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Inflammatory papillomatous hyperplasia and epidermal necrosis in a transgenic rat for HIV-1

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Abstract

Background—Skin lesions commonly affect AIDS patients. The pathogenesis of certain dermatologic disorders primarily associated to HIV-1 is unclear, and better forms of therapy for these conditions need to be discovered. Transgenic animal models represent a novel approach for the study of these disorders and for the quest of more effective forms of treatment.

Objective—Characterize this HIV-1 transgenic rat as a model to study skin diseases related to HIV/AIDS.

Methods—A transgenic rat was developed, using an HIV-1 construct with deleted gag and pol genes. Morphological and genotypical evaluations were followed by cytokine profile characterization of the lesions.

Results—We report the characterization of a colony of HIV-1 transgenic rats that developed skin lesions in a frequency of 22.5%. Cutaneous expression of functional HIV-1 transgenes correlated precisely with the severity of the phenotype. In early stages, rats manifested localized areas of xerosis and dispersed papulosquamous lesions. These hyperplastic manifestations were observed in conjunction with an increased epidermal expression of *tat* protein and a Th1/Th2 profile of cytokines. As the lesions progressed, they formed inflammatory plaques that subsequently ulcerated. Histologically, these lesions displayed a profound lymphocytic infiltrate, epidermal necrosis, and a marked increase of both Th1 and Th2 derived cytokines. Moreover, the presence of circulating IgG antibodies against HIV-1 *gp120* was detected.

Conclusion—This animal model as other HIV-1 transgenic mice described in the past, is not able to fully explain the myriad of skin findings that can occur in HIV-infected humans; however, it represents a potential animal model system for the study of immune-mediated inflammatory skin diseases.

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Keywords

HIV-1; Transgenic; Skin; Erythema multiforme; Cytokines

1. Introduction

In the first years of the AIDS epidemic and before the introduction of the highly active antiretroviral therapy (HAART), virtually all AIDS patients had skin compromise [1]. After HAART became widely available, the prevalence of opportunistic infections and Kaposi's sarcoma dramatically declined [2,3]. Nevertheless, the prevalence of most primary inflammatory complications related to HIV-1 remained steady [4]. Inflammatory skin disorders primarily related to HIV-1 include pruritus, xerosis, atopic-like dermatitis, psoriasis, and eosinophilic folliculitis [4]. These diseases resemble those seen in the seronegative counterpart, but presented in a more severe, extensive and recalcitrant fashion.

The presence of dermatologic disorders of co-infectious and neoplastic origin in AIDS patients is directly associated with the decline in the CD4⁺ lymphocyte count, a fact that augments the vulnerability of the host [5]. However, the pathogenesis of most skin disorders primarily related to HIV-1 still remains to be elucidated.

The use of transgenic (Tg) animal models for the study of the pathogenesis of HIV-1 associated complications represent a safe, reproducible, and cost effective approach; as they develop similar pathology as that seen in HIV-1/AIDS patients. Several HIV-1 Tg mice models have been reported to develop skin lesions [6]. Distinct skin phenotypes, including epidermal hyperproliferation [7], papilloma formation [8], psoriasiform and Kaposi's-like lesions [9] have been described to occur in these models. However, a major flaw encountered in most of these murine prototypes relies in that the viral replication is not regulated by the HIV-1 long terminal repeats (LTR). This defect relies on an unproductive interaction between the HIV-1 *tat* protein and the cyclin T of mice, leading to an absent expression of the transgenes in lymphocytes and monocytes, main target cells of HIV-1 [10,11].

In 2001, we reported the first HIV-1 Tg rat made from a provirus with deleted *gag-pol* genes and regulated by HIV-1 LTR (Fig. 1a and b) [10]. This non-infectious animal model certainly represents a bigger species than mice, with a greater genetic homology to the human being. Moreover, spliced and unspliced viral transcripts were expressed in lymph nodes, spleen, thymus, and peripheral blood cells suggesting a functional *tat* [10].

In this manuscript we describe the main dermatological features of the HIV-1 Tg rat, highlighting the expression of the transgenes, certain cytokines, as well as the presence of circulating autoantibodies against *gp-120* that correlate with the development of the skin phenotype.

2. Materials and methods

2.1. HIV-1 Tg rats

Animal care was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The whole description of the HIV-1 Tg rat was broadly depicted in a previous publication [10]. In summary, the animal model was generated using a modification of the HIV-1 provirus pNL4-3. A 3-kbp SphI-MscI fragment encompassing the 3' region of the *gag* and the 5' region of *pol* genes was removed from the proviral plasmid to generate the non-infectious clone pNL-3:d1443 (Fig. 1a and b) [10]. A 7.4-kbp

EaeI-NaeI fragment containing the provirus and host cell flanking region was microinjected into fertilized one-cell Sprague–Dawley × Fisher 344/NHsd F1 eggs. The detection of the HIV-transgene was performed by Southern blot analysis of the tail DNA as previously described [10]. Non-transgenic Fisher/Sprague–Dawley littermates were used as controls.

2.2. Experimental design

A total of 40 HIV-1 Tg rats and their non-transgenic siblings were followed longitudinally since birth up to 6 months of age. Both groups of study were isolated to avoid contact with any infectious pathogens. Animals were routinely examined twice a week for the recognition of new clinical features.

2.3. Definitions

A rat was defined as affected by the recognition of a distinct skin phenotype compared to their non-transgenic siblings. The rats were arbitrarily classified according to the extent of body surface area involved into five different categories: wild type, Tg non-lesional, mildly affected, moderately affected and severely affected rats (Fig. 2). In this particular case, we refer to non-lesional skin as the skin Tg rats with no lesions whatsoever and the healthy skin of Tg rats with lesions elsewhere. Papular skin lesions with minor inflammation that developed over the course of 14 days or less are defined as early lesions. Late lesions are defined as chronic papulosquamous plaques with erosion, typically covered by a hemorrhagic crust in an erythematous background. Non-tg rats are also referred as wild type (WT) rats.

2.4. Histological and immunohistochemical procedures

Multiple skin punch biopsies (5mm) from lesions and non-lesional areas of Tg and WT rats were taken between the 12th and the 24th weeks of life. Tissue samples were fixed in 10% neutral buffered formalin for 36 h and embedded in paraffin. Five micrometer sections were stained with hematoxylin and eosin (H&E), as well as used for immunohistochemistry. Immunohistochemistry was performed by using a horseradish-peroxidase (HRP) anti-mouse IgG detection kit (BD Biosciences, USA). Sections were dewaxed in three changes of xylene (5 min each) and rehydrated twice in 200-proof ethanol (5 min each), once in 190-proof ethanol, 140-proof ethanol and 100-proof ethanol (3 min each). Endogenous peroxidase was blocked with 3% H₂O₂ in PBS for HIV-1 proteins and with 0.3% H₂O₂ in PBS for CD4 and CD8. Endogenous biotin was blocked using the avidin/biotin blocking kit (Zymed, USA). The panel of antibodies used in this study included the mouse anti-HIV-1 *gp120* mAb (NEN, Boston, MA; NEA 9301) diluted 1:100, incubated overnight at 22 °C; the mouse anti-HIV-1 *tat* protein mAb (Advanced Biotechnologies, Columbia, MD) diluted 1:100 incubated overnight at 4 °C; the mouse anti-CD4 mAb (OX-38 Chemicon International Temecula, CA, USA) diluted 1:50, incubated overnight at 22 °C; and the mouse anti-CD8 alpha chain mAb (OX-8 Chemicon International Temecula, CA, USA) diluted 1:50 incubated for 1 h at 22 °C. The biotinylated anti-mouse Ab, streptavidin-HRP, diaminobenzidine (DAB) and chromogen, were utilized from the detection kit (BD Biosciences, USA). Methyl green was used for counterstaining following the standard technique.

2.5. RNA extraction and cDNA synthesis

Full thickness 5 mm punch biopsies from the dorsal area were snap-frozen in liquid nitrogen. A total of 10 samples (WT = 3, non-lesional Tg rats = 4, early lesions = 3 and late lesions = 3) were obtained. Frozen samples were immediately homogenized in 700 µl of RLT buffer (RNeasy kit, Qiagen, Valencia, CA) using a rotor-stator homogenizer. Total RNA was obtained by the mini-column procedure, following the manufacturer's guidelines

(RNeasy kit, Qiagen, Valencia, CA). First-strand cDNA was synthesized using 1 µg of total RNA and bringing it to a total volume of 20 µl with RNease-free water. Bulk first-strand reaction mix, DTT solution, and primer pd(N)₆, all from the first-strand cDNA synthesis kit (Amersham Biosciences, Buckinghamshire, UK) yielded a total of 33 µl of per reaction.

2.6. Quantitative RT-PCR

To determine quantitatively if the skin lesions of HIV-1 Tg rats expressed the same cytokine milieu that the one found in the human patient [12], SYBR-green real time RT-PCR was performed. All primers and probes for amplifying IL-4, IL-5, IL-6, IL-10, TNF- α , IFN- γ and 18S (housekeeping gene) were obtained from Super-array Bioscience Corporation (Frederick, MD). Primers to detect HIV-1 *env* (LA8 F and LA64 R) and *tat* (LA45 F and LA41 R) mRNA were synthesized as previously published [13,14]. Each PCR system (25 µl) consisted in 1 µl of cDNA, 25 µl of SYBR green master mix (Bio-Rad, Hercules, CA, USA), 1 µl of each primer, and the necessary ddH₂O to obtain the desired final volume. Identical thermal cycling conditions were used for all genes: 15 s at 95 °C and 1 min at 60 °C with a total of 40 cycles followed by a melting curve data collection set up to identify the non-specific products or primer-dimers. The reverse transcription was performed in an iCycler (Bio-Rad) by triplicate. Relative quantification of gene expression was performed using standard curves and Livak comparative Ct method, expressing the data in folds of increase over that of the WT group.

2.7. Serum anti-HIV-1 gp120 Ab ELISA

Serum samples from freshly drawn blood were analyzed for presence of antibodies against HIV-1 *gp120* in the four groups of rats previously described (WT, $n = 3$; Tg rats with no skin lesions, $n = 3$; mildly affected, $n = 2$; moderately affected, $n = 4$; severely affected, $n = 3$). Samples were measured on ELISA plates (Nunc MaxiSorp, eBioscience, USA) coated with 10 ng/ml of Ba-L gp120 (courtesy of Institute of Human Virology, Lot #137864A) overnight at 4 °C. After three washes with 0.5% Tween-20/PBS, plates were blocked with 200 µl of 5% Blotto/PBS for 60 min at 22 °C. Following washing, 100 µl samples and standards were applied to plates, incubated at 37 °C for 2 h and then washed four times with 5% Tween-20/PBS. The presence of antibodies against HIV-1 *gp120* was determined by incubation with gp120 specific sheep (for standard) and rat (for samples) peroxidase labeled anti-IgG (H + L) (KPL Cat#14-23-06 and 14-16-12, Lots # VL031 and XH085 100 µl, 1:1000 in 1% Blotto with 5% lamb serum at 37 °C for 60 min. Plates were next washed 3 $\times$ and then incubated with TMB substrate (BioFX, MD, USA) for 30 min. Reactions were stopped by addition of 50 µl of 2NH₂SO₄, and then read at 450–570 nm. Results are reported in ng/ml.

2.8. Statistical analysis

All experimental data were expressed as mean $\pm$ S.D. The significance of variation among different groups was determined by one-way ANOVA analysis. P -value ≤ 0.05 was considered to be significantly different.

3. Results

3.1. Genotypical and phenotypical analysis

The presence of the HIV-1 genome was confirmed by the identification of a dominant 7.4-kbp HIV-1 band in DNA extracted from tail tips, and analyzed by Southern blot as previously described [10].

Tg rats exhibit scaly and inflammatory papules as early as the first 3 weeks of life. The rate of skin involvement in a colony of 40 HIV-1 Tg rats was of 22.5% ($n = 9$). Gender

distribution in the affected group exhibited six males and three females. According to the severity of the phenotype, the transgenic group had the following distribution: three severely affected, four moderately affected and two mildly affected Tg rats (Fig. 2). None of the rats in the wild type group developed skin lesions throughout the entire time of study.

The topography of the lesions followed a constant pattern that involved the haired portion of the dorsal area, the tail, the feet, the anus, the scrotum, and the vagina (Fig. 1c). The type of skin lesions varied according to the severity of the phenotype. In mildly affected rats, the lesions presented as discrete areas of dry skin, predominantly on the tail. A few papules (early lesions) were localized in the dorsal area. These spontaneously resolved in terms of 2–3 weeks, leaving areas of hyperpigmentation. In moderately affected rats, erythematous papules increased in number usually forming plaques. Seldom, a small number of lesions persisted exhibiting lichenification and erosion. Severely affected rats manifested a larger number of papulosquamous pruritic plaques with erosion and ulceration, typically being covered by a hemorrhagic crust (late lesions). Numerous subcutaneous nodules were perceived adjacent to the lesions, suggesting regional lymphadenopathy. Evident areas of gangrene were also observed in the tail and feet of the severely affected animals (Fig. 1d). Concomitantly, HIV-1 Tg rats showed cataracts with different degrees of opacity that directly correlated with the extent of skin involvement (Fig. 1e). All severely affected rats showed marked wasting and renal compromise that resulted on premature sacrifice.

3.2. Histopathological analysis

Skin pathology showed a progressive spectrum of lesions (Fig. 3). Early lesions consisted on diffuse hyperkeratosis accompanied by scattered dermal infiltrates containing lymphocytes, eosinophils and mast cells. Late lesions showed marked papillomatous hyperplasia and parakeratosis, accompanied by hemorrhage, and by a lymphocytic infiltrate regularly localized in the epidermal-dermal junction, and adjacent to hair follicles. In severely affected rats, numerous sections showed vacuolization and necrosis of keratinocytes, accompanied by the formation of intraepidermal vesicles containing degraded cells and inflammatory mononuclear cells (Fig. 3). No bacterial, fungal or parasitic infection (both endo/ecto) was evidenced either by direct light microscopy, H&E, Periodic acid-Schiff, gram or KOH staining.

3.3. Immunohistochemical analysis of inflammatory infiltrate

Immunostaining for the epidermal and dermal inflammatory infiltrates consistently showed abundant expression of the CD8 marker in both, early and late lesions. Lymphocytes expressing the CD4 marker were found in very limited number in both types of lesions (Fig. 4).

3.4. HIV-1 transgene expression analysis

The transgene expression analysis was quantified by real time RT-PCR and demonstrated by immunohistochemistry. Significantly higher expressions of the two main functional HIV-1 genes, *env* and *tat*, were detected in late lesions when compared to those found in early lesions, $P = 0.007$ and $P = 0.006$, respectively (Fig. 5). Expression of both transgenes was undetectable in the skin of wild type (WT) rats, as well as in non-lesional skin of Tg rats. Immunoreactivity for HIV-1 *tat* protein was visualized throughout all layers of the epidermis, as well as in fibroblasts and endothelial cells of early and late lesions (Fig. 3). Interestingly, sections of normal skin from WT rats and healthy skin of Tg rats showed the exact same pattern of staining as that of the affected Tg rats (Fig. 3). Immunoreactivity for HIV-1 *gp120* was shown exclusively in the upper layers of the epidermis of Tg rats. These findings are compatible with those previously described for the HIV-1 Tg mice [15].

Sections from non-lesional Tg and WT rats did not show any immunoreactivity for HIV-1 *gp120* (Fig. 3).

3.5. Cytokine expression analysis

Quantitative analysis of cytokine expression was performed by real-time RT-PCR. Early appearing papular lesions were associated with significant gene expression of IL-4 ($P = 0.04$), IL-5 ($P = 0.05$), and the pro-inflammatory cytokine IFN- γ ($P = 0.02$) compared to that of non-lesional skin of Tg and WT rats (Fig. 6). In later appearing crusted hemorrhagic lesions, we observed a strikingly high expression of a mixed cytokine milieu, with further up-regulation of IL-5 ($P = 0.004$), IL-6 ($P = 0.009$), IL-10 ($P = 0.012$), and TNF- α ($P < 0.001$) when compared to that expressed in early appearing inflammatory papules (Fig. 6).

3.6. Expression of antibodies against HIV-1 *gp120*

Antibodies against HIV-1 *gp120* were quantified by serum ELISA in four subsets of rats according to the severity of the phenotype. Severely affected rats manifested remarkably high levels of circulating antibodies compared to those with mild and moderate skin involvement ($P < 0.01$) (Fig. 7). These data indicate a progressive immune response against a transgene product that correlated with the skin disease phenotype.

4. Discussion

Numerous Tg animal models have been described for the study of the pathogenesis of HIV-1 associated skin diseases [16]. In previous publications, we broadly depicted the benefits of the HIV-1 Tg rat compared to the HIV-1 Tg murine models [10], making it a more reliable model for the study of the pathogenesis of HIV-1/AIDS pathology. As in previous HIV-1 Tg models, the HIV-1 Tg rat also developed skin lesions. We classified these skin lesions into two different stages. The first stage consists in the expression of discrete epidermal hyperplastic changes accompanied with minor inflammatory infiltrates. The second stage represents an ulcerative and hemorrhagic phase, accompanied with augmented secretion of TNF- α , abundant expression of cytotoxic lymphocytes, and the presence of circulating antibodies against HIV-1 *gp120*. In this stage, there is a massive destruction of the upper layers of the epidermis, evidenced by vacuolization and necrosis of keratinocytes, and the formation of intraepidermal vesicles. These histological features are observed in a variety of dermatological conditions including; erythema multiforme (EM), toxic epidermal necrolysis (TEN) and graft versus host disease (GVHD), which are HIV-1 associated hypersensitivity reactions (EM, TEN) to medications.

In most human studies, the etiology of most pathology related to HIV-1 relies on the activity of two gene products: *gp120* and *tat*. HIV-1 *gp160* is the main gene product of the envelope (*env*) gene [17]. HIV-1 *gp160* is cleaved by a protease into two main glycoproteins, *gp120* and *gp41*. HIV-1 *gp120* mediates binding and entry of HIV-1 to cells expressing the CD4 marker, while *gp41* mediates membrane fusion [18]. HIV-1 *gp120* has cytotoxic activities that have been associated to the development of AIDS dementia complex [19], HIV nephropathy [20], HIV cardiomyopathy [21], as well as in the induction of apoptosis on CD4⁺ cells [22]. On the other hand, HIV-1 *tat* is the product of the HIV-1 transactivator (*tat*) gene. HIV-1 *tat* interacts with the long terminal repeats (LTR) leading to the production of new virions, and it also regulates the transcription of numerous other genes [23]. Furthermore, it has been associated to the angiogenic changes of Kaposi's Sarcoma [24], the up-regulation of growth factors and certain cytokines [25–28], and in the epithelial hyperproliferative changes found in HIV-nephropathy [29].

In concordance with previous Tg murine models, our Tg rat exhibits epidermal hyperplasia that might be triggered by the expression of *tat* protein. In 1992, *in vitro* studies suggested that *tat* protein might be responsible of the epidermal hyperplasia seen in dermatologic disorders of AIDS patients [30]. Since then, numerous Tg murine models including the HIV-1 *tat* transgene have consistently shown the expression of epidermal hyperproliferative lesions [9,15,31,32]. In our model, we demonstrate the cutaneous expression of *tat* mRNA and protein by real-time PCR and by immunohistochemistry, respectively. In addition to the expression of *tat* protein, early lesions also exhibited a mononuclear infiltrate predominantly composed by CD8⁺ cells, mast cells and eosinophils, as well as a significant up-regulation of IL-4, IL-5, and IFN- γ .

From previous studies in our Tg rat model, we knew that the affected animals exhibited a peripheral Th2/Th1 imbalance [33]. Th2 cytokines and specifically IL-4 have been associated with the development of most HIV-1 primary skin diseases in humans (pruritus, atopic-like dermatitis and eosinophilic folliculitis), in part because of the up-regulation of IL-4 receptors induced by the HIV-1 *tat* protein [28]. Nevertheless, the cause-effect relationship between *tat* and IL-4 being the triggers of the hyperplastic changes in our model have not been proven yet.

In the context of HIV-1 infection, CD8⁺ T cells play a key role in the pathogenesis of HIV-1 progression. Peripheral CD8⁺ T cells of HIV-1/AIDS patients are known to produce IL-4 and IFN- γ [34], and the same phenomenon may be occurring in our model. However, this fact has never been reported to occur in any of the HIV-1 related skin pathologies, and might be the target of our future studies.

The features displayed in the ulcerative/hemorrhagic plaques are far more complex to understand. The hallmark in late-stage lesions is the destruction of the epidermis, evidenced by vacuolization/necrosis of the keratinocytes, abundant CD8⁺ cells, and increased secretion of TNF- α . All of these changes are invariably localized in the upper layers of the epidermis, same localization of the immunostaining for HIV-1 gp120. These findings encouraged us to perform an ELISA to detect the presence of circulating antibodies against this retroviral glycoprotein. Indeed, significant amounts of these antibodies were quantified peripherally in the severely affected rats when compared to the less affected subsets of Tg rats ($P < 0.01$). However, more studies have to be performed in order to associate the presence of these antibodies with the pathogenesis of the epidermal destruction. The cause of the loss of tolerance to a major product of the introduced transgenes is not known and needs to be explored in future studies.

Another important finding in our study was the cross-reactivity of *tat* protein found in keratinocytes, fibroblasts and endothelial cells of non-lesional skin (Fig. 3). Molecular mimicry has been described in the past for HIV-1 *gp120* and cell components of the central nervous system [35]. Immunoreactivity for *tat* protein by histochemistry in skin of HIV-1 seronegative individuals has also been described in the past, limiting the utility of this technique [36,37]. However, the HIV-1 *tat* protein is known to promote vascular cell growth by acting synergistically with the basic fibroblast growth factor (β FGF) in the pathogenesis of KS [38], so it is possible that HIV-1 *tat* protein may show true resemblance to proteins endogenously produced by fibroblasts.

To conclude, we must say that this HIV-1 Tg rat is similar to most of the HIV-1 Tg mice models previously described. The pathology exhibited by our model does not correlate precisely with any of the inflammatory cutaneous diseases primarily correlated with HIV-1 infection. However, there are important HIV-1-associated skin diseases in which CD8⁺ T cells attach epidermal cells. For instance EM minor/major, and TEN in HIV-1/AIDS

patients, are examples where CD8⁺ T cells are major effectors of inflammatory skin reactions.

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Fig. 1. Construct and phenotype of the HIV-1 Tg rat. (a) Genome of the HIV-1 provirus pNL4-3 highlighting the 2 deleted genes: *gag* and *pol*, (b) the non-infectious plasmid was generated by removing the 3-kbp SphI-MscI fragment encompassing the 3' region of the *gag* and the 5' region of *pol* genes, (c) topography of the lesions shown in the phenotype of the HIV-1 Tg rat, (d) tail of a severely affected Tg rat showing areas of epidermal necrosis and (e) opaque cataract manifested by rats with severe phenotype.

Fig. 2. Classification of the skin phenotype according to the body surface area involved. (a) Wild type/non-lesional, (b) mild, (c) moderate and (d) severe. Dorsal fur was shaved for a better exposure of the lesions.

Fig. 3.

Histology and immunohistochemistry for HIV-1 *gp120* and *tat*. Histologic sections of skin biopsy specimens from WT rats were stained with H&E (a), for *gp120* (b), and for *tat* (c). Sections from non-lesional Tg rats were stained with H&E (d), for *gp120* (e), and for *tat* (f). Sections from early lesions were stained with H&E (g), for *gp120* (h), and for *tat* (i). Sections from late lesions were stained with H&E (j), for *gp120* (k), and for *tat* (l). Magnification of epidermal changes seen in late lesions stained in H&E (m), for *gp120* (n), and for *tat* (o). Magnification of dermis of Tg rats stained in H&E (m), for *gp120* (n), and for *tat* (o). Scale bar: 100 μ m (a–l) and 50 μ m (m–r). Immunohistochemistry sections were counterstained with methyl green.

Fig. 4. Immunohistochemistry for the inflammatory infiltrate. (a) Histologic sections of skin biopsy specimens show abundant CD8+ cells infiltrating the dermis and epidermis. Asterisk indicates a higher magnification on the morphology of the staining (inset). Scale bar: 100 μ m, inset (25 μ m). (b) Section shows minimal CD4+ cells interspersed in the dermis. Magnification of the dermis showing immunoreactivity (arrow) is shown in the inset. Scale bar: 100 μ m, inset (5 μ m). All sections counterstained with methyl green.

Fig. 5.

Real-time RT-PCR quantitative analysis of the cutaneous expression of the HIV-1 transgenes. Total RNAs were extracted from full-thickness WT rats ($n = 3$), non-lesional skin ($n = 4$), early lesions ($n = 3$), and late lesions ($n = 3$), and then were reverse transcribed. We used SYBR green mix with transgene-specific primers (*env* and *tat*) with identical thermal cycling conditions for all genes. Gene expression was normalized to housekeeping gene 18S. Comparative quantification was performed by Livak Ct method, and results were expressed in fold increase compared early lesions and late lesions. Error bars represent mean values $\pm$ S.D. of three independent experiments. (*) Significant increase compared to WT rats; (**) null expression of both transgenes in WT and non-lesional skin was observed.

Fig. 6.

Real-time RT-PCR quantitative analysis of the expression of the cytokine milieu in the skin. Total RNAs were extracted from full-thickness WT ($n = 3$), non-lesional skin ($n = 4$), early lesions ($n = 3$), and late lesions ($n = 3$), and then were reverse transcribed. We used SYBR green mix with cytokine gene-specific primers (IL-4, IL-5, IL-6, IL-10, IFN- γ and TNF- α) using identical thermal cycling conditions for all genes. Gene expression was normalized to housekeeping gene 18S. Comparative quantification was performed by Livak Ct method, and results were expressed in fold increase compared to WT rats and early lesions. Error bars represent mean values $\pm$ S.D. of three independent experiments. (*) Significant increase compared to WT; (λ) significant increase compared to early lesions; (&) significant increased compared to WT and early lesions.

Fig. 7.

Quantification of circulating antibodies against HIV-1 gp120. Serum samples from fresh drawn blood of the five subsets (wild type rats, $n = 3$; Tg rats without skin phenotype, $n = 3$; mild, $n = 2$; moderate, $n = 4$; severe, $n = 3$) were analyzed by ELISA to quantify presence of antibodies against HIV-1 gp120. Error bars represent mean values $\pm$ S.D. of three independent experiments. (*) Significant increase compared to the other three subsets ($P < 0.01$).