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T-cell receptor γδ-expressing intraepithelial lymphocytes are present in normal and chronically inflamed human gingiva

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SUMMARY

The phenotypic profile of leucocytes in diseased and normal gingival tissue was studied *in situ* and in isolated gingival mononuclear cell (GMC) preparations. T-cell receptor (TcR)$\gamma\delta^+$ cells showed preferential localization to epithelium, both in normal and inflamed gingiva, and were present in crevicular as well as oral epithelium. In normal gingiva $\geqslant 30\%$ of the isolated leucocytes expressed TcR$\gamma\delta$, of which the majority were CD4$^-$ CD8$^-$, and expressed CD45RA. The proportion of TcR$\gamma\delta^+$ cells in GMC from periodontitis tissue varied between 2 and 32%. In contrast to normal gingiva the majority of TcR$\gamma\delta^+$ cells in diseased tissue were CD8$^+$ and expressed CD45RO. Thus expression of the CD8 antigen on gingival TcR$\gamma\delta^+$ cells is probably a consequence of immune activation. Numerous Langerhans' cells and keratinocytes expressing the major histocompatibility complex (MHC) class I-like antigen, CD1, were present within normal and inflamed gingival epithelium in close proximity to the TcR$\gamma\delta^+$ cells. Most CD1a$^+$ cells were scattered within oral epithelium. CD1c$^+$ cells were localized close to the basal layer of crevicular epithelium. No CD1b$^+$ cells were found. TcR$\alpha\beta^+$ cells, CD4$^+$ and B cells were restricted to lamina propria of periodontitis lesions. The presence of intraepithelial TcR$\gamma\delta^+$ cells in normal gingiva suggests that they constitute the 'first line of defence' against the potentially harmful microflora in the oral cavity. Induction of CD8 and CD45RO antigens on TcR$\gamma\delta^+$ cells in periodontitis tissue indicate that they play a significant role in the disease. CD1 molecules on Langerhans' cells and keratinocytes may be the restriction elements for the CD8$^+$ TcR$\gamma\delta^+$ cells.

INTRODUCTION

The majority of the peripheral T lymphocytes express T-cell receptor (TcR)$\alpha\beta$ which is used by the cell to recognize antigens in the context of self major histocompatibility complex (MHC) molecules. A second TcR, termed $\gamma\delta$, has been identified on a small subset of cells in thymus and peripheral blood.[1] TcR$\gamma\delta^+$ cells have also been found in many human foetal and adult tissues, including intestinal epithelium and skin.[2,3] The biological role and repertoire of TcR$\gamma\delta^+$ cells is poorly understood. TcR$\alpha\beta^+$ cells generally express either CD4 or CD8, the accessory surface molecules associated with MHC class II and class I restricted antigen recognition, respectively. In contrast, the majority of TcR$\gamma\delta^+$ cells are CD4$^-$ CD8$^-$.[1,2] However, murine intestinal intraepithelial lymphocytes as well as a subset of human peripheral blood and spleen lymphocytes have been shown to be CD8$^+$ TcR$\gamma\delta^+$.[3-5] A small number of CD4$^+$ TcR$\gamma\delta^+$ clones have also been isolated from human peripheral blood. Polymorphic MHC class I and II antigens do not seem to be the restriction elements for TcR$\gamma\delta^+$ cells. Murine TcR$\gamma\delta^+$ cells restricted for non-polymorphic MHC class I molecules have been demonstrated[6] and human CD4$^-$ CD8$^-$ TcR$\gamma\delta^+$ peripheral blood lymphocytes specific for the non-MHC encoded CD1 antigen have been described.[7,8]

TcR$\gamma\delta^+$ cells seem to play a role in antibacterial responses. Thus TcR$\gamma\delta^+$ cells can be activated to proliferation, interleukin production and/or cytotoxicity by bacterial heat-shock proteins, mycobacterial antigens and a variety of bacteria.[9-11] It is probable that infected or transformed epithelial cells can be killed by TcR$\gamma\delta^+$ cells after activation by bacterial heat-shock proteins cross-reacting with autologous heat-shock proteins on 'stressed' cells. Alternatively, cytotoxic TcR$\gamma\delta^+$ cells could recognize microbial antigen(s) in the context of non-polymorphic MHC on infected cells.[12,13] Increased numbers of TcR$\gamma\delta^+$ cells have been found in different infectious disease lesions[14] and in chronic inflammatory diseases such as rheumatoid arthritis and coeliac disease.[15-18]

Periodontitis is a chronic inflammatory disease caused by bacterial plaque in the periodontal pocket. Immune cells accumulating in the subepithelial tissue are considered to play an important role in the disease process.[19,20] However, the characteristics and function of leucocytes residing within the epithelial structures have been overlooked.

Here we report on the presence of intraepithelially located TcR$\gamma\delta^+$ cells in normal and periodontally diseased human

Abbreviation: GMC, gingival mononuclear cells.

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gingiva. To our knowledge this is the first demonstration of TcR$\gamma\delta^+$ cells in normal human oral mucosa.

MATERIALS AND METHODS

Gingival samples

Human gingival tissues were obtained from 21 patients, 13–67 years old (12 women and nine men) undergoing periodontal surgery at the Dept. of Periodontology, University of Umeå, Sweden. Sites subjected to surgery belonged to one of the following diagnosis categories; adult periodontitis, juvenile periodontitis and rapidly progressive periodontitis. Three sites were diagnosed as normal (clinically healthy) gingiva. The diagnoses were based on clinical and radiographic criteria according to AAP consensus 1989. Surgery was performed as full-thickness flap, split-thickness flap or modified Widman technique. A piece of tissue was snap frozen in precooled isopentane and used for immunohistochemical studies.

Isolation of gingival mononuclear cells (GMC) and peripheral blood mononuclear cells (PBMC)

GMC were isolated from 24 gingival samples. The wet weight of the tissue samples ranged from 100 to 799 mg (mean $\pm$ 1 SD was 268 $\pm$ 192 mg). Tissue was immediately placed on ice in Tris-buffered Hanks' salt solution (TH; pH 7·2) containing 100 U penicillin g/ml (ASTRA, Södertäljeh, Sweden), 100 μg streptomycin/ml (Glaxo, Greenford, U.K.) and 2·5 mg amphotericin B/ml (Gibco, Paisley, U.K.). The tissue was extensively washed until visible blood was removed, diced and incubated at 37° with vigorous shaking for 60 min in RPMI-1640 (Gibco) containing 290 U collagenase (Type IV; Worthington Biochemical Co., Freehold, NJ)/ml, 5 mM $CaCl_2$, 12·5 mM HEPES and antibiotics. Detached cells and tissue fragments were gently pressed through

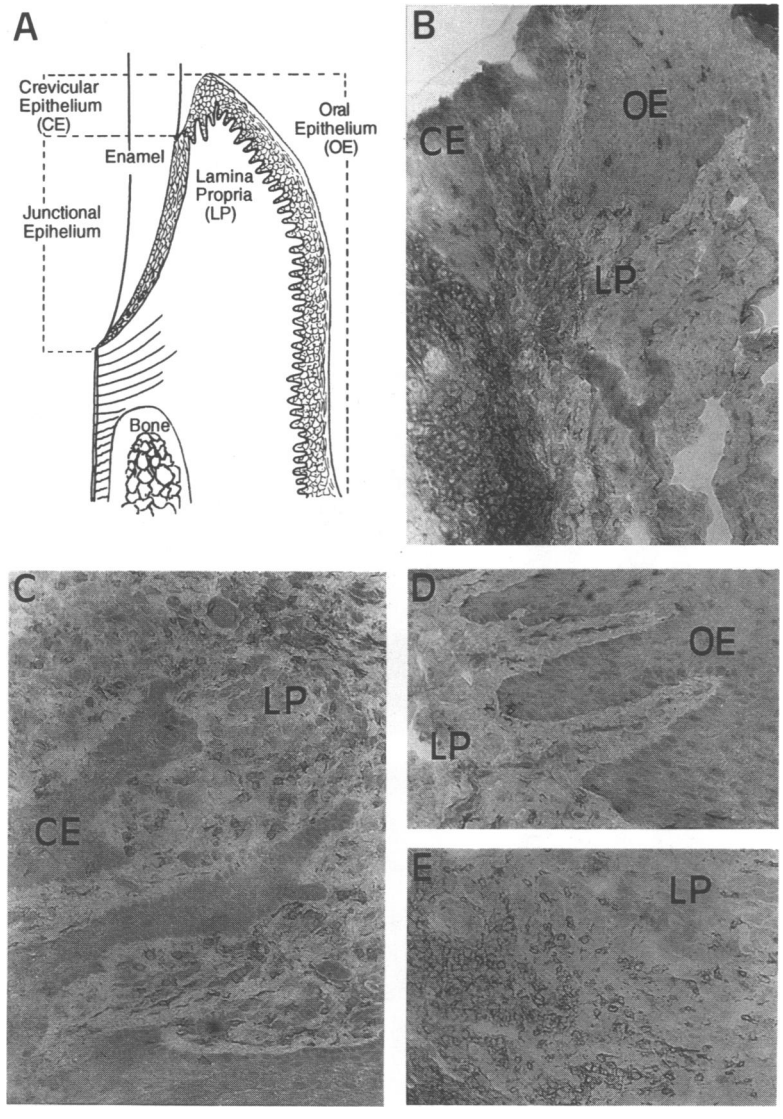

Figure 1. (A) Schematic drawing describing the anatomy of the gingival tissue. Immunoperoxidase staining of cryosections of adult periodontitis gingiva with (B) anti-CD3 mAb T3-4B5 (original magnification × 32), (C) anti-CD4 mAb MT310 (original magnification × 51), (D) and (E) anti-CD15 mAb C3D-1 (original magnification × 51). LP, lamina propria; OE, oral epithelium; CE, crevicular epithelium.

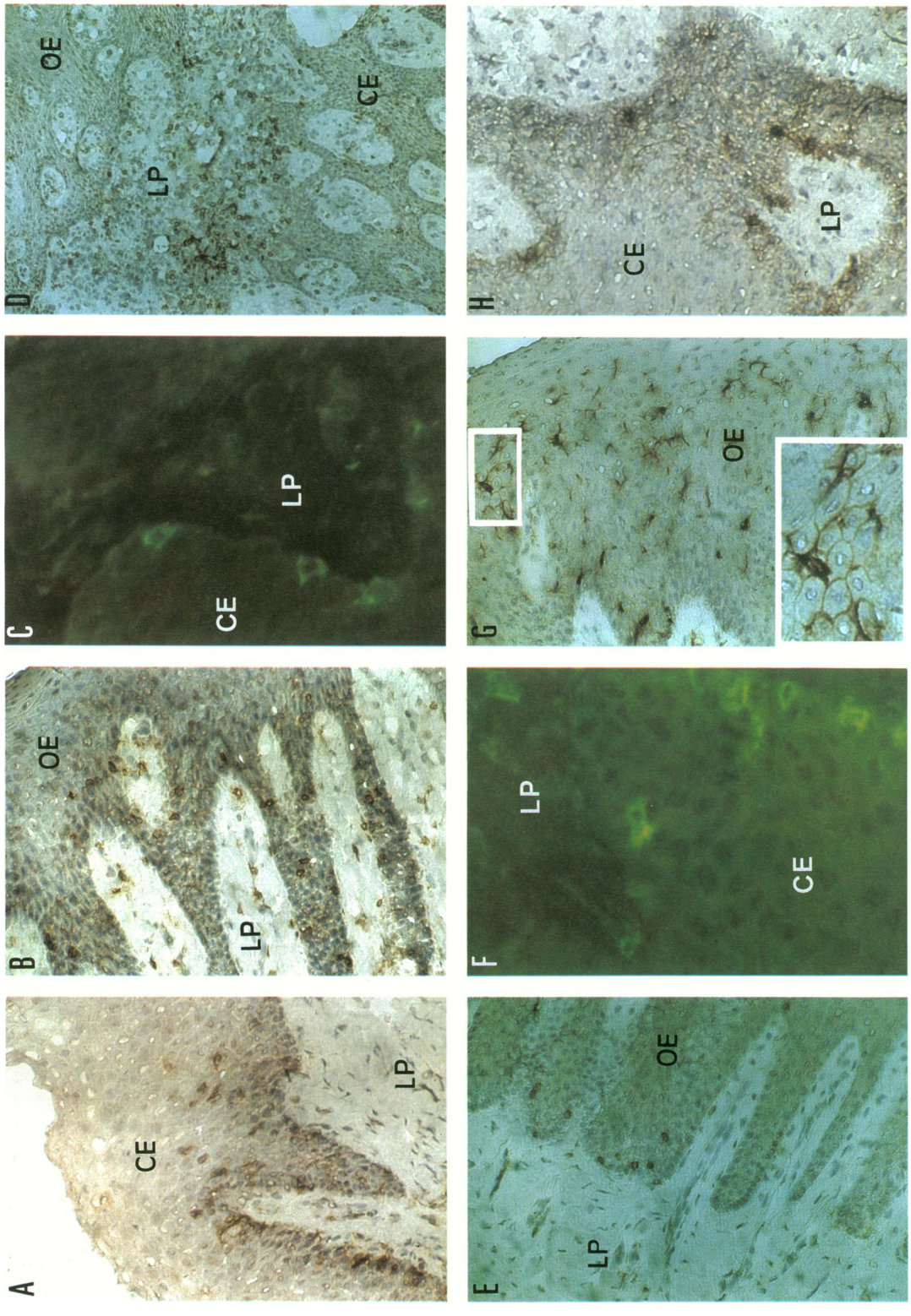

Figure 2. Immunohistological staining of cryosections of adult periodontitis (A–D, G, H) and normal (E, F) gingiva with (A), (B) and (E) anti-CD8 mAb DK25 (original magnification ×51), (C) and (F) a mixture of anti-TcR$\gamma\delta$ mAb TcRδ1 and δTCS1 (original magnification ×160), (D) anti-TcR$\alpha\beta$ mAb βF1 (original magnification ×32), (G) anti-CD1a mAb NA1/34 (original magnification ×51, insert original magnification ×128) and (H) anti-CD1c mAb L161 (original magnification ×51). Sections were stained by indirect immunoperoxidase technique (A, B, D, E, G, H) or indirect immunofluorescence (C, F). LP, lamina propria; OE, oral epithelium; CE, crevicular epithelium.

a stainless steel grid (60 mesh). Passed cells were washed with TH containing human serum albumin (HSA), suspended in 67% Percoll (Pharmacia, Uppsala, Sweden), layered under a grandient with 44 and 20% Percoll and centrifuged for 25 min at 500 *g*. The interphase between 67 and 44% Percoll, containing mononuclear cells, was collected. An average of $3{\cdot}7 \times 10^3$ GMC were recovered per mg of tissue. Viability, as determined by Trypan blue exclusion, was $\geq 95\%$. Cytocentrifuge smears of isolated GMC were air dried overnight and stained with May-Grünwald/Giemsa.

Venous blood was collected from eight patients during surgery. PBMC were isolated by Ficoll–Isopaque (Lymphoprep, Nycomed Pharma, Oslo, Norway) gradient centrifugation.

Monoclonal antibodies (mAb)

The following mAb were used: anti-TcRαβ (βF1 and BMA 031, both from T cell Diagnostics, Cambridge, MA and WT31, Becton Dickinson, Mountain View, CA); anti-TcRγδ (TcRδ1 and δTCS1, both from T cell Diagnostics and BB3, a kind gift from Dr L. Moretta, Istituto Nazionale per la Ricerca sul Cancro, Genua, Italy); anti-CD3 (T3-4B5, Dakopatts, Glostrup, Denmark and OKT3, purified culture supernatant of hybridoma cell line from ATCC, Rockville, MD); anti-CD4 (MT310); anti-CD8 (DK25); and anti-CD45RO (UCHL-1, Dakopatts); anti-CD45RA (Leu-18, Becton Dickinson); anti-CD20-like (B1, Sera Lab, Crawley Down, UK); anti-CD14 (Leu-M3, Becton Dickinson); anti-CD57 (NC-1, Immunotech, Marseilles, France); anti-CD15 (C3D-1) and anti-CD45 (a mixture of PD7/26 and 2B11, Dakopatts); anti-CD1a (NA 1/34, Dakopatts); anti-CD1b (4A7-6) and anti-CD1c (L161, Immunotech).

Immunohistochemical staining

Serial, 5 μm thick, cryostat sections, were air dried for 30 min at room temperature and fixed in acetone at −20° for 10 min.

For immunoperoxidase staining, endogenous peroxidase activity was blocked by incubation with 0·0003% H_2O_2 in 1 mM NaN_3 at 37°. Thereafter the sections were incubated with 0·2% bovine serum albumin (BSA) in 0·02 M phosphate-buffered saline (PBS; pH 7·2), followed by mAb and finally by horseradish peroxidase conjugated F(ab′)$_2$ fragments of sheep anti-mouse Ig (Amersham International, Amersham, U.K.). MAb and conjugate were diluted in PBS containing 0·2% BSA. The sections were developed with 0·05% 3′,3′-diaminobenzidine (Sigma Chemical Co., St Louis, MO) solution in Tris buffer, pH 7·6, containing 0·03% H_2O_2 and counter stained with Mayers Haematoxylin.

TcRγδ could only be revealed by immunofluorescence. For immunofluorescence staining, sections were treated and incubated with mAb as above without blocking of endogenous peroxidase activity. Thereafter fluorescein isothiocyanate (FITC)-labelled rabbit anti-mouse Ig (Dakopatts) was added followed by FITC-labelled swine anti-rabbit Ig (Dakopatts). Sections were finally mounted in 50% glycerol in TH and immediately evaluated in a fluorescence microscope.

Immunoflow cytometry

Cells ($0{\cdot}5$–1×10^5) were suspended in TH containing 0·4% HSA and 0·02% NaN_3 and incubated with mAb on ice, followed by incubation with FITC or phycoerythrin (PE) conjugated F(ab′)$_2$ fragments of affinity-purified goat anti-mouse IgG and IgM (Jackson Immunoresearch Laboratories Inc., West Grove, PA). In two-colour staining experiments a final incubation with directly conjugated mAb was performed. After each incubation the cells were centrifuged once through foetal calf serum. Cells incubated with irrelevant mAb of the same isotype served as controls. Ten thousand viable cells/sample were analysed by flow cytometry on FACScan (Becton Dickinson). Viable cells were identified by exclusion of propidium iodide (0·5 μg/ml; Sigma).

RESULTS

The phenotypic profile of leucocytes from periodontitis lesions was compared to that of normal gingival tissue *in situ* using immunohistochemistry, and in isolated GMC preparations using immunoflow cytometry.

Table 1. Surface marker profiles of GMC preparations from sites with different clinical diagnoses

	Surface marker				
Sample	CD3	TcRαβ	TcRγδ	CD20	Ratio CD4$^+$/CD8$^+$
Adult periodontitis					
1	52*	42	17	4	0·8
2A†	52	34	10	29	0·8
2B	38	31	7	10	1·0
3	37	17	18	11	0·8
4	41	36	4	10	1·3
5	63	55	5	25	1·5
6	28	24	5	14	1·4
7	19	24	32	8	1·8
8	56	51	24	ND‡	0·7
9	30	19	7	ND	0·7
10	28	19	3	ND	1·0
11	54	39	28	ND	0·4
12A	62	39	17	ND	1·1
12B	55	31	13	ND	1·0
Juvenile periodontitis					
1	54	46	8	50	2·0
2A	44	23	8	17	1·0
2B	37	32	24	55	0·9
2C	25	22	4	14	1·4
3	36	35	2	28	1·6
Rapidly progressive periodontitis					
1	32	11	20	60	1·3
2	37	36	24	29	1·1
Normal					
1	67	18	35	10	0·8
2	56	22	30	ND	0·7
3	ND	42	32	ND	1·6

* Values are calculated as percentage of CD45$^+$ cells in each sample.

† Suffix letters A, B and C indicate that the samples were obtained from the same individual at different occasions and from different sites.

‡ ND, not determined.

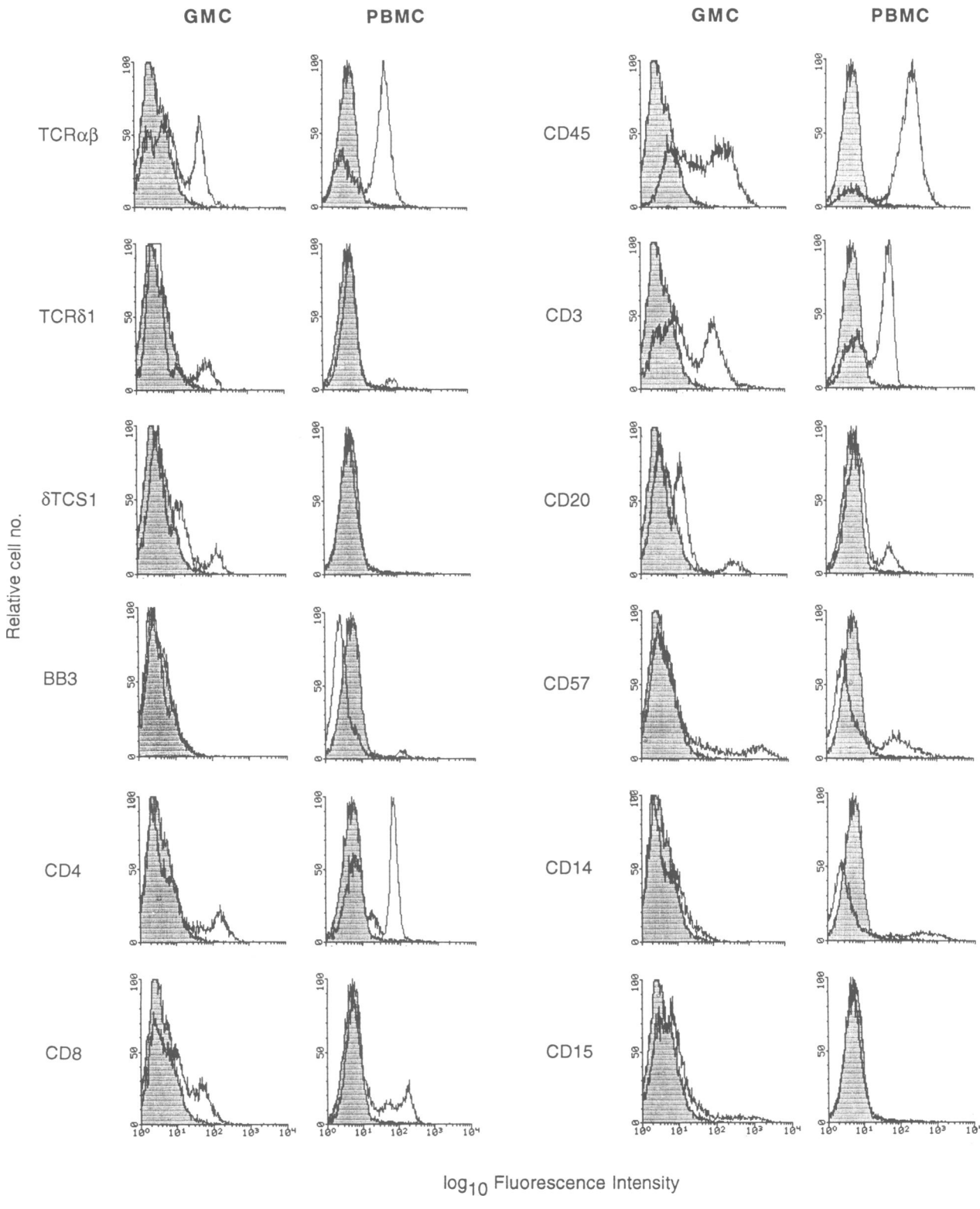

Figure 3. Flow cytometric analysis of cell surface molecule expression by GMC and PBMC isolated from a patient with adult periodontitis. The control is superimposed in the graph as a shaded area.

TcRγδ$^+$ cells reside exclusively within epithelium

Most cells expressing the common leucocyte antigen (CD45) in normal gingiva were located in the oral and crevicular epithelium. CD45$^+$ cells in periodontal disease tissues were present in lamina propria as well as scattered within the epithelium. The majority of the intraepithelial leucocytes were T cells (CD3$^+$ cells; Fig. 1B). TcRγδ$^+$ cells were localized exclusively within crevicular and oral epithelium, both in normal as well as in diseased gingiva (Fig. 2C and F). TcRαβ$^+$ cells were not detected in epithelium of normal gingiva but were occasionally found within the crevicular epithelium in periodontal disease tissues (Fig. 2D). Intraepithelial CD8$^+$ cells were frequent in inflamed gingiva and located at positions corresponding to those of TcRγδ$^+$ cells (Fig. 2A and B). Occasional intraepithelial CD8$^+$ cells were also seen in normal gingiva mainly in the basal part (Fig. 2E). No CD4$^+$ cells were detected within the epithelium. Intraepithelial neutrophil granulocytes (CD15$^+$ cells) were detected in periodontitis lesions (Fig. 1D), but not in normal tissues.

TcRαβ$^+$ cells and B cells reside in lamina propria of periodontitis tissue

The lamina propria infiltrate contained mainly CD3$^+$ cells (Fig. 1B) and CD15$^+$ cells (Fig. 1E). These T cells expressed TcRαβ (Fig. 2D) and no TcRγδ$^+$ cells were detected. All CD4$^+$ cells were located subjacent to the epithelium mostly in clusters (Fig. 1C). CD8$^+$ cells were scattered throughout lamina propria (Fig. 2A and B). B cells (CD20$^+$ cells) were localized to perivascular areas in lamina propria and not in contact with epithelial structures (not shown). In contrast to T cells, the number of CD20$^+$ cells varied extensively between different samples. Periodontitis lesions have been reported to be dominated by B cells;[20] however, we found T-cell dominance in most samples (Table 1).

CD1-expressing cells and TcRγδ$^+$ cells co-localize within epithelium

As CD1 has been suggested to be a restriction element for TcRγδ$^+$ cells we investigated the localization of CD1$^+$ cells in gingiva. The CD1 locus encodes five distinct non-polymorphic MHC class I-like molecules.[21] CD1a–c antigens are mainly expressed on cortical thymocytes while CD1d is expressed on normal intestinal epithelial cells. CD1a is also expressed on certain dendritic cells including Langerhans' cells in gingiva.[22] In this study mAb against CD1a–c were utilized. CD1a stained numerous cells within the basal and middle layer of the oral epithelium and very few cells within the crevicular epithelium. The majority of the CD1a$^+$ cells had the morphology of Langerhans' cells (Fig. 2G). CD1a$^+$ keratinocytes were also identified predominantly in sites with severe disease (Fig. 2G, insert). CD1c$^+$ cells were localized in the basal part of crevicular epithelium (Fig. 2H). No CD1b$^+$ cells were detected.

Composition of GMC

GMC were isolated by a combination of mechanical and enzymatic disintegration of gingival tissue samples followed by Percoll gradient centrifugation. The phenotype of GMC was determined by flow cytometry and differential counting of May–Grünwald/Giemsa stained cytospin smears. Seventy to 97% of the cells expressed CD45. CD3$^+$ cells constituted 19–54% of the leucocyte population and the number of CD20$^+$ cells varied from 4 to 61% (Table 1). In addition, 6–50% plasma cells, 6–39% natural killer (NK) cells (CD57$^+$ cells), 8–25% granulocytes (CD15$^+$ cells) and 2–9% monocytes/macrophages (CD14$^+$ cells) were present. The majority of the granulocytes were neutrophils but up to 2% basophils were also present. Epithelial cells were not found.

Table 2. Expression of accessory molecules and isoforms of CD45 by TcRγδ$^+$ cells isolated from normal and periodontitis gingiva

TcRγδ phenotype	Mean ± SD	Individual samples							
*Adult and rapidly progressive periodontitis gingiva**									
CD4$^+$	15 ± 9†	24	16	17	31	0	14	16	5
CD8$^+$	52 ± 38	82	96	83	69	0	14	75	0
CD4$^-$/CD8$^-$	34 ± 43	0	0	0	0	100	71	8	95
CD45RO$^+$	52 ± 38	ND‡	ND	ND	100	35	0	91	32
CD45RA$^+$	50 ± 39	ND	ND	ND	0	75	100	8	68
*Normal gingiva**									
CD4$^+$	1 ± 1	0	3	0					
CD8$^+$	10 ± 7	19	4	6					
CD4$^-$/CD8$^-$	89 ± 6	81	92	94					
CD45RO$^+$		7	7	ND					
CD45RA$^+$	84 ± 7	74	92	85					

* The number of TcRγδ$^+$ cells ranged from 14 to 28% of the leucocytes in the samples from periodontitis gingiva and from 30 to 35% of the leucocytes in the samples from normal gingiva.

† Percentage of TcRγδ$^+$ cells with indicated phenotype.

‡ ND, not determined.

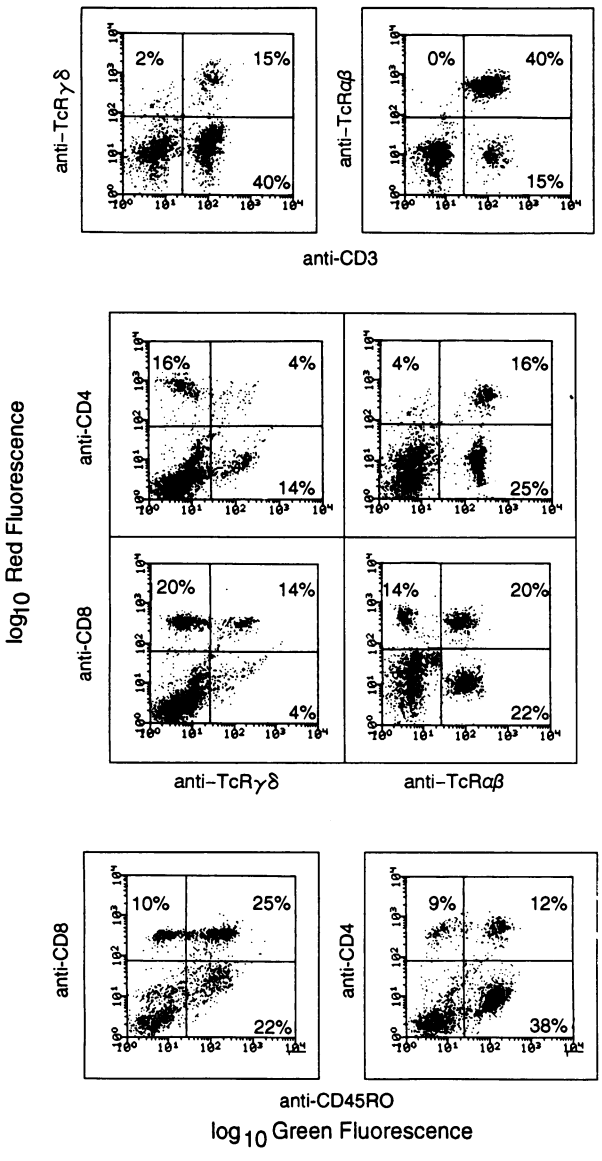

Figure 4. Flow cytometric analysis with two-colour immunofluorescence of GMC isolated from a patient with adult periodontitis. The mAb used were FITC-conjugated anti-CD3 mAb OKT3; a mixture of unconjugated or FITC-conjugated anti-TcR$\gamma\delta$ mAb TcRδ1 and δTCS1; unconjugated or FITC-conjugated anti-TcR$\alpha\beta$ mAb pan $\alpha\beta$; FITC-conjugated anti-CD45RO mAb UCHL-1; PE-conjugated anti-CD8 mAb DK25 and PE-conjugated anti-CD4 mAb MT310; unconjugated TcR$\alpha\beta$ and TcR$\gamma\delta$ mAb were revealed by PE-conjugated F(ab')$_2$ fragments of goat anti-mouse Ig.

Phenotype of isolated T cells

In three GMC preparations from normal gingiva $\geqslant 30\%$ of the leucocytes were TcR$\gamma\delta^+$ (Table 1). In most samples from periodontitis tissue the majority of the T cells were TcR$\alpha\beta^+$ (18/21; Table 1). However, TcR$\gamma\delta^+$ cells were more frequent in GMC than in PBMC from the same patient with a TcR$\gamma\delta^+$/TcR$\alpha\beta^+$ ratio of $0{\cdot}3 \pm 0{\cdot}2$ in GMC compared to $0{\cdot}06 \pm 0{\cdot}03$ in PBMC ($P < 0{\cdot}005$, Student's paired t-test, $n = 8$; Fig. 3). Variations between GMC preparations in TcR$\gamma\delta^+$/TcR$\alpha\beta^+$ ratio may reflect variations in the proportions of epithelium and granulomatous tissue in the surgical samples obtained. In five experiments mAb δTCS1 (Vδ1-Jδ1/Vδ1-Jδ2 specific) and BB3 (Vδ2 specific) were used separately to estimate Vδ region usage of gingival TcR$\gamma\delta^+$ cells.[23] In all cases the proportion of δTCS1 stained cells was larger than that of BB3 stained cells, suggesting preferential usage of Vδ1 (Fig. 3).

Two-colour staining experiments showed that the majority of the TcR$\gamma\delta^+$ cells from normal gingiva were CD3$^+$ CD4$^-$ CD8$^-$ and that $\geqslant 74\%$ expressed the CD45RA antigen (Table 2). In contrast, the majority of TcR$\gamma\delta^+$ cells in most GMC from periodontitis tissue (5/8) were CD8$^+$ and the remaining TcR$\gamma\delta^+$ cells were CD4$^+$ (Table 2 and Fig. 4). In samples containing high numbers of CD45RO expressing TcR$\gamma\delta^+$ cells the majority of these co-expressed CD8. In a few (3/8) GMC from periodontitis tissue the TcR$\gamma\delta^+$ cells had the same phenotype as in normal gingiva (CD4$^-$ CD8$^-$ CD45RA$^+$), indicating that accessory molecules are expressed only on activated or primed TcR$\gamma\delta^+$ cells (Table 2). Periodontitis has a periodic progression, with short bursts of active disease followed by periods of remission. Variations in the proportion of CD45RO$^+$ CD8$^+$ TcR$\gamma\delta^+$ cells probably reflect that samples were taken at different stages of disease. TcR$\alpha\beta^+$ cells were either CD4$^+$ or CD8$^+$ (Fig. 4).

In accordance with earlier studies[24] the CD4$^+$/CD8$^+$ cell ratio in GMC from periodontitis tissue was significantly reduced ($1{\cdot}1 \pm 0{\cdot}3$) compared to PBMC from the same patient ($2{\cdot}0 \pm 0{\cdot}5$; $P < 0{\cdot}02$, Student's paired t-test, $n = 8$). The majority of the CD3$^+$ cells from periodontitis tissue were memory/activated cells ($83 \pm 19\%$ CD45RO$^+$ cells, $n = 8$). A high proportion of both CD4$^+$ and CD8$^+$ cells expressed CD45RO, indicating that both types of T cells are involved in the inflammatory process (Fig. 4).

DISCUSSION

In mice the majority of the intraepithelial lymphocytes in the intestine are TcR$\gamma\delta^+$ and most if not all of the dendritic murine epidermal lymphocytes express TcR$\gamma\delta$, indicating a preferential localization of TcR$\gamma\delta^+$ cells in the epithelium.[4,6] At present it is less clear if this is also the case in humans. The results of Groh *et al.* suggest that TcR$\gamma\delta^+$ cells are distributed along with the TcR$\alpha\beta^+$ cells throughout the human lymphoid system[2] while others have found preferential intraepithelial localization for TcR$\gamma\delta^+$ cells.[3,18,25] Our results show that TcR$\gamma\delta^+$ cells reside selectively within the oral and crevicular epithelium in normal and chronically inflamed gingiva. The presence of intraepithelial TcR$\gamma\delta^+$ cells also at sites with no clinical inflammation suggests that they constitute a 'first line of defence' against the massive bacterial load in the oral cavity. In contrast, intraepithelial neutrophil granulocytes (CD15$^+$ cells) seem to be recruited to sites of inflammation since no CD15$^+$ cells were detected in epithelium of normal gingiva while they were presented both in epithelium and lamina propria in periodontitis lesions. In GMC from normal gingiva the majority of TcR$\gamma\delta^+$ cells were double-negative and expressed the CD45RA antigen, indicating that they are naive/unprimed cells. In contrast, most TcR$\gamma\delta^+$ cells from inflamed gingiva were CD8$^+$ and expressed the CD45RO antigen, suggesting that they play a role in periodontal disease. Furthermore, expression of CD8 on gingival TcR$\gamma\delta^+$ cells seems to be a consequence of immune activation.

Gingival TcR$\gamma\delta^+$ cells preferentially use Vδ1. A high frequency of Vδ1$^+$ cells has also been described in other

epithelia, e.g. normal intestine,[18,25] leprosy skin lesions[26] as well as in other chronic inflammatory diseases such as rheumatoid arthritis and coeliac disease.[15–18] Furthermore, both CD1c-restricted TcR$\gamma\delta^+$ clones studied so far were found to use Vδ1.[7,8] CD1 antigens were expressed in gingival epithelium on Langerhans' cells and in periodontitis tissue also on keratinocytes. CD1a expression has also been shown to be induced in gingival epithelial cells after exposure to interleukins *in vitro*.[27] Thus, keratinocytes may respond to infection or inflammation by induction of CD1 antigen expression.

We found that the CD8$^+$ TcR$\gamma\delta^+$ cells co-localized with CD1a$^+$ and CD1c$^+$ Langerhans' cells and keratinocytes within the gingival epithelium. An interesting possibility is that CD1 antigens serve as restriction elements for gingival TcR$\gamma\delta^+$ cells specific for bacterial antigens or stress proteins.

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