Different Species of Enteroviruses (EV) in Peripheral Blood Leukocytes (PBL) of Children at the Clinical Onset of Type 1 Diabetes

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Purpose: EV have long been suspected to trigger T1D. To verify this hypothesis, EV infectivity and genome were searched for in PBL of pediatric patients on the day of clinical diagnosis.

Methods: 112 children developing diabetes at two Pediatric Endocrinology Centers of Northern and Central Italy (median age 9.5 yrs; range 2-16 yrs) were studied. EV-susceptible cell lines (RD, HeLa, AV3, CaCo) were immediately co-cultured with the patients` PBLs. Primers covering the 5`UTR, VP4, and 3D genome regions of 100 EV types were used in highly sensitive RT-PCR assays that were run both on plasma and tissue culture medium from cell lines exposed to patients` PBLs. Expression of viral capsid proteins was evaluated in "infected" cell cultures with mAbs directed to the capsid protein VP1. Immunoassays were used to quantify cytokines released by cultured cells. Routine methods were used to measure levels of blood glucose, HbA1c, C-peptide (time 0 and 6 min after glucagon stimulation), diabetes related auto-Abs (GAD65, IA2, ZnT8, IAA), and - one year after diagnosis - the insulin requirement (IU/Kg/day).

Summary of Results: EV infectivity and genome fragments were found in PBLs of 89/112 (79%) children, versus 2/69 (2.8%) matched non-diabetic controls. EV of the B species were predominant (58% of positives). Viruses of the A, C, and D species were also detected. Tests on infected cell lines confirmed the intracellular production of viral capsid proteins and of the MCP1 chemokine. As compared to EV-negative children, EV-positive patients had significantly reduced levels of glucagon-stimulated C-peptide and significantly higher levels of HbA1c at diagnosis. However, titers of diabetes-related auto-Abs were not different and - one year after diagnosis - the insulin requirements of the two groups were comparable.

Conclusions: The presence of EV in blood is a frequent and significant biomarker of early stage T1D. Collaborative studies are needed to identify the EV types associated with T1D in different geographic areas. We thank: CARIPLO Foundation (IT), VIDIS Group (UK), Gianni Valcavi, Attorney.