

Human Chorionic Gonadotropin Hormone Prevents Wasting Syndrome and Death in HIV-1 Transgenic Mice

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Abstract

At birth, transgenic mice, homozygous for the HIV-1 provirus pNL4-3, deleted in *gag/pol*, are normal in appearance and weight. Within several days after birth, the pups develop a syndrome characterized by dry, scaly, hyperkeratotic skin, growth failure, and death. The possibility that the homozygous embryos are being protected during gestation by a maternal factor led us to treat the newborn animals with various pregnancy-related hormones including human chorionic gonadotropin (hCG), estrogen, progesterone, and dexamethasone. Treatment with hCG prevented death, led to normal growth, and markedly reduced skin lesions. In contrast to the skin of the untreated homozygous pups, which expressed high levels of HIV mRNA and proteins (i.e., gp120 and Nef), the skin of the hCG-treated pups showed a marked reduction in both HIV mRNA and proteins. Discontinuation of hCG resulted in the reappearance of HIV transcripts and proteins, skin lesions, and growth failure resulting in death. In addition, HIV transcripts and proteins were reduced significantly in heterozygous mothers during pregnancy, but reappeared after parturition. Similarly, hCG treatment resulted in a decrease of HIV proteins in the skin of nonpregnant heterozygous transgenic mice. These findings suggest that the inhibiting effect of hCG on HIV expression may be clinically useful in the treatment of HIV infections, and may be responsible, during pregnancy, for the low transmission of HIV from infected mothers to their offspring. (*J. Clin. Invest.* 1997. 99:1484–1491.) Key words: estrogen • progesterone • dexamethasone • gp120 • Nef

Introduction

It is estimated that the mother-to-child transmission rate of HIV infection is between 18% and 25% (1–3). Studies in some developing countries suggest that HIV-1 infection may affect fetal growth and infant weight (4, 5). Similar effects, however,

were not found in the USA (6) or Europe (7–9). Spinillo et al. (9), for example, reported that there was no difference in birthweight or gestational age when infected and uninfected newborns were compared. This was based on information collected from 559 HIV-1 seropositive women delivered in 13 Italian hospitals, where the mother-to-child transmission rate of HIV infection was 18.2%. After birth, however, a cachexia syndrome characterized by severe wasting with failure to thrive has been described in HIV-1-infected patients with congenital AIDS (10, 11). Severe weight loss, mainly as a result of muscle mass depletion, is thought to be one of the causes of death. The maintenance of fetal health and normal weight up to the time of birth suggest that the pregnant mother may provide factors that protect the infected fetus.

In the present study we used an HIV-1 transgenic mouse model to study the effect of pregnancy-related hormones on the growth and health of HIV-1 transgenic (Tg)¹ mice. The Tg26 line carries a 7.4-kb HIV-1 construct which lacks a 3-kb sequence overlapping the *gag/pol* region of provirus pNL4-3 (12–16). Tg26 mice express high levels of HIV transcripts in muscle and skin, and lower levels in brain, lung, intestine, kidney, spleen, and thymus (17). Up to the time of birth, the homozygous Tg mice are normal in appearance and weight. Shortly after birth, the homozygous Tg mice develop a debilitating cachexia comparable to that of human AIDS patients, and die within 3–6 wk of age (15, 16). Also, shortly after birth, the skin of homozygous animals show diffuse scaling and psoriasis-like lesions. In the present study we tested the effect of several pregnancy related hormones (i.e., human chorionic gonadotropin [hCG], progesterone, estrogen, and dexamethasone) on the development of the cachexia syndrome. hCG was found to have a profound protective effect.

Methods

Transgenic mice. Noninfectious pNL4-3:d1443 transgenic mice were established by microinjecting FBN/N mouse oocytes as previously described (12). The 7.4-kb transgene construct was engineered from an infectious clone of an integrated provirus, pNL4-3, by deleting 3.1 kb of sequence overlapping *gag* and *pol*, but containing *env* and the other accessory genes *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu*, together with the 5' and 3' long terminal repeats (LTRs). Both heterozygous and homozygous transgenic mice were characterized previously in detail (12–17). Heterozygous transgenic mice maintain growth rates close to that of nontransgenic mice, but develop kidney lesions (12, 13) and

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1. Abbreviations used in this paper: hCG, human chorionic gonadotropin; LTR, long terminal repeats; Tg, transgenic.

hyperproliferative skin disorders manifested as spontaneous papillomas (14). Homozygous mice suffer from growth retardation and skin disorders manifested as diffuse epidermal hyperplasia. In addition, homozygous mice suffer from muscular dystrophy, thymic hypoplasia, lymphoproliferation, splenomegaly, and severe wasting, and die within 3–6 wk (15, 16). In this study, heterozygous mice were crossbred to obtain homozygous, heterozygous, and nontransgenic mice. Southern blot hybridization was performed to confirm their genotypes. Animals were maintained in accordance with NIH Guidelines.

Treatment protocols. Heterozygous transgenic mice (7–10 wk old) were mated, and pregnant mice were monitored. 1 d after the pups were born, an Alzet mini-osmotic pump (model 2002), containing one of several hormones, was implanted in the mother subcutaneously between the scapulae. The pumping rate was 0.5 μ l/h and pumps were replaced every 10 d. Hormones (hCG, α hCG, β hCG, estrogen, progesterone, and dexamethasone) were obtained from Sigma Chemical Co., St. Louis, MO. The mini-osmotic pumps were filled with 400 U/ml hCG, 20 μ g/ml β hCG, 20 μ g/ml α hCG, 5 mg/ml dexamethasone, 1 mg/ml estrogen, and 4 mg/ml progesterone. Unless stated otherwise, the HIV transgenic pups received the hormones through the mother's milk. These concentrations of hormones were found to have no effect on normal pups when administered in an identical way (i.e., through the mother's milk). Beginning at 20 d of age, some of the HIV transgenic pups were given subcutaneous injections of 0.1 ml β hCG (20 μ g/ml) twice a week. hCG levels were measured by a sandwich ELISA according to the manufacturer's instructions (United Biotech, Inc., Mountain View, CA) and the lower limit of detection by this method was 5 mU/ml.

Southern blot analysis. To confirm the genotypes of the transgenic mice, Southern blot analyses were performed (16). Briefly, 10 μ g of extracted tail DNA were digested with EcoRI, electrophoresed through agarose gels, transferred to a nylon membrane and cross-linked by a UV cross-linker (Stratagene, Inc., La Jolla, CA). Transferred DNA in the nylon membrane was hybridized using [32 P]-labeled cDNA encoding HIV-1 *nef* gene as a probe (18). cDNA was radiolabeled using [32 P]dCTP (New England Nuclear/DuPont-NEN, Boston, MA) by the random primer method (Boehringer Mannheim Biochemicals, Indianapolis, IN). Hybridization was performed overnight at 43°C in hybridol (Oncor, Inc., Gaithersburg, MD) containing 50% formamide. The probe was added after 2–3 h of prehybridization in the same buffer. The filters were washed twice at room temperature and twice at 53°C in 2 \times SSC, 0.2% SDS. Washed filters were exposed

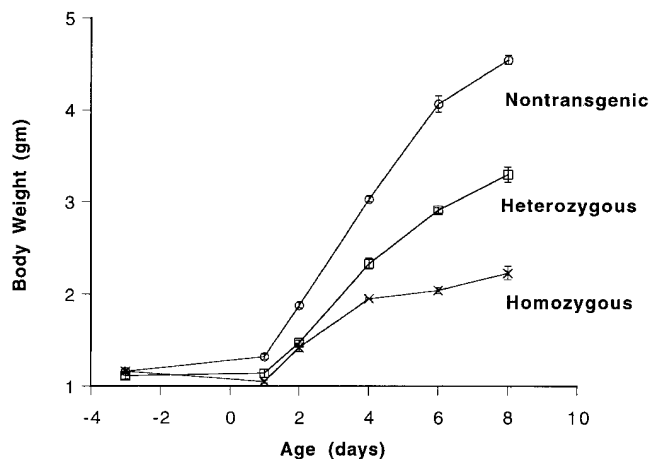


Figure 1. Mean body weight of transgenic mice before and after birth. Homozygosity versus heterozygosity determined on each mouse by Southern blot. Number of mice in each group—before birth: 4 homozygous, 12 heterozygous, and 9 nontransgenic; after birth: 8 homozygous, 12 heterozygous, and 14 nontransgenic.

for autoradiography at -70°C . Blots were scanned on a Phosphorimager (Molecular Dynamics, Sunnyvale, CA), and the intensity of the transgene construct in homozygous mice was found to be twice that in heterozygous mice.

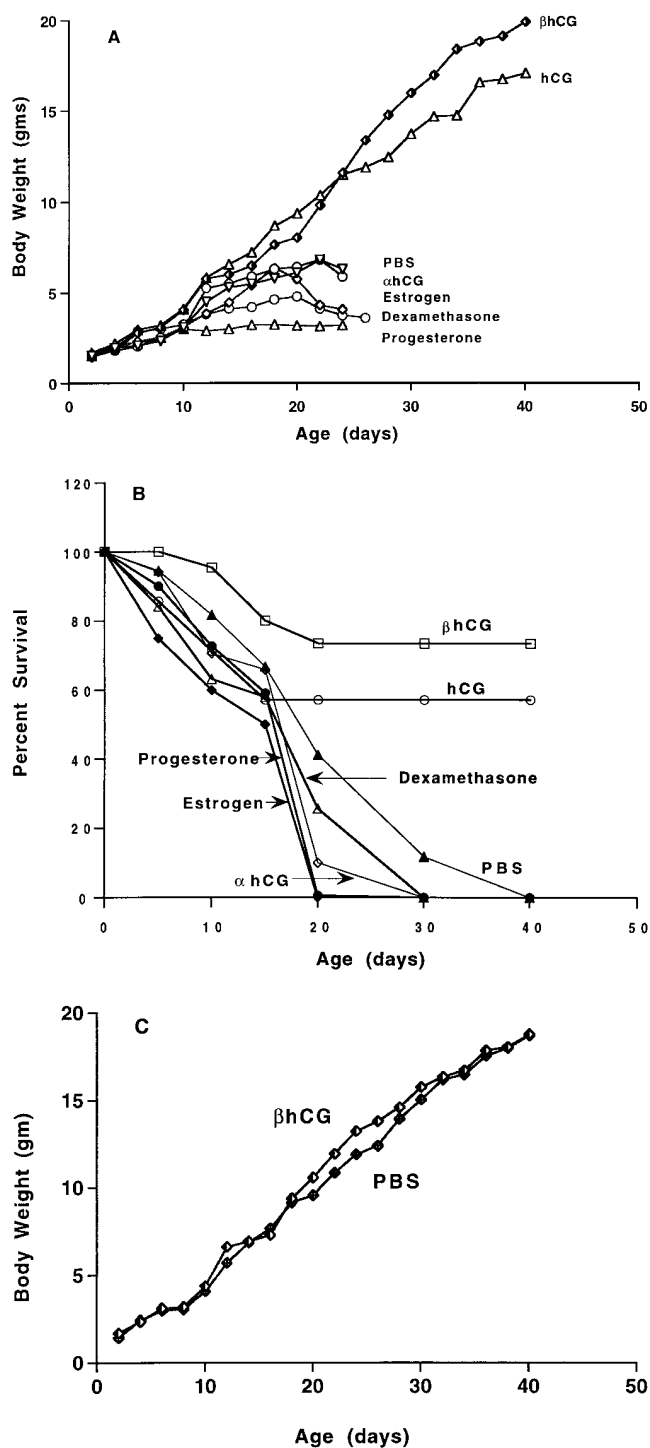


Figure 2. Effect of hormones on: (A) growth; (B) survival of homozygous; and (C) heterozygous transgenic mice. Within 24 h after birth of pups, a peristaltic pump containing PBS or hormone (hCG, estrogen, progesterone, dexamethasone, α hCG, or β hCG) was placed in the mother's body. Pups received the hormones through maternal milk while nursing and then by injection (see Methods). Each group consisted of 8–12 homozygous pups.

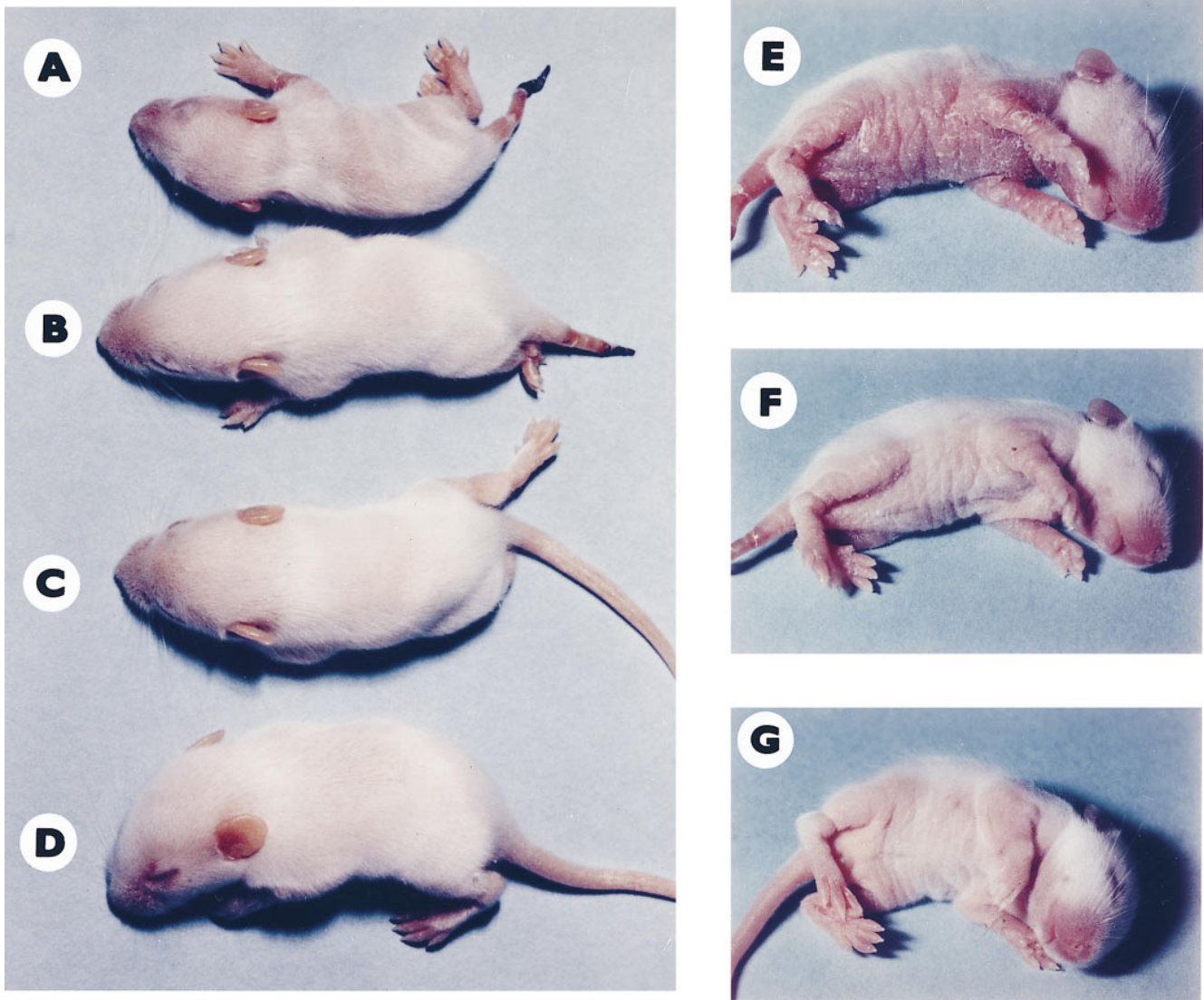


Figure 3. Appearance of homozygous mice (A) not treated; and (B) treated for 9 d with hCG. Heterozygous (C) and nontransgenic (D) littermates served as controls. At 9 d of age, the ventral surface of untreated homozygous mice appears dry and scaly (E). Treatment with hCG largely prevents the appearance of skin lesions (F). Untreated heterozygous mice served as controls and display normal appearing skin (G).

Isolation of total RNA and Northern blot hybridization. RNA was extracted using the acid guanidinium thiocyanate-phenol-chloroform extraction procedure (19). Tissues were snap frozen in liquid nitrogen, homogenized in solution containing guanidine isothiocyanate, sodium citrate, and sarcosyl, and then extracted with phenol and chloroform-isoamyl alcohol mixture. RNA was precipitated from the aqueous phase with ethanol to remove DNA. The final RNA pellet was dissolved in water. Northern blot analysis was performed as described in reference 20. In brief, RNA (20 μ g) was denatured at 60°C and separated by electrophoresis in a 1% agarose gel containing 1 \times Mops buffer (20 mM Mops, 5 mM sodium acetate and 1 mM EDTA) and 2.2 M formaldehyde. The gel was transferred to a nylon membrane and fixed by UV cross-linking (Stratagene, Inc.). Hybridization of the transferred RNA was performed using radio-labeled HIV-1 *nef* cDNA as described in the Southern blot hybridization procedure. In all experiments, duplicate gels were stained with acridine orange to ensure the integrity of the RNA samples, and to confirm that equal amounts of RNA had been loaded onto each lane. Following hybrid-

ization and washing, filters were exposed for autoradiography at -70°C from 24 to 72 h depending on the sample. Hybridized filters were scanned on a Phosphorimager and the intensity of the transcripts was quantified.

Immunolocalization of HIV-1 gp120 and HIV-1 Nef in the skin. Tissues were fixed with methanol Carnoy's solution (60% methanol, 30% chloroform, 10% acetic acid) for 8 h and paraffin sections were prepared. Sections (4 μM) were deparaffinized and digested with 0.5% trypsin for 60 min at 37°C. Immunoperoxidase staining was performed by the streptavidine-biotin complex technique, using a Histostain-SP kit (Zymed, Burlingame, CA) according to the manufacturer's protocol. Polyclonal sheep anti-HIV-1 gp120 antibody was obtained from Dr. M. Phelan through the AIDS Research and Reference Reagent Program (ARRRP) and used at a 1:2000 dilution. Polyclonal rabbit anti-Nef antiserum was used at a 1:1000 dilution and obtained from Dr. B. Cullen through the ARRRP. As a control, sections were incubated with normal serum of the species of the primary antibody. All sections were counterstained with hematoxylin.

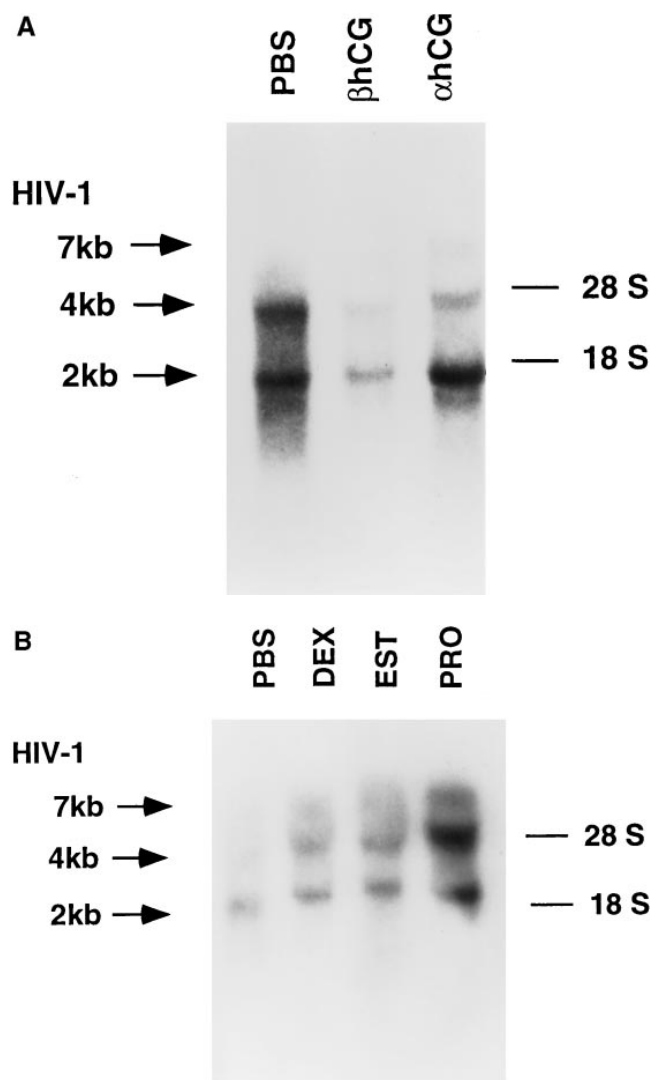


Figure 4. Effect of (A) β hCG and α hCG; and (B) dexamethasone, estrogen, and progesterone on HIV mRNA expression in homozygous transgenic mice. Pups received hormones through the mother's milk as described in Methods. RNA was extracted from muscle or skin on day 12 and analyzed by Northern blot. Duplicate gels were stained with acridine orange to ensure integrity of the RNA and to confirm that equal amounts of RNA had been loaded onto each lane.

Results

Postnatal growth failure of transgenic mice. The weight of HIV transgenic mice before and after birth is illustrated in Fig. 1. Immediately before and after birth, the heterozygous, homozygous, and nontransgenic mice were of approximately equal weight. Within a couple of days, however, the heterozygous, but in particular the homozygous, mice showed marked retardation of growth as compared to the nontransgenic mice (Figs. 1 and 2). At 8 d after birth, there was nearly a 60% decrease in the weight of the homozygous transgenic as compared to the nontransgenic mice.

Effect of reproductive hormones on growth of homozygous transgenic mice. To see if some of the hormones associated with gestation might be responsible for maintaining the body

weight of the transgenic mice while in utero, and to determine whether these hormones would restore and/or maintain bodyweight postnatally, homozygous mice were treated with hormones (obtained through the mother's milk and then by injection as described in Methods) beginning within 24 h after birth. As seen in Fig. 2 A, homozygous mice that received PBS, α hCG, estrogen, dexamethasone or progesterone, died within 3–4 wk of age. In fact, the weight loss at death was somewhat more pronounced in the animals given estrogen, dexamethasone, and progesterone than in the animals given PBS or α hCG. In marked contrast, the majority of the animals receiving hCG (60%) or β hCG (75%) survived (Fig. 2 B) and showed progressive increase in bodyweight approaching that of the nontransgenic mice (Fig. 3, A–D). Protection from wasting was somewhat greater in the β hCG treated group than the hCG group. β hCG had no effect on the weight of heterozygous transgenic mice (Fig. 2 C).

Treatment with hCG also prevents the appearance of the skin lesions which develop in homozygous mice after birth. Fig. 3 E shows the dry scaly lesions of an untreated homozygous mouse 9 d after birth. In contrast, mice treated with hCG beginning at birth (Fig. 3 F) show nearly normal skin comparable in appearance to untreated heterozygous controls (Fig. 3 G).

Effect of hormone treatment on the induction and suppression of HIV-1 mRNA in transgenic mice. Expression of HIV mRNA in transgenic mice is tissue dependent. Highest expression is found in muscle and skin. The 2- and 4-kb messages predominate as compared to the 7-kb message and homozygous animals express 5–10-fold more HIV mRNA than heterozygous animals (17). Fig. 4 A shows that β hCG, but not α hCG, treatment suppressed by 70–90% the expression of the 2- and 4-kb messages in the muscle. In contrast, dexamethasone, estrogen, and progesterone increased the expression of the HIV mRNA in muscle (not shown) and skin (Fig. 4 B). The 2- and 4-kb messages were increased 2–8-fold, and the 7-kb message 8–50-fold. The increased expression of the HIV-1 message correlates with the accelerated death of mice treated with these hormones (Fig. 2) as compared to PBS. The experiments described in Fig. 4, A and B were repeated three times with homozygous mice from different mothers with essentially similar results.

Expression of gp120 and Nef proteins inhibited by hCG and β hCG: reappearance after discontinuation of hormones. Within a few days after birth, homozygous transgenic mice develop dry, scaly skin (Fig. 3 E) characterized histologically by diffuse epidermal hyperplasia with prominent hyperkeratosis (Fig. 5, A and E). In contrast, the skin of β hCG-treated animals showed almost normal appearing skin (Fig. 3 F), although the hyperplasia and hyperkeratosis were still quite apparent (Fig. 5 B, C, F, and G). In the untreated mice, HIV gp120 and Nef were highly expressed in the keratinocyte layer (Fig. 5, A and E). Treatment for 30 d with hCG (Fig. 5, B and F), and in particular β hCG (Fig. 5, C and G), markedly inhibited the expression of both of these proteins. Discontinuation of the β hCG for 10 d (days 30 to 40) resulted in the reappearance of gp120 and Nef (D and H). Similarly, HIV mRNA (2, 4, and 7 kb) showed a marked increase in expression after discontinuation of hCG (not shown).

hCG-mediated down-regulation of HIV-1 gp120 in heterozygous transgenic mice. To examine the therapeutic effect of hCG on heterozygous mice, 25 d old mice were treated with 100 U of hCG, subcutaneously, three times per week. The ani-

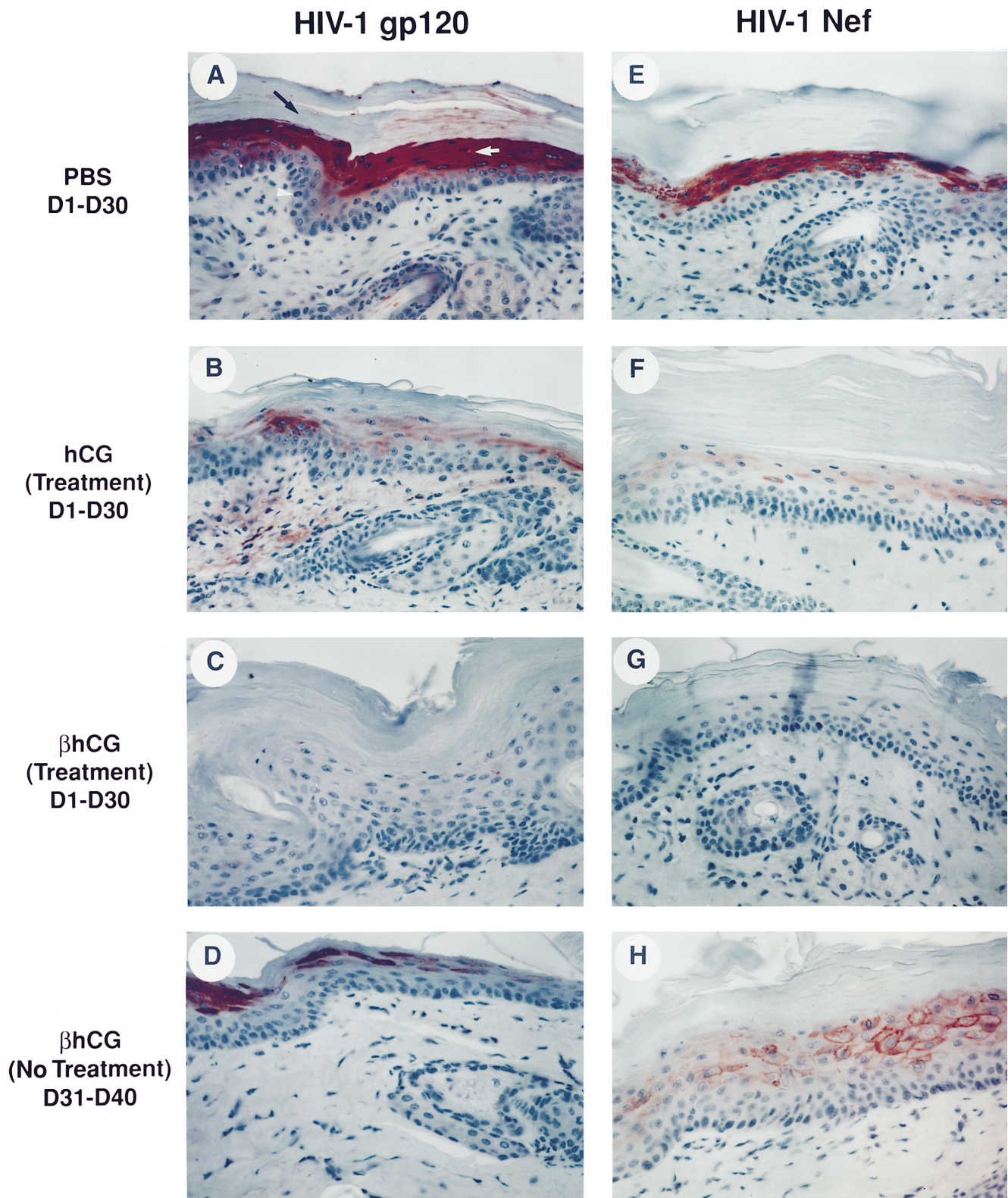


Figure 5. Inhibition of expression of gp120 and Nef proteins in the skin of homozygous transgenic mice. Pups received hCG or β hCG for 30 d (D1-D30) through the mother's milk and then by injection as described in Methods. Skin samples were fixed and immunoperoxidase stained with specific antibodies to HIV-1 gp120 (A-D) and HIV-1 Nef (E-H). Three homozygous animals were tested with each treatment and representative results are presented. Discontinuation of β hCG treatment for 10 d (D31-40) resulted in the reappearance of both HIV proteins in the skin (D and H).

HIV-1 gp120

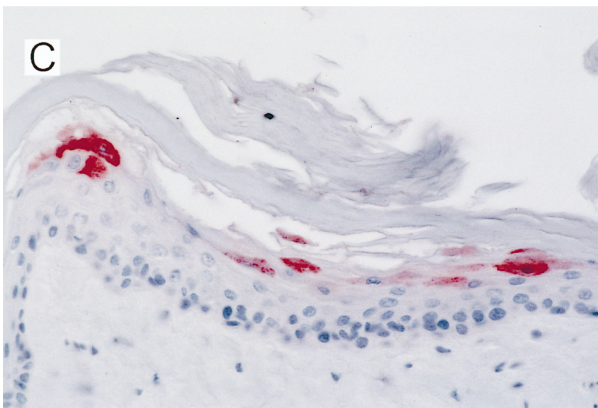
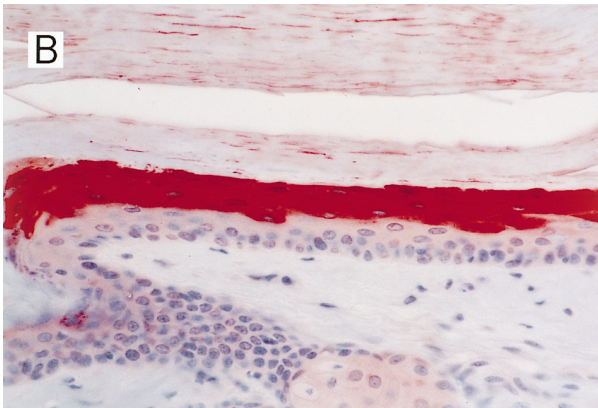
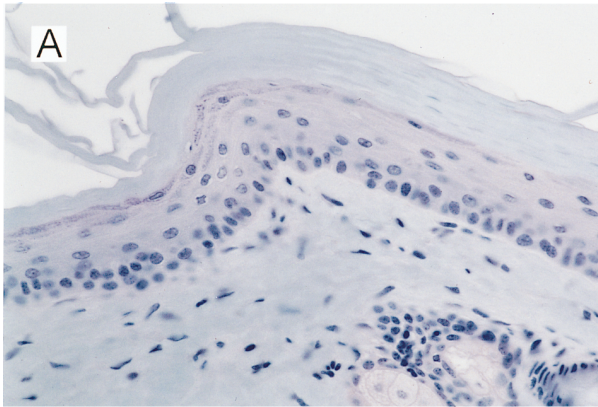


Figure 6. Inhibition of expression of gp120 in the skin of adult heterozygous animals treated for 15 d with hCG. Skin samples were fixed and immunoperoxidase stained with antibody to gp120. 10 heterozygous animals were tested for each treatment and representative results are presented. (A) nontransgenic control mice; (B) untreated heterozygous (PPS) mice expressing high levels of gp120 (intense red staining); and (C) heterozygous mice treated with hCG.

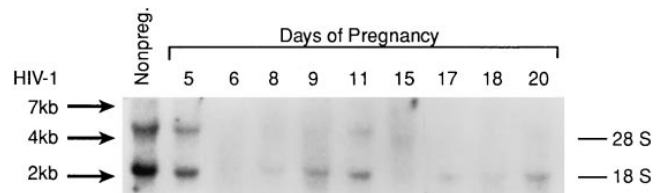


Figure 7. HIV-1 gene expression suppressed, during pregnancy, in the skin of heterozygous transgenic mothers as analyzed by Northern blot. The gestational age of the embryo was calculated from the day on which a vaginal plug was detected. Nonpregnant heterozygous female skin served as the control. Results are from two separate experiments.

mals were killed after the sixth injection on day 40. Skin and muscle were processed for HIV mRNA by Northern blot analysis and for HIV gp120 by immunohistochemistry. As seen in Fig. 6, nontransgenic control mice did not show any gp120. Heterozygous mice (PBS-treated) showed high expression of gp120 in the keratinocyte layer (Fig. 6 B). Treatment with hCG for 15 d resulted in a marked decrease of gp120 expression. Similarly, hCG treatment resulted in a substantial decrease in HIV-1 transcripts (data not shown).

HIV gene expression in pregnant versus nonpregnant heterozygous mice. To determine what, if any, effect pregnancy had on the expression of HIV mRNA in the tissues of heterozygous transgenic mothers, skin and muscles were analyzed by Northern blots at different stages during pregnancy. As seen in Fig. 7, the 2-, 4-, and 7-kb transcripts were almost completely suppressed within 6–8 d after fertilization and remained largely suppressed throughout pregnancy.

Discussion

The demonstration that homozygous HIV transgenic mice appear normal before and at birth, but deteriorate rapidly thereafter with a syndrome characterized by skin lesions, wasting, and death, suggests that a maternal hormone(s) might be responsible for protecting the fetus from developing the HIV wasting syndrome. It is known that in humans, the maternal hormonal profile changes dramatically from gestation to lactation. During early pregnancy, the corpus luteum, and later on the placenta, synthesize large quantities of steroid hormones including estrogen, progesterone, and hCG. Maternal estrogen and progesterone plasma levels gradually increased by more than 100-fold at term. Plasma levels of hCG also rise rapidly during normal pregnancy, doubling every 2–3 d (21–23), and reach a peak between 60 and 90 d of gestation (24). Among the other hormones, luteotropic hormone (LH), prolactin placental lactogen I, and placental lactogen II predominate during gestation (25–27). At birth and during lactation, the concentration of all these hormones declines to the basal level and hCG is virtually undetectable (28, 29).

The present study shows that hCG prevents the wasting syndrome and death in homozygous HIV-1 transgenic mice. hCG is a member of a family of glycoprotein hormones that includes LH, follitropin, and thyrotropin. These four hormones are heterodimers, with a common α subunit and unique β subunits (30). Recently, it was shown that hCG can suppress HIV-1 reverse transcriptase activity in chronically infected cells (i.e.,

lymphocytes and monocytes) and can block viral transmission resulting from cell-to-cell contact between virus-carrying lymphocytes and placental trophoblasts (31, 32). It also has been reported that the β subunit of hCG can inhibit p24 gag protein synthesis in virus-producing ACH-2 lymphocytes and U1 monocytes (33). In addition, Lunardi-Iskander et al., demonstrated that the β subunit of hCG can destroy Kaposi's sarcoma-derived cells for AIDS patients and can inhibit tumor growth by these cell lines in nude mice (34). Our studies in transgenic mice show that the β subunit, and not the α subunit, of hCG inhibits HIV gene transcription and that the β subunit is perhaps even more effective than the entire hCG molecule. The mechanism by which hCG inhibits HIV gene transcription, however, is not known. One possibility is that hCG and/or its breakdown products act on the regulatory elements of the HIV-LTR. Another possibility is that hCG and/or its breakdown products activate cellular mediators, such as cytokines, lymphokines, or other hormones, which in turn inhibit HIV gene transcription. Experiments to test these and other possibilities are now in progress.

In our initial experiments, hCG was administered by direct injection into newborn pups. Although some protection was observed, the injections and the handling of the pups resulted in high mortality from cannibalism making clear-cut interpretation of the data difficult. By administering hCG through maternal milk, cannibalism was markedly decreased. hCG was not detected in untreated animals. However, 2–5 d after implantation of the hormone-containing osmotic pump into the mother, hCG levels ranged from 150 to 300 mU/ml in the stomach and from 40–50 mU/ml in the circulation. hCG levels in the circulation then gradually decreased over the next several days presumably because of the increased inability of intact proteins to cross the gastrointestinal mucosa into the circulation of the maturing pup. The fact that not all the pups were protected (Fig. 2B) by hCG in maternal milk may be explained by insufficient hCG crossing into the circulation of the pups. In fact, Fig. 5 shows that discontinuation of β hCG leads to the reappearance of HIV-1 transcripts within 10 d followed by wasting and death. Taken together, these findings suggest that a more effective method of administering hCG may yield even better protection.

Treatment with other hormones (i.e., estrogen, progesterone, and dexamethasone) did not protect the homozygous mice from wasting and death. In fact, these hormones increased HIV mRNA levels and accelerated mortality. It is known that the expression of the HIV long terminal repeat (LTR) is enhanced by glucocorticoids in tissue culture cells and by pregnancy in placental and uterine tissue of transgenic mice carrying the HIV-LTR chloroamphenicol acetyltransferase (CAT) transgene (35). In fact, two potential glucocorticoid responsive elements have been identified in the HIV-1 LTR and *vif* open reading frames, and dexamethasone has been shown to increase the steady-state levels of HIV-1 specific mRNAs in latently infected ACH-2 cells (36).

Our studies show that hCG acts not only on homozygous, but also on heterozygous mice. Heterozygous mice do not develop the acute skin lesions or wasting characteristic of homozygous mice, but develop kidney and other lesions later in life (12–14). Heterozygous animals also express moderate to high levels of HIV mRNA and protein in the skin and muscle and as shown in Fig. 6, treatment with hCG profoundly suppresses this expression. We also found that expression of HIV

mRNA and protein in the skin and muscle of the heterozygous mother is profoundly suppressed during pregnancy, but is readily re-expressed post partum. Based on our knowledge of hCG levels in humans during pregnancy (21–24, 37, 38), our findings in the mouse suggest that the decreased expression of HIV mRNA and protein during pregnancy may be because of the presence of a CG-like molecule. We stress “CG-like” because CG mRNA has not been found in murine placenta during gestation (39–41). In fact, the CG β gene is found only in certain mammals such as horses, baboons, and humans (42–44). Thus, it appears that either a CG-like molecule or a still unknown pregnancy-related factor is responsible for the protection observed in newborn transgenic mice.

In conclusion, hCG directly or indirectly inhibits HIV gene expression when administered to transgenic mice. The inhibitory effect of hCG on HIV gene transcription may have clinical application at the human level and may be one of the factors responsible for the low level of transmission of HIV from mother to child (1–3).

Acknowledgments

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