ORIGINAL ARTICLES

Oligoclonal Expression of T-Cell Receptor Beta Variable Genes in Normal Human Endometrium

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ABSTRACT

In spite of their key role in various immunological processes occurring in the endometrium, T cells- especially $\alpha\beta^+$ subtype- residing in this mucosal tissue, have not been extensively explored. We present here the profile of expressed genes for variable region of β chain of T cell receptor (TCR) in normal endometrium as compared to peripheral blood. Samples from endometrium were taken from normal fertile women during routine check-up by Pipelle pipette or after hysterectomy operation. Total RNA from both blood and endometrial samples was extracted and RT-PCR using BV gene specific primers was performed. After southern blotting, hybridization with radiolabelled specific probe and autoradiography, relative expression of each BV family was determined. Clonal expansions of the over-expressed genes were studied by determining their CDR3 length polymorphism. A total of 12 blood and 14 endometrial samples were collected. Only one TCRBV gene (TCRBV7) was expressed significantly more and 3 genes less frequently in the endometrium compared to blood. Also, two other genes (TCRBV10 and 12) were found marginally more frequent in the endometrium. As for their clonality, all 3 TCRBV genes examined here showed a rather restricted (oligoclonal) and in some cases, very restricted (probably monoclonal) pattern in the endometrium in contrast to polyclonal patterns in the blood. Our results indicate the similarities between T cells residing in different mucosal tissues and support their common recruitment and functional potentials. Moreover, our findings provide a basis for future investigations about endometrial T cell involvement and their antigen specificities in different gynecological problems.

Keywords: Endometrium; Genes, T-Cell Receptor beta; T Cell

Abbreviations: TCR (T Cell Receptor); TCRBV or BV (genes for variable segment of β chain of TCR); GALT (Gut-Associated Lymphoid Tissue); NALT (Nasal-Associated Lymphoid Tissue); uNK cells (uterine Natural Killer cells); IDO (Indoleamine 2,3 Dioxygenase); RT-PCR (Reverse Transcriptase Polymerase Chain Reaction); CDR (Complementarity Determining Region); PBMC (Peripheral Blood Mononuclear Cells); RNA (Ribonucleic Acid); cDNA (Complementary Deoxyribonucleic Acid)

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INTRODUCTION

Endometrium represents the mucosal lining of the uterine cavity. As a member of common mucosal immune system and as a frontier line for encountering foreign antigens, endometrium shares the important role of orchestrating two seemingly contradictory events of generating proper response to prevent infections and tumoral growth while providing tolerance and unresponsiveness to sperm and fetal alloantigens. This capacity to distinguish between, and appropriately respond to, all foreign entities to which endometrium is exposed could only be achieved through a highly specialized local immune system.[1,2]

Compared to other mucosal immune systems such as GALT (Gut-Associated Lymphoid Tissue) or NALT (Nasal-Associated Lymphoid Tissue), there exists such differences as lack of organized lymphoepithelial aggregates, considerably low number of B cells and variations in immune cell population in response to hormonal changes during menstrual cycles and pregnancy[3,4] which all have given endometrium the unique immunological environment and flexibility for achieving its exceptional function.

The quality and direction of immune responses in virtually every tissue are strictly contingent upon the relative abundance and activity of immune cell populations present in that tissue. Apart from the period of menstrual bleeding when polymorpho-nuclear cells migrate massively to endometrium, three cell populations –uNK (uterine Natural Killer) cells, T cells and macrophages-comprise nearly 90% of all endometrial leukocytes. In contrast to other cell populations in endometrium, it is rather surprising how little is known about endometrial T cells despite their important role as the key orchestrator of any immune activity or tolerance induction in mucosal immune system.[5,6]

It has long been known that human endometrial T cells are scattered through endometrium as isolated stromal cells, intraepithelial lymphocytes and rare discrete lymphoid follicles near basal layer of endometrium. The majority of endometrial $CD3^+$ T cells in humans express αβ heterodimer surface receptor and are of cytotoxic ($CD8^+$) subtype.[7-9] Surprisingly, even in the absence of any obvious infection, they express activation markers and are presumed to be in a state of recent and persistent activation but at least in $CD8^+$ population of endometrial T cell aggregates, cytotoxicity has been shown to be extensively reduced.[10,11] There are, therefore, many reasons to assume that $\alpha\beta^+$ T cells in endometrium are phenotypically and functionally distinct. Unlike uNK cells, endometrial αβ T cells do not seem to respond to hormonal changes during menstrual cycle or early pregnancy in human.[12] There is, however, direct support for their role in preventing spontaneous abortion in animal models[13,14] and indirect evidence in favor of their key part in maintaining paternal antigen-specific tolerance during pregnancy by either producing Th2 cytokines or other yet unidentified mechanisms at the site of pregnancy.[15,16]

In spite of their implicit but very crucial role in providing the required flexibility for varied uterine immune activity ranging from infection elimination to pregnancy tolerance, endometrial T cells have not been much investigated. Since all αβ T cells rely upon the recognition of specific antigen in the context of self MHC molecules by their surface receptors (TCR) for activation and proliferation,[17] one of the very first steps in characterization of any T cell population would be the determination of the type of their receptor. Likewise, a more comprehensive picture of the exact nature of endometrial αβ T cells and their possible involvement in pathological conditions such as endometriosis, recurrent abortion, and unexplained infertility would only emerge after a detailed study of their surface receptors and antigen specificities.

We, therefore, aimed in this study to determine first, the relative expression of different families of BV genes (genes for variable segment of β chain of TCR receptor) in the normal endometrium compared to peripheral blood and second, to evaluate clonality of the over-expressed genes in the endometrium.

MATERIALS AND METHODS

Study Population and Sampling

Women who had at least one successful term pregnancy and visited for routine gynecological check-up or underwent operations for unrelated procedures such as tubal ligation or tubal re-opening or had hysterectomy for removing myomas not affecting the endometrium were included in this study.

After informing the cases about the study and obtaining a written consent, blood and endometrial samples were taken simultaneously. Venous blood was collected in tubes containing EDTA 2mg/ml of blood. Peripheral blood mononuclear cells (PBMC) were extracted by gradient separation on Ficoll-paque (Amersham Biosciences, Uppsala, Sweden) and immediately used. Endometrial samples were taken either in operation room by curetting with No. 1 sharp curette after cervix dilatation or during gynecological exam by pipelle endometrial suction. All samples were taken during last days of menstrual cycle (LH+8-12) for abundance of tissue. Endometrial samples were washed profusely with sterile normal saline and immediately stored in RNA later (Ambion, USA) or frozen in liquid nitrogen.

RNA Extraction and First Strand cDNA Synthesis

Total RNA was extracted from 2 -4 × 10^6 PBMC or from endometrial tissues of at least 1mm×1mm dimensions after complete homogenization, using RNABee (Biogene,UK) based on the guanidine thiocyanate phenol-chloroform extraction method.[18] After 1 μg of total RNA for blood and 4-5 μg for tissue were denatured at 90°C for 5 minutes, they were used for first-strand cDNA synthesis in a reaction mixture containing 40 U of murine Molony leukemia virus reverse transcriptase (Fermentas, Lituania) in its 1× reaction buffer (250 mM Tris-Hcl, 250 mM KCl, 20 mM Mgcl2, 50 mM DTT), 1 mM dNTP (Roche, Switzerland), 1 pM random hexamer primers (Amersham Biosciences) in the final volume of 20 μL for blood and 60 μL for tissue. The reaction mixture was incubated at the following profile according to the manufacturer's recommendation: 25°C for 10 minutes, 42°C for 60 minutes followed by 10 minutes at 70°C to inactivate RT, and then stored at -20°C.

Normalization of TCR Specific cDNA Concentration

The cDNA concentrations used in all samples were normalized using a TCR beta chain constant region (BC)-specific polymerase chain reaction (PCR). Briefly, a set of forward (5' -BC) and reverse (3'- BC) primers (Table1) was used in a 30-cycle PCR with serial dilutions of cDNA. The PCR products were then electrophoresed on 1.5% ethidium bromide-stained agarose gels and photographed using gel documentation device (UVP, USA). The density of each TCR BC-specific band was then quantified by Labworks software (UVP, USA). Approximately equal amounts of cDNA were used in each TCRBV-BC PCR according to the obtained density of different cDNA concentrations.

TCRBV-BC PCR

Analysis of BV gene usage was carried out by amplification of BV-specific cDNA using a panel of 28 TCR BV-specific 5' primers and a common BC 3' primer (Table1). Our BC primer recognized sequences in both BC1 and BC2 genes. PCR mixtures for blood samples contained 1 unit DNA Taq polymerase (Cinnagen, Iran) in recommended 1× buffer (10 mM Tris-HCl, 50 mM KCl), 0.2 mM final concentration of each of dNTPs (Roche, Switzerland), 1.5 mM MgCl2 and 0.6 μM of each primer in the final volume of 25 μl for each reaction. For tissue samples, 0.5 unit of Redhot Taq DNA polymerase (Abgene, UK) in its recommended 1x reaction buffer (75 mM Tris-Hcl, 20mM (NH4) 2SO4, 0.1% Tween 20) was used. All samples were amplified in a thermocycler (Eppendorf, Germany) for 35 cycles for blood and 45 cycles for tissue samples. Temperature profile was 94°C for 2 minutes for initial denaturation (for tissue), 94°C for 20 second (for blood), 55°C for 30 seconds and 72°C for 30 seconds. The last extension was continued for an additional 9 minutes to ensure complete extension of the products. For visualization of PCR products, 8-10 μL of each of the products was electrophoresed on 1.5% ethidium bromide-stained agarose gel and photographed.

Southern Blotting of TCRBV-BC PCR Products

PCR products were electrophoresed on 1.5% agarose gels. The gels were denatured using a solution containing 0.5 M NaOH and 1.5 M NaCl for 15 minutes and then neutralized in 0.5 M Tris-HCl and 1.5 M NaCl for 15 minutes, followed by incubation for 20 minutes in 10× SSC. The products were transferred onto nylon membranes (Amersham Biosciences). Membranes were air dried and baked for 2 hours at 80°C and stored for the next steps.

5' End-Labeling of BC Oligonucleotide Probe and Hybridization to PCR Product

A TCR BC-specific oligonucleotide probe (BC reporter) (Table 1), recognizing both BC1 and BC2

upstream of BC primer was used it was 5'-end labeled using T4 polynucleotide kinase enzyme (Invitrogen, UK) and γ-^{32}P-adenosine triphosphate (ATP) (Amersham Pharmacia Biotech). The baked nylon membranes were first prehybridized in a buffer containing 2× SSPE, 5× Denhardt's solution, and 0.5% sodium dodecyl sulfate (SDS) for 3 hours at 42°C followed by hybridization in the same buffer with 1×10^6 cpm/mL of the 5'-end labeled BC reporter overnight at 42°C. They were then washed twice at 42°C in a washing solution containing 0.2 × SSPE and 0.5% SDS and were subsequently exposed to Hyper film MP (Amersham Biosciences) for 6-18 hours at 70°C. The intensity of the signals exerted by specific probing of the TCR BV-BC amplified products on the films was quantified by gel scanning. The relative usage of each BV gene was determined using Labworks analysis software (UVP, USA) as the percentage of the density of each BV amplified gene to total density of the whole sample.

Table 1. Oligoclonal expression pattern of T-cell receptor BV genes.

TCR BV5'	
TcR BV1	5'-GCA CAA CAG TTC CCT GAC TTG CAC-3'
TcR BV2	5'-TCA TCA ACC ATG CAA GCC TGA CCT-3'
TcR BV3	5'-GGG GTA CAG TGT CTC TAG AGA GA-3'
TcR BV4	5'-ACA TAT GAG AGT GGA TTT GTC ATT-3'
TcR BV5s1	5'-ATA CTT CAG TGA GAC ACA GAG AAA C-3'
TcR BV5s2-3	5'-TTC CCT AAC TAT AGC TCT GAG CTG-3'
TcR BV6s1-3	5'-AGG CCT GAG GGA TCC GTC TC-3'
TcR BV7	5'-CCT GAA TGC CCC AAC AGC TCT C-3'
TcR BV8	5'-ATT TAC TTT AAC AAC AAC GTT CCG-3'
TcR BV 9	5'-CCT AAA TCT CCA GAC AAA GCT CAC-3'
TcR BV10	5'-CTC CAA AAA CTC ATC CTG TAC CTT-3'
TcR BV11	5'-TCA ACA GTC TCC AGA ATA AGG ACG-3'
TcR BV12	5'-AAA GGA GAA GTC TCA GAT-3'
TcR BV13s1	5'-CAA GGA GAA GTC CCC AAT-3'
TcR BV13s2	5'-GGT GAG GGT ACA ACT GCC-3'
TcR BV14	5'-GTC TCT CGA AAA GAG AAG AGG AAT-3'
TcR BV15	5'-AGT GTC TCT CGA CAG GCA CAG GCT-3'
TcR BV16	5'-AAA GAG TCT AAA CAG GAT GAG TCC-3'
TcR BV17	5'-CAG ATA GTA AAT GAC TTT CAG-3'
TcR BV18	5'-GAT GAG TCA GGA ATG CCA AAG GAA-3'
TcR BV19	5'-CAA TGC CCC AAG AAC GCA CCC TGC-3'
TcR BV20	5'-AGC TCT GAG GTG CCC CAG AAT CTC-3'
TcR BV21s1	5'-CTG GTT CAA TTT CAG GAT GAG AGT-3'
TcR BV21s2	5'-GAT TCG ATA TGA GAA TGA GGA AGG-3'
TcR BV21s3	5'-TCT GAT TCA GTT TCA GAA TAA CGG-3'
TcR BV22	5'-AAA GAG GGA AAC AGC CAC TCT G-3'
TcR BV23	5'-CGC TGT GTC CCC ATC TCT AAT C-3'
TcR BV24	5'-CAG TGA CCC TGA GTT GTT CTC A-3'
TCR BC primers	
3' BC	5'-GTG CAC CTC CTT CCC ATT -3'
5' BC	5'-GTC GCT GTG TTT GAG CCA TCA GAA-3'
BC primer	5'-TTC TGA TGG CTC AAA CAC-3'
BC-FITC	5'-X-GTG CAC CTC CTT CCC ATT-3'
TCR probe	
BC reporter	5'-CAC AGC GAC CTC GGG TGG GAG CAC-3'

TcR BV, T-cell receptor beta chain variable; BC, beta chain constant region; FITC, fluorescein isothiocyanate.

Analysis of CDR3 Length Polymorphism

cDNA samples with concentrations used in BV-PCR were amplified in 35-45 cycles of PCR using BV 7, 10 and 12 primers as 5' primers and 3' BC-FITC (Table1) as the 3' primer. The products were then checked on 1.5% ethidium bromide-stained agarose gels. An aliquot was loaded onto 6% denaturing polyacrylamide sequencing gels, and the electro-phoresis was performed in the presence of fluorescent 50-500 bp size markers (Amersham Biosciences), in an ALF-DNA sequencing machine (Amersham Biosciences).

The data were fed into a computer and analyzed by the "Fragment Manager" software program (Amersham Biosciences) according to criteria set by pannetier et al.[19]

Statistical Analysis

Data were collected, saved and analyzed by SPSS 11.0 software program. For comparing two related quantitative variables (blood and endometrium) nonparametric Wilcoxon signed rank test was used. Fisher Exact test was employed to compare the frequencies between two sites. P-value<0.05 was considered to be statistically significant. All data are shown as mean ±SD.

RESULTS

A total of 14 normal cases took part in this study of whom 10 blood and 12 endometrial (8 blood-endometrium matched) samples were taken. Mean age of our study population was 35.92±4.46 years and none of them had any history of recent gynecological problem or current drug usage.

Usage of TCRBV Genes in Blood and Endometrium

As shown in Figure 1, there is a slight restriction in the number of TCRBV genes used in endometrium of each sample compared to blood but there was no statistically significant difference observed between the two sites. It is worth emphasizing that in none of our tissue samples all TCRBV genes were expressed ; also, no case was detected expressing less than half of the studied genes with most cases falling in between. Moreover, we did not observe any one family of TCRBV genes not being expressed in all of our endometrial samples, though some families like TCRBV23, TCRBV21s1 or TCRBV13s2 had very low level of expression in the endometrium.

There was a skewed usage toward some TCRBV genes like TCRBV7,10 and 12 in the endometrium as compared to blood compartment. The difference was, however, significant in case of TCRBV7 which was expressed more than twice the rate observed for blood (10.8% vs 4.7%) ($p<0.01$). If other criteria such as cut-offs of more than 2 SD (standard deviation) of mean blood expression were considered, other TCRBVs (eg; TCRBV 10 and 12) could have been found over-expressed in endometrium (Figure 2). Less striking but significant differences between the two anatomical sites were observed in the expression of TCRBV13s2,18 and 23 in favor of blood ($p<0.05$).

Polymorphism of CDR3 length of TCRBV transcripts

Determining CDR3 length of transcripts of any TCRBV gene in blood or tissues provides a clue to the pattern of clonal expansion of T cells bearing that variable segment of β chain. Therefore, arbitrarily and for ease of discussion we chose to categorize the patterns observed into 3 main groups according to previous reports; these groups were unrestricted diversity of transcript lengths (polyclonal pattern), restricted transcript diversity with dominance of at least one peak occupying more than 50% of all surface area of the curve accompanied by considerable suppression of other peaks (oligoclonal pattern) and very restricted diversity resulting in only one sharp peak in CDR3 polymorphism curve (probably monoclonal pattern).

We studied the polymorphism of only those TCRBV genes that were found over-expressed in the endometrium, ie TCRBV7, 10and12, to clarify their clonal origin. All these three genes were found to have much more limited transcripts in terms of their size in the endometrium than in blood.

TCRBV7 was the most frequently expressed gene in the endometrium. Nearly two thirds (72%) of our endometrial samples showed oligoclonal pattern in the expression of TCRBV7, while all related blood samples but one had polyclonal pattern (Figure 3a,b). Such unusual pattern as single and discrete peaks located fairly far apart but well inside the limits of normal CDR3 length, that is 8-11 amino acids or 24-33 base pairs, was occasionally observed in the endometrium.

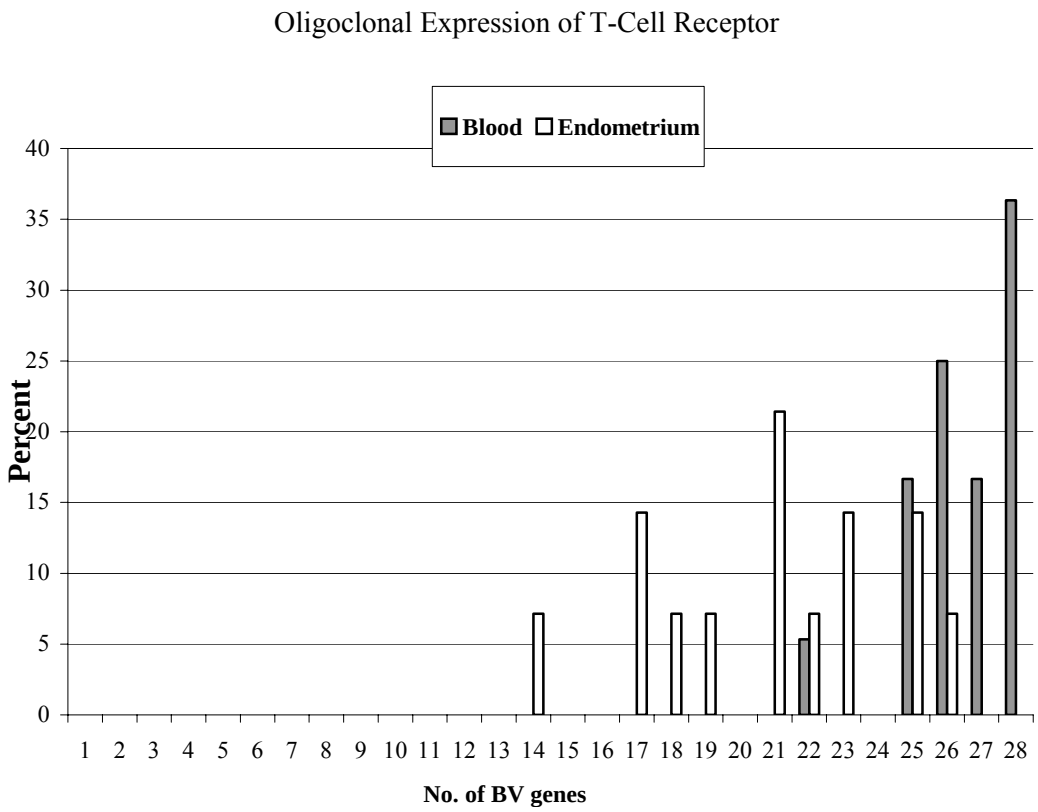

Figure 1. Number of the expressed BV gene families in endometrium and blood of normal controls.

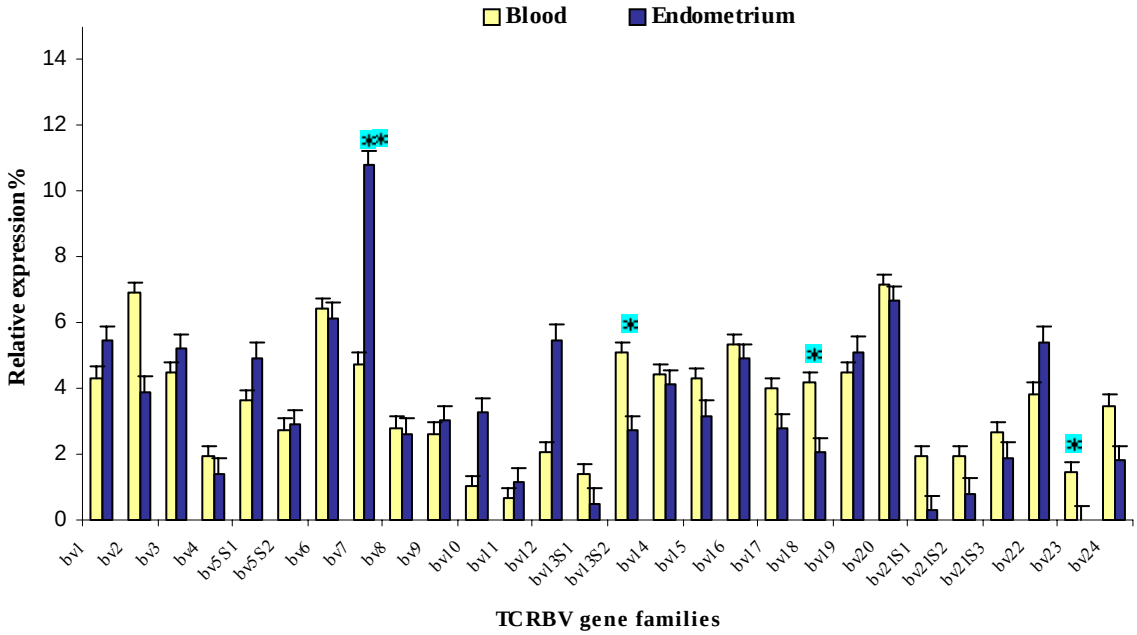

Figure 2. TCRBV genes expression in blood and endometrium of normal controls.
(* p<0.05, ** p<0.01)

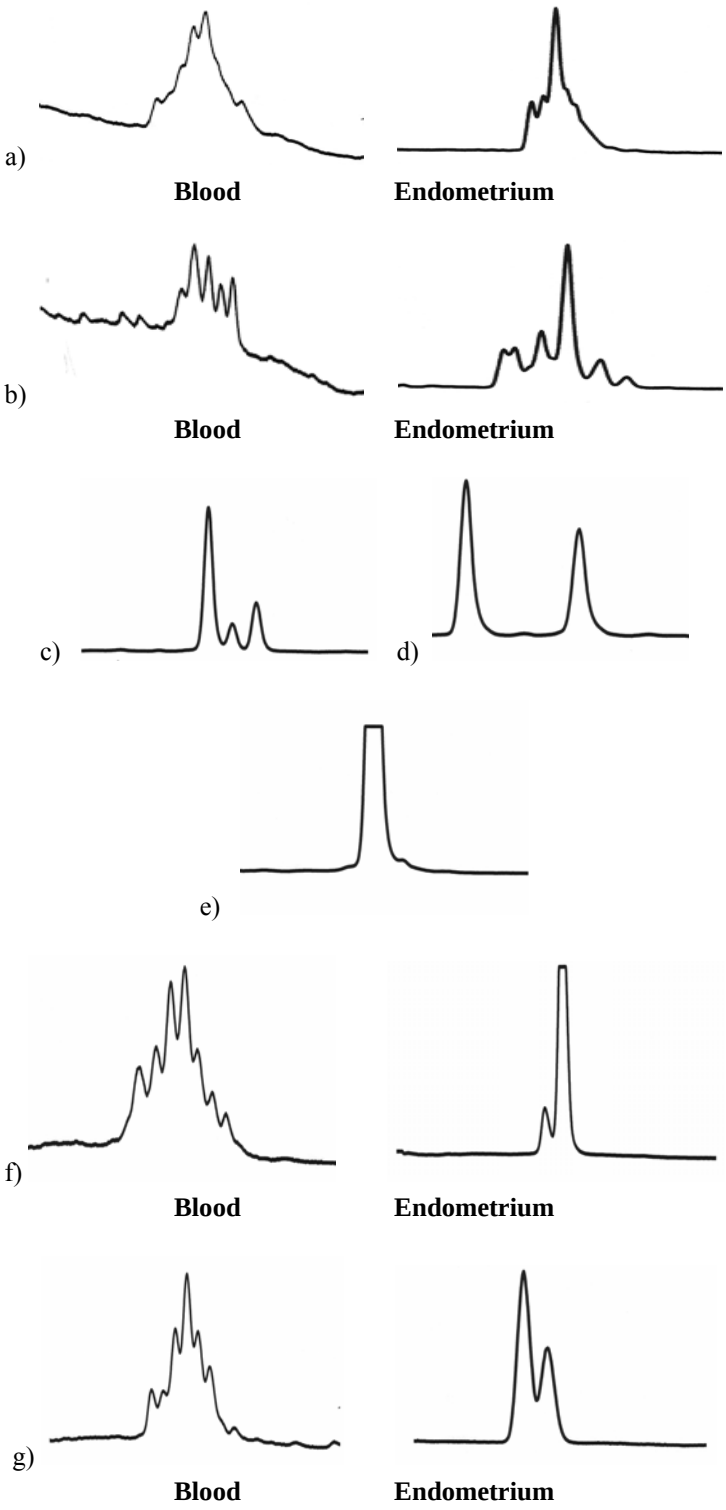

Figure 3: CDR3 length polymorphism curves a,b)of TCRBV7 transcripts in blood and endometrium; c,d) showing restricted pattern of transcripts of TCRBV10 in endometrium; e)showing very restricted pattern of transcripts of TCRBV10 in endometrium; f,g) of TCRBV12 transcripts in blood and endometrium.

TCRBV10 had more limited expression and the least diverse transcripts of all three genes studied, as all cases (100%) showed the oligoclonal pattern for expression of this gene (Figure 3c,d) and one case had even very restricted (probably monoclonal) pattern in the endometrium (Figure 3e), with all having polyclonal transcripts in the blood. The unique feature of CDR3 length polymorphism observed in TCRBV7 transcripts in the endometrium with sharp, discrete and distant peaks was also present for TCRBV10 transcripts in the endometrium of one of our cases.

As for clonal pattern of its transcripts, TCRBV12 showed a moderate level of oligoclonality. Three out of five endometrial samples studied at this stage (60%) had oligoclonal pattern, while in the same cases the distribution of CDR3 length of blood transcripts were mostly polyclonal (Figure 3f,g).

In order to evaluate whether oligoclonal pattern of expression is an invariable feature of endometrium, we studied the pattern of transcripts of another gene, TCRBV23, in selected cases and observed a rather more polyclonal pattern of expression in the endometrium, though the number of peaks seemed more limited in the endometrium than in blood (data not shown).

Overall, no correlation was observed in the type of pattern of CDR3 length distribution of any of the three genes studied between blood and endometrium of each case ($p>0.05$).

DISCUSSION

Using semiquantitative RT-PCR and CDR3 length polymorphism analysis, we provided here, for the first time, evidence for biased expression of TCRBV genes in the endometrium and the existence of a very restricted and clonal population of T cells in terms of expressing nearly similar transcripts of at least three genes for variable segment of β chain (TCRBV7, 10 and 12).

Although any claim about the clonality or exact nature of residing T cell populations in the endometrium would be void without results from quantitative and more decisive evaluations like flowcytometry or molecular clonig and sequencing, we sought as the first step in characterizing one of the most prevalent and most influential cell populations in the endometrium, that is T cells, to determine the differential usage of variable genes and the homogenicity of their transcripts in this mucosal site compared to blood. The results of similar methodology , though more relative and less precise have been proven to be in close harmony with more advanced, complicated and costly investigations, in terms of both time and money, and thus have been proposed as an appropriate first-line approach for examining unknown T cell population.[20,21]

The fine specificity of T cell responses relies mainly on the recognition of foreign antigens in the context of self MHC molecules by their specific receptors which are clonally expressed on their surfaces and consist of a heterodimer of either αβ or γδ composition. αβ T cells comprise the dominant lymphocyte population in blood and nearly every other tissue including endometrium, in humans.[22,23] Whereas the hypervariable complementarity-determining region 2 (CDR2) of both TCRα and TCRβ chains contact the MHC molecule, the CDR3 regions interact with the MHC-bound peptide.[24] Thus CDR3 is the loop with the highest potential diversity. The βCDR3 (CDR3 of β chain), is generated by somatic recombination of the TCR variable (TCRBV), diversity (TCRBD) and joining (TCRBJ) gene segments and further N-region diversification and thereby contributes substantially to antigen specificity of TCR.[25,26] Chracterization of TCRBV repertoire in blood, tissues or in the course of pathological conditions, therefore, has been utilized successfully as an estimate of T cell repertoire. Other than thymic selection, exposure to environmental antigens and microbial superantigens has great impact on shaping T cell repertoire and can thus dramatically alter the usage of particular V gene segment usage.[27]

Based on such estimation, we reached the conclusion in our study that although endometrial T cells are able to potentially express nearly all TCRBV genes, endometrial usage of these genes is much more limited in each person than the blood. In addition, the endometrial usage of the TCRBV genes is skewed towards the expression of some genes, most prominent of which was TCRBV7 and to a lesser extent TCRBV10 and TCRBV12. As shown in previous studies, TCRBV repertoire restrictions do not necessarily result from disease-associated activation of tissue T cells, but it seems to be a common feature of totally healthy and normal cutaneous and mucosal surfaces. In normal skin of adults, a bias toward usage of TCRBV2,3,6 and 12 has been reported with limited TCR transcript heterogeneity in some of these genes suggesting a preferential T cell selection or in-situ proliferation.[28] A

very similar pattern has been repetitively reported in the gastrointestinal tract. T cells in the intestinal mucosa are mainly located in two distinct areas, lamina propria and intra epithelial. It is in the intra-epithelial lymphocytes (IEL) population that skew toward one or several TCRBV genes with oligoclonal or even monoclonal usage pattern has been observed in several investigations.[29-34] Similarities between IELs and endometrial T cells are numerous and indisputable; both sites are the most superficial layers of parts of common mucosal immune system with direct and first-hand contact with foreign antigens, both T cell groups are composed of mainly $CD8^+$ cells in close proximity of epithelial cells, the function of both cell group is to provide and maintain tolerogenic responses to foreign antigens which are food allergens in case of IELs and sperm or fetal antigens in endometrium. Regarding such level of similarity, it is by no means surprising that the restricted TCRBV usage is shared by T cells at both sites.

In contrast to microbial superantigens which bind to a specific β variable domain outside CDR3 region and thus elicit a rather nonspecific and polyclonal proliferation of T cells, mono or oligoclonal expansion of T lymphocytes exclusively requires stimulation by nominal (conventional) antigens or neoplastic transformation.[35] Concerning the presence of activation markers on the surface of most endometrial T lymphocytes and our finding of limited heterogeneity of over expressed TCRBV genes in the endometrium, one may reasonably conclude that continuous stimulation by a small number of foreign antigens, probably sperm antigens, or unknown self stress antigens, might have led to clonal proliferation of a limited number of T cells in the endometrium.

On the other hand, the fact that no T cell specific antigens have so far been recognized on human sperm[36] and the relative homology of endometrial T cell repertoire between different individuals in the face of their different MHC alleles raise another possibility that endometrial T cells may have selectively been recruited and concentrated in this tissue by recognizing a nonpolymorphic MHC-like molecule(s) expressed specifically in endometrium or in at a larger scale, in the whole mucosal system. Such molecules as CD1 or TL (Thymus Leukemia) have been implicated in the same process in the intestine.[32,33] This notion is particularly attractive because it supports a comprehensive and linked mucosal circulation and training for T cells at various mucous membranes analogous to what is currently accepted for mucosal B cells[1] but with the main mission of prevailing immunological tolerance rather than active response. Any compromise in migration or activation of this mucosal $CD8^+$ T cell population would conceivably lead to different problems such as food allergy in intestine or abortions in the genital tract.

Finally, our findings that restriction in T cell variable gene usage is a constitutive characteristic of normal endometrium would provide a ground for future detailed studies addressing the exact nature and antigen specificities of endometrial T cells in health, as well as in various gynecological problems such as endometriosis, recurrent abortions, unexplained infertility and many other endometrial abnormalities in which T cells are putatively involved.

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