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

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 hypo-antigenic 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 dissent 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 alleles, 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 exposure 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.

**Study formula**

Each study formula is a nutritionally complete infant formula (Nutramigen™ powder), manufactured by one company (Mead-Johnson Nutritional), 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
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 auto-antibodies 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
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 fasting 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). Ethylenediaminetetraacetate 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.

**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 European 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
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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 (≥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. Akerblom 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. Duprê); 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
The TRIGR Study Group

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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References

1. Elliott RB, Martin JM. Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? Diabetologia 1984: 26: 297–299.
2. Atkinson MA, Winter WE, Skordis N, Beppu H, Riley WM, Maclaren NK. Dietary protein restriction reduces the frequency and delays the onset of insulin dependent diabetes in BB rats. Autoimmunity 1988: 2: 11–19.
3. Elliott RB, Reddy SN, Bibby NJ, Kida K. Dietary prevention of diabetes in the non-obese diabetic mouse. Diabetologia 1988: 31: 62–64.
4. Scott FW, Daneman D, Martin JM. Evidence for a critical role of diet in the development of insulin-dependent diabetes mellitus. Diabetes Res 1988: 7: 153–157.
5. Coleman DL, Kuzava JE, Letter EH. Effect of diet on incidence of diabetes in nonobese diabetic mice. Diabetes 1990: 39: 432–436.
6. Hoofar J, Scott F, Cloutier HE. Dietary plant materials and development of diabetes in the BB rat. J Nutr 1991: 121: 908–916.
7. Hoofar J, Buschard K, Dagnaes-Hansen F. Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice. Br J Nutr 1993: 69: 597–607.
8. Hermitt L, Atlan-Gepner C, Payan MJ, Meheller M, Viallettes B. Dietary protection against diabetes in NOD mice: lack of a major change in the immune system. Diabetes Metab 1995: 21: 261–268.
9. Li XB, Scott FW, Park YH, Yoon JW. Low incidence of autoimmune type 1 diabetes in BB rats fed a hydrolysed casein-based diet associated with early inhibition of non-macrophage-dependent hyperexpression of MHC class I molecules on beta cells. Diabetologia 1995: 38: 1138–1147.
10. Reddy S, Bibby NJ, Wu D, Swinney C, Barrow G, Elliott RB. A combined casein-free-nicotinamide diet prevents diabetes in the NOD mouse with minimum insulitis. Diabetes Res Clin Pract 1995: 29: 83–92.
11. Scott FW, Cloutier HE, Kleemann R et al. Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats: dose, timing, early effect on islet area, and switch in infiltrate from Th1 to Th2 cells. Diabetes 1997: 46: 589–598.
12. Karges W, Hammond-Mckibben D, Cheung RK et al. Immunological aspects of nutritional diabetes prevention in NOD mice. A pilot study for the cow’s milk-based IDDM prevention trial. Diabetes 1997: 46: 557–564.
13. Diabetes Prevention Trial-Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002: 346: 1685–1691.
14. The European Nicotinamide Diabetes Intervention Trial (ENDIT) Group (Including Knip M). Intervening before the onset of type 1 diabetes: results of the European Nicotinamide Diabetes Intervention Trial (ENDIT). Lancet 2004: 363: 925–931.
15. Diabetes Prevention Trial-Type 1 Diabetes Study Group. Effects of oral insulin in relatives of patients with type 1 diabetes. Diabetes Care 2005: 28: 1068–1076.
16. The EURODIAB ACE Study Group and the EURODIAB ACE Substudy 2 Study Group. Familial risk of type 1 diabetes in European children. Diabetologia 1998: 41: 1151–1156.
17. Thomson G, Robinson WP, Kuhnner MK et al. Genetic heterogeneity, modes of inheritance, and risk estimates for a joint study of Caucasians with insulin-dependent diabetes mellitus. Am J Hum Gen 1988: 43: 799–816.
18. Barnett AH, Eff C, Leslie RDG, Pyke DA. Diabetes in identical twins. Diabetologia 1981: 20: 87–93.
19. Kaprio J, Tuomilehto J, Koskenvuo M et al. Concordance for type-1 (insulin-dependent) and type-2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 1992: 35: 1060–1067.
20. Johnston C, Pyke DA, Cudworth AG, Wolf E. HLA-DR typing in identical twins with insulin-dependent diabetes: difference between concordant and discordant pairs. Br Med J 1983: 286: 253–255.
21. Warram JH, Krolevs AS, Gottlieb MS, Kahn CR. Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. N Engl J Med 1984: 311: 149–152.
22. Tinil H, Koberling J, Age-corrected empirical genetic risk estimates for first-degree relatives of IDDM patients. Diabetes 1987: 36: 93–99.
23. Tuomilehto J, Lounamaa R, Tuomilehto-Wolf E et al. The Childhood Diabetes in Finland (DiMe) Study Group: epidemiology of childhood diabetes mellitus in Finland – background of a nationwide study of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1992: 35: 70–76.
24. Thorsby E, Ronningen KS. Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type-1 (insulin-dependent) diabetes-mellitus. Diabetologia 1993: 36: 371–377.
25. She JX. Susceptibility to type I diabetes: HLA-DQ and DR revisited. Immunol Today 1996: 17: 323–329.
26. Becker KG, Simon RM, Bailey-Wilson JE et al. Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. Proc Natl Acad Sci U S A 1998: 95: 9979–9984.
27. Becker KG. Comparative genetics of type 1 diabetes and autoimmune disease. Common loci, common pathways? Diabetes 1999: 48: 1353–1358.
67. Virtanen SM, Lääра E, Hyppönen E et al. Cow’s milk consumption, HLA-DQB1 genotype, and type 1 diabetes. Diabetes 2000: 49: 912–917.
68. Kostraba JN, Cruickshanks KJ, Lawler-Heavner J et al. Early exposure to cow’s milk, and solid foods in infancy, genetic predisposition and risk of IDDM. Diabetes 1993: 42: 288–294.
69. Perez-Brafo F, Carrasco E, Gutierrez-Lopez MD, Martinez MT, Lopez G, Garcia de los Rios M. Genetic predisposition and environmental factors leading to the development of insulin-dependent diabetes mellitus in Chilean children. J Mol Med 1996: 74: 105–109.
70. Ziegler A-G, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA 2003: 290: 1721–1728.
71. Norris JM, Barriga K, Klingensmith G et al. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. JAMA 2003: 290: 1713–1720.
72. Cooper JJ, Steele C, Beresford S et al. Lack of association between duration of breast-feeding or introduction of cow’s milk and development of islet autoimmunity. Diabetes 1999: 48: 2145–2149.
73. Kimpimaki T, Erkkola M, Korhonen S et al. Short exclusive breast-feeding predisposes young children with increased genetic risk of type 1 diabetes to progressive beta-cell autoimmunity. Diabetologia 2001: 44: 63–69.
74. Akerblom HK, Virtanen SM, Ilonen J et al. Dietary manipulation of beta cell autoimmunity in infants at increased risk of type 1 diabetes: a pilot study. Diabetologia 2005: 48: 829–837.
75. American Diabetes Association Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997: 20: 1183–1197.
76. Ringquist S, Alexander AM, Rudert WA, Styche A, Trucco M. Pyrosequence-based typing of alleles of the HLA-DQB1 gene. Biotechniques 2002: 33: 166–170, 172, 174–175.
77. Ringquist S, Alexander AM, Styche A, Pecoraro C, Rudert WA, Trucco M. HLA class II DRB high resolution genotyping by pyrosequencing: comparison of group specific PCR and pyrosequencing primers. Hum Immunol 2004: 65: 163–174.
78. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974: 2: 1279–1282.
79. Lernmark A, Molenaar JL, van Beers WA et al. The fourth international serum exchange workshop to standardize cytoplasmic islet cell antibodies. Diabetologia 1991: 34: 534–535.
80. Greenbaum CJ, Palmer JP, Nagataki S et al. Improved specificity of ICA assays in the fourth international immunology of diabetes serum exchange workshop. Diabetes 1992: 41: 1570–1574.
81. Williams AJK, Bingley PJ, Bonifacio E, Palmer JP, Gale EAM. A novel micro-assay for insulin autoantibodies. J Autoimmun 1997: 10: 473–478.
82. Savola K, Sabbah E, Kulkula P, Vahasalo P, Ilonen J, Knip M. Autoantibodies associated with type 1 diabetes mellitus persist after diagnosis in children. Diabetologia 1998: 41: 1293–1297.
83. Savola K, Bonifacio E, Sabbah E et al. IA-2 antibodies – a sensitive marker of IDDM with clinical onset in childhood and adolescence. Diabetologia 1998: 41: 424–429.
84. Savilahit E, Saukkonen TT, Virtala ET, Tuomilehto J, Akerblom HK. Increased Levels Of Cow’s milk and beta-lactoglobulin antibodies in young children with newly diagnosed IDDM. The Childhood Diabetes in Finland Study Group. Diabetes Care 1993: 16: 984–989.
85. Saukkonen T, Savilahit E, Vaarala O, Virtala ET, Tuomilehto J, Akerblom HK. Children with newly diagnosed IDDM have increased levels of antibodies to bovine serum albumin but not to ovalbumin. Childhood Diabetes in Finland Study Group. Diabetes Care 1994: 17: 970–976.
86. Kimpimaki T, Kulmala P, Savola K et al. Disease-associated autoantibodies as surrogate markers of type 1 diabetes in young children at increased genetic risk. J Clin Endocrinol Metab 2000: 85: 1126–1132.
87. Schenker M, Hummel M, Ferber K et al. Early expression and high prevalence of islet autoantibodies for DR3/DR4 heterozygous and DR4/4 homozygous offspring of parents with type I diabetes: the German BABYDIAB study. Diabetologia 1999: 42: 671–677.
88. Rewers M, Eisenbarth GS, Elsey C et al. Target population for prevention trials of beta-cell autoimmunity in early childhood. Diabetologia 1998: 31(Suppl.1): A86 (Abstract).
89. kalbfleish JD, Prentice RL. The statistical analysis of failure time data. New York: Wiley, 1980.
90. Lan KK, Demets DL. Changing frequency of interim analysis in sequential monitoring. Biometrics 1989: 45: 1017–1020.
91. Lan KK, Demets DL, Halperin M. More flexible sequential and non-sequential designs in long-term clinical trials. Commun Stat Ser A 1984: 13: 2339–2353.
92. Lan KK, Lachin JM. Implementation of group sequential log-rank tests in a maximum duration trial. Biometrics 1990: 46: 759–770.
## Appendix: List of TRIGR investigators*

| Administration                                      | Last name | Initials | Role                                      |
|-----------------------------------------------------|-----------|----------|-------------------------------------------|
| **International Coordinating Center, Helsinki, Finland** | Åkerblom   | HK       | PI of the study                           |
|                                                      | Knip      | M        | Deputy PI                                 |
|                                                      | Koski     | K        | Study monitor for Europe                  |
|                                                      | Koski     | M        | Study monitor for Europe                  |
|                                                      | 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        | Chair                                     |
|                                                      | Lernmark  | A        |                                           |
|                                                      | 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 |
|                                                      | Schmidt   | I        | Canadian Institutes of Health Research    |
| **Countries**                                       | Study centers |       |                                           |
| Australia                                           | AU01 – Children’s Hospital at Westmead |       |                                           |
|                                                     | Bongiorno | R        |                                           |
|                                                     | Fraser    | G        | 2                                         |
|                                                     | Howard    | N        | 1                                         |
|                                                     | Lloyd     | M        |                                           |

*(The full list of investigators is provided in Appendix 5 of the original paper.)*
| 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                         | Bere     | LJ 2    |
| CAN01 – St Joseph's Health Care Centre, London | 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       |
| Administration | Last name | Initials |
|----------------|-----------|----------|
| CAN07 – Toronto Mount Sinai Hospital/Hospital for Sick Children | Tawagi | GF |
| | Kane | D |
| | Ranieri | M |
| | 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 |
| | Kensonole | A |
| | Knight | B |
| | Lackie | E |
| | Lewis | V |
| | Martin | MJ |
| | Maxwell | C |
| | McLeod | L |
| | Oliver | G |
| | Panchum | P |
| | Sermer | M |
| | Shilietto | 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 |
| | Frenette | L |
| | Fraser | W |
| | Garrido-Russo | M |
| | Latulippe | D |
| | Imbeault | J |
| | Marc | I |
| | Morin | V |
| | Olivier | G |
| | Weisnagell | J |
| CAN09 – Atlantic Health Sciences Corporation, Saint John | Dornan | J |
| | Heath | K |
| | MacSween | MC |
| | McGibbon | A |
| | Ramsay | C |
| | Sanderson | F |
| | Sanderson | S |
| CAN10 – L’Hôpital Sainte-Justine, Montreal | Anctil | C |
| | Benabdesselam | L |
| | Bureau | N |
| | Gonthier | M |
| | Huot | C |
| | Legacé | M |
| | Lortie | L |
| | Roy | C |
Appendix. Continued

| Administration                  | Last name | Initials |
|---------------------------------|-----------|----------|
| **CAN11 – Montreal Children’s Hospital** | Thibeault | M 2      |
| 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         |
| Lokashi                          | 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**               | Cerna     | M         |
| Cerny                            | M         |
| Kandralova                       | J         |
| Koukalova                        | R         |
| Mendlova                         | P 2       |
| Sitova                           | R         |
| Stechova                         | K         |
| Vavrinec                         | J 1       |
| Zlatohtlavkova                   | 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      |
| Administration | Last name | Initials |
|----------------|-----------|----------|
| Estonia        | CZE06 – Faculty Hospital Plzen | Krauseova | H 1 |
| EST01 – Tallinn Children’s Hospital | Mockova | A |
|                | Einberg | U |
|                | Jakovlev | U |
|                | Krassotkina | S |
|                | Rannaste | E |
|                | Raukas | R |
|                | Riikjärvi | MA 1 |
| EST02 – Tartu University Children’s Hospital | Astover | V |
|                | Kirss | A |
|                | Ormisson | A 1 |
|                | Retpap | J |
|                | Taht | E |
|                | Tillmann | V |
|                | Vahtra | S 2 |
| Finland        | FIN01 – Hospital for Children and Adolescents, University of Helsinki | Äkerblom | HK 1 |
|                | Heikkilä | M |
|                | Kleemola | P |
|                | Luoppajärvi | K |
|                | Pigg | HM |
|                | Puttonen | H |
|                | Renlund | M |
|                | Suomalainen | H |
|                | Tenkula | T 2 |
|                | Teramo | K |
|                | Järvenpää | AL 1 |
| FIN02 – Department of Obstetrics and Gynecology, University of Helsinki | Hämäläinen | AM 1 |
| FIN03 – Jorvi Hospital | Haavisto | H 1 |
| FIN04 – Kymenlaakso Central Hospital | 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 E |
|                | Laino | L E |
|                | Marjamäki | L E |
|                | Suominen | S |
|                | Ylitalo | S |
| FIN08 – Central Finland Central Hospital | Lounamaa | R 1 |
|                | Matikainen | M |
|                | Nuuja | A |
|                | Salo-Edwards | H 2 |
|                | Alanne | S |
| FIN09 – South Ostrobotnia Central Hospital | Kultti | T 2 |
|                | Linjama | H |
|                | Sauna-aho | M |
| Administration                      | Last name         | Initials |
|-------------------------------------|-------------------|----------|
| FIN10 – Hyvinkaa Hospital           | Talvitie          | T 1      |
| FIN11 – Department of Pediatrics,  | Backman           | M 1      |
| Kuopio University Hospital          | Hanhijärvi        | R 2      |
| FIN11 – Department of Pediatrics,   | Koivula           | P 2      |
| Oulu University Hospital            | Lindström         | K 1      |
| FIN11 – Department of Pediatrics,   | Martikainen       | A 1      |
| Kuopio University Hospital          | Nurmi             | P 1      |
| FIN12 – Department of Pediatrics,   | Björk             | A 2      |
| Oulu University Hospital            | Komulainen        | J 1      |
| FIN12 – Department of Pediatrics,   | Lehtomäki         | S 1      |
| Kuopio University Hospital          | Muikku            | E 1      |
| FIN12 – Department of Pediatrics,   | Pesola            | J 1      |
| Oulu University Hospital            | Sankilampi        | U 1      |
| FIN12 – Department of Pediatrics,   | Tuovinen          | M 1      |
| Oulu University Hospital            | Väätäinen         | RL 1     |
| FIN13 – Kanta-Hame Central Hospital | Alar              | A 2      |
| FIN13 – Kanta-Hame Central Hospital | Jovio             | S 2      |
| FIN13 – Kanta-Hame Central Hospital | Korpela           | P 1      |
| FIN13 – Kanta-Hame Central Hospital | Mäkinen           | E 1      |
| FIN14 – Vaasa Central Hospital      | Hietanen          | L 2      |
| FIN14 – Vaasa Central Hospital      | Kääär             | ML 1     |
| FIN15 – South Carelian Central     | Laaksonen         | N 1      |
| FIN15 – South Carelian Central     | Luoto             | M 2      |
| Hospital                             | Virransalo        | R 1      |
| FIN16 – Mikkeli Central Hospital    | Nykänen           | P 1      |
| FIN16 – Mikkeli Central Hospital    | Pääajanen         | S 2      |
| FIN16 – Mikkeli Central Hospital    | Parkkinen         | S 1      |
| FIN16 – Mikkeli Central Hospital    | Särkkä            | T 1      |
| Germany                              | Aschemeier        | B 2      |
| GER01 – Kinderkrankenhaus auf der   | Bektas            | S 1      |
| Bult, Hanover                        | Danne             | T 1      |
| GER02 – Klinik Für Allgemeine       | Kordonouri        | O 1      |
| Pädiatrie Charité, Berlin           | Lüptke            | K 1      |
| GER02 – Klinik Für Allgemeine       | Nestoris          | C 1      |
| Pädiatrie Charité, Berlin           | Sadeghian         | E 1      |
| GER02 – Klinik Für Allgemeine       | Semler            | K 1      |
| Pädiatrie Charité, Berlin           | Deiss             | D 1      |
| GER02 – Klinik Für Allgemeine       | Müller            | B 2      |
| Pädiatrie Charité, Berlin           | Arató             | A 1      |
| Hungary                              | Krikovszky        | D 2      |
| HUN01 – Semmelweis Medical University, Budapest | Madácsy | L 1 |
| Hungary                              | Nobilis           | A 1      |
| ITA01 – University Campus Bio-Medico of Rome | Beretta Anguissola | G 1 |
| Italy                                | Bizzarri          | C 1      |
| ITA01 – University Campus Bio-Medico of Rome | Cherubini | V 1 |
| Italy                                | Giordano          | C 1      |
| ITA01 – University Campus Bio-Medico of Rome | Khazraï | YM 2 |
| Italy                                | Napoli            | A 1      |
| ITA01 – University Campus Bio-Medico of Rome | Piergiorgianni | F 1 |
| Administration                  | Last name      | Initials |
|---------------------------------|----------------|----------|
| SAR01 – St Michele Hospital, Cagliari | Pitocco        | D        |
|                                 | Pozzilli       | P        |
|                                 | Suraci         | T        |
|                                 | Valente        | L        |
|                                 | Visalli        | N        |
|                                 | Carboni        | MB       |
|                                 | Casu           | A        |
|                                 | Cavallo        | R        |
|                                 | Porceddu       | M        |
|                                 | Putzu          | C        |
|                                 | Songini        | M        |
| Luxembourg                      | de Beaufort    | C        |
|                                 | Peters         | D        |
|                                 | Schierloh      | U        |
| The Netherlands                 | Bisschoff      | M        |
|                                 | Blonk          | L        |
|                                 | Bruining       | GJ       |
|                                 | Lappenschaar   | T        |
|                                 | Manai          | B        |
|                                 | Seesink        | M        |
|                                 | Sperling-Conrad| M        |
|                                 | Zoethout       | JA       |
| Poland                          | Basiak         | A        |
|                                 | Chalas         | M        |
|                                 | Gramza         | A        |
|                                 | Iwankiewicz    | J        |
|                                 | Wasikowa       | R        |
|                                 | Ciechanowska   | M        |
|                                 | Dziatkowiak    | H        |
|                                 | Gorska         | A        |
|                                 | Kaim           | I        |
|                                 | Tokarska       | L        |
|                                 | Wolanin        | M        |
|                                 | Chucherco      | D        |
|                                 | Deja           | G        |
|                                 | Firek-Pedras   | M        |
|                                 | Jarosz-Chobot  | P        |
|                                 | Kalina         | M        |
|                                 | Kutrowska-Adamusiak | I     |
|                                 | Minkina-Pedras | M        |
|                                 | Muchacka-Bianga| M        |
|                                 | Bodalski       | J        |
|                                 | Mlynarski      | W        |
|                                 | Szadkowska     | A        |
|                                 | Cieslak        | A        |
|                                 | Cypryk         | K        |
|                                 | Dziatosz       | K        |
|                                 | Wilczyński     | J        |
|                                 | Zawodniak-S zalapska | M   |
|                                 | Aguayo         | A        |
|                                 | Bilbao         | JR       |
|                                 | Castano        | L        |
|                                 | Chueca         | M        |
|                                 | Cortazar       | A        |
|                                 | Gonzalez       | T        |
|                                 | Jimenez        | P        |
|                                 | Martul         | P        |
|                                 | Moreno         | A        |
|                                 | Oyarzabal      | M        |
### Administration

| Administration | Last name | Initials |
|----------------|-----------|----------|
| SPA02 – Hospital Clinico San Carlos, Madrid | Rica | I |
| SPA03 – Hospital Gregorio Marañon, Madrid | Leon Luis | J |
| SPA03 – Hospital Gregorio Marañon, Madrid | Ortiz Quintana | L |
| SPA03 – Hospital Gregorio Marañon, Madrid | Pintado Recarte | P |
| SPA03 – Hospital Gregorio Marañon, Madrid | Rodriguez Arnau | D |
| Sweden | SWE01 – University of Linkoping, Linkoping | Bodén | S |
| Sweden | SWE01 – University of Linkoping, Linkoping | Fredriksson | J |
| Sweden | SWE01 – University of Linkoping, Linkoping | Isacsson | E |
| Sweden | SWE01 – University of Linkoping, Linkoping | Lock | C |
| Sweden | SWE01 – University of Linkoping, Linkoping | Ludvigsson | J |
| Sweden | SWE01 – University of Linkoping, Linkoping | Sandström | AM |
| Sweden | SWE02 – Uddevalla Hospital | Dahlström | U |
| Sweden | SWE02 – Uddevalla Hospital | Hanås | R |
| Sweden | SWE02 – Uddevalla Hospital | Lundqvist | K |
| Sweden | SWE03 – The Queen Silvia Children's Hospital, Gothenburg | Lindbladh | B |
| Sweden | SWE03 – The Queen Silvia Children's Hospital, Gothenburg | Odenman | I |
| Sweden | SWE03 – The Queen Silvia Children's Hospital, Gothenburg | Pettersson | C |
| Sweden | SWE03 – The Queen Silvia Children's Hospital, Gothenburg | Sundqvist | M |
| Sweden | SWE05 – Halmstad Hospital | Bellman | I |
| Sweden | SWE05 – Halmstad Hospital | Lydén | GB |
| Sweden | SWE05 – Halmstad Hospital | Nilsson | NO |
| Sweden | SWE06 – Trollhattan Hospital | Bengtsson | M |
| Sweden | SWE06 – Trollhattan Hospital | Fors | H |
| Sweden | SWE07 – Vrinnevi Hospital, Norrkoping | Andersson | AC |
| Sweden | SWE07 – Vrinnevi Hospital, Norrkoping | Hellgren | G |
| Sweden | SWE07 – Vrinnevi Hospital, Norrkoping | Nilsson | M |
| Sweden | SWE07 – Vrinnevi Hospital, Norrkoping | Nordwall | M |
| Sweden | SWE08 – Boras Hospital | Lindh | A |
| Sweden | SWE08 – Boras Hospital | Samuelsson | C |
| Sweden | SWE09 – Karlskrona Hospital | Wiik | A |
| Sweden | SWE10 – University Hospital Orebro | Åman | J |
| Switzerland | SWT01 – University Children's Hospital, Zurich | Beccarelli | A |
| Switzerland | SWT01 – University Children's Hospital, Zurich | Damagro-Elias | ME |
| Switzerland | SWT01 – University Children's Hospital, Zurich | Franciscus | M |
| Switzerland | SWT01 – University Children's Hospital, Zurich | Gilmour | C |
| Switzerland | SWT01 – University Children's Hospital, Zurich | Lain | C |
| Switzerland | SWT01 – University Children's Hospital, Zurich | Salerno | D |
| Switzerland | SWT01 – University Children's Hospital, Zurich | Vats | K |

The TRIGR Study Group

Appendix. Continued

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| Administration                                      | Last name  | Initials |
|-----------------------------------------------------|------------|----------|
| USA02 – Pugent Sound Health System, Seattle         | Munns M    | 2        |
|                                                     | Palmer JP   | 1        |
|                                                     | Siernion W  |          |
|                                                     | Taculad R   |          |
|                                                     | Van Horn K  |          |
| USA03 – St Louis, Washington University             | Chambliess 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    |          |
|                                                     | Martiinez-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                                        | Ilonen J    |          |
| HLA-Typing Laboratory, Turku, Finland               | 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  |          |
|                                                     | Härkönien T |          |
|                                                     | Joutsjoki L |          |
|                                                     | Knip M      |          |
|                                                     | Latva-Koivist 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.