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Difference in the Number of Insulin Binding Sites between Cortisol-Sensitive and Cortisol-Resistant Lymphoma P1798 Cells (39291)

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The corticoid-sensitive (CS) and corticoid-resistant (CR) strains of lymphosarcoma P1798 differ markedly in cell surface characteristics. Thus, Behrens *et al.* (1) showed that CS cells possess a negative surface charge which is absent in CR cells. In addition, plasma membrane glycopeptides from CS lymphocytes differ in their chromatographic behavior from those of the resistant tumor (2).

Insulin receptors are generally thought to be located on the cell surface (3). We have found that lymphoma P1798 cells specifically bind [^{125}I]insulin and we have used this binding as a probe to explore further the surface differences between CS and CR tumor lymphocytes.

Materials and methods. Crystalline porcine insulin, crystalline porcine glucagon, and porcine proinsulin were gifts from Lilly Laboratories. Porcine somatotrophin, B grade, was from Calbiochem and Synacten was generously supplied by Ciba Laboratories. Na^{125}I was purchased from New England Nuclear Corp. Bovine serum albumin, fraction V, was from the Sigma Chemical Co.

CS and CR strains of P1798 lymphosarcoma were maintained by serial sc transplantation in female BALB/c mice (Mammalian Genetics and Animal Production Section, Drug Research and Development, N.C.I.) as described previously (4). Cell suspensions were prepared using the technique of Stevens *et al.* (5), except that the tumor strips were teased in 50 m*M* Tris-HCl, pH 7.8, containing 10 m*M* dextrose, 1 m*M* EDTA, 1.4 m*M* Na-acetate, 0.5 m*M* KCl, 120 m*M* NaCl, 1.2 m*M* $MgSO_4$, and 1% bovine serum albumin (6), instead of in Dulbecco's medium. Broken and dead cells were removed from the cell suspension by washing once with the same buffer. The cells were then resuspended in buffer and viability (usually 90% or more) determined at the beginning of each experiment by trypan blue exclusion. [^{125}I]Insulin of high specific activity (300-350 μCi/μg) was prepared as described by Cuatrecasas (7).

Triplicate 0.5-ml aliquots of cells in teasing buffer (10^7 cells/ml) were incubated for 15 min at 15° in the presence or absence of 1×10^{-5} *M* native insulin. [^{125}I]Insulin was then added to each tube at a final concentration of 1×10^{-10} *M* and the incubation continued for 1 hr. At the end of incubation, 0.4 ml of the cell suspension was layered onto 0.6 ml of buffer in plastic microtubes. After centrifugation in a Brinkman microfuge, the pellet was washed with 10% aqueous sucrose and radioactivity determined in a Beckman gamma counter, with 63% efficiency. Specific insulin binding was obtained by subtracting from the total radioactive uptake, the amount not displaced by 10^{-5} *M* native insulin (7).

Results. Effect of cell concentration. Figure 1 shows that binding of [^{125}I]insulin by CS lymphocytes is directly proportional to cell concentration between 0.3 and 1.5×10^7 cells/ml. Deviation from linearity at higher cell concentrations probably results from insulin degradation since 20% of the [^{125}I]insulin was not precipitable with 10% trichloroacetic acid after incubation with 3×10^7 cells/ml. No insulin degradation was observed with CR lymphocytes at concentrations up to and including 1.5×10^7 cells/ml. Therefore, the lower insulin binding by CR cells described below is not due to insulin breakdown during incubation.

Influence of pH on insulin binding. Binding of [^{125}I]insulin by CS cells occurs over a relatively narrow pH range as illustrated in Fig. 2. Maximal binding took place at pH 7.8, as reported by Gavin *et al.* (8) and Olefsky and Reaven (9) for human lymphocytes. Similarly, binding of [^{125}I]insulin by

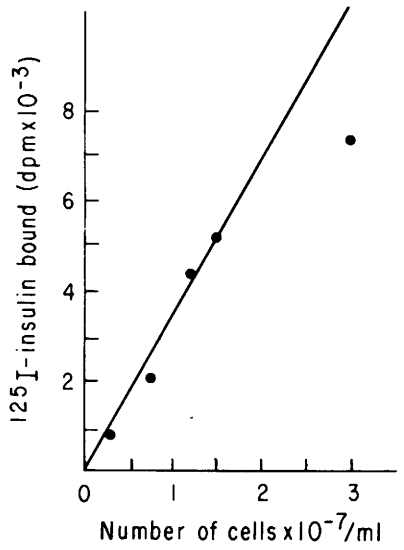

FIG. 1. Specific binding of [^{125}I]insulin by CS lymphocytes as a function of cell concentration. Specific binding was determined as in Methods.

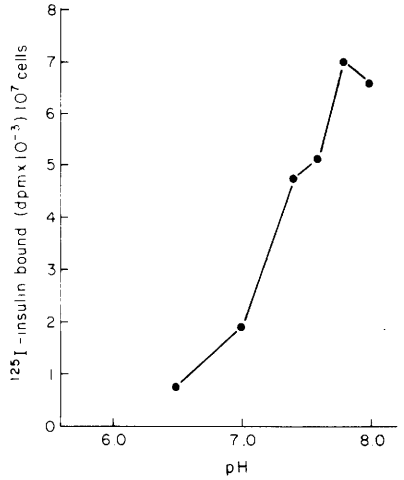

FIG. 2. Influence of pH on specific binding of [^{125}I]insulin by CS cells. Determination of specific binding is described in Methods. Buffers used were: pH 6.5, 0.1 *M* Na acetate-acetic acid; all others were 50 m*M* Tris-HCl. [^{125}I]Insulin was 2 × 10^{-10} *M*.

CR lymphocytes was much higher at pH 7.8 (4900 dpm/10^7 cells) than at pH 7.6 (3400 dpm/10^7 cells). These values are 30–35% lower than the levels obtained with CS cells, assayed under identical conditions. It was not possible to study insulin binding above pH 8.0 because of cell breakage in the alkaline medium. Consequently, all incubations were performed at pH 7.8.

Effect of time and temperature on insulin binding and degradation. Figure 3 shows that binding of [^{125}I]insulin to the cells is time and temperature dependent. At 15°, binding was rapid and reached a constant value after 1 hr. Under these conditions, the binding was stable for at least 2 hr more. A similar curve was obtained at this temperature for the CR lymphocytes, except that the levels of [^{125}I]insulin binding were 40–50% lower at all the time points studied. At 5°, insulin bound more slowly and failed to reach a plateau after 3 hr. Binding was extremely low at 0°. At 25°, insulin binding plateaued at a much lower level than at 15° probably due to extensive degradation of the hormone since 50% of the [^{125}I]insulin had been rendered acid-soluble by 60 min. Similar results were obtained by Gavin *et al.* (8) and Olefsky and Reaven (9) with human lymphocytes and by Goldfine *et al.* (17) with rat thymocytes.

Effect of unlabeled insulin on binding of [^{125}I]insulin. When 1 × 10^7 CS cells/ml were incubated with 1 × 10^{-10} *M* [^{125}I]insulin, 1.12 ± 0.05% of the total radioactivity was bound compared to only 0.62 ± 0.01% in the CR cells (average of 19 separate experiments). As shown in Fig. 4, 50% of specifically bound [^{125}I]insulin was competed for by 2 to 5 × 10^{-8} *M* native insulin in both CS and CR lymphocytes. Twenty percent of the bound radioactivity in CR cells and 15% in the CS cells could not be competed by 1 × 10^{-5} *M* insulin and was considered to be nonspecific binding.

Specificity of insulin binding. Figure 5 shows that specific binding of [^{125}I]insulin to

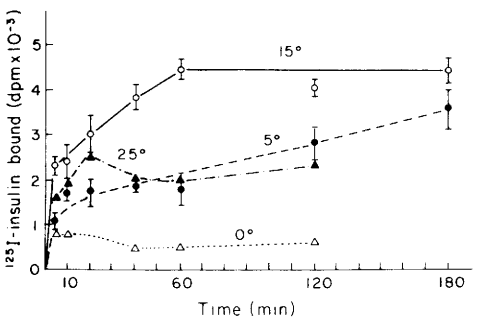

FIG. 3. Effect of time and temperature on insulin binding by CS lymphocytes. Determination of specific binding is described in Methods. Results are expressed as dpm specific binding/10^7 cells. Vertical bars represent SE of three experiments.

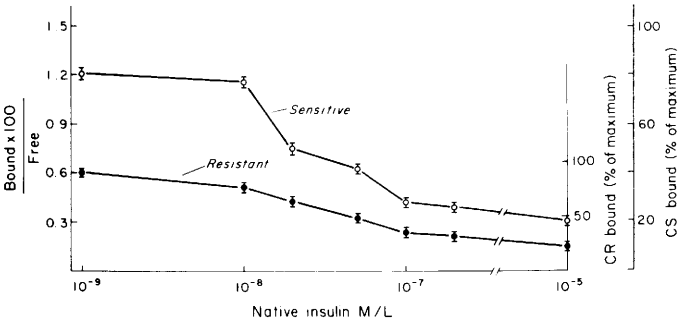

FIG. 4. Effect of unlabeled insulin on binding of [^{125}I]insulin to CS (○) and CR (●) cells. Left: percentage of total bound/free [^{125}I]insulin is plotted as a function of the native insulin concentration in the medium. Right: percentage of maximum [^{125}I]insulin bound by cells. Each point represents the mean ± SE of triplicate determinations. Details of assay procedure are described in Methods.

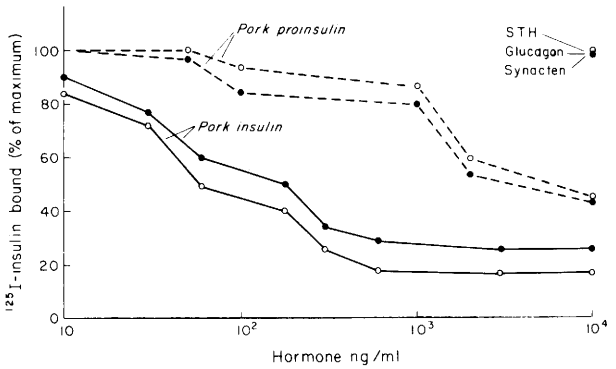

FIG. 5. Specificity of insulin binding. Effect of unlabeled insulin and other hormones on binding of [^{125}I]insulin by CS (○) and CR (●) lymphocytes. The inhibition of [^{125}I]insulin binding, expressed as a percentage of maximum, is plotted as a function of concentration of unlabeled hormones. Details of assay procedure are described in Methods.

CS and CR lymphocytes is unaffected by preincubation with somatotrophin, glucagon, and Synacten, and is partially inhibited by pork proinsulin.

Effect of macrophages on insulin binding. It has been reported that macrophages and monocytes possess a relatively large number of insulin binding sites. Hormone binding by CS and CR tumor cell suspensions was shown not to involve macrophages by performing assays before and after macrophage removal. This was achieved by incubating the tumor cells in plastic tissue culture flasks at 37° for 1.5 hr, as described by Rinehart *et al.* (11). The nonadherent cells (lymphocytes) were removed and assayed for insulin binding, while the adherent population (macrophages) was visualized by reincubating the flask with 10% trypan blue in 0.15 *M* NaCl for 30 min or with 1% carbon in Eagle's minimal essential medium for 120 min. Insulin binding by CS and CR cells was not influenced by this procedure.

Scatchard analysis. [^{125}I]insulin binding to CS and CR P1798 lymphocytes was studied under steady-state conditions in the presence of varying concentrations of labeled hormone. Saturation was reached at 10^{-9} *M* [^{125}I]insulin. When bound/free [^{125}I]insulin was plotted as a function of insulin bound according to Scatchard (12) (Fig. 6), straight lines with similar slopes were observed in both strains of tumor, indicating similar dissociation constant for CS and CR cells. In contrast, the total number of binding sites was significantly lower in the CR lymphocytes ($P < 0.02$). Our results suggest that CR cells may have a lower concentration of sites per unit surface area since no difference in size distribution was found between lymphocytes obtained from both strains of tumor (Table I). This does not

TABLE I[a]

P1798 cells	No cells with less than 5 μm diameter	No cells between 5 and 7 μm diameter	No cells between 7 and 11 μm diameter
Sensitive	4000 ± 1490	7133 ± 152	14648 ± 1169
Resistant	1652 ± 546	6699 ± 1304	15875 ± 113

[a] Size distribution of CS and CR P1798 lymphocytes. Cell size was determined with a Bio/Physics system Cytograf. Mean ± SE of two separate experiments.

exclude the possibility that reduced binding by CR cells may be related to the previously described differences in cell surface between CS and CR P1798 lymphocytes (1).

Discussion. This study demonstrates that lymphoma P1798 cells bind insulin specifically. In addition, we have found a highly reproducible difference in number of binding sites between the CS and CR tumor lymphocytes; thus, CR cells bind 40% less insulin than CS cells. The apparent dissociation constant for binding was very similar for both strains of tumor and was comparable to that of mouse thymocytes (10) but considerably higher than the corresponding value for human lymphocytes (8, 9). This is not unexpected since lymphoma P1798 is of thymic origin (13). Chang *et al.* (14) suggested that apparent changes in insulin binding in obese animals result from alterations in membrane glycoproteins. The reduced number of insulin binding sites in CR P1798 lymphocytes may be a reflection of differences between CS and CR cells with regard to coat structure and membrane glycopeptides (1, 2). The role of insulin in lymphocytes is unknown, but effects on glucose transport (15) and metabolism (16), activation of membrane ATPase (15) and transport of aminoisobutyric acid (17) have been described. In P1798 lymphosarcoma, Rosen *et al.* (18), in a single experiment, observed that insulin had no influence on glucose uptake in CS cell suspensions and that cortisol plus insulin did not significantly alter the inhibition produced by cortisol alone. In another variant of this tumor, Stevens *et al.* (19) found that exogenous glucose was not required for cortisol to exert metabolic effects on CS cells in the presence of amino acids in the medium.

The existence of insulin binding sites in human leukemic cells (20) and in lymphoma P1798 (this study) suggests that proliferation of certain malignant lymphocytes may be under insulin control. We are currently exploring the possibility that the difference in insulin binding between CS and CR strains of P1798 lymphocytes may be related to the response of this tumor to glucocorticoid therapy.

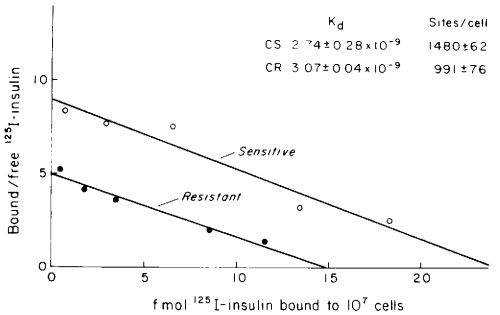

FIG. 6. Scatchard plot of [^{125}I]insulin binding by CS (○) and CR (●) cells. Assays were performed at 1×10^{-10}, 5×10^{-10}, 1×10^{-9}, 5×10^{-9} and 1×10^{-8} *M* [^{125}I]insulin. Details of assay procedure are described in Methods. Average K_d and number of binding sites represent the mean ± SE of three experiments.

Summary. Cortisol-sensitive and cortisol-resistant lymphoma P1798 cells specifically bind [^{125}I]insulin. Resistant lymphocytes bind 40% less insulin than sensitive cells. These results suggest that insulin (or insulin-like substances) may play a role in growth regulation and/or response of this tumor to glucocorticoid therapy.

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