

## C-Peptide Does Not Parallel Increases of Serum Levels of Substances Immunologically Cross-Reactive With Insulin in Non-Hodgkin's Lymphoma Patients

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In 28 of 45 patients suffering from non-Hodgkin's lymphoma, the blood levels of substances detectable by the insulin-specific radioimmunoassay were supranormal, while the C-peptide levels were almost normal or even low. Such a ratio of these substances was also found in a diabetic non-Hodgkin's lymphoma patient. Two sera with

**C**LINICAL<sup>1-3</sup> and experimental<sup>4</sup> evidence reveals that tumors can be accompanied by hypoglycemia and by elevated levels of nonsuppressible (by anti-insulin antibodies) insulin-like protein(s)<sup>1</sup> and by entity(ies) immunologically cross-reactive with insulin.<sup>2-4</sup> Elevated levels of this substance(s) are induced by the tumors and they enhance tumor growth.<sup>4-6</sup> We examined the levels of glucose, substances immunologically cross-reactive with insulin (SICRI), and C-peptide in the blood of 45 non-Hodgkin's lymphoma patients. The serum levels of SICRI were dramatically elevated and the corresponding values of C-peptide were low, sometimes even subnormal. As it is normal that peripheral levels of this peptide are quantitatively related to those of peripheral insulin,<sup>7,8</sup> our data reveal an altered status of insulin (SICRI) and C-peptide metabolism in lymphoma patients. Moreover, the finding of a diabetic patient suffering from non-Hodgkin's lymphoma with supranormal levels of SICRI and subnormal C-peptide is not incompatible with the idea of ectopic SICRI production. In addition, the gel filtration data in this article are the first evidence of the molecular properties of SICRI.

### MATERIALS AND METHODS

The sera of 26 lymphoma patients in the relapse phase and of 19 in the remission phase of disease were examined for levels of glucose by the *o*-toluidine method<sup>9</sup> and for SICRI and C-peptide by radioimmunoassay (RIA).<sup>10,11</sup> For SICRI determination, the commercial RIA kit was obtained from Sorin (Saluggia, Italy; sensitivity less than 5  $\mu$ U of insulin/1 ml of serum) and for the C-peptide from Byk-Mallinckrodt (Dietzenbach, Germany). Therefore, the SICRI concentrations are relative, expressed as insulin equivalents. Sera of patients N.N. (28  $\mu$ U/ml), K.M. (36  $\mu$ U/ml), M.M. (1040  $\mu$ U/ml), and D.P. (560  $\mu$ U/ml) were titrated by RIA and the results were compared with the titration data of insulin standard.

A portion of one serum sample (patient K.M., 36  $\mu$ U SICRI/ml) was incubated with radioiodinated insulin (Novo, Copenhagen, Denmark) for 3 days and chromatographed on a Sephadex G50 (1  $\times$  60 cm) column.<sup>12</sup>

The sera of two patients had exceptionally high SICRI levels. The serum of patient D.P. contained 560 and of patient M.M. 1040  $\mu$ U SICRI/ml. These sera were chromatographed on a 0.9  $\times$  65 cm

the highest concentrations of these substances were investigated by gel filtration. In one case, the insulin cross-reactive material was eluted at the position of the insulin marker; in the other serum, its molecular mass was approximately 120,000.

Sephadex G100 column in 20 mM sodium phosphate containing 0.15 M NaCl, pH 7.4, at 4°C. Also, a portion of the serum of patient M.M. was chromatographed on a 1  $\times$  55 cm Sepharose 6B column at 4°C using the same mobile phase. The columns were calibrated with Blue Dextran, <sup>125</sup>I-insulin, potassium hexacyanoferrate, human serum albumin, and human immunoglobulin G. The fractions were assayed for SICRI by RIA.

One portion of patient M.M.'s serum was mixed with an equal volume of 2.0 M acetic acid. The resulting precipitate was separated from the supernatant by centrifugation and both samples were freeze-dried. They were reconstituted in 20 mM sodium phosphate containing 0.15 M NaCl, pH 7.4, and were assayed for SICRI by RIA.

One of the patients was diabetic and none suffered from an insulinoma. Patient B.E. (age 46) was found to be diabetic in 1966 and began treatment with oral antidiabetics at that time. In 1977, non-Hodgkin's lymphoma was diagnosed. In October 1979, she was admitted to the Military Medical Academy in Belgrade, during the relapse phase of disease; since then she stopped the antidiabetic therapy. On October 10, 1979, glucose in her preprandially drawn blood was 7.8 mM and SICRI was 73.7  $\mu$ U/ml. The next morning the oral glucose tolerance test was performed: at zero-time, when the patient ingested 50 g glucose in 300 ml water, glucose in blood was 7.33 mM (mean normal value 5.56 mM), 1 hr after oral glucose intake it was 12.78 mM (normal 8.89 mM), and 2 hr after intake it was 9.44 mM (normal 6.67 mM). At zero-time, SICRI in blood was 68.5  $\mu$ U/ml. On October 15, 1979, glucose in blood was 12.78 mM and SICRI 70.0  $\mu$ U/ml. After cyclophosphamide treatment (6 mg/1 kg body weight every second day for 5 wk), glucose in the preprandially drawn blood was 3.0 mM and SICRI 64  $\mu$ U/ml on June 5, 1980.

The patients received no medication at least 30 days before glucose, SICRI, and C-peptide determinations and did not take any food overnight (at least 8 hr) before blood sampling.

### RESULTS

The data on the correlation of SICRI and C-peptide concentrations in peripheral blood of lymphoma

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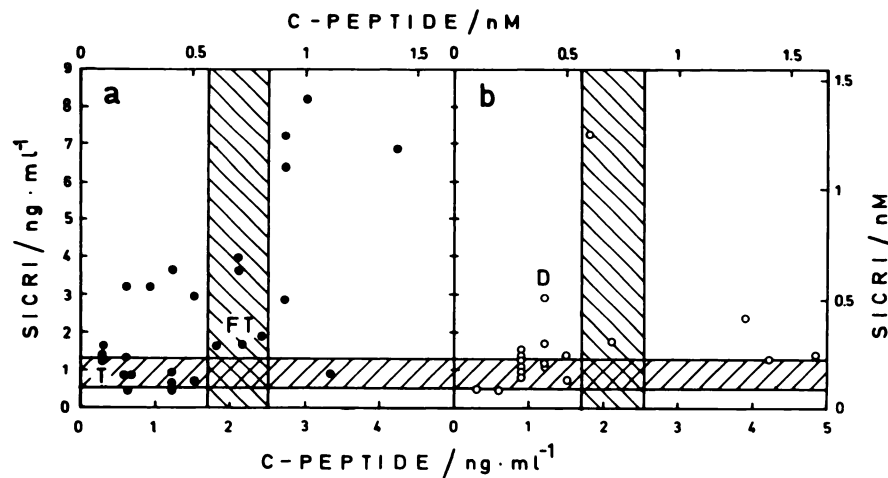


Fig. 1. The correlation of the serum levels of substance(s) immunologically cross-reactive with insulin (SICRI) and C-peptide in (A) 26 patients in relapse and (B) in remission phase of non-Hodgkin's lymphoma. Shaded areas denote the ranges of normal values of substances detectable by insulin and C-peptide specific radioimmunoassays in our laboratory, respectively. *D* denotes results obtained with blood of the diabetic patient B.E.; *T* stands for sera with which full RIA titrations of SICRI were performed; and *F* for the serum that was gel-filtered to check for the presence of insulin-binding macromolecules. The linear least-square regression analysis of data in (A) yields the relation (SICRI) = 3.32 (C-peptide) - 2.75 with  $r = 0.636$ ; for data in (B),  $r = 0.346$ ; both concentrations expressed in ng/ml.

patients are presented in Fig. 1. Of 26 patients in relapse, 16 (62%) had elevated SICRI (Fig. 1A). Of 19 patients in remission, 9 (47%) displayed SICRI levels above normal (Fig. 1B). (The normal range is defined as the range between the mean minimal and maximal values determined in 200 healthy persons.) The highest SICRI level was seven times that of the normal concentration of immunoreactive insulin. Most of the patients with elevated SICRI were hypoglycemic (Fig. 2).

Contrary to the high percentage of patients with supranormal levels of SICRI, the C-peptide levels were above the mean level of normal in 9 of 45 patients (20%) only; the highest value was less than twice above normal (Fig. 1).

The RIA titration curves of the sera of patients N.N. and K.M. were parallel to those of insulin standard above the concentrations of  $5 \mu\text{U}/\text{ml}$  (sensitivity limit). On the other hand, the RIA titration curves of the two sera with very high SICRI (patients D.P. and M.M.) were not strictly parallel with the standard dilution curve (Fig. 3). Moreover, the elution profile of the radioiodinated insulin preincubated with a patient's serum, obtained from the Sephadex G50 column, revealed a single peak at the position of insulin standard (Fig. 4). This shows that neither aggregation nor binding of SICRI to other molecules (e.g., antibodies) occurs and that insulin tracer is not degraded in the serum.

The chromatograms of two sera with very high

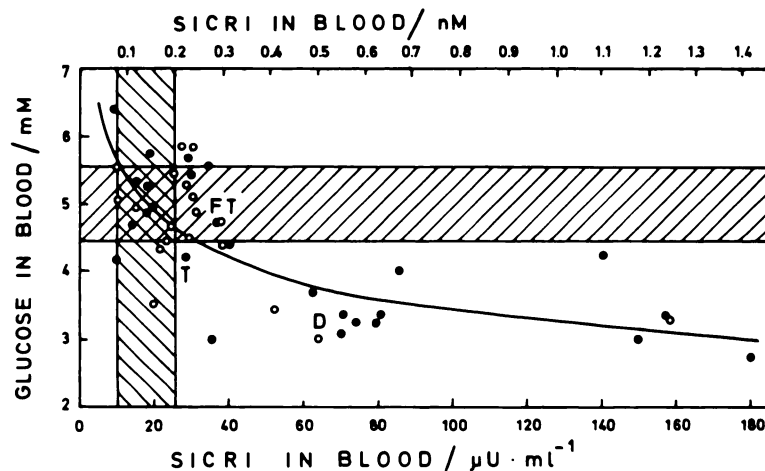


Fig. 2. The correlation of levels of blood glucose and SICRI in patients suffering from non-Hodgkin's lymphoma. The meaning of symbols and letters is the same as in Fig. 1. A nonlinear least-square regression analysis yielded the relation (Glucose) = 0.74 (SICRI)<sup>-0.22</sup> with  $r = 0.727$ ; both concentrations expressed as molarities.

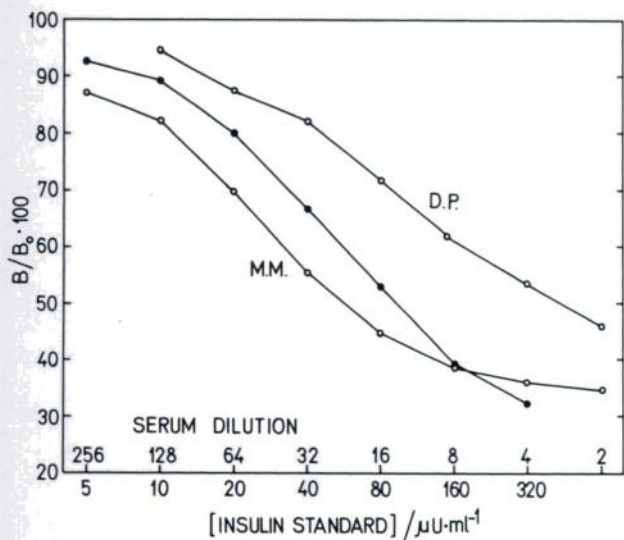


Fig. 3. The percent of  $^{125}\text{I}$ -insulin bound ( $B/B_0 \times 100$ ) as a function of insulin-standard concentration (full circles) and dilution of the SICRI-containing sera of the non-Hodgkin's lymphoma patients M.M. and D.P. in the radioimmunoassay titration experiment.

SICRI levels (patients D.P. and M.M.) in Fig. 5 demonstrate the SICRI of the molecular mass similar to insulin in the case of patient D.P. and of the molecular mass of around 120,000 in the serum of patient M.M. It is important to note that the recovery of SICRI from columns was almost complete (80% and 100% for sera M.M. and D.P., respectively, in Fig. 5A, and 70% in Fig. 5B), indicating SICRI homogeneity within a given serum.

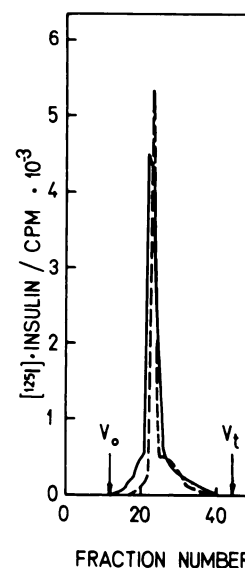


Fig. 4. The Sephadex G50 elution profile of  $^{125}\text{I}$ -insulin preincubated for 3 days with the SICRI-containing serum ( $36 \mu\text{U}/\text{ml}$ ) of the patient K.M. (full line) compared with the corresponding profile of the serum-free  $^{125}\text{I}$ -insulin (dashed line).  $V_0$  denotes the void volume and  $V_t$  the total bed volume of the gel in a  $1 \times 60 \text{ cm}$  column. The flow rate was  $10 \text{ ml} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ . Other conditions as in ref. 12.

The serum of patient M.M. was treated with  $1.0 \text{ M}$  acetic acid to check whether the high molecular mass SICRI could be dissociated into smaller insulin-like molecules. No assayable material was detected either in the acid-free reconstituted supernatant or precipitate, demonstrating that SICRI of the patient M.M.

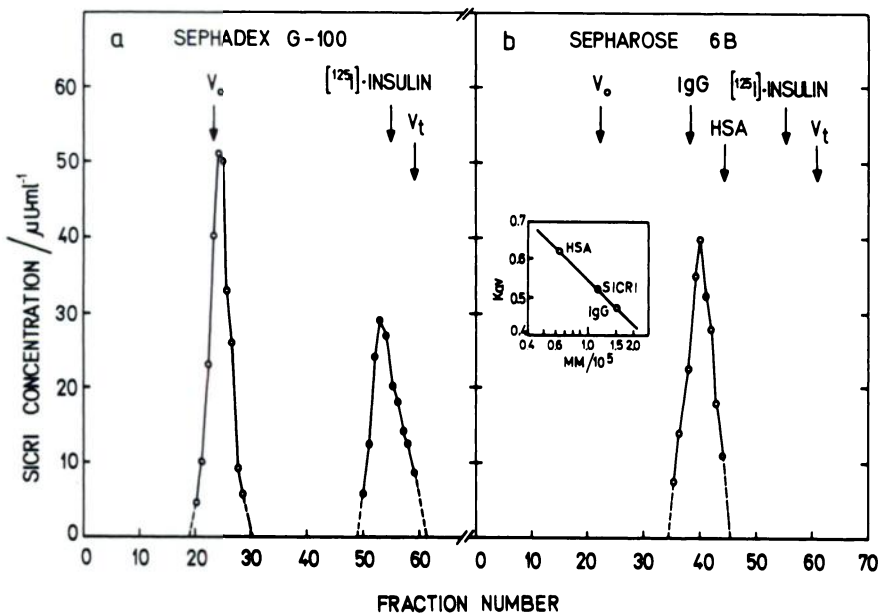


Fig. 5. The chromatograms of sera of the non-Hodgkin's lymphoma patients M.M. (open circles) and D.P. (full circles) on the  $0.9 \times 65$  Sephadex G100 column and  $1 \times 55 \text{ cm}$  Sepharose 6B column at  $4^\circ\text{C}$  using  $20 \text{ mM}$  phosphate containing  $0.15 \text{ M}$  NaCl, pH 7.4, as the mobile phase. The fractions were assayed by insulin-specific RIA sensitive down to  $5 \mu\text{U}$  insulin/ml. The arrows denote positions of human immunoglobulin G (IgG), human serum albumin (HSA), and  $^{125}\text{I}$ -insulin used as molecular mass markers. Inset to (b): The estimation of the molecular mass of SICRI using a  $K_{AV}$  versus log molecular mass plot.  $K_{AV} = (V_0 - V_e)/(V_t - V_0)$ , where  $V_e$  denotes the respective elution volume.

was probably irreversibly denatured by the acid and retained in the precipitate. In case of acid-induced dissociation, the small insulin-related molecules would be recovered from the supernatant and renatured into RIA-detectable material.<sup>13</sup>

#### DISCUSSION

In two-thirds of patients suffering from non-Hodgkin's lymphoma, the blood levels of substances immunologically cross-reactive with insulin were elevated. At the same time, the levels of C-peptide, a split-off of the proinsulin molecule, were not elevated accordingly. Normally, due to the slower C-peptide catabolism, basal molar concentrations of peripheral C-peptide always exceed those of insulin<sup>8</sup> (cf. Fig. 1).

Elevated levels of RIA-detectable material could be attributed to the increased concentrations of proinsulin if they were not accompanied by hypoglycemia (which is not produced by proinsulin). Moreover, proinsulin is cross-reactive with the anti-C-peptide antibodies<sup>8,13</sup> and, therefore, the increase of proinsulin concentration also elevates the level of RIA-detectable C-peptide to some extent. Hence, if it is pancreatic insulin that accounts for the increased levels of SICRI in patients with non-Hodgkin's lymphomas, the ratio of insulin and C-peptide catabolism must be diminished. However, morphological and histologic changes that might reflect a decreased metabolic activity of liver in non-Hodgkin's lymphoma patients have not been reported.<sup>14</sup>

The molecular mass of SICRI in patient M.M.'s

blood is approximately 120,000, and in acid, it is not dissociated into the smaller insulin-like molecules. From this finding, it appears that some of the high molecular mass molecules with insulin-like activity<sup>1</sup> can immunologically cross-react with insulin. An extensive study of molecular properties of SICRI, now underway, will help to resolve these questions.

It is of importance to note that elevated SICRI levels were found in patient B.E., suffering from non-Hodgkin's lymphoma, who was also diabetic. We have no data that could point to the site of SICRI synthesis in this patient. However, in cases of Hodgkin's disease<sup>2</sup> and of renal adenocarcinoma,<sup>3</sup> as well as in cultured murine aplastic carcinoma,<sup>15,16</sup> we have shown that the sites of SICRI synthesis are tumorous cells themselves.

The fact that in the relapse phase of non-Hodgkin's lymphoma the average SICRI levels are higher than in remission parallels similar observations in patients suffering from Hodgkin's lymphoma<sup>2</sup> and the stage-dependent SICRI levels in patients with carcinoma of the cervix or corpus uteri.<sup>17</sup> Therefore, it is relevant that the tumor-generated SICRI is utilized by the tumor as a growth factor<sup>6</sup> in both mice<sup>4,5</sup> and humans,<sup>2,17</sup> thus playing a role within the mechanism of self-control of tumorous growth.<sup>18</sup>

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