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T-cell receptor heterogeneity of γδ T-cell clones from human female reproductive tissues

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SUMMARY

γδ T cells were isolated from human decidua parietalis, decidua basalis and cervix and cloned in the presence of interleukin-2 (IL-2). T-cell receptor (TcR) expression was then analysed and compared with that of a panel of γδ T-cell clones from peripheral blood. Only 17/40 (42·5%) clones from decidua parietalis were $V\gamma9^+/V\delta2^+$ as compared to 68/94 (72%) of peripheral blood clones ($P<0·005$). Conversely, 50% of clones from decidua parietalis but only 15% of clones from peripheral blood were $V\delta1^+$ ($P<0·001$). At least seven distinct TcR types were identified among the panel of clones from decidua parietalis and at least six different types were expressed by the panel of 17 clones from cervix. This receptor heterogeneity was not a result of interdonor variation as in all instances where more than one clone was obtained from a single sample, individual clones having between two and five receptor types were identified. However, 23/24 (95·8%) of clones from decidua basalis were $V\gamma9^+/V\delta2^+$. Most clones from decidua parietalis and cervix, whether $V\gamma9^+/V\delta2^+$ or $V\delta1^+$, were positive for the mucosal lymphocyte marker, HML-1, but expression was often heterogeneous within a single clone. In contrast, almost all γδ T-cell clones from peripheral blood were HML-1^-. Thus, unlike the mouse, γδ T cells within these human female reproductive tissues have a diverse TcR repertoire which, in decidua parietalis, is distinct from that of peripheral blood.

INTRODUCTION

In man, most peripheral blood T cells express an αβ T-cell receptor (TcR) for antigen but a small subset, comprising 1–10% T cells, expresses a cell surface γδ TcR.[1] The function of these cells is not fully known but reactivity with heat-shock proteins has been reported, suggesting that they may have a surveillance function against stressed or transformed cells.[2] In the mouse, γδ T cells are relatively enriched in epithelial tissues and it has been suggested that their primary function is in epithelial immunity.[1,3] In man, although γδ T cells are present in most epithelial tissues studied, it is clear that there is not a substantial enrichment with respect to αβ T cells[4–8] except for intraepithelial lymphocytes (IEL).[9–12] In some types of inflammation of the bowel numbers of γδ T cells are increased at the site of tissue damage.[13,14]

The human γδ TcR has only a limited degree of combinatorial diversity; the majority of γδ T cells in peripheral blood of most normal individuals express the Vγ9/Vδ2 form of the receptor[15–17] while in a minority of cases most cells express Vδ1[15–17] or Vγ8/Vδ3.[18] Cells expressing a Vγ9/Vδ2 encoded receptor react with a diverse group of antigens including *Mycobacterium tuberculosis*,[19–22] staphylococcal enterotoxin A,[23] *Plasmodium falciparum* merozoites[24] and the Daudi cell line.[25] In at least some cases these responses are not major histocompatibility complex (MHC) restricted[20,24,25] and are independent of TcR γ and δ junctional region sequences.[20–22]

In the mouse, γδ T cells expressing the same Vγ and Vδ genes and having minimal junctional diversity are found in the skin.[26,27] A discrete subset of cells expressing the same Vδ gene but a different Vγ gene from that of epidermal γδ T cells, is located in epithelia of the vagina, uterus and tongue.[28,29] However, TcR of intestinal γδ T cells show a greater range of both combinatorial and junctional diversity.[30,31] It has also been shown that cells expressing different forms of the γδ TcR develop at different stages in thymic ontogeny and leave the thymus in temporally discrete 'waves' to populate different epithelial tissues.[32,33] Similar thymic waves have been described in man, with $V\gamma9^+/V\delta2^+$ cells appearing early in foetal ontogeny and $V\delta1^+$ cells later in development.[34] Although $V\gamma9^+/V\delta2^+$ cells predominate in peripheral blood, $V\delta1^+$ cells are relatively enriched in intestinal epithelium.[6,12–14]

Immunohistochemical studies have revealed the presence of small numbers of γδ T cells in human female reproductive

Abbreviations: G, germ line; HML-1, human mucosal lymphocyte antigen-1; IEL, intraepithelial lymphocytes; IL-2, interleukin-2; PCR, polymerase chain reaction; PHA, phytohaemagglutinin; TcR, T-cell receptor.

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tissues, namely uterus, cervix, vagina and ovary, in the presence of inflammation,[7] and within the intraepithelial compartment[35] as well as total cellular extracts[36] of first-trimester decidualized endometrium. However, it is unclear whether these cells express essentially homogeneous receptors as has been found in the mouse. We have previously isolated small numbers of γδ T-cell clones from decidua and cervix and found that a lower proportion of these were Vγ9$^+$/Vδ2$^+$ than in peripheral blood.[18] Here, we have analysed more extensive panels of clones from human decidua and cervix, revealing that unlike the mouse there is considerable γδ TcR diversity as regards V and J gene usage in both tissues and that patterns of TcR expression differ from that commonly found in peripheral blood.

MATERIALS AND METHODS

Lymphocyte preparation and cloning

Peripheral blood was obtained by venepuncture from normal donors and anti-coagulated with preservative-free heparin. Blood was layered onto lymphocyte separation medium (Flow, Irvine, U.K.) and centrifuged at 400 ***g*** for 20 min. Cells accumulating at the interface were washed twice in phosphate-buffered saline (PBS) prior to use. Macroscopically normal cervix tissue, comprising both ecto- and endo-cervix, was obtained from total abdominal hysterectomy specimens from patients with menorrhagia. Decidua parietalis, the part of the decidualized endometrium with abembryonic location, was obtained from first trimester elective terminations of pregnancy. In some cases, only decidual tissue associated with placental bed (decidua basalis), identified by its whitish opaque appearance, was obtained and this was dissected free from placental tissue before use. Tissues were washed free of blood and minced with opposing scalpels and digested for 30 min in 0·1% collagenase (Type IV, Sigma, Poole, U.K.). Digests were filtered through fine gauze and the cells washed twice before depleting of αβ T cells.[18] Both peripheral blood and tissue γδ T cells were purified by labelling for 30 min at 4° with the monoclonal antibody TcR1 against a framework determinant of the αβ TcR–CD3 complex (1:20, Becton Dickinson, Cowley, U.K.). After washing in ice-cold PBS, cells were incubated with magnetic microbeads coupled to sheep anti-mouse Ig (Dynal, Wirral, U.K.) for 30 min at 4° with agitation at a ratio of beads:cells of 40:1. Cells bound to the beads were removed with a magnet and the remaining cells washed and counted. Cell populations depleted of αβ T cells were then cloned by limiting dilution as previously described.[18,37] Briefly, cells were plated in 96-well round-bottomed plates in RPMI-1640 containing: 10% autologous or allogeneic plasma, 2×10^5 autologous or allogeneic peripheral mononuclear cells/ml and 1×10^5 B-lymphoblastoid cells/ml (both given 5000 rads γ-irradiation), 100 U/ml interleukin-2 (IL-2; Biotest, Dreieich, Germany), 1 μg/ml phytohaemagglutinin (PHA; Wellcome, Beckenham, U.K.) and 1 μg/ml indomethacin (Sigma). Clones were maintained in the same feeder mixture but without PHA for 2–3 weeks prior to analysis.

Phenotypic analysis

Aliquots of *c.* 10^5 cloned cells were analysed for TcR and T-cell antigen expression using a panel of monoclonal antibodies: TcR1 (pan-αβ T cell, 1:20), TcRδ1 (pan-γδ T cell, 1:20), δTCS1 (Vδ1J1/2, 1:20; both T Cell Sciences, Cambridge, MA), A13 (Vδ1, 1:1000), BB3 (Vδ2, 1:1000; both from Professor L. Moretta, Genova, Italy), TiγA (Vγ9, 1:500; Dr T. Hercend, Villejuif, France), HML-1 (1:20; Dianova, Hamburg, Germany)[38] and MLA (1:100; Dako, Copenhagen, Denmark) (both recognizing a β7 integrin present on human mucosal lymphocytes[39] and anti-CD3 (1:100; Dako). After incubating for 30 min at 4°, cells were washed with cold PBS and incubated for a further 30 min at 4° with fluorescein-conjugated goat anti-mouse Ig (1:40; Sigma). Cells were then washed with cold PBS prior to analysis using an EPICS C flow cytometer (Coulter Electronics, Luton, U.K.).

DNA extraction and Southern blotting

Cloned cells (5×10^6 to 5×10^7) were lysed in the presence of proteinase K and SDS and extracted with phenol and chloroform/isoamyl alcohol. DNA was precipitated with ammonium acetate and ethanol and redissolved in TE buffer, pH 7·6.[40] Ten to 15 μg aliquots were digested with an appropriate restriction enzyme (*Eco*RI, *Hin*dIII, *Kpn*I or *Xba*I) and electrophoresed in 0·7% agarose gels. DNA was denatured, neutralized and transferred to nylon filters (Hybond N, Amersham International, Amersham, U.K.). These were then hybridized overnight at 68° with DNA probes labelled with α^{32}PdCTP (Amersham), using a random oligonucleotide priming kit (Boehringer Mannheim, Mannheim, Germany) and washed in 2 × SSC followed by 0·2 × SSC at 68°.

Polymerase chain reaction (PCR)

Briefly, rearrangements involving the Vγ1 subgroup genes were amplified from DNA (1 μg) from γδ T-cell clones using an adaptation of the PCR.[41] The primers used were a consensus Vγ1 primer and either a primer complementary to the Jγ1 and Jγ2 genes[41] or to JγP1 and JγP2. The latter primer had the sequence 5′ AGG TGA AGT TAC TAT GAG CTT AGT CCC TT 3′, which is exactly complementary to the JγP1 gene segment and has a 2 base pair (bp) mismatch with JγP2.[42] Both combinations of primers give rise to an approximately 400 bp fragment from the relevant rearranged genomic DNA. Following amplification, PCR products were electrophoresed in 2% agarose gels and visualized by ethidium bromide staining. Aliquots of amplified products were then digested with a panel of restriction enzymes (*Kpn*I, *Alu*I, *Hae*III, *Hpa*II, *Sca*I, *Taq*I and *Dde*I), permitting the identification of Vγ1 gene segments by virtue of the characteristic band patterns in 2% agarose gels in clones with mono- or biallelic rearrangements.[41]

DNA probes

The following probes were used in Southern blot analysis: pH 60 (Jγ1; Dr T. H. Rabbitts, MRC LMB, Cambridge, U.K.),[43] Vγ1 (Professor M.-P. Lefranc, Montpellier, France),[44] Jδ1, Jδ3, Vδ1, Vδ2 and Vδ3 (Dr M. S. Krangel, Dana Farber Cancer Institute, Boston, MA).[45] Patterns of rearranged bands were analysed as previously described.[18,42–46]

Statistical analysis

Differences between proportions of clones expressing different forms of the γδ TcR were analysed using a χ^2-test.

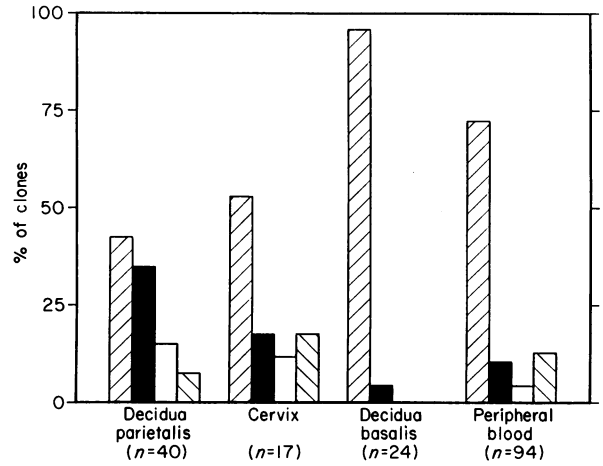

Figure 1. Phenotype of γδ T-cell clones isolated from human female reproductive tissues and peripheral blood on the basis of staining with monoclonal antibodies against Vγ and Vδ gene products. Histograms represent per cent clones having the phenotype: Vγ9$^+$/Vδ2$^+$ (■); Vγ9$^-$/Vδ1$^+$ (■); Vγ9$^+$/Vδ1$^+$ (□); and others (▨), comprising Vγ9$^+$/Vδ2$^-$ and Vγ9$^-$/Vδ1$^-$2$^-$ clones.

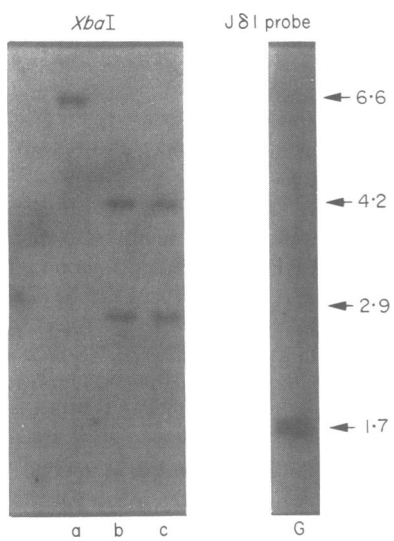

Figure 2. Southern blot of DNA from γδ T-cell clones digested with *Xba*I and hybridized with the Jδ1 probe. Lane a, clone D23.1D; lane b, clone D23.5A; lane c, clone D23.5B; G, germ line.

RESULTS

TcR expression by γδ T-cell clones from decidua parietalis

Forty TcRδ1$^+$ clones were isolated from 15 different first-trimester samples of decidua parietalis. Seventeen (42·5%) were Vγ9$^+$/Vδ2$^+$ clones as assessed by a combination of monoclonal antibody staining and Southern blot analysis (Figs 1, 2; Table 1). Three out of 11 tested (27%) expressed Vδ2 in association with joining gene segments other than Jδ1 (Table 1). Decidua parietalis yielded a lower proportion of Vγ9$^+$/Vδ2$^+$ clones than peripheral blood, from which 68/94 (72%) of γδ T-cell clones, from a panel of 10 different donors, were Vγ9$^+$/Vδ2$^+$; this difference was statistically significant ($\chi^2 = 9{\cdot}77$, df = 1, $P < 0{\cdot}005$). Furthermore, most of these had Vδ2Jδ1 rearrangements, with only 2/42 tested (5%) having rearrangements of Vδ2 to other Jδ genes, in both cases to Jδ3 (data not shown).

A much higher proportion of γδ T-cell clones from decidua parietalis (50%) were Vδ1$^+$ than for peripheral blood (15%; $\chi^2 = 16{\cdot}4$, df = 1, $P < 0{\cdot}001$) and 6/20 also expressed Vγ9, a configuration which was rare in peripheral blood clones. Two other Vγ genes, Vγ4 and Vγ5, were expressed by one or more Vγ9$^-$/Vδ1$^+$ clones and several clones had two VγI rearrangements, including one clone with both chromosomes rearranged to JP1 (D5.10; Table 1). Jγ gene usage in this and other clones was determined following *Kpn*I digestion and Southern blotting with a Jγ1 probe. Vγ gene usage was determined following *Eco*RI digestion and Southern blotting with a Jγ1 probe or in some cases by PCR with the consensus VγI primer and the appropriate Jγ primer (Jγ1/2 or JP1/2) and restriction enzyme digestion of the products. Vγ gene usage was assigned as previously described.[41] Clone D5.10 had biallelic Vγ2 and Vγ8JP1 rearrangements (Fig. 3a), clone D30.5 had a monoallelic Vγ5J1/2 rearrangement and clone D30.8 had biallelic Vγ2 and Vγ5 rearrangements to Jγ1 or Jγ2 (Fig. 3b).

Thus, including Vγ9, a minimum of four and a maximum of six different Vγ genes were expressed by one or more clones from decidua parietalis. Most Vγ9$^+$ clones expressed the disulphide-linked Cγ1 form of the γδ TcR but one of the Vγ9$^+$/Vδ1$^+$ clones (D7.1) expressed the non-disulphide-linked Cγ2 form. Most Vδ1$^+$ clones expressed Cγ2 but one clone (D5.10) expressed Cγ1. At least three Vδ genes were expressed with three clones being negative for all three Vδ markers A13, δTCS1 and BB3 (Table 1). A distinct pattern of γδ TcR expression was found in clones from decidua basalis. All but one of 24 TcRδ1$^+$ clones were Vγ9$^+$/Vδ2$^+$ (Fig. 1); the remaining clone expressed Vδ1 together with Vγ8J2 (data not shown).

TcR expression by cervical γδ T-cell clones

Seventeen clones were isolated from nine separate cervix samples. Again, a smaller proportion of these (53%) were Vγ9$^+$/Vδ2$^+$ than for peripheral blood (Fig. 1) but this was not statistically significant. Only 5/17 (29%) were Vδ1$^+$, of which two also expressed Vγ9. The remaining three clones expressed neither Vδ1 nor Vδ2 (Table 2); one of these expressed Vδ3J1. This and a second clone also expressed Vγ9JP and the third clone expressed either Vγ2 or Vγ4. All of the Vδ1$^+$ clones for which conclusive rearrangement data were obtained expressed the non-disulphide-linked Cγ2 form of the γδ TcR.

Expression of human mucosal lymphocyte antigen-1

The human mucosal lymphocyte marker, HML-1, is present on most IEL but on a smaller proportion of lamina propria lymphocytes and only on 1–3% peripheral blood lymphocytes.[11,38] HML-1 expression by some γδ T-cell clones obtained from cervix and decidua parietalis was therefore tested as this might have indicated whether they were derived from the epithelial compartment rather than from contaminating peripheral blood. In contrast to anti-TcR monoclonal antibodies, which essentially labelled either all or none of the cells from each clone, there was considerable heterogeneity of HML-1 staining

Table 1. TcR expression by decidual γδ T-cell clones. Underlining indicates the rearranged gene expressed

Clone	TcRδ1	TiγA	BB3	A13	δTCS1	γ genes	δ genes	Cγ
Vγ9⁺/Vδ2⁺								
D5.6	+	+	nt†	nt	−	V9JP V2/4J1/2	V2J1	1
D6.4	+	+	nt	nt	−	V9JP VI/IIIJP2	V2J1 V5J1	1
D6.9	+	+	nt	nt	−	V9JP V8J1/2	V2J? DJ1	1
D8.7	+	+	nt	nt	−	V9JP VI/IIIJP2	V2J3? DJ1	1
D22.5C	+	+	+	−	−		V2J1 VxJ1	
D22.10B	+	+	+	−	−	V9JP G*	V2J? DJ1	
D23.5A	+	+	+	nt	−	V9JP VI/IIIJP2	V2J1 DJ1	1
D23.5B	+	+	+	nt	−	V9JP G	V2J1 DJ1	1
D26.5A	+	+	+	nt	−	V9JP	V2J1	1
D30.6	+	+	+	−	nt	V9JP		1
D30.7	+	+	+	−	nt			
D30.12	+	+	+	−	nt			
D30.15	+	+	+	−	nt			
D31.10E	+	+	+	−	−	V9JP	V2J1	1
D32.5A	+	+	+	−	−	V9JP	V2J1	1
D33.10C	+	+	+	−	−			
D34.5J	+	+	+	−	nt			
Vγ9⁺/Vδ1⁺								
D6.12	+	+	nt	nt	+	V9J1/2 V2/4J1/2	V1J1 DJ1	
D7.1	+	+	nt	nt	+	V9J2 V3J2	V1J1 V2J1	2
D26.20D	+	+	nt	nt	+			
D29.20F	+	+	−	+	+			
D30.10	+	+	−	+	+			
D33.20L	+	+	−	+	+			
Vγ9⁻/Vδ1⁺								
D5.10	+	−	nt	nt	+	V2JP1 V8JP1	V1J1 V2J2	1
D8.11	+	−	nt	nt	+	V5J1/24 V7J1/2	V1J1	
D23.1D	+	−	−	nt	+	V4J2 V8J2	V1J1	2
D25.20C	+	−	−	nt	+	V5J1/2 V2JP1/2		
D26.20A	+	−	−	nt	+			
D29.20C	+	−	−	+	+			
D29.20D	+	−	−	+	+			
D29.20E	+	−	−	+	+			
D30.3	+	−	−	+	nt	V4J1/24 V7J1/2		
D30.5	+	−	−	+	nt	V5J1/2		
D30.8	+	−	−	+	nt	V2J1/2 V5J1/2		
D33.20F	+	−	−	+	+			
D34.5A	+	−	−	+	nt			
D35.10K	+	−	−	+	nt			
Vγ9⁻/Vδ1⁻2⁻								
D25.5A	+	−	−	nt	−			
D29.5A	+	−	−	−	−			
D29.10A	+	−	−	−	−			

* G, germ line.
† nt, not tested.

within individual clones. Three categories of labelling were therefore used in which <33%, 33–67% and >67% of cells within a clone were positive. Most γδ T-cell clones from decidua parietalis and cervix showed some staining with HML-1, whether they expressed the Vγ9/Vδ2 form or the Vδ1 form of the γδ TcR (Table 3). Similarly, most αβ T cell and CD3⁻ clones derived from cervix and decidua parietalis were HML-1⁺ (data not shown). In contrast, most peripheral blood γδ T-cell clones were HML-1⁻. The only clone in which >67% of cells were positive was a Vδ1⁺ clone but the remaining three Vδ1⁺ clones were negative for HML-1. All but one of the Vγ9⁺/Vδ2⁺ clones from peripheral blood were also HML-1⁻. These differences between HML-1 expression by decidual and peripheral blood clones were statistically significant ($\chi^2 = 16{\cdot}0$, df = 2, $P < 0{\cdot}001$).

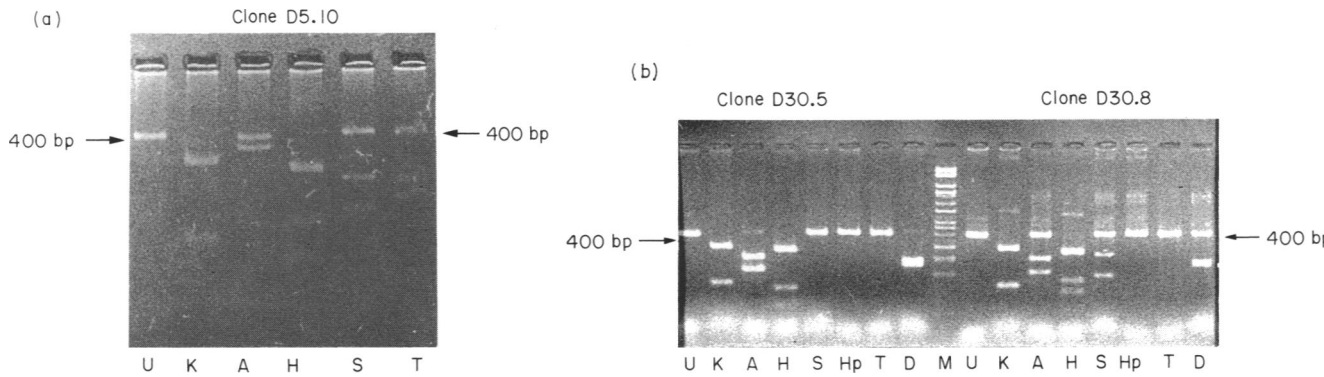

Figure 3. Restriction enzyme digestion of PCR products from decidual γδ T-cell clones. DNA was amplified with VγI and JγP1 primers (a) or VγI and Jγ1 primers (b) and aliquots digested with a panel of restriction enzymes as indicated: (a) clone D5.10; (b) clones D30.5 and D30.8. U, undigested; K, *Kpn*I; A, *Alu*I; H, *Hae*III; Hp, *Hpa*II; S, *Sca*I; T, *Taq*I; D, *Dde*I; M molecular weight markers.

Table 2. TcR expression by cervical γδ T-cell clones. Underlining indicates the rearranged gene expressed

Clone	TcRδ1	TiγA	BB3	A13	δTCS1	γ genes	δ genes	Cγ
Vγ9⁺/Vδ2⁺								
C2.18	+	+	+	nt	−			
C2.20	+	+	+	nt	−			
C2.21	+	+	+	nt	−			
C2.22	+	+	+	nt	−			
C3.5C	+	+	+	nt	−			
C6.1A	+	+	+	−	−			
C7.10D	+	+	+	−	−	V9JP	V2J?	1
C9.2A	+	+	+	−	nt	V9JP	V2J1	1
C9.5F	+	+	+	nt	nt		V2J1	
Vγ9⁺/Vδ1⁺								
C3.5B	+	+	−	nt	+			
C4.5C	+	+	−	nt	+	V9J2		2
Vγ9⁺/Vδ1⁻2⁻								
C1.6	+	nt	nt	nt	nt	V9JP, VJ1/2	V3J1, DJ1	1
C8.5E	+	+	−	−	−	V9JP, V2J1/2		1
Vγ9⁻/Vδ1⁺								
C2.16	+	−	−	nt	+	V2/4J2, V5J2	VJ1	2
C5.5B	+	−	−	nt	+			
C7.13	+	−	−	+	nt	V4J2, V5JP2		2
Vγ9⁻/Vδ1⁻2⁻								
C8.1C	+	−	−	−	−	V2J1/2, V4J1/2		

Table 3. HML-1 staining of γδ T-cell clones

% staining	Cervix		Decidua parietalis			Peripheral blood	
	Vγ9⁺/Vδ2⁺	Vδ1⁺	Vγ9⁺/Vδ2⁺	Vδ1⁺	Other	Vγ9⁺/Vδ2⁺	Vδ1⁺
<33%	1	2	4	4	—	13	3
33–67%	2	1	4	1	2	1	—
>67%	2	1	3	9	1	—	1

DISCUSSION

TcR expression by γδ T-cell clones from both decidua parietalis and cervix was heterogeneous with seven and six receptor types, respectively, identified using a combination of monoclonal antibody staining, Southern blotting and PCR. As these clones were obtained from a panel of donors it is possible that some of the heterogeneity was a result of interdonor variation. However, in all instances where more than one clone was obtained from a single decidual sample, clones with at least two, and up to five (D30) different types of receptor were identified. These findings suggest that γδ T cells localizing in human female reproductive tissues do not express invariant TcR and that even within a single individual cells expressing several different receptor forms may be present.

This is in contrast to the mouse in which vaginal epithelial γδ T cells express exclusively the Vγ4/Vδ1 form of the receptor and furthermore have almost no junctional diversity.[29] The reasons for this difference are unclear but may be related to the use of inbred mouse strains, raised in relatively pathogen-free conditions. As there is experimental evidence for intrathymic selection of γδ T cells in the mouse[47] the classical (or non-classical) MHC haplotype of the individual may influence the receptor specificity of mature γδ T cells. If positive selection on the basis of MHC recognition operates during intrathymic development of human γδ T cells, heterozygotes would be expected to select for receptor specificities appropriate to both MHC haplotypes. This may partially explain the well-known variation between donors in γδ T-cell repertoire in peripheral blood[15-17] and may also extend to reproductive tissues. Antigenic exposure may have a major influence on γδ T-cell repertoire and sexually active women would be expected to encounter a range of infectious agents and antigens within seminal fluid or placenta which may result in local expansion or infiltration of γδ T cells with different TcR types. In this context, the first-trimester decidual samples studied in the present experiments were inevitably from sexually active women. Also, the cervical samples were mainly from women aged 35–50 who would potentially have come into contact with a variety of microbial or other antigens. Thus the outbred nature of human populations, together with the comparatively greater antigenic exposure may be additional factors leading to heterogeneity of γδ TcR expression within female reproductive tissues as compared with inbred, relatively pathogen-free, virgin mice.

The source of γδ T-cell clones in the present experiments was a crude tissue digest, presumably comprising subepithelial and stromal tissue as well as the epithelial compartment. It is therefore uncertain what proportion of the clones was actually derived from IEL populations. Clones were tested for the expression of the mucosal lymphocyte antigen recognized by the monoclonal antibody HML-1,[38] which labels 10–15% freshly isolated decidual leucocytes,[36] although it is unclear whether these constitute decidual IEL. As most γδ T-cell clones from decidua and cervix expressed the HML-1 antigen, this would indicate that the majority were derived from within the tissue rather than from contaminating peripheral blood. Although the HML-1 antigen has been reported to be induced on peripheral T cells stimulated with PHA,[48] almost all (89%) of γδ T-cell clones isolated from peripheral blood and cultured under the same conditions as those from decidua and cervix remained negative for HML-1. However, it has been suggested that the novel β7 integrin recognized by the HML-1 antibody is induced locally on IEL rather than constituting a specific homing receptor;[49] the present results suggest that its expression is stable on most γδ T-cell clones maintained in IL-2. However, some γδ T-cell clones from decidua parietalis and cervix were HML-1$^-$, suggesting either that some negative cells are present in these tissues or in contaminating peripheral blood, or that the antigen can be lost in culture. The reason for the heterogeneity of staining for HML-1 within individual clones is unclear but could be related to the stage of the cell cycle or whether the cloned cells were actively proliferating. Immunohistochemical studies have revealed very small numbers of intraepithelial γδ T cells in human decidual tissue[35] and dual staining with HML-1 and TcRδ1 would clarify what proportion of γδ T cells in decidua and cervix are HML-1$^+$ IEL.

A higher proportion of γδ T-cell clones from both cervix and decidua parietalis expressed Vδ1 than was found in peripheral blood clones from a large panel of donors, indicating that Vδ1$^+$ cells are relatively enriched in reproductive tissues in comparison with peripheral blood. The excess of Vδ1$^+$ cells is in concordance with the majority of reports of anti-Vδ1 staining of intestinal lymphocytes.[12-14] This enrichment could be a result of preferential homing to epithelial tissues of Vδ1$^+$ as compared to Vγ9$^+$/Vδ2$^+$ cells or of increased local expansion of Vδ1$^+$ cells. Vδ1$^+$ cells are greatly increased in gastrointestinal epithelium of patients with inflammation of the bowel,[13,14] indicating that both mechanisms may be operative. The possibility remains that there are superantigen-like stimuli which specifically activate Vδ1$^+$ cells as has been found for Vγ9$^+$/Vδ2$^+$ cells[19-25] and it has recently been reported that a subset of Vγ9$^+$Vδ1$^+$ cells is stimulated by the Daudi cell line.[50]

In contrast to the findings with cervix and decidua parietalis, almost all γδ T-cell clones from decidua basalis were Vγ9$^+$/Vδ2$^+$. The reasons for this are unclear but as this region is anatomically more intimately associated with a large volume of maternal blood than the decidual tissue lining the remainder of the uterus (decidua parietalis) there would be a greater likelihood of peripheral blood contamination or extravasation of γδ T cells. Alternatively, some of these cells may be of foetal rather than maternal origin in view of the early thymic wave of Vγ9$^+$/Vδ2$^+$ cells,[34] although there is no evidence for this.

It has been suggested that the presence of γδ T cells with invariant receptors in murine vagina and uterus is to combat a commonly encountered infectious agent[28,29] but it is uncertain whether this is the exclusive function of these cells in human tissues. As the cervix is a frequent site of neoplastic transformation, these cells may mediate epithelial surveillance against virally infected or transformed cells. One of the best-studied functions of γδ T cells is their non-MHC-restricted cytotoxic activity and we have found that decidual γδ T-cell clones, including Vδ1$^+$ clones, which are normally less cytotoxic than Vγ9$^+$/Vδ2$^+$ clones,[51] can mediate this function.[18] Alternatively, γδ T cells may exhibit MHC-restricted functions and although clones have been described which are restricted to classical MHC antigens, reactivity with non-classical MHC antigens has been reported.[52] In this context, MHC expression by trophoblast, the foetal tissue in closest contact to maternal decidua, is confined to the novel class I-like antigen HLA-G,[53] suggesting the possibility that decidual γδ T cells may be restricted by HLA-G molecules. Future work will investigate this possibility.

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