

FIG. 2. Vaginal histology of *C. albicans*-infected mice. (A) PAS stain of a vaginal section from a control mouse shows a florid infection with pseudo hyphae (PH) and blastoconidia (B) penetrating the cornified epithelium. The lumen (L), mucosa (M), and submucosa (SM) are denoted for orientation. Magnification, $\times 83$. (B) H&E stain of a vaginal section from the same mouse as in panel A. Neutrophils are present in the mucosa and have formed a microabscess (MA) in the cornified epithelium. Significant edema and submucosal fibroblastic disorganization are also present. Magnification, $\times 83$. (C) PAS stain of a vaginal section from a neutrophil-depleted mouse. Pseudo hyphae (PH) are present in the cornified epithelium in numbers similar to those of control mice. No blastoconidia are seen, and the pseudo hyphae form short stubby chains. Magnification, $\times 83$. (D) H&E stain of a vaginal section from a neutrophil-depleted mouse shows a profound lack of neutrophils in either the cornified epithelium or mucosa. Some edema has occurred, but the severity of inflammation is significantly less than that in the control animals. Magnification, $\times 83$. (E) H&E stain of a vaginal section from a noninfected mouse 3 days after estrogen administration. No microabscesses are evident, and PMN infiltration is minimal. Magnification, $\times 83$.

with recurrent vaginitis and was routinely typed by the rapid yeast plus test (Innovative Diagnostic Systems Inc, Norcross, Ga.) and analyzed for germ tube formation in serum. On day 8 mice were washed vaginally with 40 μ l of PBS. The washes were serially diluted, plated on Sabouraud dextrose agar plates containing chloramphenicol, and incubated for 24 h at 37°C, and the colonies were enumerated. For evaluation of inflammation, vaginal tissues (day 8) were fixed in buffered formalin and 2- μ m sections of the entire vagina were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff base

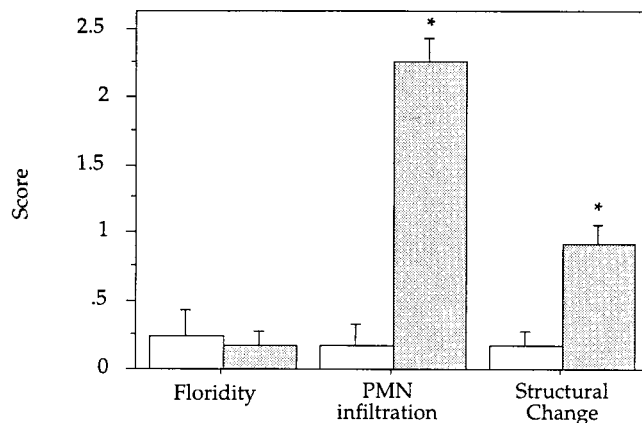


FIG. 3. Neutrophil depletion decreases vaginal inflammation. Histological sections were graded as described in the text. No statistical difference in floridity between control and neutrophil-depleted mice was observed; however, both the level of PMN infiltration ($P < 0.001$) and the degree of inflammation ($P < 0.05$) were significantly decreased in the neutrophil-depleted mice. The average scores \pm standard errors (indicated by error bars) are graphed ($n = 2$). Results are representative of two separate experiments. Open bars, neutrophil-depleted mice; shaded bars, control mice. Asterisks indicate significant differences.

(PAS) and scored for inflammation and extent of infection. The grading scale for the level of fungal burden was 0, no detectable yeast in vaginal section by PAS; 1, mild to moderate infection—sporadic (1 to 50) pseudohyphae and/or blastoconidia in the vaginal lumen and/or cornified epithelial layer; and 2, florid infection—high levels of detectable yeast (50+ CFU) in the vaginal lumen as well as pseudohyphae and yeast in the deeper cornified epithelial layers. The level of inflammation was scored according to influx of neutrophils and degree of ultrastructural change. The scale for neutrophilic influx was 0, no neutrophils present; 1, mild—1 to 10 neutrophils present in the lumen and one to five microabscesses present in the epithelial layer; 2, moderate—numerous neutrophils in the lumen and numerous microabscesses and neutrophils present in submucosa; and 3, severe—large abscess formation, microabscesses coating the cornified epithelial layer, and numerous neutrophils in submucosa. The scale for ultrastructural changes was 0, no change—well-defined circular blood vessels and layered submucosal fibroblasts; 1, moderate—vessels were constricted or ovoid from edema, and fibroblasts were arranged chaotically; and 2, severe—fibroblasts were depleted and necrosis was present in submucosa.

The significance of differences in vaginal yeast burden and inflammation between experimental and control groups was determined by Mann-Whitney analysis.

RB6-C85-induced PMN depletion is maintained for only 5 days (16) so the numbers of recoverable yeast cells in vaginal washes were determined at 5 days postinfection. No significant difference in the number of recoverable *Candida* was seen between control and PMN-depleted animals (Fig. 1). Twenty-five percent of the control animals had completely cleared the infection compared to 16% of the PMN-depleted animals, but this difference was not significant. In both control (Fig. 2A) and PMN-depleted (Fig. 2C) mice infection was limited to the cornified epithelial layers and consisted primarily of pseudohyphae. In PMN-depleted animals pseudohyphae appeared less filamentous and more “stubby.” Blastoconidia were common in vaginal sections from control animals but were rarely seen in vaginal sections from PMN-depleted mice. Numerous neutro-

phils in both the lumen and lamina propria were obvious in control animals, and microabscesses were frequently observed (Fig. 2B). Luminal PMNs in control mice appear to have emigrated from the cervix. PMNs were not observed in RB6-C85-treated animals (Fig. 2D). In noninfected, estrogen-treated animals PMN infiltration into the vagina was minimal and microabscesses were not observed (Fig. 2E), demonstrating that the observed effects were due to *Candida* infection rather than to the induction of estrus.

Histological grading of vaginal sections from PMN-depleted and control mice (Fig. 3) showed no difference in the floridity of infection, supporting the counts of recoverable yeast cells shown in Fig. 1 and the expected absence of PMN infiltration in RB6-C85-treated animals. Interestingly, structural changes associated with inflammation were significantly decreased in PMN-depleted animals ($P < 0.05$), suggesting that the neutrophil influx associated with infection contributes to inflammation.

Our data show that while neutrophils are recruited into the mouse vaginal mucosa during induced estrus they appear not to play a major role in clearance of *C. albicans* from this site. Depletion of PMNs did, however, result in decreased inflammation in the vagina, suggesting that neutrophils contribute to the symptomatology of vaginitis. As mice are only infectable during induced estrus it may be that PMNs recruited into the mucosa cannot enter the lumen due to the cornified epithelium. Hence, degranulation at this site would enhance inflammation but be ineffective in clearing luminal *Candida*. Mice will, however, spontaneously clear the infection if allowed to cycle normally. Thus, the growth of *C. albicans* in this model is favored by conditions found at estrus where the cornified epithelium not only provides dead epithelial cells to which yeast can adhere and invade but may also isolate the *Candida* from the innate immune system.

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