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Retrovirus-Induced Immunodeficiency in Mice Exacerbates Gastrointestinal Candidiasis

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Dysfunction of neutrophils in patients infected with human immunodeficiency virus is at least partly responsible for secondary microbial diseases in these individuals, including invasive gastrointestinal (GI) candidiasis. Immunoregulatory disturbances associated with the development of AIDS in human immunodeficiency virus-infected patients exacerbates *Candida albicans* infection of the upper GI tract and frequently leads to oropharyngeal and esophageal candidiasis. In this article, we present the first report of a murine model of invasive GI candidiasis associated with an AIDS-related murine immunodeficiency syndrome that results from infection of C57BL/6 mice with a previously described retrovirus complex (LP-BM5). Mice of the inbred strain were infected with *C. albicans* by oral-intragastric inoculation as infants and with the retrovirus by the intraperitoneal route 30 days later. Control mice of the same strain were infected with *C. albicans* as above and subsequently infected with the avirulent, ecotropic helper virus (MBI-5). Animals were killed 90 days after retroviral challenge. Total and differential blood cell counts, $CD4^+$ T-cell counts in the spleen, and the histopathology of the gastric mucosa of experimental and control animals were determined. The virulent LP-BM5-infected animals developed murine AIDS and showed eruptive and suppurative lesions, with associated *C. albicans* mainly in regions of the cardial-atrium fold of the stomach. Well-defined abscesses with entrapped *C. albicans* hyphae were observed in the region of the cardial-atrium fold of control mice. A significant increase in the number of *C. albicans* CFU in homogenized and plated segments of the GI tract was recognized in mice with murine AIDS versus the control animals. The murine model of GI candidiasis reported here permits examination of the nature of *C. albicans* interaction with the gastric mucosa both in the immunocompetent host under conditions in which the yeast exists predominantly as a commensal organism and in the immunosuppressed host during progressive stages of AIDS induced by a retroviral infection.

Candida albicans is an opportunistic fungal pathogen which frequently occurs as a component of the indigenous microflora in the gastrointestinal (GI) tract of apparently immunocompetent humans (31). The mechanisms by which *C. albicans* is able to persist in this hostile environment of the host are unknown. Localized foci of colonization by *C. albicans* in the GI tract are a potential threat to the health of the individual. The *C. albicans* foci in the GI mucosa may serve as reservoirs of the fungus from which it may proliferate and possibly invade submucosal tissue to cause systemic infection when the host is immunosuppressed (9, 11). In neutropenic patients, foci of GI candidiasis have been suggested to be important avenues of entry of the pathogen, leading to systemic *Candida* infection (29). Oropharyngeal and esophageal candidiasis are frequently diagnosed in patients with AIDS (22). Gastric candidiasis may also occur in these individuals, especially if extensive esophageal *Candida* infection is involved (37). However, systemic candidiasis is rarely observed in AIDS patients, at least in the early stages of the syndrome (37). The frequency of oropharyngeal candidiasis is estimated at present to be 45% among AIDS patients in the United States, while the occurrence of candidal infection at some time in the course of human immunodeficiency virus infection is recognized in approximately 75% of these cases (37). The histopathologic lesions which result from the interaction between *C. albicans* and the host mucosa under conditions of human immunodeficiency virus-induced immunosuppression have not been explored, partly because of the lack of suitable animal models which would permit examination of the development of GI candidiasis exacerbated by a retrovirus-induced immunodeficiency syndrome (18, 23, 24, 28, 38).

In this article, we present the first report of a murine model of concurrent GI candidiasis and a retrovirus-induced immunodeficiency. C57BL/6 mice were first infected with *C. albicans* by the oral-intragastric route and then immunosuppressed by infection with a previously described murine leukemia virus (MuLV) mixture (7). This retrovirus complex was first isolated by Latarjet and Duplan (21); it causes a

TABLE 1. Absolute counts of leukocytes, lymphocytes, and neutrophils in peripheral blood at death[a]

Inoculation	Mean absolute count ($10^3/mm^3$) ± SD[b]		
	Leukocytes	Lymphocytes	Neutrophils
Culture medium	14.22 ± 1.22	11.80 ± 0.96	2.27 ± 0.05
Avirulent virus (MBI-5)	13.41 ± 2.10	10.59 ± 1.70	2.68 ± 0.11
Virulent virus (LP-BM5)	4.22 ± 0.62	1.98 ± 0.22	1.94 ± 0.02

[a] Animals were killed 120 days after oral-intragastric infection with *C. albicans* (90 days after inoculation with medium or avirulent or virulent virus).
[b] Absolute counts are equal to the total count multiplied by the decimal percentage of the differential count. Differential counts were performed on blood smears by standard methods. Values are means ± standard deviations for five mice per group.

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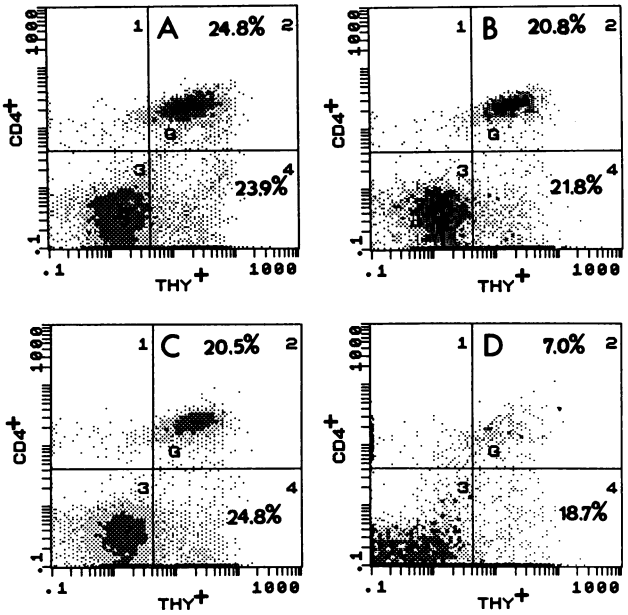

FIG. 1. Expression of Thy 1.2 and CD4 on splenic cells. Ficoll-purified mononuclear cells from spleens of untreated animals (A), mice infected with *C. albicans* and subsequently inoculated with medium (B), mice infected with *C. albicans* and subsequently inoculated with the avirulent (MBI-5) virus (C), and mice infected with *C. albicans* and then with the MAIDS (LP-BM5) virus (D) were examined by FACS analysis as described in Materials and Methods. The upper right-hand portion (quadrant 2) of each panel depicts the percentage of $CD4^+$ cells, while the lower right-hand portion (quadrant 4) depicts total Thy^+ cells minus $CD4^+$ cells.

lymphoproliferative disease primarily involving the lymph nodes and spleen. A cell line derived from murine bone marrow (BM5) was used as a source of the virus for subsequent studies (25). The continuous cell line produces a mixture of replication-competent, ecotropic, and mink cell focus-inducing MuLV and a replication-defective virus with a 4.8-kb genome (7). It appears that the defective virus is the disease-producing component of the mixture, but it also requires the replication-competent helper virus for cell-to-cell dissemination in the host. The ecotropic mink cell focus-inducing MuLV alone, which was biologically cloned from the mixture, did not induce disease (7). Other investigators have presented evidence that inbred C57BL/6 mice infected with the virus mixture develop a syndrome termed murine AIDS (MAIDS), which demonstrated many characteristics of human AIDS (19, 20). Although the murine and human syndromes are induced by retroviruses of different classes, the disease characteristics of the LP-BM5 MuLV infection include polyclonal B-cell activation, hypergammaglobulinemia, late-onset aggressive B-cell lymphoma, and increased susceptibility to infection (20). Both B-cell and T-cell functions in these retrovirus-infected mice have been found to be abnormal.

Impaired T-cell-proliferative response has been suggested to be related to an intrinsic defect of $CD4^+$ T cells but not of $CD8^+$ T cells (6). We have shown that C57BL/6 mice are also susceptible to long-term *C. albicans* infection when challenged with the yeast as infants by the oral-intragastric route. *C. albicans* remains in the GI tract, apparently as a commensal organism, for at least 120 days postchallenge when the animals are not exposed to immunocompromising

TABLE 2. *C. albicans* recovered from organ homogenates of yeast-infected, retrovirus-inoculated mice[a]

Group (inoculum)	Esophagus		Stomach		Intestines	
	Mean CFU/organ (range)[b]	No. of positive cultures/no. tested	Mean CFU/organ (range)	No. of positive cultures/no. tested	Mean CFU/organ (range)	No. of positive cultures/no. tested
G1 (culture medium)	2.88×10^1 (1.00×10^1–1.80×10^2)	11/18	1.89×10^5 (1.20×10^4–4.40×10^5)	18/18	7.80×10^4 (3.10×10^3–1.70×10^5)	18/18
G2 (avirulent virus MBI-5)	0.95×10^1 (1.00×10^1–5.00×10^1)	11/20	2.29×10^5 (2.40×10^4–1.20×10^6)	20/20	8.71×10^4 (1.00×10^4–2.30×10^5)	23/23
G3 (virulent virus LP-BM5)	1.25×10^2 (1.00×10^1–2.50×10^3)	18/26	4.27×10^6 (3.60×10^4–4.10×10^7)	26/26	1.58×10^5 (1.20×10^4–1.10×10^6)	26/26

[a] Six-day-old mice were infected with 10^8 yeast cells. Thirty days later, they were challenged intraperitoneally with Dulbecco's medium or with virulent or avirulent virus. The mice were killed 120 days after *C. albicans* infection.

[b] Range for positive samples.

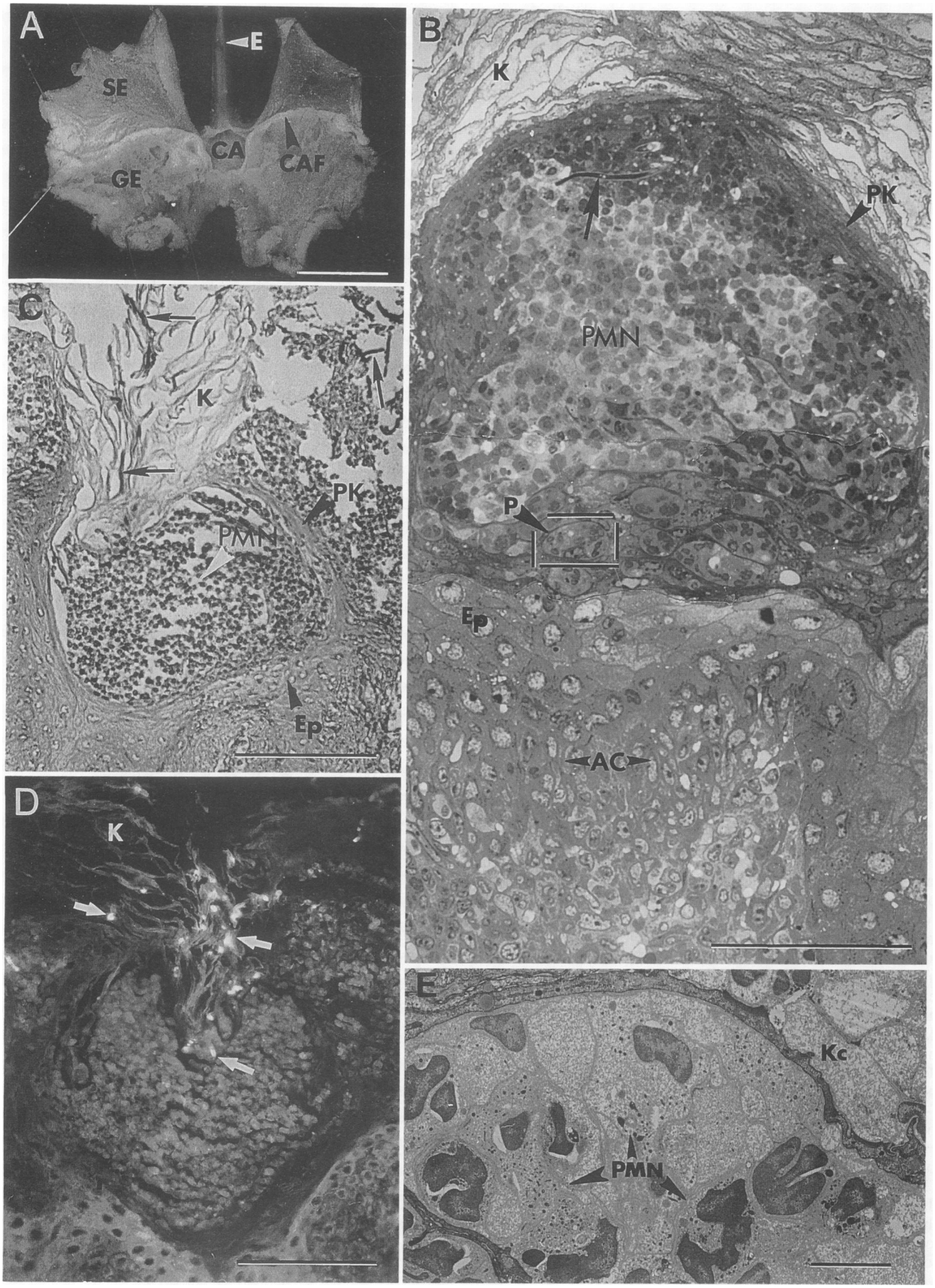
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FIG. 2. Gross and histologic preparations of the stomachs of mice infected with *C. albicans* and inoculated with the avirulent helper virus MBI-5. (A) Macroscopic features of a longitudinally bisected stomach. The CAF is slightly thickened. CA, cardiac antrum; E, esophagus; GE, glandular epithelium; SE, squamous epithelium. (B) Medial section of an intraepithelial abscess in the region of the CAF. The neutrophilic aggregate (PMN) is well circumscribed, sharply demarcated from the epithelial layer (Ep) below. Hyphae of *C. albicans* (arrow) are occasionally observed within the upper region of the abscess. AC, region of acanthosis; K, keratin; P, packet of PMN (the region within the box is shown in panel E); PK, region of parakeratosis. (C) Periodic acid-Schiff-stained paraffin section of intraepithelial abscess. *C. albicans* cells (arrows) were found within the stratum corneum. (D) Paraffin section corresponding to panel C, which was reacted with anti-*C. albicans*-FITC conjugate. Hyphae (arrows) are visible in the upper region of the abscess and in the keratin layers (K). (E) Thin section of packet of PMN (enclosed by box in panel B) which shows both granulated and degranulated cells surrounded by keratinocytes (Kc) and keratin. The bars in panels A through E represent 5 cm, 50 μm, 100 μm, 100 μm, and 5 μm, respectively.

agents or antibacterial antibiotics. After infection of these mice with the MAIDS virus, however, *C. albicans* assumes the role of an opportunistic pathogen. The murine model of concurrent GI candidiasis and MAIDS described here has been used to examine the histopathology of *C. albicans* invasion of the gastric mucosa of mice at a late stage in development of the retrovirus-induced immunodeficiency syndrome.

MATERIALS AND METHODS

Fungus. *C. albicans* CA30 (11) was grown on Sabouraud's dextrose agar (SDA; Difco) slants at 37°C overnight, harvested, washed three times in sterile, nonpyrogenic saline (Travenol; Travenol Laboratories Inc., Deerfield, Ill.), and counted in a hemacytometer. A suspension of 10^8 yeast cells per ml was prepared from this stock in saline and used for preparation of the inoculum. A quantitative check of the final suspension was performed by determinations of CFU cultured on SDA plates.

Viruses. Frozen stock preparations of the pathogenic LP-BM5 MuLV and nonpathogenic ecotropic helper virus (MBI-5) were obtained from G. L. Gilmore, Medical Biology Institute, La Jolla, Calif. Virus pools were obtained from infected SC-1 cell cultures as reported before (3), assayed for ecotropic MuLV by XC plaque tests in SC-1 cells (19), and tested for their ability to induce MAIDS in 4- to 6-week-old C57BL/6 mice (19). The stock preparation of the nonpathogenic virus (MBI-5) was obtained from the BM-5 cell line and determined to have a titer of 5×10^5 PFU/ml of cell culture medium (Dulbecco's modified Eagle's medium; Sigma Chemical Co., St. Louis, Mo.). The stock of pathogenic virus (LP-BM5) had a titer of 5×10^6 PFU/ml, and 0.1 ml of a 1:10 dilution of this preparation in saline was shown to be sufficient to cause mortality in 90% of 4-week-old C57BL/6 mice after 24 to 28 weeks.

Animals and inoculations. Inbred C57BL/6 mice, purchased from Jackson Laboratories (Bar Harbor, Maine), were used to establish a breeding colony, and the offspring were used in the reported experiments. Infant mice of both sexes (6 days old) were isolated from their mothers 3 to 4 h before inoculation with *C. albicans* and held at 35°C, as reported previously (13). The yeast cell suspension described above was used to inoculate mice by the oral-intragastric route as reported earlier (11). *C. albicans*-infected mice were each later challenged intraperitoneally (i.p.) with a single inoculum of 0.2 ml of 2×10^4 PFU of either LP-BM5 or MBI-5 virus at 30 days after yeast inoculation. A control group of *C. albicans*-infected mice were each inoculated with Dulbecco's medium (0.2 ml) alone. Mice challenged with the yeast were chosen for further study on the basis of the presence of *C. albicans* in their fecal pellet homogenates at 29 days after oral-intragastric inoculation, as previously described (11). Animals were killed at 120 days postinoculation with *C. albicans* by asphyxiation with carbon dioxide. The mice were immediately dissected to remove the GI tract and body organs for homogenization and dilution plating, fluorescence-activated cell sorting (FACS) analyses, or histological studies, as described below.

Blood cell counts. Blood samples were collected by retroorbital venipuncture just before death. Total leukocyte counts were made on a Coulter 5770 blood counter (Coulter Electronics, Hialeah, Fla.). Differential counts were made on blood smears by standard methods.

FACS. Single-cell suspensions of the spleen from untreated mice and mice killed 120 days after inoculation with *C. albicans* alone or in combination with retrovirus challenge were prepared for FACS analysis as described before (34). Briefly, cell suspensions from spleens were purified on a Ficoll-Hypaque gradient (Sigma). Two million cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-Thy 1.2 (Sigma) and phycoerythrin-conjugated anti-CD4 (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) to estimate relative numbers of pan T cells (Thy^+) and $CD4^+$ T cells, respectively. The cells were diluted 1:100 in phosphate-buffered saline (PBS) in a volume of 100 μl on ice for 45 min and washed three times with cold PBS containing 5% fetal calf serum. Dead cells were excluded from analysis by setting an appropriate threshold trigger on the forward-angle and 90° light scatter parameters. A minimum of 10,000 cells were counted from each sample in an Epics Elite flow cytometer (Coulter).

Enumeration of *C. albicans* in GI tract and body organs. Isolation of GI tract segments (esophagus, stomach, and intestines [duodenum, small and large intestine, cecum, and rectum]) and body organs (liver, lungs, spleen, and kidneys) was performed under aseptic conditions as described previously (11). The CFU of *C. albicans* in homogenized tissue were determined by dilution plating on SDA containing chloramphenicol (50 μg/ml; Sigma). Total counts were calculated for each organ, based on the volume of the homogenate. The plates were incubated at 37°C for 48 h. Enrichment cultures (Sabouraud's broth) of body organ homogenates were prepared to amplify possible low numbers of *C. albicans* cells by the method reported previously (11). Homogenates of GI tract segments were dilution plated onto SDA without this amplification step. It was not difficult to distinguish between *C. albicans* and the indigenous yeast *Candida pintolopesii* in estimating total CFU of the former in organ homogenates (11).

All statistical comparisons were performed by Student's *t* test. $P < 0.05$ was considered significant.

Histology. We performed necropsies for five animals from each group of *C. albicans*-infected mice which were subsequently inoculated with the virulent virus LP-BM5, the avirulent virus MBI-5, or culture medium, and selected organs were prepared for histological examination as de-

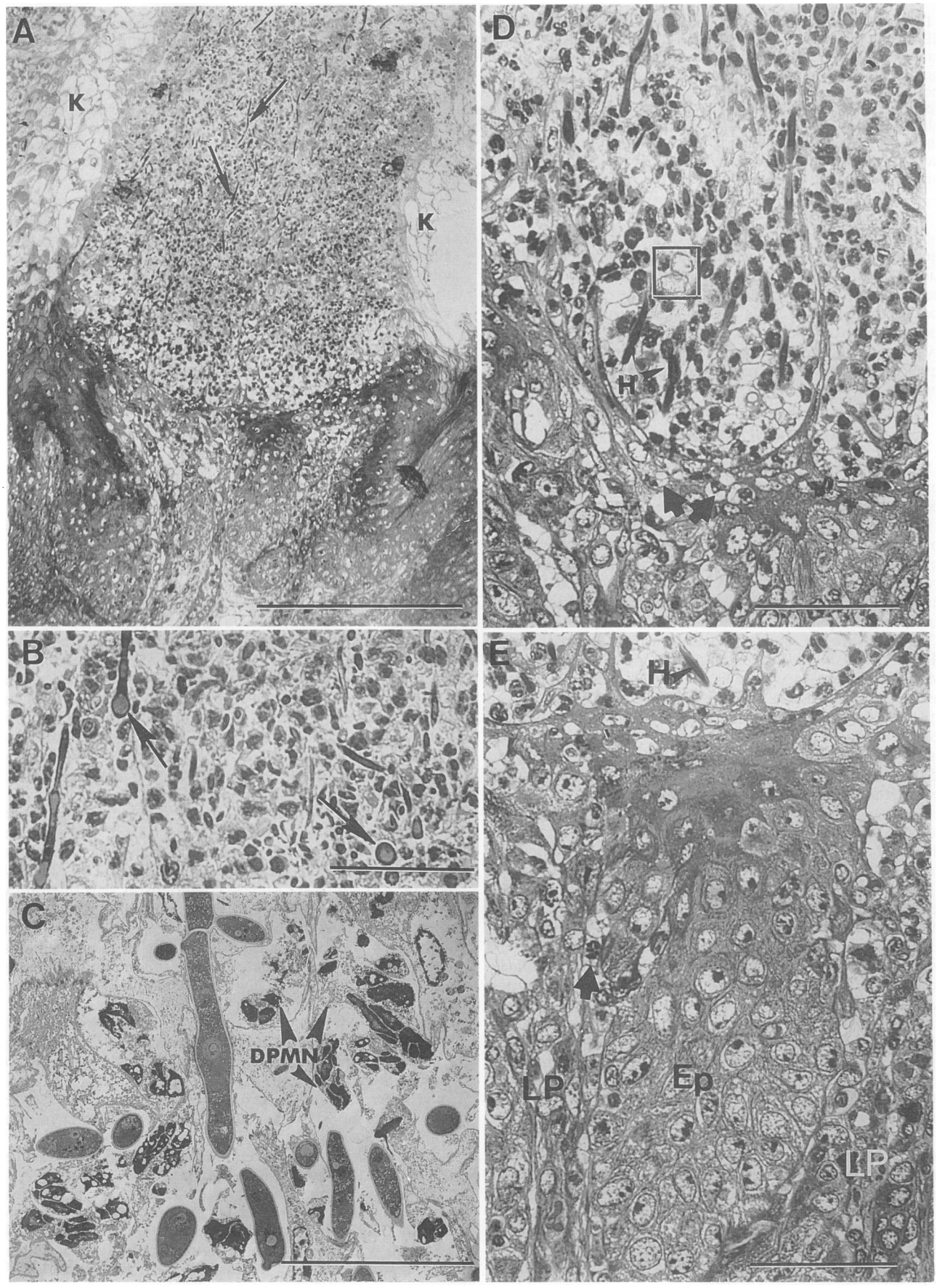
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FIG. 3. Histological sections of the stomachs of mice infected with *C. albicans* and the MAIDS (LP-BM5) virus. (A) Thick section of gastric mucosa in the region of the CAF, which shows a volcanolike eruption due to purulent suppurative inflammation involving the epithelial layer. Arrows indicate *C. albicans*. K, keratin. (B) Thick section of eruptive *C. albicans* abscess, which shows chlamydosporelike cells (arrows) admixed with numerous neutrophils. (C) Thin section of eruptive abscess, which shows degranulated PMN (DPMN) and adjacent intact and apparently viable *C. albicans*. (D) Thick section of eruptive abscess, which shows transition between aggregate of neutrophils and epithelial lining. Note that *C. albicans* hyphae (H) have invaded the epithelial lining. The epithelial cells demonstrate vacuolation, which is suggestive of degeneration. Lysed epithelial cells were visible within the abscess (boxed). The epithelium at the base of the abscess also revealed transepithelial migration of neutrophils (arrows). (E) Thick section of stomach in the region of the CAF, showing degeneration of epithelium (Ep) and neutrophils (arrows) in the lamina propria (LP). The bars in panels A through E represent 20, 40, 20, 40, and 40 μm, respectively.

scribed previously (9, 11, 13). Tissue sections were examined by both light microscopy and transmission electron microscopy, as reported before (11, 13). Sections of tissue embedded in paraffin were stained with periodic acid-Schiff reagent; sections of plastic-embedded tissue were stained as previously reported for light microscopic examination (9).

Immunofluorescence. Sections of paraffin-embedded tissue from *C. albicans*-infected, virulent virus- and avirulent virus-inoculated mice were examined by immunofluorescence light microscopy for localization of the fungal pathogen and specific host cells. For detection of the fungus, sections were reacted with anti-*C. albicans* (strain CA30) cell wall serum raised in rabbits and then with goat anti-rabbit immunoglobulin G (IgG)-FITC conjugate, as reported earlier (11, 13). This antiserum was not reactive with the indigenous yeast *C. pintolopesii* (13). Murine macrophages were detected in sections reacted with rat anti-mouse Mac-1 surface antigen monoclonal antibody (IgG2b isotype) (clone M1/70.15; Caltag Laboratories, San Francisco, Calif.), followed by goat anti-rat IgG-FITC conjugate (Caltag). The primary anti-Mac-1 antibody was diluted 1:100 in 0.1 M PBS (pH 7.6) with 1% bovine serum albumin. The secondary antibody was diluted 1:50 in PBS alone. Sections were incubated with the first and second antibody at 24°C. Sections incubated with the second antibody only or FITC alone served as controls.

RESULTS

Effects of LP-BM5 infection on peripheral blood cell counts. A summary of blood cell counts for the *C. albicans*-infected control and virus-inoculated animals is presented in Table 1. No significant difference in counts was recognized between animals inoculated with culture medium or avirulent virus (MBI-5). However, both leukopenia and lymphocytopenia were evident in animals inoculated with the virulent (LP-BM5) virus. A less dramatic but significant decrease in the number of neutrophils in this group of mice was also demonstrated.

FACS analysis of spleen cells. The results of a representative FACS analysis of spleen cell preparations are presented in Fig. 1. The relative numbers of Thy 1.2-positive T cells (Thy^+) and CD4-positive T cells ($CD4^+$) were compared for untreated, age-matched control mice (A), *C. albicans*-infected and medium-inoculated mice (B), and mice coinfected with *C. albicans* and either the avirulent virus (C) or the virulent virus (D). The mice were killed 120 days after oral-intragastric challenge with *C. albicans*. The percentage of labeled $CD4^+$ cells is shown in quadrant 2 of each panel. The percentage of Thy^+ cells in quadrant 4 excludes the $CD4^+$ cells and is therefore an estimate of the percentage of $CD8^+$ cells (4). Only the animals with concurrent *C. albicans* and LP-BM5 infections showed a marked reduction in relative numbers of $CD4^+$ cells (Fig. 1D). The estimated percentage of $CD8^+$ spleen cells in these animals appeared to be less affected by concurrent infection with *C. albicans* and the virulent (LP-BM5) retrovirus mixture.

Effects of LP-BM5 infection on GI candidiasis. The distribution and numbers of *C. albicans* CFU in control and test animals are shown in Table 2. In all cases, no yeast cells were detected in the liver, lungs, spleen, or kidneys at the time of death, even after homogenates of these organs were examined by the culture amplification procedure. No significant difference was noted between numbers of *C. albicans* detected in homogenates of the esophagus, stomach, and intestines of mice between treatment groups G1 (inoculated with culture medium) and G2 (inoculated with the avirulent virus MBI-5). In contrast, *C. albicans* CFU determined from dilution plates of the esophagus, stomach, and intestines of mice also infected with LP-BM5 (treatment group G3) were significantly higher ($P < 0.001$ to < 0.01) than corresponding values for the two groups of control mice (G1 and G2; Table 2). The number of animals with concurrent *C. albicans* and LP-BM5 infections which revealed persistent *C. albicans* in their esophagus increased by 15% compared with positive-control mice.

Histopathology. The focus of our histological examinations was the cardial-atrium fold (CAF) of the stomach (Fig. 2A), since this had been reported to be the principal region of *C. albicans* infection of the gastric mucosa in immunocompromised and nonimmunocompromised mice (10, 11, 27). The CAF is a keratinized fold of mucosal tissue which is located at the gastroesophageal junction (cardiac antrum) and is visible in longitudinally bisected murine stomachs along the greater curvature as a distinct zone that separates the squamous and glandular epithelial regions (Fig. 2A).

(i) Avirulent virus (MBI-5)-infected mice. Thick and thin sections of the gastric mucosa of *C. albicans*-infected mice which had been subsequently inoculated with the avirulent virus revealed well-defined, intraepithelial abscesses in the region of the CAF (Fig. 2B through D). These abscesses were characterized by infiltration and accumulation of primarily polymorphonuclear neutrophils (PMN). Occasionally, *C. albicans* hyphae were observed within the upper region of the abscess (arrow in Fig. 2B). The results of ultrastructural examinations of the entrapped hyphal elements suggested that destruction of cytoplasmic components of the fungal cells had occurred (data not shown). The central and peripheral regions of the intraepithelial abscess contained numerous PMN which had apparently undergone or were in the process of degranulation (Fig. 2B). The intraepithelial abscess was spatially defined by a thickened keratinized layer above and a basal epithelial layer (Fig. 2B and C). The keratinized layer demonstrated parakeratosis, while the basal epithelial layer revealed acanthosis in these animals with persistent GI candidiasis (Fig. 2B). Periodic acid-Schiff-stained sections showed fungal hyphae associated with the keratinized tissue adjacent to the intraepithelial abscesses (Fig. 2C). These fungal elements were clearly

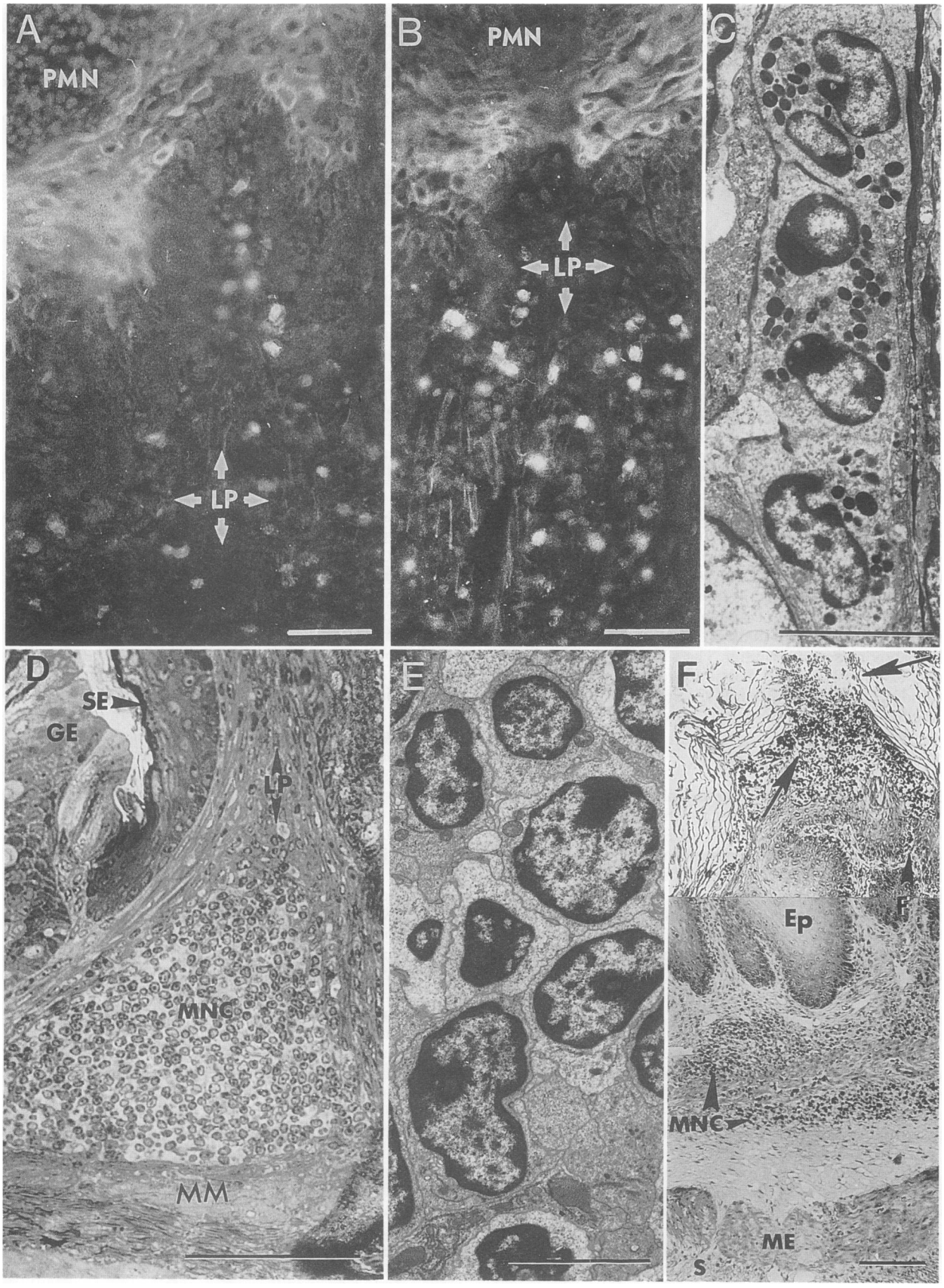
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FIG. 4. Histological sections of stomachs of mice infected with *C. albicans* and inoculated with either the avirulent helper virus (A, D, and E) or MAIDS virus (B, C, and F). (A and B) Immunofluorescence microscopy of paraffin-embedded sections reacted with monoclonal antibody directed against the murine Mac-1 surface antigen. Note the presence of macrophages in the lamina propria (LP) juxtaposed to an abscess of the gastric mucosa in mice inoculated with either avirulent virus (A) or MAIDS virus (B). (C) Neutrophils in the lamina propria apparently migrating toward the site of *C. albicans* infection. (D and E) Thick section (D) and thin section (E) of mononuclear cell (MNC) aggregate in the lamina propria of the CAF. GE, glandular epithelium; MM, muscularis mucosa; SE, squamous epithelium. (F) Paraffin-embedded section which shows inflammation throughout the gastric wall. Note eruptive abscess (arrows) at mucosal surface, focal erosion (F) of epithelial layer, and confluent aggregates of mononuclear cells (MNC) in lamina propria and submucosa. Scattered inflammatory cells were also seen in the muscularis externa (ME) and serosa (S). The bars in panels A through F represent 50, 50, 5, 100, 5, and 100 μm, respectively.

visible by immunofluorescence microscopy (Fig. 2D). Packets of neutrophils were commonly visible at the base of the abscess (Fig. 2B, box). These were surrounded by thin layers of keratin, keratinocytes, and remnants of epithelial cells (Fig. 2E).

(ii) Virulent virus (LP-BM5)-infected mice. A striking feature of the histopathologic lesions in mice with concurrent *C. albicans* and MAIDS virus infections was the presence of multiple focal areas of ulcerative, purulent gastritis in the CAF region (Fig. 3A). These abscesses were characterized by a central core of degenerate neutrophils admixed with abundant *C. albicans* hyphae (Fig. 3A through C). Sections of the lesions also revealed thick-walled, vacuolate cells of *C. albicans* (arrowheads in Fig. 3B), which were reminiscent of the chlamydosporelike cells found in the gastric mucosa of immunocompromised mice (12). No such fungal cells were found in sections of the stomachs of avirulent virus (MBI-5)-infected mice. The thickened keratinized layer which partially enclosed the abscesses observed in mice infected with *C. albicans* and the avirulent virus was absent from these lesions. The representative abscess in Fig. 3A is a volcanolike suppurative eruption of the gastric mucosa. The thickened basal epithelial layer revealed irregular acanthosis and degeneration (Fig. 3D and E). Neutrophils were visible in the lamina propria below the eroded region of the basal epithelial zone (Fig. 3E). It appeared that the neutrophils had migrated to sites of *C. albicans* infection by way of the lamina propria.

Identification and localization of neutrophils and macrophages in the gastric mucosa. Both groups of *C. albicans*-infected animals which were subsequently infected with either the avirulent or virulent virus showed accumulation of mononuclear cells in the lamina propria and submucosa (Fig. 4). Immunofluorescence microscopy was conducted with a monoclonal antibody directed against the Mac-1 murine surface antigen. Sections of the gastric mucosa in the region of the CAF showed that a significant number of these mononuclear cells were macrophages (Fig. 4A and B). Thin sections through the lamina propria of the MAIDS virus-infected animals also revealed that abundant neutrophils were present, often in a linear arrangement adjacent to the epithelial zone in the *C. albicans*-infected region of the mucosa (Fig. 4C). In the avirulent virus (MBI-5)-inoculated animals, mononuclear cells in the lamina propria consisted predominantly of lymphocytes and macrophages (Fig. 4D and E) and were found adjacent to the well-defined intraepithelial abscesses. In contrast, a more extensive inflammatory response to *C. albicans* was evident in the MAIDS virus-infected animals. In a segment of the gastric mucosa which includes part of the CAF in Fig. 4F, large numbers of host inflammatory cells are visible extending from the serosa to the suppurative lesion at the keratinized surface. Abundant mononuclear cells were visible in the submucosa and lamina propria of these animals. Extensive and irregular acanthosis of the squamous epithelium was also demonstrated.

DISCUSSION

We have shown that C57BL/6 mice challenged with *C. albicans* by oral-intragastric inoculation at infancy demonstrate persistent colonization of their gastrointestinal tract by the opportunistic pathogen. Infection of the same mice with the LP-BM5 MuLV complex (MAIDS virus) at 30 days after *C. albicans* inoculation led to severe lymphoproliferative disease and immunodeficiency, which resulted in the onset of invasive gastrointestinal candidiasis over the subsequent 90 days. The results of peripheral blood cell counts and FACS analyses of spleen cell preparations of mice with concurrent *C. albicans* and MAIDS virus infections indicated that the animals were immunosuppressed. These observations are in agreement with the data of other investigators who have characterized the retrovirus-induced immunodeficiency syndrome in C57BL/6 mice (2, 6, 25). A significant reduction in the number of $CD4^+$ cells in MAIDS virus-infected animals compared with control mice was demonstrated by the results of our FACS analyses. Animals infected with *C. albicans* alone showed approximately the same number of $CD4^+$ T cells as untreated mice. It is known that functional $CD4^+$ T cells and B lymphoid cells are required at the time of infection by LP-BM5 for development of MAIDS (6). However, marked reductions in the number of $CD4^+$ cells as well as functional defects in these immune cells have been observed in advanced stages of this disease (20). Nude mice inoculated with the LP-BM5 virus do not develop MAIDS because functional T cells are absent (26). Virulent-retrovirus-inoculated mice treated with the immunosuppressive drug cyclophosphamide failed to develop MAIDS, presumably because of depletion of the number of infected target cells (36). Expansion of the target cell population in the retrovirus-infected C57BL/6 mice has been proposed to "initiate a cascade of events that leads to immunodeficiency" (36). Reports of clinical studies have suggested that cell-mediated immunity provides protection against mucosal candidiasis and that $CD4^+$ lymphocytes play a critical role in this process (5, 32). The LP-BM5-induced immunosuppression in C57BL/6 mice with persistent GI candidiasis leads to a significant increase in the number of *C. albicans* cells in the esophagus, stomach, and intestines and to invasion of the gastric submucosal tissue in the region of the CAF. We suggest that this model simulates the conditions in certain patients infected with the human immunodeficiency virus, who present early with chronic GI candidiasis and then gradually develop more extensive candidal infection of their upper GI mucosa as AIDS progresses.

The differences in the histopathology of *C. albicans*-infected gastric mucosa between control mice and mice with a retrovirus-induced immunodeficiency have been sum-

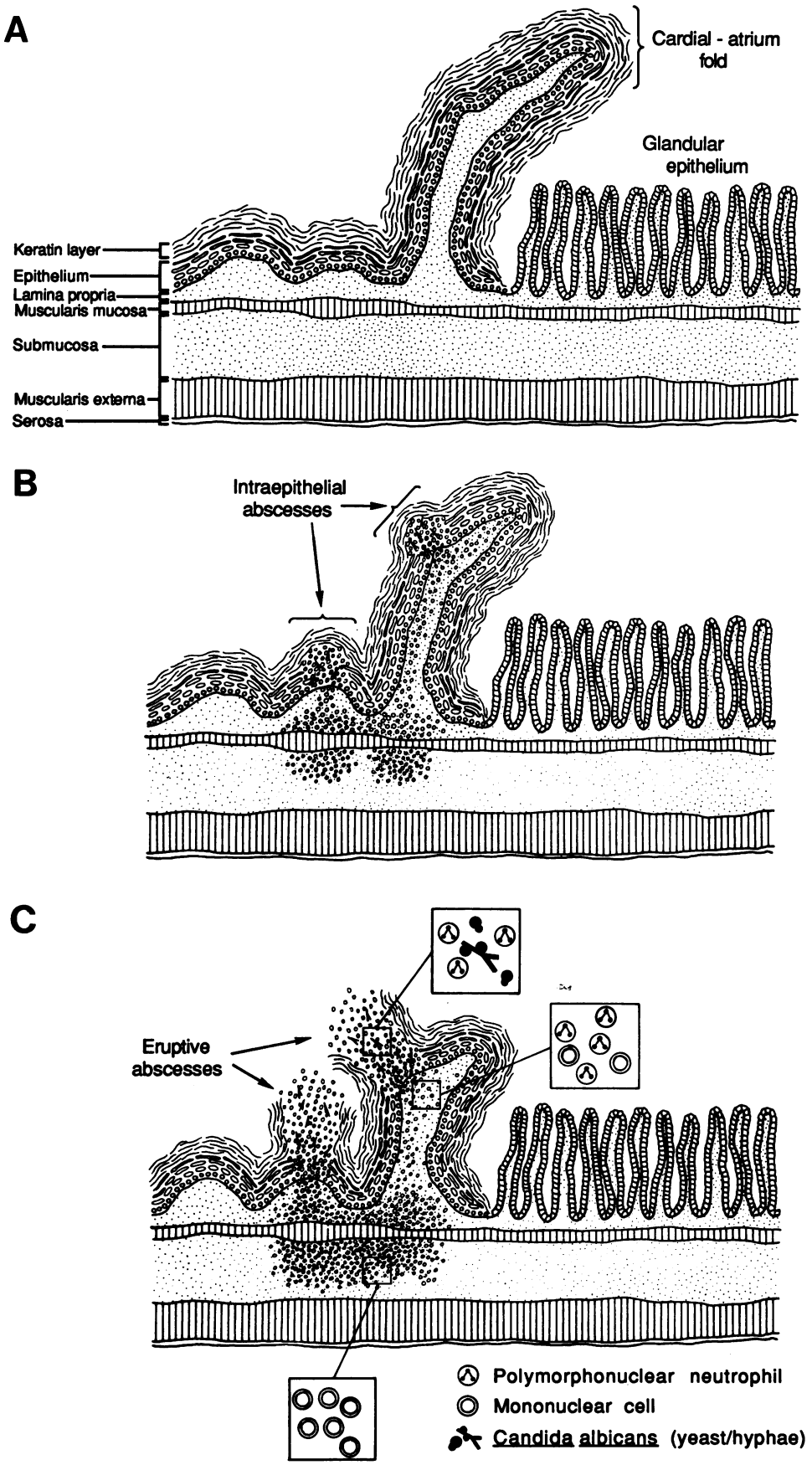

FIG. 5. Diagrammatic comparison of the histology of the gastric mucosa in the region of the CAF in an untreated mouse (A), and histopathology associated with *C. albicans* infection by the oral-intragastric route and intraperitoneal inoculation 30 days later with either the avirulent (MBI-5) helper virus (B) or virulent MAIDS (LP-BM5) virus (C). The latter two groups of mice were killed and examined histologically 90 days after retrovirus inoculation.

marized in Fig. 5. Animals with persistent GI candidiasis which were inoculated with the avirulent helper retrovirus alone consistently developed well-defined, intraepithelial abscesses (Fig. 5B). These abscesses occasionally contained *C. albicans* hyphae, some of which, upon ultrastructural examination, appeared to be nonviable. Phagocytosis as well as the accumulation of toxic products of host degenerate neutrophils within the abscess may contribute to the death of the opportunistic pathogen and gradual clearance of GI candidiasis. However, viable hyphae and yeast cells associated with keratin layers in the region of the CAF were also found in the control animals coinfected with *C. albicans* and avirulent virus. It appeared that these fungal cells had not evoked an inflammatory response and therefore may account for the high numbers of CFU of the opportunistic pathogen in C57BL/6 control mice even at 120 days after *C. albicans* inoculation.

Mice which were infected with *C. albicans* and subsequently challenged with the MAIDS virus developed ulcerated abscesses in the gastric mucosa (Fig. 5C). Large numbers of yeast cells, hyphae, and chlamydosporelike cells (12) were visible between the outer keratin layers and epithelium. An intense inflammatory cell response was evident in histological sections of the gastric mucosa of these animals in conjunction with the proliferation and invasiveness of the opportunistic pathogen. In contrast to the control animals, extensive mononuclear cell and neutrophil responses were evident in the lamina propria and submucosa of the LP-BM5-infected mice adjacent to sites of *C. albicans* invasion. The presence of neutrophils in the lamina propria suggests that at least some of these cells retained in vivo chemotactic function in the *C. albicans*-infected mice which developed MAIDS. No information was obtained in this study on the effect of virulent-retrovirus infection on the candidacidal activity of peripheral blood PMN.

We suggest that the inflammatory cell response in our immunodeficient murine model is due to several factors. The pathogen releases chemotactic factors (16) as well as lytic enzymes capable of causing tissue damage (14). Lysis of keratinocytes occurs at the squamous epithelial surface of the GI tract, which potentiates the release of interleukin-1, a well-known chemotactic factor for PMN and mononuclear cells (15). In addition to these innate host defense mechanisms, *C. albicans* is known to release antigens which induce an immune response (32). This acquired host defense mechanism normally evokes local immunoprotection at the mucosal level, mediated by secretion of specific IgA and the presence of sensitized T lymphocytes (5, 17). Evidence has been presented that T lymphocytes play an important role in the process of abscess formation (30, 35), although the specific nature of T-cell involvement in this pathogenic process has not been defined. Several possible dysfunctions of the cell-mediated immunity may exist in mice which have concurrent *C. albicans* and MAIDS virus infections. These may include T-cell activity in abscess formation as well as phagocytosis and killing of *C. albicans* by macrophages and neutrophils in the GI tract. The immunoregulatory disturbances associated with MAIDS exacerbate *C. albicans* infection of the gastric mucosa. In support of this interpretation, Moors and coworkers (23) have reported that increased susceptibility to systemic infection by the opportunistic fungal pathogen in a related murine model of concurrent infection with a retrovirus (Friend leukemia virus) and *C. albicans* may have been due to dysfunction of T cells which secondarily affected PMN activity. The depression of neutrophil function reported for human immunodeficiency virus-infected patients is at least partly responsible for secondary microbial infection in these individuals (1). Alterations in the expression of immunoregulatory cytokines have also been suggested to be important in the pathogenesis of both MAIDS and AIDS (8, 33).

A murine model of retrovirus-induced immunosuppression and concomitant increased susceptibility to GI candidiasis is described here for the first time. The animal model will be useful for further studies of the relative importance of mucosal immune cell components to host defense against opportunistic infection by *C. albicans*.

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REFERENCES

1. **Abramson, J. S., and E. L. Mills.** 1988. Depression of neutrophil function induced by viruses and its role in secondary microbial infections. Rev. Infect. Dis. **10**:326–341.
2. **Aziz, D. C., Z. Hanna, and P. Jolicoeur.** 1989. Severe immunodeficiency disease induced by a defective murine leukaemia virus. Nature (London) **338**:505–508.
3. **Buller, R. M., R. Y. Yetter, T. N. Fredrickson, and H. C. Morse.** 1987. Abrogation of resistance to severe mousepox in C57BL/6 mice infected with LP-BM5 murine leukemia viruses. J. Virol. **61**:383–387.
4. **Cantor, H., and E. A. Boyse.** 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. J. Exp. Med. **141**:1376–1389.
5. **Cantorna, M. T., and E. Balish.** 1991. Role of $CD4^+$ lymphocytes in resistance to mucosal candidiasis. Infect. Immun. **59**:2447–2455.
6. **Cerny, A., A. W. Hugin, K. L. Holmes, and H. C. Morse.** 1990. $CD4^+$ T cells in murine acquired immunodeficiency syndrome: evidence for an intrinsic defect in the proliferative response to soluble antigen. Eur. J. Immunol. **20**:1577–1581.
7. **Chattopadhyay, S. K., H. C. Morse, M. Makino, S. K. Ruscetti, and J. W. Hartley.** 1989. Defective virus is associated with induction of murine retrovirus-induced immunodeficiency syndrome. Proc. Natl. Acad. Sci. USA **86**:3862–3866.
8. **Cheung, S. C., S. K. Chattopadhyay, J. W. Hartley, H. C. Morse, and P. M. Pitha.** 1991. Aberrant expression of cytokine genes in peritoneal macrophages from mice infected with LP-BM5 MuLV, a murine model of AIDS. J. Immunol. **146**:121–127.
9. **Cole, G. T., K. T. Lynn, and K. R. Seshan.** 1990. Evaluation of a murine model of hepatic candidiasis. J. Clin. Microbiol. **28**:1828–1841.
10. **Cole, G. T., K. T. Lynn, and K. R. Seshan.** 1990. An animal model for oropharyngeal, esophageal, and gastric candidosis. Mycoses **33**:7–19.
11. **Cole, G. T., K. T. Lynn, K. R. Seshan, and L. M. Pope.** 1989. Gastrointestinal and systemic candidosis in immunocompromised mice. J. Med. Vet. Mycol. **27**:363–380.
12. **Cole, G. T., K. R. Seshan, M. Phaneuf, and K. T. Lynn.** 1991. Chlamydospore-like cells of *Candida albicans* in the gastrointestinal tract of infected, immunocompromised mice. Can. J. Microbiol. **37**:637–646.
13. **Cole, G. T., K. R. Seshan, L. M. Pope, and R. J. Yancey.** 1988. Morphological aspects of gastrointestinal tract invasion by *Candida albicans* in the infant mouse. J. Med. Vet. Mycol. **26**:173–185.
14. **Cutler, J. E.** 1991. Putative virulence factors of *Candida albicans*. Annu. Rev. Microbiol. **45**:187–218.
15. **Dinarello, C. A.** 1984. Interleukin-1 and the pathogenesis of the acute-phase response. N. Engl. J. Med. **311**:1413–1418.

16. **Domer, J. E.** 1989. *Candida* cell wall mannan: a polysaccharide with diverse immunologic properties. Crit. Rev. Microbiol. **17:**33–51.
17. **Epstein, J. B., L. H. Kimura, T. W. Menard, E. L. Truelove, and N. N. Pearsall.** 1982. Effects of specific antibodies on the interaction between the fungus *Candida albicans* and human oral mucosa. Arch. Oral Biol. **27:**469–474.
18. **Gardner, M. B.** 1989. SIV infected rhesus macaques: an AIDS model for immunoprevention and immunotherapy. Adv. Exp. Med. Biol. **251:**279–293.
19. **Hartley, J. W., T. N. Fredrickson, R. A. Yetter, M. Makino, and H. C. Morse.** 1989. Retrovirus-induced murine acquired immunodeficiency syndrome: natural history of infection and differing susceptibility of inbred mouse strains. J. Virol. **63:**1223–1231.
20. **Jolicoeur, R.** 1991. Murine acquired immunodeficiency syndrome (MAIDS): an animal model to study the AIDS pathogenesis. FASEB J. **5:**2398–2405.
21. **Latarjet, R., and J. F. Duplan.** 1962. Experiment and discussion on leukaemogenesis by cell-free extracts of radiation-induced leukaemia in mice. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. **5:**339–344.
22. **Meyer, R. D., and K. Holmberg.** 1989. Fungal infections in HIV-infected patients, p. 79–100. *In* K. Holmberg and R. D. Meyer (ed.), Diagnosis and therapy of systemic fungal infections. Raven Press, New York.
23. **Moors, M. A., S. M. Jones, K. K. Klyczek, T. J. Rogers, H. R. Buckley, and K. J. Blank.** 1990. Effect of Friend leukemia virus infection on susceptibility to *Candida albicans*. Infect. Immun. **58:**1796–1801.
24. **Mosier, D. E.** 1986. Animal models for retrovirus-induced immunodeficiency disease. Immunol. Invest. **15:**233–261.
25. **Mosier, D. E., R. A. Yetter, and H. C. Morse.** 1985. Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57BL/6 mice. J. Exp. Med. **161:**766–784.
26. **Mosier, D. E., R. A. Yetter, and H. C. Morse.** 1987. Functional T lymphocytes are required for a murine retrovirus-induced immunodeficiency disease (MAIDS). J. Exp. Med. **165:**1737–1742.
27. **Myerowitz, R. L.** 1981. Gastrointestinal and disseminated candidiasis. Arch. Pathol. Lab. Med. **105:**138–143.
28. **Namikawa, R., H. Kaneshima, M. Lieberman, I. L. Weissman, and J. M. McCune.** 1988. Infection of the SCID-hu mouse by HIV-1. Science **242:**1684–1686.
29. **Narayanan, R., W. A. Joyce, and R. A. Greenfield.** 1991. Gastrointestinal candidiasis in a murine model of severe combined immunodeficiency syndrome. Infect. Immun. **59:**2116–2119.
30. **Nulsen, M. F., J. J. Finlay-Jones, and P. J. McDonald.** 1986. T-lymphocyte involvement in abscess formation in nonimmune mice. Infect. Immun. **52:**633–636.
31. **Odds, F. C.** 1988. *Candida* and candidosis, 2nd ed. Bailière Tindall, London.
32. **Pankhurst, C., and M. Peakman.** 1989. Reduced $CD4^+$ T cells and severe oral candidiasis in absence of HIV infection. Lancet **i:**672.
33. **Pitha, P. M., D. Biegel, R. A. Yetter, and H. C. Morse.** 1988. Abnormal regulation of IFNα, -β, and -γ expression in MAIDS, a murine retrovirus-induced immunodeficiency syndrome. J. Immunol. **141:**3611–3616.
34. **Saha, K., and P. K. Y. Wong.** 1992. *ts*1, a temperature-sensitive mutant of Moloney murine leukemia virus TB, can infect both $CD4^+$ and $CD8^+$ T cells but requires $CD4^+$ T cells in order to cause paralysis and immunodeficiency. J. Virol. **66:**2639–2646.
35. **Shapiro, M. E., D. L. Kasper, D. F. Zaleznik, S. Spriggs, A. B. Onderdonk, and R. W. Finberg.** 1986. Cellular control of abscess formation: role of T cells in the regulation of abscesses formed in response to *Bacteroides fragilis*. J. Immunol. **137:**341–346.
36. **Sinard, C., and P. Jolicoeur.** 1991. The effect of anti-neoplastic drugs on murine acquired immunodeficiency syndrome. Science **251:**305–308.
37. **Tanowitz, H. B., D. Simon, and M. Wittner.** 1992. Gastrointestinal manifestations. Med. Clin. N. Am. **76:**45–62.
38. **Wu-Hsieh, B., D. H. Howard, and R. Ahmed.** 1988. Virus-induced immunosuppression: a murine model of susceptibility to opportunistic infection. J. Infect. Dis. **158:**232–235.