Mucosal Candidiasis in Transgenic Mice Expressing Human Immunodeficiency Virus Type 1

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The availability of CD4C/HIV MutA transgenic (Tg) mice expressing human immunodeficiency virus type 1 in immune cells and developing an AIDS-like disease has provided the opportunity to devise a model of mucosal candidiasis that closely mimics the clinical and pathologic features of candidal infection in human AIDS. After intraoral infection with Candida albicans, oral burdens were strikingly elevated in the Tg mice, compared with non-Tg littermates (P < .05), during primary infection, a 6–10-week carrier state, and a marked terminal outgrowth preceding death. The chronic carrier state was absent in the non-Tg mice because of clearing of C. albicans. Candida hyphae penetrated the epithelium of the oral cavity, esophagus, and cardial-atrium fold of the stomach, accompanied by a mononuclear cell infiltrate. Immunohistochemical analysis suggested that decreased frequencies of major histocompatibility complex class II–expressing cells, combined with reduced CD4+ cells, may underlie the susceptibility to mucosal candidiasis in these Tg mice.

Oropharyngeal candidiasis (OPC) is a frequent opportunistic fungal infection among human immunodeficiency virus (HIV)–infected patients [1], although a declining incidence has been noted since the inception of highly active antiretroviral therapy [2]. Clinically, OPC in HIV infection has been classified as exhibiting pseudomembranous and erythematous variants [3]. OPC is frequently complicated by esophageal candidiasis, which may lead to markedly reduced food intake and weight loss in HIV-infected patients [4]. Furthermore, clinical and in vitro resistance to antifungal azoles frequently occurs in OPC when CD4+ cell counts fall to <200 cells/mm3 of blood, either by selection or acquisition of resistant strains of Candida albicans or by infection with inherently resistant species of Candida other than C. albicans [5–8].

The predisposition for OPC among HIV-infected patients, attributed to T cell impairment, is enigmatic [9–14]. Candida colonization of the keratinocyte surface takes place without invasion of the submucosa, and, therefore, the occurrence of this superficial fungal disease in a T cell–poor environment has not been adequately explained. The onset of lesions depends on imbalances between Candida virulence attributes and impaired host oral immune defenses in the sequential development of HIV infection; however, the exact pathways leading to this imbalance are still unclear. Limited studies of HIV-infected patients with OPC have identified an impairment of terminal differentiation of Langerhans’ cells and reduced expression of major histocompatibility complex (MHC) class II alloantigens and the adhesion molecules lymphocyte function–associated antigen (LFA)–1 and intercellular adhesion molecule (ICAM)–1, suggesting that abnormal processing and presentation of antigen to CD4+ cells may at least partially explain the mucosal outgrowth of C. albicans in HIV infection [12]. OPC in HIV-infected patients also has been associated with low salivary levels [15] of the candidacidal protein calprotectin [16], produced by neutrophils, monocytes, macrophages, and keratinocytes [10, 16].

Controlled studies on the pathogenesis of mucosal candidiasis in HIV infection have been hampered by the lack of a relevant animal model. Here, we describe a novel experimental model of mucosal candidiasis in transgenic (Tg) mice expressing HIV-1 and developing an AIDS-like disease.

Materials and Methods

Generation of Tg mice expressing HIV-1. The CD4C/HIV MutA Tg mice have been described elsewhere [17]. CD4C/HIV MutA mutant DNA harbors mouse CD4 enhancer and human CD4 promoter elements to drive the expression of HIV-1 genes. Founder mouse F21388 was bred on the C3H background, and progeny mice were genotyped and routinely examined for signs of disease. Animals from this line express moderate levels of the transgene, with 50% survival at 3 months [17]. Several HIV-1 genes (gag, pol, vif, vpr, tat, and vpu) are mutated in the CD4C/HIV MutA DNA, whereas env, rev, and nef are intact. nef was found to be required and suffi-
cient to elicit an AIDS-like disease in these Tg mice. This disease is characterized by failure to thrive, wasting, severe atrophy and fibrosis of lymphoid organs, loss of CD4$^\text{+}$ cells, interstitial pneumonitis, and tubulointerstitial nephritis [17]. These changes are not observed in control non-Tg littermates [17]. Specific pathogen–free male and female Tg mice and non-Tg littermates were housed in a protective environment at the University of Montreal Animal Care Unit, in sterilized individual cages equipped with air filter hoods. The animals were supplied with sterile water and were fed with sterile mouse chow. In addition to the non-Tg littermates, male and female 5–6-week-old C3H mice (17–18 g), obtained from Charles River Canada, were used as immunocompetent controls.

Animal model of mucosal candidiasis. Oral inoculation with C. albicans LAM-1 was done as described elsewhere [18]. In brief, C. albicans was grown to late-log phase in Sabouraud dextrose broth (Difco Laboratories) for 18 h at 30°C with rotary agitation. Yeast cells were washed twice in sterile 0.01 M PBS (pH 7.4) and counted in a hemacytometer. Mice were anesthetized with 0.1 mL of a solution containing 1 mL of Hypnorm (0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone; Janssen Pharmaceutica), 1 mL of Versed (5 mg/mL midazolam; Hoffmann–La Roche), and 2 mL of water, by intraperitoneal injection. They were then inoculated by topical application of 10$^5$ pelleted blastocconidia recovered on sterile calcium alginate Calgiswabs (Fisher Scientific).

Mice were observed daily for signs of morbidity, and their weights were recorded at the beginning and at the end of the experiment. Moribund animals were scored as nonsurvivors and euthanized by CO$_2$ inhalation or were anesthetized with Hypnorm and Versed, exsanguinated with PBS, and then perfusion-fixed with periodate-polylysineraformaldehyde fixative [19].

A longitudinal quantification of C. albicans in the oral cavities of individual mice was done from day 1 until euthanizing of the animals. Calgiswabs used for sampling were dissolved in 2-mL volumes of Ringer’s citrate buffer [20], and serial dilutions were prepared in PBS (0.05 M pH 7.4) and plated on Sabouraud dextrose agar supplemented with chloramphenicol (0.05 g/L). Plates were incubated for 24 h at 37°C.

To determine burdens of C. albicans in the gastrointestinal tract and the potential for systemic dissemination, brain, lungs, liver, kidney, stomach, small intestine, large intestine, and esophagus were counted in a hemacytometer. Mice were anesthetized with 0.1 mL of a solution containing 1 mL of Hypnorm (0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone; Janssen Pharmaceutica), 1 mL of Versed (5 mg/mL midazolam; Hoffmann–La Roche), and 2 mL of water, by intraperitoneal injection. They were then inoculated by topical application of 10$^5$ pelleted blastocconidia recovered on sterile calcium alginate Calgiswabs (Fisher Scientific).

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To determine burdens of C. albicans in the gastrointestinal tract and the potential for systemic dissemination, brain, lungs, liver, kidney, stomach, small intestine, large intestine, and esophagus were removed aseptically at the time of death, weighed, and homogenized in 5 mL of PBS. Homogenates were serially diluted, plated on Sabouraud dextrose agar supplemented with chloramphenicol (0.05 g/L), and incubated for 24 h at 37°C.

To evaluate the extent of mucosal candidiasis and to assess possible systemic spread of C. albicans, the following tissues were removed from perfusion-fixed animals and prepared for histologic analysis: anterior sagittal section of the oral cavity (containing hard palate, gingivae, teeth, tongue, and cheeks), soft palate, pharynx, esophagus, stomach (including the cardial-atrium fold), small intestine, large intestine, kidney, and liver. The tissues were embedded in paraffin, sectioned, and stained with hematoxylin, phosphine, and safranin or by the Gomori-Grocott methenamine silver procedure [21].

Flow cytometry. Heparinized blood was collected from both Tg mice and non-Tg littermates, 74–83 days old, by retro-orbital puncture under anesthesia with Hypnorm and Versed. Blood (50 μL) was incubated with 1 μg of fluorescein isothiocyanate–conjugated anti-CD3 or anti-CD4 or phycoerythrin–conjugated anti-CD8 or anti-CD45 (leukocyte common antigen) antibodies (PharMingen Canada) for 30 min at 4°C. After monoclonal antibody staining, red blood cells were lysed by addition of 2 mL of PHARMLYSE solution (PharMingen). After 3 washes in 0.01 M PBS (pH 7.2), cells were fixed with 2% paraformaldehyde in PBS and stored at 4°C until analysis on a FACStar flow cytometer (Becton Dickinson Immunocytometry Systems) equipped with Consort 30 software. A minimum of 10,000 cells from each sample was counted to establish the percentage of positive cells.

In situ hybridization. In situ hybridization was done on paraffin-embedded tissues, with the use of $^{35}$S-UTP–labeled antisense and control sense RNA probes, as described elsewhere [19, 22]. A mixture of 2 probes was used: the 1.4-kbp HindIII-Sac I fragment (nt 8131–9566) of the pNL4-3 clone (GenBank accession no. M19921) and the 627-bp HindIII fragment (nt 407–1034) of the HIV-1 BH10R3 clone (GenBank accession no. M15654). Tissues from non-Tg control animals hybridized with antisense probes, as well as Tg animal tissues hybridized with sense probes, failed to exhibit any specific hybridization signal.

Immunohistochemistry. Tg mice and control non-Tg littermates, infected or uninfected with C. albicans, were euthanized by CO$_2$ inhalation. From each animal, the spleen, tongue, esophagus, and stomach, including the cardial-atrium fold, were dissected and placed in Peel-Away base molds (PharMingen) partially filled with PBS, and then perfusion-fixed with periodate-polylysinaformaldehyde fixative [19].

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To evaluate the extent of mucosal candidiasis and to assess possible systemic spread of C. albicans, the following tissues were removed from perfusion-fixed animals and prepared for histologic analysis: anterior sagittal section of the oral cavity (containing hard palate, gingivae, teeth, tongue, and cheeks), soft palate, pharynx, esophagus, stomach (including the cardial-atrium fold), small intestine, large intestine, kidney, and liver. The tissues were embedded in paraffin, sectioned, and stained with hematoxylin, phosphine, and safranin or by the Gomori-Grocott methenamine silver procedure [21].
Spleens from non-Tg mice and thymus tissues from normal 4-week-old CD-1 mice (Charles River) were used as positive controls, and negative controls were obtained by omitting primary antibodies from these tissues, as well as from oral mucosa. All slides were read independently by 2 observers. The densities of positively stained cells were determined for $\geq 20$ microscopic fields (magnification, $\times 400$) and expressed as the number of positive cells among 100 epithelial cells (for intraepithelial cells) or 100 subepithelial cells to compare the densities of these cell types [23].

Statistical analysis. Differences in oral burdens of $C. \text{albicans}$ in Tg and control non-Tg mice were determined by using SPSS software (SPSS). Repeated-measurements analysis of variance was conducted with 2 factors, 1 between (group) and 1 within (time). Significant interactions ($P < .05$) were further analyzed, and significant differences ($P < .05$) between the 2 group means at fixed times were determined by use of the 2-sample, 2-tailed Student's $t$ test for independent samples. The Mann-Whitney $U$ test [24] was used to analyze peripheral blood lymphocyte populations and body and kidney mass data. Differences were considered to be significant at $P < .05$.

Results

Mucosal candidiasis in Tg mice expressing HIV-1. CD4C/HIV$^{\text{MutA}}$ Tg mice and non-Tg littermates were inoculated orally with $C. \text{albicans}$ LAM-1, and longitudinal assessments of oral burdens were done by sampling the oral cavities. In 3 separate experiments (figure 1A, 1B, and 1C), oral burdens of $C. \text{albicans}$ were strikingly elevated in the Tg mice, compared with control non-Tg animals. Beginning 4 days after inoculation, significant ($P < .05$) and sustained enhancement of infection in the Tg mice was maintained during primary infection, a 6–10-week carrier state, and a marked terminal outgrowth during the later stage of infection, which correlated with visible thrush on the dorsal aspect of the tongue. In contrast, primary infection in the non-Tg mice was self-limited, with significantly lower ($P < .05$) burdens of $C. \text{albicans}$, compared with those seen in Tg animals, and uniform clearance of $C. \text{albicans}$ from the oral cavities within 7 days after inoculation. Oral burdens during primary infection in

Figure 1. Oral burdens of $Candida \text{albicans}$ in CD4C/HIV$^{\text{MutA}}$ transgenic (Tg) mice and non-Tg controls. Mice were inoculated intraorally with $10^8$ cfu of $C. \text{albicans}$ LAM-1, and burdens were assessed longitudinally by sampling the oral cavity. In 3 separate experiments (A–C), oral burdens were strikingly elevated in Tg mice (colored lines), compared with control non-Tg mice (black lines), during primary infection, 6–10-week carrier state, and marked terminal outgrowth preceding death. In contrast, $C. \text{albicans}$ was rapidly cleared from oral cavities of non-Tg mice immediately after primary infection. Oral burdens during primary infection in control non-Tg (A–C) and intact C3H mice (D; mean $\pm$ SD) were not significantly different ($P > .05$). There were 3 Tg and 2 non-Tg mice in panel A, 3 Tg and 4 non-Tg mice in panel B, and 6 Tg and 6 non-Tg mice in panel C. There were 42 C3H mice in panel D.
control non-Tg (figure 1A, 1B, and 1C) and intact C3H mice (figure 1D) were not significantly different (P > .05), demonstrating unaltered resistance of these mice to C. albicans.

High burdens of C. albicans were found consistently in stomach and small and large intestine of the Tg mice and were uniformly absent in the non-Tg animals (table 1). Lower burdens of C. albicans were found in the esophagus of the Tg mice. Dissemination to brain, lungs, liver, and kidneys occurred in 4 of 9 Tg mice (table 1). However, evidence of dissemination was limited to a single organ (liver or kidneys) in 2 of these 4 mice or to 2 organs (brain and lungs or liver) in the other 2 animals. Burdens of C. albicans remained low, especially in brain and kidneys.

Histopathology investigation of 3 Tg mice during terminal outgrowth at a mean age of 135 days consistently demonstrated penetration by Candida hyphae of the stratified squamous epithelium of the oral cavity (tongue, palate, gingivae, and cheeks; figure 2), esophagus (figure 3), and cardiac-atrium fold of the stomach (figure 4), accompanied by an abundant mononuclear cell infiltrate. In contrast to the oral cavity and esophagus, lesions in the region of the cardiac-atrium fold of the stomach were markedly more extensive and not confined to the superficial keratin layer (figure 4). Extensive erosion of the mucosal surface was seen at the junction of the stratified squamous and glandular epithelium, and numerous Candida hyphae penetrated beyond the epithelium to deep within the lamina propria (figure 4F). A massive mononuclear cell infiltrate extended from the superficial keratin layer to the submucosa (figure 4A). No Candida hyphae or inflammatory cell infiltrate were found in the epithelium of the small and large intestine, in kidneys or liver of the Tg mice (data not shown), or in the oral cavities (figure 2F), esophagus (figure 3C), or stomach (figure 4B) of age-matched control non-Tg mice inoculated with C. albicans.

Gated lymphocytes in the peripheral blood of Tg mice were severely depleted relative to non-Tg age-matched controls (3691 ± 627 and 5685 ± 481/10,000 events, respectively [mean ± SD]; P = .004), as were CD3⁺, CD4⁺, and CD8⁺ T cells (table 2). The CD4:CD8 ratio was also significantly decreased in the Tg mice (table 2), as observed elsewhere [17].

Moderate wasting and marked renal atrophy were noted in the Tg mice (table 3), which is consistent with previous observations [17]. The Tg mice sustained an average loss of 6% of body mass over a mean duration of 100 days, in striking contrast to the non-Tg littermates, which gained an average of 69% of body mass over the same period (table 3).

Expression of the transgene evaluated by in situ hybridization. A mononuclear cell infiltrate in the epithelium and lamina propria of the tongue was negative for transgene expression (figure 5A and 5C). However, transgene expression was specifically detected in cells exhibiting the morphologic features of macrophages underlying the mononuclear cell infiltrate (figure 5C). A similar pattern of transgene expression was also observed in the cardiac-atrium fold of the stomach (figure 5E, 5F, and 5G).

As in the tongue, the transgene was expressed in cells with the features of macrophages in the lamina propria (figure 5E), underlying a partly degenerated mononuclear cell infiltrate that did not express the transgene. In contrast to the tongue, however, infiltrates of mononuclear cells located more deeply in the submucosa did express the transgene (figure 5G). Together, these results are consistent with the previously demonstrated expression of the transgene in cells of the macrophage lineage and in infiltrating mononuclear cells in the lamina propria of the intestine [17, 19].

Immunohistochemistry. The oral, esophageal, and gastric mucosae were analyzed by immunohistochemistry to assess alterations in the incidence and distribution of T cells and levels of activated T cells.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tg</th>
<th>Control non-Tg</th>
</tr>
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<tbody>
<tr>
<td>No. inoculated</td>
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<td>10</td>
</tr>
<tr>
<td>Age at assessment, mean days (range)</td>
<td>148 (129–171)</td>
<td>154 (133–192)</td>
</tr>
<tr>
<td>Organs culture positive for C. albicans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>C. albicans count, mean cfu/g</td>
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<td>NA</td>
</tr>
<tr>
<td>Range</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lungs</td>
<td></td>
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</tr>
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<tr>
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</tr>
<tr>
<td>Range</td>
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<tr>
<td>Liver</td>
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<tr>
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<tr>
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<tr>
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<td>Kidneys</td>
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<td>Range</td>
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</tr>
<tr>
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NOTE: NA, not applicable.

* Mice studied included Tg and control non-Tg offspring derived from founder mouse F21388.

* Assessment on day of death due to disease or euthanization because of severe illness. Control non-Tg littermates were euthanized on the same day as Tg mice.
MHC class II expression, which has been associated with mucosal candidiasis in HIV-infected patients [12]. In the lingual mucosa of mice uninfected with \textit{C. albicans}, intraepithelial I-A$^{K^+}$ cells were consistently absent in the Tg mice but occurred at a low frequency in the basal layer of the epithelium of non-Tg littermates (table 4). In addition, the frequency of I-A$^{K^+}$ cells in the lamina propria was lower in the Tg mice than in non-Tg animals (2.2–6.5 and 5.5–14 per 100 subepithelial cells, respectively). These results suggest that decreased densities of MHC class II-expressing cells and/or defective expression of MHC class II alloantigens may be at least partially responsible for the enhanced mucosal proliferation of \textit{C. albicans} in the Tg mice. MHC class II alloantigens are a marker for Langerhans’ cells in the oral mucosa [25, 26], and the location of I-A$^{K^+}$ cells in the basal layer of the epithelium and in the superficial portion of the lamina propria concurred with previous observations [25, 27].

In addition to reduced frequencies of cells expressing MHC class II molecules, CD4$^{+}$ T cells were consistently absent in the epithelium and lamina propria of the lingual mucosa of the uninfected Tg mice (table 4), which correlated with their depletion in peripheral blood (table 2), spleen, and lymph nodes of the Tg animals [17]. Finally, CD8$^{+}$ T cells were uniformly undetectable in the epithelium and lamina propria of the uninfected Tg mice and the non-Tg littermates (table 4).

Frequencies of CD4$^{+}$ cells and I-A$^{K^+}$ cells were dramatically increased in the mucosa of control non-Tg mice infected with \textit{C. albicans}, compared with uninfected animals (table 4). In the Tg mice, infection with \textit{C. albicans} also resulted in a substantial

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**Figure 2.** Histopathology of oral candidiasis in CD4C/HIV$^{\text{AIDS}}$ transgenic (Tg) mice. \textit{A–C}, \textit{Candida} hyphae penetrate superficial keratin layer of stratified squamous epithelium of tongue (To), palate (P), and gingiva (G) surrounding tooth (T). \textit{D} and \textit{E}, Mononuclear cell infiltrate in lamina propria (arrowhead) and superficial keratin layer (arrow) of dorsal surface of tongue (D), with \textit{Candida} hyphae confined to keratinized layer (E). \textit{F}, Stratified squamous epithelium of palate of control non-Tg mouse infected orally with \textit{Candida albicans}, showing no mononuclear cell infiltrate. No hyphae were observed by Gomori-Grocott staining. Stains: \textit{A–C} and \textit{E}, Gomori-Grocott methenamine silver; \textit{D} and \textit{F}, hematoxylin, phloxine, and safranin. Magnifications: \textit{A}, ×100; \textit{B}, ×250; \textit{C}, ×500; \textit{D–F}, ×1000.
increase in frequencies of mucosal I-AK$^+$ cells and a more modest enhancement in CD4$^+$ cells, demonstrating the ability of the Tg mice to partially respond to mucosal infection despite the reduction in these cell populations (table 4). Interestingly, 1 of 4 infected Tg mice had much faster disease progression [17], a complete absence of CD4$^+$ cells in spleen, tongue, esophagus, and stomach, and scarce I-AK$^+$ cells only in spleen, tongue, and esophagus. The ability to recruit and attract CD4$^+$ and I-AK$^+$ cells to the mucosa of Tg mice infected with C. albicans may thus occur only temporally before the severe depletion of thymocytes and peripheral lymphocytes and the loss of architecture of the lymphoid organs and fibrosis observed in the CD4C/HIVMutA Tg mice [17]. CD8$^+$ cells were rarely identified in the epithelium and lamina propria of infected Tg and non-Tg mice (table 4).

**Discussion**

In this study, we have developed and characterized a novel model of mucosal candidiasis in Tg mice expressing HIV-1, which is strikingly similar to the clinical and pathologic features of candidiasis in HIV-infected patients. The CD4C/HIVMutA Tg mice exhibit AIDS-like disease, characterized by failure to thrive, wasting, severe atrophy and fibrosis of lymphoid organs, loss of CD4$^+$ T cells, interstitial pneumonitis, and tubulointerstitial nephritis [17]. The following features of mucosal candidiasis in the Tg mice were identical to those in patients with HIV infection: a sustained enhancement of oral burdens of C. albicans, becoming more manifest in the later stage of HIV disease [28–30]; penetration by Candida hyphae of the stratified squamous epithelium of the oral cavity and esophagus limited to the superficial epithelial layer [10–12]; a low incidence of systemic dissemination of C. albicans [31–33]; and a mononuclear inflammatory cell infiltrate of the mucosa [10, 12]. In addition to closely mimicking the features of mucosal candidiasis in patients infected with HIV, the model obviates problematic procurement of tissue samples from human patients, avoids the potentially confounding effect of mixed infections by different strains of C. albicans or different Candida species, often found in HIV-infected patients [6], and allows for longitudinal observations at fixed times during progression of HIV infection, by direct comparison with non-Tg littermates. The Tg mice thus provide a novel opportunity to study the pathogenesis of mucosal candidiasis in HIV infection under controlled conditions in a small laboratory animal.

A large body of work conducted in congenitally immunodeficient mice [13, 34–40] has demonstrated that functional T cells play a role in resistance to C. albicans colonizing or infecting mucosal surfaces and that an added defect of phagocytes is required to produce dissemination of C. albicans from the gastrointestinal tract [36, 38]. Further investigation showed that, although Th1 and Th2 CD4$^+$ cells are involved in recovery from primary gastrointestinal candidiasis in immunocompetent mice, activation of a Th1 response occurs in animals that show delayed-type hypersensitivity to Candida and protection after a second gastrointestinal inoculation [41]. Studies of B cell–knock out mice demonstrated that antibodies do not play a role in protection against mucosal candidiasis nor dissemination
from the gastrointestinal tract [42]. However, a protective role of antimannan antibodies has been demonstrated in experimental vaginal candidiasis [43, 44]. Overall, these investigations have produced the current paradigm of a central role for a Th1 CD4+ response in host defense against mucosal candidiasis [41, 45, 46]. However, approaches that use congenitally immunodeficient mice [13, 34–40] or the depletion of specific factors (CD4+ cells or interferon-γ) in intact [47] or knockout [48] mice have important inherent limitations. First, the role of only single or a few factors potentially involved in mucosal protection against Candida can be addressed in these animal models, and their involvement may be masked or underestimated by intact complementary defense mechanisms. Second, although these animal models can provide significant insights into the mechanisms that protect the healthy host against Candida at mucosal sites, they do not allow for the identification of specific defects that pre-

**Figure 4.** Histopathology of gastric candidiasis in region of cardial-atrium fold (F) in CD4CHIVMucA transgenic (Tg) mice. A. Extensive erosion (E) of mucosal surface at junction of stratified squamous (S) and glandular (G) epithelium, accompanied by massive inflammatory cell infiltrate extending from superficial keratin layer (arrow) to submucosa (arrowhead). B. Region of cardial-atrium fold in control non-Tg mouse infected orally with *Candida albicans*, showing absence of erosion and cell infiltrate. No hyphae were observed by Gomori-Grocott staining. C and D. Abundant partly degenerated cell infiltrate (C) and numerous *Candida* hyphae (D) seen in superficial layer (arrow in panel A). E. Cell infiltrate composed of mononuclear cells and occasional polymorphonuclear leukocytes present in submucosa (arrowhead in panel A). F. Penetration by abundant *Candida* hyphae extends from superficial keratin layer to deep within lamina propria. Stains: A–C, E, Hematoxylin, phloxine, and safranin; D and F, Gomori-Grocott methenamine silver. Magnifications: A and B, ×250; C–F, ×1000.
Langerhans’ cells are the purported initial in HIV infection, including macrophages and dendritic gens appears to be a general feature of antigen-presenting cells. However, a low expression of MHC class II alloantigens and adhesion molecules LFA-1, and ICAM-1, and their products to directly induce maturation of dendritic cells, also has been found in antigen-presenting dendritic cells from mucosal Langerhans’ cells [69], resulting either directly from infection on antigen-presenting cells by coculture with C. albicans or from cytokines produced by the T cell response to infection [69]. Maturation of dendritic cells, including mucosal Langerhans’ cells, is characterized by a strong up-regulation of MHC class II expression, secretion of interleukin (IL)–12, and the expression of the adhesion molecules LFA-3, ICAM-1, and B7-2 [69]. Enhancement of MHC class II expression on antigen-presenting cells by coculture with C. albicans or exposure to C. albicans antigen has been demonstrated in vitro [70], in accordance with the ability of microbial pathogens and their products to directly induce maturation of dendritic cells [69, 71]. Cytokines such as IL-1, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor–α, as well as the T cell ligand CD40L, which binds CD40 on dendritic cells, may also have contributed to maturation of Langerhans’ cells in the C. albicans–infected mice [69]. However, the latter disposition to mucosal candidiasis in HIV infection. Consequently, an animal model of the natural disease (HIV infection) that most frequently predisposes to OPC in humans is a powerful new tool to further our understanding of the pathogenesis of mucosal candidiasis.

Oral candidiasis has been shown to be a significant marker, independent of the CD4+ cell count, in the progression of HIV disease [49]. However, the precise impairments that favor Candida outgrowth on mucosal surfaces in the sequential development of HIV infection have not been defined. In the present study, the incidence of CD4+ T cells and I-AK+ cells was sharply reduced in the oral mucosa of uninfected Tg mice, compared with non-Tg littermates. These results suggest that defective expression of MHC class II alloantigens by altered Langerhans’ cells and/or decreased densities of Langerhans’ cells may interfere with normal processing and presentation of Candida antigens to CD4+ cells, which are themselves depleted in HIV infection. Interestingly, decreased expression of MHC class II molecules also has been found in antigen-presenting dendritic cells from spleen, lymph nodes, thymus, and bone marrow of these Tg mice, as well as loss of dendritic cells in lymph nodes and thymus of these animals [50]. These alterations in the Tg mice concur with the results of limited studies of HIV-infected patients with or without OPC, which demonstrated an impairment of terminal differentiation of Langerhans’ cells, reduced expression of MHC class II alloantigens and adhesion molecules LFA-1 and ICAM-1, and depletion of CD4+ lymphocytes in the oral mucosa, compared with control patients uninfected with HIV [12]. Langerhans’ cells from rat [51] or human [52] oral mucosa function as dendritic antigen–presenting cells and thus occupy a strategic position in the induction of immune responses to oral pathogens. However, a low expression of MHC class II alloantigens appears to be a general feature of antigen-presenting cells in HIV infection, including macrophages [53] and dendritic cells [54]. Langerhans’ cells [55, 56] are the purported initial cellular targets in HIV infection, the site of active viral replication in vitro [57–59] and in vivo [60, 61], and the vectors of infection for CD4+ T cells [59, 62]. The densities of Langerhans’ cells are diminished in the oral [63], esophageal [64], and cervical [65] mucosa of HIV-infected patients. HIV and its transcriptional transactivator (tat) block the expression of MHC class II genes [66], resulting in impaired antigen presentation to CD4+ T cells [67] and defective primary immune responses [68]. Interestingly, the open-reading frame of the tat gene is interrupted in the CD4C/HIVMutA mice [17], suggesting that alternate mechanisms may be responsible for defective expression of MHC class II alloantigens and/or a low density of MHC class II–expressing cells in this animal model.

Although the frequencies of cells expressing MHC class II alloantigens were reduced in the uninfected Tg mice, infection with C. albicans enhanced their frequencies in the epithelium of both Tg and control non-Tg animals. The observed enhancement in frequencies of cells expressing MHC class II likely indicates the maturation and mobilization of antigen-presenting mucosal Langerhans’ cells [69], resulting either directly from exposure to C. albicans or from cytokines produced by the T cell response to infection [69]. Maturation of dendritic cells, including mucosal Langerhans’ cells, is characterized by a strong up-regulation of MHC class II expression, secretion of interleukin (IL)–12, and the expression of the adhesion molecules LFA-3, ICAM-1, and B7-2 [69]. Enhancement of MHC class II expression on antigen-presenting cells by coculture with C. albicans or exposure to C. albicans antigen has been demonstrated in vitro [70], in accordance with the ability of microbial pathogens and their products to directly induce maturation of dendritic cells [69, 71].

### Table 2. Cell surface marker analysis of peripheral blood lymphocytes from CD4C/HIVMutA transgenic (Tg) mice inoculated orally with Candida albicans.

<table>
<thead>
<tr>
<th>Mice* inoculated</th>
<th>No. at inoculation</th>
<th>Age at assessment (mean days)</th>
<th>CD45+</th>
<th>CD3+</th>
<th>CD4+</th>
<th>CD8+</th>
<th>CD4:CD8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg</td>
<td>8</td>
<td>79 (74–83)</td>
<td>96.3 ± 0.7</td>
<td>14.6 ± 3.5</td>
<td>2.1 ± 0.6</td>
<td>8.2 ± 1.8</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>Control non-Tg</td>
<td>4</td>
<td>79 (74–83)</td>
<td>97.6 ± 0.9</td>
<td>54.5 ± 2.5</td>
<td>36.3 ± 1.5</td>
<td>16.7 ± 1.5</td>
<td>2.18 ± 0.16</td>
</tr>
</tbody>
</table>

* Mice studied included Tg and control non-Tg offspring derived from founder mouse F21388.

### Table 3. Wasting and renal atrophy in CD4C/HIVMutA transgenic (Tg) mice inoculated orally with Candida albicans.

<table>
<thead>
<tr>
<th>Mice* inoculated</th>
<th>No. at inoculation</th>
<th>Age at inoculation (mean days)</th>
<th>Body mass, mean g ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg</td>
<td>9</td>
<td>50 (46–57)</td>
<td>21.0 ± 1.6</td>
</tr>
<tr>
<td>Control non-Tg</td>
<td>10</td>
<td>50 (46–57)</td>
<td>22.3 ± 1.8</td>
</tr>
</tbody>
</table>

* Mice studied included Tg and control non-Tg offspring derived from founder mouse F21388.
Figure 5. Transgene expression in CD4C/HIV<sup>mutA</sup> transgenic (Tg) mice inoculated with Candida albicans. Transgene expression was detected by in situ hybridization with human immunodeficiency virus type 1 (HIV-1)–specific riboprobes. A–D, Tongue hybridized with HIV-1 antisense probe (A and C) or sense probe (B and D) as control. Mononuclear cell infiltrate in epithelium and lamina propria showed absence of cell-specific hybridization signal (A and C). However, transgene expression was specifically detected in cells exhibiting morphologic features of macrophages (arrow) underlying mononuclear cell infiltrate (C). Transgene expression was undetectable after hybridization with control sense probe (B and D).

E–G, Cardial-atrium fold of stomach hybridized with HIV-1 antisense (E and G) or sense (F) probes. As in tongue, transgene was expressed in cells with features of macrophages (arrow in panel E) in lamina propria underlying partly degenerated mononuclear cell infiltrate that did not express transgene. In contrast, infiltrates of mononuclear cells located more deeply in submucosa were positive by in situ hybridization (arrow in panel G). Magnifications: A and B, ×680; C–G, ×430. Counterstain was hematoxylin-eosin.
and LFA-3 [75] are directly involved in II alloantigens [73–75] and the adhesion molecules ICAM-1 with the reduced CD4 with more advanced disease. These potential defects, combined adhesion molecules by Langerhans' cells from infected Tg mice activities. Further studies will be needed to characterize potential de-
at least 70 days after clearance of II–expressing cells persisted in the infected non-Tg mice for

There is a need to further investigate the mechanisms that underlie the predisposition to mucosal candidiasis in HIV-infected patients by use of this novel model in Tg mice.

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References


66. Kanazawa S, Okamoto T, Peterlin BM. Tat competes with CIITA for the binding to P-TEFb and blocks the expression of MHC class II genes in HIV infection. Immunity 2000;12:61–70.


