antigen X, which would be present on JY cells and M1 cells but not on the murine cells. Matzinger and Bevan (*13*) have argued that alloreactivity always involves recognition by the T-cell receptor of a cell surface antigen X plus an MHC product. Thus, antigen X must be a structure that is shared by a human B lymphoblastoid cell line and a transformed fibroblast cell line. Although this explanation cannot be ruled out, we would suggest that the structures on human M1A2 cells which bind to the CTL glycoproteins LFA-1, T8, and T11 are absent from (or different on) murine L cells. In other words, a species-specific barrier exists between the CTL clone and the target cell, which does not allow for the appropriate cell adhesion required for the precise interaction of the T-cell receptor with antigens.

Note added in proof: Similar results have recently been obtained by others (*13a*).

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β-Adrenergic Mechanism of Insulin-Induced Adrenocorticotropin Release from the Anterior Pituitary

Abstract. *Intraperitoneal administration of insulin to control rats and to rats with pituitary stalk transections or with lesions of the median eminence resulted in increased plasma adrenocorticotropin (ACTH) levels. The insulin-induced stimulation of ACTH release was blocked in both the control and lesioned animals by prior treatment with either the β-adrenergic antagonist propranolol or the glucocorticoid analog dexamethasone. The direct application of insulin to primary cultures of the anterior pituitary did not evoke ACTH release or affect the maximal ability of corticotropin-releasing factor or epinephrine to stimulate ACTH secretion. The results suggest that insulin stimulates ACTH release by a mechanism in which catecholamines of peripheral origin act directly on the anterior pituitary.*

Insulin is vital in maintaining the normal metabolic state of the body. It is released from the pancreas and reduces circulating glucose by enhancing the uptake of glucose into cells. The hypoglycemia induced by insulin is stressful and causes a rise in plasma adrenocorticotropin (ACTH) and cortisol (*1*). To stimulate ACTH secretion, insulin may act (i) centrally to effect the release of corticotropin-releasing factor (CRF), (ii) by direct stimulation of the anterior pituitary, or (iii) at a peripheral site. We showed that peripheral administration of the β-adrenergic agonist isoproterenol results in the release of ACTH from the adenohypophysis in rats whose pituitary is separated from the brain either by transection of the pituitary stalk or by lesioning of the median eminence (*2*). The effect of isoproterenol on ACTH release is blocked by propranolol, a β-receptor antagonist, and by dexamethasone, an agent that blocks the synthesis and release of ACTH from the anterior pituitary (*3*). These results indicate that catecholamines can release ACTH by direct stimulation of β-adrenergic receptors on the anterior pituitary.

Insulin stress causes a marked increase in plasma epinephrine (*4*), and administration of epinephrine to rats increases ACTH release through a β-adrenergic mechanism (*5*). We now report that insulin-induced hypoglycemia, a physiologic stimulus, releases ACTH by a peripheral β-adrenergic mechanism.

When rats were injected with insulin, a threefold rise in plasma ACTH immunoreactivity was observed (Fig. 1). To examine whether a β-adrenergic mechanism was involved in the insulin-induced release of ACTH, we injected propranolol prior to the insulin administration. Propranolol blocked the rise in plasma ACTH induced by insulin. Propranolol did not affect basal ACTH levels in nonstressed rats.

ACTH can be released from either the anterior or intermediate lobe of the pituitary. Because glucocorticoids selectively block ACTH release from the anterior

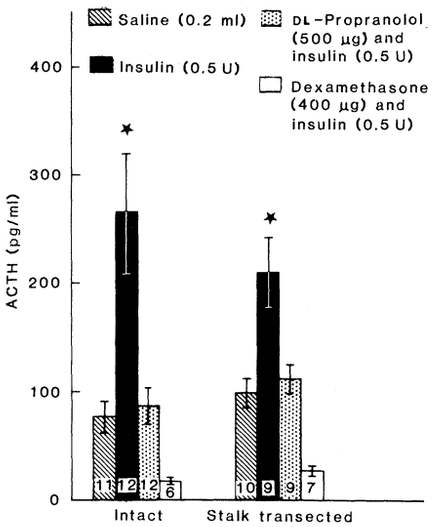

Fig. 1. Effect of insulin on ACTH release in intact rats and rats in which the pituitary stalk was transected. The animals received 0.2 ml of saline or 0.2 ml (0.5 U) of insulin intraperitoneally 45 minutes before they were decapitated. One group of animals received 400 μg of dexamethasone 16 hours before the insulin injection, and another group was given 500 μg of propranolol 15 minutes before the insulin injection. ACTH was extracted from plasma and was measured by methods described earlier (*2*). The number of animals in each experimental group is indicated, and values are given as the mean ± S.E.M. for each group. The star designates a difference from saline control values with $P \leq 0.05$.

but not the intermediate lobe of the pituitary (*3*), we used glucocorticoids to determine the source of the ACTH released after insulin injection. Treatment of rats with the glucocorticoid analog dexamethasone inhibited the insulin-induced ACTH secretion (Fig. 1).

To establish whether the action of insulin on ACTH release is centrally or peripherally mediated, we administered insulin to a group of animals in which the pituitary stalk had been transected. This lesion removes all central input to the pituitary and does not compromise the blood supply enough to affect normal pituitary function (*6*). Insulin injection increased plasma ACTH by a factor of 2.5 in rats with stalk transections. Propranolol blocked the stimulation of ACTH release in these animals. Treatment with dexamethasone also abolished the ACTH release response to insulin in these animals (Fig. 1). To exclude the possibility that these results might be due to the regeneration of the pituitary stalk, we repeated the treatments in a group of animals in which the medial basal hypothalamus (MBH) was destroyed 36 hours before the experiment. The results after this procedure were similar to those in the groups with stalk transections [saline, 187 ± 38 (n = 4); insulin, 350 ± 31 (n = 5); propranolol + insulin, 162 ± 22 (n = 7); values are in picograms of ACTH per milliliter of plasma and are expressed as standard error of the mean (S.E.M.) of n determinations].

To test whether insulin might act directly on corticotrophs to stimulate ACTH release, we used primary cultures of the rat anterior pituitary. Insulin did not release ACTH from monolayer cultures by itself, nor did it alter the maximal ability of CRF or epinephrine to stimulate ACTH secretion (Table 1).

ACTH release can be provoked by numerous humoral and environmental factors. CRF is the most effective ACTH secretagogue and is important in mediating central effects of corticotrophs (*7*). Catecholamines, both of brain and peripheral origin, also contribute to the control of ACTH release (*8*). We showed earlier that the peripheral administration of the β-adrenergic agonist, (−)-isoproterenol, stimulates ACTH release in vivo in intact rats and rats with pituitary stalk transections (*2*). Since stalk transection prevents the direct central control of ACTH release, the above experiment—as well as others—shows that catecholamines of peripheral origin can act directly on the anterior pituitary, possibly through β_2-adrenergic receptors to evoke ACTH secretion. A similar mechanism appears to mediate in part the increase in ACTH release induced by insulin. This hypothesis is supported by the following findings: (i) insulin stimulates ACTH release in rats with pituitary stalk transections (and MBH lesions); (ii) the β-adrenergic antagonist propranolol blocks insulin's effect on ACTH release in the lesioned rats; and (iii) insulin does not act directly on the anterior pituitary to increase ACTH secretion. The latter finding is in agreement with data showing that insulin-induced hypoglycemia is responsible for the rise in plasma ACTH, rather than insulin itself (*9*). A site affected by insulin-induced hypoglycemia to release ACTH could be the hypothalamus (*9, 10*). Two neuronal pathways—the hypothalamo-hypophyseal system (the central CRF-ACTH system), and the projection of hypothalamic fibers to the spinal cord which affects the activity of the splanchnic nerves innervating the adrenal medulla—could mediate insulin's ACTH releasing activity (*1*). Our experiments eliminate the first of these alternatives but not the second. Thus, the hypoglycemia induced by insulin could stimulate the hypothalamus to activate the sympathoadrenal medullary system to liberate epinephrine from the adrenal, which then reaches the pituitary to stimulate corticotrophs. Consistent with this hypothesis are the observations that insulin injection increases the firing activity of the splanchnic nerve (*9*), and severing the splanchnic nerve blocks insulin-induced epinephrine release (*11*).

Table 1. Effect of insulin on ACTH release from primary cultures of the anterior pituitary. Primary cultures of male rat anterior pituitaries were prepared as described (*13*). Culture medium containing insulin alone or either synthetic ovine CRF (Peninsula) or epinephrine (in the presence of ascorbic acid) alone or in combination with insulin was incubated with the cells for 3 hours. Portions of medium were then removed, and ACTH immunoreactivity was measured as described (*13*). Results are means ± S.E.M. of six separate determinations.

Insulin (U/ml)	ACTH immunoreactivity (ng/well)
Insulin only	
0 (basal)	0.96 ± 0.14
0.1	1.01 ± 0.07
0.5	1.24 ± 0.07
1.0	1.36 ± 0.29
With 10^{-7}M CRF	
0	13.0 ± 1.34
0.01	12.7 ± 0.92
0.1	11.2 ± 0.52
0.5	13.4 ± 0.39
1.0	13.0 ± 0.96
With 10^{-6}M epinephrine	
0	2.08 ± 0.19
0.01	2.28 ± 0.05
0.1	1.78 ± 0.18
0.5	2.66 ± 0.23
1.0	2.91 ± 0.19

Although other forms of stress also increase plasma catecholamines, they seem to affect ACTH release predominantly through central mechanisms (*8, 12*). In contrast, the hypoglycemia caused by insulin appears to be an example of a physiologic condition in which peripheral catecholamines are induced to stimulate ACTH release.

These studies support the hypotheses that ACTH secretion is under multihormonal control, and that various combinations of ACTH secretagogues derived from both central and peripheral sources mediate the effect of various stresses on the ACTH release response.

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Autoimmunity and Increased c-*myb* Transcription

Abstract. *A single recessive gene,* 1pr, *induces an autoimmune-lymphoproliferative syndrome in several strains of mice. The lymphoid organs of* 1pr/1pr *mice contained cells with increased amounts of* myb *RNA, which codes for a protein found in the nucleus. A similar human lymphoproliferative disorder also had an increase in c-*myb *expression. Mouse T cells induced by mitogens to proliferate did not express large amounts of* myb *RNA, indicating that marked* myb *expression is not a general feature of lymphocyte activation and proliferation.*

The generalized autoimmune diseases in mice and humans are a diverse group of disorders of unknown etiology characterized by excessive proliferation of T or B lymphocytes and production of autoantibodies (*1, 2*). In mice, a single recessive gene, *lpr*, induces autoantibody production and massive lymphocyte proliferation (*1*). Similarly, among the human autoimmune diseases, angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) is also characterized by autoantibody production and lymphocyte proliferation (*3, 4*). In our study of the molecular events responsible for these disease states, we examined the oncogene RNA products in involved lymphoid cells.

Oncogenes are the genetic elements in acute transforming retroviruses that are associated with the ability of these viruses to induce neoplastic transformation of cells (*5*). Cellular DNA sequences (c-*onc* genes) homologous with viral oncogenes (v-*onc* genes) have been conserved through evolution and can be found in most eukaryotic cells (*5–8*). Quantitative and qualitative alterations in different oncogene RNA's have been found in a large number of human and murine cancers (*9, 10*). We wondered whether a mechanism similar to this, but lacking a neoplastic transformation step, could play a role in the abnormal lymphocyte proliferation that characterizes most autoimmune diseases. To test this hypothesis we used oncogene probes to hybridize blots of electrophoretically separated polyadenylated [poly(A)$^+$] RNA from human peripheral blood mononuclear cells (PBMC's) and bone marrow or from spleens and lymph nodes from mice with and without autoimmune diseases. We found increases in the expression of the oncogene c-*myb* in both of these autoimmune diseases.

Three of the first oncogenes used as probes have been associated with lymphoid tumors: *abl, myc*, and *myb* (*9–11*). A fourth oncogene probe, *raf*, was studied because it has been mapped to chromosome 6 in the mouse, which contains the κ immunoglobulin light-chain gene (*12*) and has been associated with small cell lung carcinomas (*13*). Almost no difference in c-*abl* RNA expression was noted in a comparison of autoimmune and nonautoimmune mice (data not shown). The spleens of autoimmune NZB and BXSB mice, however, contained two to three times more c-*myc* RNA than those of nonautoimmune mice, and Southern blot hybridization showed no evidence of *myc* gene rearrangements or amplification (data not shown). The most striking and consistent finding was an elevation in the amount of c-*myb* RNA in tissues from subjects with a subset of autoimmune diseases, namely the spleens of MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice and PBMC's and bone marrow cells of a patient with AILD.

The c-*myb* RNA's appeared as a major hybridizing band of 3.8 kilobases (kb) plus a fainter band of 4.2 kb (Fig. 1). The lowest amount of *myb* RNA was consis-

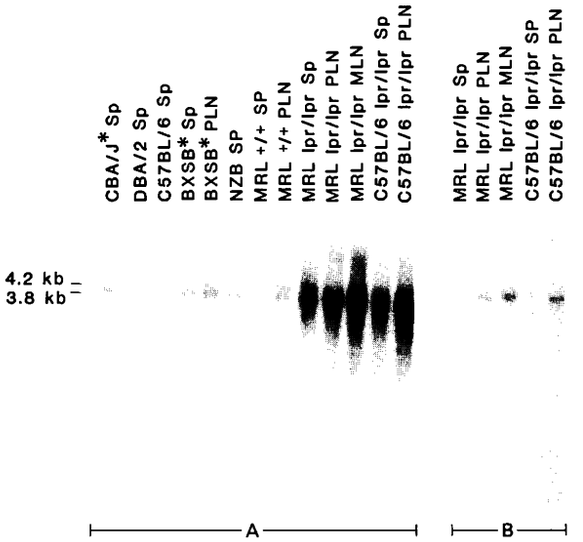

Fig. 1. Hybridization of v-*myb* probe to poly(A)$^+$ RNA (10 μg) from spleens (Sp), peripheral lymph nodes (PLN), and mesenteric lymph nodes (MLN) of mice (~6 months old) of indicated strain. The guanidine thiocyanate method (*22*) was used to rupture cells and to denature ribonuclease simultaneously. The RNA was separated from DNA and proteins by centrifugation through cesium chloride (*23*). For final purification, the RNA was extracted once in chloroform-phenol and once in chloroform. The poly(A)$^+$ RNA fraction was obtained by passing the total purified RNA twice over an oligo(dT)-cellulose column (*24*). Poly(A)$^+$ RNA (10 μg) was denatured in 14 m*M* methylmercury hydroxide and subjected to electrophoresis in 1.5 percent agarose gels containing 5 m*M* methylmercury hydroxide. The RNA was blotted onto *O*-diazophenyl thioether paper and prehybridized and hybridized with a v-*myb* DNA probe that had been labeled with ^{32}P by nick translation to a specific activity of 1.4×10^8 to 2.0×10^8 count/min per microgram (*9*). The films were scanned with a CS300 scanning densitometer (Hoefer Scientific). In some cases, after photography of the autoradiographs certain lanes were cut out and juxtaposed with others from the same gel to illustrate more clearly important comparisons. The v-*myb* probe was a 1.3-kb Kpn I–Xba I fragment isolated from cloned avian myeloblastosis virus (*11*) and subcloned in pBR322. (A) Autoradiographs of the v-*myb* probe hybridized to blots of spleen and lymph node poly(A)$^+$ RNA after a 12-hour exposure. (B) The right-most six lanes of (A) after a 1-hour exposure. All mice were female except those designated by an asterisk (*).