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Immune Cell-Mediated Protection against Vaginal Candidiasis: Evidence for a Major Role of Vaginal CD4$^+$ T Cells and Possible Participation of Other Local Lymphocyte Effectors

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The protective roles of different lymphocyte subsets were investigated in a rat vaginal candidiasis model by adoptive transfer of vaginal lymphocytes (VL) or sorted, purified CD3$^+$ T cells, CD4$^+$ or CD8$^+$ T cells, or CD3$^-$ CD5$^+$ B cells from the vaginas of naïve or immune rats following three rounds of *Candida albicans* infection. The adoptive transfer of total VL from nonimmune animals did not alter the course of vaginal candidiasis of the recipient rats. In contrast, the animals receiving total VL or CD3$^+$ T cells from immune rats showed a highly significant acceleration of fungus clearance compared with animals which received nonimmune VL. The animals with vaginal CD3$^-$ CD5$^+$ B cells transferred from immune rats also had fewer *Candida* CFU than the controls, but fungal clearance was significantly retarded with respect to the animals administered immune T cells. Sorted, purified CD4$^+$ and CD8$^+$ vaginal T cells from immune rats were also adoptively transferred to naïve animals. Although both populations were seen to accelerate the clearance of the fungus from the vagina, CD4$^+$ T cells were much more effective than CD8$^+$ T cells. Overall, there was no difference between the antifungal effects of immune vaginal CD4$^+$ T cells and those achievable with the transfer of whole, immune VL. Histological observations of the vaginal tissues of rats with adoptively transferred immune T cells demonstrated a remarkable accumulation of lymphocytes in the subepithelial lamina propria and also infiltrating the mucosal epithelium. These results strongly suggest that distinct vaginal lymphocyte subsets participate in the adaptive anti-*Candida* immunity at the vaginal level, with the vaginal CD4$^+$ T cells probably playing a major role.

Vulvovaginal candidiasis, mostly caused by *Candida albicans*, is a common mucosal infection affecting a large proportion of women of child-bearing age (34). Antifungal therapy is effective for individual attacks but may be unable to prevent recurrences (19, 20, 25, 26). However, the pathogenesis of this frequent clinical condition and the vaginal immune response to the fungal attack remain largely unknown. There is substantial agreement that local rather than systemic immunity plays a critical role (17, 19), but the mechanisms underlying this site-defensive response have not been defined. This particularly concerns the relative contributions of B- and T-cell compartments, together with locally released soluble products, such as cytokines and chemokines, and the interplay between immune and other cells of the vaginal canal (15, 32, 33, 35).

In order to understand the contribution of each of the above-mentioned critical factors to the host response against vaginal candidiasis, we have long been employing a rat model of vaginal infection that has a remarkable immunological similarity to human disease in the ratio of vaginal CD4$^+$ to CD8$^+$ T cells (12). In this model, we have demonstrated the pathogenic role of secreted aspartyl-proteinases (Sap) (8, 10), as well as the efficacy of anti-Sap and other antibodies in protection against infection (6, 9). Importantly, the analysis of the T-cell responses in immunized and protected rats demonstrated relevant changes in the ratio of CD4$^+$ to CD8$^+$ T cells during the infection, as well as elicitation of a *Candida*-specific vaginal T-cell response (13). However, the type and function of the T cells possibly mediating, or participating in, the protection were not precisely assessed. In an attempt to fill this gap, we have now determined the protective roles of different lymphocyte subsets in rat vaginal candidiasis by experiments of adoptive transfer of naïve or immune vaginal lymphocytes (VL).

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MATERIALS AND METHODS

Microrganisms and growth conditions. The yeast used throughout this study was *C. albicans* SA-40, first isolated from the vaginal secretions of women with acute vaginitis (7). For the experimental infection (see below), a stock strain from Sabouraud-dextrose agar (Difco Laboratories, Detroit, Mich.) was grown in YEPD medium (yeast extract, 1%; neopeptone, 2%; dextrose, 2%) for 24 h at 28°C under mild shaking at 200 rpm; it was then harvested by centrifugation (3,500 × *g*), washed, and suspended to the required concentration in saline solution.

Animals. Oophorectomized female Wistar rats (80 to 100 g; Charles River Breeding Laboratories, Calco, Italy) were used throughout this study. Animal maintenance and overall care were as described elsewhere (6, 9).

Experimental rat vaginitis. All rats were maintained under pseudoestrus by injection of estradiol benzoate (Estradiolo; Amsa Farmaceutici srl, Rome, Italy). Six days after the first extradiol dose, the animals were inoculated intravaginally with 10^7 yeast cells in 0.1 ml of saline solution. The cells in the vaginal fluid were counted by culturing 1-μl samples (using a calibrated plastic loop [Disponoic; PBI, Milan, Italy]) taken from each animal on Sabouraud agar containing chloramphenicol (50 μg/ml) as previously described (6, 9). Rats were considered infected when at least 1 CFU was present in the vaginal lavage, i.e., a count of

$\geq 10^3$ CFU/ml. Some other vaginal samples were also stained by a periodic acid-Shiff–van Gieson method for microscopic examination.

Collection of VL. To elicit a sufficient number of vaginal T cells, some with anti-*Candida* specificity, we resorted to an immunization schedule consisting of a round of three consecutive infections, after resolution of each preceding infection, as previously described (13). At the end of the third infection, the vagina was aseptically removed from each sacrificed rat; the vaginal tissue was cut longitudinally and minced with a sterile scalpel in complete medium consisting of RPMI 1640 supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), L-glutamine (2 mM), sodium pyruvate (2 mM), 2-mercaptoethanol (5×10^{-5} M), and 5% heat-inactivated fetal calf serum (all from Life Technology International, Paisley, Scotland) with 25 mM HEPES buffer. The minced tissues were digested in complete medium with sterile 0.25% collagenase D (Boehringer Mannheim, Mannheim, Germany) following incubation in a shaker at 37°C for 30 min. Before, during, and immediately after the incubation period, samples were mixed in a stomacher homogenizer (Lab Blender 400; PBI). After digestion, tissues and cells were filtered through a sterile gauze mesh, washed with RPMI 1640 medium, and centrifuged three times (200, 800, and $1,800 \times g$, respectively, for 15 min each time). Finally, the cells were collected from the supernatant and resuspended in Hanks buffered salt solution and counted by trypan blue dye exclusion. About 80% of these cells were VL, as judged by their morphology in Giemsa-stained smears. At least 2×10^5 VL were collected from each rat.

Antibodies. Phycoerithrin (PE)- and fluorescein isothiocyanate (FITC)-conjugated antibodies specific for rat CD3 (pan-T), CD4, CD8α (T-cell subsets), CD5 (T cells and a subset of B cells in Wistar rats), immunoglobulin M (IgM) H chain (B cells), αβ T-cell receptor (TCR), and γδ TCR were from Pharmingen Corp. (San Diego, Calif.). Mouse FITC- and PE-conjugated antibodies (Becton Dickinson, Mountain View, Calif.) were used as negative controls.

Immunofluorescence, sorting, and flow cytometric analysis. Standard methodology was used for direct single and double VL immunofluorescence. Briefly, 2×10^5 VL from *C. albicans*-infected rats (see above) were suspended in complete medium, pelleted, and then incubated with the appropriate antibody or negative control for 30 min at 4°C. After three washes with cold phosphate-buffered saline, the VL were analyzed for relative fluorescence intensity. For double immunofluorescence, PE- or FITC-conjugated antibody was incubated for an additional 30 min on ice with the respective FITC- or PE-conjugated antibody and similarly washed with cold phosphate-buffered saline. The percentage of positively stained cells, determined over 10,000 events, was analyzed by a FACScan cytofluorimeter (Beckton Dickinson). The fluorescence intensity was expressed in arbitrary units on a logarithmic scale. Cells incubated with mouse FITC-IgG1 and PE-IgG2a control antibodies were used to determine the background fluorescence. The compensation for each fluorochrome was determined by parallel single-color analysis of cells labeled with one of the fluorochrome-conjugated antibodies.

Sorting of $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ vaginal T-lymphocyte subsets and the $CD5^+$ IgM^+ vaginal B-lymphocyte population from estrogenized and infected rats was performed on a FACStar-Plus cytometer (Becton Dickinson) equipped with an enterprise laser emitting 150 mW at 488 nm. VL (2.5×10^7) labeled with FITC-conjugated anti-CD3 monoclonal antibody (MAb) underwent two rounds of sorting by gating on fluorescence-triggered cells using FACStar-Plus software. Thus, $CD3^+$ and $CD3^-$ VL were sterilely collected and further sorted. The degree of purification of the sorted $CD3^+$ VL was about 98%, as analyzed with Cell Quest software (see Results). For each experiment in VL purification and sorting, around 80 rats were used.

Adoptive transfer of VL. VL were collected by the method described above from rats consecutively infected three times with *C. albicans* (13), separated by FACScan cytofluorimeter cell sorting, and injected (5×10^4 cells/rat) intravenously into naive, oophorectomized, estradiol-treated rats. Control rats received the same number of VL taken from uninfected animals. After 24 h, all of the rats were infected with 10^7 cells of *C. albicans*, and the course of infection was followed by CFU counts, as described above.

Histology. The rats were sacrificed, and the vaginas were removed and immediately fixed in 10% (vol/vol) neutral buffered formalin. After dehydration in a graded ethanol series and clearing with xylene, the material was embedded in paraffin and 8-μm-thick sections were stained with hematoxylin-eosin for observation under the light microscope.

Data assessment and statistics. With the exception of the single experiment of adoptive transfer of vaginal $CD4^+$ and $CD8^+$ T cells, all other experiments were repeated once and are here reported as typical experiments or as pooled determinations. Statistical comparisons among various groups of animals undergoing adoptive cell transfer were carried out by analysis of variance (ANOVA) followed by Bonferroni's multiple *t* tests. The statistical significance of the differences was always set at a *P* value of ≤0.05, two tailed.

TABLE 1. Phenotype characterization of $CD3^+$ VL after three rounds of infection with *C.albicans*[a]

Phenotype	% Expression in vaginal T cells from:	
	Uninfected estrogenized rats	Infected rats
$CD3^+$ $\alpha\beta^+$	71 ± 1.3	81 ± 1.3
$CD3^+$ $\gamma\delta^+$	29 ± 1.2	19 ± 2.0
$CD4^+$ $CD8^-$	39 ± 1.2	51 ± 1.5
$CD8^+$ $CD4^-$	61 ± 1.3	45 ± 2.2
$CD4^+$ $\alpha\beta^+$	18 ± 1.0[b]	41 ± 1.7[b]
$CD8^+$ $\alpha\beta^+$	53 ± 1.3[b]	38 ± 0.7[b]
$CD4^+$ $\gamma\delta^+$	17 ± 1.1	11 ± 0.8
$CD8^+$ $\gamma\delta^+$	12 ± 0.9	10 ± 1.5

[a] Measured after pooling the VL of 10 rats in each category. The average ratio of $CD4^+$ to $CD8^+$ T cells in the blood of nonestrogenized, noninfected rats was 3 (more or less 10% variation) (13). Both groups of rats were under similar estrogen treatments.

[b] Fraction of $CD4^+$ (or $CD8^+$) T cells with the indicated TCR phenotype out of all $CD3^+$ T cells with that phenotype.

RESULTS

Phenotypic characterization of VL populations. In initial investigations, VL were isolated from collagenase-digested vaginal tissue on day 7 of the third infection round, as well as on the corresponding day of estrogen treatment in uninfected rats, and their phenotypes were characterized by FACscan. The $CD3^+$ T cells represented about two-thirds of the total viable cells harvested from rat vaginas. The $CD3^-$ VL were not extensively characterized, but the majority of them expressed the CD5 marker, which is present on certain B-lymphocyte subsets in Wistar rats (23, 27) (see below for further details). Background fluorescence, as determined by cellular staining with mouse PE-conjugated IgG1 and FITC-conjugated IgG2b, was negligible and had no effects on VL staining (data not shown).

Following dual labeling with FITC- or PE-conjugated anti-CD3 antibodies coupled with PE-conjugated anti-αβ TCR or FITC-conjugated anti-γδ TCR MAbs, about 80% of the $CD3^+$ VL from infected, immunized rats expressed the αβ TCR and the remainder expressed the γδ receptor (Table 1). Moreover, as evaluated by dual labeling with PE- or FITC-conjugated anti-αβ (or anti-γδ) TCR antibody together with either anti-CD4 or anti-CD8 MAb, about 50% of both $CD4^+$ and $CD8^+$ VL subsets of infected rats expressed αβ or γδ TCR (Table 1). In the VL from rats after the third round of infection, the $CD4^+/CD8^+$ ratio was confirmed to approach 1/1. Confirming previous results (13), the vaginal T-cell population, which appeared to expand more following the three rounds of infection and the consequent immunization, was made up of $CD4^+$ $\alpha\beta^+$ TCR cells, which almost doubled with respect to their number in the vaginas of uninfected rats (Table 1) (13).

Protection against *C. albicans* vaginal challenge by adoptive transfer of VL from *Candida*-infected, immunized rats. To assess the extent to which the VL collected from *Candida*-infected rats could play a role in protection against a vaginal challenge by *C. albicans*, we performed adoptive-transfer experiments. Thus, total VL and sorted, purified $CD3^+$ T cells, $CD4^+$ or $CD8^+$ T cells, or $CD3^-$ $CD5^+$ IgM^+ B cells from immunized rats were injected intravenously into nonimmunized, uninfected rats as a single administration 24 h before the usual intravaginal *C. albicans* challenge. The controls were

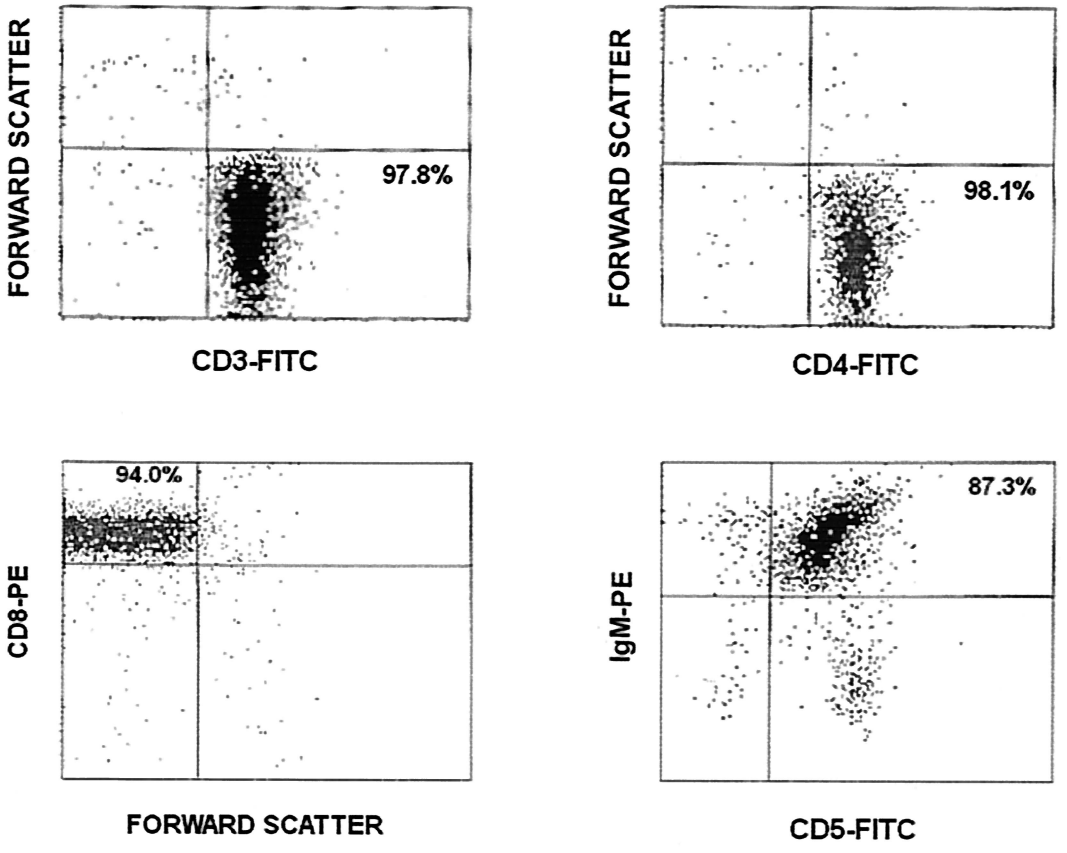

FIG. 1. Fluorescence-activated cell sorting of total VL and sorted T-cell subsets from *C. albicans*-infected rats. Total, $CD3^+$, and $CD3^-$ VL from *C. albicans*-infected rats, labeled with FITC-conjugated anti-CD3; FITC-conjugated anti-CD4; or PE-conjugated anti-CD8, FITC-conjugated anti-CD5, and PE-conjugated anti-IgM MAbs, respectively, were sorted by gating on fluorescence-triggered cells using FACStar-Plus software. The number in each quadrant represents purified sorted $CD3^+$, $CD4^+$, $CD8^+$, and $CD5^+$ IgM^+ VL as analyzed with Cell Quest software. For details, see the text.

animals receiving VL from uninfected rats or *C. albicans* cells only. The degree of purification of the sorted cell populations was assessed cytofluorimetrically and shown to be around 98% for both total $CD3^+$ and $CD4^+$ T cells and 94 and 87.3% for $CD8^+$ T cells and $CD5^+$ IgM^+ B cells, respectively (Fig. 1).

Figure 2 shows the kinetics of fungal clearance from the rat vagina in one of two independent experiments in adoptive transfer of total VL or $CD3^+$ T cells or $CD5^+$ IgM^+ B cells. Control rats receiving only *C. albicans* cells had the usual course of vaginal infection (9, 13), with a high *Candida* burden in the first week of infection followed by a progressive decline of fungal CFU and spontaneous healing of the infection after the third week. The same kinetics of infection was shown by the animals which received nonimmune VL from uninfected rats. In contrast, the animals receiving immune VL or immune $CD3^+$ T cells showed a significant acceleration of fungus clearance, as early as 1 day after challenge. The acceleration persisted for the whole course of infection so that at each measurement of fungal clearance a statistically significant difference was detected between the animals which received naïve VL and those which received immune VL or $CD3^+$ T cells (Fig. 2).

A comparison of the infection course in rats receiving total immune VL with that in rats receiving transferred immune $CD3^+$ T cells showed an apparently more rapid decline of vaginal *Candida* CFU in the rats with the transferred T cells, but the difference never reached statistical significance. The animals with transferred vaginal immune B cells had fewer CFU than the controls, but their initial rate (in the first 4 to 6 days) of vaginal CFU decline was significantly lower than that measured in animals administered total immune VL or T cells. Nonetheless, the clearance curves tended to overlap after the initial period, and no statistical significance was detected any longer between the numbers of vaginal CFU of rats with any transferred immune cell population.

In another experiment, the animals received adoptively transferred $CD4^+$ or $CD8^+$ vaginal T lymphocytes and were compared with animals given nonimmune VL as a control. As shown in Fig. 3, adoptive transfer of $CD4^+$ cells caused a much higher rate of vaginal CFU decline, mostly in the first week postchallenge, than that in the rats administered nonimmune VL or $CD8^+$ vaginal T cells. Importantly, at variance with all other adoptive transfers (Fig. 2), the infection was substantially cleared (<1 CFU/μl of vaginal fluid) at the end of the second week postchallenge. $CD8^+$ vaginal T cells, however, still caused a small but statistically significant reduction in the *Candida* vaginal burden compared to that in the animals given VL from nonimmunized rats.

By pooling all the data from adoptive-transfer experiments and making a single statistical comparison limited to the first

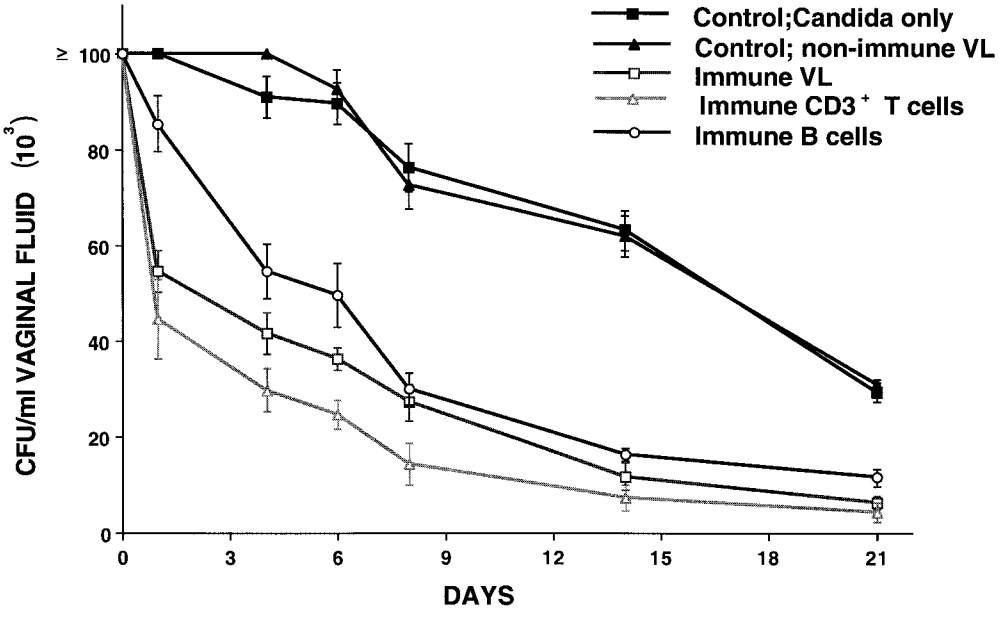

FIG. 2. Kinetics of vaginal infection by *C. albicans* in oophorectomized estradiol-treated rats intravenously injected (24 h before intravaginal *C. albicans* challenge) with saline (control), nonimmune VL, immune VL, immune $CD3^+$ T cells, and immune B cells, as indicated. Each curve represents the mean (± standard error) of the fungal CFU of five rats. Twenty nonimmunized and 80 *C. albicans*-immunized rats were used in this and an independent, similar experiment (data not shown) to obtain the cells for transfer. There was a highly significant reduction ($P < 0.01$; ANOVA and Bonferroni's multiple *t* tests) in CFU counts in animals receiving immune total VL, immune $CD3^+$ T cells, or immune B cells compared to either control. There was also a statistically significant ($P < 0.05$) difference between the CFU counts of rats given transferred T cells and those which received B cells on days 1, 4, and 6. No other significant difference was detected.

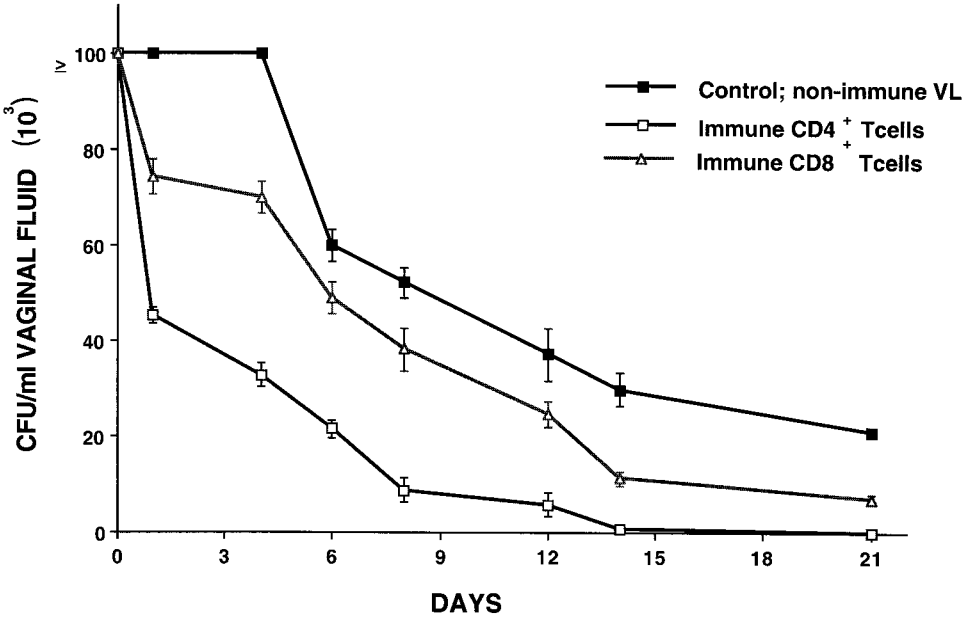

FIG. 3. Kinetics of vaginal infection by *C. albicans* in oophorectomized estradiol-treated rats intravenously injected (24 h before intravaginal *C. albicans* challenge) with nonimmune VL, immune $CD4^+$ T cells, or immune $CD8^+$ T cells as indicated. Each curve represents the mean (± standard error) of the fungal CFU of five rats. Ten rats were used to obtain the nonimmune VL, whereas 40 *C. albicans*-immunized rats were used to obtain and sort $CD4^+$ and $CD8^+$ T VL. There was a highly significant reduction ($P < 0.01$; ANOVA and Bonferroni's multiple *t* tests) between the CFU counts of animals receiving immune $CD4^+$ T cells and those receiving nonimmune VL. The animals with transferred immune $CD8^+$ T cells also had fewer CFU than the control rats ($P < 0.05$). There was also a significant difference ($P < 0.05$) between the vaginal CFU counts in the animals receiving $CD4^+$ T cells and those receiving $CD8^+$ T cells on days 1, 4, 14, and 21.

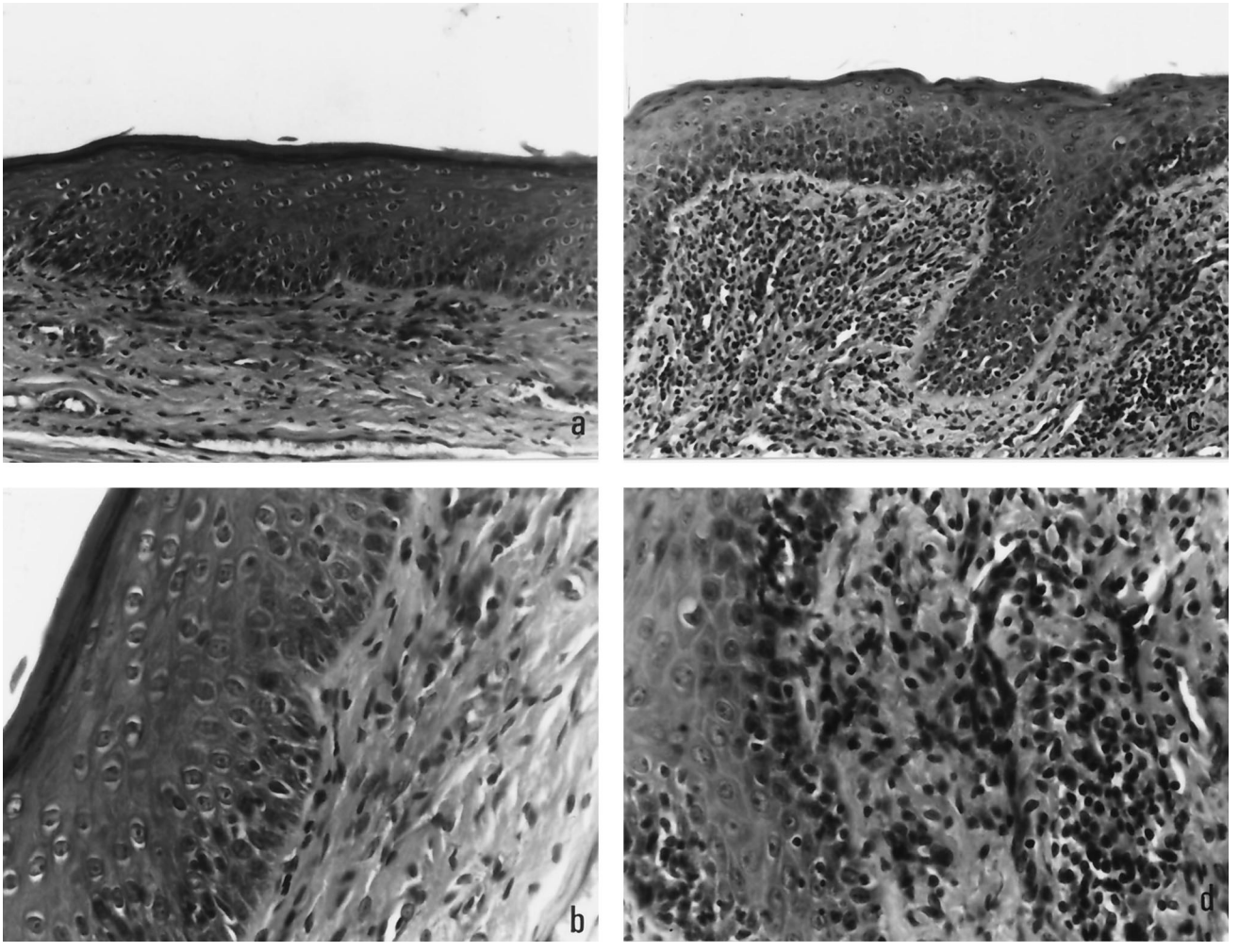

FIG. 4. Sections of rat vagina taken on day 3 after intravenous injection of naïve (a and b) or immune (c and d) VL. The sections were stained with hematoxylin-eosin. Magnification, ×170 (a and c) and ×340 (b and d).

week of infection (the rapid-clearance period), the rank of anti-*Candida* efficacy of adoptive transfer was as follows: $CD4^+$ $CD3^+$ T cells > $CD3^-$ $CD5^+$ B cells > $CD8^+$ T cells.

Histology. Sections of the vaginal tissues were examined for evidence of cell infiltration and other histological changes after adoptive transfer of VL from naïve and immune rats into uninfected rats. No or very few mononuclear cells were detected within the mucosal tissue in animals receiving naïve VL (Fig. 4a and 4b). In contrast, in the animals receiving immune VL, numerous infiltrating inflammatory cells, mostly lymphocytes based on their aspect and staining, were observed in the subepithelial lamina propria of the vaginal tissue and also infiltrating the epithelium of the mucosal tissue (Fig. 4c and d).

DISCUSSION

There are many clinical observations and a number of experimental studies which suggest that in the acquired resistance to *Candida* infection, the participation of T lymphocytes is critical (3, 4, 22, 30, 31, 32). However, the exact role, played by these cells, and their specific subsets, to combat each of the multifaceted diseases caused by this fungus is not known. This situation is particularly complicated in the case of vaginal candidiasis. Vaginal tissue does not contain an organized lymphoid tissue, but it appears to be highly responsive to local (18, 19) or distant mucosal (14) or even systemic immunization with suitable *Candida* antigens (21, 37, 38) so that a protective antimicrobial response at the vaginal level can easily be achieved. Interestingly, however, this local protection can occur in the absence (17) or in the presence (13) of modifications in the vaginal T cells with (13) or without (17) evidence of a specific locally induced (13) or systemically derived (28) anti-*Candida* immune response. From studies by Mulero-Marchese et al. (28), it is also clear that immune responses at the vaginal level are under tight genetic control and may be quite distinct in even genetically close strains of mice (5, 29), probably also explaining the clear-cut differences in the immune responses underlying protection in mouse (17), rat (13), and human vaginal candidiasis.

We have long been studying a model of rat vaginal candidiasis which is mostly characterized by the following features of immune responses to *Candida*: (i) acquired resistance to reinfection after clearance of the primary infection, (ii) activation

and expansion of local T cells, and (iii) induction of both humoral and cellular *Candida*-specific immunity (13). In particular, antibodies against specific virulence factors and immunodominant cell surface antigens were produced during infection or active immunization, and these antibodies could passively transfer anti-*Candida* protection (6, 9).

Since in congenitally athymic nude rats antibodies were not produced and protection was not achieved after healing of the primary infection (9), a critical role for T cells in the induction of immunity at the vaginal level was postulated (9), in agreement with previous results in mice (4). This assumption was subsequently strengthened by the demonstration of *Candida*-specific T-cell responses, restricted to the vaginal level and associated with protection, in *Candida*-immunized animals (13).

Along this line, we have further investigated the nature and phenotype of vaginal T cells in immunized and protected rats and used these cells for adoptive-transfer experiments. An interesting and somewhat unexpected outcome of these experiments is the discovery that a variety of VL populations appear to participate in anticandidal protection at the vaginal level. This multiactor participation seems to be highly efficient, as demonstrated by the fact that a single inoculation of a relatively low ($<10^5$) number of vaginal immune cells, given 1 day before challenge, was sufficient to achieve a degree of protection. The high potency of the adoptive transfer may depend on the fact that vaginal, not splenic, lymphocytes were transferred and that a large proportion of the immune vaginal T cells were probably *Candida* specific, as suggested by the extent of vaginal cell proliferation in response to *Candida* antigen (reference 13 and data not shown). Among the VL, however, the $CD4^+$ T cells were apparently exerting the most extensive protection, as the accelerated clearance of the fungus from the vaginas of rats with adoptively transferred immune $CD4^+$ T cells was greater than the clearance observed in rats infused with an equal number of $CD8^+$ T cells or $CD5^+$ B cells. Moreover, only the infusion of $CD4^+$ T cells caused the substantial elimination of the fungus from the vagina as early as 2 weeks after challenge.

That protection at the vaginal level could be mediated by local effectors is a notion that has long been circulating in the field, but to our knowledge this is the first formal demonstration that vaginal $CD4^+$ T cells, and other VL, do indeed exert a protective role against vaginal candidiasis. Interestingly, splenic $CD4^+$ T cells from subcutaneously immunized BALB/c mice also conferred protection against candidal vaginitis (28, 29), in clear contrast to other data (1). The data of Mulero-Marchese et al. (28, 29) clearly imply that peripheral $CD4^+$ T cells in immunized mice can migrate to the vagina and there exert a protective role. A similar event probably occurs in T-cell-deficient mice, in which the adoptive transfer of T cells from *Candida*-primed or even nonimmune normal mice confers an accelerated fungal clearance from the oral cavity (16). In our experimental model, the rats are naturally immunized by the healing of a primary vaginal infection, which makes them resistant to all subsequent rounds of infection (6, 9). In these animals, the immunization (and protection) is totally restricted to the vaginal level, and no *Candida* antigen-responsive T cells are found in the blood or in the spleen (13). Despite these remarkable differences, one common and important trait of our data and those of Farah et al. (16) and Mulero-Marchese et al. (28, 29) is the major role assigned to the $CD4^+$ T cell in oral and vaginal protection, consistent with the importance of this cell subset in mucosal candidiasis (3, 31). A recent observation by Wormley et al. (39) would indirectly support the concept that $CD4^+$ $\alpha\beta$ TCR rather than $\gamma\delta$ TCR cells exert the protection, as suggested by the resistance of $\gamma\delta$-T-cell knockout mice to experimental vaginal candidiasis. However, the remarkable differences in local immune responses to *Candida* in mouse and rat models have already been remarked upon (13).

While these studies emphasize the protective role of VL against vaginal candidiasis, the exact mechanisms whereby these cells confer protection are far from being defined. Work performed mostly with systemic models of candidiasis does clearly indicate a central mechanism of protection in the production of gamma interferon (IFN-γ) and responsiveness to that and other cytokines (30). We have previously shown that IFN-γ is indeed present, in nanogram amounts, in the vaginal fluid of protected rats (13). While clinical and experimental evidence clearly supports this role in several mucosal infections by *Candida*, this could not be the case in vaginitis. For instance, human immunodeficiency virus-positive (HIV^+) subjects with low $CD4^+$-T-cell counts, who are exquisitely susceptible to oral candidiasis, do not differ from HIV^+ women or HIV^- women at risk of HIV infection in the prevalence of vaginal candidiasis (11, 25). In a well-studied mouse vaginal-candidiasis model (36), it has been difficult to establish a role for any specific cytokine in the substantial absence of those effector cells (e.g., neutrophils and macrophages) which would benefit most from IFN-γ production by $CD4^+$ T cells for their anticandidal activity (31, 32). In our rat model, which has a remarkable degree of resemblance to humans in the ratio of vaginal $CD8^+$ to $CD4^+$ T cells (13, 14), passive transfer of antibodies, directed against principal virulence factors and T-cell-dependent antigens of the fungus, was clearly protective (6, 9). Thus, it can also be envisaged that a main role of $CD4^+$ T cells is to provide help to vaginal B cells for antibody formation.

Indirect support for this hypothesis comes from the partial protection conferred by the $CD3^-$ $CD5^+$ B cells, although no demonstration has been given here that those cells are indeed producing antibodies. It must also be considered that $CD4^+$ and $CD8^+$ vaginal T cells could be endowed with direct *Candida* inhibition properties. In particular, Mathews et al. have repeatedly shown that $CD8^+$ T cells inhibit hyphal growth of *C. albicans* when activated by interleukin 2 (IL-2) (2, 24). Remarkably, hyphal growth is required for vaginal infection (12), IL-2 is well represented in the vaginal fluid of protected rats (13), and the proportion of vaginal $CD4^+$ and $CD8^+$ T cells bearing the IL-2 receptor is elevated in protected rats (13), demonstrating that the great majority of these cells are indeed in an activated state. It is therefore possible that several concurrent, though not equivalent, mechanisms are involved in anticandidal protection at the vaginal level.

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