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Occurrence of Antibodies Against Insect Virus Proteins in Mammals: Simple Model to Differentiate Between Passive Exposure and Active Virus Growth

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Antibodies against an "enterovirus-like" virus of insects, cricket paralysis virus, occur in the sera of domestic animals. When these antibodies were used in combination with the immunoprecipitation of radiolabeled virus proteins from infected *Drosophila* cells in culture, it could be demonstrated that the animals were exposed to preformed virus.

Antibodies which react with small RNA viruses of insects occur in a wide range of domestic and wild animals and humans (2-5, 9; F. MacCallum and T. W. Tinsley, unpublished data). The obvious problem is whether these animals have been exposed to viral proteins (either the test insect virus or a serologically related virus) by passive exposure or active infection. A procedure to differentiate between exposure to preformed virus or active virus growth within the animal is possible, using an insect picornavirus (cricket paralysis virus [CrPV]).

CrPV has many characteristics in common with mammalian picornaviruses (6-8). It contains three major structural proteins with molecular weights of about 30,000 (Fig. 1), two of which can be separated only on high-percentage polyacrylamide gels or by isoelectric focusing. Minor amounts of another protein, VP0, occur in the purified virus, which, by analogy with the mammalian picornaviruses, is the immediate precursor of VP2 and VP4. VP4 is not adequately characterized in this insect virus. It has not been detected in the pure virions, but has possibly been identified in the infected cell. Infection of *Drosophila* cells with CrPV results in the suppression of host cell protein synthesis and the appearance of virus-induced polypeptides. Figure 1 demonstrates the [^{35}S]methionine-labeled proteins occurring in infected and noninfected cells. The viral structural proteins VP1, VP2, and VP3 and the precursor VP0 are clearly visible. Small amounts of precursor proteins and low-molecular-weight, apparently nonfunctional, virus-coded proteins are also apparent. The structural proteins of CrPV are formed from high-molecular-weight precursors in the classical piconavirus manner by multiple proteolytic cleavages (6). The virus-induced proteins can thus be divided into three major groups: (i) precursor proteins, (ii) viral structural proteins, and (iii) apparently nonfunctional, wasted low-molecular-weight proteins arising from the multiple proteolytic cleavage.

By using these divisions of the virus-induced proteins, it is then possible to develop a model to differentiate between infection and exposure. (i) Antibodies raised in rabbits (passive exposure) against CrPV cause the precipitation of group 1 (precursors) and group 2 (structural) proteins but not group 3 (low-molecular-weight) proteins. (ii) If the antibodies occurring in the vertebrate caused precipitation of groups 1 and 2, this would indicate that the animal had been exposed to CrPV (or a serologically related virus), but active virus growth in the animal had not occurred. (iii) However, if the serum caused the precipitation of the nonstructural low-molecular-weight proteins (group 3) as well as groups 1 and 2, then it could be suggested that the virus had actually replicated in the animal, i.e., antibodies were produced against all the virus-coded proteins.

The assumption has to be made that the low-molecular-weight proteins are released from the infected cells. The foreign or non-self proteins are produced in equimolar amounts to the viral structural proteins with this form of replication, and the normal cycle of maturation of CrPV from *Drosophila* cells involves lytic release. Therefore, it follows that there should be a corresponding antigenic response with the low-molecular-weight proteins. However, it is possible that replication in mammalian cells may result in nonlytic virus production which could result in a low (short-term) reaction to the nonstructural low-molecular-weight proteins.

Figure 2 shows that the immune precipitation of the proteins from *Drosophila* cells with cattle serum results in the precipitation of only classes 1 and 2 and no low-molecular-weight protein. The same pattern of precipitation is also obtained with rabbit hyperimmune serum. This experiment was repeated with the sera from

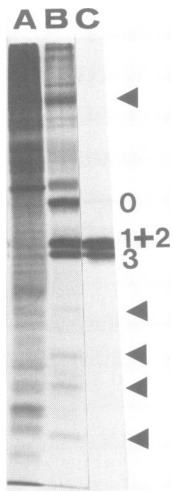

FIG. 1. *Comparison of radiolabeled uninfected (A) and CrPV-infected (B) Drosophila cells and purified CrPV polypeptides (C). CrPV was used to infect confluent monolayers of Drosophila melanogaster cells at a multiplicity of infection of approximately 25 plaque-forming units per cell for 1 h at 28°C. The inoculum was removed, and infected (and mock-infected) cells were incubated with Schneider maintenance medium (7) for 4 h at 28°C. Cells were washed with and incubated in the presence of methionine-deficient medium before pulsing with 50 μCi of [^{35}S]methionine per ml for 20 min; 2 μg of actinomycin D per ml was present throughout the experimental procedures. The major structural proteins of CrPV are indicated by the numbers 1, 2, and 3. Two of the polypeptides comigrated on this gel system. With pure CrPV, minor amounts of VP0 could be detected, which is the immediate precursor of VP2 and VP4. VP0 is a major protein detected in the infected cell (B), and minor amounts of this protein are found in the mature virions (C). A group of three high-molecular-weight proteins could be detected in infected Drosophila cells (7) at the position near the origin of the gel (top arrowhead). Four low-molecular-weight, wasted virus-coded proteins are indicated by the remaining arrowheads. These are cleavage products which are not incorporated in the virus and which do not, therefore, contain antigenic determinants in common with the viral structural proteins. All solubilized proteins were electrophoresed on 12.5% polyacrylamide slab gels with a discontinuous buffer system (1).*

sheep and horses which gave immune precipitates in double-diffusion plates against CrPV structural proteins, and no evidence was found for the precipitation of class 3 proteins from the infected cell. The evidence suggests that CrPV is not eliciting the production of antibodies through an actual infection. This model is further supported by pulse-chase experiments which suggest that the low-molecular-weight nonstructural proteins of CrPV are relatively stable in the infected cell (6, 7; Moore, unpublished data).

This model for differentiation between infection and passive exposure will have to be tested in a mammalian virus-host system with a virus which codes for nonstructural proteins. Preferably, a picornavirus such as poliovirus could be used as it theoretically generates equimolar amounts for all proteins. Influenza virus, for example, might be an unsuitable system to use as an animal model because of the potential

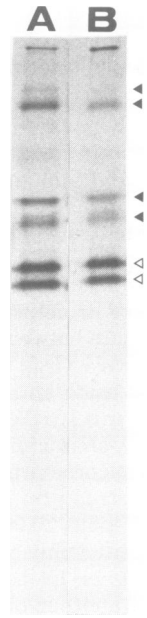

FIG. 2. *Comparison of the polypeptides precipitated from [^{35}S]methionine-pulsed Drosophila cells by hyperimmune rabbit serum (A) and cow anti-CrPV serum (B). Radiolabeled infected Drosophila cells were lysed in detergent containing TNE buffer (0.5 M tris(hydroxymethyl)aminomethane - hydrochloride, pH 7.4, 0.1 M NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Nonidet P-40, 0.5% sodium deoxycholate). The supernatant from a 12,000 × g centrifugation at 4°C was mixed with serum for 3 h at 37°C, left overnight at 4°C, and then applied to a protein A-Sepharose CL-4B column. (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The column was repeatedly washed with phosphate-buffered saline before elution with 0.6% acetic acid in saline. Bovine serum albumin was added to the eluate as a carrier, and the radioactive proteins were precipitated with 10% trichloroacetic acid on ice for 30 min. Precipitated proteins were washed with 10% trichloroacetic acid and acetone before boiling in solubilizer solution containing 2% sodium dodecyl sulfate, 2% 2-mercaptoethanol, 15% glycerol, and bromophenol blue as an electrophoresis marker. Group 1 precursor proteins (▲) and viral structural proteins (△) are indicated on the 12.5% polyacrylamide slab gel.*

unequal production of structural and nonstructural proteins resulting from a sequential genome. However, it should be possible to readily differentiate between, for example, antibodies resulting from immunization against influenza and active production of live virus within an individual.

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