Virus in the pancreas, Sugar in the blood?

Is there a link?

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Outline

- Virus in the pancreas
  - Which viruses show islet/ β cell tropism
  - Which cell types are infected
  - Mechanisms of damage
  - EV infection of insulin producing cells
- Sugar in the blood
  - Evidence for infections in diabetes aetiology
  - VIGR (**V**irus **I**nfections in the **G**enetically at **R**isk)
- Future directions

Hypothesis

- Infection and replication of viruses in pancreatic β-cells contributes to the development of type 1 diabetes
  - Mediated by pro-inflammatory cytokines (produced by β cells and other inflammatory cells, eg macrophages)
  - Infection triggers apoptosis

Pancreatotropic viruses

- Humans
  - Rubella
  - Mumps
  - Enterovirus
  - CMV, EBV
  - Rotavirus
  - Adenovirus
  - Reovirus
  - Ljungan virus
- Animal
  - Rubella
  - Encephalomyocarditis virus
  - Enterovirus
  - CMV
  - Rotavirus
  - Reovirus
  - Kilham rat virus
  - Ljungan virus

Characteristic	Rubella	EV	CMV	Rotavirus
Time of infection	*In utero*	*In utero*, perinatal, pre-diabetes, at onset	*In utero*, perinatal	Pre-diabetes
HLA association	DR3 (DR2-)	DR3, DR4, or non DR3/4	No	DR3, DR4
Cell Tropism	Pancreas and beta cells	Beta cells	Pancreas and beta cells	Pancreas and beta cells
Mechanisms for diabetes	Direct infection; autoimmune? (molecular mimicry)	Direct infection; autoimmune (bystander, mimicry, other)	Direct infection; autoimmune	Direct infection; autoimmune

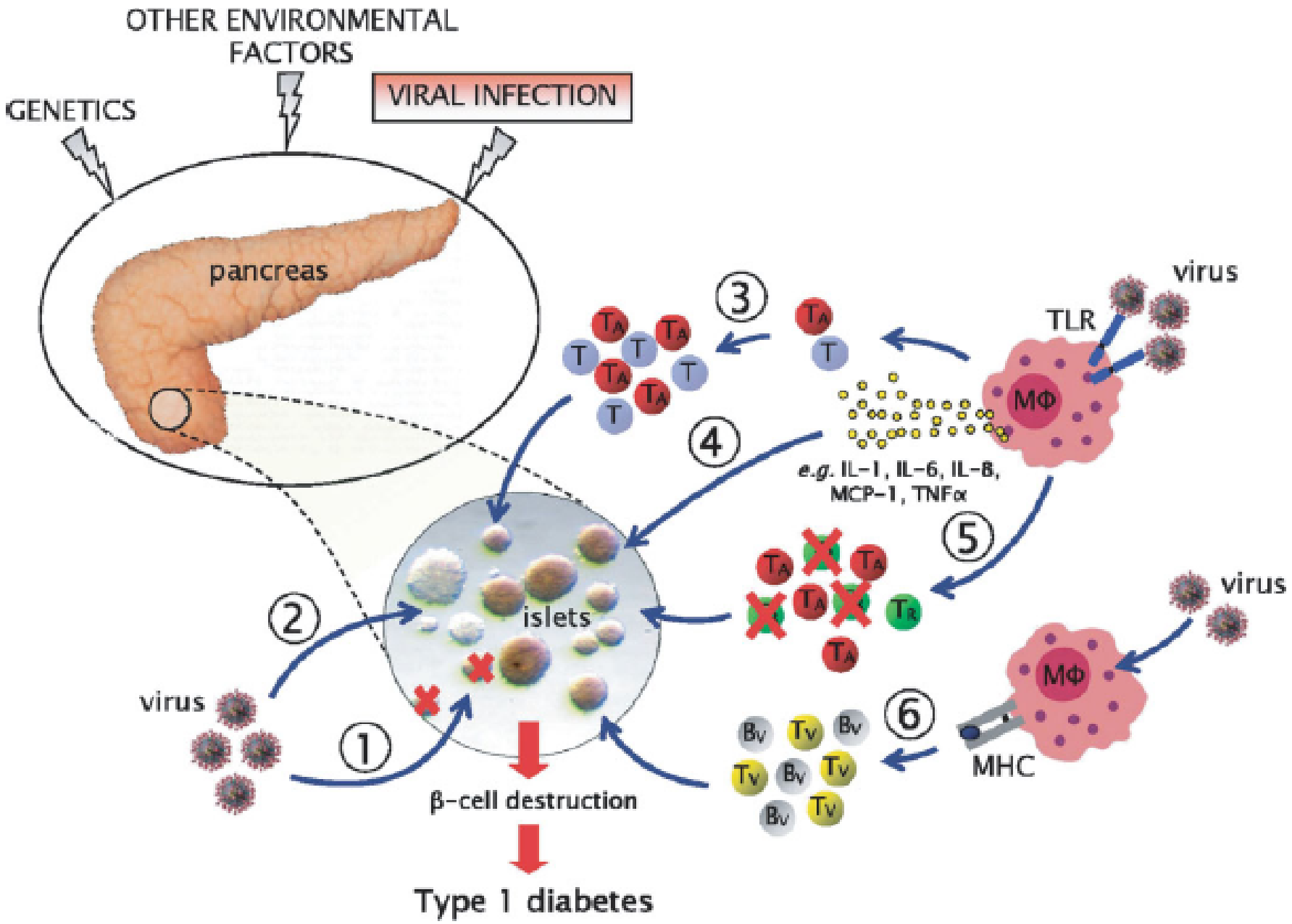
Adapted from Honeyman, *Current Opinion Immunology* 2005

Viral aetiology of diabetes

- Concepts
  - Sporadic infection with diabetogenic virus
    - In genetically susceptible host (HLA, other genes)
    - or in any individual?
  - Multiple or persistent infection with ubiquitous virus
    - Interaction with other factors (eg diet, vitamin D)
    - Role of host susceptibility?

Mechanisms of infection

- Direct infection, cytolysis
  - *In utero*
  - Postnatally, in the pre-clinical period
    - As an initiator of autoimmunity
  - At diagnosis
    - Rapid disease onset (in those not at genetic risk?) or an accelerator of autoimmunity
- Other
  - Role of cytokines, autoreactive T cells, T Regs
- Role of EV receptors
  - Up-regulation of receptors on beta cells?



Diabetes Metab Res Rev 2007; 23: 169–183.

Congenital Rubella and Diabetes

- ~20 published case reports
  - Autopsies performed in 2 cases
    - Insulitis in a child with DKA
    - Atrophic islets consistent with long term diabetes
- Case series from NSW
  - 20% reported to have diabetes
    - Forrest, *Pediatrics* 1969; Forrest *Lancet* 1971
  - After 60 years, 7/40 still alive had diabetes (22%)
    5 type 2 diabetes, 2 LADA, none had type 1
    - Forrest, *MJA* 2002

Other countries

- USA
  - Diabetes 15/242 (6%), IGT in 14/242
- UK
  - 2/616, ie no observed excess
- Canada
  - 12/100 diabetes
- Japan
  - 3/200 diabetes

Congenital Rubella and Diabetes

Study	M/F	Birthweight (g)	Age at diagnosis of diabetes	DKA	Insulin-dependent	Other features
Forrest et al., 1969 [19]	F		18 months	+	+	Glaucoma
	F		12 years	–	+	Cataract, PDA
	M		12 years[a]	–	+	Deaf, rubella retinopathy
	M		24 years[a]	–	–	Cataracts, pulmonary stenosis
	M		28 years	–	–	Cataracts
Plotkin and Kaye, 1970 [21]	M	2,200	12 months	–	+	Deaf, PDA
	M		19 months	+	+	Deaf
Johnson and Tudor, 1970 [22]	M	2,300	3 years	–	+	Deaf, PDA
	M	2,400	20 months	–	+	Cataracts, PDA
Halvorson, 1977 [23]	M	2,510	7 years	–	+	Deaf, pulmonary stenosis
Smithells et al., 1978 [24]	M	1,941	3.5 years	?	?	Deaf
	F	1,913	2.5 years	?	?	Deaf
Floret et al., 1980 [25]	M	3,050	17 years	–	+	Mental retardation, hyperthyroidism
Jenson et al., 1980 [26]	M		4 years	+	+	Died in DKA aged 11 years
Patterson et al., 1981 [27]	M		14 months	+	–	Died in DKA, insulitis, cataract
Schopfer et al., 1982 [28]	F	2,270	4 years	–[b]	+	Deaf, PDA, hypothyroid
	F	1,900	11 years	–[b]	–	Deaf, microcephaly, cataracts
Takasu et al., 2005 [29]	F		21 years	+	+	Deaf, cataracts, hypothyroidism
	F		13 years	+	+	Deaf, cataracts, mutism
	M		18 years	–	+	Deaf, cataracts, atrial septal defect

Gale, *Diabetologia* 2008

Islet damage in fatal viral infections

- 45 with congenital rubella
  - Only 2 had histological abnormalities
- 4/7 with Coxsackie B4
- 20/45 with CMV
- 14 with VZV
  - Jenson et al, *Lancet* 1980

- Rubella less likely than other viruses to cause beta cell damage??

Rotavirus and type 1 diabetes

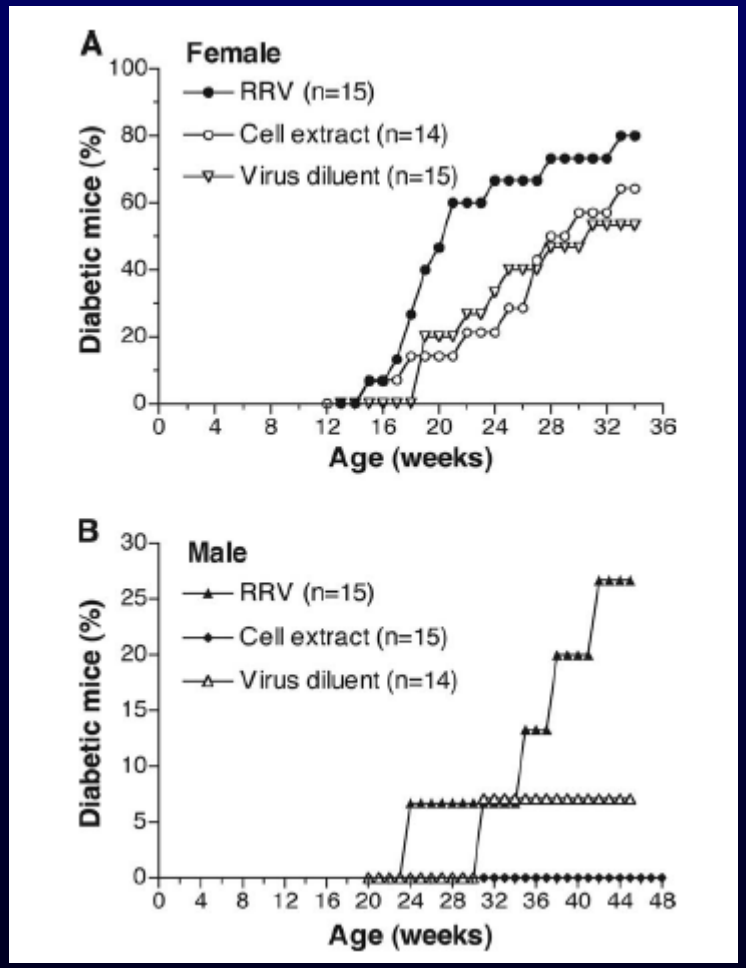
Evidence in Humans

- Childhood rotavirus infections have been associated with islet autoimmunity in children with HLA risk genes for diabetes
  - Honeyman et al, *Diabetes* 2000
    - Data not replicated by other cohort studies
- Increased antibody responses to dietary bovine insulin after rotavirus infection
  - Makela et al, *J Autoimmunity*, 2006

Rotavirus and type 1 diabetes

Evidence in Mice

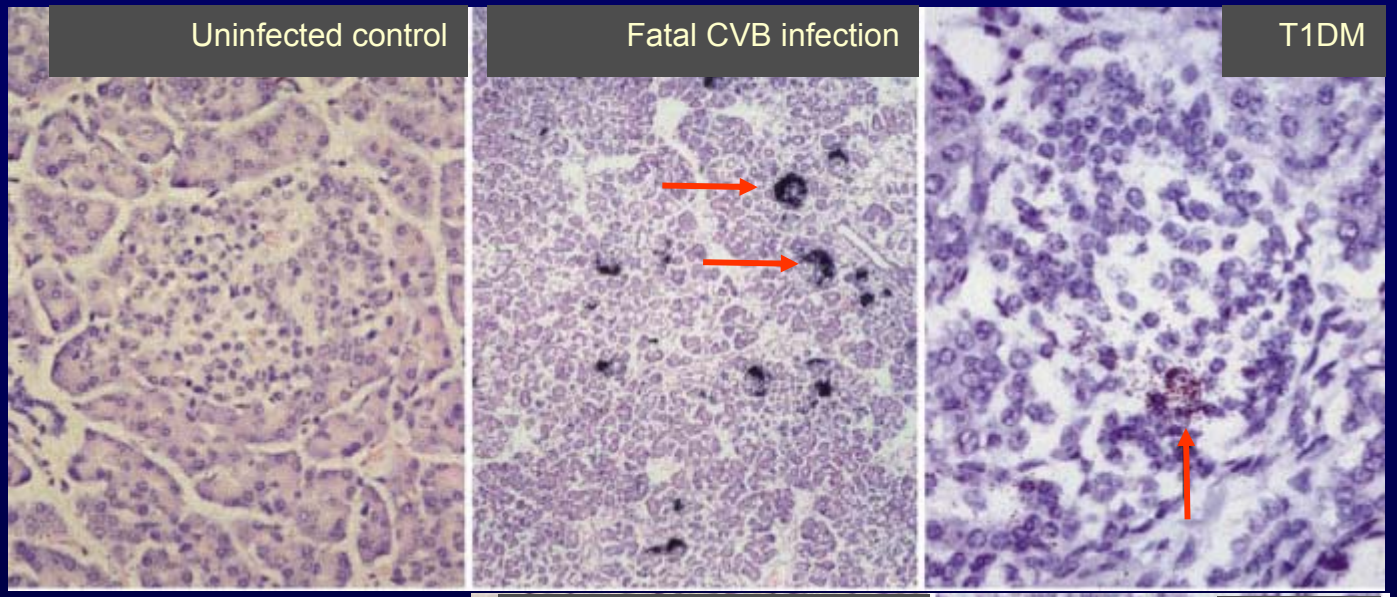
- Rotavirus infection, *after β-cell autoimmunity is established* , affects insulitis and exacerbates diabetes
  - Graham et al, *J Virology* 2008
- Timing of infection relative to mouse age and degree of insulitis determines whether diabetes onset is delayed, unaltered, or accelerated

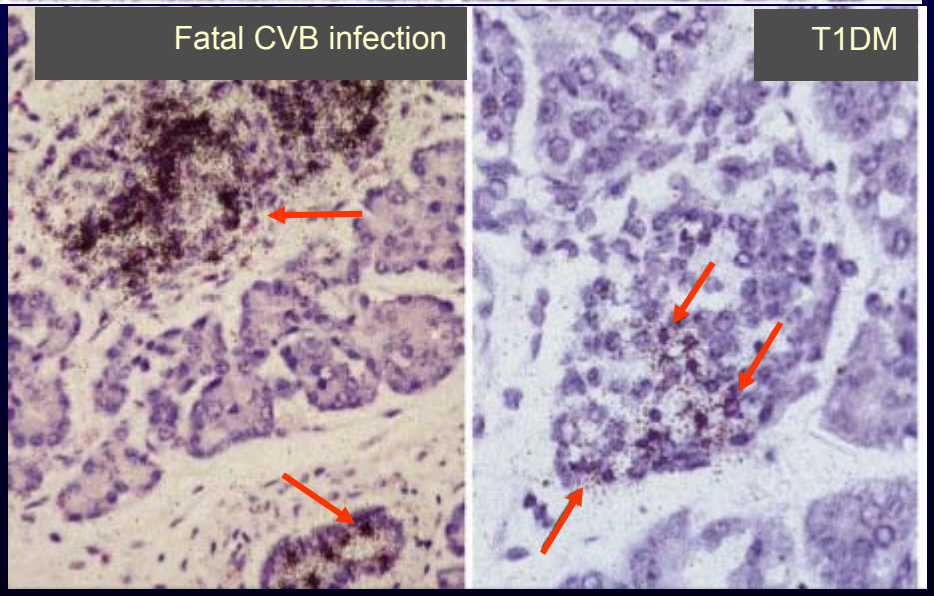


Enterovirus infection of β-cells

- Enterovirus infection demonstrated in pancreatic islets and duct cells in autopsy specimens from 7/12 infants who died of fulminant coxsackievirus infection
  - & pancreata from 4/65 type 1 diabetic patients
- No infected cells in exocrine tissue
- No EV detected in 40 control pancreata
  - Ylipasto et al *Diabetologia* 2004

Direct EV Infection of Pancreas





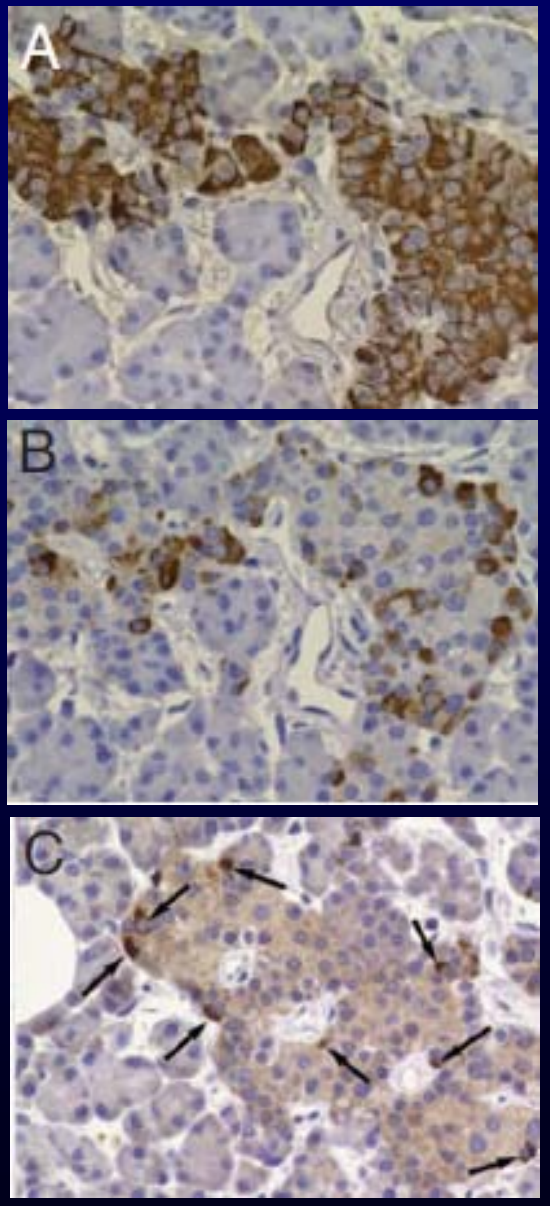
Ylipaasto *et al* (2004) *Diabetologia* 47:225-39

Postulated mechanisms

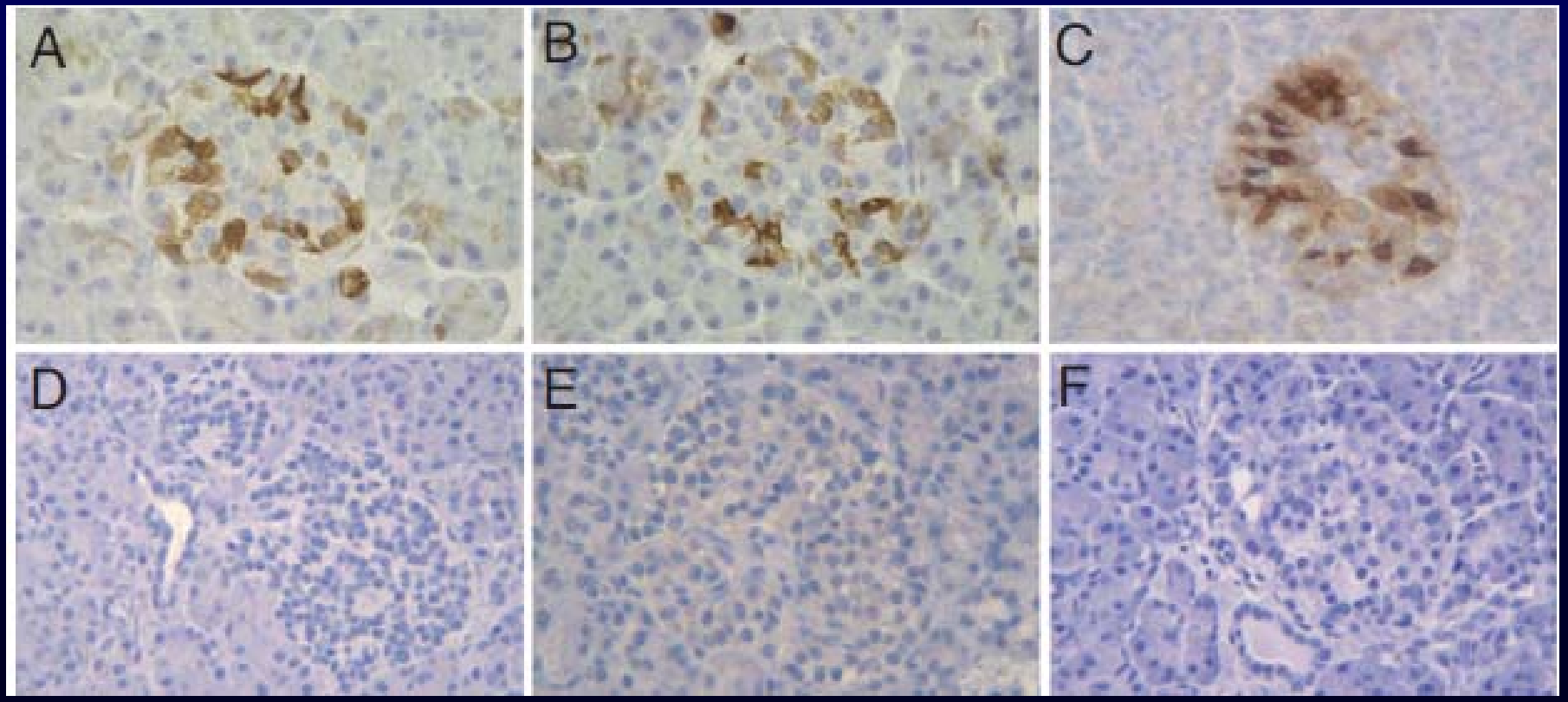
- Poliovirus Receptor and integrin αvβ3
  - these allow entry of enteroviruses in established cell lines and are expressed in primary human islets
- No expression of DAF, a regulator of complement activity, which acts as a receptor for several EVs known to replicate in islet cell lines

Further direct human evidence

- EV infection with a novel CVB4 "Tuscany Strain“ was found in 3/6 TIDM patients
  - Dotta et al, *PNAS* 2007
- Viral infection was β-cell specific and caused β -cell dysfunction (impaired glucose-stimulated insulin secretion)
- Nondestructive islet inflammation with NK cell infiltration and IFNα infiltration



Patient no.	Age, yr	Sex	Time from diagnosis	β cell function	Enteroviral infection of β cells	β cell destruction	Insulitis
1	26	F	—*	Impaired	Yes	No	Minor; dominated by NK cells; no evidence for autoreactive T cells
2	19	M	9 months	Partially lost	Yes	No	Minor; dominated by NK cells; no evidence for autoreactive T cells
3	15	F	1 week	Lost	Yes	Limited	Minor; dominated by NK cells; no evidence for autoreactive T cells
4	14	F	8 months	Lost	No	Yes	Moderate T cell infiltrate; no NK cells
5	5	M	1 week	Lost	No	Yes	Moderate T cell infiltrate; no NK cells
6	4	F	1 week	Lost	No	Yes	Moderate T cell infiltrate; no NK cells



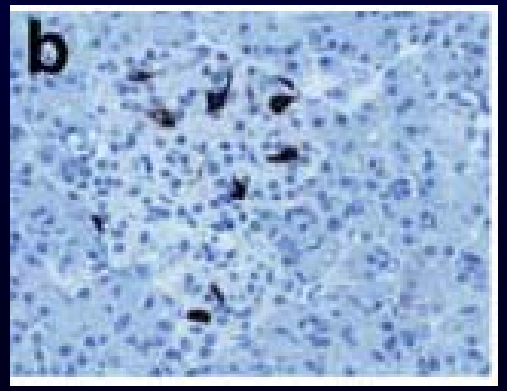
Immunohistochemistry shows reactivity to VP-1 EV peptide

Further analysis

- Pancreata of CVB4 positive T1DM patients:
  - insulitis characterised by NK cells (CD94+) and few CD3+ T-cells
- IFN-α and FAS detected in the majority of pancreatic islets by immunohistochemestry
- Chemokine CXCL10 increased in islets, with infiltration of lymphocytes expressing the corresponding chemokine receptor CXCR3
- Both in islet and endothelial cells over-expressed class I MHC molecules

EV capsid protein VP1 + in islets

- pancreatic autopsy samples from 72 young recent-onset type 1 diabetes patients and 161 controls
- vp1-immunopositive cells detected in multiple islets of 44/72 (61%) type 1 pts
  - vs 3/50 neonatal/paediatric normal controls
  - and 10/25 type 2 diabetic patients
- Staining was specific to islets
    - Richardson *Diabetologia* 2009



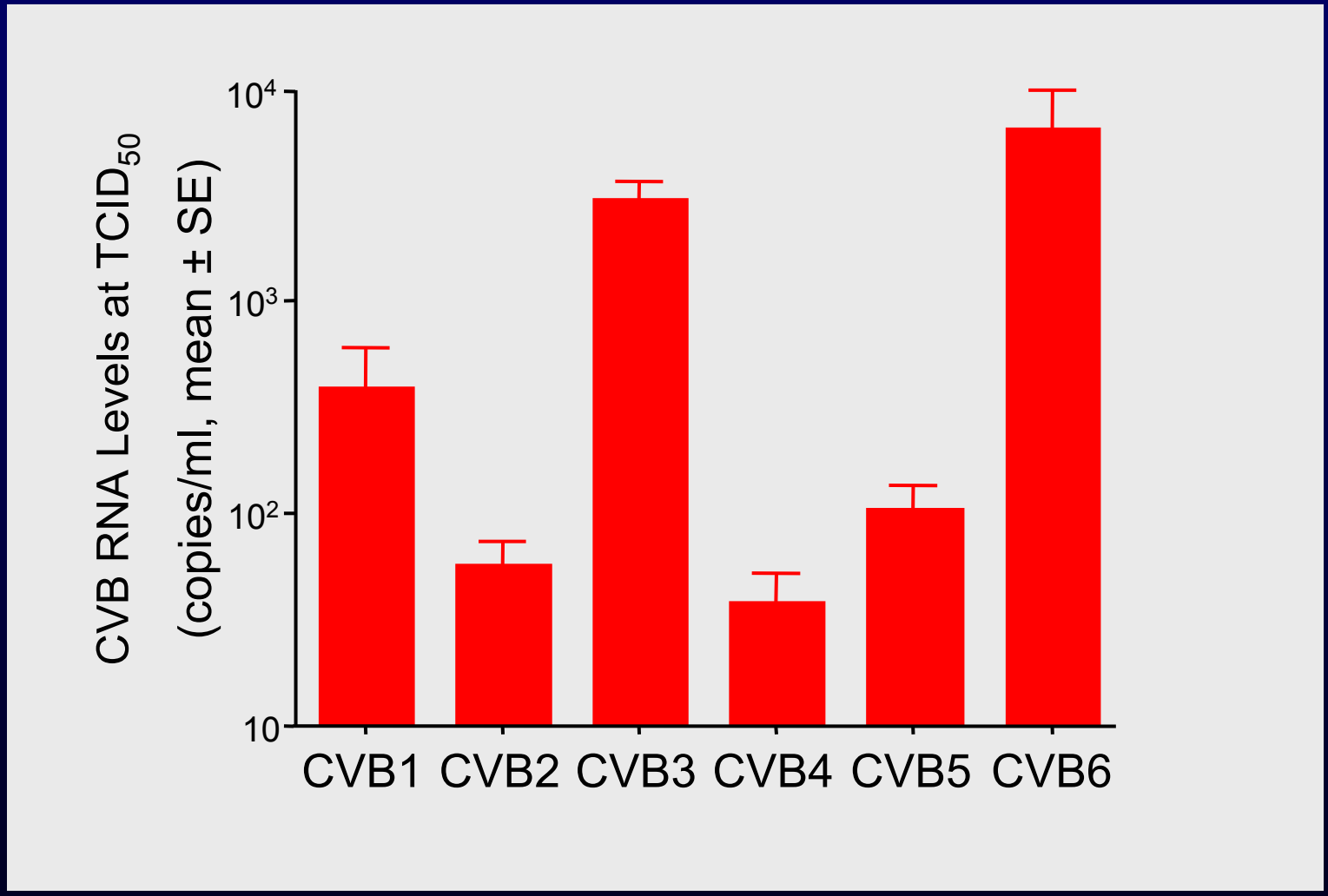
EV infection and autoimmunity

- Enterovirus VP1 capsid stained + in pancreatic tissue in a child repeatedly positive for islet cell antibodies
  - No inflammatory changes in islets
  - No reduction in beta cells
  - No insulitis, apoptosis
- Findings suggest that (chronic) EV infection contributed to positive islet cell autoantibodies
  - Oikarinen et al, *Diabetologia* 2008

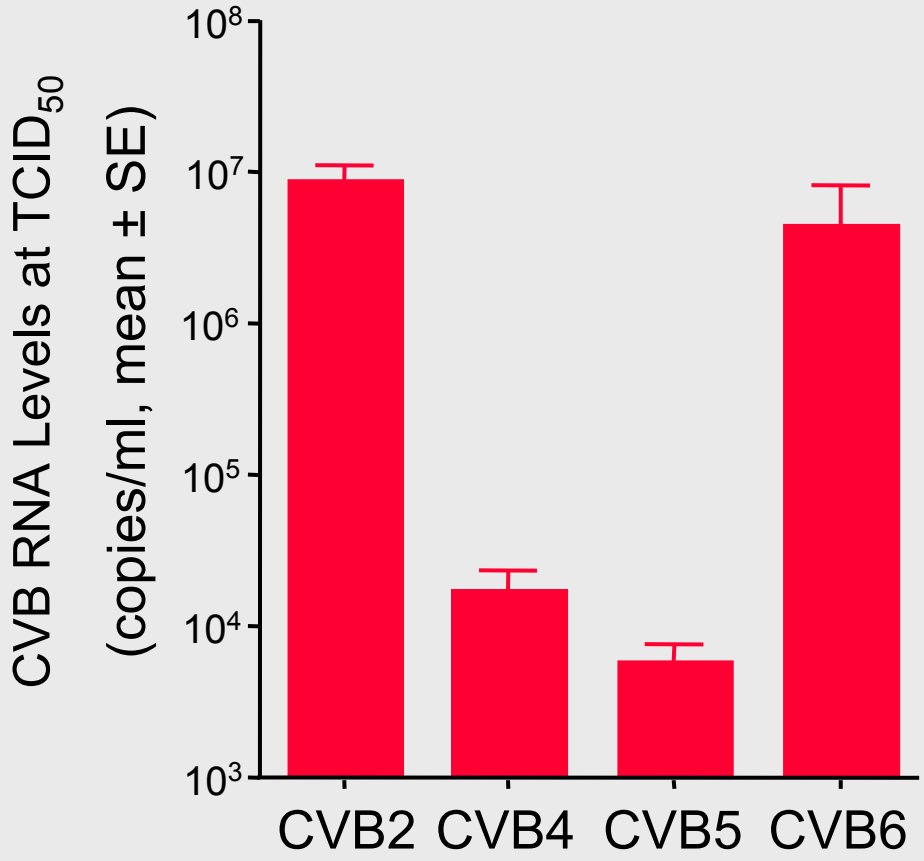
EV infection of β-cell lines

- Enteroviruses:
  - CVB1-6, ECHO4, 6, 9, 11, 18, EV70
- Cellular Models:
  - Insulin-producing cells: INS-1, RINm5F
  - Non-insulin-producing cells: HeLa, MRC-5
- Assays:
  - Cytolytic effect by TCID50 assay
  - Viral RNA in cell lysates & culture supernatants by qRT-PCR
  - Insulin, Pdx-1 and b-actin mRNA in cell lysates by qRT-PCR

Cytolytic Effects of CVBs in HeLa



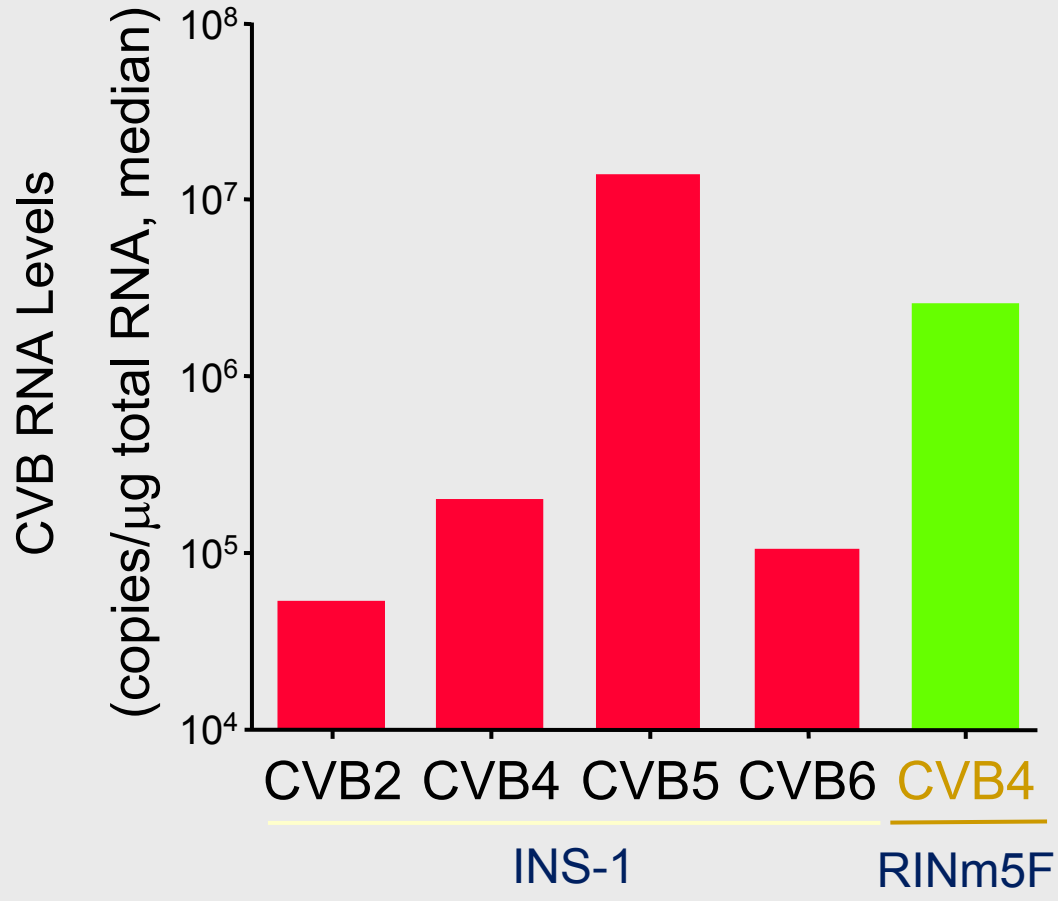
Cytolytic Effects of CVBs in INS-1



- CVB1 and CVB3 had no effect on INS-1
- CVBs did not cause cytolysis in RINm5F

CVB Replication in insulin producing Cells

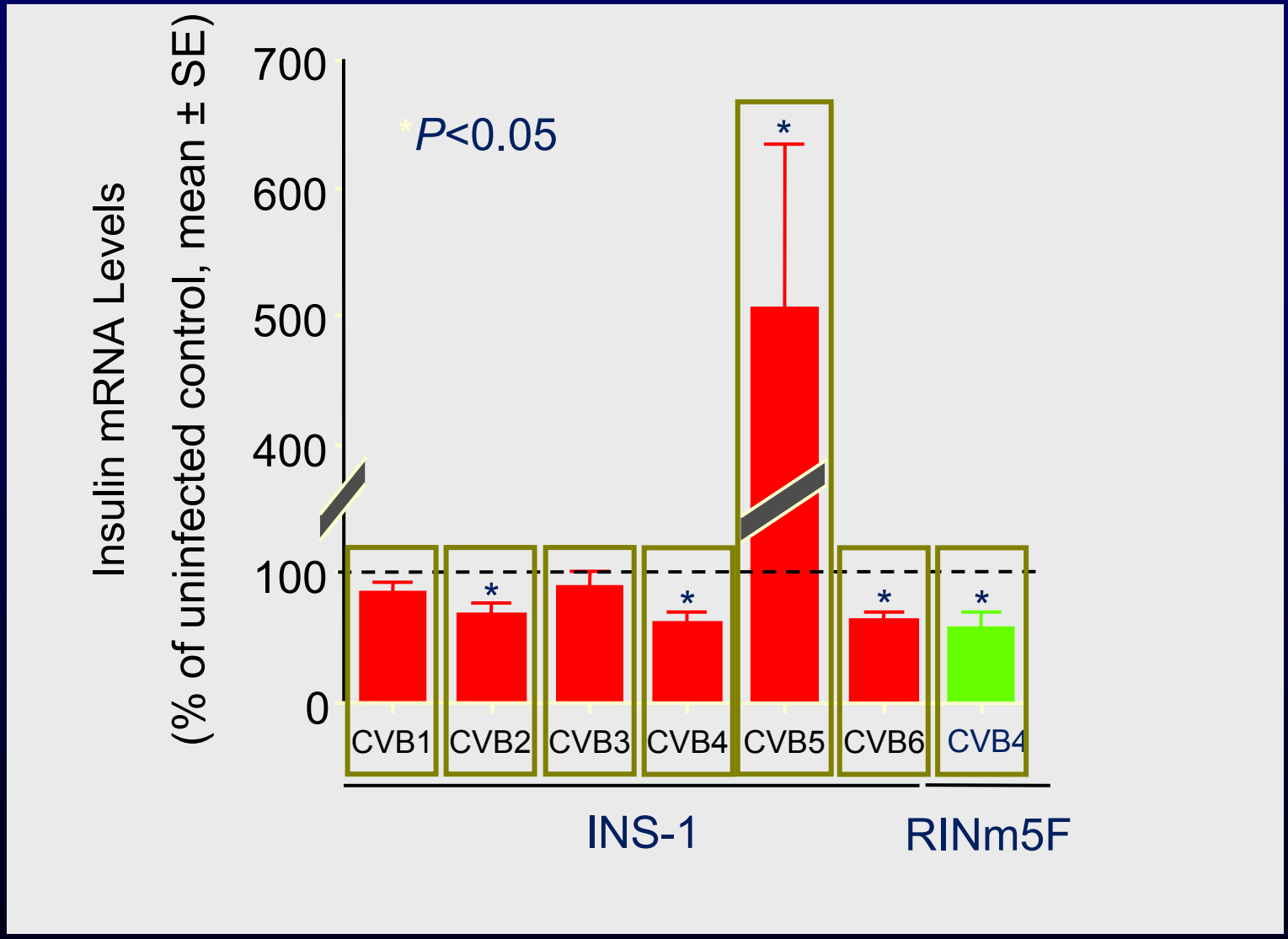
Viral RNA in Host Cell Lysates



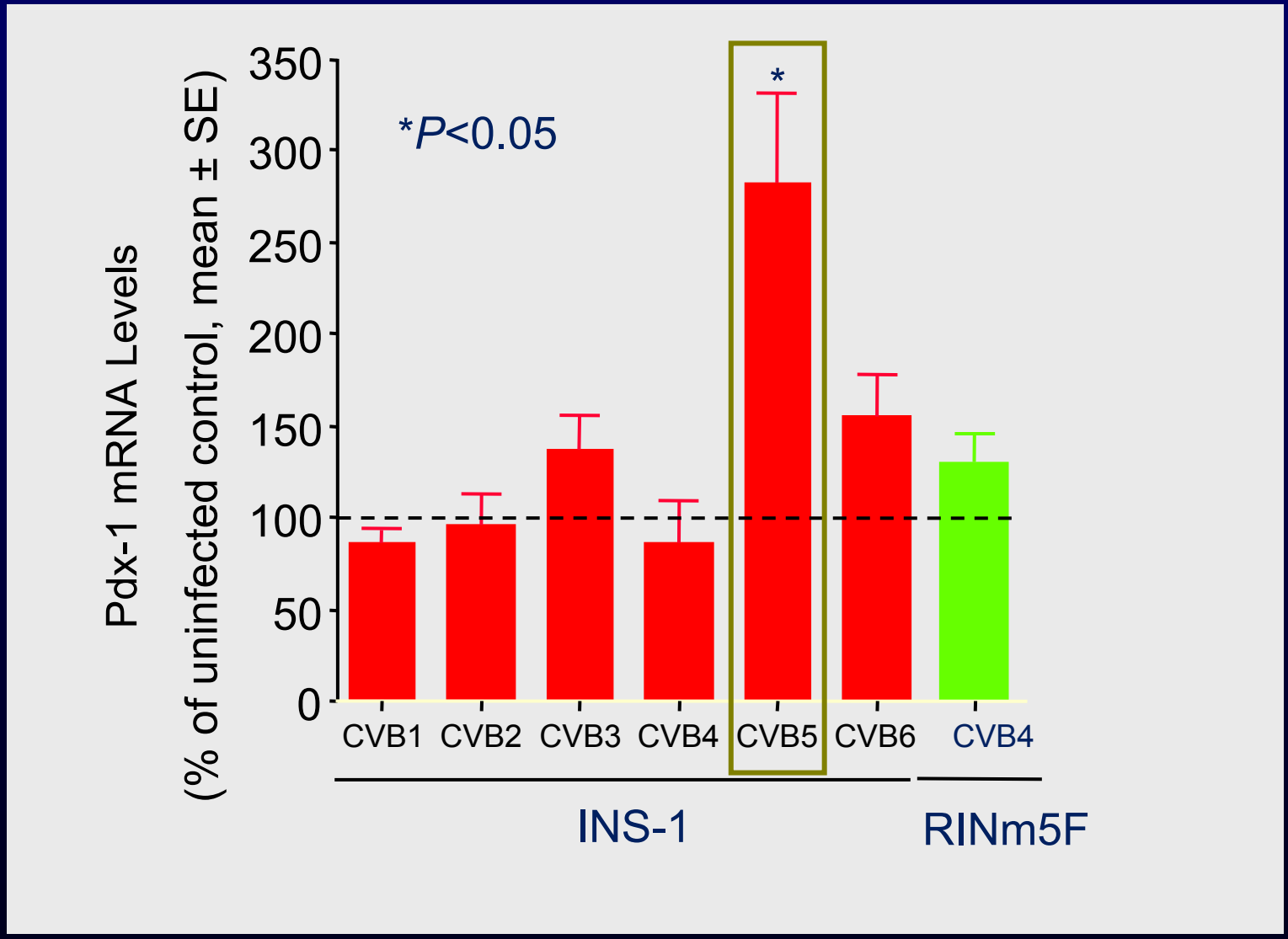
No EV RNA detected for CVB1, 3 in INS-1 cells

No EV RNA detected for CVB1-3, 5, 6 in RINm5F

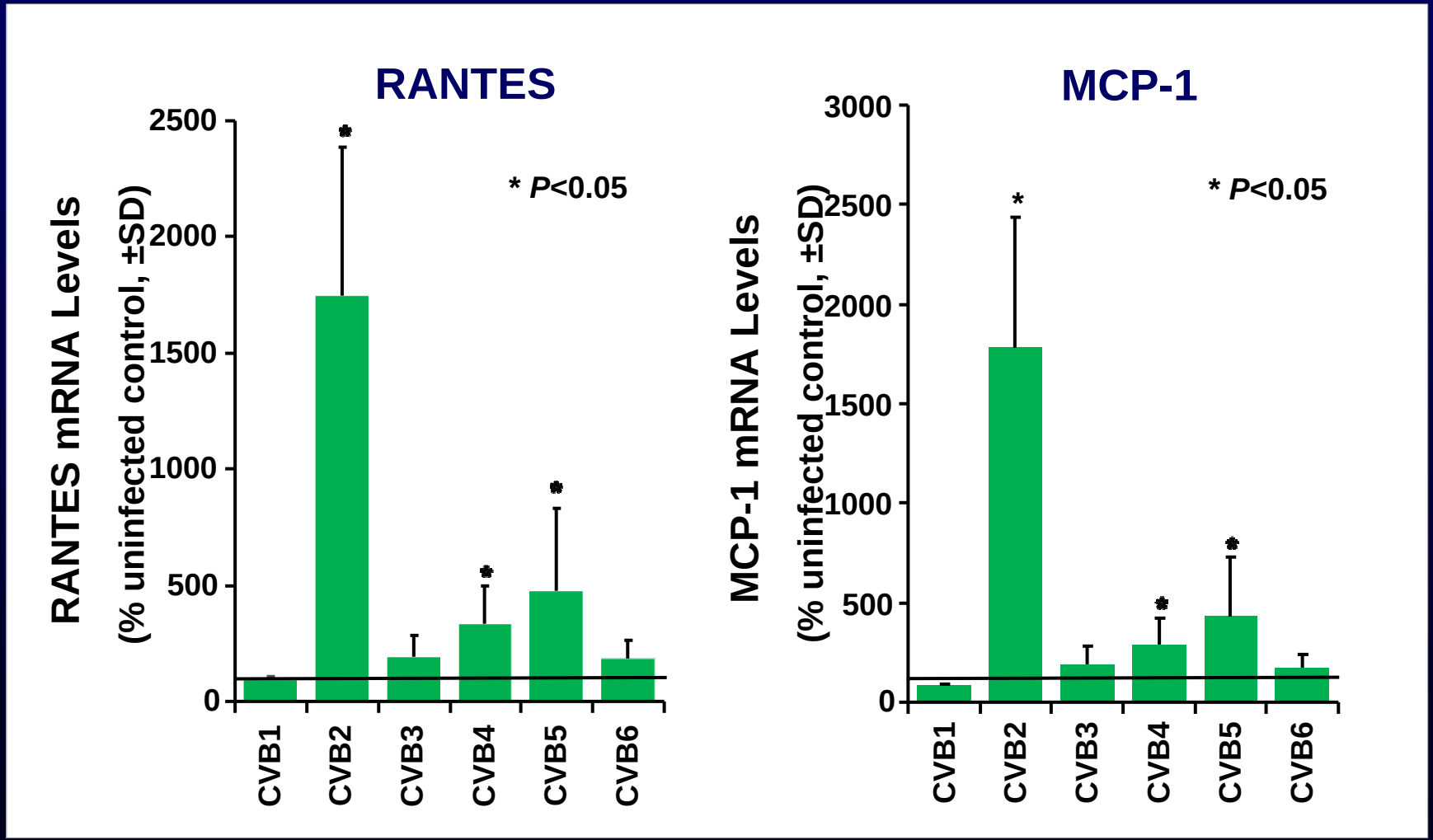
Effects of CVBs on Insulin Expression



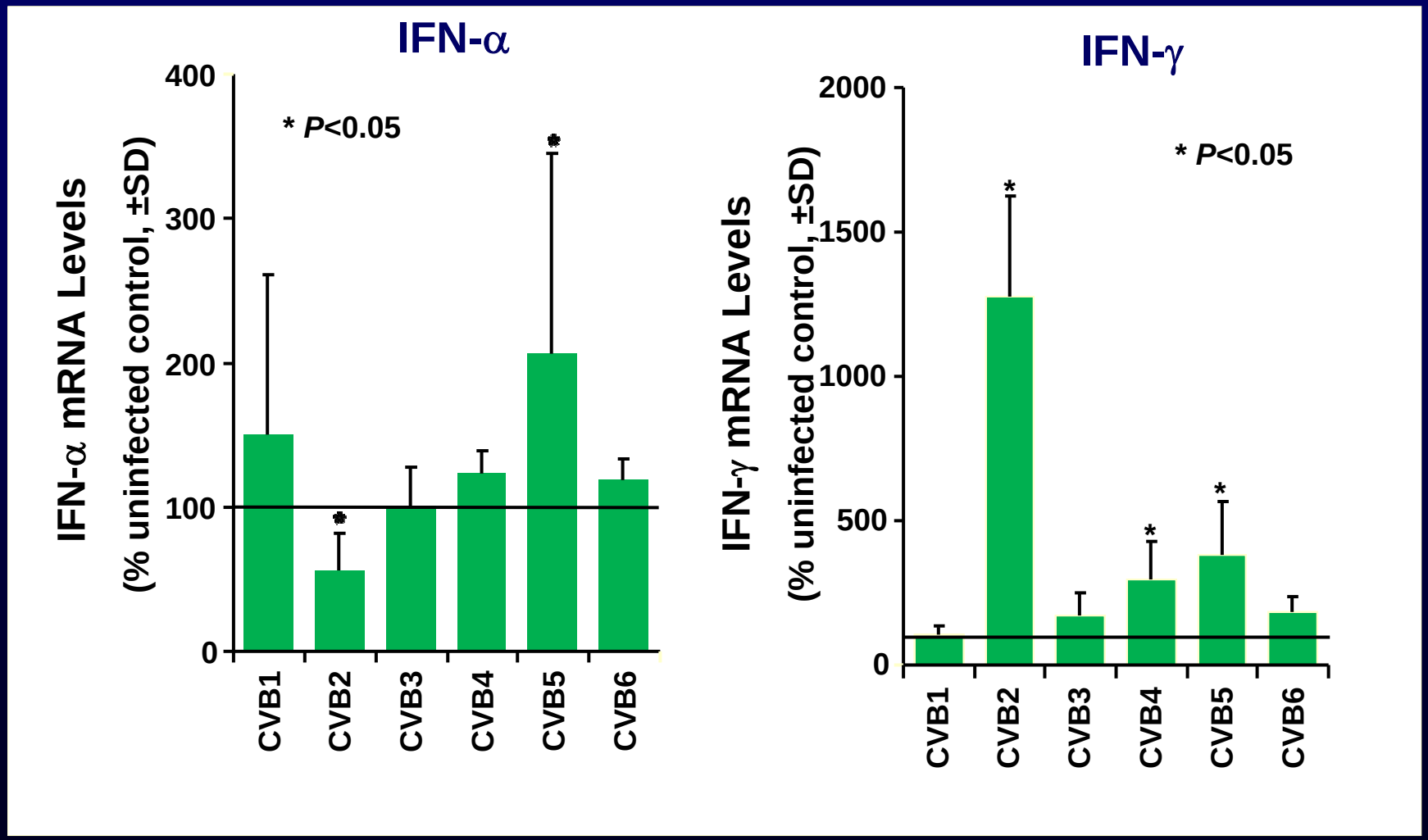
CVBs on Pdx-1 Expression



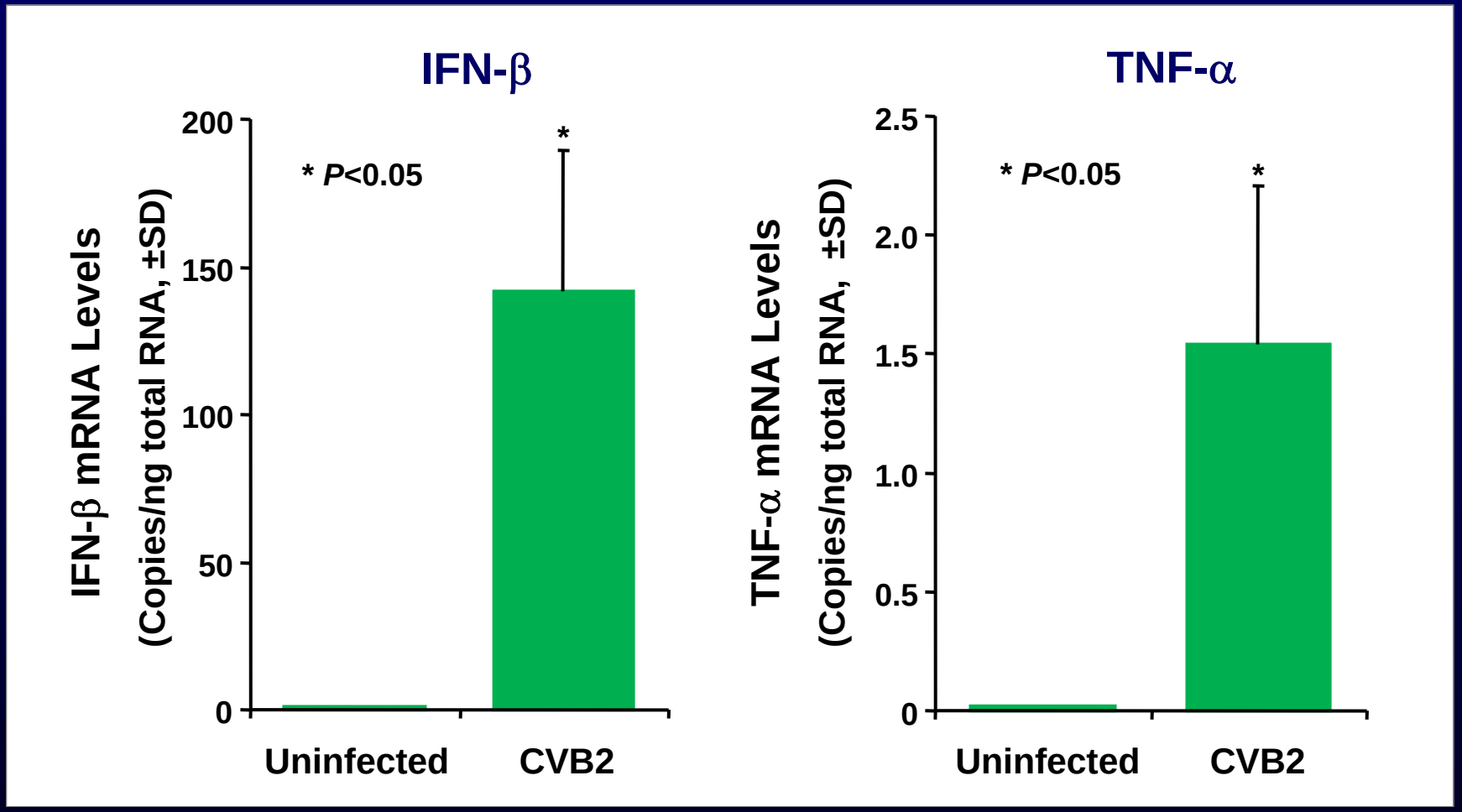
CVB effects on RANTES and MCP-1 Expression



CVBs - IFN-a and IFN-g Expression



CVB2 - IFN-b and TNF-a Expression



No effects detected with other CVBs

Summary of CVB Effects in INS-1

	Cyto-lytic
CVB1	-
CVB2	+
CVB3	-
CVB4	++
CVB5	++
CVB6	+

Summary of CVB Effects in INS-1

	Cyto-lytic	Prolifer-ation
CVB1	-	-
CVB2	+	+
CVB3	-	-
CVB4	++	++
CVB5	++	++
CVB6	+	+

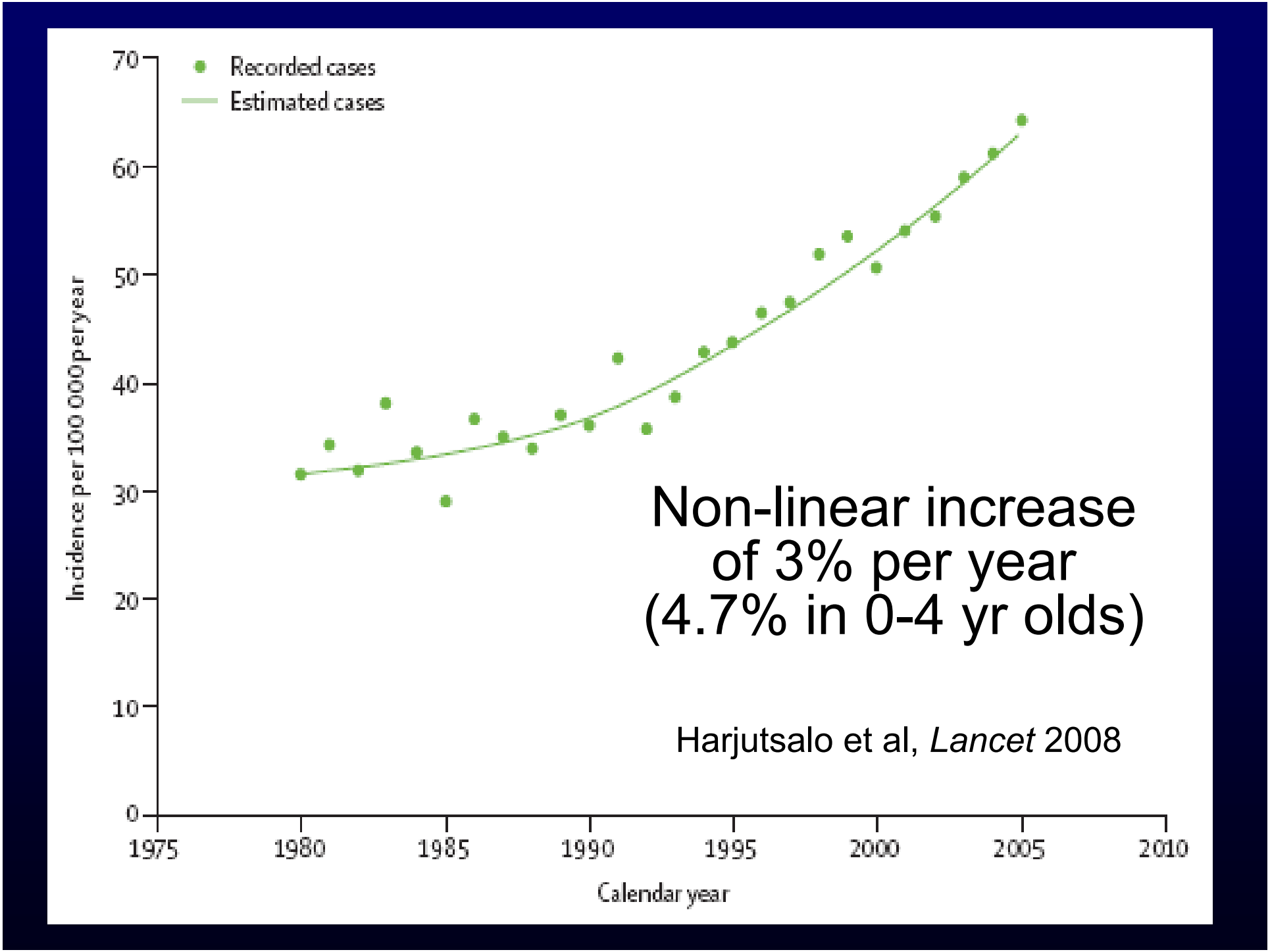
Summary of CVB Effects in INS-1

	Cyto-lytic	Prolifer-ation	Chemokines		
			IP-10	RANTES	MCP-1
CVB1	-	-	-	-	-
CVB2	+	+	+	+	+
CVB3	-	-	-	-	-
CVB4	++	++	+	+	+
CVB5	++	++	+	+	+
CVB6	+	+	+	-	-

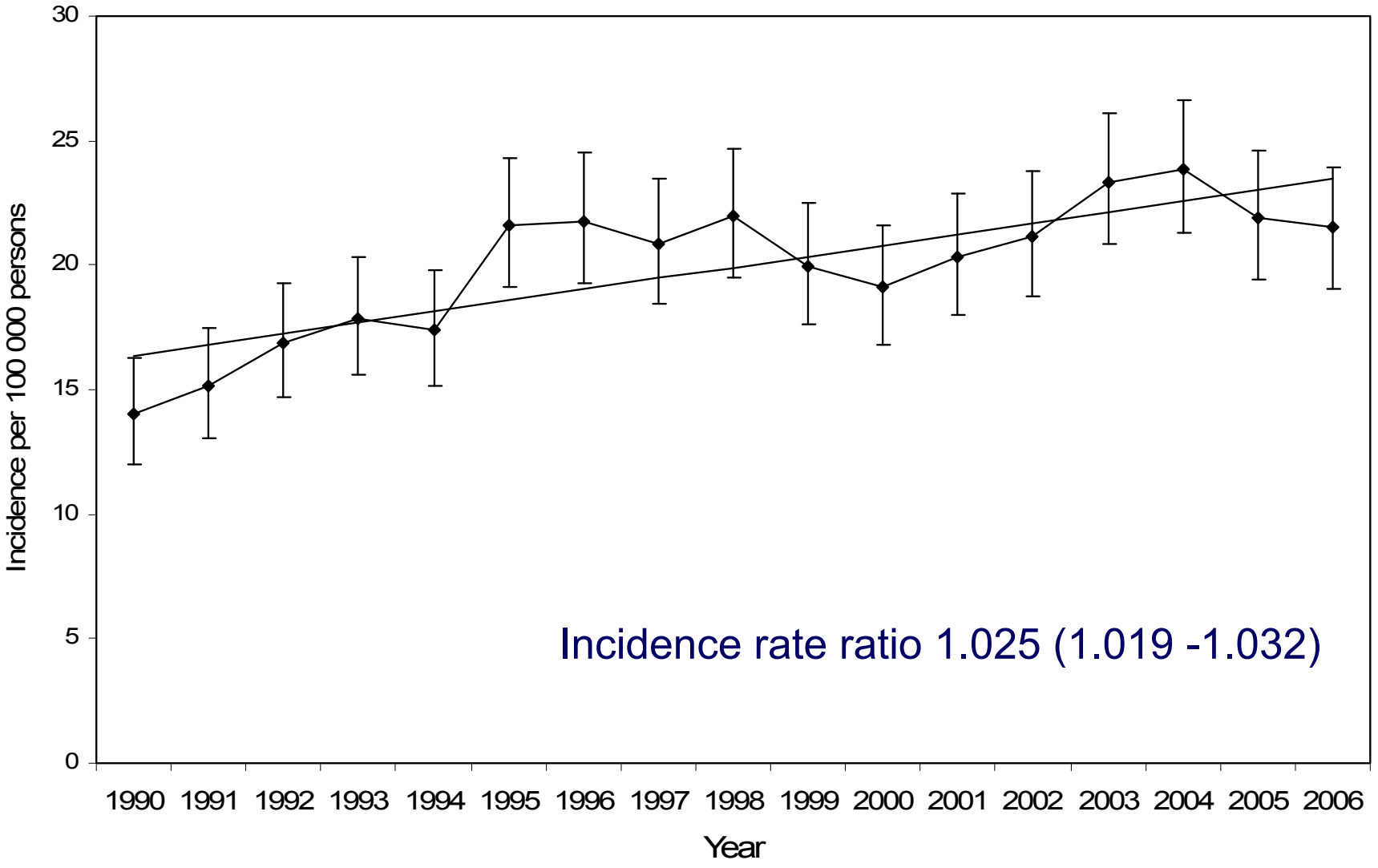
Summary of CVB Effects in INS-1

	Cyto-lytic	Prolifer-ation	Chemokines			Cytokines			
			IP-10	RANTES	MCP-1	IFN-α	IFN-β	IFN-γ	TNF-α
CVB1	-	-	-	-	-	-	-	-	-
CVB2	+	+	+	+	+	+ (↓)	+	+	+
CVB3	-	-	-	-	-	-	-	-	-
CVB4	++	++	+	+	+	-	-	+	-
CVB5	++	++	+	+	+	+	-	+	-
CVB6	+	+	+	-	-	-	-	-	-

Can enterovirus infections explain the rising incidence of type 1 diabetes?

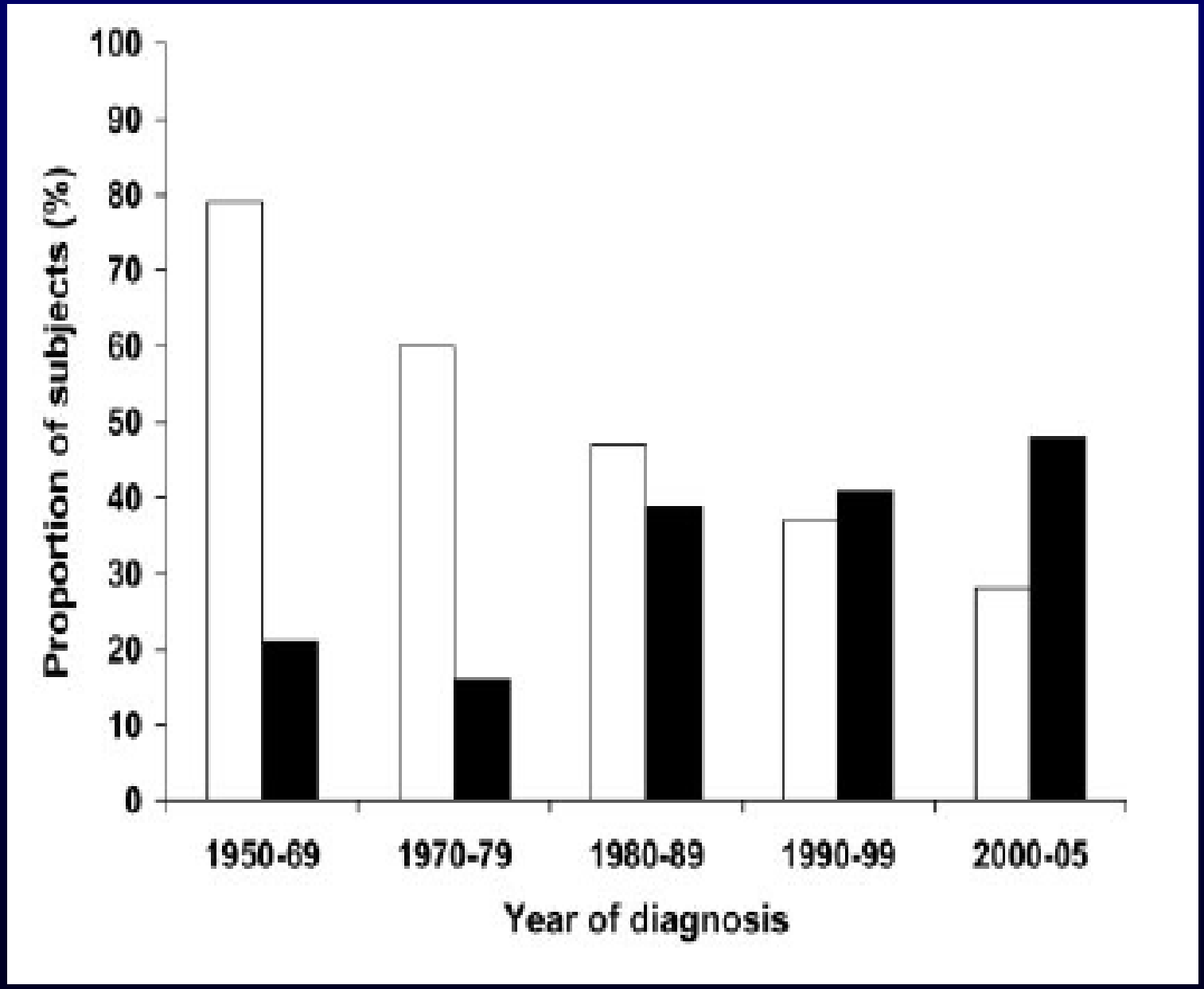

Recorded cases
Estimated cases
Incidence per 100 000 per year
70
60
50
40
30
20
10
0
1975
1980
1985
1990
1995
2000
2005
2010
Calendar year
Non-linear increase
of 3% per year
(4.7% in 0-4 yr olds)
Harjutsalo et al, *Lancet* 2008

Rising incidence in NSW 1990-2006



HLA and diabetes

- Certain HLA genotypes confer increased risk for type 1 diabetes (HLA DR3/4)
  - But < 50% of cases attributable to host genes
- % of new cases with high risk genotypes is decreasing (while actual # unchanged)
  - Suggests ↑ % of environmentally induced cases
  - ? explanation for rising incidence of diabetes
    - Fourlanos et al, *Diabetes Care* 2008
- Seasonality of diabetes restricted to individuals without high risk HLA genotypes
    - Weets et al, *Diabetologia* 2004,



Fourlanos et al, *Diabetes Care* 2008

Enterovirus infection and diabetes

- Large body of evidence from clinical and epidemiological studies
  - Serology
  - Virus culture
  - PCR
  - T cell responses
    - Limited direct evidence
- Conflicting data for role of enteroviruses in development of autoimmunity pre-diabetes
- Most studies of type 1 diabetes, few implicating EVs in ‘fulminant diabetes’

Enteroviruses	References
CVB2, 4, 5	King *et al* (83)
CVB3, 4	Clements *et* al (95)
CVB3, 4	Andreoletti *et al* (97)
CVA9, CVB1-6, ECHO1, 11	Roivainen *et al* (98)
CVB2, 3, 4	Chehadeh *et al* (00)
CVB1, 3, ECHO30, EV71	Craig *et al* (03)
ECHO16	Cabrera-Rode *et al* (03)
CVB4, ECHO11	Salminen *et* al (03)
CVB	Ylipaasto *et al* (04)
CVB4	Yoon *et al* (79)
CVB5	Champsaur *et al* (82)
ECHO6	Smith *et al* (98)
ECHO9	Vreugdenhil *et al* (00)
ECHO6	Otonkoski *et al* (00)
CVB5	Hindersson *et al* (05)
CVB4	Dotta *et al* (07)

Does EV infection persist?

Table 1 The age and the positivity of RT-PCR for enterovirus and comparison of the positivity of RT-PCR for enterovirus and the time after the occurrence of IDDM

Age (years)	Men[a]	Women[a]	Positivity of PCR	%
0-10	1/3	2/5	3/8	37.5
11-15	3/7	2/5	5/12	41.7
>15	4/15	11/26	15/41	36.6
Years after the occurrence				
0-1			5/7	71.4[b]
2-5			5/10	50.0
6-9			6/14	42.8
10-15			4/15	26.6
>15			2/11	18.1[b]
Total			23/61	37.7[b]

[a] There was no significant differences within the positivity in each age and gender.

[b] Within the ratio of positivity in years of occurrence there was significant difference between below 1 year and from 10 to 15 years old and more than 15 years-old.

Kawashima et al, *J Infection* 2004

Conclusions

- There is definite evidence that viruses demonstrate β cell specific tropism
  - Most research directed at enteroviruses
- Enterovirus infection is likely to be implicated in some cases of type 1 diabetes, and possibly other forms (type 2, fulminant)
- Effect of EVs on β cell is strain specific, but role of host factors also influence effects
  - Genetic predisposition, EV receptors, cytokine production

Future directions

- Enterovirus infection of insulinoma cell studies (apoptosis, cytokine production) in human islets
- Characterisation of ‘diabetogenic’ enteroviruses
  - using isolates from clinical cases
- Examination of autopsy specimens
- Vaccine development

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