence of endogenous and exogenous ALVs, and the REV integration into the large DNA virus, pox.

The analysis of retroviral integration into MDV in an *in vivo* situation, in which the co-infection was sequential is novel. We showed that chimeric molecules containing retroviral sequences were produced in the experimental MDV and ALV-J co-infected birds, and each bird contained more than one type of chimeric molecules.

As former findings enlightened the *cis* type of herpes-retrovirus molecular interactions, the *trans* type, the Meq/jun oncoproteins were also explored.

Agricultural and/or economic impacts of the research findings

Retrovirus integrations into MDV might lead to the emergence of new MDVs with altered properties, including tropism, spreading pattern, pathogenicity and protection by vaccination. The present study was aimed to raise the consciousness to various aspects of herpes-retrovirus integration issue in poultry. We focused and highlighted that possibility in various lectures and publications, so if unusual phenomena would occur in commercial flocks, integration analysis using the knowledge and methods that were established in the course of the present project might be considered. The study of the

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retro-herpes integrations in the avian system is important for agriculture, but also provides a natural model for analyzing the fluidity and the gene shuffling among species, that might reflect on the general virology.

Details of cooperation

The overall project objective was to analyse and characterize the extent of integration events that occur in the bird, *in vivo*. We focused on commercial flocks that were naturally-infected and on experimental trials. The cooperation among the three collaborators was vital in order to accomplish these tasks: Dr. Witter performed at ADOL the three trials of experimental infection with MDV and ALV-J and sent the samples for analysis to Dr. Davidson, and Dr. Kung collaborated in the development of the HS-cPCR assay and molecular analysis, thus making possible screening of numerous samples.

List of Publications

Articles

Borenshtain, R. and Davidson, I. (1999). Development of the hot spot-combined PCR assay for detection of retroviral insertions into Marek's disease virus. *J. of Virol. Methods* 82: 119-127. (Ch. 2 of the appendix).

Borenshtain R. and Davidson I. (2001) Marek's disease virus genome separation from feather tip extracts by Pulsed Field Gel Electrophoresis. *J. Virol. Meth.* In Press. (Ch. 6 of the appendix).

Davidson, I. and R. Borenshtain (1999) Multiple infection of chickens and turkeys with avian oncogenic viruses: Prevalence and molecular analysis. *Acta Virologica* 43: 136-142.

Davidson I. and R. Borenshtain (2001) *In vivo* events of retroviral LTR integration into MDV in commercial poultry: Detection of chimeric molecules as a marker. *Avian Diseases* 45: 102-121. (Ch. 3 of the appendix).

Davidson, I., R. Borenshtain, H-J Kung and R. L. Witter (2001) Molecular indications for *in vivo* integration of the ALV, subgroup J-long terminal repeat into the Marek's disease virus in experimentally dually-infected chickens. *Virus Genes*, In Press. (note: Ch. 4 of the appendix).

Davidson I. and R. Borenshtain (2001) The feather tips of commercial chickens are a favorable source of DNA for the amplification of MDV and ALV-J. *Avian Pathology*, In Press (Ch. 5 of the appendix).

Kamil, J and Kung H.J. (2001) Marek's disease-like viruses. The Springer Index of Viruses. In Press.

Kung, H.-J., Kamil, J., Witter, R. and Davidson, I. (2001) Retroviral insertion into herpesviruses: discovery and implications. *Evolving Scientific and Regulatory Perspectives on Cell Substrates for Vaccine Development*, Karger Publisher, Ed. A. Lewis 106: 223-229

Liu, J-L., Lin, S-F., Xia, L., Bronovskis, P., Li, D., Davidson, I., Lee, L.F. and H-J Kung (1999) Meq and v-IL8: Cellular genes in disguise? *Acta Virologica* 43: 94-101. (Ch. 8 of the appendix).