Insulin Receptors on Leukemia and Lymphoma Cells

By Po-min Chen, Siu-hoi Kwan, Tien-szu Hwang, Benjamin N. Chiang, and Chen-Kung Chou

Tumor cells obtained from leukemia and lymphoma patients were investigated for specific insulin receptors. Using radioactive 125I-labeled insulin, specific insulin binding sites were demonstrated on most acute lymphocytic leukemia (ALL) and acute myelocytic leukemia (AML) cells, including acute promyelocytic leukemia (APL), chronic myelocytic leukemia (CML), and acute monocytic leukemia (AMoL) cells. Insulin receptors were not found on chronic lymphocytic leukemia (CLL) and malignant lymphoma (ML) cells. Specific insulin binding sites were also found on monocytes and thymocytes after treatment with phytohemagglutinin (PHA-P), but not on inactivated tonsil cells, peripheral blood lymphocytes, or thymocytes. There was no inverse correlation between the content of insulin receptors and the basal level of circulating insulin. These data suggest that the insulin receptor may be a new marker of acute leukemia and chronic myelocytic leukemia.

Insulin plays a central role in the regulation of the intermediary metabolism of higher animals. The biologic action of insulin appears to be initiated by the interaction of the hormone with its specific receptor on the plasma membrane of the target cell. A specific receptor for insulin has been characterized on many tissues, such as adipose, liver, and skeletal muscle, and is also found in cells whose metabolism is not obviously altered by insulin, including fibroblasts, monocytes, erythrocytes, granulocytes, and cultured leukemic lymphoblasts.

Helderman et al. reported that the resting lymphocyte does not have insulin receptors in vivo. However, receptors do emerge on T lymphocytes after concanavalin-A treatment or allogeneic skin grafting. A similar phenomenon with B lymphocytes was observed after in vitro stimulation. Insulin has also been shown to augment glucose transport, glucose oxidation, lactate oxidation, and amino acid transport in activated T lymphocytes. It has been suggested that the lymphocyte insulin receptor may be a marker of lymphocyte activation and that insulin could play an immunoregulatory role in the cytotoxic responses of that cell.

Significant differences in insulin binding were observed between null cell and T-cell-type acute lymphocytic leukemia (ALL), and ALL and chronic lymphocytic leukemia (CLL). However, for other malignant cells, such as myelocytic leukemia and lymphoma, no results have been reported. In the present study, we examine and compare the specific insulin receptors on leukemic cells and try to illustrate the relationship of insulin receptors to various types of leukemias.

MATERIALS AND METHODS

Patients

The study group was comprised of patients of the Veterans General Hospital, Taipei, Taiwan, and consisted of 10 cases of acute myelocytic leukemia (AML), 1 case of acute promyelocytic leukemia (APL), 4 cases of acute mononocytic leukemia (AMoL), 8 cases of chronic myelocytic leukemia (CML), 10 cases of ALL, 4 cases of CLL, 3 cases of malignant lymphoma (ML), 7 cases of polycythemia vera, and 3 cases of severe infection. Patients with peripheral blood (PB), bone marrow (BM), or lymph node abnormalities with an excess of 50% leukemic cells or ML established by lymph node biopsy were chosen for this study.

Diagnosis was based on the presence of abnormal leukemic cells in the PB and/or BM revealed by conventional morphological and cytochemical criteria. Surface markers, including E, EA, EAC rosette assay, surface immunoglobulin (Slg), cytoplasmic immunoglobulin (CmIg), anti-common ALL, Ia antisera, and OKT monoclonal antibodies, were also analyzed as described.

Cell Collection

Leukemic cells were obtained from patient PB or BM by venous puncture or BM aspiration; over 80% of leukemic cells were obtained by the Ficoll-Hypaque method, with 95% viability. Neutrophils (over 95% pure) were obtained using a continuous blood cell separator. Monocytes (over 60% pure) were separated by a method previously described. Eosinophils (over 80%) were obtained from the patient with eosinophilia.

Thymocytes were obtained from the thymus during cardiac surgery; tonsil cells and lymphoma cells were obtained from tonsillectomy or lymph node biopsy of patients with ML. Each tissue was suspended in phosphate-buffered saline (PBS), and the cells separated by squeezing the organ between 2 glass slides, filtered through 2 sheets of cotton, and collected by centrifugation.

Iodination of Human Insulin

Semi-synthetic human insulin was kindly donated by the Novo Company, Copenhagen, Denmark. 125I-insulin was prepared by the chloramine-T method to a specific activity of approximately 100 μCi/μg. The radioactivity was 95%–100% precipitable by 5% trichloroacetic acid.

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**Insulin Binding Assay**

The leukemic cells were suspended in a modified Eagle's medium with 1% bovine serum albumin (pH 7.8) to a concentration of $5 \times 10^6$ cells/ml. Cells were incubated with different concentrations of $^{125}$I-insulin alone or in the presence of unlabeled insulin (1 $\mu$M) to determine the nonspecific binding. Duplicate assays were performed at each insulin concentration. After incubation with constant shaking at 25°C for 60 min, 100-μl aliquots were added to microfuge tubes containing 400 μl dibutyl phthalate ($d = 0.037$) and centrifuged at 10,000 g for 1 min. The supernatants were aspirated; the tip, which contained the cell pellet, was cut and counted in a Beckman γ-counter at 70% efficiency. In all studies, the degradation of insulin during the incubation was less than 5%, as determined by precipitation in 5% trichloroacetic acid.

Plasma glucose concentration was determined by the glucose oxidase method. Plasma insulin concentration was measured by radioimmunoassay (Hypol. Hypolab. S.A., Switzerland).

**RESULTS**

$^{125}$I-insulin was incubated with $5 \times 10^6$ myeloblast cells/ml in the absence or presence of unlabeled insulin. The time course of insulin binding at 25°C was examined. Figure 1 shows that specific insulin binding reached a plateau in about 40 min. Nonspecific binding represents 20% or less of total binding. The optimal pH for insulin binding was approximately 8 (data not shown), which is similar to many other systems reported.

Specific insulin binding to myeloblasts was detected. Figure 2 shows a typical binding curve for increasing concentrations of radiolabeled insulin. When the binding data were analyzed by Scatchard plot, a curvilinear line was obtained that is consistent with earlier studies with insulin receptors on monocytes, muscle cells, adipocytes, and liver cells. The dissociation constant, $K_d$, for insulin-labeled leukemic cells could be calculated from the slope of the Scatchard plot and is about 2nM, which represents a class of insulin receptor with high affinity. There were no significant differences in the $K_d$ for insulin in leukemic cells bearing insulin receptors (data not shown). The percentage of insulin bound to cells was low, and varied from 1% to 5%. However, the nonspecific binding never exceeded 20% of the total binding.

To further validate our binding assay, we tested specific insulin binding to monocytes, eosinophils, tonsil cells, thymocytes, T lymphocytes, phytohemagglutinin (PHA-P) treated T lymphocytes, and neutrophils from polycythemic, severely infected, and normal persons. Only PHA-P-treated activated T lymphocytes and monocytes showed specific insulin binding, consistent with other reports.

From the measurement of specific insulin binding to cells, the number of insulin receptors with high affinity could be estimated from the intercept of the Scatchard plot on the X-axis (Fig. 2). The results of the examination of insulin receptors on different leukemia cells are summarized in Table 1. Nine of 10 AML patients, all AMoL (4 cases), and 1 APL patient were positive for insulin receptors. For CML, 5 of 6 patients and 2 cases of CML with blast crisis also were positive for insulin receptors. Nine of 10 ALL patients, except 1 case with T-ALL, including null cell type, IA, cALL, pre-B and B-cell types, bound insulin specifically, whereas the CLL and ML cells showed no specific insulin binding (Table 2).

We also checked serum insulin levels of 27 patients. All of them showed normal fasting blood sugar (60–110 mg/dl), while 11 of 27 showed higher insulin levels in serum than normal (6–22 μU/ml). However, no correlation has been found between the number of insulin receptors on leukemia cells and the serum insulin level.
DISCUSSION

Helderman et al. studied insulin receptors on lymphocytes and concluded that insulin receptors may be universal surface markers for activated T and B lymphocytes. Esber et al. examined insulin binding in childhood ALL. An interesting difference was found between null cell and T-cell-type ALL. Lymphoid cells from null-cell-type ALL showed substantially higher insulin binding activity than T-cell ALL patients. The insulin binding was also studied in adult patients with ALL and CLL, and similar observations were made. In the patients with CLL, some lymphoid cells showed very low insulin binding, while others showed total absence of insulin receptors.

Insulin receptors that we have characterized on the surface of leukemic cells have a similar pH dependence, time dependency, and specificity of insulin binding to that of the classic insulin receptors on target cells, such as hepatocytes and adipocytes.

To rule out the possibility that insulin binding of tumor cells was not due to monocyte contamination in our cell preparations, we examined for the presence of monocytes in each case. ALL cell preparations contained over 80% tumor cells, and less than 3% monocytes could be detected. At the cell concentration (5 x 10⁶) used to test insulin binding activity, the assay could only detect monocyte insulin binding activity if the monocytes represented over 20% of the population under study (Fig. 3). Our results indicate that insulin receptors are present on most ALL cells, but not on CLL and T-ALL cells. This agrees with the data of Esber et al. and Thomopoulos et al. For ALL, detailed analyses of other surface markers have been done. Positive cases belong to null cell, Ia, cALL, pre-B, and B-cell types.

Insulin receptors could be detected on 5 of 6 patients with CML. These cell populations contain many immature cells, including myeloblasts, promyelocytes, and myelocytes in PB. No receptors were found. In only one case of CML were no receptors found, and this patient's cells contained mostly mature granulocytes after chemotherapy. This observation is in contrast to the report by Fussganger et al. They showed...
Table 2. Insulin Binding to Lymphoid Cells From Lymphocytic Leukemia and Lymphoma Patients

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Cases 1–10: ALL. ML.PD, malignant lymphoma, poorly differentiated.
ND, not done.
*²¹²⁵I-insulin binding is expressed as the number of binding sites per cell. This number was calculated after transformation of the binding data according to Scatchard.
†Positive to S-33 (90%) and OKT11A (92%) monoclonal antibodies.

that human granulocytes exhibit insulin binding activity if they included protease inhibitors in their assay system. However, the receptor number they detected on granulocytes was low (~1,000/cell), as was the affinity (~20nm). The nature and physiologic significance of insulin receptors on granulocytes is still questionable.

The insulin receptors demonstrated on most AML, AMoL, and CML cells support the hypothesis that insulin receptors were only present on immature myelocytic cells and disappeared during the maturation process.

Luke has suggested that human malignant lymphomas might appear as a result of a “block” or a “switch on” in a process of lymphocyte transformation. However, in our studies, 3 cases of poorly differentiated ML showed no insulin receptors at all on the surface membrane. We don't know if this means that the blockage of ML occurred at an early stage of lymphocyte transformation before the emergence of insulin receptors. Nevertheless, further follow-up in these cases might provide more clues as to the defect of ML.

We also examined the correlation between the serum insulin level and the number of insulin receptors on leukemic cells. All patients showed normal fasting blood sugar levels, and some of them showed elevated serum insulin levels. However, no correlation has been found (r = 0.31) between receptor number and plasma insulin level.

In summary, we have confirmed earlier observations on the difference of insulin receptors among null-cell-type ALL in children and ALL and CLL in adults. We further extended these observations to insulin...
of disease or tumors arising at different stages of cellular differentiation is still a question requiring more study.

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PM Chen, SH Kwan, TS Hwang, BN Chiang and CK Chou