Homing of transgenic \( \gamma \delta \) T cells into murine vaginal epithelium

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Abstract
The vaginal epithelium of normal mice contains lymphocytes of fetal thymic origin that express an invariant \( \gamma \delta \) TCR. The apparent lack of other \( \gamma \delta \) TCR species suggests that a selection mechanism might operate to regulate the localization of \( \gamma \delta \) T cells at this anatomical site. Selection might be connected to the \( \gamma \delta \) TCR or to some homing characteristic of the fetal thymic lineage that appears at day 17–18 of embryonic life. In the present studies, we investigated whether transgenic \( \gamma \delta \) cells expressing a TCR species characteristic of the subpopulation of \( \gamma \delta \) T cells found in the blood, spleen and lymph would translocate to the vaginal epithelium. We found that the transgenic \( \gamma \delta \) T cells did accumulate in the vagina of transgenic mice. Furthermore, like normal vaginal \( \gamma \delta \) T cells, the transgenic vaginal \( \gamma \delta \) T cells expressed the phenotype of recently activated memory/effector T cells (CD44\( ^{hi} \), CD62L\(^{−} \), CD45RB\(^{lo} \), CD69\(^{F} \)). Vaginal \( \gamma \delta \) T cells in normal mice do not express the CD2 and CD28 antigens, but both of these markers are present on transgenic vaginal \( \gamma \delta \) T cells. We observed that a small fraction of splenic transgenic \( \gamma \delta \) T cells had the same surface phenotype as the vaginal transgenic \( \gamma \delta \) T cells, raising the possibility that the \( \gamma \delta \) T cells present in the vaginal epithelium of transgenic mice originated from the peripheral lymphoid organs. Data in support of this possibility came from experiments in which co-incubation of splenic transgenic \( \gamma \delta \) T cells with vaginal epithelial cell suspensions induced the vaginal \( \gamma \delta \) T cell phenotype on the splenic \( \gamma \delta \) T cells. The finding of transgenic \( \gamma \delta \) T cells in the vaginal epithelium suggests that homing of \( \gamma \delta \) T cells to this site is not restricted to \( \gamma \delta \) T cells that express the \( V_{\gamma}4/V_{\delta}1 \) invariant TCR. Furthermore, these findings imply that retention of \( \gamma \delta \) T cells in the vaginal epithelium of normal mice is affected by a \( V_{\gamma}4/V_{\delta}1 \)-specific mechanism. The finding of a significant level of apoptosis in the transgenic vaginal \( \gamma \delta \) T cells, but not in the normal vaginal \( \gamma \delta \) T cells, could reflect that the mechanism of retention of \( V_{\gamma}4/V_{\delta}1 \) T cells in the vaginal epithelium involves selective survival at the site.

Introduction
The frequency of \( \gamma \delta \) T cells in certain epithelial compartments in humans and mice is higher than their frequency in the peripheral blood (1–4). These epithelial compartments include the intestine, vagina and uterus, and in mice also includes the skin and tongue. Restricted TCR gene usage by \( \gamma \delta \) T cells located at different epithelial sites in mice is a defining characteristic and the genes used are different for each site. The \( \gamma \delta \) T cells of the skin express exclusively an invariant \( V_{\gamma}3/V_{\delta}1 \) TCR (5–7), while the majority of the intraepithelial \( \gamma \delta \) T cells of the small intestine use the \( V_{\gamma}5 \) chain together with either one of the \( V_{\delta}5, V_{\delta}4, V_{\delta}6 \) or \( V_{\delta}7 \) chains in their TCR (8–10). In humans the TCR gene usage of \( \gamma \delta \) T cells at the epithelial surfaces is not as narrowly restricted as in mice, but bias in the TCR repertoire does occur as shown by the preferential display of the \( V_{\delta}1 \) chain by intraepithelial compared to blood \( \gamma \delta \) T cells (3,4). Preference in variable gene usage at an anatomic location suggests that an active process selects for the presence of a particular TCR species. A possible mechanism to account for retention of restricted TCR species of \( \gamma \delta \) T cells that translocate from the blood.
might be their encounter with cognate antigen at that site. However, transgenic T cells with TCR species characteristic of blood γδ T cells were found to accumulate both in the skin and the intestine of the transgenic mice. Thus it was concluded that the homing of γδ T cells to these epithelial surfaces is not determined by a TCR-antigen recognition-dependent process, but instead is a cell lineage characteristic (11). Since the γδ T cells that are destined for skin localization appear in the thymus only during a very narrow time window of fetal development, it was postulated that the close association between localization at a particular anatomic site and VγT chain usage might reflect a linkage between TCR gene rearrangement and the expression of specific homing receptors during ontogeny (12–14). The γδ T cells in the normal murine vagina also express a strongly biased T cell receptor repertoire (15,16). Only Vγ4/Vδ1-expressing γδ T cells have been found at this anatomic site. Vγ4/Vδ1+ γδ T cells appear as the second wave of γδ T cells in the fetal thymus at days 16–18 of development, shortly after the appearance of skin γδ T cells (17,18). We previously reported (19, 20) that normal murine vaginal γδ T cells are phenotypically activated (CD44 hi, CD62L –, CD45RBlo, CD69+) and that they contain keratinocyte growth factor mRNA, a characteristic associated with activation of epithelial γδ T cells at other sites. The presence of an activation phenotype on normal vaginal γδ T cells and the absence from the vagina of γδ T cells with other TCR species presented an interesting set of circumstances. First, it showed for vaginal γδ T cells, the curious association between restricted TCR gene usage and selective anatomical localization. In addition, it documented the novel occurrence of an activation phenotype on a TCR-restricted population of normal intraepithelial γδ T cells, a finding that raised questions about the basis for activation and the roles, if any, of γδ T cells that are present in the T cells, the transgenic γδ T cells that are present in the vaginal epithelium of normal mice.

Methods

Animals

The G8 γδ transgenic mice have a BALB/c background and express a monoclonal Vγ11Vδ2 TCR specific for the MHC class Iib T10β antigen. They were generously provided by Dr. J. Bluestone (University of Chicago) (22). The expression of the transgenic receptor was confirmed in each mouse by staining the blood lymphocytes with apan-γδ TCR-specific antibody. It has been established that T cells are most abundant in the murine vagina at the diestrous phase (23). Therefore, in order to obtain cell numbers high enough to perform FACS analysis, we selected mice in the diestrous phase of the estrous cycle on the basis of their vaginal smear (24).

Cell preparations

Mononuclear cells from the vaginas of three to five mice were prepared as described in the following protocol: the vagina was dissected from the mouse, cut into small pieces and agitated for 60 min at 37°C in HBSS containing 5% DNase I. The tissue debris was removed by passing through a CMN-74-B nylon filter sheet (Small Parts, Miami Lakes, FL). The cells were fractionated by HBSS/40/70% Percoll gradient centrifugation at 600 g for 15 min and collected from the 40/70% interface. This extraction method yielded ~1–3×10⁶ cells/mouse.

For the RT-PCR or in vitro co-culture experiments, vaginas of four mice were cut and digested in HBSS supplemented with 0.25% trypsin/5% DNase for 60 min at 37°C. Cells were removed from the digestive medium and transferred into cold HBSS supplemented with 10% calf serum. Small intestinal intraepithelial lymphocytes cell preparations were produced as described elsewhere (25).

mAb

FITC-labeled H1.2F3 (anti-CD69), 16A (anti-CD45RB), RM2-5 (anti-CD2), phycoerythrin (PE)-labeled 37.51 (anti-CD28), IM 7.8.1 (anti-CD44) and GL-3 (anti-γδ TCR) were purchased from PharMingen (San Diego, CA). The hybridoma UC3-10A6 (anti-Vγ2) was generously provided by Dr J. Bluestone (University of Chicago). The anti-Vγ2 antibody was prepared from the supernatants of HB101 cultured hybridoma by a standard protocol of saturated ammonium sulfate precipitation and then was conjugated to Cy-5 fluorochrome by a Fluorolink kit (Biological Detection Systems, Pittsburgh, PA) according to the manufacturer’s recommendation.

Flow cytometric analysis

For three-color analysis, cells in HBSS containing 10% BCS, 10 mM HEPES and 0.02% NaN₃ were stained with saturating amounts of labeled antibodies on ice for 30 min in the presence of 2.4G2 anti-FcγRI/III to prevent binding of the antibodies to IgG Fc receptors. Following the incubation step with antibodies, the cells were washed 3 times. Some samples were analyzed on a four-decade Becton Dickinson FACS 440 flow cytometer (Becton Dickinson, Mountain View, CA) and the data were analyzed using a VAX computer equipped with DESK software; other samples were analyzed on a three-decade Coulter Epics 753 flow cytometer and the data analyzed with the ELITE 4.0 software.

Apoptosis detection by FITC-labeled annexin V

The external exposure of cell surface membrane phosphatidyserine during the apoptotic process was detected by FACS...
by the binding of FITC-labeled annexin V (26,27). FITC-labeled annexin V was purchased from R & D Systems (Minneapolis, MN) and used according to the manufacturer's recommendations.

RNA isolation and RT-PCR
Poly(A) RNA was isolated using a Micro Fast Track kit (Invitrogen, San Diego, CA) according to the manufacturer's recommendations. cDNA synthesis was performed in a 40 µl volume containing 0.5 µg oligo(dT)/18mer, 40 U RNasin, 400 U MMLV reverse transcriptase and 5 mM of each dNTP. The reaction was incubated for 2 h at 42°C, then aliquots of the mixture were directly used for PCR, which was performed on a HyBond DNA Thermal Cycler under the following conditions: 94°C for 90 s, 60°C for 90 s and 72°C for 90 s. The reactions were subjected to 35 cycles followed by an extension step at 72°C for 7 min. The reaction buffer consisted of 50 mM KCl, 20 mM Tris–HCl pH 8.3, 100 µg/ml BSA, 10 pM of each primer, 0.2 mM of each dNTP and 1 U Taq polymerase. The concentration of MgCl2 was 1.5 mM.

The Vγ4, Vγ2 and Cγ1/2 primers were as described by Nandi and Allison (16): Vγ4, CCA AAG AAT TCT GTG TAG TTC; Vγ2, GGG GGG AAT TCC CTG ACC CAT ATT TTC TTT; and Cγ1/2, GAA TTC TAG ATG TCT GCA TCA AGC CT.

Cell culture experiments.
For in vitro stimulation by anti-TCR antibody, 2×10⁶ spleen cells were seeded on a 24-well-plate coated with 50 µg/ml anti-CD3 antibody in 1 ml RPMI 1640 supplemented with 10% newborn calf serum, 1 mM sodium pyruvate, 1 mM non-essential amino acids, 1 mM essential amino acids, 2 mM L-glutamine, 5×10⁻⁵ M 2-mercaptoethanol and antibiotics. After 48 h the cells were washed and re-seeded into non-coated wells in the presence of 25 U/ml rIL-2 for 24 h.

For co-culture experiments, Vγ2⁺ G8 spleen cells were enriched by magnetic cell-sorting (MACS; Miltenyi Biotec, Inc., Auburn, CA) positive selection. G8 cell suspensions were stained with biotin-labeled anti-Vγ2 TCR-specific antibody. Following a washing step, cells were resuspended in FACS buffer containing streptavidin-conjugated magnetic beads (Miltenyi Biotec). The cell density, bead titer and length of incubation were determined as suggested by Miltenyi Biotec. The magnetic bead-labeled cell suspensions were then washed, resuspended in FACS buffer and passed through the MACS apparatus. While unlabeled cells pass through the MACS column, antibody-labeled cells are retained in the apparatus. The MACS column was then removed from the magnetic separator and rinsed with 3 volumes of FACS buffer to recover the Vγ2⁺ enriched G8 spleen cells.

BALB/c vaginal cell suspensions were prepared by mild trypsinization as described above. Vaginal cells (1×10⁶) were co-incubated on a 48-well-plate with 4.5×10⁶ Vγ2⁺ enriched G8 cells at 37°C for 48 h.

Results
Vγ₂ TCR-expressing transgenic cells translocate into the vaginal epithelium
The initial experiments were performed to determine whether transgene expressing γδ T cells were present in the vaginal mucosae of G8 mice. FACS analysis of vaginal cell suspensions from G8 mice showed that all of the γδ T cells expressed the Vγ₂ transgene and that the percentage of γδ T cells in the lymphocyte gate was ~3-fold higher than in the normal mice (Fig. 1A and B). As the average total cell yield from the vagina of transgenic mice was the same as from normal mice (~20,000 cells/mouse), the absolute number of γδ T cells recovered from vaginal preparations from transgenic mice was 3-fold higher. The vaginal γδ T cells of normal mice are located exclusively above the basal lamina, surrounded by epithelial cells (15). We found that >85% of the transgenic γδ T cells in the vagina are also localized in the epithelium (data not shown). These findings show that the transgenic γδ T cells can populate the vagina and that expression of the transgenic TCR per se does not appear to influence the translocation of γδ T cells to the murine vagina.

Comparative analysis of the expression of activation markers/adhesion molecules on transgenic γδ TCR⁺ lymphocytes in the vagina and the spleen
Having established that transgene-expressing γδ T cells were present in the vaginal epithelium of G8 mice, we were interested in determining whether their pattern of expression of activation markers/adhesion molecules was similar or different from the pattern expressed by normal vaginal γδ T cells and by transgene-expressing γδ T cells located at other anatomical sites in the same mice. γδ T cells of the normal murine vagina express a CD44⁺, CD62L⁺, CD45RB⁺, CD69⁺ phenotype, a pattern characteristic of recently activated effector cells. In the G8 transgenic mice the majority of γδ T cells in the spleen express a phenotype indicative of resting, naive T cells: CD44⁺, CD62L⁺, CD45RB⁺ and CD69⁻ (Fig. 2A–D, thin line). Interestingly, all of the transgenic vaginal γδ T cells express surface markers indicative of previous activation. Similar to normal vaginal γδ T cells, the transgenic γδ T cells are CD44⁺, CD62L⁺, CD45RB⁺ and CD69⁻ (Fig. 2A–D, thick line). To test whether the observed phenotype was due to 'leaky' expression of the normal Vγ4 vaginal TCR, we performed RT-PCR with primers specific for the Vγ4 or Vγ2 chains. We did not detect Vγ4 mRNA in the vaginal samples but did observe mRNA of the Vγ2 chain transgene (Fig. 3A).

Thus the transgenic γδ T cells located in the vagina show the same surface marker phenotype with regard to the functional T cell subsets as do the γδ T cells in the vagina of normal mice.

The transgenic vaginal γδ T cells differ from their normal counterparts in the expression pattern of CD2 and CD28 antigens
To determine whether the transgenic γδ T cells showed the same pattern of co-stimulatory molecule expression as their normal counterparts, we tested for the presence of CD2 and CD28 antigens. Previously, it was shown that in normal mice CD2 is not expressed on vaginal γδ T cells, but is expressed on splenic γδ T cells (20). We were interested in whether the transgenic γδ T cells recapitulated this phenotypic pattern. We found that, in contrast to normal vaginal γδ T cells, the transgenic vaginal γδ T cells did express CD2 (Fig. 4A). Likewise, vaginal γδ T cells in normal mice do not
Transgenic γδ TCR+ cells translocate to the vaginal epithelium

Fig. 1. γδ TCR-expressing cells in the (A) normal and (B) G8 transgenic murine vagina. Cells extracted from the vaginae of four mice as described in Methods were stained for γδ TCR and Vγ2 TCR. During the FACS analysis 30,000 events/sample were collected. Percentiles of the populations within the lymphoid gate were calculated by means of DESK software. Fluorescence intensity is presented on a four-decade log scale.

Fig. 2. Expression of adhesion molecules/activation markers on γδ T cells of the spleen and vagina of G8 mice. (A) Profile for CD44. (B) Profile of CD62L. (C) Expression of CD45RB. (D) Expression of CD69 of cells gated for the Vγ2 TCR+ cells within the lymphocyte gate. Histograms with a thin line represent samples of transgenic spleen, thick line represents transgenic vagina. During the FACS analysis 30,000 events/sample were collected. Fluorescence intensity is presented on a four-decade log scale. The data are representative of four independent experiments.

Fig. 3. RT-PCR of vaginal samples of G8 transgenic and normal mice for detection of Vγ4 and Vγ2 TCR transcripts. Lanes 1 and 2 show the presence of Vγ2 TCR mRNA in G8 but not in normal vaginal samples; lanes 3 and 4 show presence of Vγ4 TCR mRNA in normal but not in G8 vaginal samples; lane 5, 100 bp DNA ladder marker. (B) β-actin control. Vaginal cells of four mice were used for RT-PCR analysis. A 35 cycle RT-PCR of murine Vγ2 and Vγ4 TCR and a 30 cycle RT-PCR of murine β-actin was visualized on 1.5% agarose gels. The size of the PCR product was estimated by comparison to a 100 bp incremental DNA ladder.
augmented by T cell activation, one possible explanation for the failure of normal vaginal γδ T cells to express CD2 and CD28 is that these cells have not been activated. However, when normal vaginal γδ T cells were activated in vivo with anti-TCR-specific antibody, CD2 and CD28 were not induced even though the IL-2 receptor α chain was up-regulated, confirming that activation had actually occurred (our unpublished observations).

A second possible explanation for the lack of CD2 and CD28 expression on normal vaginal γδ T cells is that this is a lineage determined characteristic. If this were true, then the expression of CD2 and CD28 antigens by the transgenic vaginal γδ T cells would suggest that these cells are of a different lineage than the vaginal γδ T cells in normal mice. A possible source of the transgenic vaginal γδ T cells is the splenic γδ T cells. Splenic γδ T cells belong to the pool of γδ T cells that recirculate in the blood and therefore might gain access to the vaginal epithelium from the circulation. To determine whether when activated the γδ T cells in the spleens of transgenic mice expressed levels of CD2 and CD28 antigens similar to those found on vaginal γδ T cells in transgenic mice, we stimulated the splenic γδ T cells by cross-linking their TCR with solid-phase anti-CD3 antibody. As a positive control for activation we chose the CD44 antigen since it is well established that activation increases the expression of CD44 on splenic γδ T cells in normal and G8 transgenic mice (32-34) (Fig. 5A). We found that after 72 h of in vitro incubation transgenic γδ T cells expressed CD2 at a higher level than their freshly isolated counterparts (Fig. 5B versus Fig. 4A) and that the level increased further in the presence of anti-CD3 antibody.

The in vitro incubation alone induced no change in the expression pattern of the CD28 antigen on the transgenic splenic γδ T cells. However, anti-CD3 induced a significant increase in the expression of CD28 on the transgenic splenic γδ T cells (Fig. 5C). The intensity of CD28 expression on the splenic transgenic γδ T cells was similar to the intensity expressed by the freshly isolated transgenic vaginal γδ T cells.

These data show that the patterns and levels of CD2 and CD28 expression by vaginal γδ T cells in the transgenic mice parallel the levels of expression of those antigens on activated transgenic splenic γδ T cells.

**Alteration in CD62L and CD44 expression on transgenic splenic γδ T cells co-cultured with normal BALB/c vaginal epithelial cells**

To address whether the transgenic γδ T cells acquired their phenotypic appearance in situ in the vagina, we co-cultured BALB/c vaginal epithelial cells with transgenic splenic cells enriched by magnetic sorting for the Vγ2 TCR-expressing cells. Since the vaginal cell suspension was not sorted for a selected cell type, it contained mainly (~95%) epithelial cells, and a small fraction of fibroblasts, lymphocytes and leukocytes. Co-culture induced alterations in the expression of CD62L and CD44 antigens on the transgenic γδ T cells. Co-incubation with vaginal cells increased by 2-fold the percentage of CD62L+ splenic γδ T cells (Fig. 6A) and showed a similar magnitude increase in expression of CD44 (Fig. 6B).

In control co-cultures spleen cells from normal BALB/c mice did not induce any change in expression of CD44 or CD62L on the transgenic γδ T cells (Fig. 6C and D).

### Table 1. CD2 and CD28 expression by γδ T cells of the different organs in BALB/c or G8 transgenic mice

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express CD28 (20) but, as shown in Fig. 4(B), the vaginal γδ T cells in the transgenic mice expressed CD28.

The lack of expression of the CD2 antigen by normal vaginal γδ T cells is not unique, but is a feature shared with normal small-intestinal γδ T cells, suggesting that this trait in mice might be a lineage-determined marker at these mucosal sites (30,31). Freshly isolated small intestinal γδ T cells in the transgenic mice preserved this phenotypic feature, since they did not express CD2 antigen (Table 1). In addition, like their normal counterparts they did not express CD28 antigen. Thus, with regard to CD2 and CD28 expression, transgene-expressing γδ T cells in the vagina differed from their normal counterparts, whereas those located in the small intestine showed the same pattern as their normal counterparts.

The levels of CD2 and CD28 expressed by vaginal γδ T cells in transgenic mice are similar to the levels expressed by activated splenic γδ T cells in transgenic mice

The discrepancy in CD2 and CD28 expression between normal and transgenic vaginal γδ T cells was not readily explained. Transgene-expressing γδ T cells at other sites showed patterns of CD2 and CD28 expression similar to their normal counterparts, but the vaginal γδ T cells appeared to be an exception. Since CD2 and CD28 expression is typically augmented by T cell activation, one possible explanation for
Transgenic γδ TCR+ cells translocate to the vaginal epithelium

The results of these experiments suggest that the vaginal microenvironment has the potential to alter the expression of CD44 and CD62L on a subset of transgenic splenic γδ T cells. Transgenic γδ T cells show a high level of apoptosis in the vagina, but not in the spleen

A possible mechanism that could account for the apparent exclusive population of γδ T cells in the vaginal mucosa of normal mice is that circulating γδ T cells in the blood do translocate to the vaginal mucosa in adult mice, but they do not accumulate in numbers large enough to be detected, because they either exit the vaginal mucosa or they remain there but undergo cell death. Earlier it had been reported that the transgenic γδ T cells that translocate to the skin epithelium were smaller in size and had a different morphology than their normal counterparts (11). We also observed that some of the vaginal transgenic γδ T cells were smaller than the normal γδ T cells of the vaginal epithelium (data not shown). We considered the possibility that the observed decrease in size of some of the transgenic vaginal γδ T cells might be accounted for by ongoing programmed cell death of the transgenic γδ T cells in the vaginal epithelium. To investigate this possibility we conducted FACS analysis and found a significant level of apoptosis in the transgenic vaginal γδ T cells, but not in the normal vaginal γδ T cells (Fig. 7).

Since the transgenic γδ T cells in the spleens of the same mice did not show evidence of apoptosis, we infer that the apoptosis observed in the transgenic vaginal γδ T cells is not due to the transgenic state per se, but may be a consequence of activation and/or translocation to the vagina.

Discussion

Although the γδ T cells present in the vagina of normal mice exhibit TCR gene usage restricted to Vγ4/Vδ1, the findings in
the Vγ2 transgenic mice indicate that translocation of γδ T cells to the vaginal mucosa is not restricted to T cells that express Vγ4/Vδ1 TCR. It had previously been reported that the skin and intestine of G8 mice, and of mice transgenic for a different γδ TCR (11,33,34), were populated by transgenic γδ T cells, and our present experiments show that transgenic γδ T cells also translocate to the vaginal epithelium. These findings have implications for the observed restriction of γδ TCR usage by normal vaginal γδ T cells, because if TCR species does not restrict the translocation of γδ T cells to the vaginal mucosa, then there must be other mechanisms to account for the apparent selective accumulation there of the Vγ4/Vδ1 T cells in normal mice.

It is known that in normal mice the vagina is populated by Vγ4/Vδ1 T cells that develop in the thymus on day 16–18 of fetal development and migrate via the blood to translocate into the vaginal mucosa. It is inferred that the original γδ T cell immigrants to the vagina maintain the vaginal population for life since Vγ4/Vδ1 T cells are not detected by PCR in the thymus of adult mice (35,36). Since TCR species does not appear to restrict translocation to the vagina based on our findings in γδ TCR transgenic mice, it might be predicted that the normal γδ T cells in the blood might also gain access to the vaginal mucosa.

An interesting issue is the basis for the observed activation phenotype of normal and transgenic γδ T cells present in the vaginal mucosa. The restricted TCR gene usage exhibited by normal vaginal γδ T cells coupled with their display of phenotypic markers indicative of recently activated memory/effector cells suggests the possibility of ongoing exposure to cognate antigen. One possibility is that the Vγ4/Vδ1 TCR on normal vaginal γδ T cells recognizes an injury-induced antigen expressed by vaginal epithelial cells. Since the vaginal epithelium in mice undergoes significant cell loss as part of the normal estrous cycle, repetitive exposure to an injury-induced antigen is a reasonable possibility. However, this proposed explanation would not account for the recently activated, memory/effector phenotype of the transgenic γδ T cells because they have a TCR that recognizes an antigen that is not present in the transgenic mouse.

The flow cytometry analyses show that virtually all of the transgenic vaginal γδ T cells in G8 mice exhibit the markers of recently activated, memory/effector cells. In contrast, the overwhelming majority of splenic and blood transgenic γδ T cells show the phenotype of resting, virgin T cells (33,34 and our unpublished results). These results provide strong evidence that the preparations of transgenic vaginal γδ T cells were not contaminated to any significant extent by transgenic γδ T cells from the blood. For the same reason we infer that the phenotype of vaginal γδ T cells in G8 mice reflects a site-specific process.

There are several possibilities that might account for the pattern of expression of activation markers on the transgenic vaginal γδ T cells. One consideration is that the model is ‘leaky’ and that the transgenic vaginal γδ T cells also express the authentic Vγ4 TCR chain that occurs in normal mice. The results of the analysis by RT-PCR do not support this hypothesis.

A second possibility is that the pattern of activation markers displayed on transgenic vaginal γδ T cells reflects constitutive expression (CD44, CD69), or constitutive repression (CD62L, CD45RB), and that the pattern is cell lineage determined. In normal mice the intraepithelial γδ T cells of the small intestine (30,31) and the vagina (20) do not express CD2 or CD28. If cell lineage determined the pattern of expression of activation markers on intraepithelial γδ T cells, then it would be expected that the transgenic γδ T cells would be CD2+/CD28- at both anatomical sites. However, we found that the transgenic intestinal γδ T cells did not express CD2 and CD28 in keeping with their normal counterparts, but the transgenic vaginal γδ T cells, unlike their normal counterparts, did express CD2 and CD28. This finding raised the possibility that the vaginal γδ T cells in the transgenic mice are not of vaginal lineage. The close similarity of the overall phenotype (CD44hi, CD62L-, CD69+, CD2ii, CD28ii) of the transgenic vaginal γδ T cells to that of the activated transgenic splenic γδ T cells suggests that the vaginal epithelium of these mice is populated mainly by γδ T cells translocated originally from the peripheral lymphoid organs and not from the fetal thymus.

A third possibility that might account for the activated phenotype is that the pattern of these surface markers is influenced by local environmental factors. Since the cognate antigen recognized by the transgenic γδ TCR is not present in the transgenic mice, TCR-dependent alteration in the expression of surface markers might be induced by a cross-reactive antigen or a superantigen effect. Alternatively, it is known that modulation of individual activation markers can occur independent of TCR engagement. For example, transforming growth factor-β has been reported to down-regulate CD62L, antigen without TCR-specific stimulation (38–40). Furthermore, CD69 expression can be induced by cross-linking the CD5 or CD28 antigens. IFN-α and IFN-γ also augment the expression of CD69 on certain cell lines in an antigen-independent fashion (41–43). There is no example, however, for the expression of CD44 antigen at high levels without previous TCR engagement. In the co-incubation experiments we could partly induce on splenic transgenic γδ T

Fig. 7. Transgenic vaginal γδ T cells show a higher apoptotic rate than their normal counterparts, or transgenic γδ T cells of the spleen. Vaginal cells of five BALB/c or five G8 mice were extracted and pooled as described in Methods, and stained with annexin V-FITC and Cy-5-labeled anti-Vγ2 TCR antibody, or in the case of BALB/c mice with PE-conjugated anti-γδ TCR antibody. As a control we show the apoptotic rate of transgenic splenic γδ T cells from the same mice (thin line). The thick line represents transgenic γδ T cells in the vagina. The dotted line shows γδ T cells of normal murine vagina. During the FACS analysis 30,000 events/sample were collected. Data were analyzed and calculated by means of DESK software. Fluorescence intensity is presented on a four-decade log scale.
T cells a phenotype that is similar to the in vivo displayed phenotype on transgenic vaginal γδ T cells. Thus, co-incubation of bulk vaginal cells induced the down-regulation of CD62L and up-regulation of CD44 antigens on a sub-population of transgenic splenic γδ T cells. Whether this alteration in the surface phenotype is a TCR-dependent or -independent process remains to be determined.

Recently, it was found that Thy-1+ γδ CD44+ cells belonging to both the CD4+ and CD8+ γδ TCR cell subpopulations actively traffic from the vaginal epithelium to the regional lymph node (44). Since the vaginal mucosa is not a T cell developmental area, these migratory T cells most likely translocated earlier to the vagina from a peripheral lymphoid organ. Because normal γδ T cells, particularly subpopulations such as vaginal γδ T cells, are present in such small numbers, it is difficult to investigate their migratory patterns. However, extrapolation of our findings in the γδ TCR transgenic model raises the possibility that blood-derived γδ T cells normally translocate to the vaginal epithelium, but under physiological circumstances they do not reach threshold levels necessary for detection because they undergo apoptosis in the vagina unless they express the Vγ4/Vδ1 TCR. The alternative hypothesis is that the transgenic vaginal γδ T cells in G8 mice originally migrated to that site during fetal life and except for the transgenic TCR have the properties of lineage-committed vaginal γδ T cells. This seems less likely because the transgenic vaginal γδ T cells expressed CD2 and CD28, a characteristic not exhibited by lineage-committed normal vaginal γδ T cells. With each estrous cycle there is shedding of the vaginal epithelium. The population of vaginal γδ T cells is thought to be maintained through adulthood by local proliferation of the original fetal migrants. In the transgenic vaginal γδ T cells we detected a significant degree of apoptosis. The presence of apoptosis would tend to decrease the population of vaginal γδ T cells, but we found the opposite. While the origin of the transgenic vaginal γδ T cells is still unknown, the predominance of the data in our studies suggest that they are derived from the peripheral lymphoid organs, most likely by the hematogenous route.

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Abbreviations

PE phycoerythrin

References

Transgenic γδ TCR⁺ cells translocate to the vaginal epithelium


