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Study design of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR)

The TRIGR Study Group. Study design of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR).
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Abstract: The hypothesis for this study is that weaning to an extensively hydrolyzed infant formula will decrease the incidence of type 1 diabetes (T1D), as it does in all relevant animal models for the disease. This will be tested in children who carry risk-associated human leukocyte antigen genotypes and have a first-degree relative with T1D. The trial will use a double-blind, prospective, placebo-controlled intervention protocol, comparing casein hydrolysate with a conventional cow's milk (CM)-based formula. A secondary aim is to determine relationships between CM antibodies, a measure of CM exposure, and diabetes-associated autoantibodies. To achieve an 80% power for the detection of a 40% intervention-induced difference in the development of autoantibodies and subsequent diabetes, the study requires 2032 subjects. A multicenter, international, collaborative effort is necessary to achieve recruitment targets. A collaborative international study group of 78 clinical centers in 15 countries has therefore been assembled for this purpose.

The TRIGR Study Group*

Key words: autoantibodies – hydrolyzed infant formula – primary prevention – type 1 diabetes

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*See Appendix.

Substantial progress has been made in understanding autoimmune diabetes. However, the principal mechanisms that initiate and mediate the process of β-cell destruction remain obscure. Large collaborative efforts over the last 15 yr have consolidated our view of the diabetes-prone host. Thus, nearly 90% of subjects will carry one of a small number of disease-associated human leukocyte antigen (HLA) genotypes, protective alleles are rare, and autoantibodies to different islet cell proteins accumulate progressively as strong harbingers of impending disease. The Trial to Reduce IDDM in the Genetically at Risk (TRIGR) has its roots in both human epidemiological and rodent experimental studies. Although hypothetical causes of diabetic autoimmunity remain controversial, the best strategy for disease prevention in diabetes-prone BB rats and non-obese diabetic (NOD) mice uses hypoantigenic weaning diets, based on amino acids or extensively hydrolyzed casein (1–12). The ultimate goal of animal studies is the translation of their results to the human disease. The major secondary type 1 diabetes (T1D) prevention trials are good examples (13–15), and TRIGR is the first primary prevention effort.

Genetic risk factors

The role played by genetics in T1D is illustrated by family and genetic marker studies. Compared to the general population, first-degree relatives of patients with T1D have a markedly increased risk of T1D, which varies with the risk within the background population of the region (16). Siblings of a patient may have a 6–8% average rate of diabetes development by adulthood, but this rate can reach 12–19% if the sibling and patient share the same high-risk HLA haplotypes (17). If they are identical twins, approximately 20–30% of the siblings will develop diabetes (18, 19), this rate increasing in the presence of a high-risk genotype (20). Children of affected mothers have a 1.5–2.5% diabetes risk; the rate in children of affected fathers is 3–6% (21–23). The most important genetic risk markers for T1D are located within the HLA class II region. The protein products encoded by class II genes are expressed on antigen-presenting cells. They capture and present processed peptide antigen to the T-cell receptor – a central event in the initiation of any immune response. HLA-DQ heterodimer molecules encoded by DQA1 and DQB1 genes are most strongly

associated with disease susceptibility (24). The highest risk is associated with the genotype containing two different susceptibility genotypes (DR4-DQ8/DR3-DQ2), whereas the risk associated with a double dose of individual haplotypes is not markedly higher than the single dose without protective markers. This synergism between risk haplotypes may reflect the creation of new heterodimer molecules encoded by A1 and B1 genes in *trans* position.

HLA-DR4 subtypes among DR4-DQ8 haplotypes confer different risks, and DRB1*0403 and *0406 alleles seem to be dominantly protective (25). Approximately 80% of Caucasian subjects with T1D have at least one of the HLA susceptibility haplotypes, but these are also present in about 30% of the general population. This suggests interactions with one or more of the other genes that have been identified, with broad similarities between human and syngenic regions in NOD mouse genomes (26, 27). About 20 risk loci have been mapped in humans, but only the insulin gene region on chromosome 11 and to a lesser extent CTL4 have been consistently established to affect risk in man but play a lesser role than HLA in the predisposition to diabetes (28, 29).

Environmental risk factors

A low twin concordance of 20–30% (30, 31) rapid shifts in incidence (32), as well as large variations in diabetes incidence in humans (33) and among different colonies of inbred, diabetes-prone rodents (34), all contribute to the evidence that progression of diabetic autoimmunity is driven by interactions of environmental factors with the products of predisposing and protective genes (35, 36). Risk-associated environmental factors remain largely unproved, but some infectious and nutritional agents, and toxins have been proposed (32, 37). Enteroviral infection, in particular Coxsackie B4, attracted renewed interest (38–43), and in diabetes-prone NOD mice, this virus can accelerate (but not initiate) prediabetes progression (44). There clearly are β-cell selective toxins (45–46), while the partial IL-12 antagonist, vitamin D3, has diabetes-protective effects at least in rodents (47). However, a universal relevance of these elements to autoimmune diabetes in the population at large is uncertain.

Complex diets as a prerequisite for rodent diabetes

Diabetes-prone BB rats and NOD mice are models for the human disease, and while differences exist, the list of similarities is long and detailed (48–51).

Following the early observation that amino acid-based weaning diets protected BB rats from overt diabetes (1), numerous studies analyzed dietary disease prevention in rodents (reviewed by Scott et al. (11)]. Although diverse in design, focus and conclusions, there is one common thread in these studies: complex weaning diets, such as the multicomponent standard rodent chows, are actually a prerequisite for high-incidence diabetes development (2). In contrast, hypoantigenic protein sources, such as amino acids or extensively hydrolyzed casein, prevent the development of diabetes (11). Although there can no longer be dissention that weaning diets affect diabetes development, mechanisms remain uncertain and controversial (52).

Spurred by the observation that early weaning to cow's milk (CM)-based formula is a diabetes risk factor in humans (reviewed in ref. 32, 37) and by abnormal immunity of NOD mice to CM protein (53) attempts to identify specific proteins in CM as initiators of islet autoimmunity found considerable (54), intermediate (55), or little diabetogenicity (8, 52). However, none of these studies were able to mimic the human situation of early weaning to exclusively CM proteins, and high oral doses of the whey protein, bovine serum albumin (BSA), actually showed some protection (52).

Collectively, there is one consensus conclusion common to all these studies (5, 9, 12, 52): a very clear protection from diabetes is afforded by weaning to extensively hydrolyzed casein formulas, while a partially hydrolyzed protein diet rich in potentially antigenic peptides retains diabetogenicity (7).

Despite considerable differences in study design, not a single published experiment failed to result in disease protection with hydrolyzed casein as weaning diet in high- or low-incidence, BB rat or NOD mouse colonies. This became the basis and intervention strategy for TRIGR.

Does the weaning diet affect human diabetes?

There are many indications that the pathogenetic process leading to manifest T1D may start very early in life in some cases, even *in utero* (56, 57). In addition, during childhood the incidence of the disease has shifted toward younger age in several countries over the last decades (58), providing added impetus for studies of environmental factors, including complex weaning diets in the first years of life.

The first evidence in man for an association between weaning diet and the risk of T1D comes from a case–control study showing an inverse correlation between the duration of breastfeeding (BF) and diabetes risk (59). Numerous subsequent studies explored this relationship with various study designs, power, and focus on candidate offenders such as CM protein, often the sole protein source, in particular for very young weanlings. With two exceptions, all retrospective studies used general population controls genetically

resistant to diabetes, and few distinguished between exclusive BF and formula-supplemented feeding periods (60, 61). The study by Virtanen et al. showed that age at introduction of CM was inversely related to risk of diabetes independently of both exclusive and total BF (60). Meta-analyses confirmed the positive conclusions of the majority of studies with an overall significant but small risk attributed to early formula exposure (62, 63). However, conclusions have remained controversial (64), and the complexity of issues surrounding diet and T1D was compounded by large studies that attributed elevated diabetes risk to high liquid CM exposure later in the life of previously exposed children (65–67), a factor that may obscure pure weaning effects. The risk of T1D described in two case-control studies indicates that there might be an interaction between increased genetic disease susceptibility, based upon HLA class II allels, and an early introduction of supplementary milk feeding during infancy (68, 69). Disease risk may also be affected by the interaction between genetic susceptibility and a large intake of CM later in life (67).

Data from a prospective study of 1610 German children (70) concluded that there might be an association of T1D risk and early cereal exposure, but no association of CM exposure and T1D risk. The nature and extent of CM exposures were not reported, but ~50% of infants in Germany are weaned to hydrolyzed formula. A cohort study from Denver (71) analyzed 1183 children (0.7–7 yr old) with increased genetic susceptibility to T1D. They found no association between early CM exposure and β-cell autoantibodies. In this study, 259 children had early cereal exposure (before the age of 4 months) and 13 developed autoantibodies, whereas 105 had late exposure (at the age of 7 months or later) and 7 seroconverted to autoantibody positivity. In contrast to the German study, a significant association was observed for both early (odds ratio 3.03) and late exposure (odds ratio 3.86). The numbers of infants with early exposure to gluten ($n = 85$ with four seroconverters) were too small to draw any conclusions regarding gluten as a risk factor for β-cell autoimmunity. Early CM exposure (<3 months) showed a non-significant protection from autoimmune seroconversion. The likelihood of confounding is increased by using exposures at 4–6 months as the reference group since this time period was the recommended age for introduction of cereals. These studies and their partly discordant conclusions are difficult to evaluate because of a lack of statistical power and because the main autoantibodies detected (>80%) were insulin autoantibodies (IAA).

In a third prospective study from Australia, life-table analysis failed to detect an association between CM exposure and autoantibodies. Intriguingly, opposite conclusions can be drawn of very significant risk associations caused by short BF and early CM exposure, which are apparent from data tabulated in the same paper (72). These data show that formula was introduced at a mean age of 3.5 months in children who developed at least two autoantibodies, compared to 7 months in autoantibody-negative children ($p < 0.0001$).

In the prospective population-based Finnish Type 1 Diabetes Prediction and Prevention Study (73), newborn infants from the general population were tissue typed and those with risk HLA genotypes were recruited for a nested, prospective case–control study. The first 65 children who seroconverted to islet cell antibodies (ICA) positively before the age of 4 yr (cases) were compared with 390 ICA-negative control subjects, matched for date of birth, gender, and HLA-DQB1 genotype. The risk of IA-2 autoantibody positivity or positivity for all four autoantibodies measured (ICA, insulin, GAD and IA-2 autoantibodies) was lower in children exclusively breastfed for more than 4 months compared to those who were breastfed for 2 months or less [odds ratio 0.24, 95% confidence interval (CI) 0.06–0.94, for IA-2A positivity; odds ratio 0.17, 95% CI 0.03–0.86, for positivity for four antibodies]. Infants weaned to standard CM formula before the age of 4 months were at increased risk of IA-2A positivity (odds ratio 4.37, 95% CI 1.33–14.4, for <2 months vs. ≥4 months; odds ratio 5.50, 95% CI 1.21–25.0, for 2–3.9 months vs. ≥4 months) or positivity for all four autoantibodies (odds ratio 5.02, 95% CI 1.27–19.9, for <2 months vs. ≥4 months; odds ratio 6.19, 95% CI 1.10–34.7, for 2–3.9 months vs. ≥4 months). The associations remained significant after adjustment for maternal age, duration of maternal education and 12-month relative weight and height of the child. These observations suggest that short BF and early introduction of CM-based infant formula predispose young children at increased genetic risk for diabetes to the development of progressive β-cell autoimmunity.

These results were the impetus for a double-blind, randomized pilot trial of weaning either to a casein hydrolysate (Nutramigen®, Mead Johnson Nutritionals, Evansville, IL, USA) or to a conventional CM-based formula until the age of 6–8 months in 242 infants (74). The results indicated a substantial reduction in the cumulative incidence of β-cell autoimmunity with continued follow-up to the age of 5–7 yr. After adjustment for duration of study formula feeding, life-table analysis showed protection by the intervention from the development of ICA ($p = 0.02$) and one or more autoantibodies ($p = 0.03$). A similar trend was seen in the development of overt T1D (currently three vs. nine of those exposed to the study formula). Thus, despite its limited power, the pilot study provides the first evidence ever in man that it may be possible to

deviate spontaneous β-cell autoimmunity by dietary intervention in infancy. The current international TRIGR is powered to provide a definitive test of the hypothesis that weaning to a hydrolyzed formula protects against initiation and progression of T1D.

Study design (Fig. 1)

Recruitment

Extensive recruiting strategies focus on the identification of newborn infants with a first-degree relative who has T1D. In addition to major television, newspaper, magazine, and website publicities, mothers with T1D are identified during pregnancy via endocrinologists or high-risk pregnancy services. Fathers with T1D are identified by (i) available history or data already in the medical record of pregnant women; (ii) interviewing women at prenatal maternity clinic visits; and (iii) existing registries of T1D in some centers. Pregnant women with one or more children affected by diabetes are identified through various pediatric diabetes clinics. Consent is obtained primarily during pregnancy and rarely, in some countries, at delivery. Potentially eligible subjects meet protocol inclusion and exclusion criteria (Table 1) insofar as possible prior to the baby's delivery and are reassessed after birth.

Intervention

BF is encouraged. Babies are randomized to receive either a regular CM-based infant formula or an extensively hydrolyzed CM formula upon weaning from breast milk in the first 6–8 months of life. Other formulas and cow protein products are avoided.

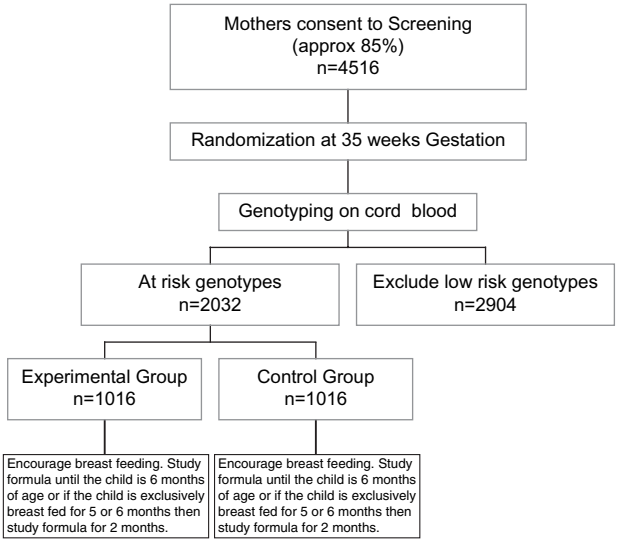

Fig. 1. TRIGR study design.

Study formula

Each study formula is a nutritionally complete infant formula (Nutramigen™ powder), manufactured by one company (Mead-Johnson Nutritionals), containing extensively hydrolyzed casein as the protein source, vegetable oils as the fat source, and glucose polymers and modified starch as the carbohydrate source. The control formula is a mixture of commercial routine CM-based formula powder made by the same company (Enfamil™) plus casein hydrolysate powder in a 4:1 ratio in order to mask the flavor and smell differences between the two study formulas. This casein hydrolysate has been shown to be non-diabetogenic in the BB rat (52) and NOD mouse models (12).

The formulas are packed in four different colors – two colors for test formula and two colors for control formula. The four-color coding scheme is well tested in infant formula studies, is found to aid the blinding process, and provides a hard control for randomization during data analysis, while avoiding accidental miss-shipments as the families recognize 'their' color. Subjects are allocated to receive one-color formula box, which is maintained throughout the study.

'Randomization' of the babies to one of the four-color coded test formulas stratified by geographic region is implemented after 35 wk of gestation or immediately after birth prior to the availability of HLA eligibility results. Randomization is assigned by the Data Management Unit (DMU) via the TRIGR website with the system available 24 h/d to the research assistant or investigator. This early randomization allows immediate use of study formula whenever BF is not available. Any subject requiring supplemental feeding in the nursery prior to randomization (e.g., unrandomized infants born at night or on weekends) is given either banked breast milk or casein hydrolysate.

'Eligibility' is determined by HLA-typing blood obtained from cord blood at birth whenever possible or alternatively by a heel prick performed within 7 d after birth. The blood sample and/or blood spot on filter paper is forwarded to the continental genotyping center, and screening is performed for the presence of the increased risk HLA genotypes listed below. Results are sent via the DMU to the central project office and the investigator within 5–7 d. Only subjects with the risk HLA genotypes (approximately 45%) are included in the nutritional prevention trial.

Implementation of intervention

All recruited mothers are encouraged to breastfeed. The planned duration of the intervention is until at least 6 months of age with the goal of a minimum of 2 months daily exposure to the study formula. If the mother chooses to exclusively breastfeed up to the age of 6 months, she will be asked, thereafter, to give the

Table 1. TRIGR inclusion and exclusion criteria

Newborn infants who have first-degree relatives with T1D (i.e., a mother, father, or sibling) and who meet the inclusion but not the exclusion criteria will be recruited:

Inclusion criteria
(i) The biological parent and/or full (not half) sibling of the newborn infant has T1D as defined by the World Health Organization.
(ii) The infant's parents or legal guardians give signed consent to participate.
(iii) The infant has one of the following genotypes:
(a) HLA-DQB1*0302/DQA1*05-DQB1*02;
(b) HLA-DQB1*0302/x (x not DQB1*02, DQB1*0301, or DQB1*0602);
(c) HLA-DQA1*05-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603);
(d) HLA-DQA1*03-DQB1*02/y (y not DQA1*0201-DQB1*02, DQB1*0301, DQB1*0602, or DQB1*0603).

Exclusion criteria
(i) An older sibling of the newborn infant has been included in the TRIGR intervention.
(ii) Multiple gestation.
(iii) The parents are unwilling or unable to feed the infant CM-based products for any reason (e.g., religious, cultural)
(iv) The newborn infant has a recognizable severe illness such as those caused by chromosomal abnormality, congenital malformation, respiratory failure needing assisted ventilation, enzyme deficiencies, etc.
(v) The gestational age of the newborn infant is less than 35 wk.
(vi) Inability of the family to take part in the study (e.g., the family has no access to any of the study centers, the family has no telephone).
(vii) The infant has received any infant formula other than Nutramigen prior to randomization.
(viii) The infant is older than 7 d at randomization (applicable to subjects randomized after birth).
(ix) No HLA sample drawn before the age of 8 d.

study formula for at least 2 months, i.e., until the age of 8 months. Similarly, if exclusive BF lasts for 5 months, the infant would receive study formula until the age of 7 months. Infant BF practices are altered as little as possible by the trial and is entirely at the discretion of participating mothers. The rationale for the 6–8 month intervention period is based on the following considerations: in early infancy, the child receives the major part of energy in liquid form, either as human milk or as CM-based formula. The period chosen provides a reasonable and practical safety margin over the 2- to 3-month 'vulnerable' time when the gut is permeable to proteins, as suggested in several epidemiological studies (reviewed in 32).

Dietary advice is provided by a dietitian member of the study team, before or soon after delivery; at the 2-wk telephone call to notify the HLA-typing result; at the 1-, 2-, 4-, and 5-month telephone calls; and at the 3- and 6-month visits. If the infant continues in the intervention after the age of 6 months, the family is called also at 7 and 8 months of age. The families receive both written and oral instructions about infant feeding during the intervention period including pamphlets, which describes the sequence and amounts of food recommended at specific ages, according to local guidelines. The use of soy-based infant formula is not encouraged because it could replace the study formula. Parents are provided with a list of all solid foods, giving choices of brand names that can be given to the infant and that do not contain CM protein; they are also provided with a list of foods not recommended which do contain CM protein. Dietary advice leaflets, field book, dietary forms, and questionnaires were translated into 11 relevant languages and adapted to national practices before the start of enrollment. Postintervention diets follow generally accepted practices. After randomization, Nutramigen is used in case of suspected or proved study formula intolerance.

Study assessments (Fig. 2)

Baseline

At the time of randomization, medical and perinatal history of the infant and mother (including birthweight and gestational age) and the results of the newborn physical examination are recorded on the case report forms.

Follow-up during and after intervention

The subjects have blood draws at the research center, or a satellite or home visit at the ages of 3, 6, 9, 12, 18, and 24 months and then annually until 10 yr of age or when clinical T1D develops. The presence or absence of diabetes is determined according to the criteria outlined (see *Outcome Assessment*). Clinical findings at each visit (e.g., weight, height) are recorded on the case report forms.

Blood samples for measurements of the β-cell autoantibodies are drawn (after the application of an analgesic cream at the venipuncture site) at each visit. In addition, the 3- and 6-month serum samples are used for measurements of CM antibodies for the assessment of dietary compliance. All serum samples

	0 mo *	½ mo Call	1 mo Call	2 mo Call	3 mo Visit	4 mo Call	5 mo Call	6 mo Visit	7 mo Call	8 mo Call	9 mo Visit	12 mo Visit	18 mo Visit	2 yr Visit	3 yr Visit	4 yr Visit	5 yr Visit	6 yr Visit	7 yr Visit	8 yr Visit	9 yr Visit	10 yr Visit
Contact number	1	2	3	4	5	6	7	8	8B	8C	9	10	11	12	13	14	15	16	17	18	19	20
Weight and height	X				X			X			X	X	X	X	X	X	X	X	X	X	X	X
Blood specimens																						
Cord blood	X																					
Venous blood					X			X			X	X	X	X	X	X	X	X	X	X	X	X
Blood glucose												X	X	X	X	X	X	X	X	X	X	X
HbA1c												X	X	X	X	X	X	X	X	X	X	X
Dietary interview	X	X	X	X	X	X	X	X	X**	X**	X											
Dietary counseling	X	X	X	X	X	X	X	X	X**	X**												
Delivery of study formula	X																					
When needed		X	X	X	X	X	X	X	X													
Examination by study doctor	X				X			X				X		X				X				X
OGTT																		X				X

* Contact number 1 will be performed in the delivery hospital, but in case of home deliveries, it will be done at home.

** For those who continue in dietary intervention

Fig. 2. Procedures at the time of birth and at each follow-up call and visit.

are aliquoted and stored centrally at −70°C. Measurement of plasma glucose and glycated hemoglobin is performed locally at 12, 18, and 24 months and then annually until 10 yr. The specimens for plasma glucose are preferentially drawn 1–2 h postprandially. If the glycated hemoglobin is higher than the normal range, an additional oral glucose tolerance test (OGTT) may be required to confirm the diagnosis. Oral glucose tolerance tests are performed in all subjects at 6 and 10 yr of age.

Monitoring of compliance and retention strategy

The diet of the child and the compliance with the avoidance of intact CM proteins are assessed at the delivery hospital by interview; by a telephone interview when the child is 2 wk and 1, 2, 4, and 5 months old; and at 3- and 6-month visits. If the infant continues in the intervention after the age of 6 months, telephone interviews are done also at 7 and 8 months of age. Dietary interviews record by means of a structured form information on the duration of total and exclusive BF, the age at introduction, duration of study formula feeding, and amount of study formula given per feeding. In addition, the families are asked about the frequency of BF and the frequency of use of study formula, several allowed foods (potato and vegetables, fruit and berries, cereals, meat, fish, dietary supplementation), and non-recommended foods and food groups (infant formulas other than the study formula, foods containing CM or beef protein). In addition, at the 2-wk interview, the feeding during the first 3 d is enquired: all types of milk feeding given and the most frequently used milk feeding. Study formula distribution form is completed always when formula is given to the family (amount and code of formula). Unused formula must be returned to the local study center at each appropriate clinical visit (6 and possibly 9 months) in order to get an estimate of total formula exposure. Compliance with the avoidance of intact CM proteins is also assessed by measuring CM protein antibody levels from sera at 3 and 6 months, and the results correlated with the information received by dietary forms.

Outcome assessment

The major outcome for the first phase will be the frequency of T1D associated autoantibodies and/or the development of clinical diabetes by the age of 6 yr. The outcome of the second phase will be the manifestation of diabetes by the age of 10 yr. The manifest diabetes outcome is assessed as the proportion of subjects in each group who develop T1D, as well as the age at diagnosis. These subjects will be classified as having T1D if they fulfill one of the following criteria:

(i) on daily insulin injections and at least one of the following:

(a) a history of ketoacidosis;

(b) at least two fasting plasma glucose levels ≥7.0 mmol/L;

(c) a random plasma glucose ≥11.1 mmol/L plus (b) or symptoms;

(d) two random plasma glucose levels >11.1 mmol/L;

(ii) a fasting plasma glucose ≥7.0 mmol/L and/or a plasma glucose of ≥11.1 mmol/L at 2 h after ingestion of 1.75 g of glucose/kg (to a maximum of 75 g) body weight [i.e., a diabetic oral glucose tolerance test according to the ADA/WHO criteria (75)]. A diabetic oral glucose tolerance has to be confirmed by a second test.

Laboratory methods

Genetic screening

The procedures used for HLA-DQ typing were specifically developed for screening relevant DQB1 and DQA1 alleles (76, 77). Ethylenediaminetetraacetic acid-treated cord blood is collected and a blood spot prepared on filter papers. Alternatively, capillary blood is taken directly on the filter paper. The filter papers are sent from the US and Canadian centers to the tissue-typing laboratory of Dr M. Trucco, University of Pittsburgh, PA, USA, and from the various European and other centers to the tissue-typing laboratory of Dr J. Ilonen, University of Turku, Finland. The results are electronically communicated to the DMU and, by them, sent by e-mail to the central project office and the hospital in question.

Autoantibodies

ICA, IAA, antibodies to the 65-kDa isoform of glutamic acid decarboxylase (GADA), and antibodies to the protein tyrosine phosphatase-related IA-2 molecule (IA-2A) are humoral markers of β-cell autoimmunity. All autoantibody analyses will be performed in the laboratory of Dr M. Knip (University of Helsinki).

ICA are analyzed with a standard immunofluorescence assay performed on sections of frozen human pancreas from a blood group O donor (78). Fluorescein-conjugated anti-human IgG (Sigma, St Louis, MO, USA) is used to detect ICA. All initially ICA-positive samples are retested to confirm antibody positivity. End-point dilution titers are identified, and the results are expressed in Juvenile Diabetes Foundation (JDF) units relative to an international reference standard (79). The detection limit is 2.5 JDF units. The sensitivity of this ICA assay was 100%, the specificity 98%, the validity 98%, and the consistency 98% in the most relevant international standardization round (80).

IAA are measured with a microassay, modified from that described by Williams et al. (81). The cut-off limit for IAA positivity [2.80 relative units (RU)] is based on the 99th percentile in 344 non-diabetic children. Samples with an initial IAA titer between the 97th and 99.5th percentiles are reanalyzed to verify the antibody status. The disease sensitivity of the IAA microassay was 44% and its specificity 98% in the CDC-sponsored Diabetes Autoantibody Standardization Program (DASP) Workshop in 2002.

GADA are detected in an immunoprecipitation radioligand assay (82). The cut-off limit for antibody positivity is set at the 99th percentile in 373 non-diabetic children and adolescents, i.e., 5.36 RU (corresponding to 20.90 WHO units/mL). All samples with an initial GADA level between the 97.5th and 99.5th percentiles are reanalyzed to verify the antibody status. This assay had a sensitivity of 82% and a specificity of 98% in the 2002 DASP Workshop.

IA-2A are detected in a similar radiobinding assay (83). A subject is considered IA-2A positive, if the serum antibody levels are equal to or exceed 0.77 RU (corresponding to 16.46 WHO units/mL), which represents the 99th percentile in 374 non-diabetic Finnish children and adolescents. Samples with an initial IA-2A level between the 97.5th and 99.5th percentiles are reanalyzed to verify the antibody status. The disease sensitivity of this assay was 66% and the specificity 100% in the 2002 DASP Workshop.

Antibodies to CM Proteins

CM antibodies (IgG, IgA, and IgM), β-lactoglobulin antibodies (IgG and IgA) (84), β-casein antibodies (IgG and IgA), and BSA antibodies (IgG and IgA) (85) are measured with modifications of the original enzyme-linked immunosorbent assay techniques by Dr E. Savilahti, Helsinki, Finland.

Biostatistical considerations

Randomization

This is a randomized double-blinded intervention study using an intention-to-treat statistical analysis to compare the incidence of predictive autoantibodies and clinical T1D in the two treatment groups. Recruitment is carried out during a 4-yr period in 6 major centers in the USA, in 18 centers in Canada, in 51 centers in 12 Europen countries, and 3 centers in New South Wales, Australia. To facilitate recruitment and to minimize any possibility of unintentional exposure to CM protein, every attempt is made to identify and randomize eligible families before the child is born. Written consent is obtained at this time; the child participates after birth if he/she meets the inclusion but not exclusion criteria. Families not

identified until just prior to the onset of maternal labor may be approached at that time.

The children are randomly assigned to the two treatment groups in a 1:1 ratio using randomly permuted blocks. Randomization is balanced within each participating center/country.

Sample size and power

The sample size estimate is based on experiences from family studies analyzing the occurrence of autoantibodies in siblings of children with T1D or in offspring of affected parents and progression to clinical disease in such young first-degree relatives. The data on the frequency of multiple ($\geq$2) autoantibodies by the age of 6 yr and the cumulative incidence of T1D by the age of 10 yr are based on 82 young siblings from the DiMe Study carrying increased genetic risk for T1D (86).

Nineteen out of 325 offspring (5.9%) with increased genetic risk according to the criteria used in the Pilot-2 trial tested positive for at least one autoantibody by the age of 2.4 yr in the German BABYDIAB study (87). Seventeen of these 19 subjects (89%) developed multiple autoantibodies during prospective observation. Assuming a constant increase in autoantibody frequency from ages 2 to 6 yr, the expected cumulative incidence of at least one antibody would be 14.8% and that of at least two antibodies 13.0% by the age of 6 yr, which is well within the 95% CI of the observed frequency of 20.7% of multiple autoantibodies in the young DiMe siblings.

The cumulative incidence of at least one autoantibody by the age of 4 yr was 10.6% in siblings and offspring of affected subjects in the DAISY study from Denver (88). Published data are not available, allowing an estimation of the frequency of at least one or two autoantibodies in subjects with increased genetic risk as defined in the Pilot-2 trial. Nevertheless, a constant increase in the frequency of at least one autoantibody from ages 4 to 6 yr would result in an autoantibody prevalence of 15.9% by the age of 6 yr in siblings and offspring irrespective of HLA-defined genetic risk. The data from the DiMe study were used for the power calculation as it includes the largest long-term follow-up of young relatives.

The projected sample size of 2032 infants to be randomized for the trial is based on the following assumptions:

(i) a confidence level of 95%;
(ii) a statistical power of 80%;
(iii) a reduction of 40% in the hazard rate of T1D in the intervention group;
(iv) a dropout rate of 20%;
(v) a frequency of 10% of exclusive BF up to the age of 6 months

These figures represent a conservative estimate because they are based on the lower 95% CI (7.6%) of the observed cumulative incidence of T1D by the age of 10 yr (15.4%) in young siblings with moderate (DQB1*0302/x) and slightly increased genetic risk (DQB1*02/y). In the Pilot-2 trial, the prevalence of the high-risk genotype (DQB1*02/0302) was about two times higher (absolute frequency 20%) among siblings of affected children than among offspring of affected parents.

To achieve that number, 4516 infants must be screened assuming a frequency of 45% of the genotypes conferring increased risk (see Fig. 1). The observed prevalence of risk genotypes was 46% among the 474 infants screened for the Pilot-2 trial.

Infants are to be recruited over a 4-yr period and the planned follow-up will be 6 yr after the last infant has been accrued for the antibody end-point and 10 yr after the last infant is accrued for the T1D end-point. Thus, all subjects will have at least 10 yr of follow-up.

Analysis plan

Two hypotheses will be tested. The first hypothesis is that the children in the group fed casein hydrolysate will have a decreased occurrence of diabetes-associated antibodies in comparison with the control group receiving conventional CM-based formula. The second hypothesis is that the group weaned to a casein hydrolysate formula has a reduced incidence of T1D.

The Kaplan–Meier method will be used to construct survival curves and the log-rank statistic to compare treatment arms with respect to time until the development of autoimmunity or type 1 diabetes mellitus (T1DM). Secondary analyses will be carried out using the proportional hazards regression model (89), including milk exposure as a risk factor and potential confounding factors as time-dependent covariates in the model. Baseline variables that will be used in stratified analyses include gender, HLA risk category, and other prognostic characteristics. If differences in baseline characteristics are observed, analyses will be conducted of the effects of the treatments on outcomes adjusting for the potential confounding effects of these baseline characteristics. If only a few differences in such baseline characteristics are identified, analyses will be conducted stratifying for these characteristics. If any more than a few baseline characteristics are identified because of small sample size, it will be necessary to use regression models to adjust the treatment comparison for the confounding effects of these characteristics.

A number of subgroup analyses are planned to help identify individuals more likely to benefit from, or to be harmed by, the treatment. Such subgroups might include gender, gender of the relative with T1DM, HLA risk

group, and other factors suspected to be associated with the event. Exploratory data derived through subgroup analyses will serve primarily to generate new hypotheses for subsequent testing, and conclusions drawn from subgroup hypotheses not explicitly stated before data analysis will have less credibility than those from hypotheses stated in the protocol.

Interim analysis

A Data and Safety Monitoring Board (DSMB) has been created by National Institutes of Health to monitor this trial. In addition to semiannual reviews of accrual and safety, the DSMB has planned two interim analyses to assess whether large treatment differences have emerged early or there is insufficient reason to expect that differences will occur. The analysis will use the approach to group sequential testing that has been presented by Lan and DeMets (90) for which neither the number of looks nor the increments between looks must be prespecified. Rather, Lan and DeMets only require specification of the rate at which the type I error, e.g., total alpha = 0.05, will be 'spent'. This procedure allows 'spending' a little of alpha at each interim analyses in such a way that, at the end of the study, the total type I error does not exceed, e.g., 0.05. An alpha or spending function is defined in terms of time. One alpha function generates boundaries that are nearly identical to the O'Brien-Fleming boundaries. Time can be thought of either as information time (the fraction of information available at any point in the study) or as calendar time (the portion of the study time already passed) (91). For ease of implementation, exposure time will be used (92). The fraction of exposure time will in general approximate the true fraction of information and, regardless of the underlying hazard functions, will provide a group sequential procedure with the desired alpha level. The boundaries will be two-sided (5%), symmetric O'Brien-Fleming-type boundaries as implemented by Lan and DeMets to minimize the likelihood of confronting a large critical value prematurely. The Lan–DeMets procedure will be formally applied to the primary analysis, which is most important in terms of leading to a decision to stop the trial prematurely because of significant differences.

Organization and administration

The trial is executed by a multinational consortium of clinical research groups. The international central coordinating center with the study principal investigator is located in Helsinki (Dr H.K. Åkerblom and his deputy, Dr M. Knip). The three major regional groups are (i) Europe and Australia, with a coordinating center in Helsinki; (ii) Canada with a coordinating center in London, ON, Canada (Dr J. Dupré); and (iii) the USA, with a coordinating center in Pittsburgh, PA (Dr D. Becker).

The essential laboratory functions required for TRIGR are conducted in three central internationally recognized study laboratories for determination of (i) diabetes-related autoantibodies (Dr M. Knip, Helsinki); (ii) compliance through measurements of CM antibodies (Dr E. Savilahti, Helsinki); and (iii) MHC typing for Europe (Dr J. Illonen, Turku) and for North America (Dr M. Trucco, Pittsburgh).

The study coordinating center is located in Tampa, FL (Dr J. Krischer). In the Helsinki center, Dr S.M. Virtanen is responsible for the follow-up and reporting of dietary compliance for the whole TRIGR based on data from Tampa center. Each region operates through its Executive Committee, which is a subcommittee of the Regional Steering Committee representing all clinical centers.

Significance

The data reviewed in the background section emphasize that diabetic autoimmunity in rodents has at least one early prerequisite for its development: exposure to complex diets. Thus, in inbred animals, living in conditions sheltered from many environmental influences and invariably breastfed, weaning diet is a critical initiator or, in the case of casein hydrolysate, preventer of progressive autoimmunity. Epidemiological data accumulated in humans are more difficult to interpret, but the results of our pilot studies emphasize the similarities. Weaning to casein hydrolysate affects the development of diabetic autoimmunity, in rodents and, apparently, in man. This strategy is the first to have such an impact in man. The TRIGR consortium reached a consensus that the TRIGR is feasible, promising, and necessary.

The consequences costs of T1D presenting in childhood are immense, not only economically and for the society but also with regard to human life. Microvascular complications develop in a considerable proportion of the patients affecting their quality of life. Those individuals manifesting T1D in childhood and adolescence may have a severalfold increased risk of macrovascular complications in adult life. It is therefore obvious that major benefits would result if we could prevent T1D even in a proportion of cases. The TRIGR constitutes the first ever primary prevention trial for T1D. In contrast to secondary prevention strategies that attempt to arrest the progression of established islet autoimmunity detected through screening, the innocuous TRIGR strategy could be applied to the general population with increased genetic risk from where some 90% of the new cases of T1D are derived.

Screening for TRIGR began on 1 May 2002, and the target enrollment was achieved by 1 September

2006. Through November 2006, the study has met or exceeded all of its planning parameters in terms of expected duration of exclusive BF (<4% at 6 months of age) and subject retention and follow-up (>95% at 3 yr). Interim results are regularly presented to a DSMB and formal analyses follow the plan outlined above. According to the study timetable, the 6-yr autoantibody results will be available in 2012 and the T1D results in 2016.

Acknowledgements

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Appendix. List of TRIGR investigators*

Administration	Last name	Initials	
International Coordinating Center, Helsinki, Finland	Åkerblom	HK	PI of the study
	Knip	M	Deputy PI
	Koski	K	Study monitor for Europe
	Koski	M	
	Merentie	K	
	Pajakkala	E	Study monitor for Europe
	Salonen	M	Study coordinator
	Terhonen	T	
	Virkkunen	S	
Data Management Unit, Tampa, FL, USA	Cuthbertson	D	
	Gainer	B	
	Krischer	JP	PI of the Data Management Unit
	Malloy	J	
	Nallamshetty	L	
	Shanker	L	
Canadian Coordinating Center, London, Ontario and Ottawa, Quebec	Dupré	J	Co-PI for Canada
	Lough	G	Coordinator for Canada
	Nielsen	D	
	Dosch	HM	
	Fraser	W	
	Lawson	ML	
	Mahon	JL	
	Taback	SP	
USA Coordinating Center, Pittsburgh, PA, and Seattle, WA	Becker	D	Co-PI for USA
	Franciscus	M	Coordinator for USA
	Nucci	A	
	Palmer	JP	
Nutritional Epidemiology Unit, Helsinki, Finland	Bärlund	S	
	Pekkala	M	
	Uusitalo	U	
	Vähätalo	L	
	Virtanen	SM	
Data Safety Monitoring Board	Arjas	E	
	Lernmark	Å	
	Mandrup-Poulsen	T	Chair
	Schmidt	B	
Scientific Officers of Funding Agencies	Akolkar	B	National Institute of Diabetes and Digestive and Kidney Diseases
	D'Amario	R	European Commission
	Eriksson	M	Juvenile Diabetes Research Foundation
	Grave	G	National Institute of Child Health and Human Development
	Jörgens	V	European Foundation for the Study of Diabetes
	Schmid	I	Canadian Institutes of Health Research
Countries / Study centers			
Australia / AUS01 – Children's Hospital at Westmead	Bongiorno	R	
	Fraser	G	2
	Howard	N	1
	Lloyd	M	

Appendix. *Continued*

Administration		Last name	Initials	
	AUS02 – John Hunter Children's Hospital, Newcastle	Crock	P	1
		Siech	K	2
		Wong See	D	
	AUS03 – Sydney Children's Hospital	Brown	C	
		Catteau	J	2
		Craig	M	1
		Johnston	A	
Canada	CAN01 – St Joseph's Health Care Centre, London	Bere	LJ	2
		Clarson	CL	1
		Mahon	JL	
		McManus	R	
		Renato	N	
	CAN02 – Children's and Women's Health Centre of British Columbia, Vancouver	Higo	D	2
		Kent	N	
		Kozak	S	
		Marshall	C	
		Metzger	D	
		Taylor	C	
		Thompson	D	1
		Tong	S	
	CAN03 – Alberta Children's Hospital, Calgary	Edwards	A	
		Lange	I	
		Mercer	J	
		Pacaud	D	
		Schwarz	W	2
		Stephure	D	1
		Swaby	C	
	CAN04 – Walter MacKenzie Health Sciences, Edmonton	Baergen-Fladager	S	
		Boer	J	
		Chatur	T	2
		Chick	C	
		Couch	B	
		Demianczuk	N	
		Girgis	R	1
		Marks	S	
	CAN05 – Health Sciences Centre, Winnipeg	Berard	L	
		Bloomfield	EAC	
		Catte	D	2
		Dean	HJ	
		Grant	L	
		Hamelin	K	
		LaForte	J	
		Murphy	L	
		Schneider	C	
		Sellers	EAC	
		Taback	SP	1
		Woo	V	
	CAN06 – Children's Hospital of Eastern Ontario and The Ottawa Hospital, Ottawa	Boland	A	
		Clark	HD	
		Cooper	T	
		Cormier	R	
		Frederick	C	
		Good	M	
		Gruslin	A	
		Karovitch	A	
		Keely	E	
		Lawson	ML	1
		Lough	G	2
		Malcolm	JC	
		O'shea	E	
		Rouatt	S	

Appendix. *Continued*

Administration	Last name	Initials	
	Tawagi	GF	
	Kane	D	
	Ranieri	M	
CAN07 – Toronto Mount Sinai Hospital/Hospital for Sick Children	Andrighetti	S	
	Aquilina	A	
	Arnold	G	
	Barrett	J	
	Bartley	C	
	Blumer	I	
	Brush	C	
	Daneman	D	
	Donat	D	
	Ehrlich	R	
	Feig	D	
	Gherson	B	
	Gottesman	I	
	Gysler	M	
	Karkanis	S	
	Kenshole	A	
	Knight	B	
	Lackie	E	
	Lewis	V	
	Martin	MJ	2
	Maxwell	C	
	McLeod	L	
	Oliver	G	
	Panchum	P	
	Sermer	M	1
	Shilletto	N	
	Simone	A	
	Skidmore	M	
	Turrini	T	
	Wong	S	
CAN08 – Centre Hospitalier Universitaire de Québec, Quebec	Allen	C	
	Bélanger	L	
	Bouchard	I	
	Chapdelaine	M	
	Ferland	S	2
	Frenette	L	
	Fraser	W	
	Garrido-Russo	M	
	Latulippe	D	
	Imbeault	J	
	Marc	I	
	Morin	V	1
	Olivier	G	
	Weisnagell	J	
CAN09 – Atlantic Health Sciences Corporation, Saint John	Dornan	J	
	Heath	K	2
	MacSween	MC	
	McGibbon	A	
	Ramsay	C	
	Sanderson	F	
	Sanderson	S	1
CAN10 – L'Hôpital Sainte-Justine, Montreal	Anctil	C	
	Benabdesselam	L	
	Bureau	N	
	Gonthier	M	
	Huot	C	1
	Legacé	M	
	Lortie	L	
	Roy	C	

Appendix. *Continued*

Administration		Last name	Initials	
		Thibeault	M	2
	CAN11 – Montreal Children's Hospital	Diatalevi	S	
		Gagné	V	
		Laforte	D	2
		Legault	L	1
		Perron	P	
	CAN12 – IWK Health Centre/Dalhousie, Halifax	Armson	A	
		Canning	P	
		Cummings	EA	1
		Hardie	R	2
		Ivanko	V	
		McLeod	L	
		McPhee	A	
		Mokashi	A	
		Morrison	L	
	CAN13 – Janeway Child Health Center, St John's	Bridger	T	1
		Crane	J	
		Crummell	C	2
		Curtis	JC	
		Dawson	C	
		Grant	M	
		Newhook	LA	
	CAN14 – Queen's University, Kingston	Begum-Hasan	J	
		Breen	A	2
		Chambers	B	
		Houlden	R	1
		Woods	M	
	CAN15 – Regina Qu'Appelle, Regina	Brown	S	2
		Carson	G	1
	CAN16 – Royal University Hospital, Saskatoon	Andreychuk	B	
		Martel	MC	
		Penner	M	2
		Sankaran	K	1
	CAN17 – Peterborough Regional Health Centre, Peterborough	Burnie	S	
		King	N	2
		White	A	1
	CAN18 – Victoria – Vancouver Island Health Research Centre	Park	M	
		Peters	D	
		Popkin	J	1
		Robson	L	2
Czech Republic	CZE01 – University Hospital Motol, Prague	Cerna	M	
		Cerny	M	
		Kandrnalova	J	
		Koukalova	R	
		Mendlova	P	2
		Sitova	R	
		Stechova	K	
		Vavrinec	J	1
		Zlatohlavkova	B	
	CZE02 – Hospital Milosrdnych Bratri, Brno	Brazdova	L	1
	CZE03 – University Hospital Olomouc	Gregorova	D	
		Kantor	L	
		Venhacova	J	1
	CZE04 – Hospital of Masryk	Cipra	A	
		Skvor	J	1
	CZE05 – Hospital Ceske Budejovice	Tomsikova	Z	1

Appendix. *Continued*

Administration		Last name	Initials	
	CZE06 – Faculty Hospital Plzen	Krauseova	H	1
		Mockova	A	
Estonia	EST01 – Tallinn Children's Hospital	Einberg	Ü	
		Jakovlev	Ü	
		Krassotkina	S	
		Rannaste	E	
		Raukas	R	
		Riikjärv	MA	1
	EST02 – Tartu University Children's Hospital	Astover	V	
		Kirss	A	
		Ormisson	A	1
		Retpap	J	
		Täht	E	
		Tillmann	V	
		Vahtra	S	2
Finland	FIN01 – Hospital for Children and Adolescents, University of Helsinki	Åkerblom	HK	1
		Heikkilä	M	
		Kleemola	P	
		Luopajärvi	K	
		Pigg	HM	
		Puttonen	H	
		Renlund	M	
		Suomalainen	H	
		Tenkula	T	2
		Teramo	K	
	FIN02 – Department of Obstetrics and Gynecology, University of Helsinki	Järvenpää	AL	1
	FIN03 – Jorvi Hospital	Hämäläinen	AM	1
	FIN04 – Kymenlaakso Central Hospital	Haavisto	H	1
		Holopainen	S	
		Kupiainen	H	
		Leeve	T	2
		Lumme	K	
		Nironen	T	
	FIN05 – Paijat-Hame Central Hospital	Keinonen	H	
		Lautala	P	1
		Vesanto	M	2
	FIN06 – Department of Pediatrics, Tampere University Hospital	Asunta	P	2
		Ikävalko	H	
		Knip	M	1
		Lähde	J	
		Mäkelä	M	
		Poutiainen	L	
		Salonsaari	T	
		Siljander	H	
	FIN07 – Satakunta Central Hospital	Haanpää	PL	2
		Holm	C	1
		Järveläinen	V	
		Laino	E	
		Marjamäki	L	
		Suominen	E	
		Ylitalo	S	
	FIN08 – Central Finland Central Hospital	Lounamaa	R	1
		Matikainen	M	
		Nuuja	A	
		Salo-Edwards	H	2
	FIN09 – South Ostrobotnia Central Hospital	Alanne	S	
		Kultti	T	2
		Linjama	H	
		Sauna-aho	M	

Appendix. *Continued*

Administration		Last name	Initials	
		Talvitie	T	1
	FIN10 – Hyvinkaa Hospital	Backman	M	
		Hanhijärvi	R	1
		Koivula	P	2
		Lindström	K	
		Martikainen	A	
		Nurmi	P	
	FIN11 – Department of Pediatrics, Kuopio University Hospital	Björk	A	2
		Komulainen	J	1
		Lehtomäki	S	
		Muikku	E	
		Pesola	J	
		Sankilampi	U	
		Tuovinen	M	
		Väätäinen	RL	
	FIN12 – Department of Pediatrics, Oulu University Hospital	Arkkola	T	
		Hekkala	A	
		Leinonen	E	
		Niittyvuopio	A	2
		Stenius	A	
		Tapanainen	P	1
	FIN13 – Kanta-Hame Central Hospital	Alar	A	
		Jovio	S	2
		Korpela	P	1
		Mäkinen	E	
	FIN14 – Vaasa Central Hospital	Hietanen	L	2
		Käär	ML	1
		Lehtimäki	P	
		Säätelä	S	
		Taittonen	L	
	FIN15 – South Carelian Central Hospital	Laaksonen	N	
		Luoto	M	2
		Virransalo	R	1
	FIN16 – Mikkeli Central Hospital	Nykänen	P	1
		Paajanen	S	2
		Parkkinen	S	
		Särkkä	T	
Germany	GER01 – Kinderkrankenhaus auf der Bult, Hanover	Aschemeier	B	2
		Bektas	S	
		Danne	T	1
		Kordonouri	O	
		Lüpke	K	
		Nestoris	C	
		Sadeghian	E	
		Semler	K	
	GER02 – Klinik Für Allgemeine Pädiatrie Charite, Berlin	Deiss	D	1
		Müller	B	2
Hungary	HUN01 – Semmelweis Medical University, Budapest	Arató	A	
		Krikovszky	D	2
		Madácsy	L	1
		Nobilis	A	
Italy	ITA01 – University Campus Bio-Medico of Rome	Beretta Anguissola	G	
		Bizzarri	C	
		Cherubini	V	
		Giordano	C	
		Khazrai	YM	2
		Napoli	A	
		Piergiovanni	F	

Appendix. *Continued*

Administration		Last name	Initials	
		Pitocco	D	
		Pozzilli	P	1
		Suraci	T	
		Valente	L	
		Visalli	N	
	SAR01 – St Michele Hospital, Cagliari	Carboni	MB	
		Casu	A	2
		Cavallo	R	
		Porceddu	M	
		Putzu	C	
		Songini	M	1
Luxembourg	LUX01 – Centre Hospitalier de Luxembourg	de Beaufort	C	1
		Peters	D	
		Schierloh	U	
The Netherlands	NET01 – Sophia Children's Hospital	Bisschoff	M	
		Blonk	L	
		Bruining	GJ	1
		Lappenschaar	T	2
		Manai	B	
		Seesink	M	
		Sperling-Conrad	M	
		Zoethout	JA	
Poland	POL01 – Medical University of Wroclaw	Basiak	A	2
		Chalas	M	
		Gramza	A	
		Iwankiewicz	J	
		Wasikowa	R	1
	POL02 – Polish-American Children's Hospital, Krakow	Ciechanowska	M	
		Dziatkowiak	H	1
		Górska	A	2
		Kaim	I	
		Tokarska	L	
		Wolanin	M	
	POL03 – Medical University of Silesia, Katowice	Chucherco	D	
		Deja	G	2
		Firek-Pedras	M	
		Jarosz-Chobot	P	1
		Kalina	M	
		Kutrowska-Adamusiak	I	
		Minkina-Pedras	M	
		Muchacka-Bianga	M	
	POL04 – Medical University of Lodz	Bodalski	J	1
		Mlynarski	W	
		Szadkowska	A	2
	POL05 – Polish Mother's Memorial Hospital (ICZMP), Lodz	Cieslak	A	
		Cypryk	K	2
		Dziatosz	K	
		Wilczyński	J	1
		Zawodniak-Szalapska	M	
Spain	SPA01 – Hospital de Cruces, Barakaldo	Aguayo	A	
		Bilbao	JR	
		Castano	L	1
		Chueca	M	
		Cortazar	A	
		Gonzalez	T	2
		Jimenez	P	
		Martul	P	
		Moreno	A	
		Oyarzabal	M	

Appendix. *Continued*

Administration		Last name	Initials	
		Rica	I	
	SPA02 – Hospital Clinico San Carlos, Madrid	Hawkins	FG	
		Herranz	L	
		Martínez-Larrad	MT	2
		Pallardo	LF	
		Saiz de Ibarra	L	
		Serrano-Ríos	M	1
	SPA03 – Hospital Gregorio Marañon, Madrid	Leon Luis	J	
		Ortíz Quintana	L	1
		Pintado Recarte	P	2
		Rodriguez Arnau	D	
Sweden	SWE01 – University of Linkoping, Linkoping	Bodén	S	
		Fredriksson	J	
		Isacsson	E	
		Lock	C	2
		Ludvigsson	J	1
		Sandström	AM	
	SWE02 – Uddevalla Hospital	Dahlström	U	
		Hanås	R	1
		Lundqvist	K	2
	SWE03 – The Queen Silvia Children's Hospital, Gothenburg	Lindbladh	B	1
		Odenman	I	
		Pettersson	C	
		Sundqvist	M	2
	SWE05 – Halmstad Hospital	Bellman	I	2
		Lydén	GB	
		Nilsson	NÖ	1
	SWE06 – Trollhattan Hospital	Bengtsson	M	2
		Fors	H	1
	SWE07 – Vrinnevi Hospital, Norrkoping	Andersson	AC	
		Hellgren	G	
		Nilsson	M	2
		Nordwall	M	1
	SWE08 – Boras Hospital	Lindh	A	1
		Samuelsson	C	2
		Wiik	A	
	SWE09 – Karlskrona Hospital	Edenwall	H	1
		Persson	IB	
		Strigard	E	2
		Svensson	BL	
	SWE10 – University Hospital Orebro	Åman	J	1
		Breivik	GE	
		Detlofsson	IL	
		Kroon	M	2
	SWE11 – Ryhovs Hospital, Jonkoping	Johansson	C	1
		Lundberg	A	2
Switzerland	SWT01 – University Children's Hospital, Zurich	Beccarelli	A	
		Rappold-Amrein	C	2
		Schoenle	E	1
USA	USA01 – Children's Hospital of Pittsburgh, Pittsburgh	Becker	D	1
		Daftary	A	
		Damagro-Elias	ME	
		Franciscus	M	2
		Gilmour	C	
		Lain	C	
		Salerno	D	
		Vats	K	

Appendix. *Continued*

Administration	Last name	Initials	
USA02 – Pugent Sound Health System, Seattle	Munns	M	2
	Palmer	JP	1
	Siemion	W	
	Taculad	R	
	Van Horn	K	
USA03 – St Louis, Washington University	Chambliss	C	
	Jones	J	
	Sadler	M	
	Tanner	M	2
	White	N	1
USA04 – Mattel Children's Hospital of UCLA, Los Angeles	Bell	C	
	Devaskar	S	
	Devaskar	U	1
	Horowitz	H	
	Rogers	L	2
	Silk	K	
USA05 – Ponce School of Medicine, Ponce	Bermudez	Z	
	Colon	R	2
	Frazer	T	
	Martinez-Nieves	B	
	Torres	J	1
	Vega	J	
USA06 – Naomie Berrie Diabetes Center, New York	Goland	R	1
	Greenberg	E	2
	Montes	J	
	Nelson	M	
	Schachner	H	
	Softness	B	
Laboratories			
HLA-Typing Laboratory, Turku, Finland	Ilonen	J	
	Suominen	R	
HLA-Typing Laboratory, Pittsburgh, PA, USA	Hyrckowian	E	
	Nichol	L	
	Stefura	C	
	Trucco	M	
Cow's Milk Antibody Laboratory, Helsinki, Finland	Karjalainen	E	
	Louhio	T	
	Sarnesto	A	
	Savilahti	E	
	Valtonen	E	
Autoantibody Laboratory, Helsinki, Finland	Davydova	B	
	Härkönen	T	
	Joutsjoki	L	
	Knip	M	
	Latva-Koivisto	M	
	Puro	H	
T-Cell Laboratory, Helsinki, Finland	Cederlöf	A	
	Kiikeri	M	
	Tsupari	S	
	Vaarala	O	
T-Cell Laboratory, Toronto, Ontario, Canada	Cheung	R	
	Dosch	HM	

*1, local investigator; 2, local coordinator; PI, principal investigator.