Is the increased prevalence of autoimmunity in Downs syndrome related to early infant feeding practice - a potential BRU study

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Incidence One of the most common genetic disorders, affecting 1 in 650-1,000.

Risk factors
Family history.
Age of mother:
  1:385 risk at 35 years
  1:106 at 40 years
  1:30 at 45 years
Autoimmunity and Down’s Syndrome

Increased risk of autoimmunity:

- Thyroid autoantibodies (~33%)
- Coeliac associated autoantibodies (10.3%)
- Type 1 Diabetes (?)
Figure 1

Age at diagnosis of diabetes in children with Down's syndrome (n=103)

Age at diagnosis of T1D in children from the general population (n=1822)
<table>
<thead>
<tr>
<th>Marker Combinations</th>
<th>Down’s Syndrome Children (106)</th>
<th>Healthy Schoolchildren (2860)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No markers</td>
<td>90 (84.9%)</td>
<td>2667 (93.3%)</td>
</tr>
<tr>
<td>Single Marker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GADA</td>
<td>2 (1.9%)</td>
<td>59 (2.0%)</td>
</tr>
<tr>
<td>IA-2A</td>
<td>3 (2.8%)</td>
<td>60 (2.1%)</td>
</tr>
<tr>
<td>IAA</td>
<td>5 (4.7%)</td>
<td>61 (2.1%)</td>
</tr>
<tr>
<td>Two markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GADA/IA-2A</td>
<td>2 (1.9%)</td>
<td>2 (0.07%)</td>
</tr>
<tr>
<td>GADA/IAA</td>
<td>4 (3.8%)</td>
<td>4 (0.14%)</td>
</tr>
<tr>
<td>IA-2A/IAA</td>
<td>0</td>
<td>2 (0.07%)</td>
</tr>
<tr>
<td>Three markers</td>
<td>0</td>
<td>5 (0.17%)</td>
</tr>
</tbody>
</table>

The prevalence of islet auto-antibodies in children with Down’s syndrome compared to healthy schoolchildren
The frequency of high risk T1D genotypes in DS and DS+D

HLA high risk genotypes in healthy controls, Down’s syndrome (DS), Down’s syndrome and diabetes (DS+T1D) and type 1 diabetes (T1D)
Figure 2. Bar chart showing the indexed distribution of BSA abs in DS (n=106) and controls (n=85) in competition assay of 0.01% BSA. Mean cold index for DS = 0.233 (standard deviation = 0.4550), mean cold index for controls = 0.042 (standard deviation = 0.075).
A BOVINE ALBUMIN PEPTIDE AS A POSSIBLE TRIGGER OF INSULIN-DEPENDENT DIABETES MELLITUS

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Abstract  Background. Cow's milk has been implicated as a possible trigger of the autoimmune response that destroys pancreatic beta cells in genetically susceptible hosts, thus causing diabetes mellitus. Studies in animals have suggested that bovine serum albumin (BSA) is the milk protein responsible, and an albumin peptide containing 17 amino acids (ABBOS) may be the reactive epitope. Antibodies to this peptide react with p69, a beta-cell surface protein that may represent the target antigen for milk-induced beta-cell-specific immunity.

Methods. We used immunoassays and Western blot analysis to analyze anti-BSA antibodies in the serum of 142 children with insulin-dependent diabetes mellitus, 79 healthy children, and 300 adult blood donors. Anti-ABBOS antibodies were measured in 44 diabetic patients at the time of diagnosis, three to four months later, and one to two years later.

Results. All the diabetic patients had elevated serum concentrations of IgG anti-BSA antibodies (but not of antibodies to other milk proteins), the bulk of which were specific for ABBOS. The mean (±SE) concentration was 8.5±0.2 kiloﬂuorescence units (kFU) per microliter, as compared with 1.3±0.1 kFU per microliter in the healthy children. IgA antibodies were elevated as well, but not IgM antibodies. The antibody concentrations declined after diagnosis, reaching normal levels in most patients within one to two years. The initial decline involved anti-ABBOS-specific antibodies almost exclusively. Much lower serum concentrations of anti-BSA antibodies were found in all 379 control subjects, but only 2.5 percent of them had small amounts of ABBOS-specific IgG.

Conclusions. Patients with insulin-dependent diabetes mellitus have immunity to cow’s-milk albumin, with antibodies to an albumin peptide that are capable of reacting with a beta-cell-specific surface protein. Such antibodies could participate in the development of islet dysfunction. (N Engl J Med 1992;327:302-7.)
Early Childhood Infections and the Risk of Islet Autoimmunity

The Diabetes Autoimmunity Study in the Young (DAISY)

OBJECTIVE—Type 1 diabetes is a common chronic childhood disease, and the incidence is increasing globally. Childhood infections are considered a potential environmental trigger of type 1 diabetes. Alternatively, improved hygiene and reduced childhood infections could explain the increase in type 1 diabetes in developed countries. The association of reported illnesses during infancy and later development of islet autoimmunity (IA) were examined in the Diabetes Autoimmunity Study in the Young.

RESEARCH DESIGN AND METHODS—Complete illness interviews through 9 months of age were collected for 1,729 children—1,174 without a family history of type 1 diabetes and 555 with a first-degree relative with type 1 diabetes. Persistent IA was defined as positive antibodies to insulin, glutamic acid decarboxylase, or tyrosine phosphatase on at least two consecutive study visits.

RESULTS—There were 109 children with persistent IA among the 1,729 children with illness records. A greater number of gastrointestinal illnesses were associated with an increased risk of IA, but only among children who were exposed to gluten-containing grains (wheat or barley) either <4 months of age (hazard ratio 1.37 [95% CI 1.22–1.55]; P < 0.0001) or ≥7 months of age (1.12 [1.05–1.19]; P = 0.0005) compared with 4–6 months of age (P for interaction = 0.02). There were no associations of upper respiratory symptoms, respiratory illnesses, or fevers with IA.

CONCLUSIONS—Specific pathogens such as enteroviruses or rotavirus may increase the risk of IA in the presence of existing inflammation induced by diet.

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Dietary Intervention in Infancy and Later Signs of Beta-Cell Autoimmunity


ABSTRACT

BACKGROUND

Early exposure to complex dietary proteins may increase the risk of beta-cell autoimmunity and type 1 diabetes in children with genetic susceptibility. We tested the hypothesis that supplementing breast milk with highly hydrolyzed milk formula would decrease the cumulative incidence of diabetes-associated autoantibodies in such children.

METHODS

In this double-blind, randomized trial, we assigned 230 infants with HLA-conferred susceptibility to type 1 diabetes and at least one family member with type 1 diabetes to receive either a casein hydrolysate formula or a conventional, cow’s-milk-based formula (control) whenever breast milk was not available during the first 6 to 8 months of life. Autoantibodies to insulin, glutamic acid decarboxylase (GAD), the insulinoma-associated 2 molecule (IA-2), and zinc transporter 8 were analyzed with the use of radiobinding assays, and islet-cell antibodies were analyzed with the use of immunofluorescence, during a median observation period of 10 years (mean, 7.5). The children were monitored for incident type 1 diabetes until they were 10 years of age.

RESULTS

The unadjusted hazard ratio for positivity for one or more autoantibodies in the casein hydrolysate group, as compared with the control group, was 0.54 (95% confidence interval [CI], 0.29 to 0.95), and the hazard ratio adjusted for an observed difference in the duration of exposure to the study formula was 0.51 (95% CI, 0.28 to 0.91). The unadjusted hazard ratio for positivity for two or more autoantibodies was 0.52 (95% CI, 0.21 to 1.17), and the adjusted hazard ratio was 0.47 (95% CI, 0.19 to 1.07). The rate of reported adverse events was similar in the two groups.

CONCLUSIONS

Dietary intervention during infancy appears to have a long-lasting effect on markers of beta-cell autoimmunity — markers that may reflect an autoimmune process leading to type 1 diabetes. (Funded by the European Commission and others; ClinicalTrials.gov number, NCT00570102.)
Increased FOXP3 expression in small-bowel mucosa of children with coeliac disease and type I diabetes mellitus

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Abstract

Objective. To determine whether the expression of FOXP3 is changed in small-bowel mucosa in coeliac disease (CD).

Material and methods. The study comprised 52 patients (mean age 8.01 ± 6.14 years) who had undergone small-bowel biopsies. CD only was diagnosed in 16 patients, and CD with type I diabetes mellitus (T1D) in 7. These 23 patients and 4 others without CD had partial or subtotal villous atrophy (PVA, SVA). Twenty-five persons without CD had normal mucosa. The transcription level of the FOXP3 gene (Hs00203958_m1) was evaluated in biopsy samples (small bowel) using TaqMan gene expression assays. FOXP3 protein in mucosal cells was evaluated with mouse anti-human FOXP3 antibodies and CD25⁺, and CD4⁺ T cells were evaluated by mouse monoclonal antibodies. Results. Expression of FOXP3 mRNA was higher in both PVA and SVA compared to normal mucosa (p = 0.007). Patients with CD and T1D had higher expression of FOXP3 mRNA than patients with CD alone (p = 0.02). The number of FOXP3⁺ cells in intestinal mucosa was higher in patients with CD, especially those with coexisting T1D, than in those with normal mucosa (p = 0.01). The results of double staining showed that, among all positive cells, FOXP3 expression alone was revealed in 25.6% of the cells, CD25 positivity in 18% and both markers simultaneously were found in 55.6% of lymphocytes (p = 0.03). Double staining for CD4 and FOXP3 showed that 87.5% of cells were CD4⁺, 2.8% were FOXP3⁺ and 9.7% of cells simultaneously expressed the CD4 and FOXP3 markers. Conclusions. A more pronounced expression of FOXP3 mRNA and also the number of FOXP3⁺ cells (with simultaneous expression of CD25 and CD4 markers) were found in the small-bowel biopsy specimens obtained from children with CD, particularly those with coexisting T1D, compared with the FOXP3 expression in normal mucosa.
Down syndrome, autoimmunity and T regulatory cells

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Summary

Autoimmune diseases are more represented in Down syndrome (DS) individuals compared to chromosomally normal people. Natural T regulatory cells (nTreg) have been considered to be primary in the role of controlling the intensity and targets of the immune response. We have investigated the phenotypical and functional alteration of nTreg in a group of DS people. The phenotypical characteristic of Treg cells of 29 DS was analysed and compared with an age-matched healthy control group. The inhibitory potential of CD4+CD25highCD127low T regulatory cells was evaluated on autologous CD4+CD25+ T cell proliferation in response to activation with a myogenic pan-stimulus (anti-CD2, anti-CD3 and anti-CD28 antibodies). The CD4+CD25high cells in the DS and control groups were 2.692 ± 0.3808%, n = 29 and 1.246 ± 0.119, n = 29, respectively (P = 0.0007), with a percentage of forkhead box protein 3 (FoxP3)-expressing cells of 79.21 ± 3.376%, n = 29 and 59.75 ± 4.496%, respectively (P = 0.0015). CD4+CD25-FoxP3+ cells were increased in peripheral blood from DS subjects (DS mean 5.231 ± 0.6065% n = 29, control mean 3.076 ± 0.314% n = 29). The majority of CD4+CD25high were CD127low and expressed a high percentage of FoxP3 (natural Treg phenotype). While the proliferative capacity of DS T cells was not altered significantly compared to normal individuals, a reduced inhibitory potential of Treg compared to healthy controls was clearly observed (mean healthy control inhibition in Tinf : Treg 1:1 co-culture: 58.9% ± 4.157%, n = 10 versus mean DS inhibition in Tinf : Treg 1:1 co-culture: 39.8% ± 4.788%, n = 10, P = 0.0075; mean healthy control inhibition in Tinf : Treg 1:0.5 co-culture: 45.10 ± 5.858%, n = 10 versus DS inhibition in Tinf : Treg 1:0.5 co-culture: 24.10 ± 5.179%, n = 10, P = 0.0177). DS people present an over-expressed peripheral nTreg population with a defective inhibitory activity that may partially explain the increased frequency of autoimmunity disease.

Keywords: autoimmunity, coeliac disease, Down syndrome, Hashimoto disease, regulatory T cells

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One further avenue by which early feeding practices may influence autoimmunity of gut and pancreas is through the microbial population present in the intestine, the so-called ‘gut microbiome’.

There have been well documented differences in this microbiome recorded both in those developing diabetes1,2 and coeliac disease 3 when compared to non-affected controls.

Furthermore, the development of the gut microbiome has been linked to early infant feeding practices such breast versus cow’s milk feeding and time of weaning 4.


If Down’s syndrome is a model of accelerated and exaggerated autoimmunity that is not simply conferred through HLA

Is there something in the way children with DS are fed, react to feeds or are susceptible to infection that increase autoimmunity risk?

If so, could DS inform the general prevention of Type 1 diabetes/ coeliac disease etc in general population?
Plan:

(1) Recruit the Downs Syndrome Association as collaborators

(2) Organise a focus group of 5-6 mothers to examine feeding issues in DS as babies are hypotonic and have increased history of infections

(3) Prospectively recruit a cohort of infants with DS through DSA
Soon after diagnosis, all parents are sent an information pack by the DSA regarding DS. The DSA have agreed to include a study information pack for the IFADS study in this primary posting. 750 children a year are born with DS of which approximately 500 per year are sent this information (Information supplied by Sheila Heslam: Policy Manager DSA). The study information sheet will include a web address by which parents can register an interest in taking part and download a consent form which can be free posted back to the Bristol Research Team. With a relatively conservative estimate of 20% interest in this study we therefore would be able to recruit 100 patients per year
Feeding practices in Down’s syndrome

(1) To document the current feeding practices of infants born with Down’s syndrome
(2) To examine the barriers and facilitators to sole breast feeding to 4 months of age such as hypotonia and DS related co-morbidities such as congenital heart disease
(3) To examine timing of critical changes in feeding practice such as weaning and introduction of gluten containing cereal products and the relationship to DS associated co-morbidities.
(4) To develop a toolkit to facilitate exclusive and sustained breast feeding in infancy and the introduction of weaning foods only after 4 months of age to be trialled nationally in years two and three.
To examine the induction of autoimmunity in Down’s syndrome and relationship to infant feeding

1. To HLA type all infants in the cohort using oral mouth wash kits examining specifically HLA haplotypes conferring increased risk of autoimmunity such as HLA DR3
2. Collect heel prick, blood tests to establish the levels of autoimmunity to thyroid, gut and pancreas as soon after recruitment as possible (baseline) and at six and twelve months.
3. Measure IgG antibodies to Bovine Serum Albumin at baseline, 6 and 12 months.
4. Collect stool samples from babies at baseline, 3,6 and 12 months for gut microbiome analysis.
5. To assess impaired gut barrier function/integrity at six and twelve months through the measurement of serum Zonulin levels.

To establish a cohort of children with DS to prospectively document the development of gut, thyroid and islet cell auto-immunity through childhood.