Elevated C-Reactive Protein Levels in the Development of Type 1 Diabetes

H. Peter Chase,1 Sonia Cooper,1 Iris Osberg,2 Lars C. Stene,1 Katherine Barriga,3 Jill Norris,3 George S. Eisenbarth,1 and Marian Rewers1,3

Elevated C-reactive protein (CRP) levels have previously been described before the onset of type 2 diabetes and gestational diabetes. We hypothesized that inflammation, as reflected by elevated CRP levels, can help predict development of islet autoimmunity or type 1 diabetes. Children at risk for type 1 diabetes and followed in the Diabetes Autoimmunity Study of the Young (DAISY) had blood samples drawn and frozen serum saved at various intervals after birth. CRP was measured using a high-sensitivity sandwich enzyme immunoassay. Islet autoantibodies (IAs) were measured using biochemical immunoassays. Elevations in CRP concentrations were significantly more frequent (P < 0.01) in children who later developed type 1 diabetes (8 of 16 children) than in children negative for IAs at their last testing (3 of 26). Children with one or more positive IA were more likely to have elevated CRP concentrations (15 of 36) than IA-negative children (3 of 26; P < 0.01). The finding of elevated CRP levels in infants and young children before the onset of type 1 diabetes adds to the evidence that the disease is an immunoinflammatory disorder. The elevated CRP levels may provide an additional marker for risk of progression to type 1 diabetes. Diabetes 53:2569–2573, 2004

RESEARCH DESIGN AND METHODS

Samples obtained from 62 children who participated in the Diabetes Autoimmunity Study of the Young (DAISY) in Denver, Colorado, were available for analysis. Blood draws were scheduled at age 9 months and then at regular intervals as part of the DAISY protocol (14). The children were determined to be at increased risk for developing type 1 diabetes because they had a first-degree relative with type 1 diabetes or they have HLA genes that put them at an increased risk for developing type 1 diabetes. All participating DAISY families signed a consent form approved by the Colorado Multiple Institutional Review Board.

HLA typing was done on cord blood samples obtained from cord blood at birth or from a heel stick if the child enrolled after birth (14). The high-risk genotype was DRB1*04, DQB1*0302/DRB1*0301, DQB1*0201 (i.e., DR3/4, DQ8). The moderate-risk genotype was DRB1*04, DQB1*0302 or DRB1*0301/0301 or DRB1*04, DQB1*0302/x (where x is neither DRB1*04, DQB1*0302, nor DRB1*0301 nor DR2). All other genotypes were classified as low risk, and in this study, the moderate- and low-risk genotypes were combined.

Biochemical islet autoantibodies (IAs) were determined in the laboratory of Dr. George Eisenbarth at the Barbara Davis Center for Childhood Diabetes as previously described (15). The IAs measured included GAD65, ICA512bc, and insulin autoantibodies using the microinsulin autoantibody assay (16). All of the assays were performed in 96-well filtration plates using protein A/G precipitation. In the most recent Diabetes Autoantibody Standardization Program workshop (2002), where initial results have been reported to laboratories with analysis of 50 patients with new-onset type 1 diabetes and 100 control subjects in blinded fashion, the antibody sensitivities/specificities from this laboratory were as follows: ICA512bc 62%/99%, insulin 62%/98%, and GAD65 90%/92% (17).

Using a nested case-control design, we selected four groups of children for this study, depending on their development of islet autoimmunity or type 1 diabetes during the current follow-up: 16 children who developed type 1 diabetes, 20 children who developed persistent islet autoimmunity (positive for at least one of three IAs), 11 children who developed transient islet autoimmunity (positive for at least one of three IAs at two or more consec-

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COX2, cyclooxygenase 2; CRP, C-reactive protein; DAISY, Diabetes Autoimmunity Study of the Young; IA, islet autoantibody; IL, interleukin.

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TABLE 1
Age (in years) at events during follow-up of the study children

<table>
<thead>
<tr>
<th></th>
<th>Consistently IA−</th>
<th>Transiently IA+*</th>
<th>Persistently IA−</th>
<th>Type 1 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>11</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Most recent visit*</td>
<td>7.6 ± 2.1</td>
<td>7.8 ± 2.7</td>
<td>8.1 ± 2.8</td>
<td>6.7 ± 3.7</td>
</tr>
<tr>
<td>First positivity for IA</td>
<td>NA</td>
<td>3.6 ± 2.4</td>
<td>5.3 ± 3.7</td>
<td>3.3 ± 3.4</td>
</tr>
<tr>
<td>First CRP testing†</td>
<td>2.7 ± 1.1</td>
<td>2.0 ± 1.6</td>
<td>3.8 ± 3.3</td>
<td>2.7 ± 3.0</td>
</tr>
<tr>
<td>Last CRP testing‡</td>
<td>7.6 ± 2.1</td>
<td>7.2 ± 2.6</td>
<td>8.0 ± 2.9</td>
<td>6.5 ± 3.8</td>
</tr>
<tr>
<td>Diagnosis of type 1 diabetes</td>
<td>NA</td>
<td>NA</td>
<td>7.0 ± 3.0</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ±SD. *Most recent visit when IAs were measured, regardless of CRP testing. †Before the first positive test for IAs. Data were available for only 7 of the 11 transiently positive individuals and for 7 of the 16 with type 1 diabetes. ‡After testing positive for IAs (except for consistently IA− groups).

RESULTS
There were 29 boys and 33 girls. Eight of the children were Hispanic, one was biracial, and the remaining children were classified as white/Caucasian. The mean follow-up of the 62 children (age at the time of their most recent IA testing) was 7.6 years (range 0.6–13.4), and this was similar in all groups. The mean age at the time of diagnosis of type 1 diabetes was 7.0 years (1.9–13.7). In individuals who developed type 1 diabetes, all CRP measurements were done before clinical diagnosis. The last CRP measurement was done on samples collected an average of 7.8 months before clinical diagnosis. There were no overweight or obese children, with BMI ranges of 13.4–22.3 kg/m² for the 62 children.

To assess whether elevated serum concentrations of CRP might predict type 1 diabetes, we compared CRP levels before and after the diagnosis of autoimmunity and of type 1 diabetes with CRP levels for individuals who did not develop type 1 diabetes. The percentage of people with elevated CRP levels for the various groups is shown in the “combined” category of Table 2. The actual values of CRP, including longitudinal values for children who were transiently IA+ or who were progressors to type 1 diabetes, are shown in Fig. 1. In the combined group of 46 children who did not develop type 1 diabetes, the last CRP test showed a positive result in 10 (21.7%). In contrast, the CRP test was positive in 50% of the 16 who developed type 1 diabetes (P = 0.05, Fisher’s exact test). The strongest contrast was obtained when comparing the combined group of children consistently negative and transiently positive for islet autoimmunity (3 of 26, or 11.5% with elevated CRP) with the combined group of children with persistent islet autoimmunity and those who developed type 1 diabetes (15 of 36, or 41.7%; P = 0.01, Fisher’s exact test).

To demonstrate that there were no changes over time as a result of increasing age, we compared the median CRP levels for the 15 children who were never positive for any IAs (control subjects, Fig. 2, right) at the first and second measurements (on average, 5 years apart). There were no differences at the different ages (P = 0.91, Wilcoxon’s signed-rank test). In evaluating whether seroconversion for islet autoimmunity was accompanied or followed by an increase in CRP levels, we compared the serum concentrations of CRP before and after seroconversion for islet autoimmunity within the children who developed islet autoimmunity (Fig. 2, right). There were no significant differences in serum CRP concentrations before and after IA seroconversion (P = 0.87).

CRP was found with similar frequencies in the high- and low-risk HLA groups (Table 2). Most children who were transiently IA+ (IA− on two or more visits and subsequently losing IA positivity) were in the low/moderate-risk HLA groups.

TABLE 2
Number of subjects with positive CRP in groups by HLA type and IAs

<table>
<thead>
<tr>
<th></th>
<th>Consistently IA−</th>
<th>Transiently IA+*</th>
<th>Persistently IA−</th>
<th>Type 1 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>11</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>High risk†</td>
<td>20% (1 of 5)</td>
<td>0% (0 of 1)</td>
<td>50% (3 of 6)</td>
<td>44% (4 of 9)</td>
</tr>
<tr>
<td>Moderate/low risk</td>
<td>10% (1 of 10)</td>
<td>10% (1 of 10)</td>
<td>29% (4 of 14)</td>
<td>57% (4 of 7)</td>
</tr>
<tr>
<td>Combined‡</td>
<td>13% (2 of 15)</td>
<td>9% (1 of 11)</td>
<td>35% (7 of 20)</td>
<td>50% (8 of 16)</td>
</tr>
<tr>
<td>P§</td>
<td>0.03</td>
<td>0.03</td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

CRP positivity was defined as a serum concentration >0.05 mg/dl. *Before the first positive test for IAs. †The high-risk and moderate/low-risk groups are defined in RESEARCH DESIGN AND METHODS. ‡High risk plus moderate/low risk. §Fisher’s exact test for difference between each combined group and the combined group with type 1 diabetes.
group (10 of 11 children). Only one of the transiently IA\(^+\) children had positive CRP, and this individual was in the low/moderate-risk group. The high-risk HLA type (DR3/DR4) tended to be most frequent in the children who developed type 1 diabetes (9 of 16 children; Table 2). The occurrence of DR3 (alone or with DR4) also tended to be more common in the type 1 diabetic group (13 of 16) and the IA\(^-\) group (11 of 20) than in the IA\(^+\) group (6 of 15) and the reverters (3 of 11). CRP was positive in 7, 3, 0, and 1 of the DR3 subjects in these four groups, respectively. Be-

![Graph showing CRP levels](image1)

**FIG. 1.** A logarithmic scale showing quantitative levels of CRP for control subjects, for initial and final samples from transiently IA\(^+\) children, for persistently IA\(^+\) children, and for initial and final samples from progressors to type 1 diabetes (T1D). The values within the boxes reflect the IA status, the number who were CRP\(^+\), and the ages at the initial and final testing.

![Graph showing CRP levels](image2)

**FIG. 2.** A logarithmic scale showing levels of CRP were not associated with age (left and right) or the development of IAs (right) in the same individuals over time. The groups on the right only include samples from children when one sample was available before the development of autoantibodies and another was again available after the development of autoantibodies. The values in the boxes are as in Fig. 1.
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cause of small numbers, statistical analyses were not possible.

Positive CRP (using the last serum samples available) was found more frequently in children with positive IAs. There was not a statistically significant difference (Fisher's exact test) when comparing the groups positive for one versus two or more IAs, respectively ($P = 0.60$; data not shown). Only 3 of 26 children had positive CRP (12%) among those who were negative or transiently positive for IAs, compared with the 7 of 16 children (44%) with two or more positive IAs ($P = 0.04$) and the 8 of 20 children (40%) with one positive IA ($P = 0.03$). When considering the two groups of IA$^+$ children together and comparing them with the children without IAs, elevated CRP concentrations were significantly more likely to be found in the IA$^+$ children ($P = 0.01$).

DISCUSSION

Type 1 diabetes is now accepted to be a chronic immune-inflammatory disorder. Because it is a disease of inflammation—both of the innate and adaptive immune systems—it is perhaps not surprising that elevated CRP levels were found. It is important to note that the elevated CRP levels were found before elevated glucose levels, so that this marker of inflammation is not related to hyperglycemia. This observation is impossible to study in people who already have type 1 diabetes.

CRP was more likely to be positive in children progressing to type 1 diabetes. CRP production is stimulated by inflammatory cytokines such as interleukin (IL)-6. Higher levels of IL-6 have been described in young adults with type 1 diabetes compared with control subjects (19). Mendall et al. (20) showed that elevated CRP levels were positively associated with elevated serum levels of the IL-6 cytokine.

Children with either one or two or more positive IAs were more likely to have elevated CRP concentrations than IA$^-$ children. Being positive for only one IA only predicts a slightly increased risk of type 1 diabetes (15). A total of 15 of the 16 children who developed type 1 diabetes in this study had two or more positive IAs before the diagnosis of diabetes. The children were still young at the time of the last sample (mean age 7.6 years), and the peak time of diabetes onset (11–12 years) had not yet been reached for the majority of the children in our study. It is therefore possible that some of the children who had only one positive IA at the last testing will develop further antibodies and type 1 diabetes with time. Further follow-up of the DAISY cohort and other longitudinal studies will help to further elucidate the role of CRP and other markers of inflammation in the development of type 1 diabetes.

Elevated levels of inflammatory cytokines and inflammatory prostaglandins (9,10) and of prostaglandin synthase 2 (COX2) (11) have been described in children before or after the onset of type 1 diabetes. A study in adults with type 2 diabetes demonstrated that treatment with aspirin, a COX2 inhibitor, resulted in reduced CRP, insulin resistance, and serum triglycerides despite a lack of change in body weight (21). If inhibitors of the COX2 enzyme are used to try to prevent type 1 diabetes in humans, monitoring levels of CRP may be helpful. Elevated CRP levels are now described before the onset of type 1 diabetes and have previously been described before the onset of type 2 diabetes (12) and gestational diabetes (3). Inflammation may be a common factor in the development of these various types of diabetes.

CRP has previously been shown to be elevated in adults with known type 1 diabetes (7,8). In the study by Kilpatrick et al. (7), the CRP levels were related to age, BMI, HbA1c, female sex, and a history of coronary heart disease in a first-degree relative. In the study by Schalkwijk et al. (8), the CRP levels were higher in 40 subjects with type 1 diabetes in comparison with control subjects. They demonstrated a relationship with chronic hepatic inflammation. Although the mechanism of the CRP elevation is unknown, they suggest it might be related to activation of macrophages, increased oxidative stress, or induction of cytokines. There is evidence that all three of these may be factors in the etiology of type 1 diabetes. Our report is the first description of elevated CRP levels in infants and young children before the onset of type 1 diabetes.

Bohmer et al. (22) followed the immune activation state looking for a final “immunologic burst” in five patients with “high-normal blood glucose” and low insulin production (<2.5 percentile) on intravenous glucose tolerance tests. They found tumor necrosis factor-α elevated in four of five patients, with no change during the study, and normal levels of serum β2 microglobulin and CRP. (The high-sensitivity CRP assay was not yet available.) They concluded that “no evidence exists for an accelerated loss of islet β-cell function or increased immunologic activity immediately before the diagnosis of insulin dependency.” Although their patients were studied in the 6 months before diagnosis of type 1 diabetes and our last CRP levels were an average of 7.8 months before diagnosis, we also did not detect a rise in CRP levels from the first to the final sample in the children who developed type 1 diabetes (Fig. 1).

The inability to suppress the inflammation, or to allow it to affect islet tissue, may be influenced by genetics, by an accelerated immune response, by nutritional or other environmental factors, or by a combination of these mechanisms. It is of note that the incidence of type 1 diabetes in children has increased concurrently with avoidance of aspirin use because of the fear of Reye’s syndrome. Similarly, the incidence has increased as we have decreased our consumption of the anti-inflammatory ω-3 fatty acids and increased our consumption of the proinflammatory ω-6 fatty acids (23). Future studies addressing the prevention of diabetes should consider the use of anti-inflammatory agents.

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