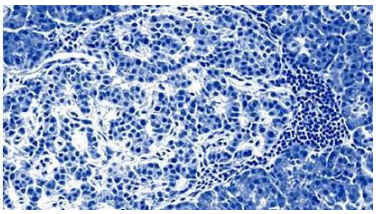
nPOD
Network for Pancreatic Organ Donors with Diabetes



Safety Studies Examining a CVB1-6 Vaccine in NOD Mice and Non-Human Primates

AUTHORS

Virginia M Stone [1], Minna M Hankaniemi [2], Marta Butrym [1], Olli H Laitinen [2], Amir-Babak Sioofy-Khojine [2], Ang Lin [3], Heikki Hyöty [2], Vesa P Hytönen [2,4], Karin Loré [3], Malin Flodström-Tullberg [1]

[1] Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[2] Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland
[3] Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.
[4] Fimlab Laboratories, Tampere, Finland

PURPOSE

An extensive concerted effort has been made by the scientific community to elucidate the causes of Type 1 diabetes (T1D), however despite these efforts the disease aetiology remains elusive. Combined with a genetic element, associations between T1D and a number of environmental factors have been described which include infections with common enteroviruses and more specifically Coxsackievirus Bs (CVBs). Despite a significant body of research examining this area, whether infections are causal is yet to be confirmed. Vaccination strategies of at-risk individuals provide a viable option to determine this. As such, we have created a polyvalent CVB1-6 vaccine and undertaken proof-of-concept studies that have demonstrated the protective capacity of this vaccine against virus induced diabetes. We have also shown that the vaccine is highly immunogenic in a non-human primate model. As an extension to these studies, in this project we address the safety of the vaccine in both the NOD mouse model for spontaneous T1D development and the non-human primate model.

METHODS

CVB1-6 serotypes were inactivated by formalin and mixed to create the polyvalent vaccine. NOD mice aged between 4.5-7.5 weeks old were vaccinated with CVB1-6 vaccine or buffer two or three times with two to three week intervals between vaccinations. Serum was collected prior to each vaccination and at the end of the study. Weight and blood glucose levels were monitored weekly. Mice were either followed to 11-13.6 weeks of age where they were sacrificed and the pancreas was collected for histological analysis of insulitis or up to 30 weeks of age for monitoring of diabetes incidence. Rhesus macaques (4 years old) were vaccinated twice with a 4 week interval between the two, with or without Alum adjuvant. Serum was collected prior to vaccination. The animals were monitored until 10 weeks after the prime vaccination and weight, body temperature, blood glucose levels were

monitored. Liver enzyme functions were assessed on days 0, 1 and 14. Serum neutralising antibody titres from all animals were assessed by serum neutralisation assay.

SUMMARY OF RESULTS

The polyvalent CVB1-6 vaccine was highly immunogenic and induced strong neutralising antibody responses in NOD mice. A strong neutralising antibody response against the six serotypes was also induced in the rhesus macaques after vaccination. Neutralising antibody responses were in general higher in the adjuvant group on days 14 and 28 than in the non-adjuvant group however the responses were equivalent after the boost vaccination. The vaccine was well tolerated in NOD mice and had no adverse effects on weight and blood glucose values. Preliminary data indicates that insulitis scores in 11-13 week old female NOD mice were comparable between buffer treated and CVB1-6 vaccinated groups and furthermore, no differences were detected in either the average age of diabetes onset or in cumulative diabetes curves between the two groups. The safety profile of the CVB1-6 vaccine was similar to that seen in the mice with no alterations in weight, blood glucose or body temperature of animals vaccinated in the presence or absence of adjuvant. There were also no changes in enzymes indicative of liver function on days 1 and 14 after the prime vaccination, which remained in normal ranges.

CONCLUSIONS

In mice and rhesus macaques the CVB1-6 vaccine had an excellent safety profile and was highly immunogenic. Moreover, the vaccine did not affect insulitis scores nor the onset of diabetes in NOD mice. These proof-of-concept studies provide necessary data to support the development of an equivalent vaccine for use in human trials to establish whether CVBs are involved in T1D and if so, provide a viable preventative treatment for the disease.