The Effects of Insulin and Insulin-Like Growth Factor-I on the Replication of the Human Immunodeficiency Virus Type 1 *In Vitro*

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ABSTRACT

Insulin and IGF-I, primarily considered to be metabolic and growth modulatory hormones, were found to inhibit HIV-1 replication in *de novo* infected CBMC and chronically infected U937 cells *in vitro*. The effect of IGF-I was seen at physiological concentrations or lower (1.3×10^{-10} M) while that of insulin was observed at supraphysiological concentrations (8×10^{-7} M). The EC_{50} for IGF-I was found to be 2.5 to 4.5×10^{-9} M and the EC_{50} for insulin was found to be 1.1 to 1.3×10^{-6} M. The insulin and IGF-I concentrations employed in these experiments were not toxic to the cells and did not inhibit the activity of purified reverse transcriptase activity *in vitro*. In contrast to insulin and IGF-I, EGF (10^{-10} to 10^{-8} M) did not inhibit HIV-1 replication in *de novo* infected CBMC. These results suggest that ICF-I under certain conditions has significant inhibitory effects on HIV-1 replication at physiological concentrations. This may prove to be of therapeutic value for people infected with HIV-1.

RÉSUMÉ

On a constaté que l'insuline et l'IGF-I, qui sont surtout considérées comme des hormones modulatories du métabolisme et de la croissance, inhibent la réplication du VIH-I dans des cellules mononucléares de sang cordonal (CMSC) infectées *de novo* et des cellules U937 infectées chroniquement *in vitro*. L'effet de l'ICF-I a été observé à des concentrations physiologiques ou à des concentrations plus faibles (1,33 x 10^{-19} M), tandis que celui de l'insuline a été observé à des concentrations supraphysiologiques (8 x 10^{-7} M). La CE_{50} de l'IGF-I était de 2,5 à 4,5 x 10^{-9} M; la CE_{50} de l'insuline était de 1,1 à 1,3 x 10^{-6} M. Les concentrations d'insuline et d'IGF-I utilisées dans ces expériences n'ont produit aucun effet toxique sur les cellules et n'ont pas inhibé l'activité de la transcriptase inverse purifée *in vitro*. Contrairement à l'insuline et à l'IGF-I, l'EGF (10^{-10} a 10^{-8} M) n'a pas inhibé la réplication de l'VIH-1 dans des CMSC infectées *de novo*. Ces résultats semblent indiquer que, dans certaines conditions, l'IGF-I presente un effet inhibitoire impotant sur la réplication de l'VIH, à des concentration physiologiques. Ces observations pourraient présenter une utilite thérapeutique pour les personnes infectées par l'VIH-1.

INTRODUCTION

1. THE ACQUIRED IMMUNODEFICIENY SYNDROME (AIDS)

1.1 History

The acquired immunodeficiency syndrome (AIDS) was first recognized as new clinical disease in North America in 1981 (Gottlieb *et al.*, 1981, Masur *et al.*, 1981 and Seigal *et al.*, 1981). The first cases of AIDS were recognized because of an unusual occurrence of diseases such as Kaposi's sarcoma and *Pneumocystis carinii* in young healthy homosexual men. AIDS cases were soon reported in other populations including intravenous drug users (Centers for Disease Control, 1982), hemophiliacs (Davis *et al.*, 1983), blood transfusion recipients (Curran *et al.*, 1984), adults from Central Africa (Clumeck *et al.*, 1983), and babies born to mothers with AIDS (Rubinstein *et al.*, 1983). It became apparent that this new disease was caused by an unknown infectious agent that could be transmitted by blood, blood products, from mother to child *in utero*, as well as semen and other body fluids.

In 1980, Gallo and his colleagues discovered the first human retrovirus associated with disease (Poiez *et al.*, 1980). This new retrovirus infects T-helper lymphocytes and was named human T-lymphotropic virus type I (HTLV-I). Similarities in transmission and immunosuppression between AIDS and HTLV-I infection led some scientists to postulate that a new retrovirus could be causing AIDS. Subsequently, in 1983, three groups of researchers independently isolated a T-lymphotropic retrovirus from patients at risk of developing AIDS. The viruses were named Lyphdenopathy-associated virus (LAV) (Barre-Sinoussi et al., 1983),

HTLV-III (Popovic *et al.*, 1984), and AIDS related virus (ARV) (Levy *et al.*, 1984). The isolates have been shown to be the same virus and now are collectively called the human immunodeficiency virus type 1, or HIV-1.

Now, 13 years since the first reported cases of AIDS, 10 years since the isolation of HIV-1, current estimates of world wide infection range between 13 million and 19.5 million people (Greene, 1993 and Jasny, 1993). Estimates of infection for the year 2000 range between 40 million and 110 million (Greene, 1993 and Jasny, 1993). The many research efforts in the last decade have led to the identification of HIV-1, the understanding of its life cycle and molecular structure, and the development of novel treatment strategies. As a result, HIV-1 is now understood more fully than any other retrovirus, however, with the rapid spread of infection, and the lack of a vaccine or satisfactory treatment, this disease remains a severe threat to human health world wide.

1.2 HIV-1 Classification

The first retrovirus to be discovered, the Rous sarcoma virus (RSV), was described in 1911 as a filterable agent that caused sarcomas in chickens (reviewed in Cullen *et al.*, 1993). Some years later a major breakthrough led to the identification of a unique enzyme, reverse transcriptase (RT), that allowed the virus to transcribe RNA into DNA and than incorporate into the host cell genome (Temin and Mizutani, 1970, Baltimore, 1970). This discovery provided unique insight into the retroviral life cycle and within one year the first human retrovirus, the human foamy virus (HFV) was isolated (Achong *et al.*, 1971). Now numerous retroviruses have been identified and they are divided into

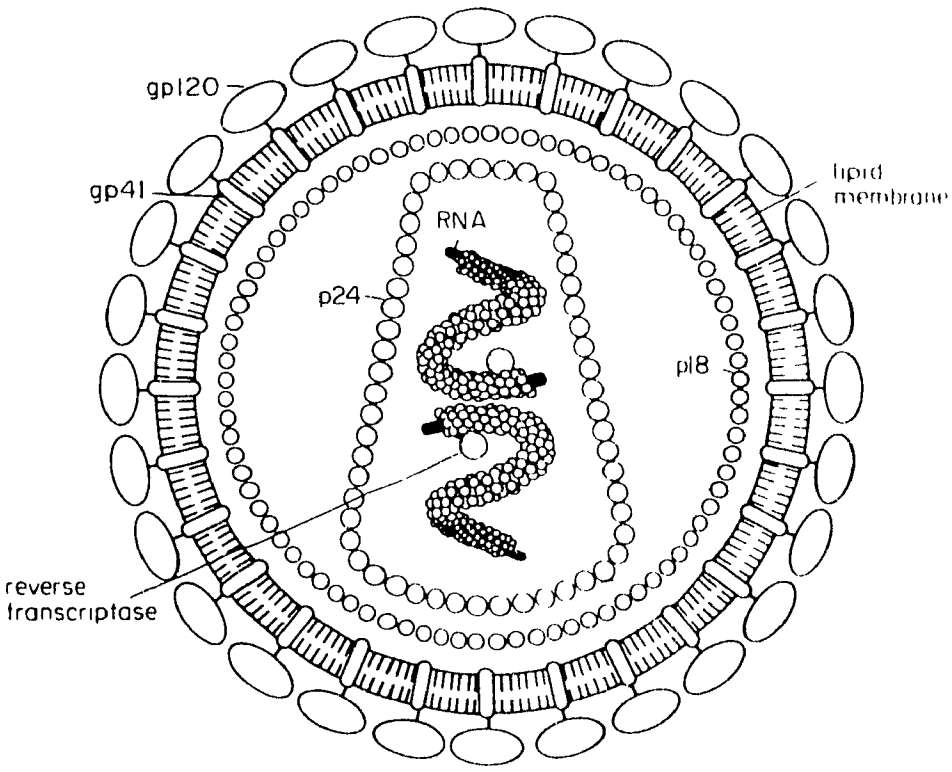
three subgroups: Oncovirinae, includes all oncogenic retroviruses and closely related non-oncogenic viruses; Lentivirinae, includes the "slow" viruses such as the visna-maedi virus, caprine arthritis encephalitis virus (CAEV), equine infectious virus (EIAV) and HIV; Spumavirinae, includes the foamy viruses that induce persistent infections without clinical disease (Teich, 1984). Like HIV-1, visna virus, CAEV and EIAV cause slow progressive wasting disorders that are often fatal (Narayan and Cork, 1985, Cheevers and McGuire, 1985). Sequence similarity and protein cross-reactivity have shown that HIV-1 is also closely related to the primate simian immunodeficiency virus (SIV) (Kanki *et al.*, 1985). One strain, SIVmac is known to cause a form of AIDS in captive macaques, where other strains including SIVsm and SIVagm are nonpathogenic to their hosts, the sooty manabey and African green monkey respectively (Fultz *et al.*, 1986, Daniel *et al.*, 1985, Letvin *et al.*, 1985). Another human T-cell lymphotropic retrovirus that shares sequence homology and serologic reactivity with HIV-1 was isolated from west Africans and is known as HIV-2. HIV-2 causes immunodeficiency and a clinical syndrome which is similar to AIDS though less pathogenic. (Clavel *et al.*, 1986, 1987, Brun-Vezinet *et al.*, 1987).

1.3 HIV-1 Structure and Genome

High resolution microscopy has shown the HIV-1 virion to be spherical and 100 nm in diameter (figure 1). The outer coat consists of a bilayer of lipid molecules derived from the membranes of human host cells. Studding the coat are 72 spikes. Each spike is a glycoprotein made up of 2 components: gp120, the extracellular portion of the glycoprotein

Figure 1: The HIV-1 Virion

Schematic representation of the HIV-1 virion including: the external envelope glycoproteins, gp120 and gp141, the nucleocapsid proteins, p24 and p18, the RNA genome and associated reverse transcriptase. (Modified from Connor and Ho, 1992).


gp120
gp41
lipid membrane
RNA
p24
p18
reverse transcriptase

that is believed to play a crucial role in binding HIV-1 to target cells; and gp41, the transmembrane portion of the glycoprotein which is involved in viral fusion. Underneath the outer coat is a layer of matrix proteins (p17) that associates with the inner surface of the lipid bilayer and possibly acts to stabilize the interior and exterior components of the virion (Greene, 1991). The nucleocapsid appears as a hollow truncated cone formed from an assembly of p24 proteins. This cone contains the two strands of viral RNA associated with several viral enzymes including RT, integrase, and protease. In addition the p7 protein binds directly to the viral RNA through a zinc finger motif and together with protein p9 forms the nucleoid core (Greene, 1991).

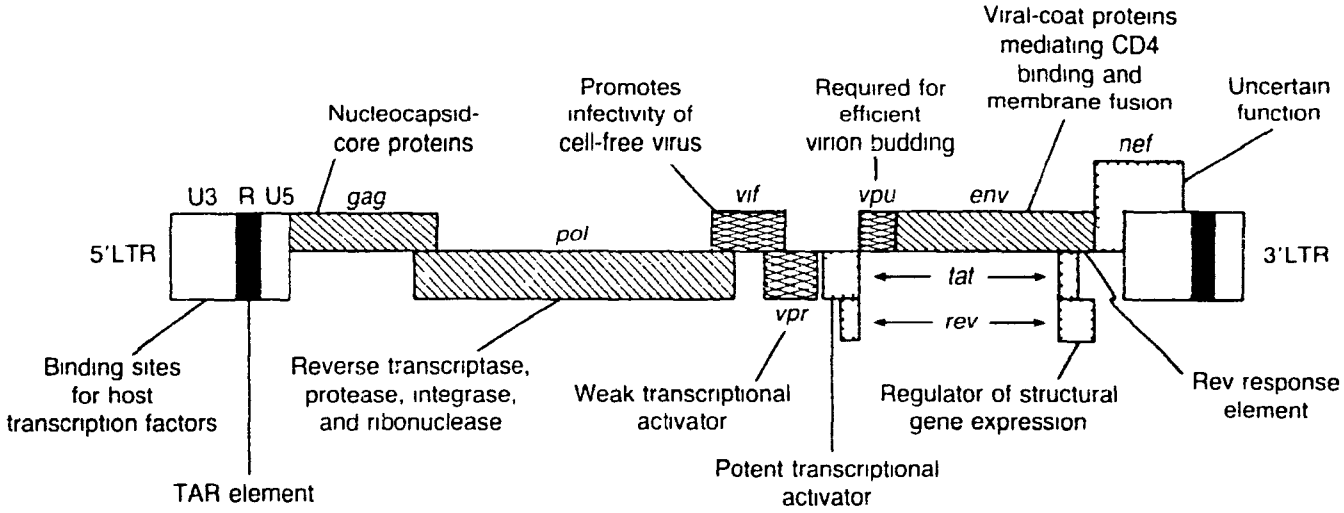
The HIV-1 genome is 9.7 kb in length (Hahn *et al.*, 1984). Molecular cloning and sequencing of HIV-1 has revealed that along with the conventional retroviral genes, *gag*, *pol*, and *env*, it encodes at least another eight. The genes encoded by HIV-1 have been classified as: the structural genes, *gag*, *pol*, and *env*; the regulatory genes, *tat*, *rev*, and *nef*; and the accessory genes, *vif*, *vpr*, *vpu*, *vpt* and *tev/tnv* (figure 2). The HIV-1 genome displays unprecedented economy in its coding potential as is evident from the presence of nine overlapping genes (Vaishnav and Wong-Staal, 1991).

The structural genes encode the viruses core proteins, glycoproteins and enzymes. The *gag* region encodes the matrix protein p17, capsid protein p24 and the nucleocapsid proteins p7 and p9. The *pol* region encodes RT p66/p51, protease p10 and integrase p32. The *env* region encodes the extracellular gp120 and transmembrane gp41 glycoproteins.

The regulatory genes play an important role in gene expression. Both *tat* and *rev* are known to enhance viral replication. The *tat* gene

Figure 2: Genomic Structure of HIV-1

This is a schematic representation showing each of the nine known genes of HIV-1 and notes their primary function. The LTR sequences, the RRE and the TAR are also indicated (Adapted from Greene, 1993).


Viral-coat proteins mediating CD4 binding and membrane fusion
Uncertain function
Promotes infectivity of cell-free virus
Required for efficient virion budding
Nucleocapsid-core proteins
nef
U3 R U5
gag
vif
vpu
env
pol
5'LTR
3'LTR
tat
rev
vpr
Binding sites for host transcription factors
Reverse transcriptase, protease, integrase, and ribonuclease
Weak transcriptional activator
Regulator of structural gene expression
Rev response element
TAR element
Potent transcriptional activator

product acts through a cis-acting sequence called the transactivation responsive element (TAR) and the *rev* gene product acts through a cis-acting sequence known as the rev-responsive element (RRE) (Sodroski *et al.*, 1986, Rice and Mathews, 1988, Olsen *et al.*, 1990). The third regulatory gene of HIV-1 is called *nef*, or negative factor. As the name implies, *nef* acts to down regulate viral replication, and this action is thought to be mediated through the interaction with a specific sequence known as the negative regulatory element (NRE) (Ahmad and Venkatesan, 1988). Unlike *tat* and *rev*, *nef* does not interact with viral RNA and is not required for viral replication (Terwilliger *et al.*, 1986).

The HIV-1 genome contains numerous accessory genes which encode proteins that are involved in many different aspects of the viral life cycle. One common characteristic of the accessory genes is that they are highly conserved in natural isolates but may become defective when extensively passaged in tissue culture. Therefore the products of these genes are probably dispensable and selected against during *in vitro* growth (Vaishnav and Wong-Staal, 1991). The *vif* (virion infectivity factor) gene product increases viral infectivity and is thought to work at the level of cell-free virus transmission but the mode of action is not understood (Fisher *et al.*, 1987). The *vpu* (viral protein U) gene product facilitates assembly and release of viral particles, but the precise mode of action is not known (Strebel *et al.*, 1988). The *vpr* (viral protein R) gene product accelerates the rate of viral protein production. It is thought to act by stimulating expression of reporter genes (Cohen *et al.*, 1990). The *vpt* (viral protein T) gene product is a fusion protein and its function is still unknown (Sonigo *et al.*, 1985). The *tnv* (tat-rev-env fusion protein) gene

product is a protein that is formed by a complex splicing event involving tat, rev and env, and has been shown to have tat and rev activities (Salfeld *et al.*, 1990).

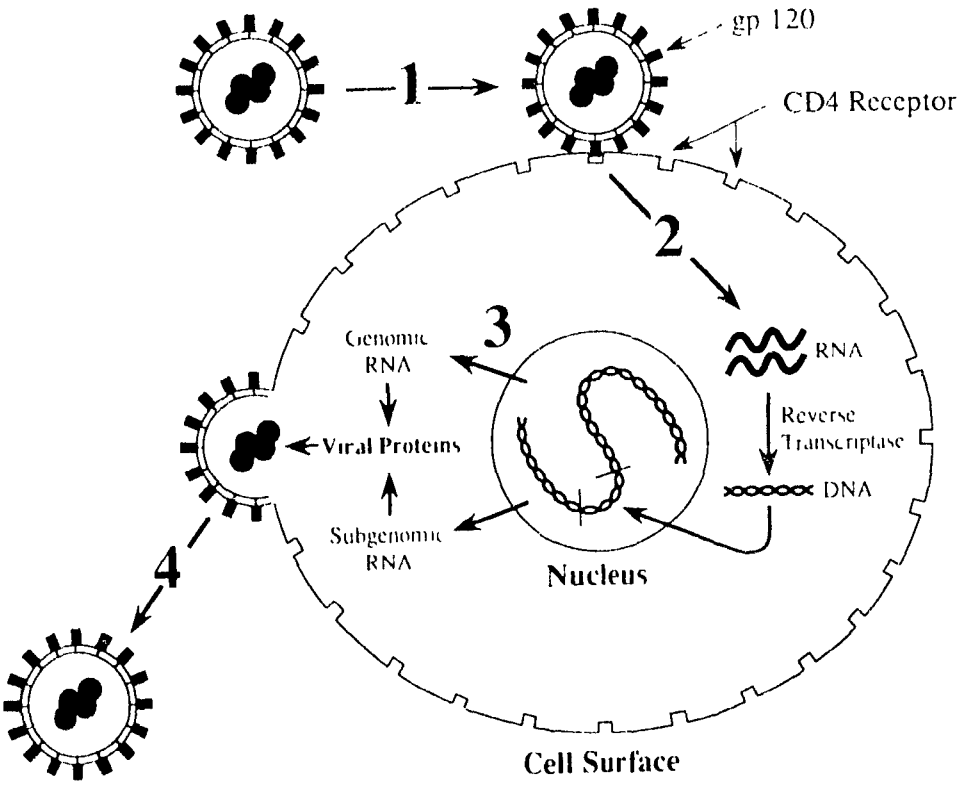
The HIV-1 genome has 5′ and 3′ ends flanked by regions called the long terminal repeats (LTR) (Starcich *et al.*, 1984). These flanking regions contain regulatory sequences that are recognized by viral and host transcriptional factors. Reverse transcription is primed by a cellular tRNA lysine hybridized with specific sequences in the 5′ LTR and proceeds towards the 5′ end of the viral RNA. Two separate and consecutive strand transfer events must take place to completely form a double stranded DNA replica of the original genome (Panganiban and Fiore, 1988).

1.4 HIV-1 Replication Cycle

The first step in the initiation of infection is the binding of a virus particle to a specific target cell surface receptor (figure 3). In the case of HIV-1, the carboxy terminal region of gp120 binds with the CD4 receptor found on immune system cell membranes. Transmembrane gp41 mediates fusion between the viral envelope and the host cell membranes (Gallaher, 1987). Following binding and internalization, the viral reverse transcriptase works to transcribe viral RNA into RNA/DNA hybrids, and then into double stranded linear DNA molecules. This newly formed HIV-1 DNA (proviral DNA) is translocated to the nucleus where it is integrated into the host cellular DNA by the endonuclease activity of the viral integrase (Brown *et al.*, 1987). Once the provirus is integrated into the host DNA, it behaves like a cellular gene and will duplicate with each replication cycle of the cell. Upon cellular activation there is an induction

Figure 3: The HIV-1 Replication Cycle

This is a schematic representation of the stages of the HIV-1 life cycle. The stages include: binding of virus to specific CD4 receptors at the cell membrane, uncoating of viral RNA, converting RNA into DNA by the actions of reverse transcriptase, integration of viral DNA into host DNA, production of viral RNA and proteins, viral assembly and release (Adapted from Wainberg and Margolese, 1992).


gp 120
1
CD4 Receptor
2
3
Genomic RNA
Viral Proteins
Subgenomic RNA
RNA
Reverse Transcriptase
DNA
Nucleus
4
Cell Surface

of a number of host transcriptional factors that bind to recognition sites within the HIV-1 enhancer element, resulting in the initiation of viral replication (Gowda *et al.*, 1989). Following translation, viral proteins are subject to post translational processing including proteolytic cleavage, glycosylation, myristilation and phosphorylation. Retroviral assembly is unique in that products of *gag* and *pol* are incorporated into virions in the form of their polyprotein precursors and then are proteolytically cleaved during or after budding to form mature particles. Assembly of the virion core which is composed of HIV-1 RNA, modified viral proteins and enzymes takes place at the plasma membrane. Mature virions are formed by budding through the cell membrane during which time they acquire an outer lipid bilayer containing the external and transmembrane envelope glycoproteins (Greene, 1991).

1.5 Cell Susceptibility and Immunopathogenesis

HIV-1 has strong tropism for cells bearing the CD4 antigen, and it has been demonstrated that CD4 is the specific receptor for the virus (Klatzman *et al.*, 1984). CD4 is commonly found on T-cells, Epstein-Barr virus (EBV)-transformed B-cells, dendritic cells and cells of the monocyte/macrophage lineage, thus making these cells susceptible to HIV-1 infection. (Gartner *et al.*, 1986 Monroe *et al.*, 1988). Monocytes appear to be able to harbour the infection for long periods of time and are thus thought to serve as a viral reservoir spreading infection by cell-to-cell contact (Roy and Wainberg 1988). In addition, infected monocytes can pass through the blood-brain barrier into the brain, where they are thought to play a role in HIV-associated neuropathogenesis (Price *et al.*, 1988).

The major basis for immunopathogenesis of HIV-1 infection is believed to be the dysfunction or depletion of CD4 expressing T-lymphocytes. A reduction in the number of CD4+ T-cells is known to result in numerous biological changes including a reduction of cytokines secreted by CD4+ T-cells, (Rook *et al.*, 1983) a loss of antigen specific responses (Fauci *et al.*, 1985), an impaired ability to induce immunoglobulin secretion from B-cells (Lane *et al.*, 1983), defective expression of interleukin (IL)-2 receptors, and a decrease of HLA-resticted cytotoxic T-cell responses (Rosenberg and Fauci, 1989). In fact, there has been a direct correlation between the increase in viral burden with the decrease of CD4+ T-cells and the development of AIDS (Schnittman *et al.*, 1990). Although only few circulating CD4+ T-cells are thought to be infected at any one time (less than 0.1% during the asymtomatic stages to 1% with symtomatic AIDS (Ho *et al.*, 1989)) evidence suggests destruction of these cells is one of the major causes of immune system failure in AIDS.

Several mechanisms for CD4+ T-cell depletion and dysfunction have been proposed.

1. Single cell killing is believed to result from the accumulation of unintegrated DNA or from the inhibition of cellular protein synthesis after HIV-1 infection (Garry, 1989).

2. Syncitia formation involves the fusion of cell membranes of uninfected cells with the cell membrane of an infected cell resulting in the formation of giant, non-functional multinucleated cells (Lifson *et al.*, 1986). Syncitia formation has been demonstrated *in vitro* and has been associated with the cytopathicity of the virus. However, syncitia has rarely been seen *in vivo*,

but it is thought to occur in the lymph nodes where HIV-1 burden is the heaviest (Pantaleo *et al.*, 1991).

3. HIV-1-specific immune responses occur when virus-infected cells expressing viral antigen are killed by cytotoxic T-lymphocytes (CTL). However, uninfected T-cells that are bound to soluble gp120 viral antigen may mistakenly become targets of sensitized CTL (Germain *et al.*, 1988).

4. The CD4 molecule is involved in antigen-specific responses as a ligand to the class II major histocompatibility (MHC) complex. Inhibitors of antigen specific responses could occur if HIV-1 infection resulted in alterations of CD4 expression. In fact, HIV-1-induced down regulation of CD4 expression in T-cells and monocytes has been observed *in vitro* (Folks *et al.*, 1985, Geleziunas *et al.*, 1991). In addition to this, it has been shown that *in vitro* exposure of CD4+ T-cells to HIV-1 proteins results in suppression of cell function, presumably by inhibiting normal cell signalling pathways (Ruegg *et al.*, 1990, Cefai *et al.*, 1990, Hofmann *et al.*, 1990).

5. HIV-1 is not thought to replicate in quiescent T-cells, but following cellular activation there is a massive burst of viral gene activation that results in cytopathology and cell death (Zagury *et al.*, 1986, Zach *et al.*, 1990). Recently, Cameron *et al.* (1992) showed that peripheral blood antigen presenting dendritic cells provide a microenvironment suitable for massive high-level T-cell activation coupled with transmission of HIV-1 infection. Continuous activation-induced cell death may contribute to the progressive loss of CD4+ T cells.

6. Considerable attention has been given to the possibility that a superantigen may play an important role in the immunopathogenesis of

HIV-1. Superantigens have been shown to bind to the variable region of the β–chain of T-cell antigen receptors causing massive stimulation and expansion of the T-cells bearing this specific β–chain. The result is a deletion or anergy of this subset of T-cells. In HIV-1 infection, rather than causing deletion of T-cell subgroups, superantigens may serve as potent activators of T-cells rendering them more susceptible to infection (Imberti *et al.*, 1991, Laurence *et al.*, 1992).

7. Programmed cell death or apoptosis is a normal mechanism of cell death that was originally described in the context of the response of immature lymphocytes to cellular activation (Duvall and Wylie, 1986). It is a mechanism used by the body to eliminate autoreactive clones of T-cells. Apoptosis in relation to HIV-1 infection has received a great deal of attention in the last few years. It is thought that the cross linking CD4 molecules with HIV-1 gp120 or gp120-anti-gp120 immune complexes primes cells for the programmed death that occurs when a MHC class II molecule in complex with an antigen binds to the T-cell antigen receptor (Ameisen and Capron, 1991).

It is unlikely that any one of the above mentioned mechanisms is solely responsible for the depletion of CD4+ T-cells, rather they most likely work in concert to produce the severe immune system dysfunction associated with HIV-1 infection.

1.6 Clinical Aspects of HIV-1 Infection

The process of infection can be divided into three phases; initial infection, asymptomatic or latent phase, and symtomatic phase or AIDS. Initial infection with HIV-1 is associated with mild flu-like symptoms such

as fever or muscle aches that last for a few weeks (Gaines *et al.*, 1988). A large amount of virus is present in the bloodstream and the immune system mounts an attack on infected cells and circulating virus. This phase lasts for only a few weeks and ends with seroconversion and the establishment of chronic verimia. The next phase, asymptomatic or latent phase can last as long as a decade. New evidence concerning the state of viral replication in this phase suggests that it may have been misnamed. Although levels of HIV-1 in the bloodstream are low, suggesting little viral replication, it is now known that during this phase there is a steady production of virus and it occurs primarily in the lymph nodes (Pantaleo *et al.*, 1991). It is not until the end of this phase that there is a rise in circulating viral particles. This is partly thought to be attributed to the break down of the lymph nodes which results in a release of virus into the circulation. Occurring with the steady increase of virus is a slow depletion of CD4+ T-cells which ultimately results in a severe suppression of most aspects of cell-mediated immunity (Fauci, 1988). In comparison to the late stage of this disease, the asymptomatic phase is relatively symptom free, however, minor symptoms such as diarrhea and night sweats persist. A progressive pattern of opportunistic infections drives the disease into its last stage where further infections, malignancies, AIDS-related dementia, and wasting eventually prove to be fatal.

Interestingly, in the late 1970's a disease in Uganda, locally known as "Slim Disease" because of associated chronic wasting, was the first documented encounter with AIDS (Serwadda *et al.*, 1985). Although the initial presentation of AIDS in North America was associated with Kaposi's sarcoma and pneumonia, now both chronic diarrhea and weight

loss are added to the AIDS case definition (World Health Organization, 1986, Centers for Disease Control, 1987). It is widely accepted that wasting and diarrhea will eventually occur during the course of AIDS. In many, deaths seem to be determined by the individuals nutritional status rather than by any particular opportunistic infection (Keusch and Thea, 1993). Therefore it is not surprising that there has been considerable interest in nutrition associated with AIDS. It is clear that strategies directed at maximizing nutrition and minimizing loss of lean body mass are important factors in fighting the disease process.

2. INSULIN AND INSULIN-LIKE GROWTH FACTOR-I

2.1 Insulin

In humans and most other vertebrates, insulin is the primary hormone involved in the control of blood glucose. Insulin acts by stimulating glucose influx and metabolism in muscle and adipocytes and by inhibiting gluconeogenesis by the liver. Some of the metabolic actions of insulin include glycogen, fat and protein synthesis and breakdown, as well as nutrient uptake, DNA synthesis, cell growth and differentiation. In addition insulin modifies the expression or activity of a variety of enzymes and transport systems in nearly all cells (reviewed in Cohen, 1993). Insulin is secreted from β–cells located in the islands of Langerhans of the pancreas and there is supporting evidence which suggests that insulin can be synthesized in the lung, intestine and central nervous system (Shuldiner *et al.*, 1991).

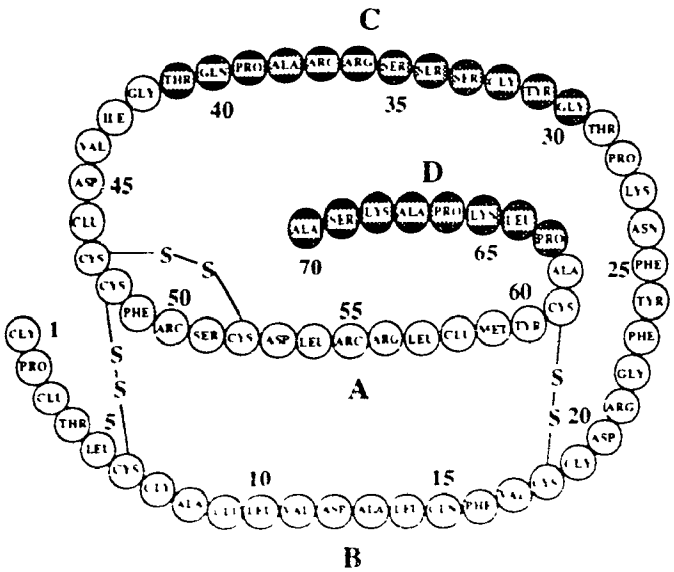
Human insulin is synthesized as a high molecular weight peptide (preproinsulin), processed as an intermediary precursor (proinsulin) and then cleaved to form a mature insulin molecule. Human insulin has a molecular weight of approximately 6000 Da and consists of two chains held together by two sulfhydryl bonds (figure 4). The A-chain consists of 21 amino acids and is responsible for the differences in amino acid composition observed between species. Amino acid 1 of the A-chain is directly involved in receptor binding and in maintaining insulin structure (Blundell, 1979). The B-chain consists of 30 amino acids and experiments have shown that amino acids 24 to 26 are important in receptor binding (Steiner *et al.*, 1990).

2.2 The Insulin Receptor

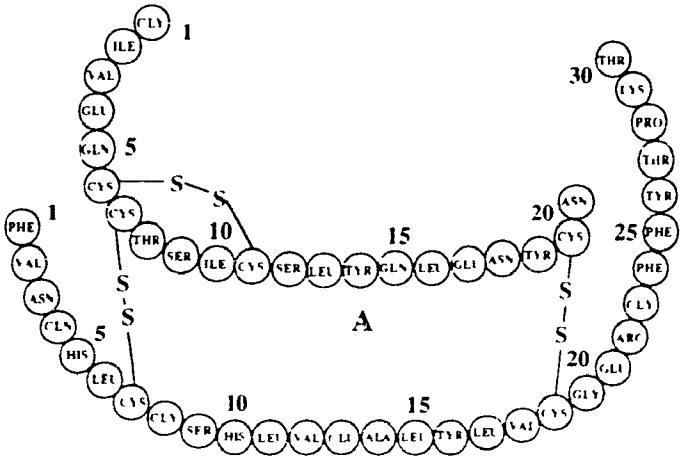
Despite the numerous biological actions insulin takes part in, all these actions are thought to be mediated through a single receptor. The insulin receptor is an integral plasma membrane glycoprotein consisting of four subunits: two α–subunits are linked by disulfide bonds to two β–subunits (figure 5) (Jacobs *et al.*, 1980). Both subunits are synthesized from a single proreceptor which is cleaved and processed prior to insertion into the plasma membrane. The α–subunits are located on the outside of the cell and contain the insulin binding domain. The extracellular part of the β–subunit is bound to the α–subunit by disulfide bonds. The intracellular portion of the β–subunit contains a juxtamembrane region (essential for signal transmission), a kinase or regulatory domain which contains an ATP binding site, and a C-terminal domain, all of which have tyrosine autophosphorylation sites (Carpentier, 1993). The insulin receptor is

Figure 4: Structures of Human IGF-I and Human Insulin

This is a schematic representation of the structures of human IGF-I and human insulin showing the A, B, C, and D chains of IGF-I and the A and B chains of insulin. The amino acid composition of the hormones as well as the internal disulfide bonds are also indicated (Adapted from Slieker *et al.*, 1993).



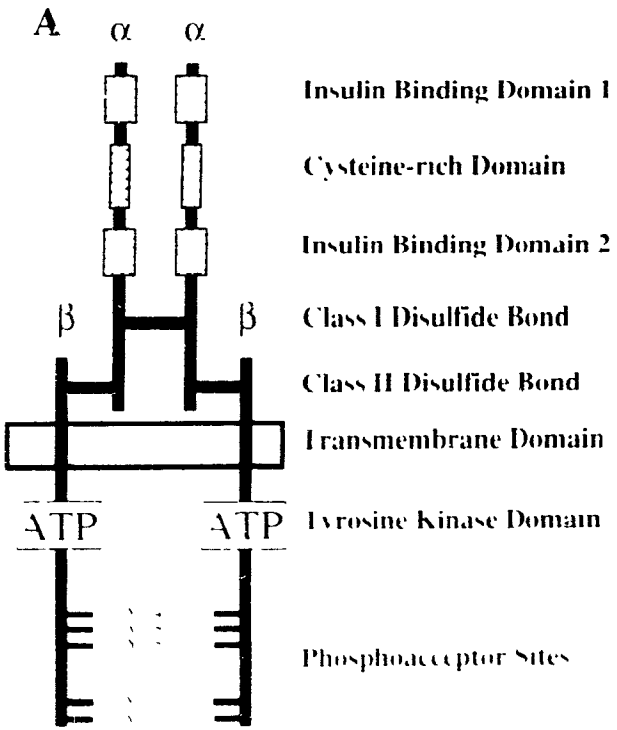
Human IGF-I



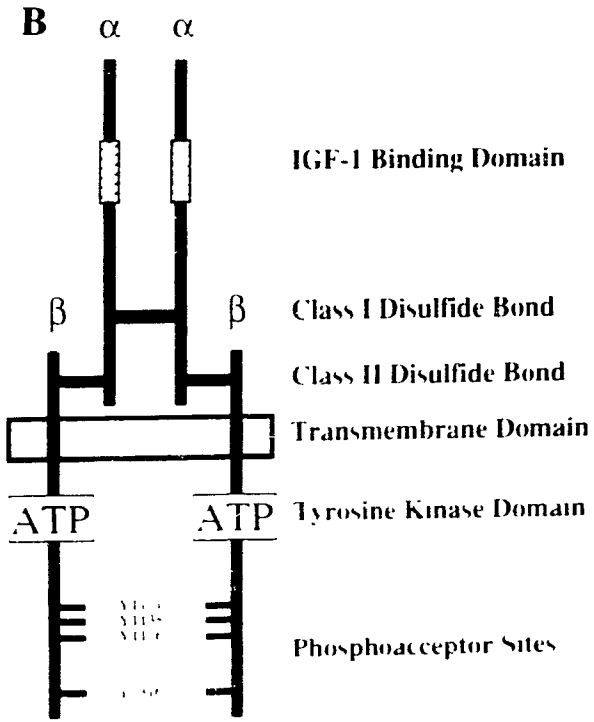
Human Insulin

Figure 5: Human Insulin and IGF-I Receptors

The models of the human insulin and IGF-I receptors show the various functional domains of the molecules including: hormone binding domains, transmembrane domains, tyrosine kinase domains, and the phosphorylation sites. The receptor α and β chains are joined by disulfide bonds as indicated (Adapted from Pessin, 1993).



hInsulin Receptor



hIGF-1 Receptor

present in most vertebrate tissues and can range in concentrations from 40 receptors on circulating erythrocytes to greater than 200 000 in adipocytes and hepatocytes (White and Kahn, 1994).

2.3 Insulin Signalling

Once insulin binds to its receptor numerous cellular events are known to occur. Determining how insulin transmits its signal to the cell has been an area of extensive research. One model of insulin signal transmission is the autophosphorylation of tyrosine residues that cause a conformational change in the β–subunit of the receptor facilitating the interaction of the receptor with cellular elements responsible for downstream signalling (White and Kahn, 1994). Many of these elements are substrate tyrosine kinases that act as second messengers used in numerous signalling cascades. This model is attractive because it is used by the growth factor receptors of epidermal growth factor (EGF) and platelet derived growth factor (PDGF), both of which interact with phopholipase C-γ, p21ras-GAP and growth factor receptor-bound protein (GRB) -2/Sem-5. The exact downstream elements responsible for the pleotropic insulin responses remains to be disclosed.

A second model has earned increased attention in the last few years. It involves the phosphorylation of a cell substrate to the insulin receptor. After insulin stimulation the receptor was shown to immunoprecipitate with a protein that contains numerous threonine/serine and tyrosine phosphorylation sites. This protein, now called insulin receptor substrate-1 (IRS-1) is known to contain threonine/serine phosphorylation sites with homology to casein kinase II, protein kinase C, the microtubule associated

protein (MAP) kinases, cdc2, and cAMP and cGMP-dependent protein kinase phosphorylation sites (Defronzo *et al.*, 1992). IRS-1 is also thought to be linked to Src homology (SH)-2 containing proteins including SH-2-containing protein tyrosine phosphatase-2 (SH-PTP-2) and GRB-2/Sem-5 (Kuhne *et al.*, 1993, Skolnik *et al.*, 1993). Tyrosine phosphorylated, IRS-1 is also known to stimulate phosphatidylinositol (PI) - 3′ kinase via the p85α subunit which possesses two SH-2 domains (Meyers *et al.*, 1992). In addition, it has been shown that over expression of IRS-1 enhances insulin mitogenesis, and insulin receptors that are mutated in the juxtamembrane region are incapable of inducing IRS-1 phosphorylation (Sun *et al.*, 1992, Backer at al., 1991).

The IRS-1 has been shown to be an *in vivo* substrate for both the insulin and IGF-I receptors, but not the EGF, PDGF, and colony stimulating factor (CSF)-1 receptors (Myers and White, 1993). Unlike the EGF and PDGF receptors, the insulin receptor does not directly associate with SH-2 containing proteins. Therefore the IRS-1 proves to be an important link to many cellular substrate kinases and it can serve as a "docking port" for many unique cellular signalling pathways that can be mediated by insulin.

2.4 The Insulin-Like Growth Factors (IGF)

Other hormones that are associated with insulin and play a crucial role in cell signalling are the family of polypeptide insulin-like growth factors (IGF). These growth factors contain structures whose amino acid sequences or primary structures contain significant homology to insulin. The best characterized members include IGF-I and II. Both IGF I and II are

circulating polypeptides which have profound effects on the proliferation and differentiation of a wide range of cell types. They are capable of exerting insulin-like metabolic effects. Unlike insulin, they are produced by most tissues in the body and are abundant in circulation (Cohick and Clemmons, 1993). IGF-II is synthesized more prominently during fetal development, whereas IGF-I synthesis persists at high levels in a wide range of adult tissues (Bennett and Schultz, 1993). IGF-I shares similarity to insulin in structure, receptor type, and, signal transduction mechanism and action.

2.5 IGF-I and the IGF-I Receptor

IGF-I was previously known as somatomedin-C because of the ability to stimulate skeletal cartilage and bone growth, as well as to increase organ size and body weight (Guler *et al.*, 1988, Isaksson *et al.*, 1987). Pituitary-derived growth hormone (GH) causes the release of IGF-I from the liver which can act in an endocrine manner by circulating to target tissues. IGF-I can also be produced locally within target tissues and in this way acts in an autocrine and paracrine manner.

IGF-I is a single chain polypeptide of 70 amino acids and has a molecular weight of 7649 Da. The A and B-chains of IGF-I are similar to the insulin A and B-chains (figure 4). In addition IGF-I has a C-chain that is representative of the polypeptide chain found in proinsulin, and a D-chain not found in insulin molecules.

In parallel with the structural homologies between IGF-I and insulin, the structure of their receptors is also highly homologous and they are believed to be derived from the same primordial gene (Ullrich *et al.*, 1986).

The IGF-I receptor consists of a heterotetrameric arrangement of two α–subunits linked by disulfide bonds to two β–subunits (figure 5). The α–subunits are extracellular and contain the IGF-I binding domains. The β–subunits span the cell membrane and their cytoplasmic domains possess intrinsic tyrosine kinase activity (Ullrich *et al.*, 1986). Ligand binding to the α–subunits stimulates protein kinase activity which leads to phosphorylation of the β–subunit as well as the phosphorylation of the cytoplasmic components of the receptor-specific signalling pathways. Despite the structural similarities, the IGF-I receptor exhibits an affinity for IGF-I that is about one hundred times higher than that for insulin. Similarly, the affinity of the insulin receptor for insulin is one hundred fold greater than for IGF-I (Czech, 1989).

2.6 IGF-I Binding Proteins

In contrast to most other peptide hormones, the IGF family appears in circulation bound reversibly and with high affinity to carrier proteins called the insulin-like growth factor binding proteins (IGFBP). Six binding proteins have been identified and designated IGFBP-1 through 6. The major site of production of the binding proteins is the liver and their circulating levels are regulated by GH. The exact role of the binding proteins is not clear but they have been shown to both inhibit and potentiate the metabolic and mitogenic effects of IGF at the cellular level (Baxter and Martin, 1989, Conover *et al.*, 1990). It has been suggested that the binding proteins can increase the circulating half life of IGF-I from thirty minutes in its free state to several hours (Guler *et al.*, 1989). It has

been shown that IGFBP-3 binds over 95% of circulating IGF-I and increases its half life by ten to fifteen hours (Chick and Clemmons, 1993).

2.7 IGF-I Actions and Clinical Applications

The *in vitro* effects of IGF-I include the stimulation of DNA, RNA and protein synthesis, the uptake of glucose and amino acids, and the promotion of cell differentiation (Cohick and Clemmons, 1993). There has been increasing information on the *in vivo* biological effects of IGF-I. For instance, IGF-I treatment of GH-deficient rats results in body weight gain, longitudinal bone growth, increase in kidney, spleen and thymus weights (Glasscock *et al.*, 1992, Lemmey *et al.*, 1991, Martin *et al.*, 1991). There is also increasing evidence that links IGF-I action through GH to the immune system. Mice with GH deficiency are known to have impaired immune systems associated with thymic atrophy, immunodeficiency and tissue wasting (Fabris *et al.*, 1971). Administering exogenous GH to these rats results in T-cell proliferation in the thymus and alters the activity of T-cells, B-cells, natural killer cells and macrophages (Timsit *et al.*, 1992, Kelley 1989). It is thought that the GH actions on the immune system are mediated by local induction of IGF-I (Tapson *et al.*, 1988, Stuart *et al.*, 1991). In fact, it has been shown that nanomolar concentration of IGF-I can promote growth in lymphocytes and be chemotactic for resting and activated T-cells (Schimpff *et al.*, 1983). Also, IGF-I has been reported to enhance the maturation of morphologically recognizable granulocytic and erythroid progenitors in suspension cultures of marrow cells (Merchav *et al.*, 1988). *In vivo*, infusion of IGF-I increases thymus and spleen weights in hypophysectomized rats and dwarf rats, as well as induce repopulation of

the atrophied thymus in diabetic rats (Guler *et al.*, 1988, Binz *et al.*, 1990). Short term administration of recombinant human (rh) IGF-I to adult normal mice causes an increase in CD4 T-cell and B-cell number as well as enhances immunoglobulin synthesis (Clark *et al.*, 1993).

In addition to the many *in vivo* and *in vitro* actions of IGF-I, it has proven to be a useful diagnostic tool. For instance it has been shown that IGF-I is decreased in malnutrition and it has been shown to be a good biological marker of endocrine disorders (Clemmons *et al.*, 1992). Recently rhIGF-I has been shown to have therapeutic potential and has been used in several clinical studies. Possible therapeutic benefits have been suggested: enhancement of wound healing by the activation of wound macrophages; growth promotion for children with chronic adrenal failure; dwarfism caused by GH resistance; controlling blood glucose levels for people with insulin dependent diabetes and insulin resistance; nitrogen retention and weight gain for patients with hypercatabolic states (Langford and Miell, 1993).

Growth retardation is a common feature of pediatric AIDS and it has been associated with hormonal deficiencies, malnutrition and overwhelming illness (Geffner *et al.*, 1993). It has been postulated the growth retardation in children with HIV-1 infection is a result of resistance to IGF-I (Geffner *et al.*, 1993). In addition there is evidence of increased insulin sensitivity in adult HIV-1 infected men (Hommes *et al.*, 1991). The effects of IGF-I on immune system function along with weight gain and increase in muscle mass makes IGF-I an attractive candidate for the treatment of HIV-1 infected patients with wasting and immmunodeficiency.

2.8 Project Rationale

Several observations have shown abnormal insulin and IGF-I levels in people with HIV-1 infection (Hommes *et al.*, 1991, Geffner *et al.*, 1993). Also, as dicussed above, IGF-I has been implicated in immune system function. For instance IGF-I has been shown to effect growth of the thymus and lymph nodes, T-cell and B-cell lymphopoiesis, and natural killer cell and macrophage activity (Clark *et al.*, 1993, Kincade, 1994, Meltzer *et al.*, 1990, Kelley, 1990).

The purpose of this study was to determine if insulin or IGF-I had any effect on HIV-1 replication in *de novo* and chronically infected cells *in vitro*. To test the specificity of the effect of these insulin-like hormones, we also measured the effect of another hormone, EGF on HIV-1 replication in *de novo* infected cells.

MATERIALS AND METHODS

1. Cell culture

Umbilical cord blood mononuclear cells (CBMC) were isolated by Ficoll-Hypaque (Pharmacia, Uppsula, Sweden) gradient centrifugation. The cells were collected, washed and stimulated with 0.1% phytohemagglutuin (P-PHA). The cells were seeded in culture flasks (2 x 10^6 cells/ml) and maintained for three days in complete RPMI-1640 culture medium (Gibco Laboratories, Toronto, Ontario, Canada) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Flow Laboratories, Toronto, Ontario, Canada), 2 mM L-glutamine, and 100 U.I./ml penicillin and 100 mg/ml streptomycin. CBMC were maintained in water-jacketed incubators at 37°C and under 5% CO_2 atmosphere. CBMC were kindly supplied by the Department of Obstetrics and Gynecology, Jewish General Hospital, Montreal, Quebec, Canada.

All cell lines were maintained in complete RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Flow Laboratories, Toronto, Ontario, Canada), 2 mM L-glutamine, and 100 U.I./ml penicillin and 100 mg/ml streptomycin. All cell lines were maintained in water-jacketed incubators at 37°C and under 5% CO_2 atmosphere.

Chronically infected U937 cells were kindly supplied by Dr. Bluma Brenner of the McGill AIDS Centre, Montreal, Quebec, Canada. MT4 cells were obtained from Douglas Richman through the AIDS Research and Reference Reagent P.ogram, Division of AIDS, NIAID, NIH, Bethesda, MD. H9 cells (Popovic et al., 1984) were provided by Dr. R. C. Gallo, NIH, Bethesda, MD.

Cell viability was assessed by trypan blue exclusion and cell proliferation was assessed by microscopy using a hemacytometer.

2. HIV-1 stock and titration of viral infectivity

The HIV-IIIB laboratory strain of HIV-1 was kindly supplied by R. C. Gallo, National Institute of Health (NIH), Bethesda, MD. Virus was concentrated by ultracentrifugation (40,000 RPM, 1h, 4°C) of clarified supernatants from chronically infected H9 cell cultures, using a L8-M ultracentrifuge and Ti-45 rotor (Beckman Instruments, Palo Alto, CA.). The viral pellet was resuspended in 1 ml of complete RPMI-1640 media and aliquots were frozen at -70°C. Stock virus titers were quantified using the infectivity assay described by Johnson and Byington (1990). Briefly, 10 fold dilutions of the viral sample were added to wells of a 96-well plate containing 4 x 10^5 CBMC/well at a final volume of 250 µl/well. The plate was incubated at 37°C for 4 days. On the fourth day 150 µl of supernatant was removed and replaced with fresh media. On the seventh day 100 µl of supernatant was removed from each well and measured for viral p24 by enzyme immunoassay (described below). The infectious titer was derived from the amount of p24 antigen present.

3. Cell infection

Direct infection of PHA-stimulated CBMC was performed with a viral titer of $TCID_{50}$=2000 for 1 x 10^6 CBMC. A maximum of 30 million cells were incubated with a maximum of 1 ml of infectious virus for 2 hours at 37°C. The cells were than washed free of unattached virus and resuspended in fresh complete RPMI media containing 10 U/ml IL-2. In

experiments testing insulin and IGF-I, the cells were resuspended in media that contained 1%, or 10% FBS. Cells were plated at 2 x 10^6 cells/ml and the appropriate concentrations of insulin (Sigma Co., St. Louis, MO), IGF-I (Gibco Laboratories, Toronto, Ontario, Canada) or EGF (Gibco Laboratories, Toronto, Ontario, Canada) were added.

4. HIV-1 detection by reverse transcriptase assay

Measurement of HIV-1 activity in cell-culture supernatants by RT assay was performed as described in Lee *et al.* (1987). Briefly, 50 µl of clarified culture supernatant from test cell cultures was added to 50 µl of reaction cocktail containing: 50 mM Tris hydrochloride (pH 7.9), 5 mM magnesium chloride, 150 mM potassium chloride, 0.5 mM ethylene glycol-bis (b-aminoethyl ether)-N,N,N', N-tetraacetic acid, 0.05% Triton X-100, 2% ethylene glycol, 5 mM dithiothreitol, 0.3 mM reduced glutathione, 20 mCi tritiated thymidine triphosphate and 50 mg/ml of template primer [poly (rA).oligo (dT)] in polypropylene tubes. The tubes were agitated and then incubated at 30°C for 22 hours. The reaction was stopped by the addition of 1 ml of cold 10% (w/v) trichloroacetic acid (TCA). Newly synthesized DNA was precipitated on ice for at least two hours, and then collected on Whatman GF/C glass fiber filters (Gelman Sciences, Ann Arbor, Michigan) and rinsed two times with cold 10% TCA and one time with absolute ethanol. Filters were dried for 20 minutes and counted in a Packard tri-carb scintillation analyzer for the incorporated radioactivity.

5. Detection of p24 antigen by EIA

Virus production was monitored by detection of p24 HIV core antigen in cell-free culture supernatants by enzyme immunoassay (EIA) using reagents and instructions provided in the EIA diagnostic kit (Abbott Laboratories, Mississauga, Ontario, Canada).

6. Experimental protocol

For most of the experiments, HIV-1 infected cells were divided into two groups. The first group was resuspended in complete RPMI-1640 supplemented with 1% FBS and the second group was resusupended in complete RPMI-1640 supplemented with 10%. The cells were plated in 96 well plates at a concentration of 4×10^5 cells/well/250µl of media. Insulin and IGF-I were added at final concentrations of 6.7×10^{-8} M, 6.7×10^{-7} M, 3.3×10^{-6} M, and 6.7×10^{-6} M for insulin and 1.3×10^{-10} M, 1.3×10^{-9} M, 2.6×10^{-9} M, and 6.5×10^{-9} M for IGF-I. The cells were cultured for various times, which are indicated in the figure legends, at 37°C and 5% CO_2. A 50% exchange of culture media was replaced twice weekly. The supernatants were collected and measured for RT activity and/or p24 antigen production. In some experiments 3'-azido-3'-deoxythymidine (AZT) and EGF were employed as additional controls.

In order to determine if different preincubation conditions could affect the inhibitory action of insulin on HIV-1 replication, either HIV-1 plus insulin, insulin plus CBMC, or HIV-1 plus CBMC were incubated for 1 hour, after which time the missing component was added and the incubation continued for another 6 hours. HIV-1 plus CBMC plus insulin incubated together for 7 hours served as the control. At the end of this

time frame the cells were washed extensively and incubated in insulin containing media supplemented with 1% FBS. Supernatants were collected and assessed for p24 antigen production on the seventh day.

To assess the infectivity and cytopathology of virus produced from cells treated with insulin, CBMC were infected and cultured in complete RPMI-1640 media or complete RPMI-1640 media containing 3.3×10^{-6} M insulin. After 7 days the supernatant was collected and used to infect MT4 cells. Over the next 5 days the MT4 cells were studied by microscopy for the production of large multinucleated cells or syncitia.

To test if insulin or IGF-I had any effects on prified RT activity, the hormones were incubated with 40 ng purified RT in a reaction cocktail containing: 50 mM Tris hydrochloride (pH 7.9), 10 mM dithiothreitol, 60 mM potassium chloride, 10 mM magnesium chloride, 5 μM tritiated thymidine triphosphate and 10 mU of template primer [poly(rA).oligo(dT)]. The tubes were incubated for 30 minutes at 37 ° C. Newly synthesized DNA was precipitated on ice for 2 hours and then collected on Whatman GF/C glass fiber filters (Gelman Sciences, Ann Arbor, Michigan) and rinsed 2 times with cold 10% TCA and one time with absolute ethanol. Filters were dried for 20 minutes and counted in a Packard tri-carb scintillation analyzer for the incorporated radioactivity. Purified RT was kindly provided by Dr. Michael Parniak of the Lady Davis Institute, Montreal, Quebec, Canada.

7. Statistical analysis

Where appropriate, statistical significance was tested using the student t-test for paired and unpaired samples, unless indicated otherwise. All data is expressed as mean +/- standard deviation or standard error of the mean as indicated.

RESULTS

1. The effect of insulin on HIV-1 replication in *de novo* infected CBMC

We began testing insulin for its potential effect on HIV-1 replication, and showed that exposure of *de novo* infected CBMC to relatively high concentrations of insulin over a period of 7 days resulted in a dramatic inhibition of HIV-1 production. At 6.7×10^{-6} M an average 60% decrease in RT activity in culture fluids was observed in comparison to control cultures (figure 6, $p<0.05$). Similar results were obtained through measurement of p24 antigen in culture fluids (figure 7) (an average of 50% decrease of p24 antigen production). This inhibitory effect was dose dependent and the 50% effective dose (EC_{50}) was calculated to be 1.1×10^{-6} on the basis of RT activity and 3.3×10^{-6} M on the basis of p24 antigen production.

In a similar experiment, we infected CBMC and cultured the cells for seven days at which time we added insulin. After a 24 hour exposure to insulin supernatant was analyzed for p24 antigen production. A concentration dependent inhibition of HIV-1 replication was observed. At an insulin concentration of 6.7×10^{-7} M a $22 \pm 10\%$ inhibition was observed and at a 6.7×10^{-6} M concentration of insulin $58 \pm 13\%$ inhibition was observed (figure 8). These results indicate that the hormonal effect seen in CBMC still occurred when HIV-1-infection was established for 7 days.

Figure 6: The Effect of Insulin on RT Activity in *de novo* HIV-1 infected CBMC Culture Supernatant

Infected CBMC were cultured in complete RPMI-1640 media supplemented with 1% FBS and treated with 0 M (□), 6.7×10^{-8} M (◆), 6.7×10^{-7} M (O), 3.3×10^{-6} M (▲) and 6.7×10^{-6} M (■) of insulin. The supernatants were collected 4 and 7 days post infection and the RT activity was assessed as described in the materials and methods. Results are expressed as mean +/- standard error (n=4).

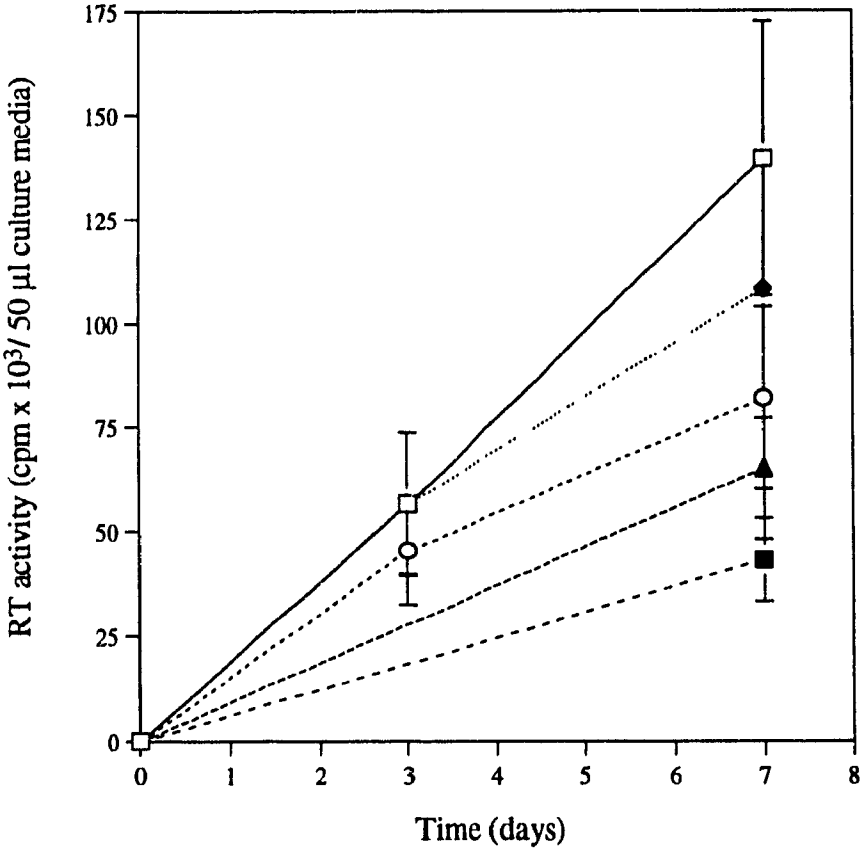

RT activity (cpm x 10^3/ 50 µl culture media)
175
150
125
100
75
50
25
0
0
1
2
3
4
5
6
7
8
Time (days)

Figure 7: The Effect of Insulin on p24 Antigen Production in *de novo* HIV-1 infected CBMC

Infected CBMC were cultured in complete RPMI-1640 media supplemented with 1% FBS and treated with 0 M (□), 6.7×10^{-8} M (◆), 6.7×10^{-7} M (○), 3.3×10^{-6} M (▲) and 6.7×10^{-6} M (■) of insulin. The supernatants were collected 7 days post infection and the p24 antigen production was measured by enzyme immunoassay. Results are expressed as mean +/- standard error (n=4). In one experiment the supernatants were also measured for p24 production on day 4.

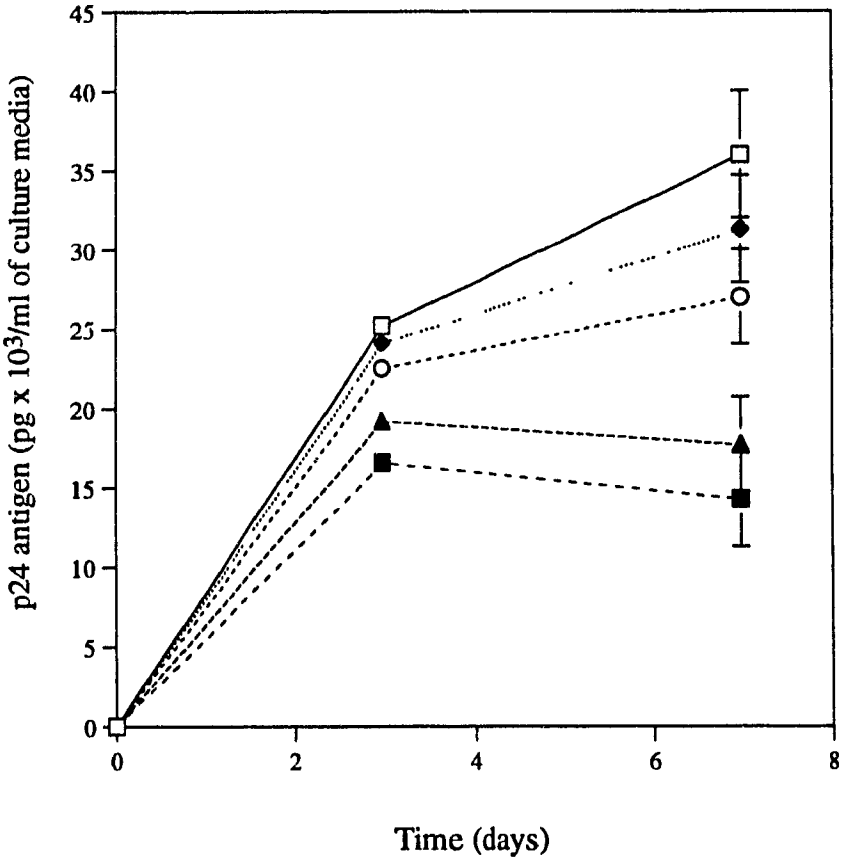
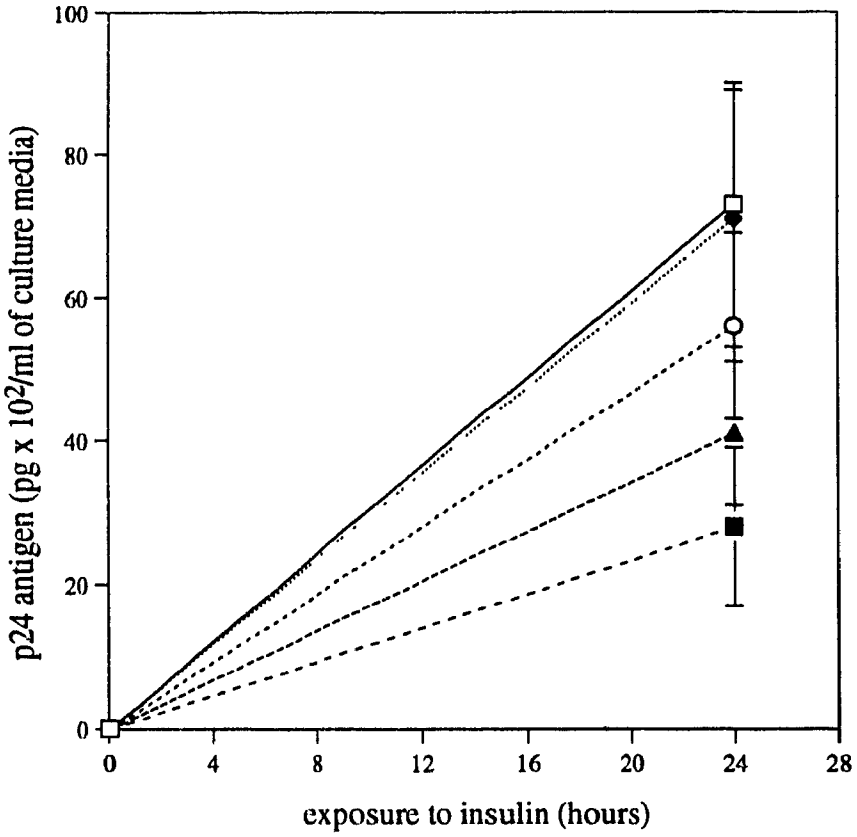

45
40
35
30
25
20
15
10
5
0
0
2
4
6
8
p24 antigen (pg x 10³/ml of culture media)
Time (days)

Figure 8: The Production of p24 Antigen in *de novo* HIV-1 Infected CBMC Exposed to Insulin for 24 Hours

Infected CBMC were cultured in complete RPMI-1640 media supplemented with 10% FBS. Seven days post infection the cells were washed to remove all free virus and then cultured in media supplemented with 1% FBS and containing 0 M (□), 6.7×10^{-8} M (◆), 6.7×10^{-7} M (O), 3.3×10^{-6} M (▲) and 6.7×10^{-6} M (■) of insulin. After a 24 hour exposure to insulin supernatant was collected and assayed for p24 antigen production. Statistical significance between groups was analyzed using a one-way anova. Results are expressed as mean +/- standard error (n=4).


100
80
60
40
20
0
p24 antigen (pg x 10²/ml of culture media)
0
4
8
12
16
20
24
28
exposure to insulin (hours)

2. The effect of IGF-I on HIV-1 replication in *de novo* infected CBMC

Previous studies have shown that insulin can bind to the IGF-I receptor and cause mitogenesis at concentrations 100 to 1000 fold higher than required for a comparable IGF-I response (Flier *et al.*, 1986, Rechler and Nissley, 1985). Given the high insulin concentrations that were necessary to achieve an antiviral effect, we reasoned insulin might be acting via the IGF-I receptor. Therefore the effect of IGF-I was also investigated. The data in figure 9 and 10 show that IGF-I also inhibited HIV-1 production in *de novo* infected CBMC and this inhibition was dose related. A 15% inhibition of p24 antigen and 30% inhibition of RT activity was seen after 7 days using an IGF-I concentration of 1.3×10^{-9} M ($p<0.05$). The physiological concentration of IGF-I, 1.3×10^{-8} M, resulted in a 92% inhibition of p24 antigen and an 88% inhibition of RT activity over the same time frame. Additionally, in the same experiment, approximately 35% inhibition of HIV-1 production by RT activity was observed after 7 days at an insulin concentration of 3.3×10^{-6} M. This same level of inhibition of HIV production was observed at a 1000 fold lower concentration of IGF-I, suggesting that the insulin effects on HIV production were most likely occurring via the IGF-1 receptor. From the data in figures 9 and 10 the EC_{50} for IGF-I was determined to be 2.5×10^{-8} M on the basis of RT activity, and 4.5×10^{-8} M on the basis of p24 production. Under similar conditions the EC_{50} for AZT was determined to be 1.7×10^{-9} M (data not shown).

Figure 9: The Effect of IGF-I on RT Activity in *de novo* HIV-1 Infected CBMC Culture Supernatant

Infected CBMC were cultured in complete RPMI 1640 media supplemented with 1% FBS and treated with 0 M (□), 1.3×10^{-11} M (◆), 1.3×10^{-10} M (○), 1.3×10^{-9} M (▲), 2.6×10^{-9} M (■), 6.5×10^{-9} M (●) and 1.3×10^{-8} M (▼) IGF-I. The supernatants were collected 4 and 7 days post infection and the RT activity was determined as described in the materials and methods. Results are expressed as mean +/- standard error (n=4).

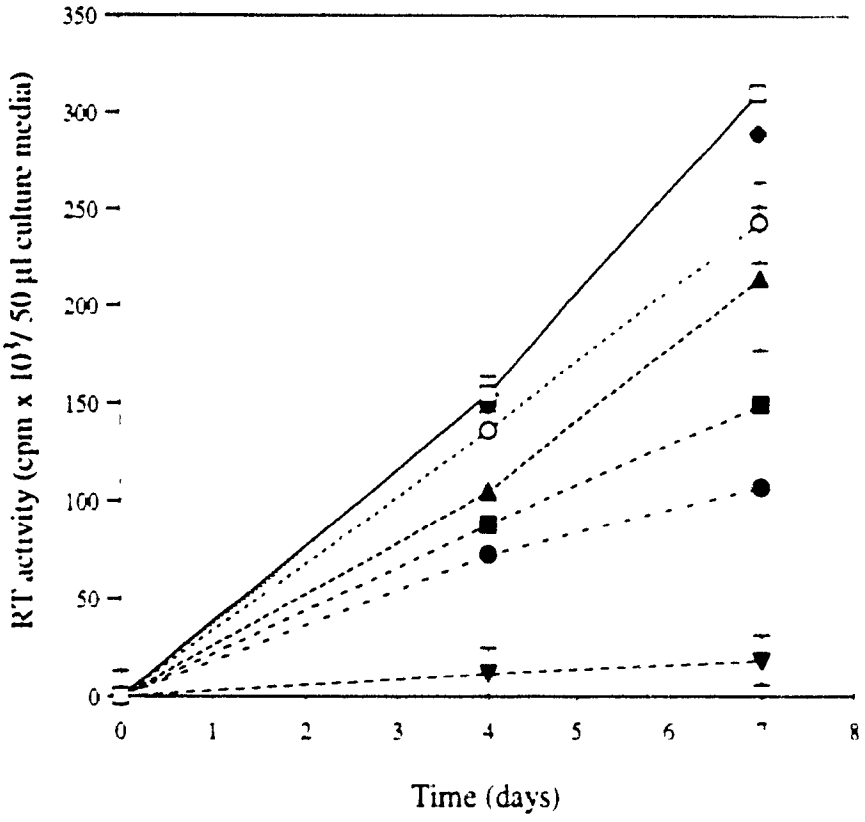
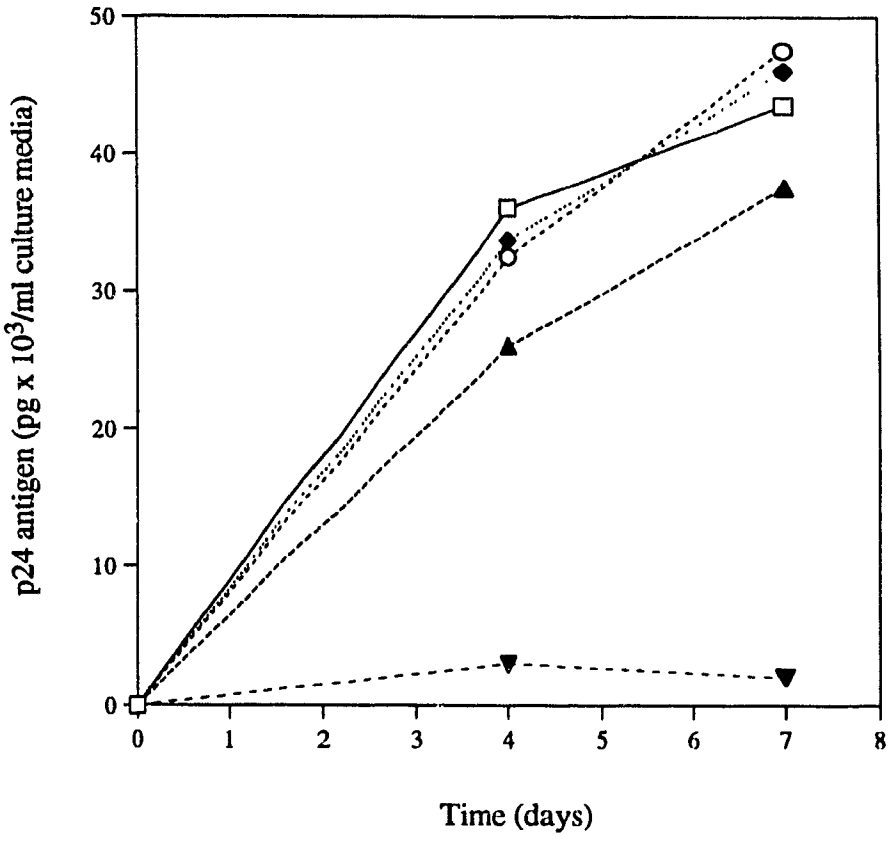

350
300
250
200
150
100
50
0
RT activity (cpm x 10^3/ 50 µl culture media)
0
1
2
3
4
5
6
7
8
Time (days)

Figure 10: The Effect of IGF-I on p24 Antigen Production in *de novo* HIV-1 infected CBMC

Infected CBMC were cultured in complete RPMI-1640 media supplemented with 1% FBS and treated with 0 (□), 1.3 x 10^{-11} M (◆), 1.3 x 10^{-10} M (O), 1.3 x 10^{-9} M (▲), and 1.3 x 10^{-8} M (▼) IGF-I. The supernatants were collected 7 days post infection and the p24 antigen production was measured by enzyme immunoassay. The data are a representative experiment.


50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
p24 antigen (pg x 10³/ml culture media)
Time (days)

3. The effect of insulin and IGF-I on cell viability and cell number

It should be noted that neither IGF-I nor insulin at the maximally effective doses employed had any inhibitory effect on CBMC viability or number over the course of the experiment. Viability of cells exposed to a maximum of 6.7×10^{-6} M insulin for 4 days was essentially identical to control cells (figure 11 A). Insulin did decrease viability of CBMC at a concentration of 3.3×10^{-6} M. Thus, insulin concentrations greater than 6.7×10^{-6} M were not employed in any of the experiments. Cell viability in all concentrations of IGF-I used were comparable to the control group after 4 days of IGF-I exposure (figure 11 B). The rate of growth, i.e. increasing cell numbers vs time was not different in control cells vs cells exposed for 4 days to insulin or IGF-I up to the maximal concentrations employed (figure 12 A and B); this includes a concentration of 1.3×10^{-8} M (not shown in figure 12B). No differences in cell viability or number were observed after 6 days of exposure to insulin or IGF-I (data not shown).

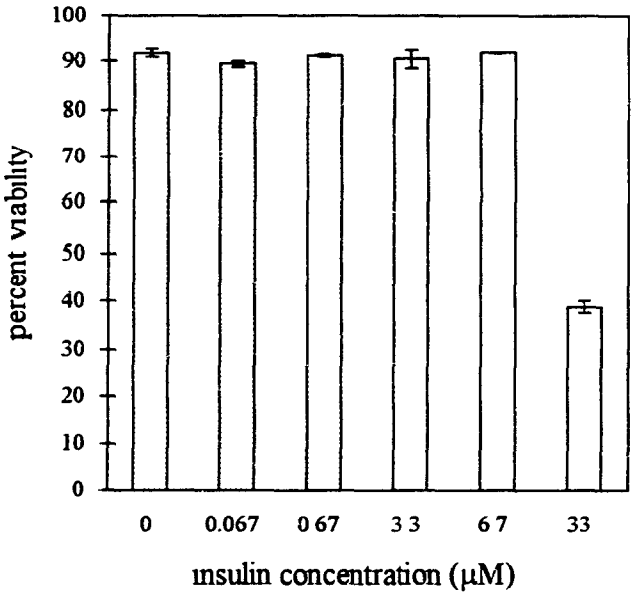
4. The effect of insulin and IGF-I on *in vitro* reverse transcriptase activity

To exclude affects of the hormone on RT in media, we investigated the effects of insulin and IGF-I on the enzymatic activity of purified RT *in vitro*. Neither hormone exhibited any inhibitory activity on RT over the range of insulin concentrations (6.7×10^{-8} to 3.3×10^{-5} M) and the range of IGF-I concentrations (1.3×10^{-10} to 6.5×10^{-9} M) employed. If any effect on RT activity was noted, it was stimulatory (figure 13 A and B).

Figure 11: The Effect of Insulin and IGF-I on the Viability of CBMC

Cells were cultured in complete RPMI-1640 supplemented with 1% FBS and treated with (A) 0 to 3.3 x 10^{-5} M of insulin or (B) 0 M to 6.5 x 10^{-9} M IGF-I. After 4 days the number of dead vs live cells was enumerated by microscopy using a hemacytometer and trypan blue exclusion. Results are expressed as mean +/- standard error (n=2).

A


100
90
80
70
60
50
40
30
20
10
0
percent viability
0
0.067
0 67
3 3
6 7
33
insulin concentration (μM)

B

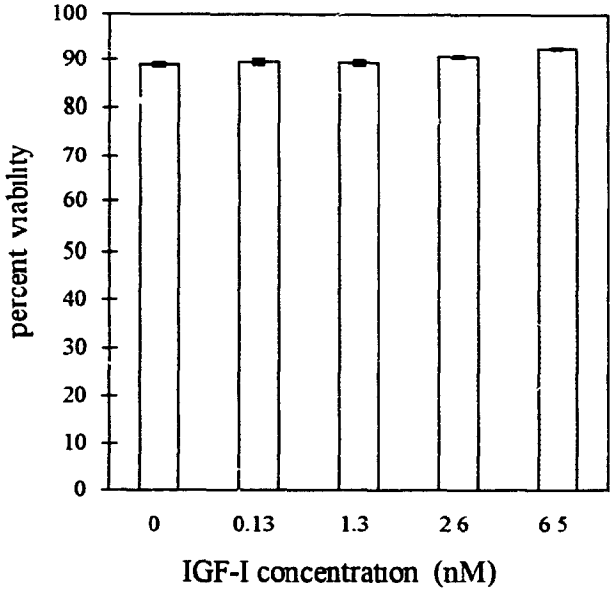

100
90
80
70
60
50
40
30
20
10
0
percent viability
0
0.13
1.3
2 6
6 5
IGF-I concentration (nM)

Figure 12: The Effect of Insulin and IGF-I on the Number of CBMC

Cells were cultured in complete RPMI-1640 supplemented with 1% FBS and treated with (A) 0 to 3.3 x 10^{-5} M of insulin or (B) 0 to 6.5 x 10^{-9} M IGF-I. After 4 days the cells were enumerated by microscopy using a hemacytometer. Results are expressed as mean +/- standard error (n=2).

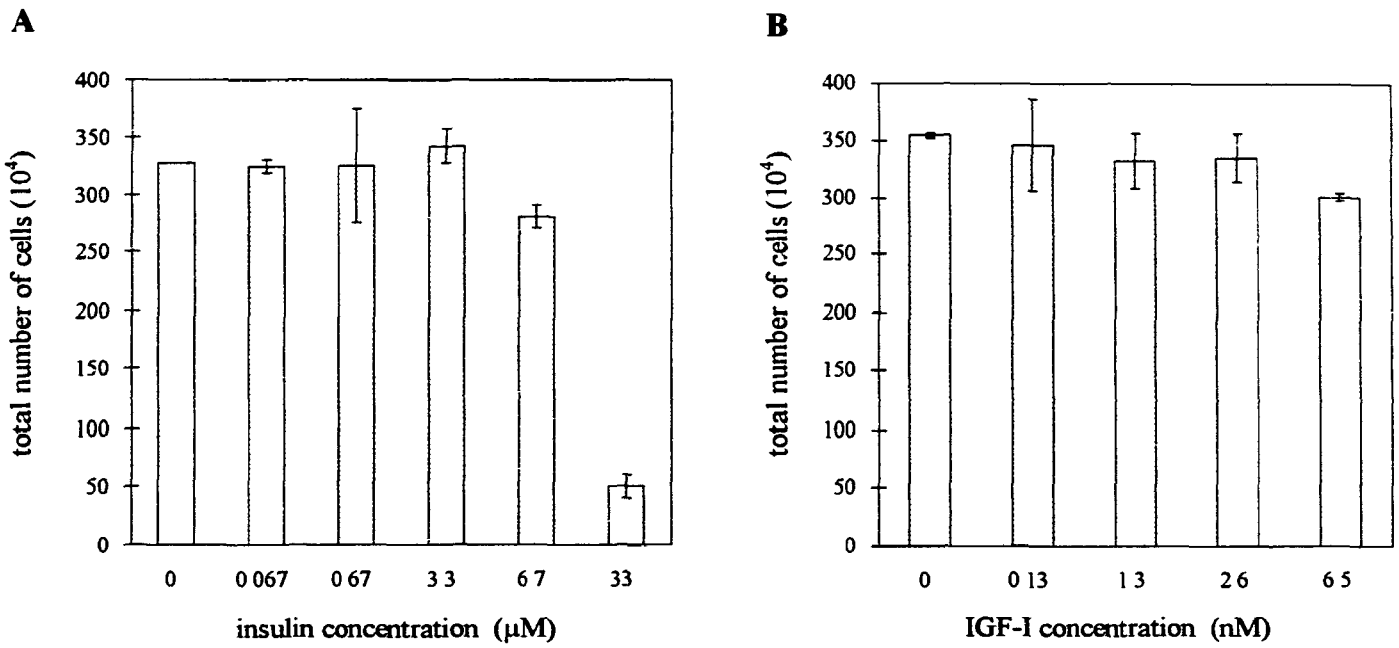
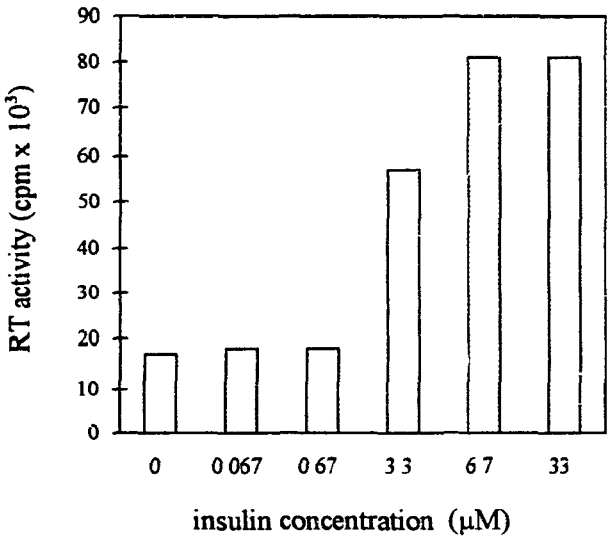

A
B
total number of cells (10^4)
400
350
300
250
200
150
100
50
0
0
0 067
0 67
3 3
6 7
33
insulin concentration (μM)
0
0 13
1 3
2 6
6 5
IGF-I concentration (nM)

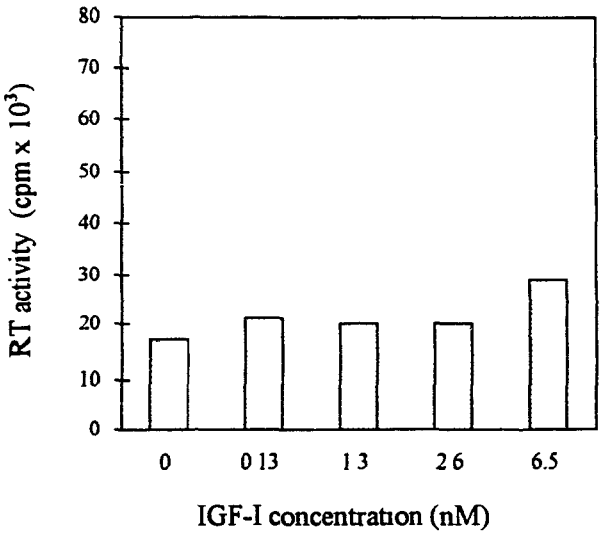
Figure 13: The Effect of Insulin and IGF-I on the Enzymatic Activity of Purified RT *in vitro*

(A) Insulin and (B) IGF-I were incubated with purified RT for 30 minutes. RT activity was measured by the incorporation of tritiated thymidine into newly synthesized DNA.

A


RT activity (cpm x 10^3)
90
80
70
60
50
40
30
20
10
0
0
0 067
0 67
3 3
6 7
33
insulin concentration (μM)

B


RT activity (cpm x 10^3)
80
70
60
50
40
30
20
10
0
0
0 13
1 3
2 6
6.5
IGF-I concentration (nM)

5. The effect of preincubation conditions on insulin's ability to inhibit HIV-1

In order to determine if different preincubation conditions could affect the inhibitory action of the hormone on HIV-1 replication, either virus plus insulin, insulin plus cells or virus plus cells were incubated for 1 hour, after which time the missing component was added and incubation continued for another 6 hours. Virus plus cells plus insulin together for 7 hours served as the control. At the end of this time frame, the cells were washed extensively and incubated in hormone containing media. Seven days later HIV-1 production was monitored in all groups. The data in figure 14 show that under all preincubation conditions, viral replication was similarly inhibited over a range of hormone concentrations. No apparent differences were observed between the control (i.e. virus plus insulin together for 7 hours) vs the other treatment groups.

6. Delayed cytopathicity as an indication of the effect insulin on infectious virus production

MT4 cells were infected with 1 ml HIV-1 produced from insulin treated and non-treated CBMC. As seen in figure 15B a low level of syncitia formation was observed in MT4 cells infected with HIV-1 produced from CBMC treated with insulin. After 3 days of infection there was some formation of syncitia-like cells and little cells lysis was evident. However, virus produced from CBMC that were not treated with insulin caused the formation of a greater number of large syncitia-like cells and a great deal of cell lysis by day 3 (figure 15C). By day 6 syncitia formation

Figure 14: The Effect of Preincubation of Cells or Virus with Insulin on p24 Antigen production in *de novo* HIV-1 infected CBMC

In the first condition (■), cells were incubated for 1 hour with 0 M, 6.7×10^{-8} M, 6.7×10^{-7} M, 3.3×10^{-6} M or 6.7×10^{-6} M insulin and than infected with HIV-1. In the second condition (●) virus was incubated for 1 hour with 0 M, 6.7×10^{-8} M, 6.7×10^{-7} M, 3.3×10^{-6} M or 6.7×10^{-6} M insulin and than used to infect cells. In the third condition (▲) cells were infected with HIV-1 for one hour and then incubated with 0, 6.7×10^{-8} M, 6.7×10^{-7} M, 3.3×10^{-6} M or 6.7×10^{-6} M insulin. After the 1 hour incubation the missing component was added and the incubation continued for another 6 hours. Cells, virus and 0, 6.7×10^{-8} M, 6.7×10^{-7} M, 3.3×10^{-6} M or 6.7×10^{-6} M insulin incubated together for 7 hours (◆) served as the control. After this time frame the cells from each condition were washed to remove free virus and cultured in media containing the appropriate concentration of insulin. After 7 days the supernatants were collected an assayed for p24 antigen production.

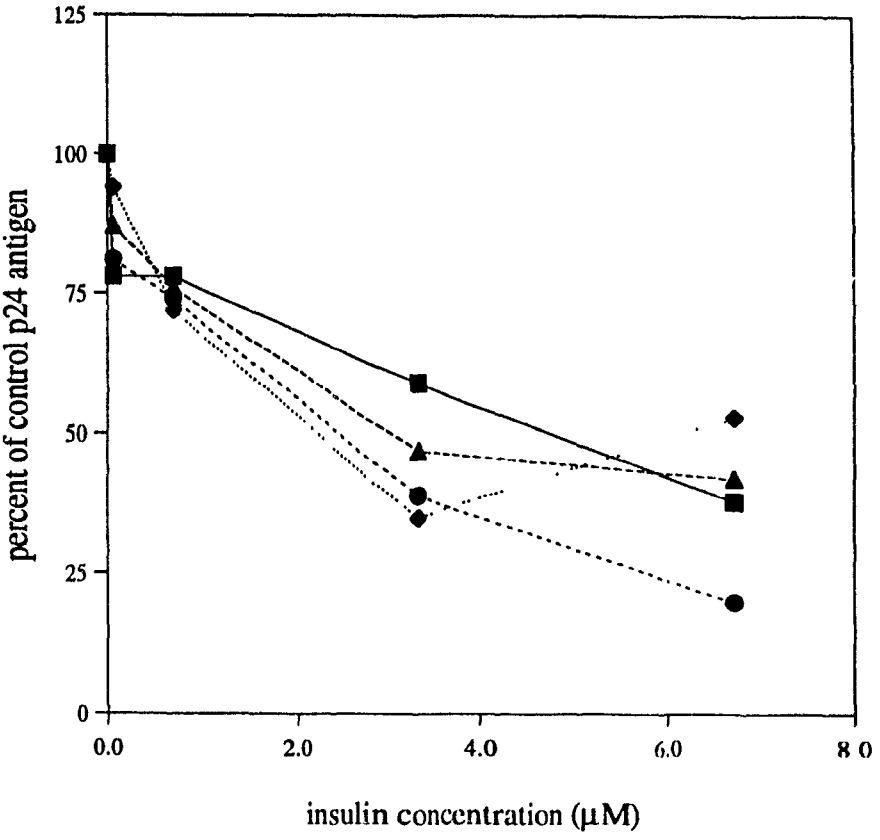
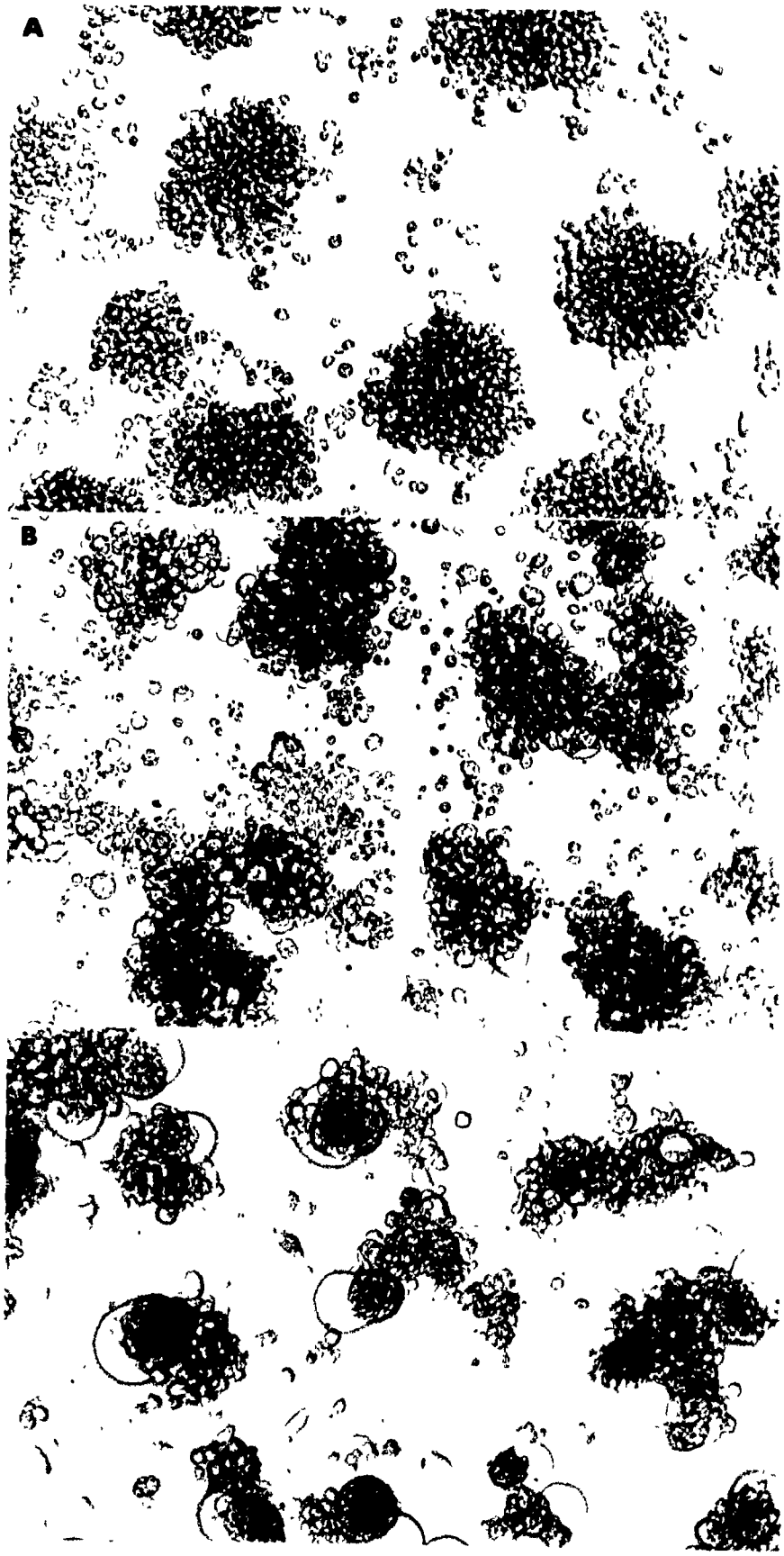

125
100
75
50
25
0
percent of control p24 antigen
0.0
2.0
4.0
6.0
8.0
insulin concentration (μM)

Figure 15: Syncitia Formation in MT4 Cells Infected with HIV-1 Produced from Infected CBMC that were Grown in the Presence or Absence of Insulin

(A) shows control healthy uninfected MT4 cells (no syncitia).

(B) shows MT4 cells that were infected with HIV-1 produced from insulin treated CBMC (low syncitia production).

(C) shows MT4 cells that were infected with HIV-1 produced from CMBCs that were not treated with insulin (high syncitia production).


A
B

was severe in both conditions. The delay of severe syncitia formation indicates that less virus was produced from the insulin treated CBMC. These results lend further support to insulin's ability to decrease HIV-1 production. The fact that HIV-1 produced in the presence of insulin is able to induce syncitia formation in MT4 cells indicates that this virus does not lose its infectivity after exposure to insulin.

7. The effect of insulin and IGF-I on HIV-1 production in chronically infected U937 cell line

As can be seen from the data in figure 16 and 17 both IGF-I and insulin can inhibit the replication of HIV-1 in chronically infected U937 cells. Cells were exposed to hormone and within 48 hours, the production of HIV-1 was inhibited 32% by 6.7×10^{-7} M insulin (figure 16) and 93% by 1.3×10^{-8} M IGF-I (figure 17). There are several key points to be made from the data. Firstly, HIV-1 replication in a different cell type (i.e. chronically infected U937 cells) can be blocked by IGF-I and insulin suggesting possible general effects of the hormones on HIV-1 replication. Secondly, the hormone effect can be seen in a chronically infected cell line suggesting that the effects of the hormones are intracellular rather than at the cell surface (i.e. viral binding).

8. The specificity of the effect of IGF-I on HIV-1 replication

Since IGF-I and insulin are mitogens for many types of cultured cells, we reasoned that the effect of these growth factors on HIV-1 replication might be related to their mitogenic potential. Thus, we compared the effects of IGF-I on HIV-1 replication with the effects of EGF, one of the most potent mitogens known, on HIV-1 replication.

Figure 16: The Effect of Insulin on the RT Activity in HIV-1 Chronically Infected U937 Cells

Chronically infected U937 cells were cultured in complete RPMI 1640 media supplemented with 1% FBS and treated with 0 M (□), and 6.7×10^{-7} M (O) insulin. The supernatants were collected 24 and 48 hours after insulin treatment and the RT activity was assessed as described in the materials and methods. Results are expressed as mean +/- standard error (n=4).

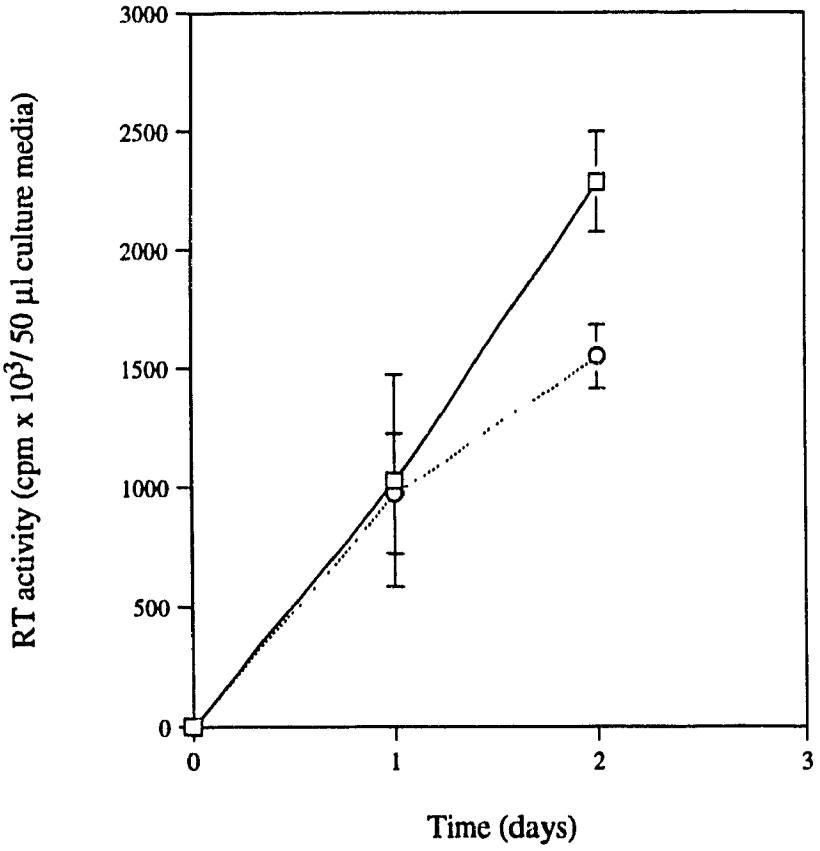
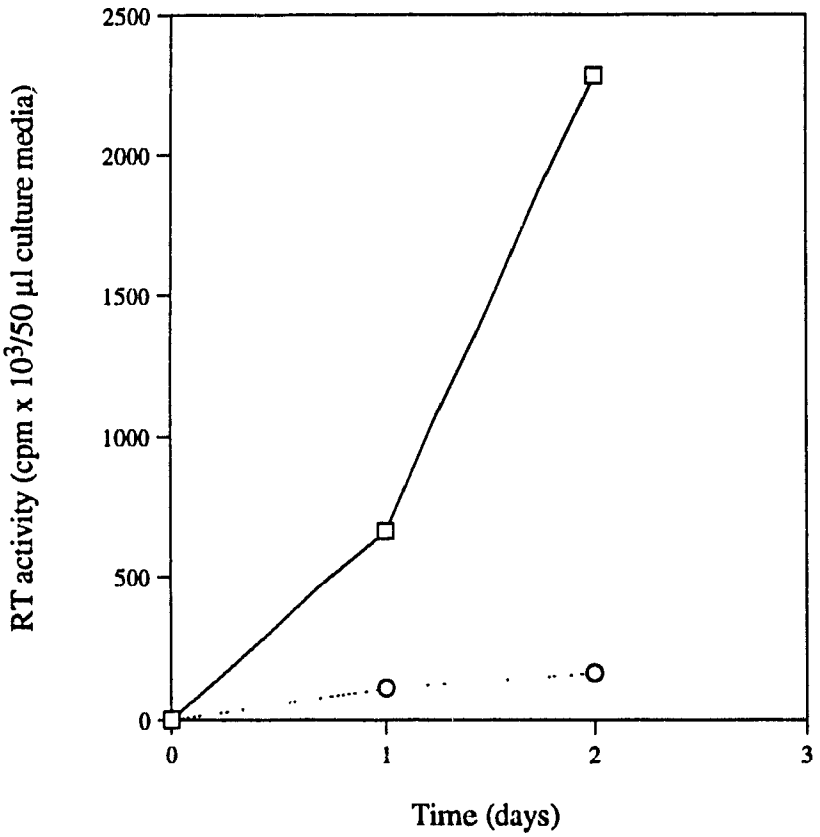

3000
2500
2000
1500
1000
500
0
0
1
2
3
RT activity (cpm x 10^3/ 50 µl culture media)
Time (days)

Figure 17: The Effect of IGF-I on the RT Activity in HIV-1 Chronically Infected U937 Cells

Chronically infected U937 cells were cultured in complete RPMI 1640 media supplemented with 1% FBS and treated with 0 M (□) and 1.3×10^{-8} M (O) IGF-I. The supernatants were collected 24 and 48 hours after insulin treatment and the RT activity was assessed as described in the materials and methods.

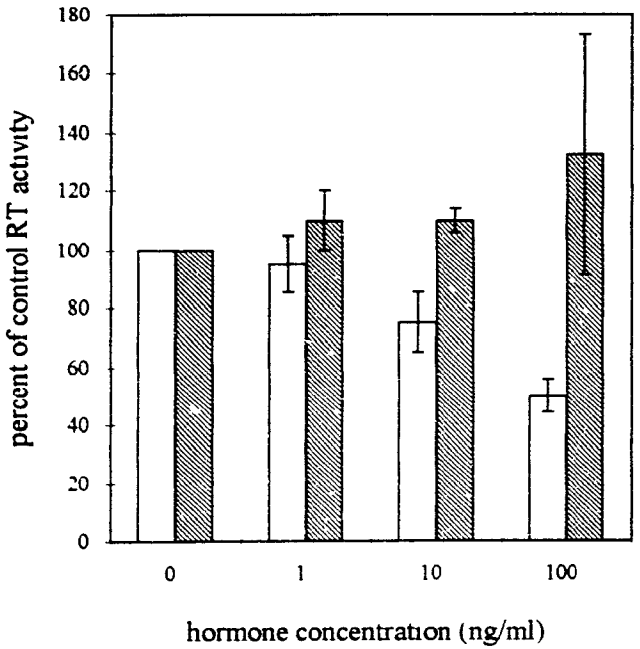

2500
2000
1500
1000
500
0
0
1
2
3
RT activity (cpm x 10^3/50 µl culture media)
Time (days)

The data in figure 18 A and B clearly demonstrate the concentration dependent effect of IGF-I on HIV-1 replication. An IGF-I concentration of 10 ng/ml (1.3×10^{-9} M) resulted in a 29% inhibition of RT activity and an IGF-I concentration of 100 ng/ml (1.3×10^{-8} M) resulted in a 50% inhibition of RT activity. Similar concentrations of EGF showed no inhibitory effect on HIV-1 replication. In fact, at the highest concentration of EGF employed, 100 ng/ml (1.6×10^{-8} M), HIV-1 replication was increased slightly above control values whether monitored by RT activity or p24 antigen production (figure 18 A and B).

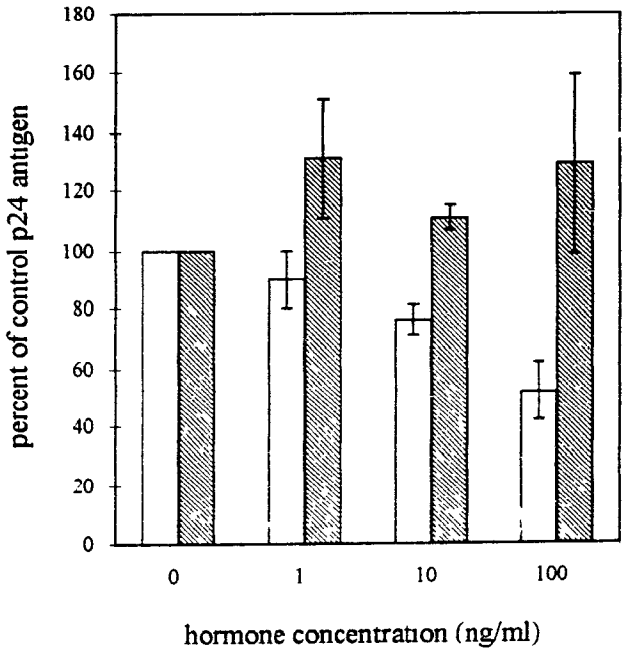
Figure 18: The Specificity of the Hormone Effect on HIV-1 Replication

Infected CBMC were cultured in complete RPMI-1640 supplemented with 1 % FBS and treated with 0, 1, 10 , 100 ng/ml of either IGF-I (clear bars) or EGF (hatched bars). The supernatant was collected 4 days after infection and the (A) RT activity and (B) p24 production were measured as described in the materials and methods. Results are expressed as mean +/- standard error (n=2). (1 ng/ml IGF-I = 1.3×10^{-10} M and 1 ng/ml EGF = 1.6×10^{-10} M).

A


percent of control RT activity
180
160
140
120
100
80
60
40
20
0
0
1
10
100
hormone concentration (ng/ml)

B


percent of control p24 antigen
180
160
140
120
100
80
60
40
20
0
0
1
10
100
hormone concentration (ng/ml)

DISCUSSION

We have reported herein that insulin and IGF-I can dramatically reduce HIV-1 replication in *de novo* infected CBMC and chronically infected U937 cells. While the overall mechanism(s) involved is not known we believe that the effects of insulin and IGF-I are proceeding via the IGF-I receptor. This conclusion is supported by the observation that the levels of insulin needed to effect HIV-1 production are relatively high, while the effects of IGF-I are seen at and below physiological concentrations of this hormone (Lieberman, 1994). This suggests a primary role for the endogenous IGF-I receptor as it is well documented that supraphysiological concentrations of insulin can activate the IGF-I receptor (Flier *et al.*, 1986, Rechler and Nissley, 1985, Maller and Koontz, 1981). In addition, others have reported that insulin (10^{-6} M) and IGF-I (10^{-9} M) effect chemotactic activity in peripheral blood mononuclear cells at concentrations similar to the concentrations we have found to inhibit HIV-1 replication (Berman and Center, 1987, Tapson *et al* 1988). Berman and Center (1987) also indicated that the effects of insulin on the chemotactic activity of peripheral blood mononuclear cells is occurring via the IGF-I receptor.

In our studies treatment with 6.7×10^{-6} M insulin resulted in a 60% reduction in RT activity and a 50% decrease in p24 antigen production in *de novo* infected CBMC (figures 6 and 7). Similarly, a 1.3×10^{-8} M IGF-I treatment resulted in an 88% reduction in RT activity and a 92% decrease in p24 antigen production in *de novo* infected CBMC (figures 9 and 10). As indicated above the high concentrations of insulin required to obtain the

same effect produced by IGF-I in these experiments suggest that insulin and IGF-I may both be acting via the IGF-I receptor.

It should be noted that human lymphocytes have been shown to express IGF-I receptors and these receptors are known to be increased in expression when PHA stimulated (Thorsen and Hiny, 1977, Kozak *et al.*, 1987, Tapson *et al.*, 1988, Kozak *et al.*, 1987). In fact, our laboratory recently showed that PHA stimulation of CBMC resulted in a nearly 2 fold increase in specific IGF-I binding when compared to non-activated CBMC (data not shown). These results indicate that activated CBMC express responsive IGF-I receptors.

In another experiment we demonstrated that insulin was able to inhibit HIV-1 production in *de novo* infected CBMC with a 7 day established infection (figure 8). A 24 hour exposure of these cells to 6.7 x 10^{-6} M insulin resulted in a 58% reduction in p24 antigen production. These results show that a short exposure time to insulin is sufficient to produce the inhibitory effect. However, preincubation of cells or virus with insulin for 1 hour before infection does not have a significant effect on viral production (figure 14).

We further demonstrated that both insulin and IGF-I reduced HIV-1 production in chronically infected U937 cells (figures 16 and 17). The inhibition of viral replication in a different cell type suggests the possible general effects of these hormones on HIV-1 replication. Also, because the effect of these hormones is seen in a chronically infected cell line suggests that they are acting intracellularly rather than at the cell surface.

Although RT activity in supernatant of *de novo* infected CBMC and chronically infected U937 cells was dramatically decreased when these

cells were exposed to insulin and IGF-I, neither hormone had an inhibitory effect on purified RT *in vitro* (figure 13). Rather a stimulatory effect on the enzyme was observed with increasing levels of insulin and IGF-I. These results suggest that insulin and IGF-I are exerting their effect on the cell rather than on the virus.

Interestingly experiments conducted using 10% FBS in the culture media attenuated the effects of insulin and IGF-I (data not shown). This indicates that there are agents in serum that could be inhibiting the effect of the hormones. For instance, IGF-I binding proteins are known to bind to free circulating IGF-I and have been shown to enhance or inhibit the effects of this hormone (Baxter and Martin, 1989, Conover *et al.*, 1990).

We also demonstrated that virus produced from insulin treated, *de novo* infected CBMC produced less syncitium when used to infect MT4 cells (figure 15). These results suggest that there was less virus produced from insulin treated cells and this lends further support to insulin's ability to inhibit HIV-1 production.

The data in figure 18 demonstrates that a different hormone, EGF was not able to inhibit HIV-1 production. These results illustrate the specificity of the actions of insulin and IGF-I on HIV-1 replication. In addition, these data further suggest that the actions of insulin and IGF-I are mediated through the IGF-I receptor and signalling cascades that are different than those used by EGF. Also, the inability of EGF, a potent mitogen, to inhibit HIV-1 replication, suggests that the effects of insulin and IGF-I are not necessarily related to their mitogenic actions.

The effects of IGF-I on lymphoid cells has been established in the last few years. For instance, IGF-I has already been demonstrated to have

significant growth and modulatory effects on cells and organs of the immune system including the T cells, B cells, natural killer cells, thymus and lymph nodes (Clark *et al.*, 1993, Kincade, 1994, Meltzer *et al.*, 1990, Kelly, 1990). Additionally, the modulatory role of growth hormone on cells of the immune system is extensive and its effects have been shown to occur via local IGF-I production (Geffner *et al.*, 1990).

Presently we do not know precisely where the block in viral replication is occurring. We feel that one possible mechanism involves the disruption of signalling events taking place during the HIV-1 infectious cycle by the IGF-I signalling cascade. This is supported by the recently demonstrated relationship between IGF-I and IL-4 signalling. IL-4 is a pluripotent cytokine intimately involved in determining the nature of an immune response to a given pathogen (Paul, 1991). IL-4 induces phosphorylation of a 170 kDa protein (4PS) which is known to associate strongly with the 85 kDa subunit of PI-3′ kinase (Wang *et al.*, 1992, Linette *et al.*, 1988). PI-3′ kinase is also known to associate with the IRS-1 which is the most prominent substrate phosphorylated in response to insulin or IGF-I. Interestingly, 4PS and IRS-1 have been shown to be antigenically related (Wang *et al.*, 1993). It has also been shown that the addition of IGF-I to myeloid cell lines resulted in tyrosine phosphorylation of 4PS, and IL-4 stimulation of a myeloid cell line transfected with cDNA encoding IRS-1 resulted in tyrosine phosphorylation of IRS-1 and its subsequent association with PI-3′ kinase. This indicates that the IL-4 receptor and IGF-I receptor have overlapping signal transduction pathways and that IRS-1 and 4PS are functionally similar. While we have no direct evidence to associate IL-4 to the observations we have made herein, the close

relationship between the IL-4 and the IGF-I signalling cascades suggests that the effect of IGF-I on HIV-1 replication probably involves interference and/or signal transactivation.

IGF-I has been implicated in growth retardation and malnutrition in pediatric AIDS and insulin sensitivity has been observed in HIV-1 infected men (Geffner *et al.*, 1993, Hommes *et al.*, 1991). Also, IGF-I levels in the blood are known to be profoundly influenced by nutritional factors and have been shown to be dramatically decreased in malnourished individuals. When one considers the extreme wasting associated with AIDS and the decreased levels of circulating hormones found in patients with AIDS, it is not unrealistic to anticipate many hormonal dysfunctions that can be associated with the pathogenesis of HIV-1 infection. Understanding the causes of these dysfunctions will help to determine strategies to modulate hormone levels to minimize the effects of the virus.

Since we believe insulin is acting via the IGF-I receptor and in view of the recent reports employing IGF-I treatment in people with AIDS to modulate immune responses (Nguyen et al., 1993) and to increase nitrogen retention in muscle (Lieberman et al., 1994), it is suggested that administration of IGF-I to selected patients via injection would be an interesting therapeutic approach. In such a study monitoring HIV-1 levels in serum and infected cells would be of considerable value.

REFERENCES

Achong, B. C., Mansell, P. W. A., Epstein, M. A., and Clifford, P. 1971. An unusual virus in cultures from a human nasopharyngeal carcinoma. J. Natl. Cancer Inst. 46: 299-307.

Ahmad, N., and Venkatesan, S. 1988. Nef protein of HIV-1 is a transcriptional repressor of HIV-1 LTR. Science 241: 1481-1485.

Ahmann, A. J. 1993. The clinical evaluation of cytokines and immunomodulators in HIV infection. Ann. N.Y. Acad. Sci. 693: 178-185.

Ameisen, J. C., and Capron, A. 1991. Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. Immunol. Today 12: 102-105.

Backer, J. M. Jr., Schroeder, G. G., Cahill, D. A., Ullrich, A., Siddle, K., and White, M. F. 1991. The cytoplasmic juxtamembrane region of the insulin receptor: a critical role in ATP binding, endogenous substrate phosphorylation and insulin-stimulated bioeffects in CHO cells. Biochemistry 300: 6366-6372.

Baltimore, D. 1970. RNA dependent DNA polymerase in virions of RNA tumor viruses. Nature 226: 1209-1211.

Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyrem M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., and Montagnier, L. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220: 868-871.

Baxter, R. C., and Martin, J. L. 1989. Binding proteins for the insulin-like growth factors: structure, regulation and function. Prog. Growth Factors Res. 1: 49-68.

Bennett, N. T., and Schultz, G. S. 1993. Growth factors and wound healing: Biochemical properties of growth factors and their receptors. Am. J. Sur. 165: 728-737.

Berman, J. S., and Center, D. M. 1987. Chemotactic activity of procine insulin for human T lymphocytes *in vitro*. J. Immunol. 138: 2100-2103.

Binz, K., Joller, P., Froesch, P., Binz, H., Zapf, J., and Froesch, E. R. 1990. Repopulation of the atrophied thymus in diabetic rats by insulin-like growth factor-I. Proc. Natl. Acad. Sci. USA 87: 3690-3694.

Blundell, T. 1979. Conformation and molecular biology of polypeptide hormones. I. insulin, insulin-like growth factor and relaxin. Trends Biochem. Sci. 4: 51-59.

Brown, P. O., Bowerman, B., Varmus, H. E., and Bishop, J. M. 1987. Correct integration of retroviral DNA *in vitro*. Cell 49: 347-56.

Brun-Vezinet, F., Rey, M. A., Katlama, C., Girard, P. M., Roulot, D., Yeni, P., Lenoble, L., Clavel, F., Alizon, M., Gadelle, S., Madjar, J. J., and Harzle, M. 1987. Lymphadenopathy-associated virus type 2 in AIDS and AIDS-related complex: clinical and virological features in four patients. Lancet 1: 128-132.

Cameron, P. U., Freudenthal, P. S., Barker, J. M., Gezelter, S., Inaba, K., and Steinman, R. M. 1992. Dendritic cells exposed to human immunodeficiency virus type -1 transmit a vigorous cytopathic infection to CD4+ T-cells. Science 257: 383-387.

Cefai, D., Debre, P., Kaczorek, M., Idziorek, T., Autran, B., and Bismuth, G. 1990. Human immunodeficiency virus-1 glycoproteins gp120 and gp160 specifically inhibit the CD3/T cell-antigen receptor phosphoinositide transduction pathway. J. Clin. Invest. 86: 2117-2124.

Centers for Disease Control. 1987. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR 36: 1S-15S.

Centers for Disease Control Task Force on Kaposi's sarcoma and opportunistic infections. 1982. Epidemiologic aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections. N. Engl. J. Med. 306: 248-252.

Cheevers, W. P., and McGuire, T. C. 1985. Equine infectious anemia virus: immunopathogenesis and persistence. Rev. Infect. Dis. 7: 83-88.

Clark, K., Strasser, J., McCabe, S., Robbins, K., and Jardieu, P. 1993. Insulin-like growth factor-I stimulation of lymphopoiesis. J. Clin. Invest. 92: 540-548.

Clavel, F., Mansinho, K., Chamaret, S., Guetard, D., Favier, V., Nina, J., Santos-Ferreira, M-O., Champalimaud, J-L., and Montagnier, L. 1987. Human immunodeficiency virus type 2 infection associated with AIDS in West Africa. N. Engl. J. Med. 316: 1180-1185.

Clavel, F., Guetard, D., Brun-Vezinet, F., Chamaret, S., Rey, M-A., Santos-Ferreira, M-O., Laurent, A. G., Dauguet, C., Katlama, C., Rouzioux, C., Klatzmann, D., Champalimaud, J. L., and Montagnier, L. 1986. Isolation of a new human retrovirus from West African patients with AIDS. Science 233: 343-346.

Clavel, F., Guyader, M., Guetard, D., Salle, M., Mantagnier, L., and Alizon, M. 1986. Molecular cloning and polymorphism of the human immunodeficiency virus type 2. Nature 324: 691-695.

Clemmons, D., Nissley, P., and Rechler, M. 1992. Insulin-like growth factors in health and disease. Annals of Internal Med. 116: 854-862.

Clumeck, N., Mascart-Lemone, F., de Maubeuge, J., Brenez, D., and Marcelis, L. 1983. Acquired immune deficiency syndrome in black Africans. Lancet 1: 642.

Cohen, P. 1993. Dissection of the protein phosphorylation cascades involved in insulin and growth factor action. Biochem. Soc. Trans. 21: 555-567.

Cohen, E. A., Terwilliger, E. F., Jalnoos, Y., Proulx, J., Sodroski, J. G., and Haseltine, W. A. 1990. Identification of HIV-1 *vpr* product and function. J. Acquired Immune Defic. Syndr. 3: 11-18.

Cohick, W. S., and Clemmons, D. R. 1993. The insulin-like growth factors. Annu. Rev. Physiol. 55: 131-153.

Connor, R. I., and Ho, D. D. 1992. Pathogenesis of human immunodeficiency virus. Sem. Virol. 3: 213-224.

Conover, C. A., Ronk, M., Lombana, F., and Powell, D. R. 1990. Structural and biological characterization of bovine insulin-like growth factor binding protein-3. Endocrinology 127: 2795-2803.

Cullen, B. R. 1993. An introduction to human retroviruses. In *Human Retroviruses.* Ed. B. R. Cullen. Oxford University Press, New York, New York. p. 1-15.

Curran, J. W., Lawrence, D. N., Jaffe, H., Kaplan, J. E., Zyla, L. D., Chamberland, M., Weinstein, R., Lui, K. J., Schonberger, L. B., Spira,

T. J., Alexander, W. J., Swinger, G., Ammann, A., Solomon, S., Auerbach, D., Mildvan, D., Stoneburner, R., Jason, J. M., Haverkos, H. W., and Evatt, B. L. 1984. The acquired immunodeficiency syndrome (AIDS) associated with transfusions. N. Engl. J. Med. 310: 69-75.

Czech, M. P. 1989. Signal transmission by the insulin-like growth factors. Cell 59: 235-238.

Daniel, M. D., Letvin, N. L., King, N. W., Kannagi, M., Sehgal, P. K., Hunt, R. D., Kanki, P. J., Essex, M., and Desrosier, S. R. C. 1985. Isolation of T cell tropic HTLV-III-like retrovirus from macaques. Science 228: 1201-1209.

Davis, K. C., Horsburgh, C. R., Hasiba, U., Schocket, A. L., and Kirkpatrick, C. H. 1983. Acquired immunodeficiency syndrome in a patient with hemophilia. Ann. Intern. Med. 98: 284-286.

Defronzo, R. A., Bonadonna, R. C., and Ferrannini, E. 1992. Pathogenesis of NIDDM: a balanced overview. Diabetes Care 15: 318-368.

Duvall, E., and Wylie, A. H. 1986. Death and the cell. Immunol Today 7: 115-119.

Fabris, N., Pierpaoli, W., and Sorkin, E. 1971. Hormones and immunological capacity. III. The immunodeficiency disease of the hypopituitary Snell-Bragg dwarf mouse. Clin. Exp. Immunol. 9: 209-225.

Fauci, A. S. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science 239: 617-622.

Fauci, A. S., Masur, H., Gelmann, E. P., Markham, P. O., Hahn, B. H., and Lane, H, C. 1985. NIH conference: the acquired immunodeficiency syndrome: an update. Ann. Intern. Med. 102: 800-813.

Fisher, A. G., Ensoli, B., Ivanoff, L., Chamberlain, M., Petteway, S., Ratner, L., Gallo, R. C., and Wong-Staal, F. 1987. The *sor* gene of HIV-1 is required for efficient virus transmission *in vitro*. Science 237: 888-893.

Flier, J. S., Usher, P., and Moses, A. C. 1986. Monoclonal antibody to the type 1 insulin-like growth factor (IGF-I) receptor blocks IGF-I receptor-mediated DNA synthesis: clarification of the mitogenic mechanism of IGF-I and insulin in human skin fibroblasts. Proc. Natl. Acad. Sci. USA 83: 664-668.

Folks, T., Benn, S., Rabson, A., Theodore, T., Hoggan, M. D., Martin, M., Lightfoote, M., and Sell, K. 1985. Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. Proc. Natl. Acad. Sci. USA 82: 4539-4543.

Fultz, P., McClure, H. M., Anderson, D. C., Swenson, R. B., Anand, R., and Srinivasan, A. 1986. Isolation of a T cell lymphotropic retrovirus from naturally infected sooty mangabey monkeys (*Cercocebus atys*). Proc. Natl. Acad. Sci. USA 83: 5286-5290.

Gaines, H., von Sydow, M., Pherson, P. O., and Lundbergh, P. 1988. Clinical picture of primary HIV infection presenting as a glandular-fever-like illness. Br. Med. J. 297: 1363-1368.

Gallaher, W. R. 1987. Detection of a fusion peptide sequence in the transmembrane protein of human immunodeficiency virus. Cell 50: 327-328.

Garry, R. F. 1989. Potential mechanisms for the cytopathic properties of HIV. AIDS 3: 683-694.

Gartner, S., Markovits, P., Markovits, D. M., Kaplan, M. H., Gallo, R. C. and Popovic, M. 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science 223: 215-219.

Geffner, M. E., Yeh, D. Y., Landaw, E. M., Scott, M. L., Stiehm, E. R., Bryson, Y. J., and Israele, V. 1993. *In vitro* insulin-like growth factor-I, growth hormone and insulin resistance occurs in symptomatic human immunodeficiency virus-1-infected children. Pediatr. Res. 34(1): 66-72.

Geffner, M. E., Bersch, N., Lippe, B. M., Rosenfeld, R. G., Hintz, R. L., and Golde, D. W. 1990. Growth hormone mediates the growth of T-lymphoblast cell lines via locally generated insulin-like growth factor-1. J. Clin. Endocrinol. Metab. 71: 464-469.

Geleziunas, R., Bour, S., Boulerice, F., Hiscott, J., and Wainberg, M. A. 1991. Diminution of CD4 surface protein but not CD4 messenger RNA levels in monocytic cells infected by HIV-1. AIDS 5:29-533.

Germain, R. N. 1988. Antigen processing and CD4+ T cell depletion in AIDS. Cell 54: 441-444.

Glassock, G. F., Hein, A. N., Miller, J. A., Hintz, R. L., and Rosenfeld, R. G. 1992. Effects of continuous infusion of insulin-like growth factor I and II alone and in combination with thyroxine or growth hormone, on the neonatal hypophysectomized rat. Endocrinology 130: 203-210.

Gottlieb, M. S., Schroff, R., Schanler, H. M., Wiseman, J. D., Fan, P. T., Wolf, R. A., and Saxon, A. 1981. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men:

evidence of new acquired cellular immunodeficiency. N. Engl. J. Med. 305: 1425-1431.

Gowda, S. D., Stein, B. S., Mohagheghpour, N., Benike, C. J., and Engleman, E. G. 1989. Evidence that T cell activation is required for HIV-1 entry in CD4+ lymphocytes. J. Immunol. 142: 773-780.

Greene, W. C. 1993. AIDS and the immune system. Scientific American 269: 99-105.

Guler, H-P., Zapf, J., Schmid, C., and Froesch, E. R. 1989. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol. 121: 753-758.

Guler, H-P., Zapf, J., Scheiwiller, E., and Froesch, E. R. 1988. Recombinant human insulin-like growth factor I stimulates growth and has distinct effects on organ size in hypophysectomized rats. Proc. Natl. Acad. Sci. USA 85: 4889-4893.

Hahn, B. H., Shaw, G. M., Arya, S. K., Popovic, M., Gallo, R. C., and Wong-Staal, F. 1984. Molecular cloning and characterization of the HTLV-III virus associated with AIDS. Nature 312: 166-169.

Ho, D. D., Moudgil, T., and Alam, M. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N. Engl. J. Med. 321: 1621-1625.

Hofmann, B., Nishanian, P., Baldwin, R. L., Insixiengmay, P., Nel, A., and Fahey, J. L. 1990. HIV inhibits the early steps of lymphocyte activation including initiation of inositol phospholipid metabolism. J. Immunol. 145: 3699-3705.

Hommes, M. J., Romijin, J. A. Endert, E., Eeftinck Schattenkerk, J. K. and Sauerwein, H. P. 1991. Insulin sensitivity and insulin clearance in

human immunodeficiency virus-infected men. Metabolism 40: 651-656.

Imberti, L., Sottini, A., Bettinardi, A., Puoti, M., and Primi, D. 1991. Selective depletion in HIV infection of T cells that bear specific T cell receptor Vβ sequences. Science 254: 860-862.

Isaksson, O. G., Lindahl, A., Nilsson, A., and Isgaard, J. 1987. Mechanism of stimulatory effect of growth hormone on longitudinal bone growth. Endocrinol. Rev. 8: 426-438.

Jacobs, S., Hazum, E., and Cuatrecasasas, P. 1980. The subunit structure of rat liver insulin receptor antibody directed against the insulin binding subunit. J. Biol. Chem. 255: 6937-6940.

Jasny, B. R. 1993. AIDS 1993: Unanswered questions. Science 260: 1219.

Johnson, V. A., and Byington, R. E. 1990. Infectivity assay. In *Techniques in HIV research.* Eds. A. Idovoni and B. Walker. Stolkton Press, New York, New York. p71-76.

Kanki, P. J., McLane, M. F., King, N. W. Jr., Letvin, N. L., Hunt, R. D., Sehgal, P., Daniel, M. D., Desrosier, S. R. C., and Essex, M. 1985. Serologic identification and characterization of a macaque T-lymphotropic retrovirus closely related to human T-lymphotropic retroviruses (HTLV) type III. Science 228: 1199-1201.

Kelley, K. W. 1989. Growth hormone, lymphocytes and macrophages. Biochem. Pharmacol. 38: 705-713.

Kelley, K. W. 1990. The role of growth hormone in the modulation of the immune response. Ann. N. Y. Acad. Sci. 594: 95-118.

Keusch, G. T. and Thea, D. M. 1993. Malnutrition in AIDS. Clinical Nutrition 77(4): 795-814.

Kincade, P. W. 1994. B lymphopoieses: global factors, local control. Proc. Natl. Acad. Sci. 91: 2888-2889.

Klatzmann, D., Barre-Sinoussi, F., Nugeyre, M. T., Dauguet, C., Vilmer, E., Griscelli, C., Brun-Vezinet, F., Rouzious, C., Gluckman, J. C., Chermann, J. C., and Montagnier, L. 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science 225: 59-63.

Kozak, R. W., Haskell, J. F., Greenstein, L. A., Rechler, M. M., Woldman, T. A., and Nissley, S. P. 1987. Type I and II insulin-like growth factor receptors on human phytohemagglutinin activated T-lymphocytes. Cell Immunol. 109: 318-331.

Kuhne, M. R., Pawson, T., Lienhard, G. E., and Feng, G. S. 1993. The insulin receptor substrate 1 associates with SH-2 containing phosphotyrosine phosphatase. J. Biol. Chem. 268: 11479-11481.

Lane, H. C., Masur, H., Edgar, L. C., Whalen, G., Rook, A. H., and Fauci, A. S. 1983. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. 309: 453-458.

Langford, K. S., and Miell, J. P. 1993. The insulin-like growth factor-I/binding protein axis: physiology, pathophysiology and therapeutic manipulation. Eur. J. Clin. Invest. 23: 503-516.

Laurence, J., Hodtsev, A. S., and Posnett, D. N. 1992. Superantigen implicated in dependence of HIV-1 replication in T cells on TCR Vβ expression. Nature 358:255-259.

Lee, M. H., Sano, K., Morales, F. E., and Imagawa, D. T. 1987. Sensitive reverse transcriptase assay to detect and quantitate human immunodeficiency virus. J. Clin. Microl. 25: 1717-1722.

Lemmey, A. B., Martin, A. A., Read, L. C., Tomas, F. M., Owens, P. C., Ballard, F. J. 1991. IGF-I and the truncated analogue des-(1- 3)IGF-I enhance growth in rats after gut resection. Am. J. Physiol. 260: E213-219.

Letvin, N. L., Daniel, M. D., Setigal, P. K.. Desrosiers, R. C., Hunt, R. D., Waldron, L. M., Mackey, J. J., Schmidt, D. K., Chalifoux, L. V., and King, N. W. 1985. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science 230:71-73.

Levy, J. A., Hoffman, A. D., Kramer, S. M., Landis, J. A., Shimabukuro, J. M., and Oshiro, L. S. 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science 255: 840-842.

Lieberman, S. A., Butterfield, G. E., Harrison, D., and Hoffman, A. R. 1994. Anabolic effects of recombinant insulin-like growth factor-1 cachetic patients with the acquired immunodeficiency syndrome. J. Clin. Endocrinol. Metab. 78: 404-410.

Lifson, J. D., Feinberg, M. B., Reyers, G. R., Rabin, L., Banapour, B., Chakrabarti, S., Moss, B., Wong-Staal, F., Steimer, K S., and Engleman, E. G. 1986. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. Nature 323: 725-728.

Linette, G. P., Hartzman, R. J., Ledbetter, J. A., and June, C. H. 1988. HIV-1 infected T-cells show a selective signalling defect after perturbation of CD3/antigen receptor. Science 241: 573-576.

Maller, J. W., and Koontz, J. W. 1981. The mitogenic actions of insulin and insulin-like growth factor-1. Dev Biol. 85: 309-315.

Martin, A. A., Tomas, F. M., Owens, P. C., Knowles, S. E., Ballard, F. J., and Read, L.C. IGF-I and its variant, des-(1-3)IGF-I enhance growth in rats with reduced renal mass. Am. J. Physiol. 261: F626-633.

Masur, H., Michelis, M. A., Greene, J. B., Onovato, I., Van de Stowe, R. A., Holzman, R. S., Wormser, G., Brettman, L., Lange, M., Murray, H. W., and Cunningham-Rundles, S. 1981. An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. N. Engl. J. Med. 305: 1431-1438.

Meltzer, M. S., Skillman, D. R., Hoover, D. L., Hanson, B. D., Turpin, J. A., Kalter, D. C., and Gendelman, H. E. Immunol. Today 11: 217-223.

Merchav, S., Tatarsky, I., and Hochberg, Z. 1988. Enhancement of human granulopoiesis *in vitro* by biosynthetic insulin-like growth factor I/somatomedin C and human growth hormone. J. Clin. Invest. 81: 791-795.

Meyers, M. G., and White, M. F. 1993. Insulin receptor substrate-1 and proteins with SH2 domains. Diabetes 42: 643-650.

Meyers, M. G. Jr., Backer, J. M., Sun, X-J., Shoelson, S. E., Hu, P., Schlessinger, J., Yoakim, M., Schaffhausen, B., and White, M. F. 1992. IRS-1 activates the phosphatidyl inositol 3'-kinase by associating with *src* homology 2 domains of p85. Proc. Natl. Acad. Sci. USA 89: 10350-10354.

Monroe, J. E., Calender, A., and Mulder, C. 1988. Epstein-Barr virus-positive and negative B-cell lines can be infected with human immunodeficiency virus types 1 and 2. J. Virol. 62: 3497-3500.

Narayan, O., and Cork, L. C. 1985. Lentiviral diseases of sheep and goats: chronic pneumonia, leukoencephalomylitis and arthritis. Rev. Infect. Dis. 7: 83-98.

Nguyen, B. Y., Clerici, M., Bauza, S., Bailey, J., Longo, D., Murphy, W., Shearer, G., Gesundkeit, N., Broder, S., and Yarckoan, R. 1993. A pilot study of recombinant human IGF-I and human growth hormone in patients with HIV infection. First Ann. Conference on Human Retro and Related Infections. p 74.

Olsen, H. S., Cochrane, A. W., Dillon, P. J., Nalin, C. M., and Rosen, C. A. 1990. Interaction of the human immunodeficiency virus type 1 rev protein with a structural region in *env* mRNA is dependent on multimer formation mediated through a basic stretch of amino acids. Genes and Development 4: 1357-1364.

Panganiban, A. T., and Fiore, D. 1988. Ordered interstrand and intrastrand DNA transfer during reverse transcription. Science 241: 1064-1069.

Pantaleo, G., Graziosi, C., and Fauci, A. S. 1993. The immunopathogenesis of human immunodeficiency virus infection. N. Engl. J. Med. 328: 327-335.

Pantaleo, G., Graziosi, C., Butini, L., Pizzo, P. A., Schnittman, S. M., Kotler, D. P., and Fauci, A. S. 1991. Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc. Natl. Acad. Sci. USA 88: 9838-9842.

Paul, W. E. 1991. Interleukin 4: a prototypic immunoregulatory lymphokine. Blood 77: 1859-1870.

Pauza, D., and Price, T. M. 1988. Human immunodeficiency virus infection of T-cell proceeds via receptor mediated endocytosis. J. Cell. Biol. 107: 959-968.

Pessin, J. E. 1993. Molecular properties of insulin/IGF-I hybrid receptors. In *Current directions in insulin-like growth factor research.* Eds. D. LeRoith and M. K. Raizada. Plenum Press, New York, New York. p. 133-144.

Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA 77: 7415-7419.

Popovic, M., Sarngadharan, M. G., Read, E., and Gallo, R. C. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS or pre-AIDS. Science 224: 497-500.

Price, R. W., Brew, B., Sidtis, J., Rosenblum, M., Scheck, A. C., and Cleary, P. 1988. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science 239: 586-592.

Rechler, M. M., and Nissley, S. P. 1985. The nature and regulation of the receptors for insulin-like growth factors. Ann. Rev. Physiol. 47: 425-442.

Rice, A. P., and Mathews, M. B. 1988. Transcriptional but not translational regulation of HIV-1 by the *tat* gene product. Nature 332: 551-553.

Rook, A. H., Masur, H., Lane, H. C., Frederick, W. J., Kasahara, T., Macher, A. M., Djeu, J. Y., Manschewitz, J. F., Jackson, L., Fauci, A. S., and

Quinnan, G. V. 1983. Interleukin-2 enhances the depressed natural killer and cytomegalovirus-specific cytotoxic activities of lymphocytes from patients with the acquired immune deficiency syndrome. J. Clin. Invest. 72: 398-403.

Rosenberg, Z. F., and Fauci, A. S. 1989. The immunopathogenesis of HIV infection. Adv. Immunol. 47: 377-431.

Roy, S., and Wainberg, M. A. 1988. Role of the mononuclear phagocyte system in the development of acquired immunodeficiency syndrome (AIDS). J. Leuk. Biol. 43:91-97.

Rubinstein, A., Sicklick, M., Gupta, A., Bernstein, L., Klein, N., Rubinstein, E., Spigland, I., Frucher, L., Litman, N., Lee, H., and Hollander, M. 1983. Acquired immunodeficiency with reversed T4/T8 ratios in infants born to promiscuous and drug-addicted mothers. JAMA 249: 2350-2356.

Ruegg, C. L., and Strand, M. 1990. Inhibition of protein kinase C and anti-CD3-induced Ca 2+ influx in Jurkat T cells by a synthetic peptide with sequence identity to HIV-1 gp41. J. Immunol. 144: 3928-3235.

Salfeld, J., Gottlinger, H. G., Sia, R. A., Park, R. E., Sodroski, J. G., and Haseltine, W. A. 1990. A tripartite HIV-1 Tat-Env-Rev fusion protein. EMBO J. 9: 965-970.

Schimpff, R. M., Repellin, A. M., Salvatoni, A., Thieriot-Prevost, G., and Chatelian, P. 1983. Effect of purified somatomedins on thymidine incorporation into lectin-activated human lymphocytes. Acta. Endocrinol. 102: 21-26.

Schnittman, S. M., Greenhouse, J. J., Psallidopoulos, M. C., Baseler, M., Salzman, N. P., Fauci, A. S., and Lane, H. C. 1990. Increasing viral

burden in CD4+ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. Ann. Intern. Med. 113: 438-443.

Seigal, F. P., Lopez, C., Hammer, G. S., Brown, A. E., Kornfeld, S. J., Gold, J., Hassett, J., Hirschman, S. Z., Cunningham, C., and Adelsberg, B. R. 1981. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. N. Engl. J. Med. 305: 1439-1444.

Serwadda, D., Mugerwa, R. D., Sewankambo, N. K., Lwegaba, A., Carswell, J. W., Kirya, G. B., Baylay, A. C., Downing, R. G., Tedder, R. S., Clayden, S. A., Weiss, R. A., and Dalgleish, A. G. 1985. Slim disease: a new disease in Uganda and its association with HTLV-III infection. Lancet 2: 849-852.

Sheppard, H. W., and Ascher, M. S. 1992. The natural history and pathogenesis of HIV infection. Annu. Rev. Microbiol. 46: 533-564.

Shuldiner, A. R., Barbetti, F., Raben, N., Scavo, L., and Serrano, J. 1991. Insulin. In *Insulin-Like Growth Factors: Molecular and Cellular Aspects.* Ed. D. LeRoith. CRC Press Inc., Boca Raton, Florida. p 181-224.

Skolnik, E. Y., Batzer, A., Li, N., Lee, C. H., Lowenstein, E., Mohammadi, M., Margolis, B., and Schlessinger, J. 1993. The function of GRB2 in linking the insulin receptor to ras signaling pathways. Science 260: 1953-1955.

Slieker, L. J., Brooke, G. S., Chance, R. E., Fan, L., Hoffman, J. A., Howey, D. C., Long, H. B., Meyer, J., Shields, J. E., Sundell, K. L., and DiMarchi, R. D. 1993. Insulin and IGF-I analogs: novel approaches to improved insulin pharmacokinetics. In *Current directions in insulin-*

like growth factor research. Eds. D. LeRoith and M. K. Raizada. Plenum Press, New York, New York. p. 25-40.

Sodroski, J., Goh, W. C., Rosen, C., Dayton, A., Terwilliger, E., and Haseltine, W. 1986. A second post-transcriptional trans-activator gene required for HTLV-III replication. Nature 321: 412-417.

Sonigo, P., Alizon, M., Staskus, K., Klatzmann, D., Cole, S., Danos, O., Retzel, E., Tiollais, P., Hasse, A., and Wain-Hobson, S. 1985. Nucleotide sequence of the visna lentivirus: relationship to the AIDS virus. Cell 42: 369-382.

Starcich, B., Ratner, L., Josephs, S. F., Okamoto, T., Gallo, R. C., and Wong-Staal, F. 1984. Characterization of long terminal repeat sequences of HTLV-III. Science 227: 538-540.

Steiner, D. F., Tager, H. S., Chan, S. J., Nanjo, K., Sanke, T., and Rubinstein, A. H. 1990. Lessons learned from molecular biology of insulin gene mutations. Diabet. Care 13: 600-609.

Strebel, K., Klimkait, T., and Martin, M. A. 1988. A novel gene of HIV-1, *vpu* and its 16-kilo dalton product. Science 241: 1221-1223.

Stuart, C. A., Meehan, R. T., Neale, L. S., Cintron, N. M., and Furlanetto, R. W. 1991. Insulin-like growth factor-I binds selectively to human peripheral blood monocytes and B lymphocytes. J. Clin. Endocrinol. Metab. 72: 1117-1122.

Sun, X-J., Miralpeix, M., Myers, M. G. Jr., Glasheen, E. M., Backer, J. M., Kahn, C. R., and White, M. F. 1992. The expression and function of IRS-1 in insulin signal transmission. J. Biol. Chem. 267: 22662-22667.

Tapson, V. F., Boni Schnetzler, M., Pilch, P. F., Center, D. M., and Berman, J. S. 1988. Structural and functional characteristics of the human T-lymphocyte receptor for insulin-like growth factor-I *in vitro*. J. Clin. Invest. 82: 950-957.

Teich, N. 1984. Taxonomy of retroviruses. In *RNA Tumor Viruses*. Eds. R. Weiss, N. Teich, H. Varmus, and J. Coffin. Cold Spring Harbor, Cold Spring Harbour Lab. 2: 25-207.

Temin, H. M., and Mizutana, S. 1970. RNA-directed DNA polymerase in virions in Rous sarcoma virus. Nature 226: 1211-1213.

Terwilliger, E., Sodroski, J. G., Rosen, C. A., and Haseltine, W. A. 1986. Effects of mutations within the 3′ *orf* open reading frame regions of human T-cell virus type III on replication and cytopathogenicity. J. Virol. 60: 754-760

Thorsen, A. V., and Hiny, R. L. 1977. Specific ^{125}I-somatomedin receptors on circulating human mononuclear cells. Biochem. Biophys. Res. Commun. 74: 1566-1571.

Timsit, J., Savino, W., Safieh, B., Chanson, P., Gagnerault, M. C., Bach, J. F. and Dardenne, M. 1992. Growth hormone and insulin-like growth factor-I stimulate hormonal function and proliferation of thymic epithelial cells. J. Clin. Endocrinol. Metab. 75: 183-188.

Ullrich, A., Gray, A., Tam, A. W., Yang-Fang, T., Tsubokawa, M., Collins, C., Henzel, W., Le bon, T., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, J., and Fujita-Yamaguchi, Y. 1986. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that determine functional specificity. EMBO J. 5: 2503-2509.

Vaishnav, Y. N., and Wong-Staal, F. 1991. The biochemistry of AIDS. Annu. Rev. Biochem. 60: 577-630.

Waife, S. O. 1980. Insulin. In *Diabetes Mellitus*. Ed. S. O. Waife. Eli Lilly and Company, Indianapolis, Indiana. p. 29-32.

Wainberg, M. A., and Margolese, R. G. 1992. Strategies in the treatment of AIDS and related diseases: the lessons of cancer chemotherapy. Cancer Invest. 10: 143-153.

Wang, L. M., Keegan, A. D., Li, W., Lienhard, G. E., Pacini, S., Gutkind, J. S., Myers, M. G., Sun, X-J., White, M. F., Aaronson, S. A., Paul, W. E., and Pierce, J. H. 1993. Common elements in IL-4 and insulin signalling pathways in factor dependent hematopoietic cells. Proc. Natl. Acad. Sci. USA 90: 4032-4038.

Wang, L. M., Keegan, A. D., Paul, W. E., Heidaran, M. A., Gutkind, J. S., and Pierce, J. H. 1992. IL-4 activates a distinct signal transduction cascade from IL-3 in factor dependent myeloid cells. EMBO J. 11: 4899-4908.

White, M. F., and Kahn, C. R. 1994. The insulin signaling system, J. Biol. Chem. 269: 1-4.

World Health Organization. 1986. Acquired immunodeficiency syndrome (AIDS). WHO/CDC case definition for AIDS. Wkly epidemiol Rec. 61: 69-76.

Zagury, D., Bernard, J., Leonard, R., Cheynier, R., Feldman, M., Sarin, P. S. and Gallo, R. C. 1986. Long-term cultures of HTLV-III infected T cells: A model of cytopathology of T-cell depletion in AIDS. Science 231: 850-853.

Zack, J. A., Arrigo, S. J., Weitsman, S. R., Go, A. S., Haislip, A., and Chen, I. Y. 1990. HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. Cell 61: 213-222.