



1

2 A Look to the Future: Prediction, Prevention,
 3 and Cure Including Islet Transplantation and
 4 Stem Cell Therapy

5 Anna Casu, MD, Massimo Trucco, MD,
 6 Massimo Pietropaolo, MD*

Q1

7 *Division of Immunogenetics, Department of Pediatrics, Rangos Research Center,*
 8 *Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, 3460 Fifth Avenue,*
 9 *Pittsburgh, PA 15213, USA*

10 Critical to the success of intervention strategies is the identification of indi-
 12 viduals at risk of developing the disease in an effort to delay or prevent the clinical
 13 onset of type 1 diabetes mellitus (T1DM). The ability to predict T1DM
 14 progression with 50% to 80% accuracy is the sine qua non for the accomplish-
 15 ment of successful intervention strategies in relatives of T1DM probands. Even
 16 with this high degree of predictability, the current comfort level with interven-
 17 tion therapies is such that there are still very few clinical trials in the preclinical
 18 phase of T1DM, with most of these trials being conducted in newly diagnosed
 19 T1DM patients.

Q2

20 **Prediction of type 1 diabetes**

21 *Islet autoantibodies*

22 T1DM is caused by autoimmune destruction or dysfunction of the insulin-
 23 secreting cells within the islets of Langerhans and represents the end point of a

This work was supported by NIH grants R01 DK53456 and R01 DK56200 (M. Pietropaolo), by NIH R01 DK24021 (M. Trucco), and by an American Diabetes Association Career Development Award (M. Pietropaolo).

* Corresponding author.

E-mail address: pietroma@pitt.edu (M. Pietropaolo).

24 progressive decline in β -cell function [1,2]. This long prodromic period before
25 overt T1DM development offers a large window of opportunity not only to
26 predict the disease onset but also to intervene with safe therapeutic agents [2].
27 Similar to many autoimmune diseases, T1DM is characterized by humoral and
28 cellular autoimmune responses directed against multiple target antigens of pan-
29 creatic islet cells. The immunologic diagnosis of autoimmune diseases relies
30 mainly on the detection of autoantibodies in the serum of T1DM patients. Al-
31 though their pathogenic significance still remains unclear, they serve as surro-
32 gate markers for specific autoimmune responses. Multiple antibodies are present
33 in most newly diagnosed T1DM patients and their presence is highly predictive
34 of disease progression in otherwise healthy first-degree relatives. The currently
35 used markers for prediction studies are islet cell antibodies (ICA), glutamic acid
36 decarboxylase autoantibodies (GAD65 AA), tyrosine-phosphatase-like protein
37 IA-2 autoantibodies (IA-2/ICA512 AA), and insulin autoantibodies (IAA).

38 During the past decade, the use of islet-related autoantibodies has allowed
39 major advances in prediction studies. All of these studies have suggested that
40 a combination of humoral immunologic markers detecting autoantibodies to
41 these islet antigens, rather than any single test, gives a high predictive value for
42 T1DM in first-degree relatives, and great sensitivity without significant loss of
43 specificity [3–7]. The detection of GAD65 AA, IA-2/ICA512 AA, and IAA is
44 now a clear prerequisite for identifying individuals at risk of developing insulin-
45 requiring diabetes. In particular, the presence of two or more of these auto-
46 antibodies to islet antigens is now used as entry criteria for intervention trials
47 aiming at mitigating the deterioration in insulin secretion after T1DM onset or
48 at preventing the disease process in first-degree relatives of T1DM probands.

49 ICA were the first disease-specific antibodies identified in patients affected
50 by T1DM. These antibodies are detected by indirect immunofluorescence using
51 human pancreatic sections [8]. The Immunology of Diabetes Society has re-
52 peatedly demonstrated a marked variability between laboratories and even within
53 the same laboratory performing ICA assays. Nonetheless, the authors pro-
54 vided evidence suggesting that ICA predict a more rapid progression to insulin-
55 requiring diabetes in GAD65 AA- and IA-2 AA-positive relatives. Despite
56 marked variability of the ICA assay formats, the authors strongly support the
57 conclusion that cytoplasmic ICA should remain part of the assessment of T1DM
58 risk for future intervention trials [9]. Their data also provided indirect evidence
59 for the presence of an important subset of ICA that apparently reacts with un-
60 identified islet autoantigens. The general view is that cytoplasmic ICA represents
61 a heterogeneous group of immunoglobulins that specifically react with a family of
62 autoantigens on frozen pancreatic sections. Thus far, the best-characterized subsets
63 of ICA react with GAD and IA-2 [10–13].

64 The availability of molecularly characterized islet autoantigens represents an
65 unlimited source of reagents that are readily used for experimental and diagnostic
66 purposes. Some of these molecules include GAD65 and IA-2, and they have been
67 used to optimize fluid-phase radioimmunoassays with much higher reproducibility
68 as compared with immunofluorescence assays used for ICA detection [14,15].

69 The presence of GAD65 and IA-2/ICA512 AA is now a prerequisite to enroll
70 subjects in prevention trials.

71 Insulin was the first identified T1DM-related autoantigen [16] and IAA are
72 detected using a radiobinding immunoassay [17–19]. They have a specificity
73 almost as high as 100%, but the sensitivity seems to be rather low (compared
74 with the sensitivity of GAD65 AA), as suggested by the results of proficiency
75 workshops organized by the Diabetes Autoantibodies Standardization Program
76 [20]. Nonetheless, IAA are detected in as much as 90% of newly diagnosed
77 patients below 5 years of age, in 71% between 5 and 10 years, and in 50% of
78 T1DM patients 10 to 15 years old [21,22].

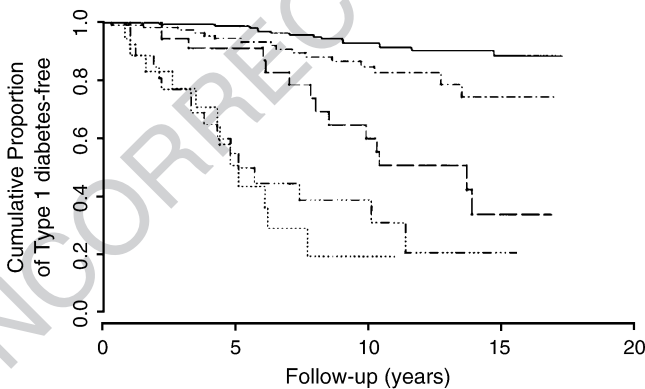
79 GAD autoantibodies were identified in 1990 [23] and they are mainly directed
80 against the 65- kd isoform of this enzyme. They are detected using a semiquan-
81 titative fluid-phase radiobinding assay, using in vitro transcribed and translated
82 ³⁵S-labeled human recombinant GAD65. The Diabetes Autoantibodies Stan-
83 dardization Program reported a median specificity of 94% and median sensi-
84 tivity of 77% [20]. GAD autoantibodies in T1DM seem to be mainly directed
85 toward disease-specific epitopes, which are localized within the middle region
86 and the COOH-terminus of the molecule. These epitopes have been identified
87 by competition assays between GAD65-positive sera and cloned GAD65-specific
88 recombinant Fab, and the results seem to be useful to improve T1DM prediction.
89 Some crucial residues for autoantibodies binding to GAD65 have been demon-
90 strated in the same area as a result of homolog-scanning mutagenesis experi-
91 ments. These studies suggested that GAD65 epitopes are conformational and
92 that the native molecule is recognized by GAD65-specific antibody responses
93 [24,25].

94 The neuroendocrine antigens ICA512 and IA-2 β (phogrin) are both targets of
95 T1DM-related autoantibody responses. A radioligand-binding assay format simi-
96 lar to that used to detect GAD65 AA is currently applied to detect autoanti-
97 bodies to IA-2 and IA-2 β . The specificity of this assay approaches 100% with a
98 sensitivity of nearly 60% [20]. All antigenic constructs used include the intra-
99 cellular portion of the molecule (IA-2ic aa. 601-979), which contains most of
100 the immunoreactive epitopes as demonstrated by binding and competition analy-
101 sis with multiple chimeric ICA512-phogrin constructs [26]. One of the immuno-
102 dominant IA-2 epitopes, termed “Fragment 1” (aa 761-964), has been proposed
103 to be one of the most significant IA-2 epitopes. Of note, autoantibodies against
104 IA-2/Fragment 1 can be detected in 16% of patients that test negative for
105 IA-2 AA measured by the conventional radioassay [27]. Recent results showed
106 that the presence of autoantibodies against the IA-2 intracellular domain epi-
107 topes conferred a cumulative risk of diabetes progression significantly higher
108 than the one in relatives with no detectable AA against Fragment 1 and IA-2ic.
109 Because the group of relatives with detectable autoantibodies against Fragment 1
110 and IA-2ic did not have different titer or prevalence of GAD65, IAA, and
111 ICA when compared with negative subjects, this means that only the presence of
112 antibodies against one of the two IA-2 epitopes conferred the increased risk
113 [28]. These data suggest that biochemical assays detecting autoantibodies against

114 IA-2 intracellular domain epitopes enhance not only sensitivity but also identify
 115 rapid progressors of T1DM onset as compared with conventional markers alone.
 116 First-degree relatives carrying IA-2ic and Fragment 1 AA should be included in
 117 the highest T1DM risk category and in the inclusion criteria for enrollment in
 118 intervention trials aimed at preventing T1DM.

119 Autoantibodies against more than 20 putative targets of islet autoimmunity
 120 have been identified in T1DM patients, but they are less well characterized.
 121 Those include ICA69, carboxypeptidase H, CD38, glima 38, the glucose trans-
 122 porter 2 the islet ganglioside GM2-1, heat shock protein 60 kd, ICA12/SOX13
 123 [12,29–31], and a number of other molecules.

124 Many well-defined epidemiologic studies are currently ongoing in the United
 125 States and around the world, such as the Bart's Windsor, Joslin, Denver, Pitts-
 126 burgh, Seattle, Gainesville, BabyDiab in Germany, and other studies [32–36].
 127 These studies provide the groundwork to understand the natural history of the
 128 disease process and to improve prediction of T1DM in first-degree relatives of
 129 T1DM probands and ultimately in the general population [21,37–40]. There is a
 130 high degree of concordance among these groups that multiple autoantibodies to
 131 islet autoantigens confer a cumulative risk of developing diabetes of 75% to 90%
 132 during 5 to 10 years of prospective follow-up in first-degree relatives (Fig. 1)
 133 [3,5,41–43]. The antibody titer also seems to be an important predictive risk
 134 factor, in that higher autoantibody titers are associated with a higher risk of de-



0Ab: 252	170	124	42
1Ab: 163	86	44	13
2Ab: 39	22	13	3
3Ab: 28	11	5	1
4Ab: 18	7	2	2

Fig. 1. Progression to insulin-requiring diabetes among first-degree relatives (N=500) in relation to the number of autoantibodies (Ab) to insulin, GAD65, IA-2, and ICA. Relatives who are positive for three or four Ab are at much greater risk of developing diabetes compared with relatives with two Ab alone. Log rank: $P = .0096$ and $.001$, respectively. (From Pietropaolo M, Becker DJ, LaPorte RE, et al. Progression to insulin-requiring diabetes in seronegative prediabetic subjects: the role of two HLA-DQ high-risk haplotypes. *Diabetologia* 2002;45:66–76; with permission.)

135 veloping the disease [4,44–46]. T1DM risk can be further stratified by taking into
136 consideration a high titer of IA-2 AA and IAA [46]. In particular, first-degree
137 relatives with titers of IA-2 AA in the upper three quartiles have significantly
138 higher diabetes risk than relatives with their IA-2 AA titer in the lowest quartile.
139 Similar results were obtained for IAA but not for GAD65 AA. High titer of
140 IA-2 AA and IAA seems to be a strong predictor of T1DM development [46].

141 To assess the predictive role of the islet-related AA, a number of studies
142 have been undertaken in the general population, particularly in schoolchildren
143 [21,37–40]. These studies concluded that ICA or other islet AA in subjects with
144 no family history of disease conferred an increased risk of T1DM development. It
145 must be emphasized that the predictive value of islet-related AA is lower in the
146 general population compared with that in first-degree relatives [21,37–39]. This
147 is because of a low prevalence of the disease in the general population. For
148 uncommon diseases like T1DM (eg, prevalence of 1.2–1.5 per 1000 in the United
149 States) [47,48], a large proportion of individuals with positive screening test
150 results, including multiple autoantibodies to islet antigens, will inevitably be
151 found not to develop the disease. According to Bayes' theorem, the positive
152 predictive value of screening tests varies dramatically based on the prevalence of
153 the disease in a given population [49,50]. For example, because the prevalence of
154 T1DM is quite low, the positive predictive value of GAD AA and IA-2 AA alone
155 or in combination is also likely to be quite low, even when using assays with high
156 sensitivity and specificity. Until proved otherwise, the only intervention strategy
157 for T1DM that should be proposed in the general population is an innocuous
158 nutritional or vaccination program, because the presence of positive markers,
159 such as ICA, does not guarantee progression to clinical disease. By contrast, with
160 a disease such as polio, it is not necessary to identify individuals at risk for the
161 development of the disease, because the benefits of a safe massive vaccination
162 program outweigh by far the devastating risk of poliomyelitis for the society [49].

163 *The role of major histocompatibility complex in type 1 diabetes mellitus* 164 *prediction studies*

165 The role of HLA in T1DM susceptibility remains unquestionable, because
166 there is convincing evidence that inherited susceptibility to T1DM is primarily
167 associated and linked with genes within the major histocompatibility complex
168 [51–53]. The HLA locus is termed *IDDM1* according to a more recent nomen-
169 clature [54]. Genome-wide scans in T1DM have identified over 18 putative loci
170 of statistical significance but only linkage to HLA seems incontestable.

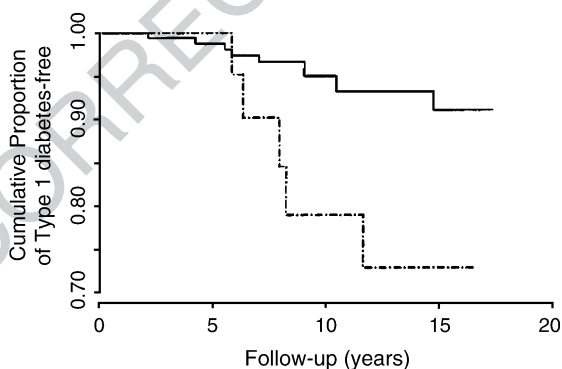
171 Early studies found that HLA-DR3 and -DR4 alleles were strongly associated
172 with T1DM susceptibility [51,55]. Approximately 95% of patients with T1DM
173 were heterozygous for DR3/4 or expressed at least one of these alleles, and
174 heterozygous individuals seemed to be more susceptible to the disease than the
175 homozygous ones. Restriction fragment length polymorphism analysis of DNA
176 from T1DM patients and nondiabetic controls showed an even stronger associa-
177 tion between the HLA-DQ locus and disease susceptibility. Analysis of the DQ β

178 chain showed that a negatively charged aspartic acid at position 57 correlated
 179 with resistance, whereas non-aspartic acid at position 57 correlated with suscep-
 180 tibility [56,57]. Also, it was shown that arginine at position 52 of the DQ α chain
 181 correlated with disease susceptibility. The ability of HLA-DQ molecules to in-
 182 fluence susceptibility or resistance to the disease is explained by the different in-
 183 teractions between DQ molecules, antigens, and T-cell receptors. The presence of
 184 susceptible HLA alleles also correlates with the presence of islet autoantibodies.

185 The addition of HLA-DQ genotypes to screening strategies does not in-
 186 crease sensitivity of combined autoantibody assays. Unidentified autoimmune
 187 phenomena may well be present in seronegative relatives who eventually develop
 188 insulin-requiring diabetes if they possess two HLA-DQ high-risk haplotypes
 189 (Fig. 2) [58,59].

190 Although HLA is not the optimal primary screening tool for T1DM and it is
 191 not sufficient alone to predict the disease onset, the evaluation of HLA gen-
 192 typing in relatives with ICA positivity can significantly improve the ability to
 193 predict T1DM progressors versus nonprogressors [60–62]. In seronegative rela-
 194 tives who developed insulin-requiring diabetes, the presence of two HLA-DQ
 195 high-risk haplotypes conferred an increased cumulative risk of developing insulin
 196 requirement (see Fig. 2) [58].

197 The prevalence of HLA-DR2 is decreased in patients with T1DM, and in
 198 many populations the DQB1*0602 allele is rarely found among patients with
 199 T1DM. This suggests that this allele may play a protective role in the disease



Number of HLA-DQ High Risk Haplotypes

0 or 1:	221	149	110	36
2 :	31	21	14	6

Fig. 2. Progression to insulin-requiring diabetes for seronegative relatives (N=252) who carry two compared with zero or one *HLA-DQ* high-risk haplotypes. Two *HLA-DQ* high-risk haplotypes conferred a cumulative risk of insulin-requiring diabetes of 27% after a follow-up of 12.5 years, compared with a risk of 6% for relatives who had zero or one *HLA-DQ* high-risk haplotypes (log rank $P = .01$) or two *HLA-DQ* high-risk haplotypes. (From Pietropaolo M, Becker DJ, LaPorte RE, et al. Progression to insulin-requiring diabetes in seronegative prediabetic subjects: the role of two HLA-DQ high-risk haplotypes. *Diabetologia* 2002;45:66–76; with permission.)

200 process. At present, carrying a protective DQB*0602 allele is considered a
201 criterion of exclusion for enrolling first-degree relatives of diabetic patients in
202 clinical trials, such as the Diabetes Prevention Trial 1 (DPT-1) or the TrialNet,
203 which is being performed in the United States. These trials have been designed to
204 use any effective therapeutic regimen that could delay and ultimately prevent
205 the clinical onset of type 1 diabetes in individuals considered at high risk of
206 developing the disease.

207 *Other genes*

208 Several studies revealed an association between the disease and 18 chromo-
209 somal regions other than major histocompatibility complex that may contain
210 susceptibility loci. Some of those associations were not confirmed in subsequent
211 studies. The main non-major histocompatibility complex loci related to T1DM
212 are the insulin gene region (INS) known as *IDDM2*, the immunoglobulin heavy
213 chain genes (*IDDM1*), and CTLA-4 (*IDDM2*). To date, none of them has been
214 shown to have any role in the prediction of T1DM [51].

215 *Pancreatic β -cell function*

216 In individuals with islet antibodies, the first-phase insulin response (FPIR)
217 after an intravenous glucose tolerance test predicts time to diabetes onset
218 [50,63–68]. The FPIR is determined as the first plus third minute plasma insulin
219 concentration (milliunit per liter) after the midpoint of intravenous injection of
220 0.5 g of glucose per kilogram body weight with a maximum of 35 g. Oral glucose
221 tolerance is not impaired until the FPIR is less than the first percentile **Q3**
222 (<50 mU/L in prepubertal children and <100 mU/L in older individuals) on
223 two occasions 3 to 6 months apart, which corresponds to a very late stage of
224 the natural history of the disease [50].

225 A low FPIR underlies an advanced autoimmune process and is the outcome
226 of a profound reduction in β -cell function. A FPIR less than the first percentile is
227 associated with an estimated risk of diabetes of 100% within a 4-year follow-up,
228 regardless of the presence of one or more islet autoantibodies [5].

229 *Proposed guidelines for screening*

230 The Immunology of Diabetes Society has proposed a number of guidelines
231 for screening and assessing diabetes risk in unaffected first-degree relatives of
232 T1DM probands. According to these guidelines, testing for GAD65 AA, IA-2/
233 ICA512 AA, and IAA can identify approximately 85% of either newly diagnosed
234 or future T1DM cases. To maximize sensitivity, IAA testing should be included
235 as primary screening strategy for children <10 years of age. Cytoplasmic ICA
236 should be included as a secondary screening methodology, because it increases
237 the predictive value of combined biochemical autoantibody markers. As a general
238 rule, subjects that are positive for one or more islet autoantibodies should be

239 further evaluated by using other markers (genetic and metabolic) to define the
240 risk more precisely. Participation of laboratories in workshop or proficiency
241 programs is strongly advised. HLA class II typing may be useful in identifying
242 infants who would be subsequently followed to document closely the occurrence
243 of islet autoantibodies and ultimately to recruit them into intervention trials. The
244 FPIR assessment is generally used to identify a subgroup of individuals with the
245 highest risk of developing diabetes within a short period of time. Findings from
246 studies in first-degree relatives should not be assumed to apply to the general
247 population [21,37–39,69] because the predictive value obtained in this population
248 is lower than those obtained for first-degree relatives. At this stage, all prediction
249 should be considered as investigational but not yet applicable to the general
250 population or general physician.

251 **Prevention strategies**

252 The rationale for undertaking prevention studies in humans stems from the
253 evidence that in animal models of autoimmune diabetes, such as the nonobese
254 diabetic (NOD) mouse and the biobreeding rat, the disease process can be
255 prevented using many different therapeutic approaches.

256 Prevention strategies can be classified into three categories based on the
257 timing of intervention: (1) primary prevention, aimed at preventing the disease
258 in high-risk populations before any serologic evidence of islet autoimmunity;
259 (2) secondary prevention, aimed at delaying and, possibly, suppressing β -cell
260 damage in euglycemic subjects with evidence of islet autoimmunity; and
261 (3) tertiary prevention, initiated after diabetes onset, aimed at inducing prolonged
262 remission or β -cell regeneration. This also seems to be useful in preserving the
263 remaining β -cell function, which has been correlated with a reduced incidence of
264 chronic complications [70–72]. First-degree relatives of patients with T1DM
265 represent an accessible and highly motivated population, for the most part, to
266 be screened and included in primary and secondary prevention trials, whereas
267 newly diagnosed diabetic patients are included in tertiary prevention strategy.

268 Several drugs, such as cyclosporine [73,74] and azathioprine [75–77], have
269 been used to preserve β -cell function after T1DM onset. These immunosup-
270 pressive therapies were administered in newly diagnosed T1DM and yielded a
271 transient beneficial effect in terms of maintaining higher C-peptide levels over
272 time compared with the placebo-treated patients. Because of the development of
273 serious side effects, these therapeutic regimens are no longer considered
274 appropriate treatments for T1DM. Other approaches have also been proposed
275 in humans (Table 1).

276 *Primary prevention*

277 Only one primary prevention trial is ongoing and it is based on the possible
278 role of cow's milk proteins in inducing diabetes. Cow's milk protein have been

t1.3	Name of the study	Strategy	Type of study	Agent	Population	Result
t1.4	TRIGR	Primary prevention	Randomized, placebo-controlled, double-blind	Hydrolyzed cow's milk formula	FDR at high genetic risk	Ongoing
t1.5	ENDIT	Secondary prevention	Randomized, placebo-controlled, double-blind	Nicotinamide	High-risk FDR: ICA+	No prevention
t1.6	DPT-1 parenteral	Secondary prevention	Randomized, no placebo	Parenteral insulin	High-risk relatives	No prevention
t1.7	DPT-1 oral	Secondary prevention	Randomized, placebo-controlled, double-blind	Oral insulin	Moderate-risk relatives	Reduced incidence of T1DM in relatives with IAA ≥ 80 U
t1.8	Cyclosporine	Tertiary prevention	Randomized, placebo-controlled, double-blind	Cyclosporine	New-onset T1DM patients	Temporary remission of T1DM
t1.9	Azathioprine	Tertiary prevention	Randomized, placebo-controlled, double-blind	Azathioprine	New-onset T1DM patients	No effect
t1.10	Azathioprine, prednisone	Tertiary prevention	Randomized, unblind	Azathioprine, prednisone	New-onset T1DM patients	Partial, temporary remission of T1DM
t1.11	Anti-CD3 treatment	Tertiary prevention	Randomized, placebo-controlled	hOKT3 γ (Ala-Ala)	New-onset T1DM patients	Prevention of loss of C peptide
t1.12	Anti-CD20	Tertiary prevention	Randomized, placebo-controlled	Anti-CD20 monoclonal antibodies	New-onset T1DM patients	To be started
t1.13	Thymoglobulin	Tertiary prevention	Randomized, placebo-controlled	Antithymocyte polyclonal antibodies	New-onset T1DM patients	To be started
t1.14	DIPP	Secondary prevention	Randomized, placebo-controlled, double-blind	Nasal insulin	High-risk subjects	Ongoing
t1.15	NBI-6024 Neurocrine	Tertiary prevention	Randomized, placebo-controlled, double-blind	Altered peptide ligand insulin B:9-23	New-onset T1DM patients	Ongoing
t1.16	Peptor HSP60	Tertiary prevention	Randomized, placebo-controlled, double-blind	Heat shock protein 60	New-onset T1DM adult patients	Ongoing; prevention of loss of C peptide
t1.17	BCG vaccination	Tertiary prevention	Randomized, placebo-controlled, double-blind	BCG vaccine	New-onset T1DM adult patients	No effect

t1.18 *Abbreviations:* BCG, bacillus Calmette-Guerin; FDR, ; IAA, insulin antibodies; ICA, islet cell antibodies; T1DM, type 1 diabetes mellitus.

279 suggested to play a role in T1DM pathogenesis following prospective studies,
280 which showed that breastfeeding was associated with a somewhat lower inci-
281 dence of children developing T1DM [78–80] as compared with children who
282 were exposed to cow's milk. A role for cow's milk proteins in diabetes has
283 also been reported in animal models of T1DM [80–82]. A decreased incidence of
284 T1DM was found in animals weaned to hydrolyzed proteins instead of intact
285 foreign proteins. Some evidence now suggests that a similar relationship may
286 exist in humans [83]. The Trial to Reduce Insulin-dependent Diabetes in
287 Genetically at Risk (TRIGR) is an ongoing randomized controlled trial aimed at
288 determining whether the absence of cow's milk proteins in the diet protects from
289 T1DM progression in first-degree relatives of T1DM patients carrying high-risk
290 HLA alleles. This is a primary prevention trial in subjects with no evidence of
291 autoimmunity. This study will also determine whether or not breastfeeding is
292 associated with a reduced T1DM risk during a 10-year follow-up. All families
293 whose offspring are included in the study receive the recommendation to
294 breastfeed for at least the first 6 months of life in accordance with the World
295 Health Organization recommendations. If a mother is unable exclusively to
296 breastfeed before the baby is 8 months of age, her child is randomly assigned to
297 one of two groups. One group receives breastfeeding supplements of a trial
298 formula based on extensively hydrolyzed protein whose fragments do not stimu-
299 late the immune system; the other group receives a trial formula containing in-
300 tact proteins. This study is designed not to interfere with infant feeding practices,
301 except to emphasize and encourage breastfeeding. What makes this intervention
302 attractive is its safety, because hydrolyzed formulas have been used for decades to
303 treat cow's milk protein allergies with no occurrence of serious adverse effects.
304 Theoretically, this intervention may be applied to the general population as a
305 form of primary intervention.

306 The results of the pilot study for TRIGR performed in Finland, Estonia, and
307 Sweden have recently been published [83]. At the end of the 2-year observation
308 period, the proportion of subjects positive for at least one autoantibody was lower
309 in the hydrolyzed group compared with the control group. In addition, during a
310 follow-up of up to 4 years (only for the Finnish subjects), the number of children
311 who developed overt diabetes was higher in the control group, although this
312 difference was not statistically significant. The completion of the larger TRIGR
313 study is needed to confirm the trends shown by the pilot study.

314 *Secondary prevention*

315 Most prevention trials have been conducted in first-degree relatives of T1DM
316 patients when many of these subjects have already developed signs of islet
317 autoimmunity documented by the presence of both humoral immunologic and
318 metabolic abnormalities. The most important secondary prevention studies are
319 the European Nicotinamide Diabetes Intervention Trial (ENDIT) and DPT-1.
320 These results have been recently reported [84,85].

321 *Nicotinamide (European Nicotinamide Diabetes Intervention Trial)*

322 Initial observations indicated that high doses of nicotinamide could prevent
323 the development of T1DM in streptozotocin-treated rats. This drug also seems to
324 prevent or delay the onset of diabetes in NOD mice, possibly preserving β -cell
325 function [86]. Initial pilot studies suggested that nicotinamide might prevent
326 diabetes development in ICA-positive schoolchildren [87]. Following these initial
327 observations, a double-blind placebo trial was undertaken in first-degree
328 relatives of T1DM patients carrying ICA autoantibodies [84,85]. More than
329 30,000 first-degree relatives were screened for cytoplasmic ICA and 552 of
330 them with ICA titer >20 Juvenile Diabetes Foundation units were randomized
331 and then nicotinamide or placebo were administered. The sample size was suffi-
332 ciently large and powered to estimate a reduction in progression to overt diabe-
333 tes from 35% to 21% assuming a 20% drop out rate. These subjects were
334 followed for 5 years with regular clinical and metabolic assessment, such as
335 intravenous glucose tolerance test, FPIR, and oral glucose tolerance test. The
336 results of this study indicated that nicotinamide treatment, at the dose used in
337 this trial (1.2 g/m² daily up to a maximum of 3 g/d in two doses), did not de-
338 crease the incidence of T1DM. The mean FPIR was not different between the
339 two groups, so nicotinamide did not stop the decrease of β -cell function. Body
340 weight and height were monitored to ascertain any possible effect of the drug
341 on growth. No differences were seen with respect to growth and no adverse
342 events were reported between the two groups [84,85].

Q5

343 *Parenteral and oral insulin: Diabetes Prevention Trial Type 1*

344 Thus far, both insulin and proinsulin are considered the only candidate pan-
345 creatic β -cell-specific autoantigens. There is evidence in both NOD mice and
346 biobreeding rats that the administration of insulin can modify the natural history
347 of autoimmune diabetes in these animals [88–90]. Although a pilot study per-
348 formed in relatives at risk of developing T1DM showed that parenteral insulin
349 administration delayed the onset of the disease, this conclusion was not con-
350 firmed by a more robust recent trial conducted by the DPT-1 diabetes study
351 group [91,92]. A number of research groups have reported the expression of
352 islet autoantigens within cells of the thymus and lymphoid organs [93–96] and
353 in peripheral lymphoid organs [97], two tissues thought to be vital to the main-
354 tenance of immunologic self-tolerance. One may postulate that disruption of ge-
355 netic elements regulating transcript and protein expression (ie, insulin) in the
356 thymus and lymphoid tissues could short circuit mechanisms necessary for
357 maintaining immune self-tolerance to endogenous antigens, such as insulin. This
358 hypothesis was corroborated by the demonstration that diabetes development is
359 accelerated in NOD mice deficient for proinsulin-2 expression within both the
360 thymus and the β -cells [98].

361 The hypothesis was further strengthened by reports that the insulin epitope
362 has experimentally blocked the development of T1DM in NOD mice and that
363 oligoclonally expanded T-cells from pancreatic lymph nodes of type 1 diabetic
364 mice recognized the insulin A:1-15 epitope.

365 In the DPT-1 trial, relatives with a risk of developing diabetes $\geq 50\%$ in
 366 5 years were enrolled in the parenteral insulin trial and randomized to receive
 367 subcutaneous insulin twice daily (0.125 U/kg of body weight) and intravenous
 368 insulin once a month or observation (a placebo was not administered) [92]. Q6
 369 Relatives with projected 5-year risk of 26% to 50% were assigned to an oral
 370 insulin trial, to assess the effect of oral insulin therapy in preventing T1DM
 371 (7.5 mg of insulin crystals per day) [99]. Starting from 1996, more than 84,000
 372 relatives were screened and 339 with a risk of developing diabetes $\geq 50\%$ in
 373 5 years underwent randomization. After a median follow-up of 3.7 years, the
 374 cumulative incidence of diabetes was similar in the two groups as was the
 375 mean C-peptide levels. Similarly, insulin taken orally did not delay or prevent
 376 T1DM in 372 relatives at moderate risk of developing the disease. Compared
 377 with the placebo group, however, a small effect of oral insulin administration
 378 in preventing T1DM was seen in relatives with confirmed high levels of IAA
 379 (≥ 80 nU/mL) (Fig. 3) [99]. It is possible that a more potent beneficial effect
 380 of oral insulin may be present in relatives enrolled in the trial with higher
 381 IAA (ie, ≥ 200 nU/mL).

382 Finally, a trial conducted by a group called Neurocrine, using an altered insulin
 383 peptide ligand of insulin B:9-23, is currently underway in humans in which
 384 the peptide is delivered without the use of an adjuvant or other immuno-
 385 modulation [100].

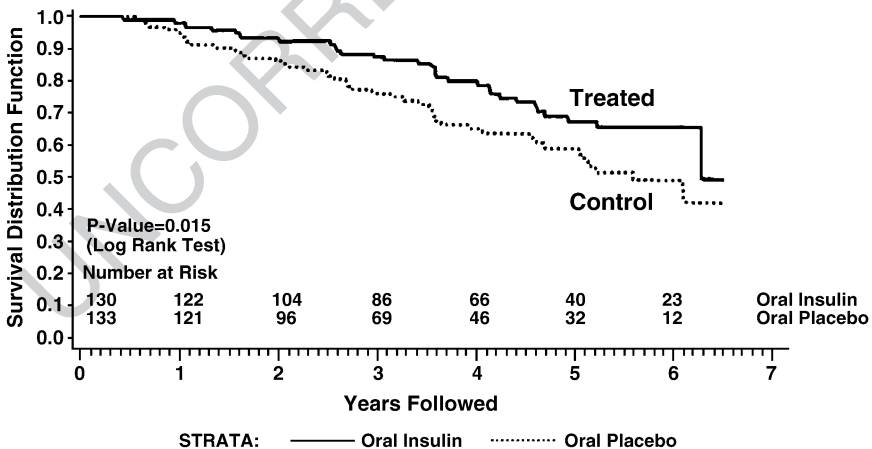


Fig. 3. Kaplan-Meier curves showing the proportion of subjects without diabetes during the trial by treatment assignment for subjects with baseline confirmed IAA ≥ 80 nU/mL. The number of subjects at risk in each group at each year of follow-up is enumerated at the bottom of the figure. The log-rank test was used for comparison between the groups, with the P values as indicated. (From Effects of oral insulin in relatives of patients with type 1 diabetes: the Diabetes Prevention Trial-Type 1. Diabetes Care 2005;28:1068–76; with permission).

386 *Lessons from secondary prevention trials*

387 Both the DPT-1 and ENDIT trials have been instrumental in paving the
388 way for designing new intervention trials. First, it was learned that large
389 preventive trials of T1DM are feasible in first-degree relatives. This major
390 commitment has also contributed in creating a large network of investigators
391 working cooperatively and collegially. Both trials re-emphasized that diabetes
392 can be predicted and the natural history of T1DM has been further elucidated.
393 It is to be noticed that both trials identified high-risk relatives on the basis of
394 ICA titer and competitive IAA assays, whereas now a better prediction is
395 achieved by using IA-2/ICA512 AA and GAD AA. A rigorous follow-up of
396 subjects to be enrolled in these trials permits an earlier diagnosis of the disease
397 with less frequency of ketoacidosis and implementation of insulin therapy when
398 higher C-peptide levels still are present. The outcome of these initial trials
399 raised questions regarding the most appropriate dose of antigen that may be
400 effective to prevent diabetes.

401 *Tertiary prevention*

402 A different preventive approach can be undertaken in newly diagnosed dia-
403 betic patients. The aims of tertiary prevention can be summarized as follows.
404 First, to preserve the remaining β -cell function. This is associated with better
405 metabolic control, less hypoglycemic events, and decrease of chronic complica-
406 tion in diabetic patients [70,101]. Second, to test the safety and efficacy of
407 preventive strategies in new-onset T1DM patients before moving to high-risk
408 populations, such as first-degree relatives.

409 The rationale for conducting these interventions is based on the assumption
410 that at least 10% of β -cells are still viable at the onset of the disease, but the
411 potential for functional recovery could even be greater. Furthermore, The Dia-
412 betes Control and Complications Trial provided evidence that preservation of
413 residual insulin production is of clinical value and that it might result in lower-
414 ing hemoglobin A_{1c} levels [70,71]. To date, the quantification of the efficacy
415 of these therapies, undertaken after the onset of insulin requirement, hinges on
416 the assessment of baseline and stimulated C-peptide levels. Although there is
417 concordance in interpreting the metabolic data (ie, C peptide), there is a paucity
418 of data addressing whether the immunotherapies induce antigen-related or regu-
419 latory tolerance. In the case of insulin trials started after diabetes onset, immune
420 responses to insulin, such as IAA (epitope, affinity, immunoglobulin subclasses)
421 and T-cell responses using the ELISPOT, were evaluated to assess whether this
422 therapy may induce changes in IAA and cytokine secretion patterns. It is impor-
423 tant to develop assays to assess whether regulatory tolerance is being generated
424 as a result of these antigen-based therapies.

425 For these reasons, the Immunology of Diabetes Society has recently es-
426 tablished Guidelines for Intervention Trials in Subjects with Newly Diagnosed
427 Type 1 Diabetes to stratify for disease risk and maximize the information gained
428 from these studies [102]. The goal for these studies is to preserve β -cell function.

429 Thus far, the only reliable screening marker for β -cell function is C peptide.
430 In fact, it is produced by cleavage of the proinsulin molecule to obtain insulin.
431 C peptide is cosecreted along with insulin, but it is subjected to less first-pass
432 clearance by the liver. Its long half-life is a prerequisite for its accurate
433 and reproducible detection in the blood. Although hemoglobin A_{1c} is a valuable
434 clinical hallmark of glycemic control, it is an insensitive marker of β -cell function.
435 Despite some limitations, C-peptide measurement under standardized conditions
436 provides a sensitive, well-accepted, and validated assessment of β -cell function.
437 It represents the most suitable primary outcome for clinical trials aimed
438 at preserving or improving endogenous insulin secretion in T1DM patients;
439 even a modest effect on preserving C-peptide secretion seems to impart benefits
440 in terms of prevention of chronic complications (Fig. 4) [70,71,101].

441 The benefits of tertiary prevention trials could be transient; also, some
442 interventions that might be effective if used in the early phase of the disease
443 might be ineffective in its terminal stage. Of note, nearly all of the prevention
444 treatments in NOD mice are of no value if started after the clinical onset of
445 the disease [86]. Treatment with anti-CD3 antibodies represents an exception to
446 this rule [103,104]. Other possible treatments in newly diagnosed T1DM patients
447 are under evaluation. The most promising ones seem to be the immunosuppressant
448 therapy with mycophenolate mofetil-daclizumab [105,106], the anti-CD20 antibody,
449 therapy with antithymocyte globulin [107], and the antigen-based vaccination using
450 the heat shock peptide DiaPep 277 [108]. Large, multicenter, controlled trials are
451 required to confirm evidence for beneficial effects in initial pilot studies and to
452 address the potential toxicity and long-term safety of the agent used.
453

454 *Anti-CD3 treatment*

455 The rationale for undertaking this approach derives from a study showing
456 a beneficial effect before and after disease onset in NOD mice treated with
457 anti-CD3 antibodies (OKT3) [103,104,109]. The remission seemed to be long
458 lasting with preservation of the capacity to mount an immune response to
459 foreign antigens. To be effective, this treatment should be started within 7 days
460 of the clinical onset, presumably when a sufficient β -cell mass is still present
461 [103,104]. Unfortunately, the use of OKT3 in humans was precluded because of
462 significant side effects as a result of massive cytokine release, particularly tumor
463 necrosis factor- α . A monoclonal antibody, termed hOKT3gl(Ala-Ala), has been
464 developed and it contains the binding region of OKT3 in which the CH2 region
465 has been modified by site-directed mutagenesis to alter FcR-binding activity.
466 Apparently, this humanized hOKT3gl(Ala-Ala) monoclonal antibody treatment
467 does not lead to major adverse effects because of release of cytokines by activated
468 T cells, such as fever, headache, hypotension, and so forth, which are common
469 following anti-CD3 monoclonal antibody treatment (OKT3). These antibodies
470 have been used successfully for the treatment of acute renal allograft rejection
471 [110] and psoriasis [111].

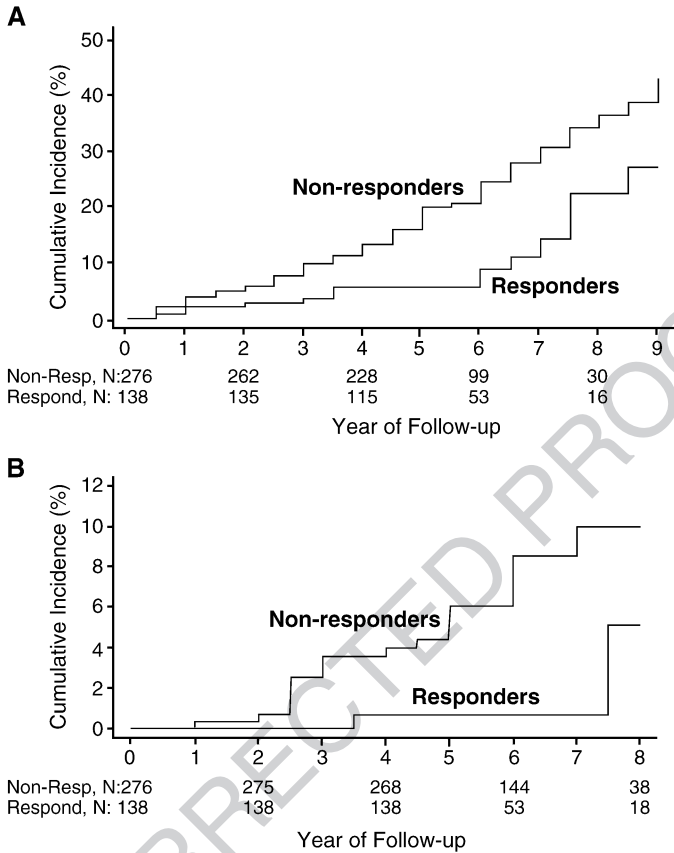


Fig. 4. (A) Cumulative incidence of any three or more step progression of retinopathy among baseline C peptide responders versus nonresponders in the intensive treatment group of the DCCT. (B) Cumulative incidence of a sustained three-step or more progression. (From Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21–22 October 2001. *Diabetes* 2004;53:250–64; with permission.)

472 A randomized placebo-controlled phase I-II trial was performed in newly
 473 diagnosed T1DM patients using this drug [112]. Twelve patients were enrolled in
 474 the intervention group and 12 were enrolled in the control group. The treatment
 475 consisted of intravenous infusion of the drug for 14 days (1.42 μg per kilogram of
 476 body weight on day 1; 5.67 μg per kilogram of body weight on day 2; 11.3 μg
 477 per kilogram of body weight on day 3; 22.6 μg per kilogram of body weight on
 478 day 4; and 45.4 μg per kilogram of body weight on days 5 through 14). The
 479 monoclonal antibody treatment resulted in a reduced decline of the C-peptide
 480 response to mixed meal tolerance test in 9 out of 12 patients (Fig. 5) and it lasted
 481 for more than 1 year. This treatment also induced a significant decrease in

Q8

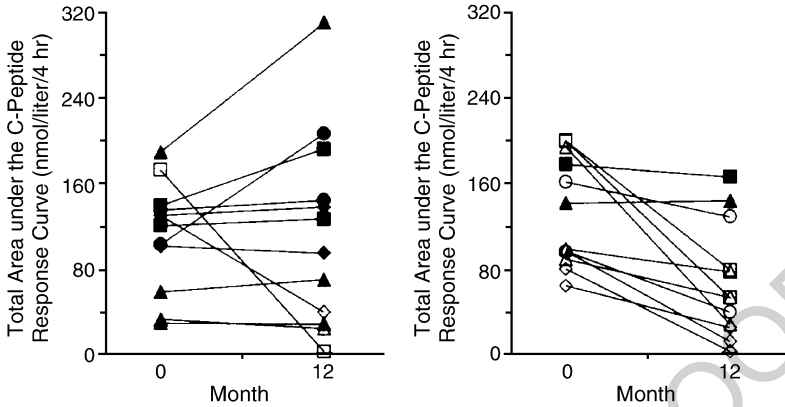


Fig. 5. Changes from study entry to 12 months in the total C peptide response to mixed-meal tolerance testing. Data from each control and antibody-treated subject are shown. Solid symbols represent patients who had a sustained or increased C peptide response, and open symbols represent patients who had a reduced response. (From Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med* 2002;346:1692–8; with permission.)

482 hemoglobin A_{1c} levels along with a significant reduction in daily exogenous
 483 insulin requirement. In the treatment group, patients experienced mild side
 484 effects, such as mild or moderate anemia, nausea, vomiting, arthralgia, headache,
 485 and urticarial rash, but with no evidence of long-term toxic effects [112]. Among
 486 patients who responded to the therapy and those who did not, there was no
 487 substantial difference in terms of clinical presentation, autoantibody titers, isotype
 488 subclasses of the autoantibodies, and HLA-DQA1 and DQB1 genotypes. No
 489 difference was found between responders and nonresponders to treatment in
 490 terms of frequency of the HLA alleles that are associated with protection or
 491 susceptibility to T1DM [112]. The mechanism of action of this drug is still
 492 unknown. It has been hypothesized that hOKT3g may affect the dynamics of
 493 regulatory T-cell populations, such as selective deletion of activated Th1 cells or
 494 activation of Th2 cells and their protective cytokines. Alterations in the number
 495 and function of regulatory cells may contribute to the generation of an auto-
 496 immune state in type 1 diabetes [109,113]. Dysfunction or loss of CD1-restricted
 497 T cells, T cells with γ/δ receptors, CD4⁺ and CD25⁺ T cells, and natural killer
 498 T cells may all theoretically contribute to disease pathogenesis through inefficient
 499 suppression of pathogenic autoreactive T cells. For instance, in monozygotic
 500 twins who are discordant for diabetes, levels of CD1-restricted T cells seem to be
 501 diminished in the affected twin. The antigens that activate regulatory T cells are
 502 unknown, and the mechanisms in which these cells exert their effect on immune
 503 responses still remains unclear. Of note, in vitro studies have demonstrated that
 504 alloantigen tolerance induced by costimulatory blockade is maintained by CD4⁺
 505 and CD25⁺ T cells [114].

506 *Antithymocyte globulin*

507 Antithymocyte globulin is being used in organ transplantation and little is
508 known about its potential beneficial effects in T1DM. Preclinical studies have
509 shown that antilymphocyte serum treatment in NOD mice with recent-onset
510 diabetes can induce disease remission. An early study that was performed in
511 newly diagnosed T1DM patients (using equine antithymocyte globulin) seemed
512 to prolong the honeymoon phase of the disease. Subsequently, to evaluate both
513 safety and efficacy of the rabbit polyclonal antithymocyte globulin, a phase II
514 study has been undertaken in newly diagnosed T1DM patients. This randomized,
515 placebo-controlled trial should determine whether antithymocyte globulin
516 (6.5 mg/kg of antithymocyte globulin is administered over 4 days) induces
517 immunologic tolerance and thereby prolongs endogenous insulin secretion in
518 adult patients affected by newly diagnosed T1DM. The primary end point is the
519 presence of residual endogenous insulin secretion at 12-month follow-up.
520 Metabolic and mechanistic studies will also be conducted over 24-month
521 intervals. The anticipated outcome is that the treated group will maintain a
522 sustained endogenous insulin secretion and will exhibit a lower daily exogenous
523 insulin requirement as compared with the control group. This study is supported
524 by both the Immuno-Tolerance Network and TrialNet [107].

Q9

525 *Anti-CD20*

526 Rituximab, an anti-CD20 monoclonal antibody, has been used for the treat-
527 ment of β -cell neoplasia and antibody-mediated autoimmune diseases. A recent
528 study showed that rituximab is effective in rheumatoid arthritis [115] and other
529 antibody-mediated autoimmune diseases. It has never been used in autoimmune
530 diseases that seem to have a cell-mediated pathogenesis. For this reason TrialNet
531 is evaluating the possibility of starting a trial to determine its safety and efficacy
532 in preserving C-peptide levels in new-onset T1DM.

533 **Pancreas and islet transplantation**

534 A major goal of clinical investigation in T1DM is to restore a physiologic
535 insulin secretion after engrafting the pancreas or the pancreatic islets into T1DM
536 diabetic recipients. Although ectopancreatic transplantation of donor pancreas
537 has proved fairly effective in normalizing blood glucose levels with partial to
538 complete restoration of C-peptide production in selected groups of patients, islet
539 transplantation gave less promising results [116]. It should be noted that pan-
540 creas and islet transplantation cannot be considered as life-saving procedures,
541 and the benefit and risk of these procedures must be thoroughly weighed, par-
542 ticularly if one is dealing with pediatric patients that need long-lasting chronic
543 immunosuppressant therapy to prevent allojection of the graft. The improve-
544 ment of new immunosuppressive regimens is an important objective to achieve

545 before considering pancreas and islet transplantation as the standard of care for
546 T1DM [117].

547 Over the past few years, Shapiro and coworkers [118] have been able to
548 reverse T1DM following islet implantation and the success of the Edmonton
549 protocol has again sparked interest for islet transplantation. Shapiro and co-
550 workers [118] administered a steroid-free immunosuppression regimen along
551 with a larger number of transplanted islets compared with previous islet
552 transplantation protocols; sirolimus (0.2 mg/kg/d orally); low-dose tacrolimus
553 (<2 mg/d orally); and daclizumab (1 mg/kg intravenously every 14 days), an anti
554 interleukin-2 receptor antibody [118]. Side effects were those related to trans-
555 hepatic puncture and intrahepatic infusion and those related to immunosuppres-
556 sion, such as ulceration of the buccal mucosa, nausea and vomiting, arthralgias,
557 diarrhea, and anemia. Some of the patients had low white blood cell count and a
558 rise in serum creatinine. After a median follow-up of 20 months, 11 transplanted
559 patients were off insulin. According to the American Diabetes Association
560 criteria for diagnosis of diabetes after oral glucose tolerance test, however, only
561 two subjects met the criteria for normal glucose tolerance and the remainder had
562 diabetes [119]. The Edmonton protocol has been widely adopted; however, data
563 on long-term follow-up are not yet available.

Q10

564 **Stem cell therapy, gene therapy, and other therapies**

565 With the initial success of the Edmonton protocol and increased interest for
566 islet transplantation [118], a number of concerns have arisen, including the
567 insufficient number of donors to yield a sufficient number of implantable islets
568 and the need for a life-long immunosuppressive therapy to prevent both allograft
569 rejection and autoimmunity recurrence. These major obstacles have shifted the
570 interest to alternative approaches to create less immunoreactive and potentially
571 endless alternative sources of islet cells, or to regenerate pancreatic β -cells
572 (reviewed in [120]). The shortage of implantable islets could be overcome by a
573 number of new approaches, including transformed insulin-producing cell lines,
574 transfection of different cell types enabled to produce insulin, in vivo trans-
575 differentiation of liver cells, and isolation of xenogeneic porcine islets [117,
576 120,121].

577 It has been hypothesized that newly formed islets derive from the duct
578 epithelium [122], or alternatively from the islet cells themselves [123] or from
579 islet neogenesis, a precursor that may be able to compensate for islet loss. Islet
580 neogenesis may be the end result of a dedifferentiation of pancreatic epithe-
581 lial cells, or newly formed islets might originate from common endocrine,
582 multipotent progenitors. These islet progenitors seem also to be present within the
583 pancreatic islet microenvironment [124]. It might be easier to derive precur-
584 sor cells from stem cells, adult or embryonic, and use them to regenerate the
585 damaged endocrine pancreas. Embryonic stem cell lines might give rise to a

586 potentially unlimited source of insulin-producing cells. Even if embryonic stem
587 cells were soon made available for the scientific community, however, their dif-
588 ferentiation toward insulin-producing cells would still remain very difficult to
589 direct. In contrast, achieving transdifferentiation into β -cells from adult stem
590 cells obtained from tissues that belong to other lineages might be a more feasi-
591 ble task. The latter could be an attractive approach to avoid the serious problems
592 related to allorejection because adult tissues can be obtained from an autologous
593 living donor. Nevertheless, this approach is not likely to prevent the recurrence of
594 autoimmunity if it is not combined with immunotherapy.

595 A similar strategy is based on converting the patient's nonislet cells of dif-
596 ferent lineages into insulin-producing cells. This has been accomplished using
597 gene-engineered hepatocytes that were able to produce insulin after transfecting
598 them with *pdx1* under the control of the rat insulin 1 promoter [125]. Although
599 not yet confirmed, this observation suggests that engineered surrogate β -cells or
600 insulin-producing cells obtained from cells of different lineages could be ex-
601 ploited to restore insulin secretion.

602 Other alternative sources of insulin-producing cells include xenogeneic donor
603 manipulated cells that could provide an indefinite supply of β -cells for trans-
604 plantation. In an elegant set of experiments, the generation of a transgenic pig that
605 was deficient for $\alpha(1,3)$ -galactosyltransferase resulted in the dissipation of the
606 hyperacute xenograft rejection response. This was a major advance that might
607 set the stage toward the attainment of the first clinical trial in humans using
608 xenogeneic islet donors [126–129].

609 Although many of these approaches seem to hold great promise as alternative
610 strategies to cure T1DM, it is unlikely that the recurrence of islet autoimmunity
611 can be prevented without an appropriate immunotherapeutic treatment. In T1DM
612 patients, the autoimmune process not only damages the pancreatic β -cells,
613 leading to the clinical onset of the disease, but also limits the regeneration of
614 newly formed β -cells that will eventually replace those cells that are lost. The
615 recurrence of autoimmunity in combination with allorejection is probably what
616 differentiates transplanted patients with T1DM from patients who received is-
617 let autotransplantation and were able to maintain long-lasting glucose homeo-
618 stasis [117,130]. Strategies aimed at blocking this autoimmune process, or at
619 manipulating potentially less immunoreactive transplanted cells, should yield
620 more encouraging results. These strategies include patient's own cells of differ-
621 ent lineages converted into insulin-producing cells; xenogeneic donor manipu-
622 lated cells; and other manipulated insulin-producing cells, which are likely less
623 immunoreactive. Furthermore, the autoimmune process is successfully averted by
624 blocking the autoreactive T cells with an anti-T-cell antibody (antilymphocyte
625 serum) and by inducing a mixed allogeneic chimerism by transplanting bone
626 marrow from a diabetes-resistant donor [131]. Hematopoietic precursors do not
627 directly participate in islet cell regeneration, although they might be necessary
628 to promote an effective regenerative process, which is independent of the ability
629 to block the autoimmune process [132]. A better understanding of the auto-
630 immune process and the ability to restrain this process not only could prevent

631 the disease, but also help restore residual islet function following islet trans-
632 plantation or regeneration.

633 **Summary**

634 There is common agreement indicating that the occurrence of multiple
635 antibodies against islet autoantigens serves as a surrogate marker of disease in
636 primary or secondary intervention strategies aimed at halting the disease process.
637 To date, a number of intervention strategies are in the pipeline and some of them
638 seem promising. These therapies include anti-CD3 humanized monoclonal
639 antibody; antilymphocyte serum; and a number of antigen-specific therapies,
640 such as oral insulin. The DPT-1 has recently performed a subgroup analysis
641 suggesting potential benefit of oral insulin for relatives with high insulin auto-
642 antibody titers. A trial conducted by Neurocrine using an altered insulin peptide
643 ligand of insulin B:9-23 is currently underway in humans in which the peptide is
644 delivered without the use of an adjuvant or other immunomodulation. Many of
645 these antigen-specific therapies for T1DM and other autoimmune diseases have
646 not been approved. There is both a growing effort and a large opportunity for
647 exploring new specific strategies alone or in combination with immunomodula-
648 tion. It is possible that gene-engineered cell therapeutics if combined with
649 immunotherapy may effectively replace the pancreatic β -cell loss in T1DM. The
650 hope to induce pancreatic islet regeneration and, ultimately, to transplant insulin-
651 producing cells with a sustained secretagogue capacity propels confidence that
652 the cure of T1DM is within reach.

653 **References**

- 654 [1] Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl*
655 *J Med* 1994;331:1428–36.
- 656 [2] Eisenbarth GS. Type I diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 1986;
657 314:1360–8.
- 658 [3] Bingley PJ, et al. Combined analysis of autoantibodies improves prediction of IDDM in islet
659 cell antibody-positive relatives. *Diabetes* 1994;43:1304–10.
- 660 [4] Verge CF, et al. Number of autoantibodies (against insulin, GAD or ICA512/IA2) rather than
661 particular autoantibody specificities determines risk of type I diabetes. *J Autoimmun* 1996;9:
662 379–83.
- 663 [5] Verge CF, et al. Prediction of type I diabetes in first-degree relatives using a combination of
664 insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 1996;45:926–33.
- 665 [6] Pietropaolo M, et al. Combined analysis of GAD65 and ICA512(IA-2) autoantibodies in organ
666 and non-organ-specific autoimmune diseases confers high specificity for insulin-dependent
667 diabetes mellitus. *J Autoimmun* 1998;11:1–10.
- 668 [7] Wiest-Ladenburger U, et al. Combined analysis and single-step detection of GAD65 and IA2
669 autoantibodies in IDDM can replace the histochemical islet cell antibody test. *Diabetes*
670 1997;46:565–71.

Q11

- 671 [8] Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with
672 autoimmune polyendocrine deficiencies. *Lancet* 1974;2:1279–83.
- 673 [9] Pietropaolo M, et al. Viva ICA: cytoplasmic islet cell antibodies 30 years later [abstract].
674 *Diabetes* 2004;53(Suppl 2):A64.
- 675 [10] Genovese S, et al. Distinct cytoplasmic islet cell antibodies with different risks for type 1
676 (insulin-dependent) diabetes mellitus. *Diabetologia* 1992;35:385–8.
- 677 [11] Gianani R, et al. Prognostically significant heterogeneity of cytoplasmic islet cell antibodies
678 in relatives of patients with type 1 diabetes. *Diabetes* 1992;41:347–53.
- 679 [12] Atkinson MA, et al. Islet cell cytoplasmic autoantibody reactivity to glutamate decarboxylase
680 in insulin-dependent diabetes. *J Clin Invest* 1993;91:350–6.
- 681 [13] Myers MA, Rabin DU, Rowley MJ. Pancreatic islet cell cytoplasmic antibody in diabetes is
682 represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. *Diabetes*
683 1995;44:1290–5.
- 684 [14] Gianani R, et al. ICA512 autoantibody radioassay. *Diabetes* 1995;44:1340–4.
- 685 [15] Grubin CE, et al. A novel radioligand binding assay to determine diagnostic accuracy of
686 isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. *Diabetologia*
687 1994;37:344–50.
- 688 [16] Palmer JP, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment.
689 *Science* 1983;222:1337–9.
- 690 [17] Williams AJ, et al. A novel micro-assay for insulin autoantibodies. *J Autoimmun* 1997;10:
691 473–8.
- 692 [18] Naserke HE, et al. Comparison of a novel micro-assay for insulin autoantibodies with the
693 conventional radiobinding assay. *Diabetologia* 1998;41:681–3.
- 694 [19] Vardi P, et al. Competitive insulin autoantibody assay: prospective evaluation of subjects at
695 high risk for development of type 1 diabetes mellitus. *Diabetes* 1987;36:1286–91.
- 696 [20] Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay
697 proficiency evaluation. *Diabetes* 2003;52:1128–36.
- 698 [21] Bingley PJ, et al. Prediction of IDDM in the general population: strategies based on com-
699 binations of autoantibody markers. *Diabetes* 1997;46:1701–10.
- 700 [22] Feeney SJ, et al. Evaluation of ICA512As in combination with other islet cell autoantibodies
701 at the onset of IDDM. *Diabetes Care* 1997;20:1403–7.
- 702 [23] Baekkeskov S, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as
703 the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 1990;347:151–6.
- 704 [24] Padoa CJ, et al. Recombinant Fabs of human monoclonal antibodies specific to the middle
705 epitope of GAD65 inhibit type 1 diabetes-specific GAD65Abs. *Diabetes* 2003;52:2689–95.
- 706 [25] Schwartz HL, et al. High-resolution autoreactive epitope mapping and structural modeling
707 of the 65 kDa form of human glutamic acid decarboxylase. *J Mol Biol* 1999;287:983–99.
- 708 [26] Kawasaki E, et al. Definition of multiple ICA512/phogrin autoantibody epitopes and detection
709 of intramolecular epitope spreading in relatives of patients with type 1 diabetes. *Diabetes*
710 1998;47:733–42.
- 711 [27] Farilla L, et al. Application of phage display peptide library to autoimmune diabetes: identifica-
712 tion of IA-2/ICA512bcd dominant autoantigenic epitopes. *Eur J Immunol* 2002;32:1420–7.
- 713 [28] Casu A, et al. Humoral autoimmunity to IA-2 intracellular domain epitopes increases the
714 cumulative risk of type 1 diabetes (T1D) progression [abstract]. *Diabetes* 2005;OR121.
- 715 [29] Pietropaolo M, et al. Islet cell autoantigen 69 kD (ICA69)L: molecular cloning and char-
716 acterization of a novel diabetes-associated autoantigen. *J Clin Invest* 1993;92:359–71.
- 717 [30] Horvath L, et al. Antibodies against different epitopes of heat-shock protein 60 in children
718 with type 1 diabetes mellitus. *Immunol Lett* 2002;80:155–62.
- 719 [31] Lieberman SM, DiLorenzo TP. A comprehensive guide to antibody and T-cell responses in
720 type 1 diabetes. *Tissue Antigens* 2003;62:359–77.
- 721 [32] Kostraba JN, et al. Incidence of insulin-dependent diabetes mellitus in Colorado. *Epidemiology*
722 1992;3:232–8.
- 723 [33] Barneier H, et al. Risk for developing type 1 (insulin-dependent) diabetes mellitus and the
724 presence of islet 64K antibodies. *Diabetologia* 1991;34:727–33.

Q12

- 725 [34] Riley WJ, et al. A prospective study of the development of diabetes in relatives of patients
726 with insulin-dependent diabetes. *N Engl J Med* 1990;323:1167–72.
- 727 [35] Maclaren NK. How, when, and why to predict IDDM. *Diabetes* 1988;37:1591–4.
- 728 [36] LaPorte RE, et al. The Pittsburgh Insulin-Dependent Diabetes Mellitus (IDDM) Registries:
729 the descriptive epidemiology of racial differences. In: Mimura G, et al, editors. Clinico-genetic
730 genesis of diabetes mellitus. International Congress Series. Amsterdam, The Netherlands:
731 Excerpta Medica; 1982. p. 66–77.
- 732 [37] Strebellow M, et al. Karlsburg type I diabetes risk study of a general population: frequencies
733 and interactions of the four major type I diabetes-associated autoantibodies studied in 9419
734 schoolchildren. *Diabetologia* 1999;42:661–70.
- 735 [38] LaGasse JM, et al. Successful prospective prediction of type 1 diabetes in schoolchildren
736 through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes
737 Prediction Study. *Diabetes Care* 2002;25:505–11.
- 738 [39] Kulmala P, et al. Beta-cell autoimmunity, genetic susceptibility, and progression to type 1
739 diabetes in unaffected schoolchildren. *Diabetes Care* 2001;24:171–3.
- 740 [40] Schatz D, et al. Islet cell antibodies predict insulin-dependent diabetes in United States school
741 age children as powerfully as in unaffected relatives. *J Clin Invest* 1994;93:2403–7.
- 742 [41] Maclaren N, et al. Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2
743 and IA-2beta predict immune-mediated (type 1) diabetes in relatives. *J Autoimmun* 1999;12:
744 279–87.
- 745 [42] Hummel M, et al. Brief communication: early appearance of islet autoantibodies predicts
746 childhood type 1 diabetes in offspring of diabetic parents. *Ann Intern Med* 2004;140:882–6.
- 747 [43] Bonifacio E, et al. IDDM1 and multiple family history of type 1 diabetes combine to identify
748 neonates at high risk for type 1 diabetes. *Diabetes Care* 2004;27:2695–700.
- 749 [44] Verge CF, et al. Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody,
750 insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet
751 Autoantibody Workshop. *Diabetes* 1998;47:1857–66.
- 752 [45] Kulmala P, et al. Prediction of insulin-dependent diabetes mellitus in siblings of children with
753 diabetes: a population-based study. The Childhood Diabetes in Finland Study Group. *J Clin
754 Invest* 1998;101:327–36.
- 755 [46] Achenbach P, et al. Stratification of type 1 diabetes risk on the basis of islet autoantibody
756 characteristics. *Diabetes* 2004;53:384–92.
- 757 [47] Dorman JS, et al. Risk factors for insulin-dependent diabetes. In: *Diabetes in America*. NIH
758 Publication No. 95–1468, 1995. p. 165–77. **Q13**
- 759 [48] LaPorte RE, Matsushima M, Chang Y-F. Prevalence and incidence of insulin-dependent
760 diabetes. In: *Diabetes in America*. NIH Publication No. 95–1468, 1995. p. 37–46. **Q14**
- 761 [49] Pietropaolo M, Becker DJ. Type 1 diabetes intervention trials. *Pediatr Diabetes* 2001;2:2–11.
- 762 [50] Harrison LC. Risk assessment, prediction and prevention of type 1 diabetes. *Pediatr Diabetes*
763 2001;2:71–82.
- 764 [51] Pietropaolo M, Trucco M. Major histocompatibility locus and other genes that determine
765 the risk for development of type 1 diabetes mellitus. **Q15**
- 766 [52] Noble JA, et al. The role of HLA class II genes in insulin-dependent diabetes mellitus:
767 molecular analysis of 180 caucasian, multiplex families. *Am J Hum Genet* 1996;59:1134–48.
- 768 [53] Rewers M, et al. Newborn screening for HLA markers associated with IDDM: diabetes
769 autoimmunity study in the young (DAISY). *Diabetologia* 1996;39:807–12.
- 770 [54] Todd JA, Farrall M. Panning for gold: genome-wide scanning for linkage in type 1 diabetes.
771 *Hum Mol Genet* 1996;5(Spec No):1443–8.
- 772 [55] Wolf E, Spencer KM, Cudworth AG. The genetic susceptibility to type 1 (insulin-dependent)
773 diabetes: analysis of the HLA-DR association. *Diabetologia* 1983;24:224–30.
- 774 [56] Morel PA, et al. Aspartic acid at position 57 of the HLA-DQ beta chain protects against
775 type I diabetes: a family study. *Proc Natl Acad Sci U S A* 1988;85:8111–5.
- 776 [57] McDevitt H. Closing in on type 1 diabetes. *N Engl J Med* 2001;345:1060–1.
- 777 [58] Pietropaolo M, et al. Progression to insulin-requiring diabetes in seronegative prediabetic
778 subjects: the role of two HLA-DQ high-risk haplotypes. *Diabetologia* 2002;45:66–76.

- 779 [59] Cornell CN. Absence of islet autoantibodies at diabetes onset does not rule out type 1A
780 diabetes. *Diabetes* 2000;49(Suppl 1):A69.
- 781 [60] Lipton RB, et al. Autoimmunity and genetics contribute to the risk of insulin-dependent
782 diabetes mellitus in families: islet cell antibodies and HLA DQ heterodimers. *Am J Epidemiol*
783 1992;136:503–12.
- 784 [61] Hahl J, et al. Costs of predicting IDDM. *Diabetologia* 1998;41:79–85.
- 785 [62] Dahlquist G. Potentials and pitfalls in neonatal screening for type 1 diabetes. *Acta Paediatr*
786 *Suppl* 1999;88:80–2.
- 787 [63] Greenbaum CJ, et al. Relationship of beta-cell function and autoantibodies to progression
788 and nonprogression of subclinical type 1 diabetes: follow-up of the Seattle Family Study.
789 *Diabetes* 1999;48:170–5.
- 790 [64] Becker DJ, et al. High risk DQ alleles improve IDDM prediction in ICA + ve first-degree
791 relatives with decreased insulin secretion [abstract]. *Diabetologia* 1995;38(Suppl 1):A42.
- 792 [65] Pietropaolo M, et al. Autoantibodies to GAD65 and ICA512/IA-2 improve IDDM prediction in
793 first-degree relatives with decreased first phase insulin response and ICA positivity [abstract].
794 *Autoimmunity* 1996;24(Suppl 1):51.
- 795 [66] Eisenbarth GS, et al. Dual-parameter model for prediction of type I diabetes mellitus. *Proc*
796 *Assoc Am Physicians* 1998;110:126–35.
- 797 [67] Colman PG, et al. The Melbourne Pre-Diabetes Study: prediction of type 1 diabetes mellitus
798 using antibody and metabolic testing. *Med J Aust* 1998;169:81–4.
- 799 [68] Vardi P, Crisa L, Jackson RA. Predictive value of intravenous glucose tolerance test insulin
800 secretion less than or greater than the first percentile in islet cell antibody positive relatives
801 of type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1991;34:93–102.
- 802 [69] Bingley PJ, et al. Proposed guidelines on screening for risk of type 1 diabetes. *Diabetes*
803 *Care* 2001;24:398.
- 804 [70] Group TDC. Effect of intensive therapy on residual beta-cell function in patients with type 1
805 diabetes in the diabetes control and complications trial: a randomized, controlled trial. *Ann*
806 *Intern Med* 1998;128:517–23.
- 807 [71] Group TDR. Effects of age, duration and treatment of insulin-dependent diabetes mellitus on
808 residual beta-cell function: observations during eligibility testing for the Diabetes Control and
809 Complications Trial (DCCT). *J Clin Endocrinol Metab* 1987;65:30–6.
- 810 [72] Rosenbloom AL, et al. Therapeutic controversy: prevention and treatment of diabetes in
811 children. *J Clin Endocrinol Metab* 2000;85:494–522.
- 812 [73] Bougneres PF, et al. Limited duration of remission of insulin dependency in children with
813 recent overt type I diabetes treated with low-dose cyclosporin. *Diabetes* 1990;39:1264–72.
- 814 [74] Group. T.C.-E.R.C.T. Cyclosporin-induced remission of IDDM after early intervention: asso-
815 ciation of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes* 1988;37:
816 1574–82.
- 817 [75] Cook JJ, et al. Double-blind controlled trial of azathioprine in children with newly diagnosed
818 type I diabetes. *Diabetes* 1989;38:779–83.
- 819 [76] Harrison LC, et al. Increase in remission rate in newly diagnosed type I diabetic subjects treated
820 with azathioprine. *Diabetes* 1985;34:1306–8.
- 821 [77] Silverstein J, et al. Immunosuppression with azathioprine and prednisone in recent-onset
822 insulin-dependent diabetes mellitus. *N Engl J Med* 1988;319:599–604.
- 823 [78] Akerblom HK, Knip M. Putative environmental factors in type 1 diabetes. *Diabetes Metab*
824 *Rev* 1998;14:31–67.
- 825 [79] Akerblom HK, et al. Environmental factors in the etiology of type 1 diabetes. *Am J Med Genet*
826 2002;115:18–29.
- 827 [80] Gerstein HC. Cow's milk exposure and type I diabetes mellitus: a critical overview of the
828 clinical literature. *Diabetes Care* 1994;17:13–9.
- 829 [81] Elliott RB, Martin JM. Dietary protein: a trigger of insulin-dependent diabetes in the BB
830 rat? *Diabetologia* 1984;26:297–9.
- 831 [82] Elliott RB, et al. Dietary prevention of diabetes in the non-obese diabetic mouse. *Diabetologia*
832 1988;31:62–4.

Q16

Q17

- 833 [83] Akerblom HK, et al. Dietary manipulation of beta cell autoimmunity in infants at increased
834 risk of type 1 diabetes: a pilot study. *Diabetologia* 2005;48:829–37.
- 835 [84] Group ENDIT. Intervening before the onset of type 1 diabetes: baseline data from the European
836 Nicotinamide Diabetes Intervention Trial (ENDIT). *Diabetologia* 2003;46:339–46.
- 837 [85] Gale EA, et al. European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised
838 controlled trial of intervention before the onset of type 1 diabetes. *Lancet* 2004;363:925–31.
- 839 [86] Atkinson MA, Leiter EH. The NOD mouse model of type 1 diabetes: as good as it gets? *Nat*
840 *Med* 1999;5:601–4.
- 841 [87] Elliott RB, et al. A population based strategy to prevent insulin-dependent diabetes using
842 nicotinamide. *J Pediatr Endocrinol Metab* 1996;9:501–9.
- 843 [88] Zhang ZJ, et al. Suppression of diabetes in nonobese diabetic mice by oral administration of
844 porcine insulin. *Proc Natl Acad Sci U S A* 1991;88:10252–6.
- 845 [89] Muir A, Schatz D, Maclaren N. Antigen-specific immunotherapy: oral tolerance and subcu-
846 taneous immunization in the treatment of insulin-dependent diabetes. *Diabetes Metab Rev*
847 1993;9:279–87.
- 848 [90] Gottlieb PA, et al. Insulin treatment prevents diabetes mellitus but not thyroiditis in
849 RT6-depleted diabetes resistant BB/Wor rats. *Diabetologia* 1991;34:296–300.
- 850 [91] Keller RJ, Eisenbarth GS, Jackson RA. Insulin prophylaxis in individuals at high risk of type I
851 diabetes. *Lancet* 1993;341:927–8.
- 852 [92] Group DPTTDS. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl*
853 *J Med* 2002;346:1685–91.
- 854 [93] Pugliese A, et al. The insulin gene is transcribed in the human thymus and transcription levels
855 correlated with allelic variation at the INS VNTR-IDD M2 susceptibility locus for type 1
856 diabetes. *Nat Genet* 1997;15:293–7.
- 857 [94] Vafiadis P, et al. Insulin expression in human thymus is modulated by INS VNTR alleles at
858 the IDDM2 locus. *Nat Genet* 1997;15:289–92.
- 859 [95] Sospedra M, et al. Transcription of a broad range of self-antigens in human thymus suggests
860 a role for central mechanisms in tolerance toward peripheral antigens. *J Immunol* 1998;161:
861 5918–29.
- 862 [96] Pietropaolo M, Giannoukakis N, Trucco M. Cellular environment and freedom of gene ex-
863 pression. *Nat Immunol* 2002;3:335 [author reply: 336].
- 864 [97] Pugliese A, et al. Self-antigen-presenting cells expressing diabetes-associated autoantigens
865 exist in both thymus and peripheral lymphoid organs. *J Clin Invest* 2001;107:555–64.
- 866 [98] Jaecckel E, Lipes MA, von Boehmer H. Recessive tolerance to preproinsulin 2 reduces but
867 does not abolish type 1 diabetes. *Nat Immunol* 2004;5:1028–35.
- 868 [99] Skyler J, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: the Diabetes
869 Prevention Trial-Type 1. *Diabetes Care* 2005;28:1068–76.
- 870 [100] Alleva DG, et al. Immunological characterization and therapeutic activity of an altered-peptide
871 ligand, NBI-6024, based on the immunodominant type 1 diabetes autoantigen insulin B-chain
872 (9–23) peptide. *Diabetes* 2002;51:2126–34.
- 873 [101] Palmer JP, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical
874 trials to preserve beta-cell function: report of an ADA workshop, 21–22 October 2001. *Di-*
875 *abetes* 2004;53:250–64.
- 876 [102] Greenbaum CJ, Harrison LC. Guidelines for intervention trials in subjects with newly
877 diagnosed type 1 diabetes. *Diabetes* 2003;52:1059–65.
- 878 [103] Chatenoud L, Primo J, Bach JF. CD3 antibody-induced dominant self tolerance in overtly
879 diabetic NOD mice. *J Immunol* 1997;158:2947–54.
- 880 [104] Chatenoud L, et al. Anti-CD3 antibody induces long-term remission of overt autoimmunity
881 in nonobese diabetic mice. *Proc Natl Acad Sci U S A* 1994;91:123–7.
- 882 [105] Hao L, Chan SM, Lafferty KJ. Mycophenolate mofetil can prevent the development of diabetes
883 in BB rats. *Ann N Y Acad Sci* 1993;696:328–32.
- 884 [106] TrialNet. MMF/DZB study. 2004.
- 885 [107] Gitelman SE. Immune Tolerance Network. Trial of Thymoglobulin for treatment of new onset
886 type 1 diabetes mellitus. 2005.

Q18

Q19

- 887 [108] Raz I, et al. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-
888 shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet*
889 2001;358:1749–53.
- 890 [109] Bach JF. Immunotherapy of insulin-dependent diabetes mellitus. *Curr Opin Immunol* 2001;13:
891 601–5.
- 892 [110] Woodle ES, et al. Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody,
893 huOKT3gamma1(Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation*
894 1999;68:608–16.
- 895 [111] Utset TO, et al. Modified anti-CD3 therapy in psoriatic arthritis: a phase I/II clinical trial.
896 *J Rheumatol* 2002;29:1907–13.
- 897 [112] Herold KC, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl*
898 *J Med* 2002;346:1692–8.
- 899 [113] Kukreja A, et al. Multiple immuno-regulatory defects in type-1 diabetes. *J Clin Invest*
900 2002;109:131–40.
- 901 [114] Taylor PA, Noelle RJ, Blazar BR. CD4(+)CD25(+) immune regulatory cells are required for
902 induction of tolerance to alloantigen via costimulatory blockade. *J Exp Med* 2001;193:1311–8.
- 903 [115] Edwards JC, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with
904 rheumatoid arthritis. *N Engl J Med* 2004;350:2572–81.
- 905 [116] Bottino R, et al. Pancreas and islet cell transplantation. *Best Pract Res Clin Gastroenterol*
906 2002;16:457–74.
- 907 [117] Bottino R, et al. Islet/pancreas transplantation: challenges for pediatrics. *Pediatr Diabetes*
908 2002;3:210–23.
- 909 [118] Shapiro AM, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a
910 glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000;343:230–8.
- 911 [119] Ryan EA, et al. Successful islet transplantation: continued insulin reserve provides long-term
912 glycemic control. *Diabetes* 2002;51:2148–57.
- 913 [120] Trucco M. Regeneration of the pancreatic beta cell. *J Clin Invest* 2005;115:5–12.
- 914 [121] Trucco M. Stem cells and diabetes. *Pediatr Diabetes* 2004;5(Suppl 2):2–4.
- 915 [122] Bonner-Weir S, Sharma A. Pancreatic stem cells. *J Pathol* 2002;197:519–26.
- 916 [123] Dor Y, et al. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell
917 differentiation. *Nature* 2004;429:41–6.
- 918 [124] Seaberg RM, et al. Clonal identification of multipotent precursors from adult mouse pancreas
919 that generate neural and pancreatic lineages. *Nat Biotechnol* 2004;22:1115–24.
- 920 [125] Ferber S, et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes
921 in liver and ameliorates streptozotocin-induced hyperglycemia. *Nat Med* 2000;6:568–72.
- 922 [126] Dai Y, et al. Targeted disruption of the alpha1,3-galactosyltransferase gene in cloned pigs.
923 *Nat Biotechnol* 2002;20:251–5.
- 924 [127] Koike C, et al. Isolation of the regulatory regions and genomic organization of the porcine
925 alpha1,3-galactosyltransferase gene. *Transplantation* 2000;70:1275–83.
- 926 [128] Koike C, et al. Molecular basis of evolutionary loss of the alpha 1,3-galactosyltransferase
927 gene in higher primates. *J Biol Chem* 2002;277:10114–20.
- 928 [129] Phelps CJ, et al. Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science* 2003;
929 299:411–4.
- 930 [130] Tzakis AG, et al. Pancreatic islet transplantation after upper abdominal exenteration and liver
931 replacement. *Lancet* 1990;336:402–5.
- 932 [131] Zorina TD, et al. Distinct characteristics and features of allogeneic chimerism in the NOD
933 mouse model of autoimmune diabetes. *Cell Transplant* 2002;11:113–23.
- 934 [132] Zorina TD, et al. Recovery of the endogenous beta cell function in the NOD model of
935 autoimmune diabetes. *Stem Cells* 2003;21:377–88.
- 936