unambiguous identification of each isoform may not be determined as precisely by hybridization methods as by the primer extension assay. Alternatively, this discrepancy may reflect differences in the avian and murine muscle tissues analyzed. Chicken breast muscle is a pure fast muscle (*8*), whereas the fetal mouse tissue is a mixture of slow and fast muscle types (*3*).

In contrast to Ordahl *et al.* (*10*), we find no evidence for the expression of the muscle-specific sarcomeric actin isoforms in embryonic chick brain. In the absence of sequence data on the α-actin probe used by these authors, the presence of coding regions homologous to β- or γ-cytoplasmic actin cannot be rigorously excluded and may account for this finding.

We have used this primer extension assay technique to measure the expression and regulation of the chicken actin gene isoforms transfected into mouse myogenic and nonmyogenic cells (*11*). Even though the primers described here have some homology to the mouse actin mRNA's, the length of the extended primer is unique for each gene; thus one can simultaneously measure the regulation and expression of both the mouse and chicken actin genes in mouse cells.

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1,25-Dihydroxyvitamin D_3: A Novel Immunoregulatory Hormone

Abstract. *The hormonal form of vitamin D_3, 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$], at picomolar concentrations, inhibited the growth-promoting lymphokine interleukin-2, which is produced by human T lymphocytes activated in vitro by the mitogen phytohemagglutinin. Other metabolites of vitamin D_3 were less effective than 1,25(OH)$_2$D$_3$ in suppressing interleukin-2; their order of potency corresponded to their respective affinity for the 1,25(OH)$_2$D$_3$ receptor, suggesting that the effect on interleukin-2 was mediated by this specific receptor. The proliferation of mitogen-activated lymphocytes was also inhibited by 1,25(OH)$_2$D$_3$. This effect of the hormone became more pronounced at later stages of the culture. These findings demonstrate that 1,25(OH)$_2$D$_3$ is an immunoregulatory hormone.*

The importance of 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] in mineral and skeletal metabolism is well established (*1*). However, the widespread distribution of receptors for 1,25(OH)$_2$D$_3$ in tissues not regarded to participate in mineral metabolism has made it apparent that 1,25(OH)$_2$D$_3$ plays a wider biologic role than was previously thought (*2*). We and others have found that 1,25(OH)$_2$D$_3$ receptors are present in normal human monocytes and malignant lymphocytes but are absent from resting T and B lymphocytes (*3*). Nevertheless, T and B lymphocytes obtained from normal human subjects express the 1,25(OH)$_2$D$_3$ receptor when the cells are activated in vitro by mitogenic lectins and Epstein-Barr virus, respectively (*3*). The mitogenic lectin phytohemagglutinin (PHA) stimulates T lymphocyte proliferation (*4*) and induces the production of various lymphokines, including interleukin-2 (IL-2), which is important for the growth of T cells (*5*). In the present investigation we report that 1,25(OH)$_2$D$_3$ suppresses IL-2 and inhibits the proliferation of PHA-stimulated lymphocytes.

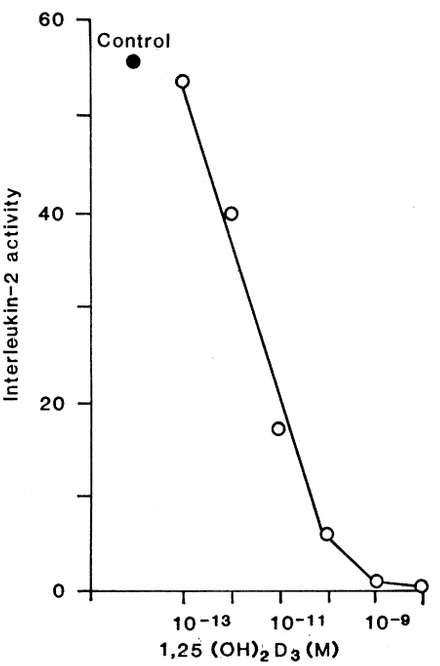

Fig. 1. Effect of 1,25(OH)$_2$D$_3$ on IL-2. Human peripheral blood mononuclear cells (1×10^6), purified by Ficoll-Hypaque gradient, were cultured with various concentrations of 1,25(OH)$_2$D$_3$ in 1 ml of medium RPMI 1640 supplemented with PHA (1 percent), fetal bovine serum (2 percent), 2-mercaptoethanol (5.6×10^{-5} *M*), indomethacin (1 μg/ml), Hepes buffer (10 m*M*), penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 m*M*), sodium pyruvate (1 m*M*), and ethyl alcohol (1 percent). Control cultures were prepared similarly, but in the absence of 1,25(OH)$_2$D$_3$. After 2 days of culture at 37°C in a humidified atmosphere of 5 percent CO_2, the media were assayed for IL-2 content with the CTLL-2 murine cell line as described (*5*). Interleukin-2 activity is expressed as the inverse of the medium dilution giving 50 percent of maximal control culture growth.

Peripheral mononuclear leukocytes were isolated by Ficoll-Hypaque gradients from blood samples obtained from normal adults. The cells were cultured for 2 days in medium containing PHA (1 percent) alone or in the presence of increasing concentrations of 1,25(OH)$_2$D$_3$ (10^{-13} *M* to 10^{-8} *M*). The IL-2 content of the media was determined by means of a bioassay that involves titration of the media on the proliferation of the murine cell line CTLL-2; proliferation of the CTLL-2 cells depends strictly on the presence of IL-2 (*5*). Using this assay method, we found that the media from activated lymphocytes grown in the presence of 1,25(OH)$_2$D$_3$ for 2 days showed reduced IL-2 activity. This effect was dependent on the concentration of 1,25(OH)$_2$D$_3$. Fifty percent reduction of IL-2 activity in the medium, in comparison with activity in media from control cultures grown without 1,25(OH)$_2$D$_3$, occurred at 2×10^{-12} *M* 1,25(OH)$_2$D$_3$ (Fig. 1). This effect was reproduced in four additional experiments with blood cells from different normal individuals. The concentration of 1,25(OH)$_2$D$_3$ causing 50 percent reduction in IL-2 activity in the four experiments was 4×10^{-12} *M*

in experiment 1, $7 \times 10^{-12}M$ in experiment 2, $10 \times 10^{-12}M$ in experiment 3, and $0.7 \times 10^{-12}M$ in experiment 4. The inhibitory effect of $1,25(OH)_2D_3$ could have occurred either on the IL-2–producing cell during mitogenic stimulation or on the IL-2–detecting cell in the quantitation assay. We therefore tested the effect of a known amount of IL-2 on the proliferation of CTLL-2 detector cells in the absence of $1,25(OH)_2D_3$ or in the presence of various concentrations ($10^{-12}M$ to $10^{-9}M$) of $1,25(OH)_2D_3$. We found that the presence of $1,25(OH)_2D_3$ in the CTLL-2 cultures did not affect the IL-2–induced proliferation. These results clearly indicated that the reduction of IL-2 content was not the result of interaction of the hormone with IL-2 or with the detector cells. Thus the effect of $1,25(OH)_2D_3$ must be exerted on the IL-2–producing cell.

To determine the specificity of the effect of $1,25(OH)_2D_3$ on IL-2 and to elucidate the role of the receptor in mediating this action, we examined the effects of other metabolites of vitamin D_3 and of vitamin D_3 itself. $1,24R,25(OH)_3D_3$ was 1.3 percent and 25-OH-D_3 was 0.2 percent as effective as $1,25(OH)_2D_3$ in inhibiting IL-2. $24R,25(OH)_2D_3$ and D_3 itself had no measurable effects at concentrations as high as $10^{-8}M$ (Table 1). The order of potency of these analogs on IL-2 suppression corresponds closely with their order of potency in other biological effects (*6*), as well as with their order of affinity for the specific $1,25(OH)_2D_3$ receptor (*7*) in various tissues. This evidence suggests that the effect of $1,25(OH)_2D_3$ on IL-2 is probably mediated by the specific receptor.

Since IL-2 is the growth-promoting lymphokine for activated lymphocytes (*5*), we tested the effect of $1,25(OH)_2D_3$ on their proliferation. We observed that after 2 days of culture the inhibitory effect of the hormone on cellular proliferation was less potent than its inhibitory effect on IL-2. However, significant inhibition of proliferation was more pronounced after 3 days of culture (Fig. 2). In experiment 2 (Fig. 2), we also noticed a significant increase of proliferative response at $1 \times 10^{-12}M$ $1,25(OH)_2D_3$. However, this augmentation was not a reproducible phenomenon, and it was not seen after 3 days of incubation. These findings suggest a difference in the time kinetics in the manifestation of the effects of $1,25(OH)_2D_3$ on IL-2 and on proliferation. Since the appearance of the $1,25(OH)_2D_3$ receptor in lymphocytes requires at least 24 hours after mitogenic activation (*3*), it seems thus

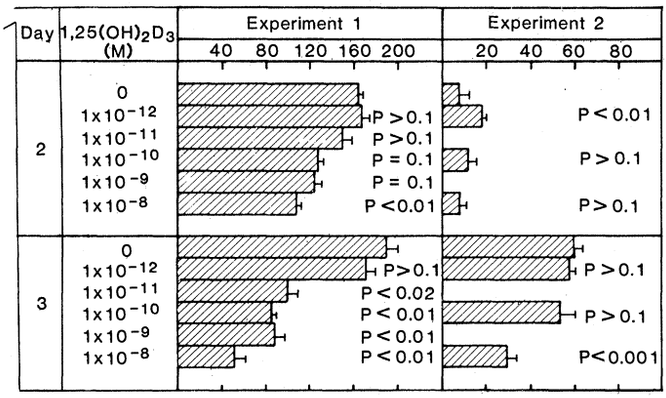

Fig. 2. Effect of $1,25(OH)_2D_3$ on lymphocyte proliferation. Peripheral blood mononuclear cells, 2×10^5, were cultured in flat-bottom microtiter tray wells for 2 or 3 days in 0.2 ml of medium containing PHA (1 percent) and various concentrations of $1,25(OH)_2D_3$ (see legend of Fig. 1). Control cultures were prepared similarly, but without $1,25(OH)_2D_3$. Proliferation was determined by adding [^{3}H]thymidine (1 μCi) in each well during the last 5 hours of incubation and measuring the incorporated radioactivity. Values shown represent the mean ± standard deviation of triplicate determinations in counts per minute. Two experiments with cells from different donors are shown. Statistical analysis was performed by Student's *t*-test.

conceivable that the above difference could be due to the time interval during which the cells are unresponsive to the effects of the hormone but sensitive to the effects of the mitogen. It is important, however, to emphasize that the effect of $1,25(OH)_2D_3$ is primarily on IL-2 and the effect on proliferation is probably secondary. This notion is supported by our studies indicating that $1,25(OH)_2D_3$ also inhibits the generation of cytotoxic T lymphocytes and antibody production by B cells.

Our results corroborate evidence obtained earlier for the expression of $1,25(OH)_2D_3$ receptors in T lymphocytes activated with mitogenic lectins (*3*) and the effect of $1,25(OH)_2D_3$ on the activated cells. Several possibilities can account for the inhibition of IL-2: (i) $1,25(OH)_2D_3$ may exert its effect directly on IL-2 by inhibiting its production or secretion, or both; (ii) $1,25(OH)_2D_3$ may increase "consumption" of IL-2 by T cells with IL-2 receptors because of changes in the IL-2 receptor concentration; or (iii) $1,25(OH)_2D_3$ may inhibit IL-2 production by promoting the differentiation of suppressor lymphocytes. Effects of $1,25(OH)_2D_3$ on differentiation of leukocytes have been observed in myeloid leukemia cells (*8*) and in normal resting monocytes (*3*). It has been suggested that calcium translocation is involved in the mitogen-induced activation of lymphocytes (*9*). In view of the well-established calcitropic effects of $1,25(OH)_2D_3$ in its classical target tissues (*1*), it is possible that the suppressive effect of this hormone on IL-2 could be mediated by an influence on calcium translocation.

We have presented evidence that $1,25(OH)_2D_3$ has a regulatory role in immune phenomena in vitro. This evidence adds a new link to the relation between the endocrine and the immune systems as exemplified by the immunosuppressive effects of corticosteroids (*10*).

Table 1. Inhibition of IL-2 by various vitamin D_3 metabolites. Human peripheral mononuclear leukocytes isolated by Ficoll-Hypaque gradient were incubated for 2 days in medium with PHA (1 percent) and various concentrations of D_3 metabolites. The IL-2 content of the media was determined as described in the legend of Fig. 1. The concentration of the metabolite causing 50 percent inhibition of control IL-2 activity is shown.

Metabolite	Concentration for 50 percent inhibition
$1,25(OH)_2D_3$	2×10^{-12}
$1,24R,25(OH)_3D_3$	1.5×10^{-10}
25-OH-D_3	1×10^{-9}
$24R,25(OH)_2D_3$	$>10^{-8}$
D_3	$>10^{-8}$

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Virus Persists in β Cells of Islets of Langerhans and Is Associated with Chemical Manifestations of Diabetes

Abstract. *Molecular hybridization, monoclonal antibody, and electron microscopic analyses showed lymphocytic choriomeningitis virus (strains Armstrong and WE) persisently infecting cells of the islets of Langerhans in BALB/WEHI mice. When monoclonal or monospecific antibody conjugated with two different fluorochrome dyes was used to mark insulin-containing β cells or viral antigens, viral nucleoprotein was identified predominately in β cells. Electron microscopy confirmed these findings by showing virions budding from the β cells. Persistent infection was associated with chemical evidence of diabetes (hyperglycemia, abnormal glucose tolerance, and normal or low-normal concentrations of insulin). Concentrations of cortisol and insulin-like growth factor in blood were normal, as was the level of growth hormone in the pituitary gland. The virus-infected islet cells showed normal anatomy and cytomorphology. Neither cell lysis nor inflammatory infiltrates were routinely seen. Thus a virus may persistently infect islet cells and provide a biochemical and morphological picture comparable to that of early adult-onset diabetes mellitus in humans.*

Diabetes mellitus, one of the most common metabolic disorders in humans, may be caused by a deficiency of insulin in genetically predisposed individuals (*1*) or may result from increased resistance of such individuals to the action of insulin (*1*, *2*). The deficiency of insulin, at least in juvenile-onset diabetes, may result from destruction of β cells in the islets of Langerhans by virus or by autoimmune constituents. Suspicion that viruses cause some cases of juvenile-onset diabetes originated from epidemiologic and pathologic surveys of humans and from animal studies (*3*). Recently, Yoon *et al.* (*4*) provided direct evidence for a viral etiology by isolating and identifying Coxsackie B4 virus from the pancreas of a child with acute diabetes. Transfer of this isolate into mice led to injury of islet cells and hyperglycemia.

The cause of diabetes beginning in adulthood is more obscure than that of juvenile diabetes. One theory implicates faulty nutrition. Because viral infection may cause juvenile-onset diabetes by destroying islet β cells, we sought to determine whether a virus can persist in the β cells of adults, perhaps altering the synthesis or function of insulin. We found that a virus, lymphocytic choriomeningitis virus (LCMV), can indeed persist in β cells of adults and be associated with aberrations in blood chemistry similar to those found in the early stages of adult-onset diabetes.

Newborn BALB/WEHI mice were inoculated intracerebrally with 60 plaque-forming units (PFU) of LCMV strain WE (30 mice) or LCMV Armstrong strain 1371 (30 mice), each strain having been cloned three times. Over 80 percent of the mice were alive 90 days later and all were persistently infected with LCMV in their blood and organs (5×10^3 to 5×10^5 PFU per milliliter), despite mounting antiviral immune responses (*5*).

Three methods were used to determine whether LCMV persisted in islet cells: molecular hybridization to localize LCMV nucleic acid, immunochemical staining to identify specific viral polypeptides, and electron microscopy to characterize virions. LCMV is a negative strand virus containing two RNA segments designated L and S, with approximate lengths of 9 and 4 kilobases (kb), respectively (*5*, *6*). The viral genome codes for three known structural polypeptides (*7*). Two of these are glycosylated and are designated GP1 and GP2 (molecular weights, 43,000 and 36,000, respectively). The third viral polypeptide is not glycosylated and constitutes the nucleoprotein (molecular weight, 63,000). GP1 and GP2 are expressed on the surfaces of infected cells or virions, being derived in equimolar amounts from a common precursor polypeptide, GPC (*7*), that is also expressed on the cell surface. A fourth suspected polypeptide, the polymerase, has not been thoroughly characterized.

The L and S RNA segments of LCMV Armstrong 1371 were isolated, reverse-transcribed into complementary DNA (cDNA), and cloned into pBR322 vector (*8*). When labeled with ^{32}P by nick translation (*8*, *9*), these cDNA probes hybridized specifically to LCMV Armstrong 1371 L or S RNA extracted from virions

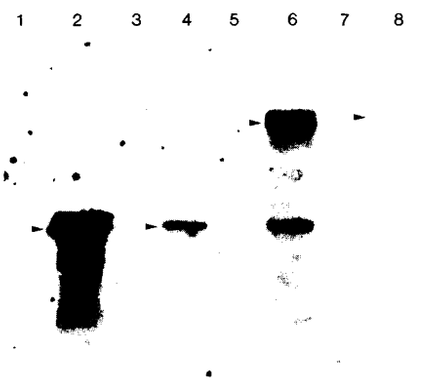

Fig. 1. Demonstration of LCMV L and S RNA segments in islet cells from 90-day-old BALB/WEHI mice persistently infected with LCMV Armstrong strain 1371 (clone 3b). When less than 18 hours old, these mice received 60 PFU of virus injected intracerebrally. At 90 days of age all the mice carried infectious virus, as determined by PFU assay or by intracerebral transfer of their serum into susceptible mice 4 to 6 weeks old. Pancreases from ten infected and ten uninfected mice were pooled separately, minced, treated with collagenase (5 mg/ml), and dissolved in Hanks balanced salt solution. After gentle stirring for 30 minutes at 37°C, dissociated cells obtained by low-speed centrifugation were placed on a discontinuous 40 percent gradient (*10*). Islet cells remained in the upper fraction and were recognized by their morphology and ability to secrete insulin in vitro and by their insulin content. RNA from the islet cells and from hybridoma 1-1-3 was obtained after treatment with guanidinium thiocyanate and analyzed by Northern blots for LCMV Armstrong 1371–specific sequences by using cDNA probes for LCMV Armstrong RNA segments L and S. Such probes do not bind to RNA extracted from uninfected cells or from cells infected with LCMV WE. Shown are sequential hybridization reactions with the same nitrocellulose filter. A cDNA probe for LCMV RNA segment S was used first (lanes 1 to 4), then a probe for segment L (lanes 5 to 8). Lanes 1 and 5 represent uninfected 1-1-3 cells; lanes 2 and 6, LCMV Armstrong–infected 1-1-3 cells; lanes 3 and 7, LCMV WE–infected islet cells; and lanes 4 and 8, LCMV Armstrong–infected islet cells. The bands in lanes 2 and 4 (arrows) represent genome-sized S RNA; the bands in lanes 6 and 8 (arrows), genome-sized L RNA. Under these conditions of hybridization this L probe shows some homology to S-sized RNA.