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1 **Coxsackievirus and type 1 diabetes:**

2 **diabetogenic mechanisms and implications for prevention**

3
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24 **Abstract**

25 The evidence for an association between Coxsackievirus B (CVB) infection, pancreatic islet
26 autoimmunity and clinical type 1 diabetes is increasing. Results from prospective cohorts and
27 pancreas histopathology studies have provided a compelling case. However, the demonstration of
28 a causal relationship is missing, and is likely to remain elusive until tested in humans by avoiding
29 exposure to this candidate viral trigger. To this end, CVB vaccines have been developed and are
30 entering clinical trials. However, the progress made in understanding the biology of the virus and
31 in providing tools to address the long-standing question of causality contrasts with the scarcity of
32 information about the anti-viral immune responses triggered by infection. Beta-cell death may be
33 primarily induced by CVB itself, possibly in the context of poor immune protection, or secondarily
34 provoked by T-cell responses against CVB-infected beta cells. The possible involvement of epitope
35 mimicry mechanisms skewing the physiological anti-viral response toward autoimmunity has also
36 been suggested. We here review the available evidence for each of these three non-mutually
37 exclusive scenarios. Understanding which ones are at play is critical to maximize the odds of
38 success of CVB vaccination, and to develop suitable tools to monitor the efficacy of immunization
39 and its intermingling with autoimmune onset or prevention.

40

41 **Key Words:** beta cells, Enterovirus, immune evasion, mimicry, T cell, vaccine.

42

43

44 **I. Introduction**

45 The most outstanding question in the field of type 1 diabetes (T1D) concerns the identification of
46 environmental triggers (1), a conundrum shared with many other autoimmune diseases. These
47 environmental triggers are gaining importance, as the steady progression of T1D incidence in the
48 last 50 years cannot be accounted for by a genetic drift of the human population. Rather, the
49 relative weight of predisposing gene variants (mostly mapping to HLA Class II loci) is decreasing.
50 Indeed, T1D patients diagnosed in more recent years carry HLA haplotypes considered protective
51 to a larger extent than patients diagnosed decades ago, while high-risk haplotypes are becoming
52 less common (2). This observation indirectly suggests that environmental pressure is increasing.
53 Moreover, these environmental factors exert their role very early in life, as the majority (~64%) of
54 children that later develop T1D display their first seroconversion for islet auto-antibodies (aAbs)
55 during the first 2 years of life (3,4).

56 Given the heterogeneity of disease, mostly related to age (5), a universal environmental trigger
57 underlying all T1D cases is unlikely to exist. Nonetheless, the strongest evidence for an association
58 with T1D points to Enterovirus infection, particularly to Coxsackieviruses B (CVBs) (6-8). This
59 echoes the robust evidence linking Epstein-Barr virus infection with multiple sclerosis (9-12). We
60 will discuss current evidence for the association between CVB and T1D, and the possible
61 pathogenic mechanisms at play. We contend that a better knowledge of such mechanisms is
62 essential to inform the design of T1D prevention trials based on CVB vaccination (13,14).

63

64 **II. Common features of putative diabetogenic viruses**

65 Many different viruses have been implicated as candidate triggers for T1D. These include
66 Enterovirus (both CVB and Echovirus), Parechovirus and Rotavirus. The strength of evidence for

67 each of those has been recently reviewed (15), but it is important to note that they share four key
68 features:

69 1) They are responsible for infections during the first years of life, which may be in line with the
70 early timing of aAb seroconversion in most pediatric T1D progressors (3,4). CVB infections show
71 some seasonal trends, being more common during the summer and early fall.

72 2) These infections are highly prevalent, with >95% of the general population being Enterovirus
73 seropositive. It thus needs to be explained how such prevalent infections may trigger T1D in only
74 few individuals. As discussed, genetic susceptibility, infection timing, viral clearance *vs.*
75 persistence and anti-viral immune responses may be at play.

76 3) They are transmitted mainly through the oro-fecal route and, to a lesser extent, through the
77 respiratory route. This feature is relevant because pancreatic lymph nodes drain not only the
78 pancreas itself but also parts of the intestinal tract (16), thus providing an ideal cross-road for
79 immune cells and viruses to reach the pancreas.

80 4) They can infect beta cells. They can thus exert their pathogenic mechanisms on the same cells
81 that are targeted by T1D autoimmunity.

82 CVBs are classified into 6 serotypes (1 to 6) and belong to the family Picornaviridae and the genus
83 Enterovirus, which also includes Poliovirus and Echovirus. They are small non-enveloped single-
84 stranded RNA viruses that initially replicate in the submucosal lymph tissue of the distal small
85 bowel, and of the upper respiratory tract when transmitted by aerosols. Although they usually
86 cause asymptomatic infections, further dissemination to target organs can occur following a
87 secondary viremia. This can occasionally result in severe diseases, such as meningitis,
88 encephalitis, myocarditis, and systemic neonatal infections. Some of these clinical conditions, e.g.
89 myocarditis, exemplify the capacity of CVBs to cause persistent (chronic) infections (8,17-19).

90

91 **III. Association between CVB infection and T1D: prospective and histopathology studies**

92 The association between CVB infection and T1D is supported by temporal correlations from
93 prospective cohorts and by spatial correlations documented in histopathological studies.

94 **Temporal correlations**

95 A 2011 meta-analysis of 26 case-control studies reported a significant association between
96 Enterovirus infection and islet autoimmunity (odds ratio, OR 3.7) or clinical T1D (OR 9.8) (20),
97 although some publication bias in favor of positive results is likely. The CVB serotypes more
98 frequently associated with T1D are CVB1 (21) and CVB4 (22), while some others, e.g. CVB3 and
99 CVB6, have occasionally been suggested to be protective (21). Associations between CVB
100 exposure (i.e. detection of CVB RNA in stools) and seroconversion and T1D progression were
101 provided by prospective cohorts such as the Finnish T1D Prediction and Prevention (DIPP;
102 <https://dipp.fi>) and The Environmental Determinants of Diabetes in the Young (TEDDY;
103 <https://teddy.epi.usf.edu>) studies. Based on serological evidence, i.e. neutralizing antibody (Ab)
104 titers against different CVB serotypes, the group of H. Hyöty (21) reported that the prevalence of
105 anti-CVB1 Abs was higher in children later seroconverting for islet aAbs, while that of anti-CVB3
106 and anti-CVB6 Abs was lower. When considered altogether, a positive CVB1 serology and a
107 negative CVB3/CVB6 serology conferred a 2.5 OR for subsequent islet aAb seroconversion. A
108 protective role for maternal Abs was further suggested, as a negative CVB1 serology in cord blood
109 and subsequent CVB1 seroconversion by 18 months of age was associated with a similar OR of
110 2.6. Given its focus on preclinical stage 1 (i.e. asymptomatic islet autoimmunity with normal
111 insulin secretion), this study suggested that CVB exposure may be associated with aAb
112 seroconversion rather than clinical progression. This is in line with previous studies from Cuba

113 reporting an association between epidemic Echovirus infection and aAb seroconversion in the
114 convalescent phase (23-25). This possibility was confirmed by a recent TEDDY study based on
115 the longitudinal analysis of the fecal viral metagenome enhanced through a preliminary culture
116 step (22). The risk of future aAb seroconversion was not associated with short and independent
117 infections, but rather with prolonged infections with the same Enterovirus B (mostly CVB4)
118 serotype, with a risk increase of ~20% at each positive stool sample.

119 **Spatial correlations**

120 A spatial correlation between CVB infection and insulinitis (i.e. the immune infiltration of islets)
121 has been highlighted by immunohistochemistry (26-29), reverse-transcription polymerase chain
122 reaction (29) and *in-situ* hybridization (30) using tissue specimens available through the network
123 for Pancreatic Organ Donors with T1D (nPOD; <https://www.jdrfnpod.org>), from brain-dead organ
124 donors; the UK Exeter Archival Diabetes Biobank (EADB;
125 <https://pancreatlas.org/datasets/960/overview>), from autopsy material; and the Norwegian
126 Diabetes Virus Detection Study (DiViD; [https://www.oslodiabetes.no/diabetes-virus-detection-](https://www.oslodiabetes.no/diabetes-virus-detection-study-divid)
127 [study-divid](https://www.oslodiabetes.no/diabetes-virus-detection-study-divid)), from living donors (29,31). Immunohistochemistry for the Enterovirus viral protein
128 (VP)1 revealed a co-localization with T-cell infiltration and/or HLA Class I hyper-expression,
129 which are the two hallmarks of insulinitis. When considering these immunohistochemistry studies
130 altogether (26-29), VP1+ islets were detected in ~70% of T1D cases and 14% of non-diabetic
131 controls. All these studies also conclude that only a fraction (7-29%) of islets with residual insulin-
132 containing cells are VP1+, suggesting that persistent low-grade rather than short-lived high-grade
133 infections may occur, probably long before T1D clinical onset (13).

134

135 **IV. Possible mechanisms of persistent CVB infection**

136 As discussed above, the small fraction of VP1+ cells in the pancreas of T1D patients
137 (corresponding to the late stage 3 disease in natural history) together with the temporal association
138 between prolonged fecal CVB shedding and aAb seroconversion (i.e. early stage 1 disease) suggest
139 a scenario of prolonged CVB infections. On the other hand, the few cases of aAb+ organ donors
140 analyzed to date did not show evidence of acute, extensively lytic CVB infection (5), although also
141 in this case we only have a ‘snapshot’ available, which may date well beyond the time of CVB
142 encounter. It should be noted that this does not exclude the possibility that a more limited lysis of
143 CVB-infected beta cells may occur. The TEDDY stool virome study (22) further indicates that
144 these prolonged CVB infections are the result of viral persistence (i.e. chronic infection following
145 a single viral encounter) rather than recurrence (i.e. multiple viral encounters).

146 The viral mechanisms leading to persistent CVB infections have been recently reviewed (8). A
147 first mechanism involves repeated CVB seeding from infection reservoirs in the gut (mostly
148 duodenal epithelial cells), pancreas (ductal and beta cells) and blood cells (mostly antigen-
149 presenting cells, APCs) (32,33), through infections that can be either cytolytic (with release of new
150 virions) or not (with virions released through other mechanisms, e.g. extracellular vesicles) (34).
151 A predisposing allele of the T1D risk gene *IFIH1* (interferon induced with helicase C domain 1;
152 coding for the melanoma differentiation associated protein 5 MDA5) may favor CVB persistence
153 in the blood (33). Reservoirs of viral persistence are also established in microvascular endothelial
154 cells, without causing overt cytopathic effects but inducing the upregulation of adhesion molecules
155 that may contribute to leukocyte recruitment (35).

156 A second mechanism exploits a naturally occurring deletion in the 5' non-coding region of the
157 viral genome, which leads to reduced viral replication and persistent low-grade infection (36,37).

158 Such deletions might be favored by the high mutation rate of CVBs, due to the lack of proofreading
159 in their RNA-dependent RNA polymerase (38).

160 A third mechanism may be a non-lytic CVB egress pathway active in beta cells. This is mediated
161 by extracellular vesicles, which also protect CVB from neutralizing Abs, thus also providing an
162 immune evasion mechanism (34). Another immune-evading CVB transfer mechanism via cell
163 protrusions has been described in other cells (39).

164 Immune mechanisms may also participate, and include innate type I interferon (IFN, mainly IFN-
165 α) responses (40) (another hallmark of T1D, starting from its early preclinical stages) (41,42)
166 limiting viral replication and beta-cell lysis (43); and insufficient adaptive anti-viral responses that
167 do not clear the virus, as discussed below. These mechanisms may also engage a vicious cycle,
168 with persistent infection promoting a prolonged immune activation favoring loss of tolerance,
169 possibly through re-iterative priming of autoimmune T cells. **Figure 1** summarized the timeline of
170 the immune responses triggered by CVB infection.

171

172 **V. From association to causality: possible diabetogenic mechanisms of CVB infection**

173 With these points in mind, three non-mutually exclusive mechanisms can be proposed to explain
174 the triggering effect of CVB infection on islet autoimmunity (**Figure 2**):

175 1) *Direct* (primary) pathogenic effects of *CVB* on infected beta cells. These may imply *poor*
176 immune responses unable to efficiently clear the virus.

177 2) *Indirect* (secondary) pathogenic effects of *anti-viral immune responses* on infected beta cells,
178 e.g. involving cytotoxic CD8⁺ T-cell-mediated beta-cell lysis, among others. These may imply
179 *strong* immune responses leading to viral clearance.

180 Both mechanisms would lead to the release of beta-cell antigens in a pro-inflammatory
181 environment, which may subsequently trigger islet autoimmunity.

182 3) Epitope mimicry. The physiological antiviral immune response may turn into a pathological
183 autoimmune response against beta cells due to T-cell cross-reactivity between homologous CVB
184 and beta-cell epitopes.

185 In the following sections, we will review the level of evidence for each of these mechanisms.

186

187 **VI. Direct pathogenic effects of CVB on infected beta cells.**

188 CVB enters cells primarily by binding to surface Coxsackievirus and Adenovirus Receptor (CAR).

189 Both human and murine alpha and beta cells express CAR and are CVB-permissive (43).

190 Moreover, beta cells selectively express a CAR isoform (CAR-SIV), which is localized mainly in
191 secretory granules (44). Hence, these granules may be hijacked by CVB during exocytosis and
192 subsequent recycling, possibly contributing to the high sensitivity of human beta cells to CVB
193 infection. An increased secretory demand on beta cells (e.g. during growth spurts) may thus
194 increase their susceptibility to infection, and the infectious and metabolic stress may synergize
195 toward beta-cell demise.

196 **Mouse studies**

197 Several *in-vivo* experimental models document the lytic effect of CVB on beta cells and its
198 capacity to trigger diabetes without engaging potent anti-viral T-cell responses. These effects are
199 dependent on the CVB serotype and the genetic background of the murine host. For instance,
200 infection of C57BL/6 mice with CVB3/4 does not induce diabetes, while it does in 25% of infected
201 SJL and CD1 hosts, independently of T cells (45,46). This variable susceptibility is reminiscent of
202 that observed with experimental CVB3-induced myocarditis (47). Most strains develop a severe
203 acute myocarditis, but completely recover; few others develop chronic myocarditis associated with

204 anti-cardiac myosin heavy chain aAbs that are also found in humans. This disease can be
205 recapitulated by immunizing mice with cardiac myosin.

206 A direct lytic effect is also suggested in the multiple low-dose streptozotocin (LD-STZ) model,
207 which triggers autoimmunity by inducing a moderate beta-cell lysis that releases autoantigens in
208 an inflammatory environment. While a single LD-STZ injection was not diabetogenic, its
209 combination with CVB3/4/5 inoculation induced hyperglycemia in CD1 mice (48,49). Moreover,
210 only a peri-islet insulinitis pattern was observed (49), suggesting an accessory role for T cells in
211 mediating beta-cell destruction.

212 Humanized immunodeficient mouse models further highlighted the beta-cell-lytic effect of CVB
213 infection. These models are based on NOD/*scid*/*gamma* (NSG) mice deprived of endogenous islets
214 by high-dose STZ or diphtheria toxin (DT) treatment (via a rat insulin promoter/DT-receptor
215 transgene) and grafted with human islets (50,51). Subsequent CVB4 infection led to
216 hyperglycemia in 50% of animals 28 days later. Hyperglycemia was associated with CVB4 RNA
217 and protein persistence in transplanted islets, increased endoplasmic reticulum (ER) stress and a
218 type I IFN gene signature. Notably, pancreas histopathology revealed reduced insulin content but
219 no significant islet destruction.

220 In spontaneously diabetes-prone non-obese diabetic (NOD) mice, CVB1/CVB3/CVB4 inoculation
221 precipitates diabetes only when applied at the pre-diabetic stage - starting at 9 weeks of age, when
222 invasive insulinitis is already present (52-55). At this stage, CVB replicates efficiently in islets (56).
223 On the contrary, CVB3 or CVB4 inoculation of young (<8-week-old) NOD mice with minimal
224 insulinitis has a preventative effect (52,53), which is associated with a defective ability of CVB to
225 replicate in islets, despite the detection of high viral titers in islets 1-2 days after inoculation and
226 the expression of the CVB receptor CAR (53,57). This CVB failure to replicate in islets has been

227 associated with the induction of beta-cell-intrinsic IFN responses and HIF-1 α expression. Indeed,
228 NOD mice with a beta-cell-specific HIF-1 α deficiency display accelerated diabetes upon
229 CVB1/CVB4 infection, accompanied by increased pancreatic viral loads (58). This provides a
230 mechanistic link between the susceptibility or resistance of beta cells to CVB infection and their
231 capacity to sense viral RNA and induce intracellular anti-viral, IFN-mediated defense mechanisms.
232 Accordingly, the inhibition of IFN responses with a beta-cell-specific transgene for the suppressor
233 of cytokine signaling-1 (*socs-1*) rapidly induces diabetes after CVB1/CVB3/CVB4 infection, with
234 very few residual insulin-positive cells (59-61). Also in this case, disease onset does not require T
235 cells, as the outcome is similar in immunodeficient *socs-1*-transgenic NOD/*scid* mice (60).
236 The diabetes protection in young NOD mice vs. acceleration at an older age seem at variance with
237 the notion that, in humans, CVB infection may be an early trigger that precedes islet autoimmunity
238 (21,22). Possible explanations include the fact that the persistent CVB infections that may release
239 a critical self-antigen load in humans are difficult to reproduce in NOD mice, and that the
240 magnitude and/or quality of anti-CVB immune responses may be different. In NOD mice, the
241 requirement for persistent CVB infection may be bypassed by the critical threshold of beta-cell
242 destruction already achieved by autoimmune mechanisms on their own. The relatively high CVB
243 doses used in these infection models, which are administered through the intraperitoneal rather
244 than the natural oral route, are another confounder.

245 **Human studies**

246 *Lytic effects on beta cells.* An early study assessed the lytic effect of several CVB and A strains on
247 islet cells (62). The infected outer islet cells died over a few days and detached from islets, leading
248 to smaller structures. No features of apoptosis were observed, rather suggesting necrosis with early
249 chromatin condensation (pyknosis). Nonetheless, some beta cells in close proximity to infected

250 and damaged ones remained virtually intact. This underlines the heterogeneous outcome of CVB
251 infection (62), possibly reflecting different levels of basal ER stress across beta cells and/or
252 preferential CVB replication in dividing cells and latent infection of quiescent cells (63), a feature
253 shared by many viruses (64,65).

254 *Non-lytic effects on beta cells.* CVB infection impacts beta cells on many other levels. First, the
255 double-stranded (ds)RNA replicative intermediate is recognized by pattern recognition receptors
256 (PRRs) such as toll-like receptor 3 (TLR3), retinoic-acid-inducible gene I (RIG-I) and
257 MDA5/IFIH1 that trigger the production of pro-inflammatory cytokines, notably type I IFNs.
258 Indeed, children sampled at the time of enteroviral RNA appearance in the blood display an IFN
259 response gene signature, similar to that of peripheral blood mononuclear cells (PBMCs) or islets
260 exposed *in vitro* to Enteroviruses (40). Accordingly, infection of human islets with CVB3, CVB4
261 or CVB5 induces type I IFN (mostly IFN- β) expression (43,66). These results suggest that the
262 early blood type I IFN signature of T1D (41,42) may reflect an anti-viral response (40). While this
263 IFN response limits CVB replication and spreading, it also enhances beta-cell apoptosis (43). Of
264 note, four rare single-nucleotide polymorphisms that reduce MDA5 function provide T1D
265 protection (67). Additional effects of dsRNA on beta cells have been observed in an *in vitro* model
266 using the synthetic dsRNA mimic polyinosinic-polycytidylic acid (polyI:C) on a beta-cell line
267 and on primary human islets (68). PolyI:C downregulated beta-cell-specific genes (e.g. *INS*,
268 *G6PC2*, *SLC30A8*, *MAFA*), induced *de-novo* expression of the progenitor-like transcription factor
269 *SOX9* and impaired glucose-stimulated insulin secretion. This gene expression pattern suggests a
270 dedifferentiation process and was recapitulated upon CVB5 infection. This dedifferentiated
271 phenotype seems beta-cell-specific, as glucagon mRNA levels were unaffected in infected human
272 islets (69). These polyI:C effects were triggered by the NF- κ B and IFN regulatory factor pathways,

273 and by the secretion of their downstream cytokines IFN- α and tumor necrosis factor (TNF)- α .
274 Indeed, the use of IFN- α , alone or in combination with TNF- α , led to a similar *SOX9* expression
275 (68), suggesting that IFN- α may trigger a vicious cycle by disrupting the identity of neighboring
276 cells in a paracrine fashion.

277 Other outcomes of CVB infection on different cells include intensive viral protein production,
278 inhibition of host cell protein translation, impaired cellular calcium homeostasis and ER membrane
279 modifications (70-72). Altogether, these alterations potentiate ER stress, therefore activating the
280 unfolded protein response (UPR). The UPR is a natural cellular response to stress which aims at
281 decreasing the translation rate, increasing the biosynthesis of protein-folding chaperones, and
282 inducing the degradation of misfolded proteins. If the UPR fails to resolve cellular stress, it triggers
283 apoptosis. Given their high insulin synthesis, beta cells have high basal levels of ER stress and
284 naturally adjust the UPR to survive (73). This situation is easily decompensated by triggers such
285 as CVB infection (74). Of further note, the anterograde vesicular trafficking is reduced,
286 concomitantly with increased retrograde trafficking (75). In the highly secretory beta cells, this
287 translates into reduced secretory activity and insulin stores (76), likely imposing an additional
288 stress. The disruption of protein trafficking induced by CVB infection extends to autophagy (77),
289 which is vital for beta cells to dispose of unused insulin granules (78). Like other Enteroviruses,
290 CVB3 hijacks the autophagy pathway to replicate into vesicles (77,79-81). Yet, the step at which
291 CVB disturbs the autophagic processes remains controversial. Indeed, CVB has alternatively been
292 reported to enhance the autophagic flux (80) or to drive autophagosome accumulation by inhibiting
293 the fusion of autophagosomes with lysosomes and endosomes (77,79,81). These discrepancies
294 possibly reflect methodological differences, including cell models, infection conditions and
295 autophagy readouts.

296 **Summing up**

297 Both mouse and human studies suggest that direct cytopathic effects of CVB infection are major
298 contributors to beta-cell demise. Besides cytolysis, the strategies used by CVBs to hijack cellular
299 pathways and favor its own replication impact beta-cell survival, identity and insulin secretion.
300 The same is true for the pro-inflammatory cytokine release triggered by PRRs, which enhances
301 beta-cell apoptosis. These direct, immune-independent lytic and non-lytic effects on beta cells may
302 secondarily trigger islet autoimmunity and are summarized in **Figure 3**.

303

304 **VII. Effects of CVB on other cell types relevant to T1D.**

305 *Effects on other islet cells.* In alpha cells, several T1D candidate genes regulating anti-viral
306 responses display higher expression than in beta cells (82), notably *IFIH1* (83) and its protein
307 product, the dsRNA sensor MDA5 (84). Moreover, IFN- α signalling in alpha cells leads to higher
308 expression of other anti-viral factors, e.g. *GBP1/3*, *OAS2*, *TRIM22*, *XAF1* (82). Altogether, these
309 gene signatures may explain the observation that alpha cells can clear CVB more efficiently (85).

310 *Effects on the exocrine pancreas.* A recent report (86) employed a highly sensitive single-
311 molecule-based fluorescent *in situ* hybridization method to clarify the localization of infected cells
312 in the pancreas. Although Enterovirus-positive beta cells were found at higher densities in T1D vs.
313 control donors, they were rare and outnumbered by the infected cells found scattered in the
314 exocrine pancreas. Moreover, the exocrine pancreas harboured more infected cells in both T1D
315 and aAb+ donors than in controls. Morphological signs of plasma membrane disintegration were
316 noted in virus-containing cells, suggesting a lytic infection.

317 *Effects on immune cells.* The above report (86) also documented that most of the scattered infected
318 cells in the exocrine pancreas were of hematopoietic origin (CD45+) or in close proximity to

319 CD45+ cells (possibly suggesting a combination of anti-viral immune responses and viral transfer
320 to immune cells), and that infected (CD45+) cells were largely more abundant in the spleen than
321 in the pancreas, and in T1D donors. Other reports previously documented enteroviral RNA in
322 PBMCs (32,33), mostly localized in APCs [B cells, monocytes, dendritic cells (DCs)] (33,87), but
323 not in the plasma (33). Interestingly, the prevalence of enterovirus-positive PBMCs was not
324 different between T1D and control adults, while it was higher in multiple-aAb+ children
325 considered altogether (i.e. with or without stage 3 clinical T1D). The effects elicited by enteroviral
326 infections on immune cells are largely unknown. A mouse study (88) reported that CVB3, although
327 marginally infective on DCs both *in vitro* and *in vivo*, diminished their capacity to prime naïve
328 CD8+ T cells *in vivo*. This correlated with a surge in spleen plasmacytoid DCs and the loss of
329 spleen and pancreatic lymph node conventional DCs, notably of the cross-presenting CD8 α +
330 subset, with neither downregulation of surface MHC Class I/II and co-stimulatory molecules nor
331 reduced T-cell stimulatory capacity *in vitro* on a per cell basis. This DC-depleting effect of CVB
332 is more profound than for other viruses and, given the DC resistance to infection, must be indirect,
333 likely mediated by type I IFNs, as described for lymphocytic choriomeningitis virus (89). Together
334 with the lack of infectious permissiveness in DCs, it may represent another efficient immune
335 escape mechanism. In human monocyte-derived DCs, phagocytosis of CVB3-infected islet cells
336 was shown to induce IFN-stimulated genes without ensuing viral replication (90). CVB3 infection
337 in mice further induces a transient T and B lymphopenia, which is also partly mediated by type I
338 IFNs (91).

339 *Effects on enterocytes.* The gut, mostly duodenal enterocytes, is a major entry site for
340 Enteroviruses, and provides a viral reservoir that may contribute to persistent infections. Indeed,
341 duodenal biopsies from living T1D patients yielded higher enteroviral titers than PBMCs or

342 pancreas tissues (32). Moreover, Enteroviruses are more frequently found in duodenal biopsies
343 from T1D patients (92,93), although this finding has been questioned (94). Using stem-cell-derived
344 human small intestine enteroids infected with different Enteroviruses, including CVB3, a study
345 (95) documented that epithelial cells were infected by CVB3 without inducing neither significant
346 cell lysis nor anti-viral responses, as assessed by the lack of cytokine, chemokine and IFN-
347 stimulated gene transcripts. Thus, the effects induced by CVB infection at the intestinal entry site
348 are quite different than those induced on beta cells.

349 **Summing up**

350 Alpha cells are more resistant to CVB infection than beta cells. Enteroviral RNA-positive cells,
351 largely CD45+, are instead abundant in the exocrine pancreas, and more so in T1D and aAb+
352 donors. In human circulating immune cells, enteroviral RNA is mostly detected in APCs, despite
353 the fact that DCs are poorly permissive to CVB infection. The effects of CVB on human immune
354 cells have not been investigated, although infection in the mouse boosts plasmacytoid DCs and
355 depletes conventional DCs, thus favouring immune escape. Human enterocytes are readily infected
356 but do not undergo lysis nor mount anti-viral responses.

357

358 **VIII. Indirect pathogenic effects of anti-viral immune responses on infected beta cells**

359 **Mouse studies**

360 *Innate anti-viral responses.* CVB3 was unable to precipitate diabetes in NOD mice deficient for
361 the NADPH oxidase, which display reduced superoxide production and impaired M1 (pro-
362 inflammatory) responses by macrophages (96). This underlines the importance of the
363 inflammatory microenvironment driven by CVB3, independently of the direct, viral-mediated

364 lysis. Conversely, diabetes protection may result from innate immune responses triggered by
365 invariant natural killer T cells (97), which may limit viral spreading.

366 *Ab-mediated anti-viral responses.* Anti-viral neutralizing Abs are important for CVB clearance.
367 Mirroring data in the human (21), CVB infection induces neutralizing Abs in NOD mice (98).
368 These Abs are transferred to the offspring of CVB-infected NOD mice and protect the offspring
369 from infection, and from diabetes development in *socs-1*-transgenic NOD mice (98). As discussed
370 below, an increased risk of developing T1D may be linked to a weak anti-CVB Ab response and
371 lack of protection by maternally transferred neutralizing Abs.

372 *Effects of CVB infection on antigen processing and presentation.* Beta-cell and immune-derived
373 IFN responses increase surface MHC Class I expression and upregulate several genes of the
374 antigen processing and presentation pathway. This results in an increased beta-cell visibility to
375 islet-reactive, and possibly viral-reactive, CD8⁺ T cells. Indeed, CVB3 inoculation failed to
376 exacerbate T-cell insulinitis and diabetes onset in *tlr3*^{-/-} NOD mice that mount impaired IFN
377 responses (99), despite higher viral titers than in wild-type mice (100).

378 Following CVB4 infection, while necrosis occurs in neighboring acinar cells but not in islets, beta-
379 cell engulfment by resident APCs is observed in diabetes-resistant immunodeficient NOD/*scid*
380 mice, and these APCs can prime diabetogenic islet-reactive BDC2.5 T cells *in vitro* (101). In
381 addition, adoptive transfer of macrophages from CVB4-infected NOD/*scid* mice into
382 NOD/BDC2.5 T-cell receptor (TCR)-transgenic animals triggers diabetes (101). This suggests that
383 diabetes may result from the uptake of infected beta cells by APCs and subsequent presentation of
384 islet antigens.

385 *T-cell-mediated anti-viral responses.* The mechanisms underlying diabetes protection in younger
386 NOD mice following CVB infection are unclear, but increased TGF- β -producing regulatory T

387 cells (Tregs) have been reported (102). In older NOD mice, data point to a role of CVB as a
388 diabetes accelerator through bystander activation of islet-reactive effector T-cell responses and
389 autoimmune beta-cell lysis. Only indirect clues are instead available on whether CVB-reactive T-
390 cell responses are required to trigger diabetes. For instance, CVB4-infected NOD/*scid* mice
391 develop high CVB4 titers but no diabetes (103), suggesting that CVB-mediated beta-cell lysis
392 alone is not sufficient to trigger disease without downstream autoimmune priming. Diabetes is
393 instead triggered when CVB4-infected NOD/*scid* mice are adoptively transferred with islet-
394 reactive BDC2.5 TCR-transgenic T cells (101), suggesting a requirement for islet-reactive but not
395 CVB-reactive T cells. Similarly, CVB4 induces diabetes in BDC2.5 TCR-transgenic NOD mice
396 that are otherwise diabetes-resistant (104).

397 Collectively, the diabetes acceleration induced by CVB infection in NOD mice does not seem to
398 exclusively rely on a direct, viral-mediated lytic effect on beta cells, but also on the presence of
399 autoreactive T cells and the enhanced presentation of islet antigens by APCs through phagocytosis
400 of infected beta cells. Anti-CVB T-cell responses seem instead dispensable and even protective.

401 **Human studies**

402 *Innate anti-viral immune responses.* Apart from the beta-cell-autonomous pro-inflammatory
403 response triggered by CVB infection through PRRs, direct evidence about the innate immune
404 responses that may be triggered by CVB infection in human T1D is very limited. This likely
405 reflects experimental challenges: the question could be addressed either with in-vitro co-culture
406 systems of human islets and relevant immune subsets or with histopathological studies, but neither
407 approach has been reported to date. Most histopathological studies have rather sought evidence of
408 anti-viral responses in beta cells. A recent report from the DiViD study (105) documented that the
409 pancreas from newly diagnosed living T1D donors harbored a small subset of VP1+ beta cells with

410 markedly increased expression of the viral response protein kinase R. An increased islet expression
411 of other viral response proteins, i.e. MDA5 and MxA, relative to non-diabetic controls was also
412 noted. A colocalization of IFN response markers (MxA, protein kinase R and HLA Class I) and
413 VP1, together with downregulation of genes in the insulin secretion pathway, was also reported in
414 aAb+ donors (106). An IFN response gene signature coincident with enteroviral RNA detection
415 has also been reported in the blood of children (40). Of note, Enteroviruses can mount mechanisms
416 to limit such innate responses, including the formation of replication organelles derived from the
417 host cell membranes that protect viral RNA from sensing by PRRs and viral protease-mediated
418 cleavage of PRRs and their downstream signaling molecules (72,107).

419 *Ab-mediated anti-viral responses.* CVB serology has often been used as an indirect readout of viral
420 exposure (21). A recent work by the group of E. Bonifacio measured neutralizing anti-VP1 Abs to
421 look at the magnitude of response according to islet aAb status (108). The results were striking:
422 anti-VP1 Abs against all 6 major CVB serotypes were absent in children later developing early
423 anti-insulin aAbs, which represent a fast-progressing T1D endotype (5,109) preferentially
424 associated with CVB1 infection in another study (110). This contrasted with the detection of anti-
425 VP1 Abs in children without islet aAb seroconversion and in those positive for anti-glutamic acid
426 decarboxylase (GAD) aAbs at a later age. A caveat of this study is that, although anti-VP2 Abs
427 were detected in anti-VP1 Ab-negative children, no direct evidence of prior CVB infection was
428 provided. Hence, the lack of anti-VP1 Abs could also reflect an absence of viral exposure.
429 Nonetheless, these results may suggest that weak anti-CVB Ab responses predispose to islet
430 autoimmunity. This scenario would help explain the epidemiological paradox that T1D incidence
431 is higher where CVB exposure is lower. Indeed, CVB exposure drastically dropped over the last
432 40 years, while T1D incidence steadily increased (111). Also geographically (111,112), the world

433 highest T1D incidence of Finland contrasts with its very low CVB circulation. This paradox has
434 led to the ‘Poliovirus hypothesis’ (113,114), postulating that this scenario may be similar to that
435 of the beginning of the 20th century, when reduced Poliovirus circulation due to improved sanitary
436 conditions was paralleled by an increased incidence of its severe form, poliomyelitis (115). The
437 explanation proposed is that a low frequency of CVB infection in the background population leads
438 to decreased herd immunity and transmission of protective maternal Abs. Children are thus less
439 protected during the first years of life and tend to develop higher-titer CVB infections, which favor
440 viremia and viral spreading to vulnerable organs such as the heart or the pancreas (or motor
441 neurons in the case of Poliovirus). It would be informative to know whether the incidence of CVB
442 myocarditis has also increased during the last decades, but the improved diagnostic workup, which
443 identified CVB as a major etiologic agent (116), introduces a major bias.

444 *Effects of CVB infection on antigen processing and presentation.* The CVB-induced disruption of
445 membrane trafficking (decreased anterograde flux and increased endocytosis) that perturbs insulin
446 secretion also represents an immune evasion mechanism. This disruption results in the
447 internalization of surface MHC Class I molecules and limits presentation of newly-formed
448 complexes, notably of viral peptides (75). It is plausible that internalized complexes are returned
449 to the cell surface, circumventing natural killer cell detection (117). This mechanism likely
450 involves the internalization of other surface molecules such as cytokine receptors.

451 Moreover, CVB infection distinctively alters the antigen processing and presentation pathway, as
452 observed in CVB3-induced myocarditis models (118). Also in this case, self-limited viral
453 myocarditis can lead to loss of tolerance to cardiac antigens and autoimmunity (47). Infection
454 induces IFN- α expression, which upregulates MHC Class I and promotes the switch from the
455 constitutive proteasome to immuno-proteasome (118). The immuno-proteasome has different

456 cleavage preferences to process peptides for the MHC Class I pathway, thus generating more
457 antigenic peptides for cell surface presentation. *In-vivo* studies revealed that CVB3-susceptible
458 mouse strains display a longer and higher immuno-proteasome expression post-infection (118)
459 along with higher expression of genes of the antigen processing and presentation pathway.
460 However, most proteasomal enzymatic activities were reduced upon infection (118), suggesting
461 the existence of other immune escape mechanisms to counteract the IFN- α effects. Similar
462 processes might be at play in infected beta cells to limit anti-viral T-cell responses.

463 *T-cell-mediated anti-viral responses.* A major gap in knowledge concerns the features of anti-CVB
464 T-cell responses, which is largely due to the lack of information about the viral epitopes
465 recognized. A first report by M. Atkinson in 1994 (119) identified an antigenic CVB region by
466 first analyzing T-cell responses against GAD in unfractionated PBMCs stimulated with
467 overlapping peptides. While these responses were detected in both T1D and healthy donors, a
468 GAD₂₄₇₋₂₇₉ region was preferentially recognized in T1D and at-risk individuals compared to
469 healthy controls. This region harbors a significant homology with the P2C protein of CVB (the so
470 called PEVKEK region), and this homologous CVB sequence was recognized by the same donors.
471 Although this suggests the possibility of epitope mimicry and T-cell cross-reactivity, formal proof
472 at the single T-cell level and whether of the CD4⁺ or CD8⁺ subset was not provided. Indeed, a
473 subsequent study by Schloot et al. (120) using CD4⁺ T-cell clones dismissed this possibility.

474 Anti-CVB CD4⁺ T-cell responses are likely to be elicited upon CVB infection, as they are required
475 to drive B-cell activation and Ab production. Using individual recombinant CVB proteins, Varela-
476 Calvino et al. (121) reported that the structural proteins VP1, VP2 and VP3 were preferentially
477 targeted by T cells (likely CD4⁺ T cells in the unfractionated PBMCs used in these assays), and
478 that T1D patients harbored lower frequencies of proliferative responses against VP2 but stronger

479 IFN- γ responses against VP3 and P2C. Another study (122) identified human CD4+ T-cell
480 responses against Poliovirus VP2 and VP3 peptides selected from regions conserved across
481 Enteroviruses.

482 CVB infection is also likely to recruit CD8+ T cells, as they are key players of viral clearance via
483 the cytotoxic destruction of infected cells. Using HLA-A2 binding prediction algorithms, Varela-
484 Calvino et al. (123) identified a CVB4₁₁₃₇₋₁₁₄₅ epitope (EVKEKHEFL) located in the same P2C
485 region described by Atkinson et al. This peptide was naturally processed and presented by protein-
486 pulsed APCs and recognized by IFN- γ -producing CD8+ T cells from 2 of 3 HLA-A2+ healthy
487 donors tested. While T-cell lines raised against this epitope were cytotoxic against peptide-pulsed
488 target B cells, there was no evidence of cross-reactivity with the homologous GAD₂₆₁₋₂₆₉ sequence
489 (EVKEKGMAA). Another study (124) identified an HLA-A2-restricted epitope
490 (ILMNDQEVGV) largely conserved across serotypes that was naturally processed and presented
491 by CVB3-infected PBMCs and recognized by 25% of tested donors. Responses against an
492 EVREKHEFL variant of the epitope previously described by Varela-Calvino et al. were not
493 detected. CD8+ T-cell responses, quantified by either IFN- γ ELISpot or tetramer/IFN- γ staining,
494 were overall weak, requiring a prior 12-day *in-vitro* PBMC sensitisation. Using more sophisticated
495 in-silico approaches, additional HLA-A2-restricted CVB epitopes were proposed, but cognate
496 CD8+ T-cell responses detected by IFN- γ ELISpot were marginal (125).

497 Finally, like other viruses, CVB can also mount mechanisms to escape T-cell recognition, such as
498 downregulating surface HLA Class I expression (75,117). It is unknown whether HLA Class II
499 expression is also downregulated. This is part of a more general immune evasion mechanism by
500 which CVB inhibits protein trafficking (126), thus limiting the routing toward the cell surface of
501 multiple immune receptors and the secretion of soluble immune mediators (127,128). Another

502 open question is whether CVB persistence may favor the induction of CD8+ T-cell exhaustion
503 and, ultimately, poor anti-viral memory (129-131), and whether viral persistence is a common
504 outcome following an acute CVB infection.

505 **Summing up**

506 There is very limited information about the anti-CVB CD4+ and CD8+ T-cell responses mounted
507 upon infection that may secondarily cause beta-cell damage. This gap in knowledge reflects the
508 lack of reliable epitopes to track them. Mouse studies support a role for autoimmune T cells, while
509 anti-viral T cells might be protective rather than harmful. The overall indirect, immune-mediated
510 effects targeting beta cells that may secondarily trigger islet autoimmunity are summarized in
511 **Figure 4.**

512

513 **IX. Epitope mimicry**

514 **Mouse studies**

515 CVB/GAD homologous peptides can be presented by the diabetes-predisposing I-A^{g7} MHC Class
516 II allele of NOD mice (132). Although T-cell cross-reactivity was demonstrated by *in-vitro*
517 functional assays, *in-vivo* evidence that CVB-reactive T cells can also recognize GAD peptides
518 and amplify autoimmune anti-GAD T-cell responses or transfer diabetes is lacking. Indeed, T cells
519 isolated from CVB4-infected NOD mice neither exhibited increased proliferation to GAD protein
520 or homologous GAD/CVB peptide, nor triggered diabetes (104). Molecular similarities were also
521 identified between the VP1 capsid protein of CVB and the T1D autoantigens tyrosine phosphatases
522 IA-2 and IAR, and sera from NOD mice inoculated with CVB4 showed some cross-reactivity with
523 a IAR peptide (133). However, whether such mechanism can trigger autoreactive T cells to attack
524 beta cells has not been demonstrated.

525 **Human studies**

526 Despite some initial enthusiasm on the possibility of T-cell cross-reactivity between CVB and
527 GAD peptides mapping to the PEVKEK region (119), subsequent reports dismissed this possibility
528 for both CD4+ (120) and CD8+ T cells (123). Cross-reactive responses between a Rotavirus VP7₁₆₋
529₄₉ region and sequences from the IA-2 and GAD islet antigens encompassing both CD4+ and CD8+
530 epitopes have also been described (134).

531 **Summing up**

532 Although molecular mimicry is an intriguing scenario, the evidence for a CVB cross-reactivity
533 with islet antigens that may underlie this hypothetical mechanism is limited and conflicting.
534 Moreover, the evidence for a causal role of this cross-reactivity in triggering islet autoimmunity is
535 missing altogether, in both humans and mice. These putative molecular mimicry mechanisms are
536 summarized in **Figure 4** together with the other immune-mediated effects of CVB infection on
537 beta cells.

538

539 **X. Implications for T1D prevention strategies**

540 Despite the strength of evidence for an association between CVB infections and islet
541 autoimmunity, demonstration of a cause-effect relationship is lacking, and is likely to remain
542 elusive until tested in the human by removing this candidate environmental trigger (13). The most
543 effective way to achieve this would be through vaccination against CVB. This could allow to
544 prevent the most common manifestations of this infection (common cold) and, more importantly,
545 its rare but severe complications (myocarditis, encephalitis, meningitis). This is similar to what
546 has been achieved by Rotavirus vaccination in several countries. Following this rationale, a
547 formalin-inactivated non-adjuvanted CVB vaccine has been developed. Preclinical studies using a

548 prototype CVB1 vaccine were performed in BALB/c and NOD mice (54). High titers of
549 neutralizing Abs were induced. While non-immunized NOD mice displayed accelerated diabetes
550 after CVB1 infection, this was not the case in vaccinated animals, suggesting that the vaccine itself
551 does not accelerate diabetes development. A subsequent study in CVB1-infected *socs-1*-transgenic
552 NOD mice, which harbor beta cells that are unable to respond to IFNs and thus develop diabetes
553 due to massive beta-cell destruction, documented protection against both CVB1 infection and
554 subsequent CVB1-induced diabetes (61). A hexavalent version of the vaccine comprising the 6
555 CVB serotypes was subsequently shown to induce strong neutralizing Ab responses without
556 adjuvant in both mice and non-human primates, and provided immunity and protection against
557 CVB-induced myocarditis and diabetes (135). In NOD mice, the vaccine did not accelerate
558 spontaneous diabetes, while it delayed diabetes acceleration upon CVB1 infection (55).

559 On these grounds, a formalin-inactivated non-adjuvanted pentavalent intramuscular vaccine
560 comprising the 5 most common serotypes (CVB1 to CVB5, i.e. barring the less prevalent CVB6)
561 is undergoing a phase I safety trial in CVB-seronegative and -seropositive healthy adults
562 (NCT04690426). Early timing of vaccination will be critical for subsequent trials in order to
563 intervene before CVB exposure, e.g. starting at 2 months of age as for the inactivated polio (Salk)
564 vaccine. This will also require an excellent safety profile. In this perspective, it is important to gain
565 further insights into the mechanisms by which CVB infection may trigger beta-cell autoimmunity.
566 Such mechanisms hold implications for the clinical benefit that may be expected (**Figure 2**). If
567 beta-cell destruction is mainly provoked by the secondary pathogenic effect of anti-viral immune
568 responses on infected beta cells, vaccination may increase such responses and accelerate beta-cell
569 destruction. The same could be true if epitope mimicry mechanisms contribute to this destruction,
570 unless the viral sequences at play are excised from the vaccine constructs. While this possibility

571 assumes that CVB may still reach the pancreas in vaccinated individuals, preclinical studies
572 demonstrate that the vaccine efficiently prevents infection *ab initio*, including viremia and
573 systemic spreading (54,55,135). Conversely, the scenario of a primary pathogenic effect of CVB
574 on infected beta cells leading to self-antigen release and autoimmune priming, possibly associated
575 with poor anti-CVB immune responses, would lend a strong rationale for boosting these responses
576 by vaccination.

577

578 **XI. Conclusions and Perspectives**

579 Recent advances in our understanding of the association between CVB infections and islet
580 autoimmunity, and between Epstein-Barr virus and multiple sclerosis, invite us to move one step
581 further to definitely prove or disprove causality and explore preventative interventions through
582 anti-viral vaccination. Several lines of evidence support the possibility of persistent CVB infection
583 in individuals later developing islet autoimmunity. This persistent infection status is located in beta
584 cells, in the exocrine pancreas as well as in the gut, and is favoured by multiple immune escape
585 mechanisms mounted in different immune and non-immune cell types. While this provides a
586 strong rationale for preventing such infections *ab initio*, it might also encourage efforts to eradicate
587 them by antiviral treatment. In both cases, the timing of intervention will be critical, since
588 association studies highlight a temporal sequence where CVB infection is an early event preceding
589 aAb seroconversion and autoimmune initiation. Whether autoimmune progression can be halted
590 by viral eradication once initiated remains uncertain. A better knowledge of the immune responses
591 elicited by CVB infections and vaccines is vital to optimize vaccination strategies and their
592 risk/benefit ratio. On one hand, available evidence suggests that direct CVB-induced cytopathic
593 effects are major contributors to beta-cell demise, which may be favoured by poor immune

594 responses unable to efficiently clear the virus. On the other hand, there is very limited information
595 about the anti-CVB T-cell responses mounted and about the epitopes targeted upon infection.
596 These responses may secondarily cause damage by recognizing viral antigens exposed by infected
597 beta cells and, possibly, homologous self-antigens on non-infected beta cells. The magnitude of
598 these anti-viral responses and whether they are eventually protective or harmful for beta cells
599 deserve further scrutiny. Elucidating their dynamics will also provide immune monitoring tools
600 and surrogate markers to predict vaccination efficacy and, hopefully, protection from T1D.

601

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1034 nonhuman primates. *Sci Adv.* 2020;6(19):eaaz2433.
- 1035

1036 **Figure legends**

1037 **Figure 1. Timeline of the immune responses triggered by CVB infection.** CVB infection
1038 through the digestive or respiratory route first leads to the activation of innate immune responses,
1039 including the production of type I and III interferons. CVB-reactive T and B cells are subsequently
1040 activated along with other adaptive immune cell subsets. Finally, the formation of an immune
1041 memory is well documented for B cells (as neutralizing Abs persist for decades after CVB
1042 encounter), but not for T cells, where exhaustion mechanisms may also be at play. The predicted
1043 time course of CVB load and its associated immune responses is shown in the bottom graph. APCs,
1044 antigen-presenting cells; NK, natural killer; NKT, natural killer T; Treg, T regulatory; Tfh: T
1045 follicular helper.

1046

1047 **Figure 2. Three hypothetical, non-mutually exclusive mechanisms leading to beta-cell**
1048 **autoimmunity, and implications for T1D prevention by means of CVB vaccination.** The direct
1049 (primary) pathogenic effects of CVB on infected beta cells are detailed in Figure 3. The indirect
1050 (secondary) pathogenic effects of anti-viral immune responses on infected beta cells, including
1051 epitope mimicry mechanisms, are detailed in Figure 4.

1052

1053 **Figure 3. Direct pathogenic (lytic and non-lytic) effects of CVB on infected and neighboring**
1054 **beta cells.** Infected beta cells increase the synthesis of viral proteins at the expense of endogenous
1055 proteins, resulting in a dedifferentiated phenotype and impaired insulin secretion. Insulin secretion
1056 is further impacted by a decreased anterograde and increased retrograde vesicular trafficking.
1057 Enhanced endoplasmic reticulum (ER) stress leads to the activation of the unfolded protein
1058 response (UPR). Significant beta-cell death ensues, leading to release of double stranded (ds)RNA

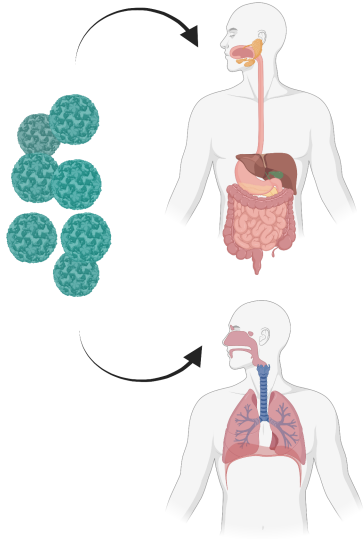
1059 and viral particles. In antigen-presenting cells like DCs and macrophages, dsRNA sensing through
1060 toll-like receptors (TLRs) leads to the activation of IFN response factors (IRF) and to type I IFN
1061 release, which inhibits viral replication. Type I IFNs are also secreted by non-infected beta cells
1062 sensing dsRNA through intracellular sensors such as MDA5 and RIG-I that activate the NF- κ B
1063 pathway. The binding of type I IFNs on surface receptors of beta cells enhances ER stress and
1064 apoptosis independently of CVB infection.

1065

1066 **Figure 4. Indirect immune-mediated pathogenic effects of CVB infection on beta cells.**

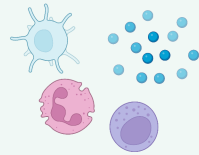
1067 Infected beta cells downregulate surface HLA Class I (HLA-I) expression, thus providing an
1068 immune escape mechanism by limiting recognition by anti-viral CD8⁺ T cells and, possibly,
1069 enhancing recognition by natural killer (NK) cells. Infected beta cells are lysed and antigens are
1070 taken up by antigen-presenting cells (APCs), thus favoring priming of both islet-reactive and CVB-
1071 reactive CD4⁺ and CD8⁺ T cells. CD4⁺ T-cell activation provides help to B cells for anti-CVB
1072 antibody production, which inhibits viral replication along with invariant (i)NK cells and
1073 macrophages. APC activation also leads to type I IFN secretion, which upregulates surface HLA-
1074 I presentation on beta cells, favoring recognition by islet-reactive CD8⁺ T cells of beta-cell
1075 endogenous epitopes, including neo-epitopes generated under these inflammatory conditions.
1076 CVB-reactive CD8⁺ T cells may also be diverted toward recognition of non-infected beta cells if
1077 epitope mimicry mechanisms are at play.

Infection



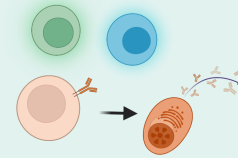
Innate immune response

- Type I and III IFNs
- APCs
- NK cells
- Neutrophils



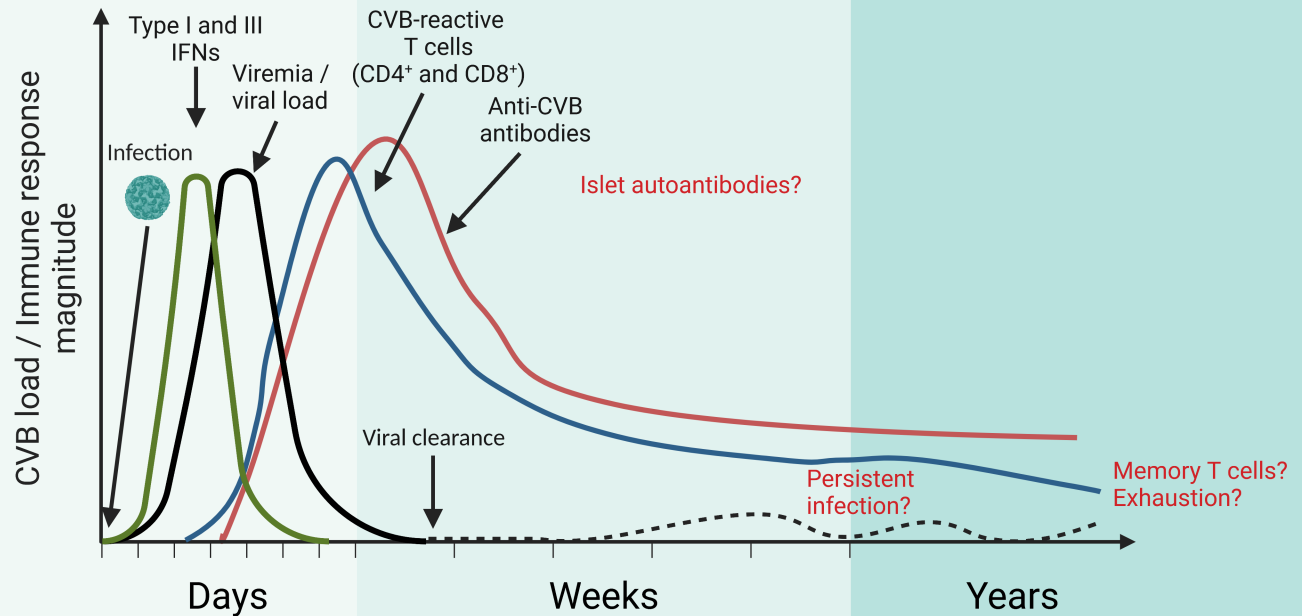
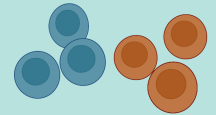
Adaptive immune response

- CD8⁺ T cells
- CD4⁺ T cells
- NKT cells
- Treg cells
- Tfh
- B cells, antibody production

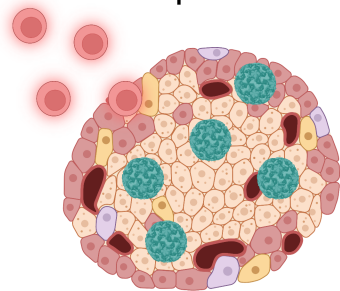


Immune memory

- Memory T cells?
- Memory B cells



1) **Direct** pathogenic effect of **CVB** on infected β cells

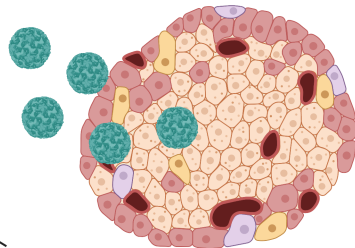


Self-antigen release

Poor anti-CVB immune-response

- Rationale **for** boosting through anti-CVB vaccination
- Potential clinical **benefit**

CVB infection of β cells

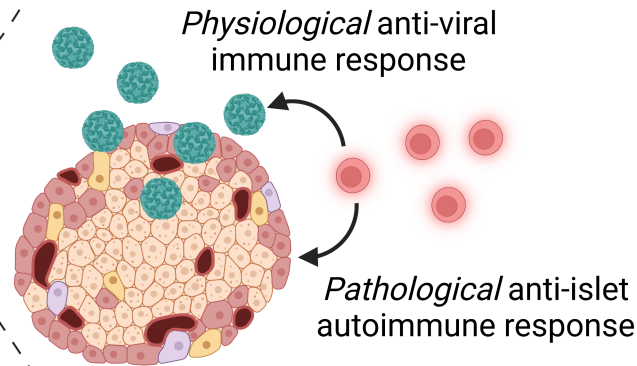


2) **Indirect** pathogenic effect of **anti-viral immune responses** on infected β cells

Self-antigen release

Strong anti-CVB immune-response

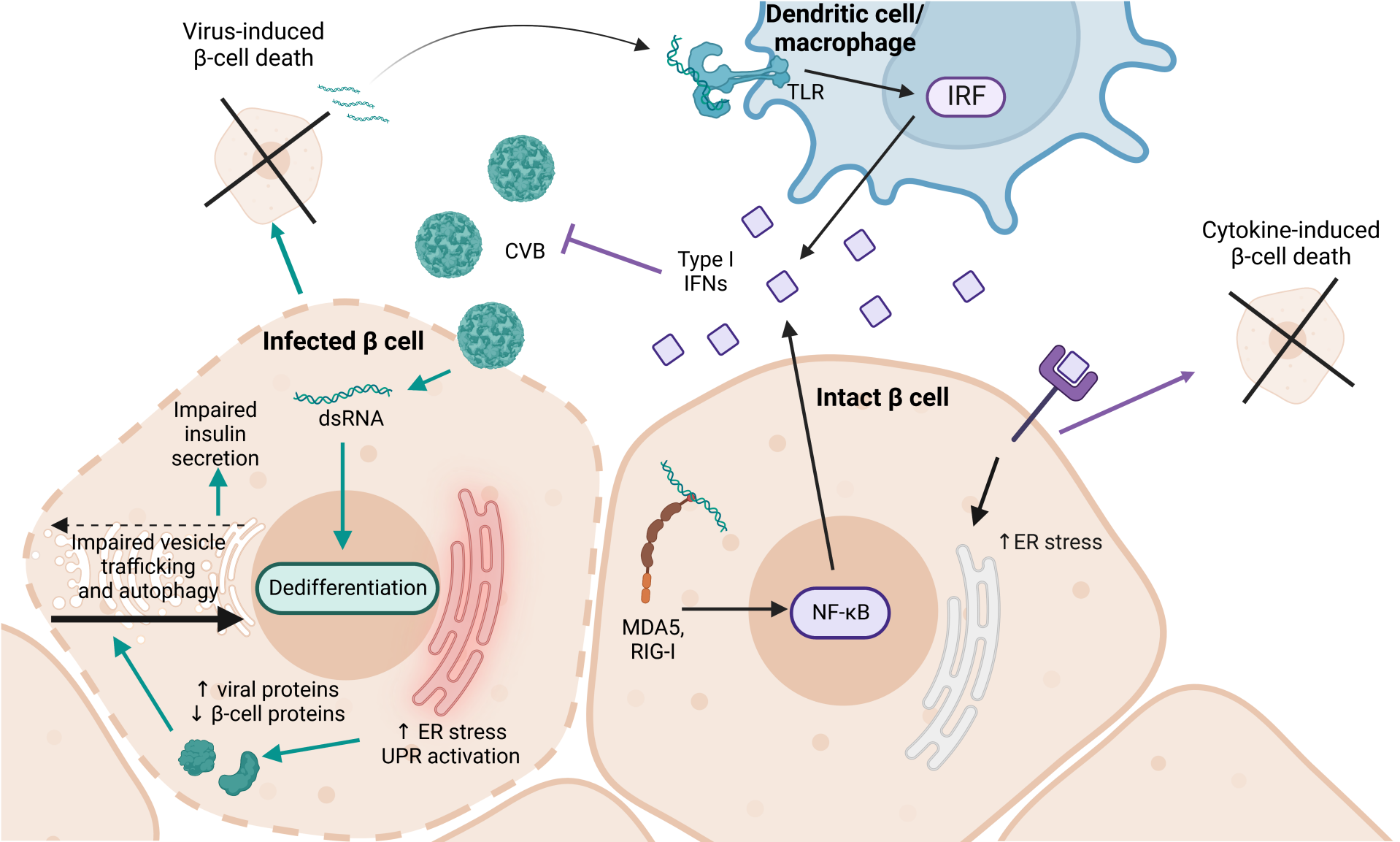
3) **Epitope mimicry**

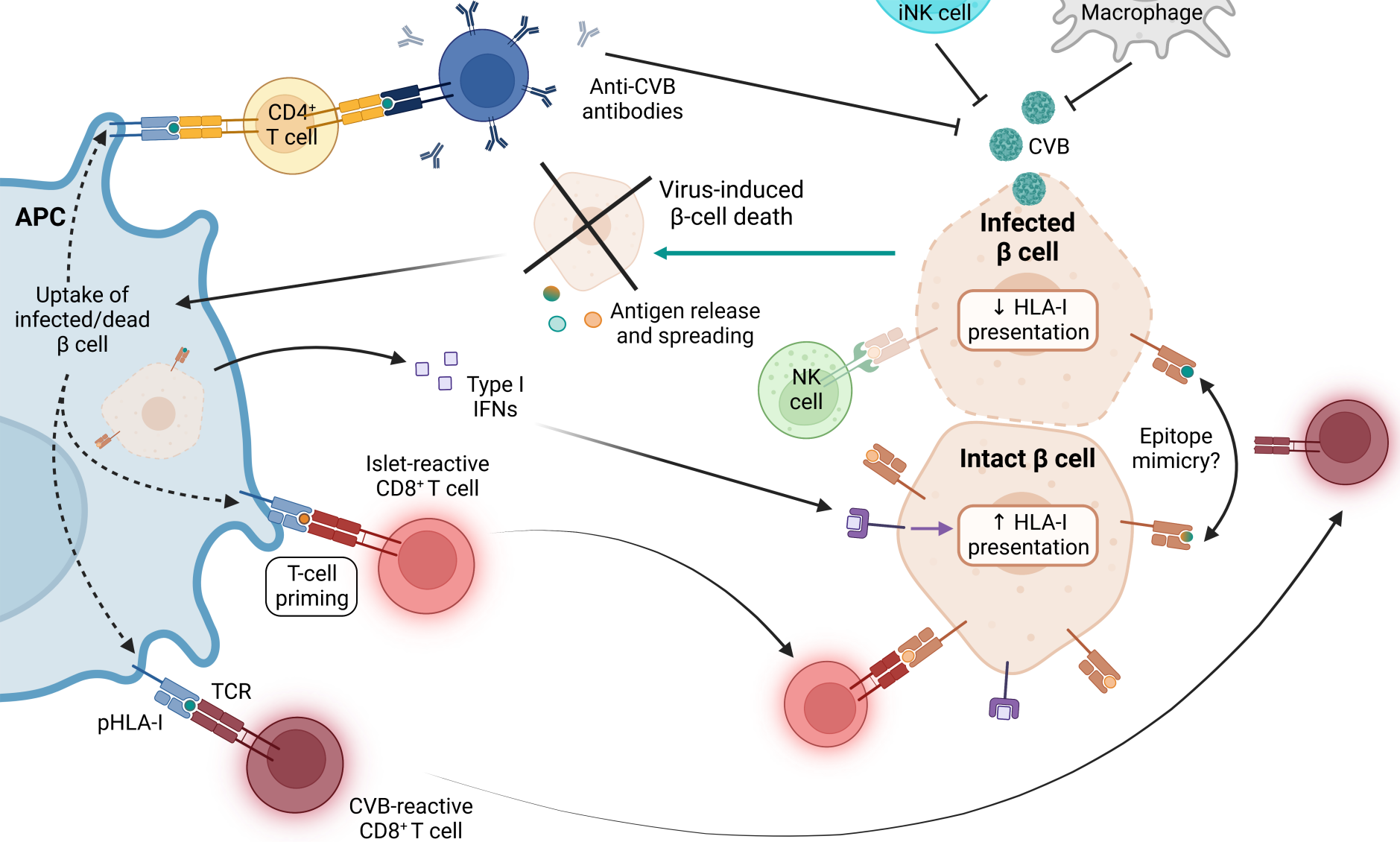


Physiological anti-viral immune response

Pathological anti-islet autoimmune response

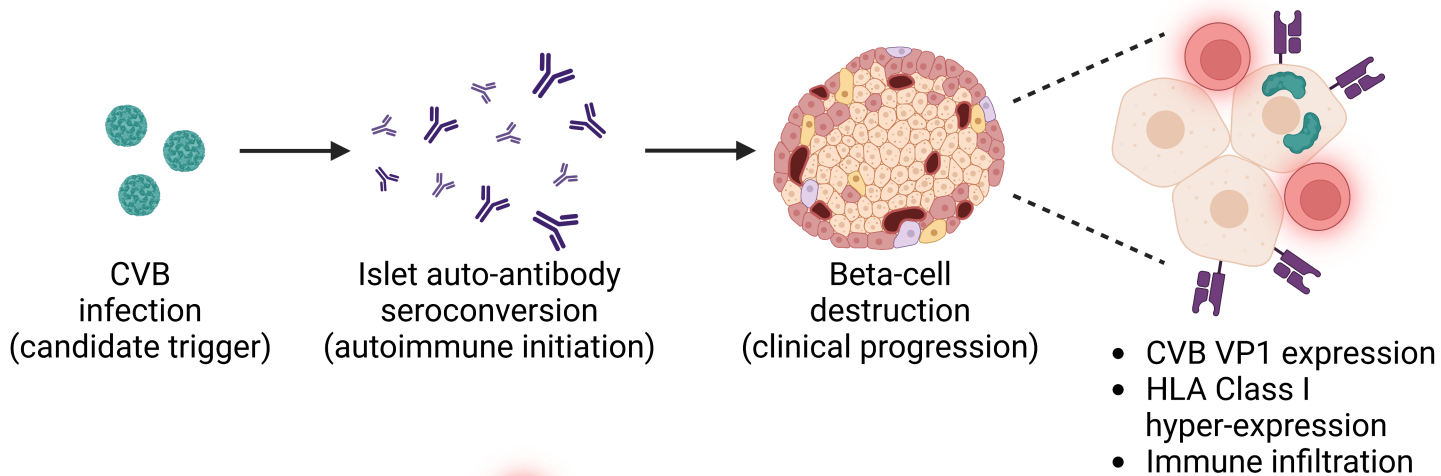
- Rationale **against** boosting through anti-CVB vaccination
- Potential clinical **risk**



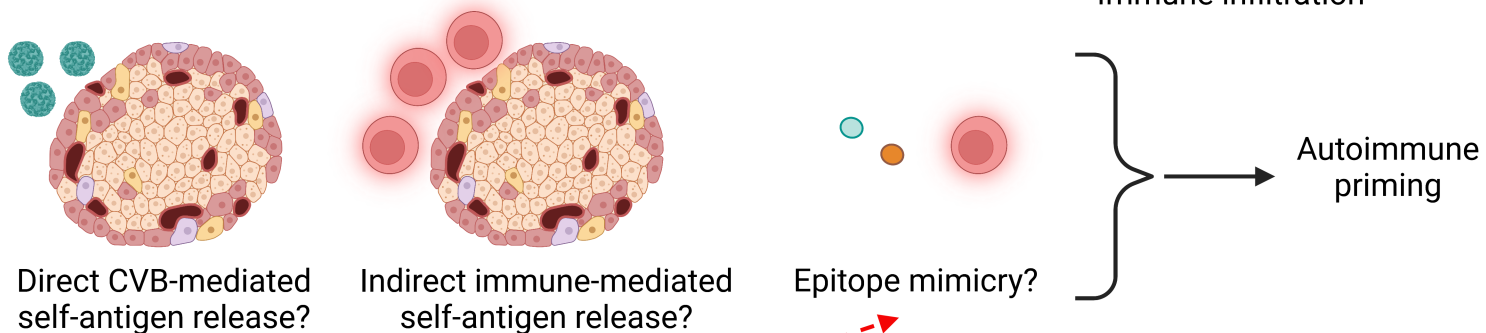


Coxsackievirus B (CVB) infection and type 1 diabetes

Association



Mechanisms?



Causality?

