A Unique Population of Extrathymically Derived αβTCR+CD4−CD8− T Cells with Regulatory Functions Dominates the Mouse Female Genital Tract

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A better understanding of the regulatory role of genital tract T cells is much needed. In this study, we have analyzed the phenotype, distribution, and function of T lymphocytes in the female genital tract of naive, pregnant, or Chlamydia trachomatis-infected C57BL/6 mice. Unexpectedly, we found that the dominant lymphocyte population (70–90%) in the genital tract was that of CD3+αβTCRintCD4−CD8− T cells. Moreover, these cells were CD90low but negative for the classical T cell markers CD2 and CD5. The CD3+B220low cells were NK1.1 negative and found in nude mice as well as in mice deficient for MHC class II, β2-microglobulin, and CD1, indicating extrathymic origin. They dominated the KJ126+Vβ8.2+ population in the genital tract of DO11.10 OVA TCR-transgenic mice, further supporting the idea that the CD3+B220low cells are truly T cells. The function of these T cells appeared not to be associated with immune protection, because only CD4+ and CD8+ T cells increased in the genital tract following chlamydial infection. Notwithstanding this, the infected, as well as the uninfected and the pregnant, uterus was dominated by a high level of the CD3+B220low cells. Following in vitro Ag or polyclonal stimulation of the CD3+CD4−CD8−B220low cells, poor proliferative responses were observed. However, these cells strongly impaired splenic T cell proliferation in a cell density-dependent manner. A large fraction of the cells expressed CD25 and produced IFN-γ upon anti-CD3 plus anti-CD28 stimulation, arguing for a strong regulatory role of this novel T cell population in the mouse female genital tract.

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Despite much interest in the mechanisms of immune protection against sexually transmitted diseases (STD), few detailed studies have been performed of the phenotype, distribution, and function of lymphocytes found in the genital tract mucosa. In particular, a better knowledge about genital tract T cells is needed to understand not only host protection against STD, but also how tolerance is established in pregnancy to avoid rejection of the fetus. It is now well established that Th1 responses are required for protection against most bacterial or viral STD (1–4). By contrast, for successful pregnancy, it is thought that Thl activity must be suppressed, because Th1 responses have been associated with miscarriage (5). Moreover, the female genital tract is under hormonal control, and this has been shown to influence both the local distribution of lymphocytes and the presence of Ig in genital tract secretions (6–8). Susceptibility to STD and responsiveness to immunization are also affected by the menstrual cycle (6, 9).

Immunohistochemical studies have indicated that the female genital tract of healthy individuals host few immunocompetent cells and lymphoid nodules (8, 10). Thus, the genital tract mucosa does not appear to share the same immune functions as, for example, the intestinal mucosa with its M cells and Peyer’s patches. Investigations conducted in humans, mice, and Rhesus macaques have demonstrated the presence of dendritic, Langerhans-like cells and T lymphocytes in the epithelium of the vagina (7, 8, 10). The majority of the CD3− T cells were found to express CD4 and the αβ TCR, while ~20% expressed the γδ TCR (11). In the uterine endometrium, intraepithelial lymphocytes (IEL) were found both at the surface epithelium and at the glandular epithelium (reviewed in Ref. 8). Although it has been shown that these IEL T cells may secrete TGF-β, IL-2, and IFN-γ (5), our knowledge about the presence, distribution, and function of lymphocytes in the endometrium is still sparse.

A large proportion of the leukocytes in the endometrium are macrophages, which appear to be independent of hormonal control (8). These macrophages and dendritic cells can be potential APC, either locally in the mucosa (12, 13) or in the draining lymph nodes as they may traffic from the vaginal mucosa to the draining iliac lymph nodes (14). The cells that dominate the human uterine mucosa at the time for implantation are uterine NK (uNK) cells. These cells express the common leukocyte Ag CD45, as well as CD56, and appear to be under strong hormonal control. In the mouse, a similar type of cells has been found in the metrial gland (15).

The unique features of the genital tract lymphocytes, involved in protection against infection as well as in allowing conception, suggest that the local immune system in the female genital tract hosts unique cell populations not seen at other mucosal sites. Most studies have documented that the normal, healthy genital tract has few immunocompetent cells and, in the virgin naive mouse, T cells are sparsely distributed in the endometrium and B cells and plasma cells are seen only occasionally (8). However, a coherent understanding of the mucosal immune system in the female genital tract is just emerging. In this study, we have analyzed the distribution of lymphocytes in female virgin or pregnant naive mice or mice recovering from a genital tract infection with Chlamydia trachomatis.
have identified a novel population of small CD3<sup>+</sup>CD25<sup>+</sup>
CD4<sup>−</sup>CD8<sup>−</sup> T cells in the mouse female genital tract that differs from
other known T cell populations in the body. These cells exhibited
impaired proliferative responses, but produced cytokine and ex-
pressed high levels of CD25 and were strongly down-regulatory on
spleenic T cell proliferation. The CD3<sup>+</sup> B220<sup>+</sup>CD4<sup>−</sup>CD8<sup>−</sup> T cells were found in the genital tract of nude mice, MHC class II<sup>−</sup>, β<sub>2</sub>-microglobulin (β<sub>2</sub>-m)<sup>−</sup>, and CD1<sup>−</sup> mice, suggesting that they
constitute a unique population of extrathymically derived regula-
tory T cells (Tr cells).

Materials and Methods

Animals

Female mice (8–10 wk old) were used at the onset of each experiment.
MHC class II-deficient (16), β<sub>2</sub>-m-deficient (17), and CD1-deficient mice (18)
were kindly provided by Dr. S. Cardell (University of Lund, Lund,
Sweden). C57BL/6 and nude mice were purchased from B&K Universal
(Sollentuna, Sweden). OVA TCR-transgenic (Tg) mice (19) were bred in
ventilated cages under pathogen-free conditions at the animal facility of
Department of Medical Microbiology and Immunology (University of
Göteborg).

Chlamydial bacteria

A human genital tract isolate of C. trachomatis serovar D was propagated
in buffalo green monkey kidney cells by conventional techniques (20) and
stored frozen at −70°C.

Bacterial inoculation of the female genital tract

The mice were inoculated as previously described (21, 22). Briefly, the mice were given s.c. injections with 2.5 mg/dose of Depo-Provera (me-
droxyprogesterone; Upjohn, Puurs, Belgium) 7 days before the intravaginal
inoculation with live chlamydial infectious elementary body. The infect-
ous dose of C. trachomatis serovar D used throughout the study for pri-
mary and challenge infections was 100 ID<sub>50</sub>, corresponding to 6 × 10<sup>4</sup>
infectious elementary body determined by culture in buffalo green monkey
kidney cells. Before evaluating host protection in immune mice following
resolution of the primary infection, all mice were tested negative for bac-
terial shedding.

Detection of genital tract infection

Mice were screened for chlamydia shedding using both conventional cul-
ture techniques and a commercial kit, the Syva MikroTrak, EIA, and im-
munofluorescence kit (Syva, San Jose, CA), according to the manufactur-
er’s instructions, as previously described (21, 23).

Pregnancy

For matings, naturally cycling virgin C57BL/6 females were caged with
C57BL/6 males. The day of detection of a vaginal plug was designated day 1
of pregnancy. On day 4 of pregnancy, the uterus was excised, as de-
scribed below in the following section.

Isolation of genital tract lymphocytes and intestinal IELs (iIELs)

The iIELs were isolated as previously described (24). This method was
modified for the isolation of genital tract lymphocytes. Briefly, the uterus and
the cervix were excised, and all surrounding fat was removed. The tissue
was cut in small pieces. The pieces were treated with collagenase (type C2139; Sigma-Aldrich, St. Louis, MO) for 1 h at 37°C with stirring.
The supernatant was collected and centrifuged at 2000 rpm for 5 min. The
supernatant was discarded and the pellet was resuspended in Iscove com-
plete medium and kept on ice until staining. The remaining tissue pieces were
once again treated with collagenase as before. This procedure was
repeated twice. After three incubations, the collected cells were washed
once, diluted in Iscove complete medium, and then counted.

FACS analysis

The cells were suspended in 0.1% BSA/PBS to a concentration of 10<sup>6</sup>
cells/ml and kept on ice. A volume of 100 μl was aliquoted in tubes. The cells were treated with anti-Fc<sub>γ</sub>-HiR Ab at 1/100 for 5 min. Thereafter,
FITC- and/or PE-labeled mAbs from BD Pharmingen (San Diego, CA)
were added to the cells and incubated for 30 min. Three corresponding
isotype control Abs were used: hamster IgG1 (Fig. 1J), rat IgG1 (Fig. 7A),
and rat IgG2a (Fig. 1K) labeled with FITC and/or PE (BD Pharmingen).

The cells were washed with 0.1% BSA/PBS twice and finally suspended in
500 μl of 0.1% BSA/PBS and analyzed in a FACScan (BD Biosciences,
San Jose, CA). Lymphocytes were analyzed by using forward and side
scatter to exclude other cells and dead cells. The gates were set using
spleenocytes, and compensation of the FL1 and FL2 channels was per-
formed to place all unstained cells in the first quadrant of the dot plot.

Immunohistochemistry

The cervix, uterus, tubes, and ovaries were removed, snap frozen, and
processed, as previously described. To block endogenous peroxidase ac-
ivity, slides were treated with 0.3% H<sub>2</sub>O<sub>2</sub> for 5 min and then stained with the following unlabeled or labeled Abs: anti-CD3 complex, FITC-anti-
CD45R, and anti-CD45R (BD Pharmingen). For CD3 and CD45R, a bi-
otin-conjugated rabbit anti-rat IgG (Vector, Burlingame, CA), diluted 1/100, was used as a secondary Ab. Thereafter, a peroxidase-conjugated
avidin (ABComplex; DAKO, Glostrup, Denmark) or a Texas red-labeled
streptavidin was added to each slide and incubated for an additional hour
at room temperature. The staining was developed using a peroxidase
3-amino-9-ethylcarbazole substrate kit (Vector), according to the manu-
facturer’s instructions. Before inspection in the microscope, the tissue was
washed and counterstained with HTX (Histolab Products, Via Fröslunda,
Sweden) and mounted in Aquamount (BDH Laboratory Supplies, Poole,
U.K.). Sections were evaluated and photographed using a Zeiss Axioskop
microscope (Zeiss, Cambridge, U.K.). The labeling was highly specific; no
staining was observed with an isotype-matched irrelevant control Ab as the
primary Ab, and no staining was observed with the second-step anti-rat IgG
(Vector) Ab used alone without a primary Ab.

Purification of B220<sup>+</sup> cells

Streptavidin-coated beads from the CELLection kit (Dynal, Oslo, Norway)
were precoated with biotinylated anti-CD45R (BD Pharmingen). The
positive selection was conducted according to the manufacturer’s instructions.
Briefly, a single-cell suspension of genital tract cells was incubated on ice
for 15 min with the appropriate amount of precocated beads allowing the
beads to attach to the B220<sup>+</sup> cells. The attached cells were sorted out using
a magnet to collect the magnetic beads. The bead-cell complexes were washed
twice and then diluted in 1% FCS/RPMI 1640. To release the cells from the
beads, an accompanying releasing buffer was added. The mixture was incu-
bated for 15 min at room temperature. Thereafter, the magnetic beads were
separated from the selected cells using the magnet. The cells were washed,
counted, and diluted in Iscove’s complete medium until further use.

In vitro culture

In a 96-well tray, single-cell suspensions from the spleen and the genital tract and positively selected B220<sup>+</sup> cells were stimulated to assess prolif-
eration and cytokine production. Spleen cells from the same animals were
used as controls. For the B220<sup>+</sup> cells, T cell-depleted spleen cells, irradi-
ated with 25 Gy, were used as APCs at a concentration of 1 × 10<sup>5</sup>
cells/well or as indicated in Fig. 7C. For cytokines, the cul-
tures were incubated in triplicate wells with the following additions: medium, 20% anti-CD3, 20% anti-CD3 plus 1 μg/ml anti-CD28, 1 μg/ml Con A, 1 mg/ml OVA,
and 1 μg/ml p223. The cultures were incubated for 72 h at 37°C and 5% CO<sub>2</sub>
with the addition of [H]thymidine for the last 6 h. Thereafter, the
plates were stored at −20°C until further analysis. For cytokines, the cul-
tures were incubated for 96 h. Thereafter, the supernatants were
analyzed using ELISA.

IFN-γ ELISA

Ninety-six-well, flat-bottom plates (Nunc, Roskilde, Denmark) were coated with
polyclonal rabbit anti-mouse IFN-γ (R&D, BD Pharmingen) in PBS at 4°C
overnight. After washing and blocking, serial dilutions of supernatants and
IFN-γ (PanData, Rockville, MD) were added to the plates and incubated
overnight. After washing, the plates were incubated with polyclonal rabbit
mouse IFN-γ serum at 1/300 dilution followed by an alkaline phos-
phatase-conjugated goat anti-rabbit Ig (Southern Biotechnology, Birming-
ham, AL) at 1/300 dilution. After washing a phosphatase substrate, p-
-nitrophenyl phosphate substrate tablets (Sigma-Aldrich) were added. The
immunoenzymatic reaction was determined as absorbance in each well
using a Titrerec Multiscan spectrophotometer (Flow; Huntsville, AL) at
405 nm. The concentrations were expressed in ng/ml as calculated from
the plotted standard curves of serial dilutions of the recombinant cytokine.
The sensitivity of detection was 1 ng/ml.

Statistical analysis

We used Student’s t test for unmatched data for analysis of significance.
FIGURE 1. A population of CD$^+$ B220$^{low}$ T cells dominates the female genital tract. UL were isolated from naive female mice using collagenase. The cells were stained with FITC- or PE-conjugated Abs and analyzed in a FACScan. The UL were compared with lymphocytes from the spleen (SP) and IEL from the same animals. UL: A, B220 (CD45R)-FITC and CD3-PE; B, TCR $\alpha\beta$-FITC and TCR $\gamma\delta$-PE; and C, CD4-FITC and CD8-PE. SP: D, B220-FITC and CD3-PE; E, TCR $\alpha\beta$-FITC and TCR $\gamma\delta$-PE; and F, CD4-FITC and CD8-PE. IEL: G, B220-FITC and CD3-PE; H, TCR $\alpha\beta$-FITC and TCR $\gamma\delta$-PE; I, CD3-FITC and CD4-PE; J, UL stained with isotype control hamster IgG1; K, UL stained with isotype control rat IgG2a; and L, CD3-FITC and CD8-PE on iIEL. The results are representative of at least 20 experiments.

Results

CD$^+$ B220$^{low}$ T cells dominate the mouse female genital tract

T cells in the mucosal immune system of the genital tract are known to play a key role in host protection against infectious diseases. Despite this, few studies have analyzed in detail the distribution and phenotype of T cells in the genital tract mucosa. To this end, we adapted and modified a method previously used to obtain lymphocytes from gut lamina propria to isolate cells from the genital tract of female mice (24). The uterus and the upper part of the cervix were excised from normal C57BL/6 mice. The tissue was cut into small pieces, which were treated with collagenase to give single-cell suspensions of UL. As assessed by FACS, only 10–20% of the cells were lymphocytes according to the preset forward- and side-scatter gates (using conventional spleen lymphocytes), and most of these cells were labeled with anti-CD3 mAb. The collagenase treatment per se did not alter the expression of surface markers as confirmed by FACS analysis of splenocytes before or after collagenase treatment (not shown). Using preset forward- and side-scatter gates, which were based on the identification of splenic lymphocytes, we found that only 10–20% of the genital tract cells were lymphocytes, and most of these cells were labeled with anti-CD3 mAb. The CD3$^+$ lymphocytes were dominated by a population of cells that was also B220$^{low}$ (70–90%), a marker found most commonly on B lymphocytes (Fig. 1A). Virtually all CD3$^+$ B220$^{low}$ cells expressed the $\alpha\beta$ TCR, which indicated that they were T cells (Fig. 1B). Less than 2% of the UL expressed the $\gamma\delta$ TCR (Fig. 1B). By comparison, splenic CD3$^+$ cells did not express B220, whereas a minor population (<20%) of gut intraepithelial CD3$^+$ cells expressed low levels of B220 (Fig. 1, D and G). Most CD3$^+$ cells from both spleen and IEL expressed higher levels of $\alpha\beta$ TCR compared with the UL (Fig. 1, E and H). Furthermore, the UL were CD4$^-$ and CD8$^-$ (Fig. 1C), in contrast to CD3$^+$ cells from the spleen and IEL, of which a majority were either CD4$^+$ or CD8$^+$ cells (Fig. 1, F, I, and L). Thus, the UL appeared to constitute a phenotypically novel and unique T cell population in the body.

Using immunohistochemistry on frozen tissue sections of the uterus, we could verify the presence of T cells in the genital tract mucosa. CD3$^+$ T cells (Fig. 2A) and B220$^{low}$ cells (Fig. 2B) were found in the endometrium of the uterus. Using double-staining, the presence of CD3$^+$ B220$^{low}$ T cells (Fig. 2C) could also be demonstrated throughout the genital tract mucosa, in the uterus as well as the cervix.

Extending the analysis, we positively selected ULs on the basis of B220 expression. Nearly all of the positively selected cells expressed CD3 as well as the $\alpha\beta$ TCR (Fig. 3A), strongly suggesting that these cells were truly T cells. Only 1% of the B220$^{low}$ cells expressed CD19 (Fig. 3B), excluding the possibility that these cells could be B cells. Moreover, of other surface markers normally expressed on conventional T cells, we found that our cell population was CD2$^+$, CD5$^+$, and CD90$^{low}$ (Thy1) (Fig. 3, C and D), while splenic CD3$^+$ cells were CD2$^+$, CD5$^+$, and CD90$^{high}$ (not shown). The NK1.1, associated with NKT cells, a subset of CD3$^+$ T cells, was expressed by only a small fraction of the UL (Fig. 3E).

FIGURE 2. CD3$^+$ B220$^+$ T cells are found throughout the genital tract mucosa. Frozen tissue sections of the uterus taken from naive mice were labeled with rat anti-CD3 complex or rat anti-B220 Abs followed by biotin-conjugated rabbit anti-rat IgG and developed using peroxidase and FITC- and PE-labeled streptavidin. Sections from naive mice exhibited CD3$^+$ cells (A) as well as B220$^+$ cells (B) scattered throughout the genital tract mucosa. CD3 and B220 were expressed by the same cells (C), as shown by the double-staining of cells using FITC-labeled anti-B220 and Texas red-labeled anti-CD3, giving rise to the orange-like cells. A and B, ×10 magnification; C, ×200 magnification.
CD3 + B220low T cells may be involved in pregnancy but appear not to affect host protection against a genital tract infection

We and others have previously shown that CD4 + T cells are critical in the clearance of a genital tract infection with C. trachomatis (3, 25, 26). Very few CD4 + T cells were found in the genital tract of naive mice (Fig. 6B). However, at 8 days following infection, we observed an increase in the CD4 or CD8 single-positive populations with a subsequent decrease in the frequency of CD3 + B220low cells (Fig. 6C). However, in the infected uterus, more cells accumulated, and therefore the decrease in frequency was not accompanied by a drop in absolute numbers of CD3 + B220low cells. After infection, at 20 days, we observed 10% CD4 + (Fig. 6D) and 8% CD8 + T cells (Fig. 6D) in the uterus. The fact that expression of CD2, CD5, and CD90 also increased following infection (not shown) suggests that the CD4 + and CD8 + T cells were recruited into the uterus and that the CD3 + B220low population took no active part in host protection against C. trachomatis disease.

Table I. Vβ chain distribution

| Vβ Chain on T Cells | Percentage of CD3 + Cells |
|---------------------|---------------------------|
|                     | Spleen | Uterus |
| 2                   |     5  |   2.5 |
| 3                   |     5  |   5    |
| 4                   |     7  |   2.5 |
| 5.1/5.2             |     7  |   <1   |
| 6                   |     7  |   <1   |
| 7                   |     2  |   <1   |
| 8.1/8.2             |     18 |   33   |
| 8.3                 |     7  |   <1   |
| 9                   |   <1   |   <1   |
| 10b                 |     5  |   <1   |
| 11                  |     5  |   2.5 |
| 12                  |     2  |   <1   |
| 13                  |     2  |   <1   |
| 14                  |     5  |   2.5 |
| 17a                 |   <1   |   2.5 |
FIGURE 5. The CD3\(^{+}\)B220\(^{low}\) T cells in the genital tract express common VB TCR chains. Genital tract lymphocytes were isolated from naive female mice. Different T cell populations have been shown to differ in their usage of VB-chains. In a normal naive mouse spleen, ~20% of the T cells express the VB 8 chain; TCR VB 8.1 8.2-FITC and CD3-PE in spleen (B); although the expression was not that defined in the UL, an increase was found: TCR VB 8.1 8.2-FITC and CD3-PE in UL (A). In OVA TCR-Tg mice, all CD3\(^{+}\) T cells both in the genital tract (C) and in the spleen (D) expressed the VB 8.2 chain. Most CD3\(^{+}\) T cells could also be detected with the Ab against KJ126, specific for the Tg TCR, in both genital tract (E) and the spleen (F).

An alternative function of these cells could be linked to pregnancy. Therefore, we pooled ULs from day-4 pregnant mice. No apparent difference in absolute numbers of CD3\(^{+}\)B220\(^{low}\) UL was observed during early pregnancy (not shown). Neither did we detect any phenotypical difference in the CD3\(^{+}\)B220\(^{low}\) population in pregnant mice compared with naive controls (Fig. 6, E and F). Furthermore, using intracellular staining of cytokines in situ, in tissue sections, we could detect increased production of neither Th1 (IFN-\(\gamma\)) nor Th2 (IL-4 and IL-10) cytokines (not shown).

**Strong down-regulation of T cell responses by the CD3\(^{+}\)B220\(^{low}\) ULs**

Tr cells may exert their function by cell-cell contact-dependent mechanisms rather than by the production of cytokines (27). Expression of CD25 may indicate a regulatory role of T cells. We found that a majority of the CD3\(^{+}\)B220\(^{low}\) population was expressing CD25 on their surface (Fig. 7A), comparable to the levels found on CD25\(^{+}\) spleen cells. Although only 7% of the spleen cells express CD25 (Fig. 7A), >30% of the ULs were CD25\(^{high}\). These findings could be indicative of a regulatory role for the CD3\(^{+}\)B220\(^{low}\) ULs.

Next, we assessed the responsiveness of this novel T cell population to polyclonal activation. We found that the CD3\(^{+}\)B220\(^{low}\) T cell population failed to proliferate in response to anti-CD3 or even anti-CD3 plus anti-CD28 mAb stimulation. Despite extended efforts, using even highly enriched CD3\(^{+}\)B220\(^{low}\) cells, we could not detect proliferation at any time. Importantly, the poor proliferation did not correlate with an enhanced frequency of cells undergoing apoptosis following anti-CD3 plus anti-CD28 mAb stimulation as confirmed by TUNEL staining (not shown). Rather, the fact that CD3\(^{+}\)B220\(^{low}\) cells, selected on the basis of B220 expression using beads (>90% purity), produced IFN-\(\gamma\) following activation, suggested a regulatory role for the CD3\(^{+}\)B220\(^{low}\) cells in the genital tract (Fig. 7B). To test this hypothesis, we took advantage of our observation that CD3\(^{+}\)B220\(^{low}\) TCR-Tg \(\times\) T cells were found in the uterus of DO11.10 mice. The responsiveness of the CD3\(^{+}\)B220\(^{low}\) TCR-Tg ULs to anti-CD3, OVA, or specific peptide p323 (not shown) was dramatically impaired (Fig. 7C). More importantly, though, when cocultured with splenocytes from the DO11.10 TCR-Tg mice, the proliferation of the splenocytes was markedly decreased in comparison to that of splenocytes cultured alone (Fig. 7C). The inhibitory effect was not cell-density dependent, but a result of down-regulation of the splenic T cell responsiveness due to presence of graded numbers of inhibitory CD3\(^{+}\)B220\(^{low}\) T cells from the genital tract (Fig. 7C). The down-regulatory effect was roughly 85% with the highest density of CD3\(^{+}\)B220\(^{low}\) cells added to the splenocyte cultures. In these cultures, IFN-\(\gamma\) was detected but not IL-10 or soluble TGF-\(\beta\) (not shown). Taken together, a majority of the CD3\(^{+}\)B220\(^{low}\) cells were CD25\(^{+}\) with striking ability to down-regulate splenic T cell
responses to Ag and polyclonal activation. These data strongly suggest that the CD3\(^+\) B220\(^{low}\) T cells are a unique and novel mucosal Tr cell population located in the female genital tract.

**Discussion**

In the present study, we report on a novel population of CD3\(^+\)CD4\(^-\)CD8\(^-\) lymphocytes expressing the B cell marker B220. The CD3\(^+\)B220\(^{low}\) cells appear to belong to the T cell lineage, because they express the \(\alpha\beta\) TCR. Moreover, using TCR-Tg mice, we could demonstrate that clonotype-specific KJ-126 CD3\(^+\)B220\(^{low}\) cells were present in the genital tract of these mice and expressed the V\(\beta8\) chain used by the Tg TCR\(^+\) cells. In normal mice, the CD3\(^+\)B220\(^{low}\) cells were found to be oligoclonal with regard to V\(\beta\) chain usage, expressing 7 of the 15 V\(\beta\)-chains that we screened for. The CD3\(^+\)B220\(^{low}\) cells were found in \(nu/nu\), MHC II-, \(\beta_2m\)-, and CD1-deficient mice, indicating that they constitute an extrathymically derived T cell population.

It is well recognized that, because of the unique ability both to protect against infections and to tolerate the semiallogenic conceptus during pregnancy, the female genital tract mucosa host highly specialized cell populations not found elsewhere. The genital tract mucosa of healthy individuals has relatively few T and B cells. This is in contrast to most other mucosal sites, e.g., the intestine where large numbers of T cells can be found. Furthermore, the genital tract mucosa differs from other mucosal surfaces in that it is under hormonal control, which has been shown to influence the presence and distribution of lymphocytes and other cells in the uterus and vagina (6, 28). Although cells like macrophages and dendritic and NK cells reside in the uterus, the CD3\(^+\)CD4\(^-\)CD8\(^-\) T cell population described in the present study accounted for a majority of the lymphocytes found. These cells did not proliferate to Ag or polyclonal activators but produced cytokine, and a large proportion of them expressed CD25. The presence of specialized cells in the genital tract mucosa is not surprising. Rather it is likely that these T cells play a regulatory role and host unique functions in the genital tract mucosa.

The CD3\(^+\)CD4\(^-\)CD8\(^-\)TCR\(\alpha\beta\) B220\(^{low}\) UL found in the present study have, to our knowledge, not been described previously. Although the phenotype may in part resemble other recently reported cell populations, the distribution of cell surface markers differs from those of previously described populations. Whereas the ULs all expressed the \(\alpha\beta\) TCR, 30–50% of iIELs express the \(\gamma\delta\) TCR (29) and only a small population of these are double-negative T cells (30, 31). The fact that the CD3\(^+\)B220\(^{low}\) cells were found in MHC class II\(^+\), \(\beta_2m\)-, as well as in nude mice, suggests that they may develop extrathymically and do not need MHC class I or MHC class II for their differentiation. Extrathy- mically derived T cells are well represented at other mucosal sites, e.g., the \(\gamma\delta\) TCR\(^+\) IELs in the intestine. Such cells appear to migrate directly from the bone marrow to the intestine as can be seen in \(nu/nu\) mice (29). Moreover, because \(\alpha\beta\) TCR\(^+\) iIELs have been found after bone marrow reconstitution of lethally irradiated mice, we know that both \(\gamma\delta\) as well as \(\alpha\beta\) TCR\(^+\) iIELs can develop extrathymically (31). In this context, it may be important that nonclassical MHC-like molecules, and in particular the CD1 molecule, is important for extrathymic T cell differentiation and Ag presentation to NKT cells. Of note, significant expression of CD1 in the pregnant uterus has recently been demonstrated (32). However, the UL in the present study, which bear some resemblance to NKT cells and therefore could be CD1 restricted, were also found in CD1-deficient mice.

A literature survey finds some resemblance between the CD3\(^+\) B220\(^{low}\) ULs and other cell populations. The ULs were selected on the basis of their expression of B220, the full-length isofrom of the tyrosine phosphate CD45, which is predominantly found on B cells. Other isoforms of CD45 are found on most
lymphocytes, but recent studies suggest that B220 may be up-
regulated on T cells undergoing apoptosis. This has been reported in
thymocytes following irradiation (33), as well as in peripheral T
cells and CD8+ T cells following activation (34). In the lpr/gld
mouse (35), Mixter et al. (36) found a large proportion of the
CD3+ cells that were also B220+, which accumulated in these
animals and were not undergoing apoptosis. Thus, an emerging
theory argues that expression of B220 on T cells may be a pre-
conditioning stage for Fas-mediated apoptosis (33). However, us-
ing TUNEL staining on frozen sections of the uterus, we could not
detect increased levels of apoptotic CD3+ cells in the genital tract
compared with spleen or gut intestine (M. Johansson, unpublished
observation). B220+CD19- cells have previously been noted in
the murine female genital tract (37) but not further investigated.
Moreover, a phenotypically unusual T cell population in the vagi-
nal tissue was described by Wormley et al. (38), using a special mAb identifying an atypical form of CD4 (11). The CD4+ cells in
the vagina were detected using 2B6 but not GK1.5, two epitope-
distinct anti-CD4 Abs that are both recognized by splenic CD4+ T
cells. However, not even with this clone (2B6) did we find any
CD4+ T cells in the uterus (M. Johansson, unpublished observa-
tion). Based on this and the other observations, we would rather
suggest that the CD3+ B220+ cells in the genital tract constitute a
novel T cell population with regulatory function.

The role of various lymphocyte subtypes in pregnancy is still
unclear. In both humans and rodents, uNK cells have been dem-
onstrated in the decidua following implantation (15, 39). Like the
ULs, the uNK cells are present in both nude and SCID mice sug-
cesting an extrathymic development (8). However, unlike the ULs,
these cells do not express CD3 on their surface, although cytosolic
CD3 has been detected (8, 15). The uNK cells appear to express
some T cell markers, such as CD2, CD7, and CD38, but are de-

fined in both mice and humans, they show some discrepancies in
their surface markers. Whether the CD3+ B220+ cells described in
the present study also are present in the human uterus remains
to be investigated.

The expression of CD25 and the inability to proliferate suggest
a more regulatory role for the CD3+ B220+ cells. Some cells with
known regulatory function, such as the CD4+CD25+ cells, have
been found to exert contact-dependent down-regulatory effects on
the microenvironment rather than cytokine-mediated effects. Al-
though the CD3+ B220+ UL produced low levels of IFN-γ upon
stimulation, we hypothesized that they were more likely to act
through contact-dependent mechanisms. This was confirmed in
the coculture experiments with Ag or polyclonal stimulation of splenic
T cells, where the CD3+ B220+ cells were much more inhibitory
compared with the low level of IFN-γ that we could detect. Neither
did we detect soluble IL-10 or TGF-β in the cultures. Although we
did not attempt to detect cell membrane-bound TGF-β, as was
recently documented to play an inhibitory role (40), we speculate
that this or some other mechanism for contact-dependent down-
regulation is operational in the activities of the CD3+ B220+ UL.
Such a possible regulatory effect by the CD3+ B220+ ULs would
be difficult to detect in the pregnant mice, which could explain why
the causative agent of tolerance in pregnancy is still unknown.

In the present study, we also hypothesized that the CD3+ B220+ ULs
could be involved in protection against STDs. We found that, fol-
lowing infection with C. trachomatis, no apparent
 change within the CD3+ B220+ UL population or other evidence
for the involvement of these cells in protective immunity
was observed. However, following a genital tract infection with C.
trachomatis, we did find CD4+ T cells in the uterus. Accumulation
of leukocytes and lymphoid aggregates has been reported follow-
ing infection (41) or insemination (42) in the uterine endometrium,
and these aggregates or infiltrates contained T cells that were re-
cruited to the genital tract mucosa as a result of inflammation.
We believe that the CD4+ T cells we observed in the genital tract of
infected animals were, indeed, recruited into the mucosa, and not
derived from the local CD3+ B220+ T cells. However, this re-
 mains to be proven.

Despite several attempts to stimulate the CD3+ B220+ ULs,
we were unable to detect significant proliferation at any time.
Following TCR stimulation in the presence of anti-CD28, the
positively selected CD3+ B220+ cells produced some IFN-γ, indicat-
ing that these cells were functionally active. In the last few years,
much interest has been focused on Tr cells mediating tolerance and
suppressing autoimmune diseases. We found that coculturing of
UL with splenocytes led to an inhibition of T cell proliferation.
This was not due to high density, because cultures of equal density
without CD3+ B220+ ULs showed no inhibition of proliferation.
The inhibitory effect was rather a direct effect of the CD3+ B220+
cell density. Taken together, these results suggest a regulatory
function for the CD3+ B220+ ULs. Although most studies on Tr
cells report on CD4+CD25+ cells, some documentation on other
populations with regulatory function can be found. A novel CD3+αβ
T cell population with regulatory function was recently described by
Zhang et al. (43, 44). These Tr cells expressed IFN-γ, TGF-β, and
TNF-α, and could inhibit T cell function in an Ag-specific manner.
The latter Tr cells showed some resemblance to the CD3+ B220+
CD4+CD8+ αβ T cell population with regulatory function, which
are described in this study and which expressed CD25 on their
surface in support of our hypothesis that cells with this phe-
notype have a regulatory function. We speculate that the
CD3+ B220+ cells are functionally active and constitute a unique
subpopulation with regulatory function in the female genital tract.

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