A Coxsackie B virus vaccine prevents virus-accelerated diabetes in NOD mice

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Conclusions:

A CVB vaccine prevents virus accelerated diabetes in the NOD mouse model.

Introduction:

The environmental causes of Type 1 diabetes (T1D) remain elusive, however enteroviruses, and in particular, CVBs, have been implicated in the disease by numerous studies. Moreover, CVBs can accelerate diabetes in pre-diabetic NOD mice. Vaccination against these viruses would provide a comprehensive strategy to examine their involvement in T1D, and such a CVB vaccine is currently undergoing phase 1 clinical trial. Previously we have shown that CVB vaccines protect against infection in NOD mice and virus-induced diabetes in a model for CVB-induced diabetes. A CVB1-6 vaccine was also immunogenic in non-human primates ([1]) and did not alter the development of insulitis or diabetes onset in NOD mice.

Aims:

To examine, in the NOD mouse model for T1D, whether a CVB vaccine can prevent a second form of virus-induced diabetes, namely virus-accelerated diabetes.

Results:

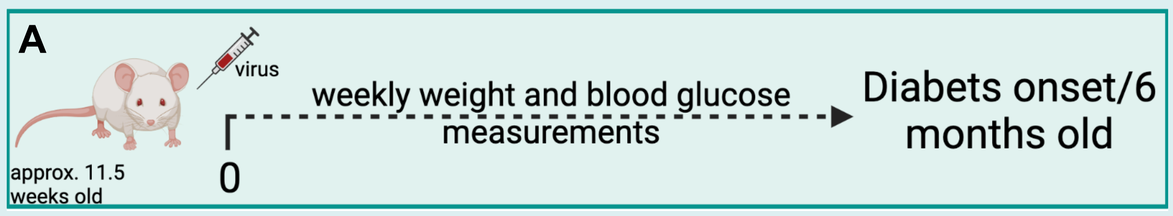
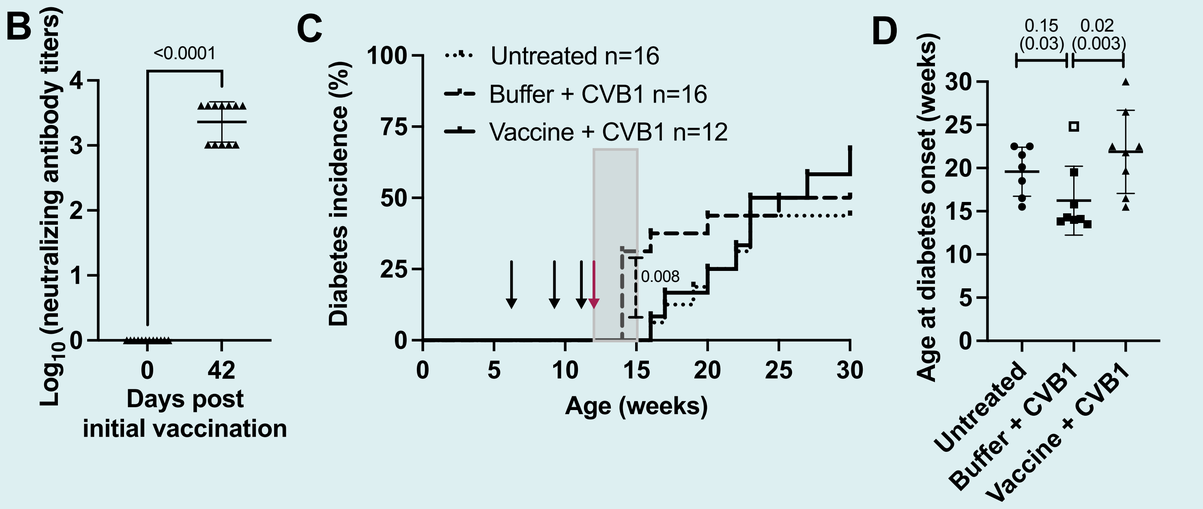


Fig.1 CVB1 accelerates diabetes onset in female NOD mice. (A) Experimental set-up. **(B)** Diabetes incidence and **(C)** mean age at diabetes onset in female NOD mice. Mice were left untreated (control n=31) or infected with CVB1 (10^7 PFU i.p. injection) between 10.5-13.5 weeks of age (CVB n=14). Diabetes onset was followed up until 30 weeks of age. (B) The red arrow indicates the mean age at infection. The grey box shows the 2-week period after virus infection. *p<0.001* when comparing the curves during this period by Gehan Breslow-Willcoxon test. (C) Individual mice are represented by a single symbol, the horizontal line shows the mean ±SD age at diabetes onset. *P <0.0001* by unpaired *t* test.

This work was published in Diabetes 2021;70:2871-2878.



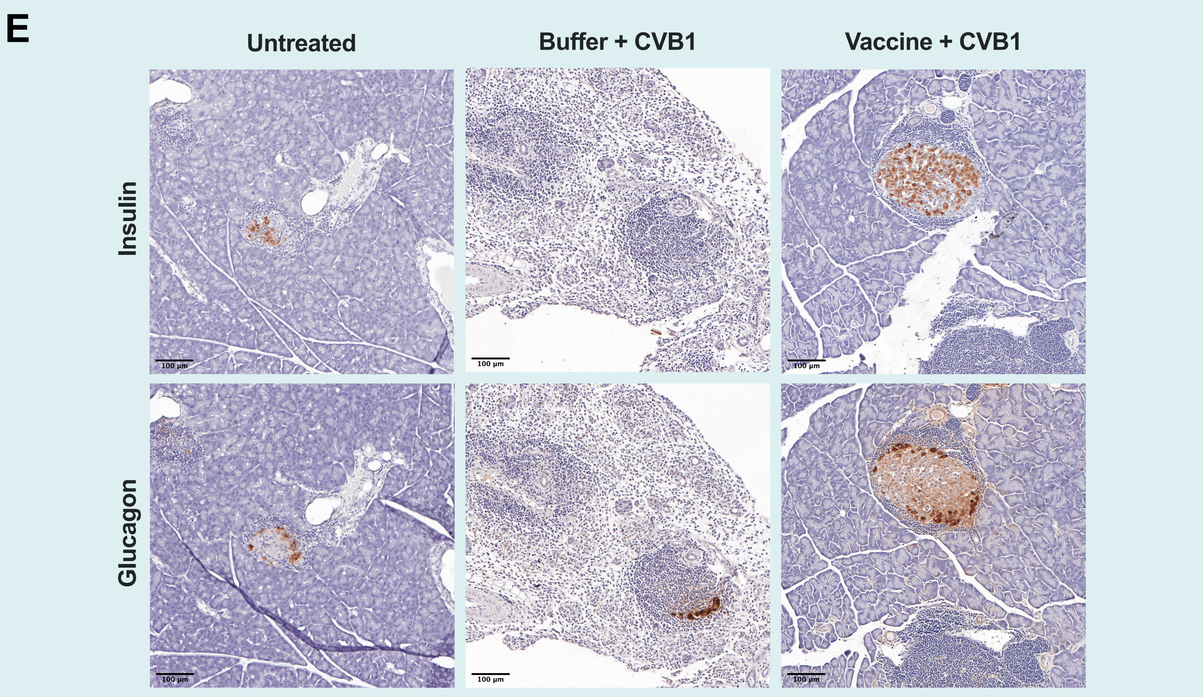


Fig.2 CVB1 protects against CVB1 accelerated diabetes in NOD mice (A) Experimental set-up. **(B-D)** Female NOD mice (6.3-6.9 weeks old) were left untreated (n=16), mock vaccinated + CVB1 infected (buffer + CVB1, n=16) or CVB1 vaccinated + CVB1 infected (vaccine + CVB1, n=12). Diabetes incidence was followed to diabetes onset/6 months old. **(B)** Neutralizing antibody titers on days 0 and 42. Mean ±SD. *p < 0.005* by unpaired t test. **(C)** Diabetes incidence curves. Black arrows show the approximate vaccination ages and the red arrow shows the average age at infection. The grey box shows the 2-week period after virus infection. *p<0.008* when comparing the curves during this period by Gehan Breslow-Willcoxon test. **(D)** Age at diabetes onset. Mean ±SD. Groups were compared by the Kruskal-Wallis test with the Dunn test for multiple comparisons. In brackets are the P values generated when one mouse, which was borderline diabetic from 15 weeks of age but did not develop overt diabetes until 25 weeks of age, was excluded (open square; buffer + CVB1). **(F)** Representative images of sequential pancreas sections stained with insulin and glucagon from mice that developed diabetes in the untreated, buffer + CVB1, and vaccine + CVB1 groups. Positive areas are stained brown. Scale = 100µm.

Methods:

- CVB1 vaccine was produced by formalin inactivation of purified CVB1 virus ([1,2]).
- Control mice received vaccine buffer or were left untreated. Regular blood glucose and weight measurements were performed.
- Neutralizing antibodies were measured by a plaque reduction assay.
- Mice were infected with 10^7 PFU CVB1 (strain CDC7) by intraperitoneal injection.
- Diabetes was diagnosed in mice after one blood glucose measurement over 18mmol/l or two measurements on consecutive days over 13mmol/l.

[1] Stone *et al* 2020 Science Advances, [2] Hankaniemi *et al* 2017 Vaccine

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