Study of T-Cell Activation in Type I Diabetic Patients and Pre-Type I Diabetic Subjects by Cytometric Analysis: Antigen Expression Defect in Vitro

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In Type I diabetes the observation of a decreased release of interleukin-2 (IL-2) and soluble IL-2 receptors by means of stimulated lymphocytes in vitro indicates that a primary immunoregulatory defect may be involved. To confirm this hypothesis we investigated the T-cell activation trend, evaluating the surface expression of IL-2 receptor (CD25), transferrin (CD71), HLA class II (DR), and CD69 phenotypes after in vitro stimulation with phytohemagglutinin (PHA; 1 and 10 µg/ml) and concanavalin A (12.5 µg/ml) in six newly diagnosed Type I diabetics and six islet cell- and insulin autoantibody-positive first-degree relatives. As controls were studied six long-standing Type I diabetics and six healthy subjects. T-cell cultures from the four groups were performed on the same day and examined at 0, 24, 48, 96, 120, and 144 hr. Cytometric analysis was performed, keeping PBMC gating constant on the basis of physical parameters (scatter and volume). Using both PHA concentrations, a lower level of CD25, CD71, CD69, and DR antigen expression was found in newly diagnosed patients at all observation times with respect to control cultures (P < 0.001). Unexpectedly, pre-Type I diabetic subjects, after 1 µg/ml of PHA, showed a significantly reduced expression of CD69 (P < 0.001) and CD71 (P < 0.001). The levels remained low, also with high PHA, at the different observation periods, while CD25 expression was found to be reduced in prediabetics only after 1 µg/ml of PHA (P < 0.001). The long-standing patients showed a T cell activation trend very close to the latter. Our data show that in Type I diabetes and in the early phases of the disease, the initial activation signal(s) appears to be affected, particularly with one or more subsequent events necessary to initiate the appearance of "activation antigens." This study suggests that the natural history of immunoregulation in pre-Type I and Type I diabetes is characterized by a primary defect in this system, which also persists in patients with long-standing disease.

KEY WORDS: Type I diabetes; pre-Type I diabetes; flow cytometry; T-cell activation; T-cell cultures; CD69+ cells; CD71+ cells; CD25+ cells; DR+ cells; interleukin-2 secretion defect.

INTRODUCTION

Today Type I diabetes is considered an autoimmune disease and several immunological abnormalities are detectable during the prodromic period preceding clinical onset. Briefly, the most widely accepted features confirming the autoimmune pathogenesis of Type I diabetes are (i) lymphocytic infiltrates in pancreatic islets (4), (ii) antibodies against pancreatic intracellular antigens (5), (iii) activated lymphocytes expressing HLA class II molecules and/or interleukin-2 (IL-2) receptors in vivo (6–9), and (iv) decreased release of IL-2 by stimulated lymphocytes (10, 11). Regarding the latter point, the observation of a decreased release of IL-2 and soluble IL-2 receptors (IL-2RS) using stimulated lymphocytes in vitro has provided further evidence for disorders in such basic regulatory events as IL-2 secretion and IL-2 receptor expression and release (12).

These results clearly indicate that T-lymphocyte activation in human Type I diabetes might also be altered when agents that activate T cells by relatively simple stimuli to the antigen receptor, such as plant lectins, are employed. In this case, activation
of human lymphocytes is associated with the expression of new antigens (13, 14). It is interesting to note that these molecules appear gradually, starting from the early hours of activation and continuing at more advanced stages. It is likely that the physiological roles of the early and late activation molecules are distinct (15, 16). “Early” molecules may be associated with growth factor receptors whose sequential expression provides the normal progression of proliferation, whereas “late” molecules may be associated with specific immunological functions. The appearance of these molecules may be considered a regulatory event responsible for both morphological and functional changes in resting T lymphocytes when these are activated in vivo or in vitro (16-18).

We evaluated in vitro T-cell activation, analyzing the surface expression of early activation phenotypes such as IL-2 receptor (CD25) and CD69 and late phenotypes such as class II (DR) and transferrin receptor (CD71) in a group of newly diagnosed Type I diabetic patients, a group of long-term Type I diabetes patients, a group of high-risk subjects with islet-cell (ICA) and insulin autoantibodies, and a group of healthy subjects. In the present study we demonstrate that in Type I diabetes, lymphocytes, although activated in the peripheral blood of newly diagnosed patients, when stimulated with lectins are characterized by a deficit in expression of T-cell activation antigens, found by evaluating both early (CD25 and CD69) and late (CD71 and DR) molecule appearance. This defect is more pronounced in patients with overt disease but is also present in subjects at risk to develop Type I diabetes, defined as pre-Type I diabetic subjects. Furthermore, our study shows that while IL-2 production is normal in individuals at risk, it is reduced in new-onset Type I diabetic patients.

MATERIALS AND METHODS

Subjects

Twelve Type I diabetic patients (seven males and five females) were studied. At the time of study, six were newly diagnosed (mean age, 20.1 ± 1.8 years) with a duration of symptoms of <3 months (mean weeks’ duration, 4.6 ± 1.4), and six were long-term diabetics with a duration of disease of >12 months (mean, 170 ± 16 weeks). They were classified in accordance with the National Diabetes Data Group (19). All patients were treated from diagnosis with human insulin, usually a combination of short and intermediate acting (Actrapid and Monotard, Novo, Copenhagen), two or three times daily.

Six pre-Type I diabetic subjects, classified in accordance with the criteria established by the “Ad Hoc Expert Committee” (20), with first-degree relatives with an ICA positivity higher than 20 JDF units and insulin autoantibody positivity (mean age, 16.2 ± 3.4 years; five males and one female), were also studied. As a control group, six normal subjects (three males and three females; mean age, 18.9 ± 4.6 years) without a family history of Type I diabetes were studied.

Glycemic values, fasting and postprandial, were determined by strips (Glucostix, Ames Miles, Milan, Italy) and were read by the reflectance method (Glucometer, Ames Miles) during the 24 hr before the study. Glycosylated hemoglobin (HbA1c) was determined by autoanalyzer (Daichi, Kagaku, Kyoto, Japan) on the day blood was drawn (normal range in our laboratory, 5.5 ± 1.2%) (Table I).

Humoral Immunity Studies

All patients were tested for insulin antibodies (125IInsulin kit, Techno Genetics, Trezzano, Milan,