Analysis of insulin receptors on human lymphoblastoid cell lines by flow cytometry

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Summary. Antibodies to the insulin receptor have provided important experimental probes of receptor structure and function. In the present study, we have characterized the insulin receptor on human lymphoblastoid cell lines using polyclonal and monoclonal anti-receptor antibodies and fluorescence flow cytometry. The cell lines were derived by Epstein-Barr virus transformation of peripheral mononuclear leucocytes from normal subjects or patients with disorders that affect the insulin receptor. Fluorescence analysis revealed a high level of specific fluorescence on lymphoid cell lines from normal individuals (mean peak fluorescence 30–50 units above the control) and was similar to the labelling of the spontaneously transformed lymphoblastoid cell line IM-9. Transformed cells

from patients with syndromes of insulin resistance, such as the Rabson Mendenhall syndrome, leprechaunism and the type A syndrome of insulin resistance and acanthosis nigricans, exhibited little or no specific fluorescence. In all cases, there was a unimodal distribution of receptors on cells. In addition, there was a good correlation between specific binding of ¹²⁵I-insulin and percentage peak fluorescence. The data indicate that fluorescence flow cytometry can be used to study the distribution of insulin receptor on different cell lines and to study cells derived from patients with disease states.

Key words: Insulin receptor, flow cytometry.

A primary approach to the characterization of the insulin receptor has been through the study of its interaction with ¹²⁵I-labelled insulin [1]. Those studies have yielded information about the structure of the receptor, its function, and its role in physiology and disease [2]. Insulin receptors change in number and affinity in response to a number of physiological regulators [3], may be modulated by anti-receptor antibodies [4], and are altered in a number of diseases [2]. In most of these studies, it has been impossible to obtain sufficient quantities of important target tissues for insulin action, and therefore, most studies have been performed with mononuclear leucocytes and erythrocytes [5]. In some cases, transformed lymphocytes or fibroblasts have also been employed and have been found to reflect the defect observed in freshly isolated cells [6].

In addition to insulin, it has been possible to detect and study the insulin receptors on the surface of cells using antibodies to the insulin receptor [7]. These studies have received relatively limited use in human disease [8]. In the present study, we have employed anti-receptor antibodies and the technique of fluorescence flow cytometry for identification of insulin receptors and for studying their expression on different human lymphoid cell lines.

Materials and methods

Anti-sera and cultured cells

Two sources of antibody to the insulin receptor used were: polyclonal autoantibodies to the insulin receptor derived from patients with the

Type B syndrome of insulin resistance, and a monoclonal antibody raised to insulin receptors purified from human placenta and grown as ascites tumours in mice (kindly provided by S. Jacobs). Fluorescein conjugated goat anti-human IgG or mouse IgG were purchased from Cappel Laboratories, Cochranville, Pennsylvania, USA.

IM-9 cells are a human lymphoblastoid cell line which has been extensively studied in terms of insulin receptors. Other human β -cell lines were obtained by transformation of peripheral blood mononuclear cells with Epstein-Barr (EB) virus [6]. The EB virus transformed lines are designated as EBVT and the description of their origin is given in Table 1. All cells were grown at 37 °C in RPMI 1640 medium supplemented with 10% fetal calf serum.

¹²⁵I-insulin binding to the lymphoid cells was measured at 15 °C using a tracer size of 0.1 ng/ml, as previously described [6].

Fluorescence flow cytometry

Cultured cells were washed twice in cold serum-free medium. The cell pellet was resuspended in cold RPMI medium plus 1% bovine serum albumin to a concentration of 2.4×10^7 cells/ml. Cells ($2 \times 10^6/100 \mu\text{l}$) were then incubated with anti-receptor antibody or a control antibody (1:100 dilution) for 1 h at 4 °C. The cells were washed twice with phosphate buffered saline at 4 °C and incubated for an additional hour at 4 °C with a fluorescein conjugated second antibody (1:30 dilution). Cells were again washed twice with phosphate buffered saline at 4 °C and analyzed using a Coulter Epics V flow cytometer (Coulter Electronics, Hialeah, Florida, USA). Cells ($10\text{--}25 \times 10^3$ per run) were analyzed by log integral green fluorescence.

Results

The fluorescence intensity distribution of insulin receptors on four human lymphoblastoid cell lines was determined by incubating the cells with monoclonal anti-

Table 1. ^{125}I -insulin binding and anti-receptor antibody binding to different human lymphoid cell lines

Cell line	Patient type	^{125}I -insulin binding (%)	Peak fluorescence Anti-receptor antibody	
			Poly-clonal (%)	Monoclonal (%)
IM-9	Multiple myeloma	100	100	100
EBVT-1	Normal female	78	62	48
EBVT-2	Diabetic insulin resistant female	22	40	27
EBVT-3	Rabson Mendenhall syndrome	2	20	0
EBVT-4	Type A extreme insulin resistance	9	7	0
EBVT-5	Leprechaun (Ark-1)	8	4	1
EBVT-6	Father of leprechaun (Ark-1)	8	24	17
EBVT-7	Mother of leprechaun (Ark-1)	65	46	46
EBVT-8	Mother of leprechaun (Minn-1)	8	22	15

body to the insulin receptor or a control antibody and analyzed using the flow cytometer (Fig. 1). Monoclonal anti-receptor antibody specifically labelled insulin receptors on IM-9 cells with mean peak fluorescence that was at least 60 fluorescent units over the peak of the control antibody. A similar pattern was seen using a human polyclonal anti-receptor antiserum (data not shown). Fluorescence analysis was then conducted using three different EB virus transformed cell lines which were known to represent a range of insulin binding. EBVT-1 cells derived from a normal female had 48%–62% of the peak fluorescence exhibited by IM-9 cells, EBVT-2 cells from an insulin-resistant diabetic female had 27% of the maximal fluorescence, and EBVT-3 cells from a patient with Rabson Mendenhall syndrome exhibited almost no specific fluorescence with monoclonal antibody. In each case, there was a close correlation between ^{125}I -insulin binding and maximal peak fluorescence (Table 1). When the human polyclonal anti-receptor serum was used, a similar pattern was seen; however, with the EBVT-2 and EBVT-3 cell lines, the polyclonal antibody showed relatively higher binding than the monoclonal antibody (Table 1), suggesting that this serum might contain antibodies other than the anti-insulin receptor antibodies which bind to human lymphocytes.

Using this technique, a more extensive evaluation of other transformed lymphocytes was performed (Table 1). Cells from a patient with leprechaunism (EBVT-5) and from a patient with the type A syndrome of insulin resistance and acanthosis (EBVT-4) exhibited almost no specific fluorescence with the monoclonal

Fig. 1. Immunofluorescence analysis of the insulin receptor on human lymphoid cell lines. Cultured cells (2×10^6 cells/100 μl) were stained for flow cytometric analysis as described in Materials and Methods. Panel a shows fluorescence histograms of IM-9 cells incubated with either a control antibody (P3) or monoclonal anti-receptor antibody. Panels b–d represent fluorescence histograms of the EBVT cell lines incubated with either P3 or monoclonal anti-receptor antibody

anti-receptor antibody and a very low binding of ^{125}I -insulin or polyclonal anti-receptor antibody. Cells from the parents of two different patients with leprechaunism [Arkansas 1 (Ark-1) and Minnesota 1 (Minn-1)] showed a wide range variation, with one parent of each pair showing markedly reduced receptor content by this technique. In all cases, there was a good correlation between the monoclonal binding and insulin binding and fluorescence analysis revealed a single peak of cells.

Discussion

Autoimmune [7] and monoclonal [9, 10] antibodies to insulin receptors have provided useful tools for purifying the insulin receptor and determining several of its properties. Human autoantibodies to the receptor inhibit insulin binding, mimic many of the actions of insulin and immunoprecipitate solubilized insulin receptors [11]. The monoclonal antibodies immunoprecipitate insulin receptor, but do not inhibit insulin binding, suggesting that these antibodies interact with the receptor at a site distinct from the insulin binding site.

In this study, we have characterized the distribution of insulin receptors on human lymphocyte cell lines and studied their expression using anti-receptor antibodies and flow cytometry. Our studies employed IM-9 cells as a model system because much of our knowledge regarding the insulin receptor was initially derived from studies of these cells [12]. Both the monoclonal and polyclonal anti-receptor antibody specifically labelled insulin receptors on these cells, with the monoclonal antibody showing the highest specific labelling.

Cells derived from patients with different receptor numbers demonstrated immunofluorescent labelling of insulin receptors. Three cell lines derived from patients with genetic defects in the insulin receptor (EBVT-3, 4, and 5) showed virtually no antibody binding and no ^{125}I -insulin binding. In addition, the father of leprechaun (Ark-1; EBVT-6) and the mother of leprechaun (Minn-1; EBVT-8) showed markedly reduced binding of anti-receptor antibodies and ^{125}I -insulin. In some cases, the binding in parents was as low as that observed in the patients, suggesting that the parent may exhibit a receptor defect which has been inherited by their child. In all cases, there was a good correlation between insulin binding and fluorescence labelling.

One feature of the insulin receptor that can be uniquely studied using the immunofluorescence technique is the question whether cell lines have a uniform distribution of receptors or may be bimodal, i.e., containing cells with a low number of receptors or some with a normal or high number of receptors. Since fluorescence is analyzed individually for each cell, it was possible to see that all cells have a proportionally high, medium, or low number of receptors depending on the line.

EB virus transformed lymphocytes have provided a useful tool for the study of human cells in culture and have been used previously to study insulin expression [13]. Since these cells grow in suspension, they provide an ideal cell type for flow cytometry analysis. However, certain caveats with regard to these cells need to be considered. First, EB virus transforms only a small percentage of lymphocytes. Thus, differences in receptor expression may reflect the genetics of transformation, the genetics of insulin receptor expression, or both. Not surprisingly, transformed lymphocytes from normal individuals exhibit a rather wide range of ^{125}I -insulin binding which may be a result of these genetic variations or even a result of the transformation process itself. With regard to the latter point, however, comparative studies measuring ^{125}I -insulin binding in cultured lymphocytes and circulating blood cells from the same individuals have revealed a close correlation [7], suggesting that transformation does not change the expression of the insulin receptor and reflects the picture seen *in vivo*.

In conclusion, insulin receptor expression can be

studied quantitatively using fluorescence flow cytometry. The experiments described in this study indicate that different populations of human lymphocytes express different levels of immunoreactive insulin receptor on their surface. These studies suggest that flow cytometry is a useful tool in the study of insulin receptors and their expression in hyper- and hypoglycaemic syndromes in man.

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