exhibits increased incorporation of ^{32}P into PIP_2 (*24*).

Invertebrates seem to use the same biochemical principles for phototransduction as vertebrates. In rods, photoexcited rhodopsin activates a cascade of chemical reactions, the first step of which is activation of a guanine nucleotide–binding protein (*10*). My model for *Limulus* suggests that the protein activated by photoexcited rhodopsin is also a guanine nucleotide–binding protein. Thus, one of the functions of rhodopsin in both vertebrates and invertebrates may be to activate guanine nucleotide–binding proteins.

My results lead me to propose the following cascade of events leading to calcium release in ventral photoreceptors. Photoisomerization of rhodopsin activates N, which stimulates phospholipase C. This activation of phospholipase C leads to the production of the intracellular messenger IP_3, which then causes the release of calcium from the endoplasmic reticulum. GDP-β-S inhibits both visual excitation and adaptation by blocking N, thereby disrupting the flow of information from rhodopsin to later stages of the cascade.

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27. A brief abstract describing this work has appeared previously [A. Fein, D. W. Corson, R. Payne, *Biol. Bull.* **167**, 526 (1984)]. Supported by NIH grants EY 01362 and EY 03793. I thank D. W. Corson, J. Lisman, and R. Payne for criticisms of an earlier version of this manuscript. IP_3 was a gift of R. F. Irvine.

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Cytotoxicity of Human *p*I 7 Interleukin-1 for Pancreatic Islets of Langerhans

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Activated mononuclear cells appear to be important effector cells in autoimmune beta cell destruction leading to insulin-dependent (type 1) diabetes mellitus. Conditioned medium from activated mononuclear cells (from human blood) is cytotoxic to isolated rat and human islets of Langerhans. This cytotoxic activity was eliminated from crude cytokine preparations by adsorption with immobilized, purified antibody to interleukin-1 (IL-1). The islet-inhibitory activity and the IL-1 activity (determined by its comitogenic effect on thymocytes) were recovered by acid wash. Purified natural IL-1 and recombinant IL-1 derived from the predominant *p*I 7 form of human IL-1, consistently inhibited the insulin response. The *p*I 6 and *p*I 5 forms of natural IL-1 were ineffective. Natural and recombinant IL-1 exhibited similar dose responses in their islet-inhibitory effect and their thymocyte-stimulatory activity. Concentrations of IL-1 that inhibited islet activity were in the picomolar range. Hence, monocyte-derived *p*I 7 IL-1 may contribute to islet cell damage and therefore to the development of insulin-dependent diabetes mellitus.

INTERLEUKIN-1 (IL-1) IS A FAMILY OF peptide hormones with a wide range of biological properties, including the ability to alter immunologic, neuroendocrine, and metabolic functions (*1*). Interleukin-1 activates a broad spectrum of cell types, such as T and B lymphocytes, neutrophils, hepatocytes, muscle cells, fibroblasts, chondrocytes, osteoclasts, and hypothalamic cells. Although the primary sources of IL-1 are blood monocytes and tissue macrophages, IL-1 is also elaborated by vascular endothelium, skin keratinocytes, gingival and corneal epithelial cells, renal mesangial cells, and brain astrocytes (*2*). The IL-1 produced by any of the latter cell types, or by tissue macrophages as a result of a localized immune reaction, probably exerts its primary effects within discrete anatomical regions. There is indirect evidence that a localized autoimmune reaction participates in the development of type 1 (insulin-dependent) diabetes mellitus (IDDM) (*3*), and infiltration by mononuclear cells is the hallmark of the histologic process affecting the islets of Langerhans (*4*). Cytotoxic macrophages that destroy cultured islet cells have been found in the mouse (*5*). Supernatants of activated human blood mononuclear cells were shown to inhibit insulin secretion and islet insulin content by a direct cytotoxic effect (*6*). Screening of partially purified lymphocyte and monocyte mediator molecules (cytokines) indicated that IL-1 contributed to this effect. We now report that this inhibition of islet cell function appears to be mediated only by the *p*I 7 form of IL-1.

The effect of IL-1 and other cytokines on the function of rat islet cells in vitro was tested as previously described (*6*). The islets were isolated from collagenase-treated pancreatic tissues and cultured for 7 days before cytokines were added. We determined by radioimmunoassay how much insulin was secreted during six subsequent days of cul-

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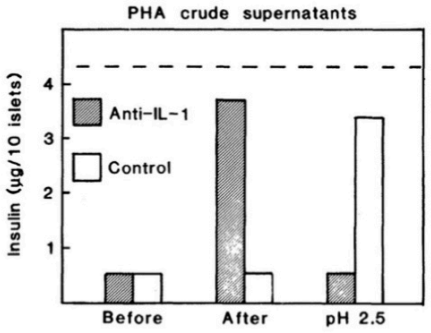

Fig. 1. Effects on insulin release (measured as micrograms per ten islets, during 6 days of culture) of crude supernatant from phytohemagglutinin-activated blood mononuclear cells. Two columns containing 1 ml of antibody to IL-1 coupled to Sepharose 4B and 1 ml of control Sepharose 4B were used (*20*). Anti–IL-1 does not react with interleukin-2, IFN-γ, lymphotoxin, or TNF (*21*). Crude pooled supernatants were dialyzed, lyophilized, and reconstituted in 0.1 volume in buffer containing 0.05*M* tris, 0.1*M* NaCl, 0.015*M* NaN_3, and 1% (by volume) heat-inactivated human serum (*p*H 7.5). Solutions (600 μl containing 100 units of IL-1) were incubated in the gels for 30 minutes at 22°C, and 1.8 ml of effluent was collected. The columns were then washed at 4°C with at least 10 ml of buffer. Bound materials were eluted at 22°C with 0.1*M* citric acid buffer, 1% (by volume) serum (*p*H 2.5). The effluent (1.5 ml) was eluted into 0.3 ml of 1*M* tris and 0.015*M* NaN_3 (*p*H 8.2) for immediate *p*H neutralization. To match the experimental dilution, the prechromatographed materials were diluted 1:3. These preparations (before), the chromatographed fractions (after), and the acid-eluted fractions (*p*H 2.5) were then dialyzed for 48 hours against Hepes-buffered RPMI 1640 (Gibco; Paisley, Scotland) at *p*H 7.4 before being tested on rat islets. The capacity of the anti–IL-1 column to remove IL-1 exceeded 800 units of purified nIL-1. Recoveries were always greater than 50%. Results are shown as means of experiments testing two different preparations of crude supernatant, each tested in duplicate. Repeated experiments with partially purified nIL-1 gave similar results. The dashed line indicates insulin release from islets cultured in medium alone. The coefficient of variation for double determinations of insulin release from different islet isolates in culture medium was 14% ($n = 9$). The interassay coefficient of variation for the radioimmunoassay of insulin was 6%. An IL-1 unit is defined as the amount of IL-1 that doubles the background proliferation of mouse thymocytes to phytohemagglutinin (*22*).

ture and the islet insulin and glucagon contents (*6, 7*).

Natural human monocyte IL-1 (nIL-1) (molecular size 17.5 kilodaltons) was obtained from *Staphylococcus albus*–stimulated blood adherent cells and purified by immunoadsorption and Sephadex G-50 (fine) gel chromatography (*8*). The production of rabbit antibody to IL-1 (anti–IL-1) and its use for purifying IL-1 have been described (*9*). This antibody was also used for immunoadsorption of crude supernatant materials, which were obtained from human blood mononuclear cells incubated with phytohemagglutinin (*10*). The recombinant IL-1 (rIL-1) used in these studies was expressed in *Escherichia coli* and consisted of polypeptide sequence 71–269 of the predominant *p*I 7 form of human nIL-1 (*11*). The rIL-1 was purified to homogeneity and contained less than 60 pg of endotoxin per microgram.

Immobilized antibody to human IL-1 removed all detectable islet-inhibitory activity from crude, cytokine-rich supernatants from polyclonally activated mononuclear cells (Fig. 1). The IL-1 activity on T cells was removed in parallel. Significant islet-inhibitory activity, along with IL-1 activity, was subsequently eluted during an acid wash. Control columns without anti–IL-1 failed to remove the islet-inhibitory or IL-1 activity; in addition, interleukin-2 and interferon-γ (IFN-γ) activities were unaffected by the anti–IL-1 immunoadsorbent.

Although cytokines have been described that arrest the cell cycle of activated lymphocytes, few have been reported to be cytotoxic to nonlymphoid cells. The best characterized of these are lymphotoxin, IFN-γ, and macrophage-derived tumor necrosis factor (TNF). Lymphotoxin comprises a complex system of related toxins of molecular sizes ranging from 12 to 200 kilodaltons (*12*). The role of lymphotoxin in lymphocyte-mediated cells lysis is not clear, and cells from various tissues and species differ markedly in their sensitivity to lymphotoxin. The fact that partly purified lymphotoxin does not inhibit islet cell function (*6*) and the data presented in Fig. 1 indicate that lymphotoxin is not responsible for the islet cell toxicity.

T-lymphocyte–derived IFN-γ and monocyte-derived TNF inhibit growth primarily of neoplastic and transformed cells (*13*). Normal cells are in some cases affected by IFN-γ, but no normal cells that are sensitive to TNF have been described, to our knowledge. The results shown in Fig. 1 indicate that IFN-γ and TNF are probably not toxic to islet cells. This suggestion is supported by the fact that purified IL-1 devoid of antiviral activity is highly toxic to rat and human islet cells (Table 1) (*6*). Furthermore, preliminary experiments show that the biological activity of recombinant TNF (rTNF) is heat resistant (to 70°C), whereas the effect of crude cytokine preparations on the elaboration of insulin is lost at 56°C.

It is unlikely that the *p*I 6 and *p*I 5 forms of IL-1 inhibit insulin production. The *p*I 5, *p*I 6, and *p*I 7 forms of IL-1 were compared for their effects on mouse thymocytes (IL-1 assay) and on islet cells (Fig. 2). Despite the

Table 1. Effects of recombinant cytokines on rat islets of Langerhans. Values are percentage of control values. Different rIL-1 preparations were used in experiments 1, 2, and 3, respectively. For assay and interassay variation, see Fig. 1.

Cytokines (unit/ml)	Experiment 1			Experiment 2			Experiment 3		
	Insulin		Glucagon content	Insulin		Glucagon content	Insulin		Glucagon content
	Release	Content		Release	Content		Release	Content	
rTNF*									
225.000				49	153	184	34	135	108
75.000	56	116	64	83	206	238	47	130	111
25.000				60	124	95	78	173	232
rIFN-γ†									
10.000	97	132	83	89	125	72			
1.000	140	123	106	106	131	120			
rIL-1‡									
30				6	26	19			
10	8	36	11				6	69	8
3				15	77	23	6	36	7
1	125	105	143				55	148	127

*L-929 fibroblast assay; 1 unit = 30 pg of rTNF. †A 549/EMCV assay; 1 unit = 50 pg of rIFN-γ. ‡Thymocyte proliferation assay; 1 unit = 10 ng of rIL-1.

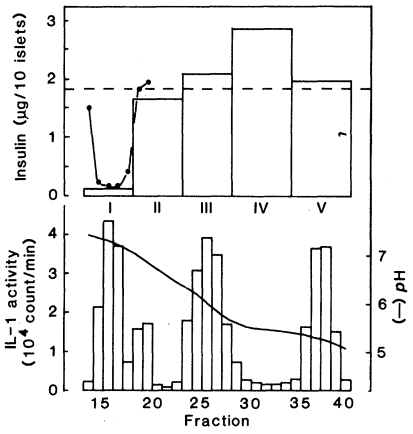

Fig. 2. Effect on insulin release of chromatofocused fractions of nIL-1. The gel (Pharmacia) was washed in endotoxin-free imidazole buffer. After immunoadsorption and gel filtration, nIL-1 was dialyzed against 0.025*M* imidazole buffer (*p*H 7.8) and applied to the gel. After washing with two column volumes of imidazole buffer, Polybuffer 94 (Pharmacia) was added to generate a *p*H gradient between 7.6 and 4.5 (lower curve). Each fraction was assayed for thymocyte costimulatory activity (bottom histogram) (*22*), and pooled fractions (I–V) were tested on rat islets (upper histogram). Individual fractions from the active pool I were then tested at different concentrations [those shown were tested at one-fourth the concentration of the pooled fractions (upper curve)]. The specific activity of pool I was approximately 6×10^6 IL-1 units per milligram of protein. The dashed line indicates insulin release in medium alone. Precipitation of [^{35}S]methionine-labeled proteins with tetrachloroacetic acid (12%) and the sodium dodecyl sulfate–polyacrylamide gel electrophoresis were performed as previously described (*8*) and revealed a homogeneous band of 17.5 kilodaltons eluting with IL-1 activity.

presence of IL-1 activity in fractions III and V, no islet-inhibitory activity was seen.

Our results suggest that the inhibitory effect on islet cells of crude cytokine preparations can be attributed to *p*I 7 IL-1. To examine this phenomenon further, we studied the effects of two different batches of rIL-1 derived from *p*I 7 IL-1, along with those of rTNF and rIFN-γ. The rIL-1 preparations inhibited insulin release as well as the insulin and glucagon contents of the islets (Table 1). The rTNF preparation also inhibited the release of insulin from the islets. This effect, however, was less pronounced, and rTNF did not affect the cellular content of insulin.

The dose-response curves for the islet-inhibitory effects and the IL-1 activities of nIL-1 and rIL-1 were similar (Fig. 3); the active concentrations of nIL-1 were in the picomolar range. We conclude that the previously described cytokine-mediated islet cytotoxicity (*6*) is primarily or solely mediated by *p*I 7 IL-1.

These results show a direct IL-1–mediated cytotoxic effect on normal cells. Preliminary findings indicate that this function of IL-1 can be found in fragments of the molecule, since tryptic digestion failed to diminish the effect of purified nIL-1. This is consistent with previous studies demonstrating bioactive IL-1 fragments (*14*).

How, and at what stage, IL-1 influences the development of IDDM, and whether IL-1 plays a role in other endocrinopathies, is unknown. The role of macrophages in the pathology of the islet inflammatory process is becoming evident (*5*), as is the role of autoreactive T lymphocytes (*3, 15*). Initial damage to the insulin-producing cells may be the result of inflammatory processes that are not specific to beta cells. For example, circulating IL-1 might selectively damage beta cells, since the microvascular supply of the beta cell core of the islet is separated from the non–beta cell mantle (*16*). This could explain signs of endothelial cell activation at the time of diagnosis of IDDM in man (*17*) and in the stage before diabetes in the spontaneously diabetic BB rat (*18*). In these early stages, the sinusoids are markedly swollen even in islets devoid of lymphocytic infiltration, and class II molecules (Ia antigens) appear on the capillary endothelium both inside and outside the islets. Such signs of activation may be seen after challenge with IL-1 (*19*). One might speculate that IL-1–mediated damage to beta cells may cause release of antigenic material, thereby initiating a self-perpetuating immune reaction as a result of the macrophage–T-lymphocyte interaction in situ. IL-1 produced during this reaction may then be responsible for further beta cell destruction. This idea does not rule out environmental agents as etiological factors, or subsequent participation of cytotoxic T lymphocytes in beta cell destruction; nor does it rule out a contributing role of specific antibodies to islet cells and of complement.

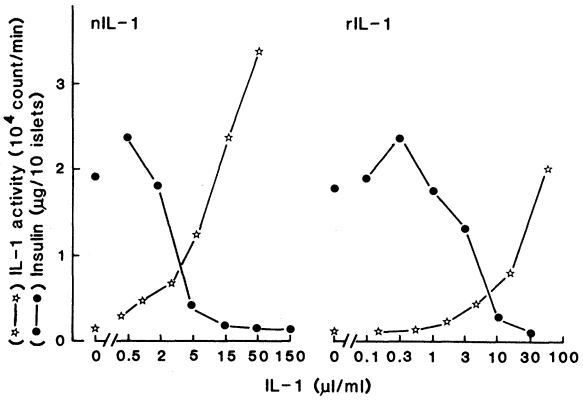

Fig. 3. Comparison between islet-inhibitory and IL-1 activities of human nIL-1 and rIL-1. IL-1 activity was tested in the mouse thymocyte proliferation assay (*22*): nIL-1, 2000 unit/ml; rIL-1, 600 unit/ml. The figure shows the data of one of three experiments on rat islets. The results were reproduced with two different batches of rIL-1.

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