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The binding of FITC-insulin to ANAE-positive cells in rat thymus

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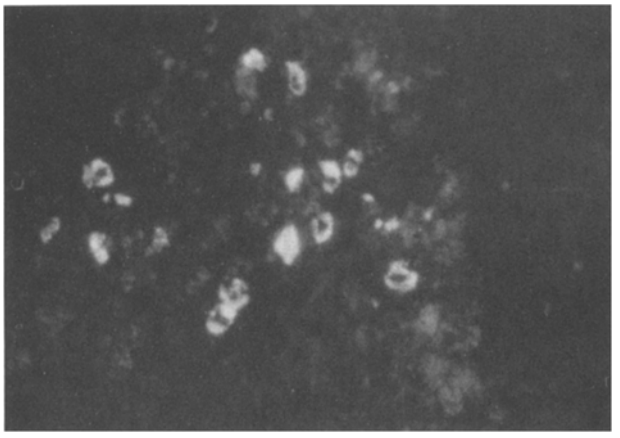
Summary. To determine if thymic macrophages have insulin receptors, alternate sections of rat thymus were stained with FITC-insulin and examined for nonspecific esterase (ANAE) activity. Cells showing a diffuse ANAE staining pattern also bound FITC-insulin. These cells were concentrated in the cortico-medullary border and increased in number following administration of cortisol. Thymic macrophages may be insulin-dependent and therefore could be malfunctional in diabetes.
Key words. Thymus; thymic macrophages; ANAE; insulin; FITC-insulin; diabetes mellitus.

T-cell mediated function has been reported to be abnormal in diabetes mellitus [1], and one of the characteristic features of experimental diabetes is an impairment of thymic lymphocyte maturation [2]. These findings suggest that insulin may play a role in T-cell development in the thymus. Although binding of ^{125}I-insulin to a mixture of thymic cells has been demonstrated [3], the cellular subtype to which the hormone binds has yet to be identified. Our studies of the pattern of binding of monofluoresceinthiocarbamyl-insulin (FITC-insulin) to frozen or fixed sections of thymus indicate that the cells in the thymus which bind insulin stain diffusely with nonspecific esterase (ANAE) suggesting that macrophages in the thymus are the cells which have insulin receptors. These cells may be important in thymic T-cell maturation and may function suboptimally in diabetes resulting in depressed T-cell development.

Materials and methods. Monofluoresceinthiocarbamyl-insulin (FITC-insulin) was prepared essentially as described [4] using crystalline bovine insulin, (23.6 IU; Lot 49C-0197) and fluorescein-isothiocyanate, isomer I on Celite obtained from the Sigma Chemical Co. Following the coupling reaction, the mono FITC-insulin was purified by chromatography on Sephadex G-25 (eluted with 0.9% NaCl-0.01 M phosphate buffer, pH 7.4), isoelectric precipitation, followed by chromatography on DEAE-Sephadex A-25 (eluted with 7 M urea in 0.01 M Tris-HCl, pH 7.6, with a 0–1 M NaCl gradient). In two separate studies, FITC-insulin was shown to have 40 or 51% of the bioassayable potency of native insulin [4, 5].

Rats (SASCO, Omaha, NE) were decapitated and the thymuses quickly frozen in O.C.T. compound (Lab-Tek Products) using 2-methylbutane and dry ice. Cryostat sections were cut at 4 μm. The mounted slices were washed 2 times with phosphate-buffered saline (PBS) (0.01 M sodium phosphate, pH 7.8) and treated for 30 min at 37 °C with 1×10^{-6} M FITC-insulin in PBS containing 1% bovine albumin. The sections were washed 2 times with PBS and photographed using a Wild fluorescence microscope. Fluoresceinisothiocyanated-bovin albumin (Sigma Chemical Co.) was dissolved in PBS and used as a negative control stain. Alpha-naphthylacetate esterase (ANAE) staining of thymus sections was performed as described [6].

Results. FITC-insulin was found to bind to a subpopulation of cells in all sections of rat thymus studied, but the number of reactive cells was significantly greater in the thymus of animals that had been treated with a lymphocytolytic steroid (e.g. 5 mg cortisol/100 g b. wt, s.c., 44 h prior to sacrifice). The figure shows a typical fluorescent micrograph of a frozen FITC-insulin-stained thymus section from a cortisol-treated rat. FITC-insulin fluorescent cells were found with the highest frequency at the cortico-medullary junction. No fluorescent cells were found in control studies using FITC-albumin as the fluorescent probe. Preincubation of the frozen tissue with unlabeled insulin (3×10^{-5} M) for 30 min at 37 °C decreased the number of fluorescent cells stained with FITC-insulin by 50–60%. Prior fixation of the tissue in 95% methanol decreased the number of cells found to stain with FITC-insulin, but a significant number of cells re-



FITC-insulin staining of frozen thymus from a cortisol-treated rat. × 400.

mained FITC-insulin-positive in the fixed sections, indicating that the staining reaction was not simply a result of phagocytosis of the FITC-insulin.

Staining of alternating thymic sections for FITC-insulin binding and ANAE activity produced labeling patterns which showed that FITC-insulin bound cells were also ANAE positive. As with FITC-insulin binding, ANAE-staining of the thymic sections was heaviest at the cortico-medullary junction and was markedly increased following cortisol administration.

Discussion. It is known that insulin receptors are located on splenic and cultured macrophages [7], and on monocytes isolated from peripheral blood [7, 8]. A mixture of thymic cells was shown to bind ^{125}I-insulin, but there has been no direct evidence to indicate which thymic cell contains the insulin receptors. Mature nonstimulated T-cells do not bind insulin [9], suggesting that the thymic cells with insulin receptors are either immature thymocytes or members of the non-lymphoid population of cells. We have shown that FITC-insulin-staining cells are also ANAE positive. Preincubation with an excess of unlabeled insulin suppressed FITC-insulin binding, indicating that the binding observed is to cells that have specific binding sites. FITC-insulin has also been shown to be useful as a probe for insulin receptors on blood monocytes [5]. Cortisol treatment of the rats, which is known to augment the thymic macrophage population [10], increased both FITC-insulin and ANAE staining which were heaviest in normal thymus at the cortico-medullary juncture. Unique nonspecific-esterase positive macrophages have been identified in this area of the thymus [11, 12].

Thymic macrophages are believed to function in T-cell maturation [13], and it has been postulated that the macrophages involved in this process are located at the cortico-medullary juncture [11]. Interleukin 1 and prostaglandin E_2, which may influence thymocyte proliferation, have been shown to be produced by thymic macrophages [14]. Furthermore these cells are believed to regulate intrathymic T-cell development [15]. The fact that diabetic animals have abnormal T-cell function [1], maturation impairment [2], and depressed thymic proliferation [16, 17] may be due to aberrant function of insulin-requiring macrophages at the thymic cortico-medullary juncture.

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Effect of niacin/nicotinamide deficiency on the diabetogenic effect of streptozotocin

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Summary. Weanling CD-1 mice were fed either a control diet or a diet deficient in niacin/nicotinamide for one month and then injected i.v. with 60, 80, 100, 120, 140, or 160 mg/kg streptozotocin. Mice on the deficient diet developed a higher incidence of diabetes and more severe hyperglycemia than those on the control diet.
Key words. Streptozotocin; diabetes mellitus; NAD; niacin; nicotinamide; tryptophan; CD-1 mice.

Streptozotocin (STZ), a methylnitrosourea with a 2-substituted glucose, is a drug which has been widely used to induce experimental diabetes mellitus [1, 2]. However, the mechanism by which STZ exerts its diabetogenic effect has not been totally elucidated. Over the past 20 years, numerous investigators have demonstrated that STZ decreases nicotinamide