Heterophile Antibodies Masquerade as Interferon-α in Subjects With New-Onset Type 1 Diabetes

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One of the potential environmental triggers in type 1 diabetes may be viruses. The mechanism of viral infections leading to β-cell destruction of the pancreas is becoming clearer, and the potential role of viruses inducing the cytokine interferon-α (IFN-α) has been implicated. It is well established that IFN-α therapy induces islet cell autoimmunity and other endocrine autoantibodies as well (1). Recent studies have indicated that IFN-α levels in serum of new-onset type 1 diabetic subjects are elevated and perhaps related to recent enteroviral infections (2,3). These studies suggest that IFN-α may be a useful surrogate marker for disease onset, perhaps due to a recent viral infection or vaccination.

At the Barbara Davis Center for Childhood Diabetes in Denver, Colorado, we screened 32 new-onset type 1 diabetic subjects and 31 healthy control subjects for serum IFN-α levels. A two-site enzyme-linked immunosorbent assay (ELISA) was used in this study as previously described (2) for IFN-α detection. We used sheep anti-human IFN-α polyclonal antibody and mouse biotin-labeled anti-human IFN-α monoclonal antibody (both from Pierce Biotechnology, Rockford, IL). We used the National Institute of Allergy and Infectious Disease IFN-α standard (no. Ga23-901-532). The detection sensitivity limit extrapolated from our standard curve was 25 pg/ml. We detected elevated IFN-α levels in 4 of the 32 type 1 diabetes serum samples and 2 of the 31 healthy control serum samples tested (Fig. 1). One diabetic subject had an average IFN-α level of 919 pg/ml when tested on 3 separate days. However, we were concerned about the possibility that we were measuring heterophile antibodies instead of IFN-α (4). Subsequently, we repeated the ELISA on the six positive serum samples using 5% mouse serum in the buffer to bind heterophile antibodies to prevent them from masquerading as IFN-α. With this modified protocol, we did not detect IFN-α in any of the previously positive serum samples.

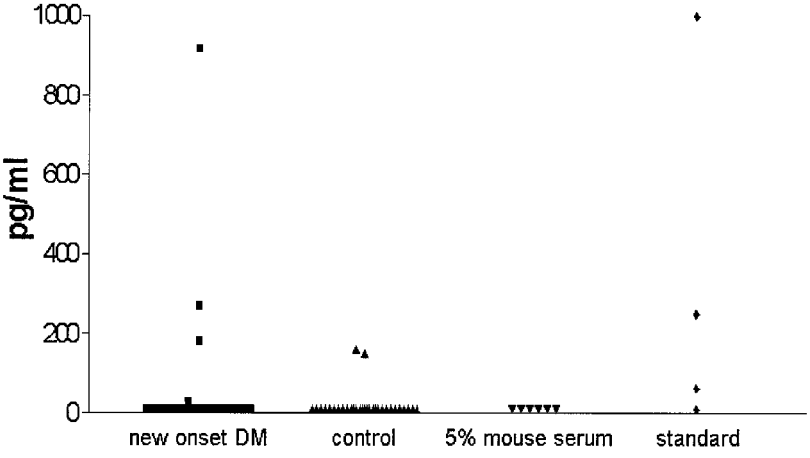

Figure 1—*Detection of IFN-α (pg/ml) in sera of new-onset type 1 diabetic subjects (DM;* n = *32), control subjects* (n = *31), six positive retested samples with 5% mouse serum assay buffer, and standard IFN-α (0, 63, 250, and 1,000 pg/ml) with 5% mouse serum.*

Heterophile antibodies are antibodies directed against animal antigens that arise in humans. They may be the result of intravenous administration of animal proteins (as used in some radiological procedures and some immunotherapy), but most commonly the cause is unknown. Therefore, it is important to consider heterophile antibodies as potential interfering substances when performing ELISA screening for cytokines, as some studies have observed false-positive levels of IFN-α in up to 27.5% of serum samples from healthy blood donors using a commercial ELISA (4,5).

There are several different ways to improve IFN-α ELISA specificity. As mentioned above, adding 5% mouse serum to the assay buffer effectively adsorbs the heterophile antibodies. Also, using rabbit $F(ab')_2$ as the coating antibody avoids heterophile antibody binding and interference (5). In light of these findings, we should reconsider previous studies reporting elevated IFN-α levels (using ELISA technique) at the onset of type 1 diabetes. Future studies attempting to detect IFN-α should take into account the possibility of heterophile antibody interference. Even though viral-induced IFN-α has been implicated in the pathogenesis of type 1 diabetes, we were unable to detect it in new-onset diabetes serum. Therefore, it may be useful to measure IFN-α in serum from individuals in the pre–type 1 diabetes phase (autoantibody positive, high-risk genotype), particularly

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Received for publication 28 January 2004 and accepted in revised form 30 January 2004.

D.D. has received research support from Eli Lilly.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFN-α, interferon-α.

during an acute viral infection or after immunization.

Acknowledgments— This research was supported by Juvenile Diabetes Research Foundation Fellowship Grant 13-2002-444 (to J.B.), National Institutes of Health Grants DK06405 (to E.L.) and DK32082, AI39213, DK55969, DK62718, AI50864, AI95380, DK50970, and AI46374 (to G.S.E.), Diabetes Endocrine Research Center Grant P30 DK57516 (to G.S.E.), and Clinical Research Center Grants MO1 RR00069 and MO1 RR00051 (to G.S.E.). G.S.E has also received support from the American Diabetes Association, the Juvenile Diabetes Foundation, and the Children's Diabetes Foundation.

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