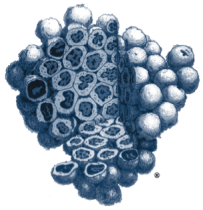

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CD45-positive Blood Cells Give Rise to Uterine Epithelial Cells in Mice

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Key Words. Adult stem cell/hematopoetic stem cell/endometrium/regeneration

Abstract

The uterine endometrium is composed of epithelial and stromal cells, which undergo extensive degeneration and regeneration in every estrous cycle, and dramatic changes occur during pregnancy. The high turnover of cells requires a correspondingly high level of cell division by progenitor cells in the uterus but the character and source of these cells remain obscure. In the present study, using a novel transgenic mouse, we show that CD45 positive hematopoetic progeniotor cells colonize the uterine epithelium, and that in pregnancy more than 80% of the epithelium can derive from these cells. Since we also found GFP positive uterine endothelial cells in long term GFP bone marrow transplanted mice we conclude that circulating CD45+ cells play an important role in regenerating the uterine epithelium.

INTRODUCTION

The lining of the uterus goes through extensive regeneration during the cycle and in pregnancy [1, 2]. Tanaka et al. studied the clonality of endometrium and demonstrated that individual uterine endometrial glands consisted of monoclonal populations of epithelial cells. Their findings indicated that single or multiple as yet unidentified stem cells with uniform clonality exist on the bottom of each endometrial gland [3]. Several investigators have used bone marrow (BM) transplants to show that bone marrow derived stem cells can repopulate a variety of organs in irradiated hosts [4-7]. The existence of a uterine endometrial stem cell has been proposed but its character, origin, and exact anatomical location have proven elusive [8]. Recently, Taylor (2004) showed that transplanted bone marrow cells give rise to uterine epithelial cells in humans [9]. Taylor's patients were exposed to full body irradiation and chemotherapy before they received BM transplants; thus, the physiological significance of his finding as well as the nature of the BM derived cells that entered the uterus and differentiated there could not be determined. Recently, he also demonstrated in lethally irradiated mice that transplanted full bone marrow gives rise to endometrial tissue including epithelium and suggested that BM mesenchymal cells might be responsible for the phenomenon [10]. Based on these data and a recent article demonstrating the presence of haematopoietic stem cells (HSCs) and lymphoid progenitors in endometrial biopsies in women [11] we asked the following questions: Do BM cells contribute to the uterine epithelium in healthy animals? If they do, what kind of BM cells populates the tissue? How extensive is their

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contribution? To answer these questions we first injected green fluorescent protein (GFP)-tagged BM cells [12] into lethally irradiated female *C57BL/6J* mice, and confirmed the fact that uterine epithelial cells can indeed arise from BM. We then created a transgenic mouse model to determine whether CD45+ cells might contribute to the epithelium.

MATERIALS AND METHODS

1. Bone marrow transplantation studies

Allogenic bone marrow transplantation was used to detect progeny of GFP+ BM derived cells. Briefly, *C57BL/6J* mice (n=6) received whole body irradiation (900 rad) and were transplanted intravenously 2 hours after irradiation with 10^6 bone marrow cells harvested from GFP+ mouse (C57BL/6-Tg (ACTB-EGFP) 1Osb/J, 003291, The Jackson Laboratory, Bar Harbor, ME, USA). At later timepoints transplanted animals were sacrificed and tissues harvested as described above.

2. Transgenic mice studies

To determine whether CD45 cell progeny contribute to the repopulation of the uterine epithelium under physiological conditions, we generated a novel transgenic mouse. First, we introduced Cre recombinase cDNA into a BAC containing the complete mouse CD45 gene (Fig. 1A). The recombinase cDNA was inserted downstream of an internal ribosomal entry site (IRES), which in turn, was placed downstream of the last coding exon of CD45. The resulting BAC was used to make transgenic mice (*CD45/Cre*). In such mice, Cre recombinase is produced in any cell that expresses CD45. First-generation heterozygous mice carrying the Cre transgene were bred. These *CD45/Cre* animals were then bred with *Z/EG* double reporter mice [13]. The latter express lacZ ubiquitously during embryonic development and adulthood. In the presence of Cre recombinase, the lacZ gene and the transcriptional stop following it are excised (Fig.1 B), activating the expression of a second reporter, enhanced green fluorescent protein (EGFP) (Fig.1 D). Only first generation Cre transgenics were used because the transgene is prone to rearrange. Thus, in subsequent generations ectopic expression, even ubiquitous expression, can be seen (unpublished observations).

Construction of recombination cassette

The recombination cassette was constructed by cloning the IRES-Cre-frt-pgk/em7/Neo/bpA-frt expression cassette from p459 (a gift from Neal G Copeland) with two flanking PCR amplified recombination fragments (A and B) into the pBC KS+ shuttle vector (Invitrogen, CA). Fragments A (263bp: 3305-3567 GenBank accession number nm_011210) and B (318bp: 3568-3886 from nm_011210) were designed to flank the insertion site for the expression cassette upstream of the stop codon at the 3' end of exon 33 of the CD45 gene (Fig. 1A). The plasmid was introduced into E. coli using heat shock, single ampicillin-resistant colonies were selected and propagated, and the plasmid was isolated by mini-prep and linearized for homologous recombination.

Homologous recombination

BAC 131H7 (#RPCI-23 Mouse BAC library (Invitrogen, CA)) was used for these studies. This BAC is approximately 190,000 bp long. There are about 30,000 bp of DNA 5' to the first exon of CD45 and 50,000 bp 3' to the last exon. The recombination cassette was inserted to the BAC by homologous recombination [14] (Fig. 1A). Briefly, the BAC was electroporated into E. coli EL250, which hosts a temperature inducible λ prophage that facilitates recombination. BAC-containing bacteria were incubated at 42ºC for 15 minutes and transformed with the linearized recombination cassette by means of electroporation. Double resistant (kanamycin-chloramphenicol) colonies were selected and propagated, and the recombinant BAC was isolated using a NucleoBond BAC Maxi Kit (BD Biosciences). Homologous recombination was confirmed by enzymatic digestion, PCR and DNA sequencing (Fig. 1A).

Production of double transgenic mice

Recombined BAC DNA was microinjected into *C57BL/6J* zygotes that were implanted into estrogen primed foster mothers and transgenic founders were selected by PCR using the following primers amplifying Cre: 5'-ccggtcgatgcaacgagtgatgagg-3' and 5'gcgttaatggctaatcgccatcttcc-3'. The resulting *CD45/Cre* mice were bred with *C57BL/6J* mice. Subsequently, *CD45/Cre* offspring were bred with heterozygous *Z/EG* double reporter mice (Jackson Laboratory, Bar Harbor, ME, USA) that express GFP upon Cre- mediated recombination, and their offspring were screened by PCR for Cre using the primers described above and for GFP (5'-gggcgatgccacctacggcaagctgaccct-3' and 5'-ccgtcctccttgaagtcgatgcccttcagc-3') (Fig. 1B). Double transgenic (*CD45/Cre-Z/EG*) animals were selected and used for the studies reported. The majority of nucleated cells in the blood of the double transgenic mice produced from all 4 founder lines were GFP+, and their progeny seemed GFP+ as well (Fig.1. C-F). Thus, while we did not study this extensively, we observed no line-to-line variation, and have no evidence that the site of integration of the recombinant BAC affected Cre expression.

Animal procedures

Animal care and procedures were approved by the Animal Care and Use Committee of NIMH, NIH. Double transgenic (*CD45/Cre-Z/EG*) animals were anesthetized, sacrificed by perfusion and examined at different ages: 18 day old embryos (n=2), 3 day old pups (n=2), 6 week old young adult (n=1), 12 week old adults (n=2), 12 week old pregnant animal (n=2) and 20 week old adult (n=1). Tissues were fixed by transcardiac perfusion or immersion (used for fixing embryos) with Zamboni's solution, harvested and processed for immunostaining.

Immunohistochemistry

Perfused tissues were cryoprotected by immersion in 20% sucrose solution and frozen. Twelve μm thick sections were cut at –24°C in a Leica Cryostat, thaw-mounted and stained using the following reagents: 1:1000 anti-GFP antibody (A11122, Molecular Probes, Eugene, OR, USA) followed by 1:1000 Alexa-488 conjugated anti-rabbit antibody (A31565, Molecular Probes), 1:100 anti-CD45 antibody (ab3088-100, Abcam Laboratories, Cambridge, MA, USA) followed by 1:1000 Alexa-594 conjugated anti-rat antibody (A11007, Molecular Probes), 1:20000 DAPI (D1306, Molecular Probes) and 1:200 biotinylated *Lotus tetragonolobus* lectin (B-1325, Vector Laboratories, Burlingame, CA, USA) followed by 1:1000 Alexa-594 conjugated streptavidin (S11227, Molecular Probes) or 1:1000 horseradish-peroxidase conjugated streptavidin and 1:10000 Alexa-350 conjugated tyramide (T20937, Molecular Probes). As a second marker we also used a wide spectrum cytokeratin antibody (Biogenex, MU-131-UC) at a 1:200 dilution followed by an anti-mouse IgG conjugated to Alexa-594 (1:1000) to confirm the characterization of epithelial cells. GFP immunostaining was confirmed by a second anti-GFP antibody (1:2000, AB16901, Chemicon, Temecula, CA, USA) that detects a different epitope of GFP and showed identical results to what is described before (data not shown). Control immunohistochemistry stainings were performed using secondary antibodies after omitting the primary antibodies. Detailed controls and variations of GFP stainings are described in Toth et al. [15]. Random areas of sections were photographed at low magnification and epithelial cells (approximately 500-1000 per animal) were counted by two independent investigators. The number of GFP positive cells was expressed as a percentage of all epithelial nuclei based on DAPI and Lotus staining.

In situ hybridization hitochemistry

Radiographic in situ hybridization was performed on 12 μm fixed sections as described before (http://intramural.nimh.nih.gov/lcmr/snge/Protocol.html). The primers that included a T7 and a T3 polymerase site were constructed to generate a template complementary to the GFP mRNA between nucleotides 1524-1823 (Accession

number: AB234879). This template was then transcribed using the Ambion (Austin, TX) Maxiscript transcription kit (Cat. # 1324) following its instructions. After hybridization the slides were dipped into autoradiographic emulsion (Kodak, NTB) and developed two weeks later. A Giemsa background staining was applied and the slides were dried and coverslipped. The sections were viewed with a Leica DMI6000 inverted fluorescence microscope and images were captured using Volocity software.

FISH in combination with GFP immunostaining

The STARFISH kit (Cat. # 1597-KD-50 Cambio, Cambridge, England) was used to detect the X chromosome in 6µm thin sections of uterine epithelium from fixed mouse sections. This allowed us to determine whether GFP+ cells were the products of cell-fusion events. Following microwave-induced antigen retrieval, GFP immunostaining was performed as described above. Then the sections were dehydrated and stained according to the Cambio's protocol. A Cy3 labeled chromosomal paint probe was used for final visualization. Random areas of 8 sections of uterine epithelium from one of the pregnant mice were chosen and 865 cell nuclei were examined focusing throughout the whole thickness of the section to count the number of X chromosomes per nuclei. Out of these nuclei 240 belonged to GFP positive epithelium, and we found no evidence of fusion, i.e. we did not see any non-dividing nucleus with 4 X chromosomes. In fact, all the nuclei that were uncut in the sections exhibited 2X chromosomes.

Flow cytometry

After pre-incubation with anti-mouse CD16/32 (Caltag) to block the Fc receptor, peripheral blood mononuclear cells were stained with the anti-mouse CD45 r-phyco-erythrin-conjugated antibody (Cat#MCD4504, Caltag). Rat r-phycoerythrin-conjugated IgG2b (Caltag) was utilized as an isotype control. CD45 staining and EGFP direct fluorescence were analyzed using a fluorescence-activated cell sorting (FACS) flow cytometer (Becton Dickinson, San Jose, CA).

RESULTS

Analysis of blood cells in the CD45/Cre/Z/EG mice. As expected, in adult CD45/Cre-Z/EG mice, CD45+ cells, such as lymphocytes (Fig. 1 D, E, F) and the derivatives of CD45+ cells, such as Kupfer cells and microglia (data not shown) were GFP positive. Most, but not all, of the white cells in blood that could be stained with an anti-CD45 antibody were GFP+. In some cells, the fluorescent signal may have been too weak to detect or excision of the floxed lacZ cassette may not have occurred because expression of the Cre recombinase was poor. FACS analysis of peripheral blood showed that the majority (65-85% in several animals tested) of the CD45 positive white blood cells of double transgenic mice expressed GFP (Fig. 1C).

Analysis of uterine histology in long term irradiated and GFP bone marrow transplanted mice. Irradiated mice are known to keep on cycling, although the length of the cycles may be more variable 16. Eight to twelve months after we transplanted GFP-tagged bone marrow cells (including hematopoetic, mesenchymal and any other BM stem cell populations) into 6 irradiated female mice, green cells were detected in the uterine endometrial epithelium and stroma of 5 out of 6 animals (Fig. 2 A,C). These bone marrow derived GFP+ epithelial cells bound Lotus tetragonolobus lectin, a marker with affinity for glycoprotein on the luminal surface of epithelial cells in the uterus 17. The GFP+ epithelial (i.e. immunopositive for Lotus tetragonolobus (Fig.2 D, E)) cells did not express the common leukocyte antigen, CD45, but numerous CD45+/GFP+ cells were detected in the uterine stroma (Fig.2. A), which is known to harbor CD45+ lymphoid cells 18. Immunostaining using a pan-cytokeratin (CK8, 18 and 19) antibody also showed colocalization with GFP (Fig.2. B) further confirming the epithelial character of the GFP positive cells.

Analysis of uterine histology in the virgin and pregnant CD45/Cre/Z/EG mice. Resident macrophages and lymphocytes in the endometrial stroma are known to express CD45 18, and indeed we saw many CD45+/GFP+ cells in the stroma of the transplanted and of the CD45/Cre-Z/EG mice (Fig 2A and 3B). We also found GFP+ cells in the epithelial layer of the endometrium (Fig. 2 and 3A). While these GFP+ cells could be stained with Lotus tetragonolobus lectin (Fig 2. D, E and Fig. 3 C, D) and with anti-keratin-8, 18, 19 antibody (Fig.2 B), another marker for epithelial cells, they could not be stained with anti-CD45 antibody (Fig. 2 A and Fig. 3 B). Expression of GFP mRNA was further confirmed by in situ hybridization histochemistry (Fig. 3. E, F). Thus, CD45+ cells appear to give rise to epithelial cells in the uterus of non-irradiated transgenic animals.

To determine the rate at which CD45+ cells contribute to the uterine epithelium and the extent of this contribution, we studied *CD45/Cre-Z/EG* animals at different ages. In mice, the estrous cycle is 5-6 days long [16, 19]. In contrast to humans, the epithelium does not shed, but it exhibits continuous degeneration and regeneration [20]. In the course of the cycle, epithelial cells undergo vacuolar degeneration and apoptosis and are replaced by newly generated cells [21]. The first cycle in mice occurs at 4-6 weeks of age. Consequently, a 1 week-old pup has not cycled yet, and 6-, 12- and 20-week old animals have gone through about 2, 10, and 26 cycles, respectively. In the double transgenic mice, we detected many CD45+/GFP+ cells in the uterine stroma (Fig.4) at all ages examined (1, 6, 12, and 20 weeks). Though no GFP+ epithelial cells could be detected in the uterine epithelium in 1 and 6 week-old animals (Fig. 4 A,B), 0.5% of the epithelial cells in 12 week-old mice and 6% in a 20 week-old animal were GFP+ (Fig. 4 C,D,E,F). The GFP+ epithelial cells were found in patches, suggesting that clonal expansion of individual progenitor cells gave rise to islands of epithelial cells. Thus, although we do not have enough animals at each timepoint to do a statistical analysis, we think that the number of uterine epithelial cells produced from CD45+ precursors increases with age and is related to the number of estrous cycles through which the animals have passed.

During pregnancy the uterus undergoes marked proliferation. The endometrial surface of the pregnant mouse is about 25 times larger than that of a non-pregnant animal and we wondered whether CD45+ cells might contribute to this increase. We were surprised to find that in one pregnant *CD45/Cre-Z/EG* mouse, the vast majority (82%) of uterine epithelial cells were also GFP+ (Fig. 4G,H). Our observations indicate that CD45+ progenitors could be the source of most of the new epithelial cells in the pregnant uterus. Based on double staining of GFP and the X chromosome, these cells seem unlikely to be the products of cell fusion events, since they never contain more than two X chromosomes (Fig.5).

DISCUSSION

Eight to twelve months following the bone marrow transplantation using GFP positive BM, the GFP positive cells were detected in the endometrial epithelium and stroma of recipient animals. Bone marrow derived GFP+ cells bound *Lotus tetragonolobus* lectin, which is an indicator of their epithelial nature. These GFP+ epithelial cells, however, did not express the common leukocyte antigen, CD45, while numerous CD45+/GFP+ cells were detected in the uterine stroma, which is known to harbor CD45+ lymphoid cells [18]. These findings clearly demonstrate that, upon transplantation, BM cells enter the uterus and differentiate into epithelial cells there. The BM cells are unlikely to have fused with uterine cells after colonizing the organ because the GFP+ cells all appear to be diploid. BM cells are heterogeneous, however, and transplantation of tagged cells did not not allow us to determine which sort of BM cell might give rise to the labeled epithelial cells in the recipient mice, or whether irradiating the animals affected what we observed.

CD45 is expressed in hematopoetic cells and their derivatives [22] including muscle satellite cells [23]. As expected, in the *CD45/Cre-Z/EG mice* CD45+ cells, such as lymphocytes (Fig. 2 D, E, F) and the derivatives of CD45+ cells, such as tissue resident macrophages, Kupfer cells and microglia (data not shown) were GFP positive. Most, but not all, white cells in the blood that could be stained with an anti-CD45 antibody were also GFP positive.

To determine the rate at which CD45+ cells contribute to the uterine epithelium and the extent of this contribution, we studied female *CD45/Cre-Z/EG* animals at different ages. The number of uterine epithelial cells produced from CD45+ precursors seems to increase with time and correlate with the number of estrous cycles through which the animals have passed.

During pregnancy, endometrial surface of the rodent uterus is about 25 times larger than that of a non-pregnant animal and this change happens in a very short time. We wondered whether CD45+ cells might contribute to this increase and were surprised to find that in a pregnant *CD45/Cre-Z/EG* mouse, the vast majority (82%) of uterine epithelial cells were also GFP+ (Fig. 4 G, H). Our observations indicate that CD45+ progenitors maybe the source of most of the new epithelial cells in the pregnant uterus.

Stem cells residing in the uterus were thought to be responsible for the expansion of epithelial cells that accompanies pregnancy [24]. The nature of these stem cells, whether they are replenished, and how replenishment might occur have been a matter of speculation, but a population of "label-retaining" putative stem cells (LRC) has recently been observed in the uterus [25, 26]. Chan described that these LRCs are frequently localized near vessels in the mouse endometrium [26]. Since our results show that CD45+ cells give rise to epithelial cells, we suggest that circulating CD45+ cells provide a renewable pool of epithelial precursors in the uterus. The fact that Chan [24] did not detect CD45 in LRCs suggests that endometrial stem cells turn the marker off after they select their fates.

Circulating hematopoetic stem or progenitor cells might enter the stroma and, when they are needed, migrate towards the lumen where they could serve to regenerate the epithelium. Alternatively, hematopoetic cells could colonize the stromal layer during fetal life and reside there from birth onwards, entering the epithelium on demand. CD45 was not thought to be produced by cells other than hematopoetic stem cells and their progeny [27], but recent work suggests that it may be made transiently by oligodendrocyte precursors during development [28] and several groups have demonstrated that lung epithelial cells might derive from hematopoetic stem cells in injury models [29-31]. Uterine epithelial progenitors might also transiently express CD45 and then turn it off before they differentiate. Were this the case, their progeny would be GFP+ in the double transgenic mice that we generated, but given the lack of GFP expression in the uterine epithelium during early postnatal development and during the first several estrous cycles, it seems unlikely that CD45 is activated in resident epithelial progenitors. Alternatively, if the BAC-based expression of Cre recombinase "leaks" briefly when the epithelial population expands, the cells could turn green. Since all of CD45's introns and long 5' and 3' flanking sequences are present in the BAC that we used (see Methods), and since several founder animals gave similar results to those reported above, we feel that leakiness is unlikely to have been a problem. Instead, we prefer the hypothesis that circulating CD45+ BM cells are responsible for the phenomena that we observed. The fact that GFP-tagged bone marrow cells transplanted into adult *C57BL/6J* mice generate patches of GFP+ uterine epithelium supports this hypothesis.

We also observed GFP+ and GFP mRNA positive uterine epithelial cells from animals studied 8-12 months after they underwent irradiation and transplantion with green bone marrow, suggesting that the colonization of the

uterus by CD45 expressing cells and their conversion into uterine epithelium can take place after development is completed. Although whole body irradiation prior to BM transplantation affects fertility and changes the length/number of estrus cycles in mice, uterine function seems to remain quite intact and turnover of the epithelium does not stop 16. This appears to be true in humans too; in fact, women who have received myeloablative doses of radiation can become pregnant and carry their fetus to term 32.

If BM progenitor cells do in fact contribute to the uterine epithelium, it is possible that alterations in these cells might result in clinical problems. For example, extramedullary hematopoesis in the endometrium, though rare, is known to occur 33 and could be caused by CD45+ cells there. Furthermore, we agree with Gargett 8 that endometriosis might be caused by misplaced endometrial stem/progenitor cells, specifically CD45+ derivatives. Du and Taylor suggested in their recent work that bone marrow derived cells contribute to the endometrium in a mouse model of endometriosis 10. This chronic, painful condition results when endometrium-like tissue grows on the uterus, ovaries, fallopian tubes, uterine ligaments, abdominal lining, or lower part of the large intestine. In addition, such tissue has been seen in the axillary glands, lung, retina, sciatic nerve, skin, and even brain.

Like the endometrium, these ectopic growths respond to hormones; they grow, swell with blood, and degenerate in a cyclic manner. Since their breakdown products have no way to exit the body, however, they cause severe menstrual, abdominal, and lower back pain, and—in as many as 40% of women suffering from it—scarring and infertility. Endometriosis is thought to have a genetic component; it is associated with autoimmune diseases, and it can be induced in rhesus monkeys by treatment with dioxin [34]. We feel that regardless of the abnormality (genetic, immunological, hormonal) that drives the process, changes in CD45+ BM derived cells could cause them to colonize sites other than the uterine lining and to differentiate and proliferate once they are there. If this is the case, one wonders whether the cells are conditioned in the endometrium and subsequently escape and take up residence in other nearby tissues [35]. Alternatively, stem cells may occasionally make inappropriate fate choices in tissues (e.g., lung, brain) where they migrate via the blood. The fact that endometriosis has been reported in males might seem to lend support to the second of these suggestions; but, it appears that in some cases the growths may arise in the prostatic utricle, a uterine remnant found in men [36].

Clearly, it would be interesting to know whether CD45+ cells are important for regenerating tissues other than the endometrium and what cues are used to attract them into any given organ and to drive their differentiation. The *CD45/Cre-Z/EG* mouse strain may serve as a useful model for such studies.

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Figure 1. GFP is expressed in CD45+ cells of the double transgenic (*CD45/Cre-Z/EG*) mice. A, Upon homologous recombination, the IRES-Cre recombination cassette flanked by two homologous fragments (A & B) was inserted into exon 33 of the CD45 gene in a BAC containing the entire coding region. The recombinant construct was confirmed to be correct by restriction mapping, PCR with primers flanking the recombination fragments, and sequencing. B, To track the fate of CD45+ cells, we created a double transgenic strain by breeding *CD45/Cre* mice with the double reporter *Z/EG* mice. In the crossbred mice, every CD45+ cell expresses the Cre recombinase that will excise the floxed lacZ cassette, thus enabling the activation of GFP. Regardless of the future fate of CD45+ cells, GFP will be expressed continously throughout their lifespans. c, FACS sorting of peripheral blood of a double transgenic animal demonstrating a high percentage (85%) of double positive blood cells using green fluorescence for GFP and red for CD45 D, E, F. White blood cells immunostained with GFP (green) (D), CD45 (red) (E) and an overlay of (D) and (E) with added DAPI (nuclear-blue) staining (F). Scale bar: 15μm.

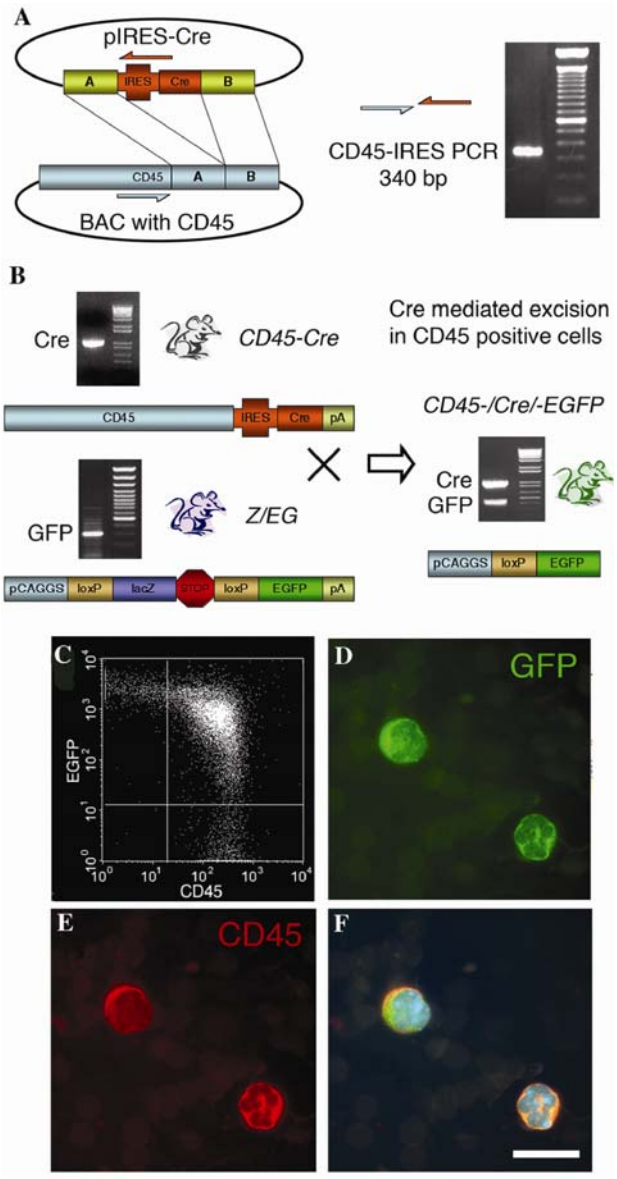


Figure 2. Endometrium of a mouse previously (10 months before) transplanted with *EGFP* BM following irradiation. GFP is in green, CD45 (in A) and Lotus tetragonolobus (in C and D) in red and DAPI, the nuclear marker, in blue. A, GFP+ and CD45- uterine epithelium in the recipient endometrium shows that BM cells can contribute to the regeneration of uterine epithelial cells. CD45+/GFP+ hematopoietic cells are present in the stromal layer. B demonstrates the colocalization of a pan cytokeratin immunostaining (in red) with GFP (in green) in epithelial cells. The image shows one level of a Z-stack in three panel view. The stack was captured at 0.5µm intervals and iterative restoration was performed using Volocity 4.0 software (Perkin-Elmers) and a Leica DMI 6000 inverted microscope. C, D, E, GFP positive nucleated (DAPI staining) cells appear in the epithelial layer (C), co-localized with *Lotus tetragonolobus*, a uterine epithelial marker (D). E, Overlay of (C) and (D). Solid arrowheads indicate GFP+; open arrowheads indicate GFP- uterine epithelial cells. Scale bar: 20 µm (A) and 14 µm (C-E).

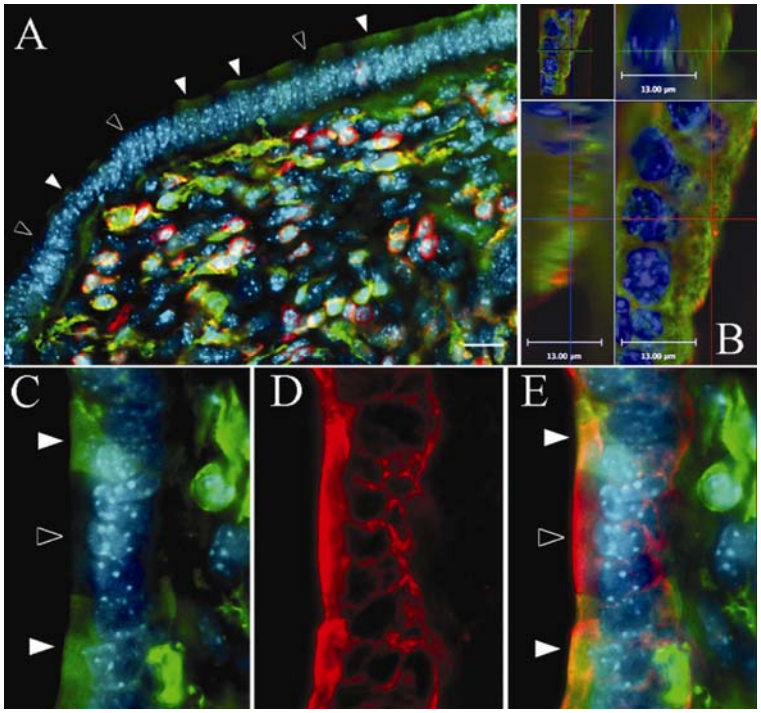


Figure 3. GFP expressing uterine epithelium of a double transgenic (*CD45/Cre-Z/EG*) mouse. We observed (A) GFP positive (green) epithelial cells in the uterus that (B) do not express CD45 (red), but (C) bind the uterine epithelial marker *Lotus tetragonolobus (blue) and* (D) is an overlay of the three stainings. Arrowhead points to GFP+/CD45- uterine epithelial cell, open arrowheads indicates GFP+/CD45+ stromal cell. (E) Uterine epithelium expressing GFP mRNA in brightfield and (F) in darkfield illumination. Scale bar: 20μm (A-D) and 50 μm (E-F)

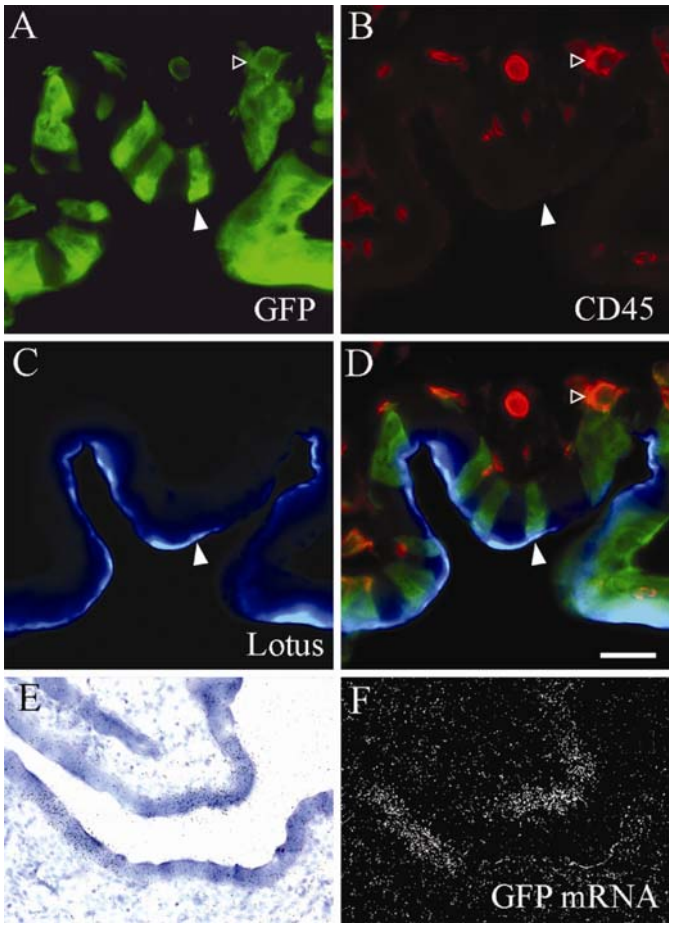


Figure 4. GFP expressing (green) uterine epithelial cells from double transgenic animals of different ages and pregnant mice. All nuclei are stained in blue with DAPI. A, B, No GFP positive (green) uterine epithelial (red staining represents Lotus tetranoglobus, the epithelial marker) cells were detected in six week-old animals. C, D, Sporadic GFP positive uterine epithelial cells were present at 12 weeks of age. Arrow indicates a GFP+ uterine epithelial cell. E, F, At twenty weeks of age, 6% of uterine epithelial cells expressed GFP. G, H, In twelve week-old pregnant mice, there was a robust increase in the number of GFP expressing cells: 82% of the uterine epithelial cells were GFP+. Scale bars: 60μm (A-G) and 20μm (B-H).

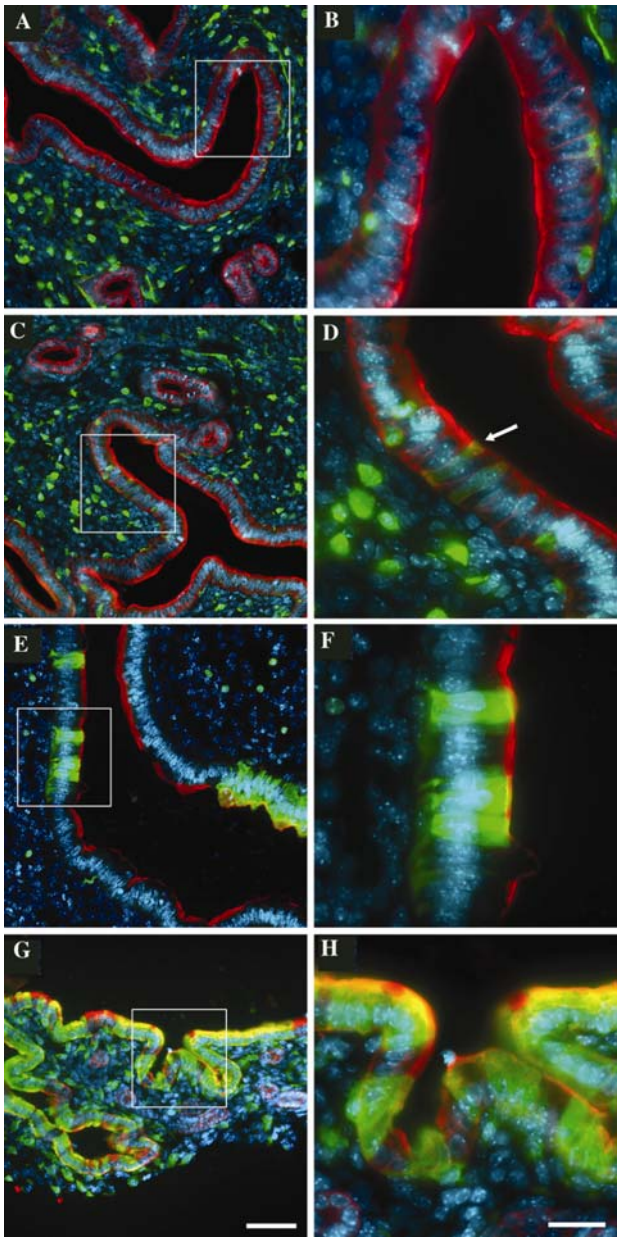
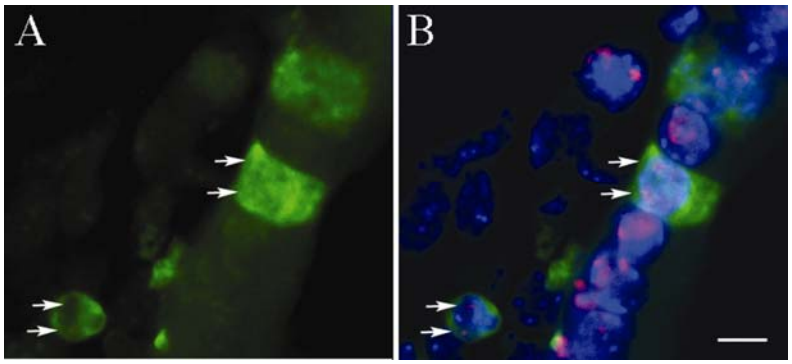


Figure 5. Arrows point to nuclei (DAPI-blue) of GFP expressing (green) uterine epithelial cells (A) from double transgenic animals that have two X chromosomes (red dots). GFP is visualized with a Tyramide-FITC conjugate, while the X chromosome is labeled by a CY3-conjugated chromosomal paint probe. Scale bar: 10μm



CD45-positive Blood Cells Give Rise to Uterine Epithelial Cells in Mice

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