for clinical use in diabetes. The study shows that sufficient concentration throughout a 24-h period may be achieved by a single subcutaneous injection of NN2211. From a clinical point of view, this is important not only because it improves compliance but also because it appears important to achieve 24-h coverage of GLP-1 to obtain sufficient clinical efficiency of the peptide [12]. Furthermore, the compound was well tolerated in healthy volunteers over a 7-day treatment period and exerted only minor side effects, mainly related to the gastrointestinal system and resembling those seen during administration of native GLP-1 [12]. No change in 24-h insulin was observed after NN2211 in the presence of reduced postprandial glucose. This would suggest that NN2211 inhibits gastric emptying, which reduces postprandial glucose in combination with a potentiated glucose-stimulated insulin secretion; these actions are similar to those observed with native GLP-1 [1–3].

Sufficient concentration throughout a 24-h period may be achieved by a single subcutaneous injection of NN2211

It should be emphasized that the study was performed in healthy volunteers and therefore an antidiabetogenic action of the compound was not examined in diabetic patients. A recent study, however, showed that NN2211 does indeed exert an antidiabetogenic effect following bedtime subcutaneous injection in subjects with type 2 diabetes [13]. Therefore, NN2211 seems to be a safe compound eliciting antidiabetogenic properties after a single subcutaneous injection in type 2 diabetic patients.

It is now important to delineate its pharmacokinetic and pharmacodynamic properties in subjects with type 2 diabetes. Furthermore, its potential use in early vs. late stages of the disease as well as in combination with other antidiabetogenic actions also needs to be defined. If these studies produce promising results, GLP-1-based treatment using the GLP-1 analogue NN2211 may well be a reality within the near future — some 20 years since the first description of GLP-1 as a gut hormone with incretin activity.

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Summary and Comment:
Bo Ahrén, Lund, Sweden

Viral infections in the pathogenesis of type 1 diabetes

Original article:
Enterovirus RNA is found in peripheral blood mononuclear cells in a majority of type 1 diabetic children at onset. Yin H, Berg A-K, Tuvemo T, Frisk G. *Diabetes* 2002; 51: 1964–71.

Summary

The occurrence of enterovirus (EV)-RNA was investigated in peripheral blood mononuclear cells of 24 children at onset of type 1 diabetes, in 19 of their siblings and in 24 matched control subjects. The detection of EV transcripts relied on reverse transcription-polymerase chain reac-

tion with two sets of primers (groups A and B) corresponding to conserved regions in the 5′ non-coding region of EV, as well as further sequencing of amplified products (amplicons).

Using group A primers, EV-RNA was detected in 12 (50%) of the 24 diabetic children, in five (26%) of the 19 siblings and in none of the control subjects. Using group B primers, EV-RNA was identified in 46% of the diabetic children, 58% of their siblings and 29% of the control subjects.

The difference in the detection patterns observed with the two sets of primers strongly suggested the existence of different diabetogenic EV strains. This was confirmed by sequencing analyses that revealed clustering of sequences in sub-branches of the Coxsackie virus B (CVB)4/VD2921 strains that differed between the diabetic children and the control subjects. Five patients also formed a branch together with the CVB4/E2 strain, four clustered with CVB5, and one with echovirus serotype.

Comment

Type 1 diabetes is an autoimmune disease that develops in individuals with a genetic predisposition determined by the balance between susceptibility and resistance alleles. Despite this genetic background, fewer than 10% of individuals with an hereditary predisposition will become diabetic. Further, the concordance rate of type 1 diabetes is approximately 40% only in homozygotic twins. These facts imply that environmental factors are necessary in type 1 diabetes pathophysiology. Such external influence may be exerted through dietary factors and even stressful life events, but documented evidence supports the influence of viral infections, in particular EV infection. Together with previous studies [1–3], the demonstration by Yin et al. that EV transcripts are present in blood cells of a majority of type 1 diabetic children strongly argues that EV infection is a crucial exogenous factor in the pathogenesis of type 1 diabetes. The higher total EV-RNA positivity in the present study (75%) probably results from a more precise design of EV primers and the isolation of peripheral blood mononuclear cells instead of whole blood samples.

A number of human viruses have been associated with type 1 diabetes including CVB, rubella virus, mumps virus, cytomegalovirus, Epstein-Barr virus and varicella zoster virus [4, 5]. Epidemiological studies have, however, provided the strongest evidence that CVB and other EV infections are very frequent events in subjects who ultimately develop insulin-dependent diabetes [6]. CVB4 is the most commonly detected strain in prediabetic and diabetic individuals. The CVB4 strain E2 has been isolated from the pancreas of an acutely diabetic deceased patient, passaged through murine islet β-cells, and then found to induce a diabetes-like disease after inoculation in mice [7].

CVB and other EV infections are very frequent events in subjects who ultimately develop insulin-dependent diabetes

Diabetogenic mechanisms of CVB infection

Much research effort is currently focused on defining the cellular and molecular mechanisms behind the epidemiological relationship between CVB4 infection and the incidence of type 1 diabetes. The hypothesis of a diabetogenic autoimmune response driven by molecular mimicry between viral antigen(s) and type 1 diabetes-related autoantigens was favoured for a long time when a significant homology was discovered between a sequence of the P2C non-structural protein of CVB4 and the glutamic acid decarboxylase 65 sequence 247–279 [8]. This hypothesis was, however, contradicted when it was found that mice with susceptible major histocompatibility complex alleles did not display acceleration of diabetes after CVB4 infection [9]. However, CVB4 inoculation in genetically modified mice with a T cell receptor transgene specific for an islet autoantigen led to the rapid development of diabetes [9]. This study strongly suggests that an autoimmune diabetogenic process follows CVB4 infection of the pancreas with local inflammation, release of islet autoantigen(s) and subsequent activation of resting autoreactive T cells. Strongly supporting this *bystander* mechanism, CVB was shown to damage human pancreatic β-cells [10, 11]. Also, CVB3 and two different CVB4 strains were demonstrated to persistently infect human pancreatic islets and to stimulate interferon-α synthesis in β-cells [12].

The CVB strain VD2921 — homologous to one sequence from type 1 diabetic patients in the present study — is also able to establish a persistent infection of human islet cells *in vitro*. In addition, VD2921 inoculation in mice induces a prediabetic state after 115 days (G. Frisk, personal communication). Some recent data also suggest that susceptibility to type 1 diabetes may be influenced by the innate

immune response of the infected host, i.e. the β-cell response to interferons [13].

CVB infection of the thymus (Fig. 1)
A novel type of mechanism was recently identified and might intervene in intimate conjunction with a bystander effect to explain the relationship between CVB4 infection and type 1 diabetes pathogenesis. Three years ago, we initiated a collaboration with D. Hober (Institute of Virology, CHRU Lille, France) to investigate the hypothesis that CVB4 is able to infect the thymus and to interfere with the intrathymic processes of T cell differentiation and central self-tolerance. Our first study confirmed this working hypothesis, since CVB4 was shown to infect cultured human thymic epithelial cells (TEC) in a persistent, productive and non-toxic way. Persistent CVB4 infection of human TEC is associated with a significant increase in TEC proliferation and secretion profiles of interleukin-6, leukemia-inhibitory factor and granulocyte-macrophage colony-stimulating factor [14]. In collaboration with C. Stoddart and J.M. McCune (Gladstone Institute for Virology-Immunology, UCSF, San Francisco, CA, USA), we are now investigating how CVB4 thymus infection interferes with T cell selection through the use of human fetal thymic organ cultures. As already shown for other viral infections [15, 16], CVB4 thymus infection could raise the level of immune tolerance to CVB4-specific antigens. This in turn will increase CVB4 infectious activity and will contribute to a more significant bystander damage of CVB4 target cells, including insulin-secreting islet β-cells. It will also be important to search for an effect of CVB4 thymus infection on central immune self-tolerance mediated by the intrathymic transcription of insulin-related genes, in particular *IGF2* [17].

Conclusions
More and more experimental evidence demonstrates that EV infection, and CVB infection in particular, plays an important role in the etiology of a still unknown percentage of type 1 diabetes cases. The question of the pathogenic mechanism is not simply theoretical but has important practical implications. In the case of molecular mimicry, an EV vaccine would be deleterious since it would initiate the diabetogenic autoimmune process. If the bystander islet damage induced by local CVB4 infection in conjunction with CVB4-induced thymus dysfunction holds true, then an EV vaccine would be able to prevent some cases of type 1 diabetes.

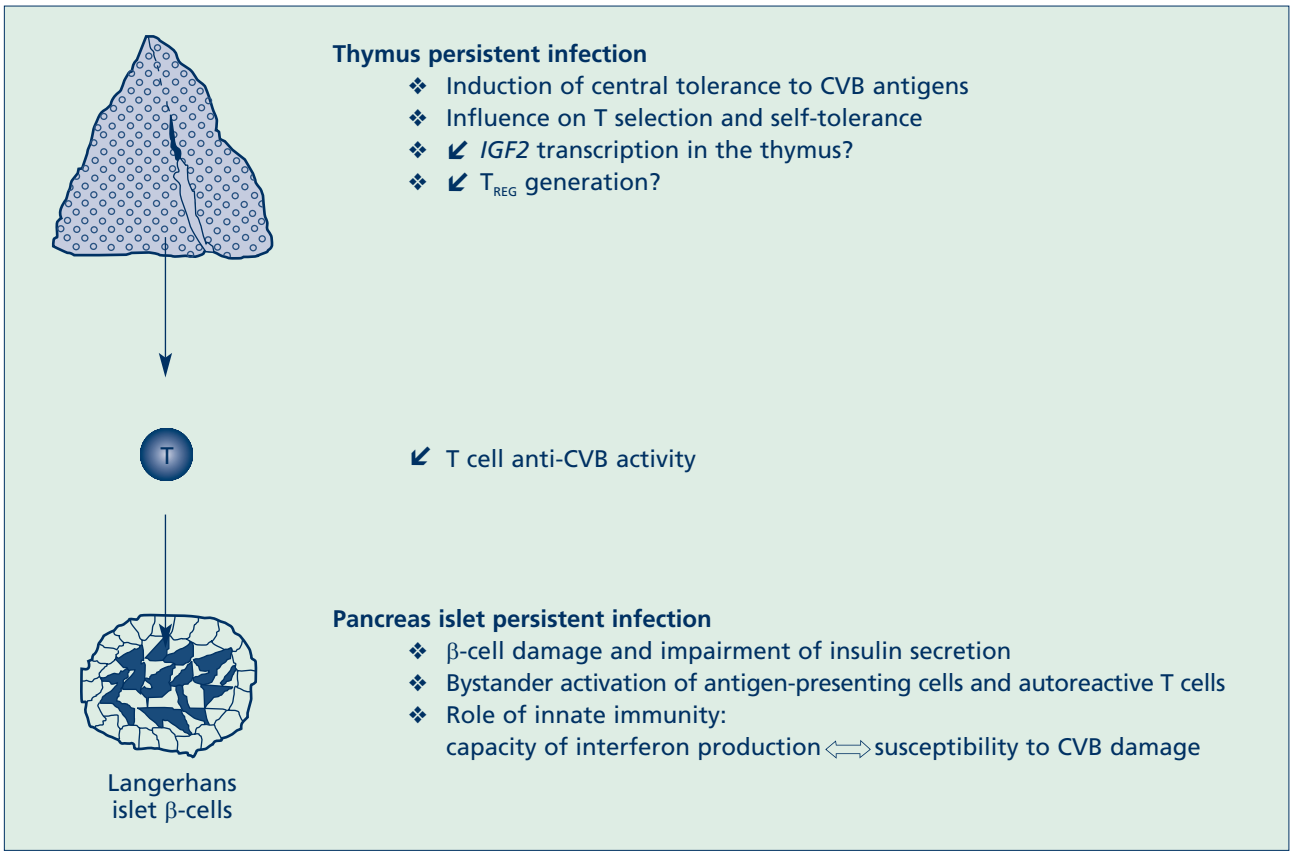

Fig. 1: *CVB infection and type 1 diabetes. T_{REG}, Regulatory T cell.*

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Summary and Comment:
Fabienne Brilot and Vincent Geenen, Liège, Belgium

Bisphosphonates in the treatment of Charcot neuroarthropathy

Original article:
Bisphosphonates in the treatment of Charcot neuroarthropathy: a double-blind randomised controlled trial. Jude EB, Selby PL, Burgess J, Lilleystone P, Mawer EB, Page SR, Donohoe M, Foster AVM, Edmonds ME, Boulton AJM. *Diabetologia* 2001; 44: 2032–7.

Summary and Comment

In this study the authors attempted to evaluate the potency of pamidronate, a bisphosphonate, in the complex therapy of acute diabetic Charcot neuroarthropathy. The incidence of this disabling diabetic complication has increased during the last decade and in Russia now affects almost 14% of diabetic patients [1, 2].

With no proven pharmacological treatment for this condition, diabetologists are faced with a difficulty when choosing the treatment strategy. Current management comprises immobilization and off-loading in a total contact, air cast or scotch cast boot. Although bisphosphonates have been recommended as first-line therapy for osteoporosis of a different origin, there is little experience with this group of drugs in the treatment of acute diabetic Charcot neuroarthropathy.

Jude et al. conducted a multicentre, double-blind, randomized, controlled trial of a group of 39 patients with acute diabetic Charcot neuroarthropathy, randomized to receive either a single infusion of pamidronate 90 mg or placebo, combined with standard Charcot foot care, i.e. immobilization. A single infusion of the drug at a dose of 90 mg seems unusual. The results were assessed during 12 months of follow-up.