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Laser-capture microdissection of oropharyngeal epithelium indicates restriction of Epstein-Barr virus receptor/CD21 mRNA to tonsil epithelial cells

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Abstract

Background: Epstein-Barr virus colonizes the oropharynx of a majority of individuals. It infects B lymphocytes and epithelial cells and can contribute to the development of both lymphoid and epithelial tumors. The virus uses CD21 for attachment to B cells which constitutively express the protein. Infection of epithelial cells *in vitro* is also more efficient if CD21 is available. However, its potential contribution to infection *in vivo* has been difficult to evaluate as discrepant results with antibodies have made it difficult to determine which, if any, epithelial cells in the oropharynx express CD21.

Methods: To reevaluate CD21 expression by an alternative method, epithelial cells were isolated by laser-capture microdissection from formalin-fixed sections of tissues from various parts of the oropharynx and mRNA was amplified with primers specific for the exons of CD21 which code for the Epstein-Barr virus binding site.

Results: CD21 mRNA was expressed in tonsil epithelium, but not in epithelium from buccal mucosa, uvula, soft palate or tongue.

Conclusions: CD21 does not contribute to infection of most normal epithelial tissues in the oropharynx, but may contribute to infection of epithelial cells in the tonsil, where virus has been demonstrated in healthy carriers.

Introduction

The human herpesvirus Epstein-Barr virus (EBV) is carried by more than 95% of the adult population worldwide. Most primary infections occur in childhood and are asymptomatic. However, infection delayed to adolescence or beyond is more frequently accompanied by acute infectious mononucleosis and long term carriage can be associated with development of lymphoid and epithelial tumors (for a review see (1)). Current models of primary infection propose that EBV is transmitted in saliva, replicates, probably transiently, in mucosal epithelial cells and ultimately accesses naïve resting B lymphocytes in Waldeyer's ring (2). Infection

drives the B cells to become proliferating blasts as a result of expression of at least nine latent proteins from the virus episome. Virus promoter usage is then thought to switch, limiting expression to a subset of viral proteins that rescue the expanded populations of B cells in the germinal center as they differentiate into memory cells. Latently infected memory cells circulate between the peripheral blood and Waldeyer's ring (3) and, in the absence of cell division, may perhaps express no EBV proteins at all (4). Further sporadic differentiation into plasmablasts is thought to induce reactivation into the productive lytic cycle (5-7) and release of virus into the oral cavity, possibly after reamplification in epithelial cells (8-10). From here it may be transmitted to a fresh host or initiate a new round of infection to replenish the B cell reservoir.

The marked tropism that EBV has for B cells and epithelial cells is at least in part a reflection of the cell proteins required for attachment and entry of the virus (11). Attachment of EBV to a B cell involves an interaction between the major virus glycoprotein gp350/220 and the complement receptor type 2, CR2 or CD21. Whether CD21 and gp350/220 also play a role in attachment of cell-free virus to epithelial cells is, however, more controversial. Certainly epithelial cells that lack expression of CD21 can be infected. Recent work has implicated the BMRF2 protein, a multispan envelope protein containing an RGD motif, as mediating attachment to integrins (12), a dimeric complex of glycoproteins gH and gL has also been shown to be critical (13-15) and EBV-specific dimeric IgA capable of binding the polymeric IgA receptor can serve an attachment function (16). However, when engineered for expression on epithelial cells CD21 proves to be a very efficient initiator of infection (13,17) and the protein is expressed, at least at low levels, on some epithelial cell lines (18,19). Early work with monoclonal antibodies also suggested that normal epithelial cells *in vivo* express CD21 (20-25), although it was compromised when at least one of the antibodies in question, HB5, was shown to cross react with an apparently unrelated epithelial protein (26). Only a subset of antibodies that recognized CD21 on B cells was found to be reactive with all epithelial cells and reports from different groups using them were not always consistent (20,23,24,27). Thus the important question of whether or not EBV can use CD21 to access normal epithelial cells during primary or persistent infection has remained open.

The ectodomain of CD21 consists of fifteen or sixteen tandem short consensus repeats (SCRs) of 60 to 70 amino acids (28) and the binding site for EBV has been precisely mapped to SCR1 and SCR2, the two amino terminal SCRs (29), which are encoded by a fused exon, exon 1,2 (30). To reevaluate whether EBV might use CD21 for attachment to normal epithelial cells *in vivo* we looked for transcripts corresponding to exon 1,2. We confirm here that epitopes recognized by antibodies to CD21 expressed on or derived from B cell cDNA are not consistently found even on subclones of a single epithelial cell lines. We also demonstrate, however, that transcripts corresponding to exon 1,2 of CD21 encoding the EBV binding site, are consistently found in epithelial cells in the tonsil and not from other regions of the oropharynx.

Materials and Methods

Cell lines

Caco2 colon carcinoma cells (ATTC) and subclones of Caco2 obtained by single cell cloning were grown in DMEM (Sigma, St. Louis, MO, USA). Media for subclone 1 of Caco2, which had been infected with recombinant EBV carrying a neomycin resistance cassette in the BDLF3 open reading frame (31), were additionally supplemented with 500 µg/ml active G418 (Mediatech). Akata Burkitt Lymphoma B cells that have lost the EBV genome, Raji Burkitt Lymphoma B cells, Molt 4 T cells were grown in RPMI (Sigma), AGS cells (ATCC), CD21-negative gastric carcinoma cells were grown in Ham's F12 medium (Invitrogen/Gibco/BRL, Carlsbad, CA, USA) and SVKCR2, an SV40 transformed keratinocyte cell line stably

transfected with a plasmid carrying a cDNA copy of CD21 and a neomycin resistance gene (17), was grown in Joklik's modified Eagle's medium supplemented with 10 ng/ml cholera toxin (Sigma) and 400 µg/ml active G418. All media were also supplemented with 10% heat inactivated fetal bovine serum (Gibco/BRL) except for SVKCR2 cell media which were supplemented with 10% heat inactivated bovine serum (Hyclone, Logan UT, USA). For flow cytometric analysis of CD21 expression, cells growing in suspension were washed in medium without serum, adherent cells were removed from dishes with trypsin and allowed to recover in medium in suspension for 30 min and both types of cell were incubated for 1 h on ice in primary antibody. Cells were then washed three times, incubated in sheep anti-mouse IgG coupled to phycoerythrin (Jackson ImmunoResearch Laboratories, Westgrove, PA, USA) and washed twice in medium and once in phosphate buffered saline before analysis.

Antibodies

Monoclonal antibodies OKB7 (32), IF8 and 6F7 (33) to CD21 were a gift of Barry Myones (Baylor College of Medicine). Monoclonal antibody HB5 (34) to CD21 was obtained from ATCC.

B cell purification

Nine ml whole blood were mixed with RosetteSep human B cell enrichment cocktail (StemCell Technologies, Vancouver, BC, Canada) at a dilution of 50 µl/ml and incubated for 20 min at room temperature. Samples were diluted with an equal volume of phosphate buffered saline containing 2% heat inactivated fetal bovine serum, layered on Ficoll-Paque (GE Healthcare Bio-Sciences AB, Piscataway, NJ, USA) and centrifuged for 20 minutes at $1200 \times g$ at room temperature. Cells at the interface were removed, washed twice with phosphate buffered saline containing 2% heat inactivated fetal bovine serum and any contaminating red cells were removed by lysis in distilled water. B cell purity was determined by flow cytometric analysis of cells stained with FITC-conjugated monoclonal antibody clone 1F8 against CD21 (Dako, Carpinteria, CA, USA).

Laser capture microdissection (LCM) and reverse transcription

Paraffin-embedded specimens including tonsils (n=6), lymph nodes (n=5), uvula (n=6), soft palate (n=4), buccal mucosa (n=1), and tongue (n=5) were sectioned to a thickness of 4-5 µm, stained with hematoxylin and eosin and kept in desiccant until used for LCM. Institutional Review Board approval was obtained. The cells of interest were identified by their morphology and captured by a PixCell IIE LCM system (Arcturus Engineering, Inc., Mountain View, CA, USA). CapSure HS LCM caps (Arcturus) coated with infrared light absorbing ethylene vinyl acetate (EVA) were placed over the tissue. The laser spot size and power were adjusted to melt the EVA film and capture cells only in the area irradiated by the very low-power infrared targeting beam which was monitored on the adjacent computer screen. The laser power was 55-60 mW, laser pulse duration was 1.5-1.8 msec, and laser spot size was 7.5 µm in diameter. Different numbers of epithelial cells (1500) and B cells (2000) from paraffin-embedded sections were captured. Each section was sampled independently 2 to 4 times. To reduce the possibility of lymphocyte contamination no cells in basal or parabasal layers were captured, but only cells that were in the keratogenous zone (granular and keratinized cells). Total RNA was extracted, purified, DNase treated, and reverse transcribed from the LCM captured cells using the Paradise Whole Transcript RT Reagent System (Arcturus) according to the manufacturer's instructions. A human formalin-fixed universal reference RNA was reverse transcribed in parallel for use as a positive control.

Real-Time Quantitative PCR

Real-time quantitative PCR (QPCR) was performed using an ABI Prism 7000 Sequence Detector with FAM-labeled target probes (Integrated DNA Technologies, Coralville, IA, USA) and a VIC-labeled probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Applied Biosystems, Foster City, CA, USA). The PCR reactions were set up in a 96-well optical plate in duplicate by adding the following reagents into each well: 3 μ l of cDNA, 15 μ l of Taqman Universal PCR Master Mix (Applied Biosystems); the final concentrations of primers were 0.3 micromol/L, and probes were 0.2 micromol/L in a final volume of 30 μ l. The PCR amplification protocol was initiated at 50°C for 2 minutes followed by 10 minutes at 95°C and 40 PCR cycles consisting of 15 seconds at 95°C followed by 60°C for 1 minute. To exclude the possibility of contamination with genomic DNA each reaction also contained a control PCR amplification of isolated RNA to which no reverse transcriptase had been added and a water only control. The sequences of the primers and probes that were used, the Genbank ID, locations, and the sizes of the PCR products of amplified target and house keeping genes are summarized in Table 1. Samples in which CK19 and CD21 but not CD20 were detected were reamplified by a more sensitive nested PCR for CD20 in which reverse-transcribed cDNA was first amplified with outer primers before being used as the template for real-time PCR.

Data Analysis

The threshold cycles (Ct) of targets and GAPDH were set to the region in the amplification plot associated with the exponential growth of the PCR product. The relative quantities of target mRNA were calculated by the comparative Ct method (35). Δ Ct represents the difference in Ct values from target genes and the GAPDH gene for the lesion at the same condition points for each sample. $\Delta\Delta$ Ct is described as $\Delta\Delta$ Ct = Δ Ct (target CD21 gene expression in different tissues and cell lines) – Δ Ct (target CD21 gene expression in the Akata B cell line was chosen as an arbitrary constant). For the $\Delta\Delta$ Ct calculation to be valid the amplification efficiencies of the target CD21 and reference GAPDH must be approximately equal. To assess if the target and reference amplicons had the same efficiency serial dilutions of cDNA from Raji cells were amplified by real-time PCR using CD21 and GAPDH primers and probes. The Δ Ct ($C_{tCD21} - C_{tGAPDH}$) was calculated for each cDNA dilution and the absolute value of the slope of the plot of the log cDNA dilution versus Δ Ct was close to zero indicating that the efficiencies of the target and reference genes were similar. This allowed the $\Delta\Delta$ Ct method for calculation of the relative amount of target to be used (35). The numerical value for $\Delta\Delta$ Ct was then used in the $2^{-\Delta\Delta Ct}$ calculation. The final result represents the differential expression of target CD21 gene in different tissues and cell lines relative to that in Akata B cells.

Results

Monoclonal antibodies to CD21 have been variously and discrepantly reported to react with epithelial cells *in vivo*. The reactivity of four of these antibodies was compared with two B cell lines, Akata and Raji, with the keratinocyte cell line, SVKCR2, which has been engineered to express CD21 from a cDNA cloned from the Raji B cell line, with the gastric carcinoma cell line AGS which has been used in many studies of EBV interactions with epithelial cells, and with several subclones of Caco2, a colon carcinoma cell line, which provided for a unique comparison of cells with an identical genetic origin. All antibodies reacted well with the B cell lines and with SVKCR2 cells and none reacted with AGS cells which have previously been reported lack expression of CR2 (14,36) (Figure 1). Monoclonal antibody OKB7, which binds close to the binding site for gp350/220 formed by SCR1 and SCR2 (37), reacted with two subclones of Caco2, subclones 1 and 2, but not with the parental Caco2 nor with subclones 3 and 4. The histogram of HB5, which binds to an epitope that maps to SCR2 and SCR3 (38), was slightly shifted relative to the isotype control for the parental line and subclone 1, but the antibody failed to react with subclones 2, 3 and 4. The histograms of 1F8 and 6F7, which bind

to distinct epitopes in SCR12-SCR15 (33,38), were both slightly and similarly shifted relative to the isotype control for subclone 1, but were discordant in their reactivity with all other subclones. Antibody 1F8 reacted strongly only with subclone 2. In contrast, antibody 6F7 reacted with parental Caco2, with each of clones 3, 4 and, to a lesser extent, also with clone 2.

Although both parental Caco2 and each of the subclones reacted with at least one of the antibodies to CD21, the failure of any of the non-engineered epithelial cell lines to react with the whole panel of antibodies indicated that no one antibody could be used to predict whether or not SCR1 and SCR2, comprising the binding site for gp350/220, might be expressed on epithelial cells *in vivo*. Reverse transcriptase QPCR (RT-QPCR) was then used to determine if mRNA transcripts corresponding to the exons encoding SCR1 and SCR2 were expressed and, if so, at what relative levels. Primers (Table 1) were designed to amplify exons 1,2 of CD21 and exons 4 and 5 of GAPDH. The relative efficiencies of amplification with each probe were compared over a range of cDNA concentrations prepared from the CD21-positive B cell line Raji, and the absolute value of the slope of a plot of log cDNA concentration versus ΔC_t ($C_{tCD21} - C_{tGAPDH}$) (5) was close to zero (Figure 2) indicating that the efficiencies of amplification of the target and reference genes were similar (35).

Expression of exons 1,2 was first examined in reverse transcribed RNA isolated from unfixed cells of lymphoid or epithelial origin. Reverse transcribed RNA was also amplified with primers to CD20, as a B cell marker, and CK19, as an epithelial cell marker. All cells of known B cell origin expressed CD20 and not CK19 and all cells of epithelial origin expressed CK19 and not CD20 (Table 2). All the cells of either cell type examined, with the single exception of the AGS gastric carcinoma cell line which had reacted with none of the Mab, expressed CD21. The $2^{-\Delta\Delta C_t}$ calculation, which converts raw C_t values to a linear form (35), was used to describe the amounts of CD21 expressed in each relative to the level of CD21 expressed in Akata B cells. The SVKCR2 cell line, which carries an integrated copy or copies of CD21 cDNA under control of a retroviral promoter, expressed the highest levels of CD21 and parental CaCo2 cells expressed the least.

Expression of exons 1,2 of CD21 was then examined in cells obtained by laser-capture microdissection of sections of formalin-fixed paraffin embedded tissue that had been stained with hematoxylin/eosin. In addition to primers for CD21, CD20 and CK19, primers that amplified the T cell specific marker CD3 were included as some subsets of T cells are known to express CD21 (39,40). The CD3 primers, which could amplify CD3 from the T cell line Molt 4, did not amplify cDNA from any of the captured cells (authors' unpublished data). All oral epithelial tissues examined were positive for CK19 and negative for CD20 (Table 3). Cells captured from the germinal centers of lymph nodes were positive for CD20 but not CK19. Relative to Akata B cells, the expression of CD21 was highest in the cells captured from lymph node germinal centers, which may have included follicular dendritic cells that have very high levels of CD21, as well as B cells. (41). Epithelial cells captured from uvula, soft palate, tongue and buccal mucosa were positive for CK19, negative for CD20 and uniformly negative for CD21. However, epithelial cells captured from tonsil epithelium, which were CK19 positive and CD20 negative, expressed CD21 at levels even higher than Akata B cells.

Discussion

Although EBV is found in almost one hundred percent of undifferentiated nasopharyngeal carcinomas and the epithelial lesion oral hairy leukoplakia is known to be driven by lytic replication of EBV, there has been considerable controversy about whether epithelial cells are infected *in vivo* during primary or persistent infection. Despite early studies that provided evidence of replication in desquamating cells from patient with acute infectious mononucleosis (42,43) and demonstration of replication in exfoliating cervical epithelium (44), the difficulty

that some had in finding infected epithelial cells in normal individuals (45-48) raised significant doubts. Resolution of the issue was not helped by the inconsistent reports of expression of CD21, the first known attachment receptor for EBV, on epithelial cells *in vivo*. More recently, however, additional studies have confirmed infection of epithelial cells *in vivo*, particularly in tonsil epithelium (9,10), virus shed in saliva has been shown to have the phenotype of virus shed from an epithelial cell (8), infection of newly explanted epithelial cells has been achieved reproducibly (12,49) and we show here that mRNA corresponding to the exons of CD21 responsible for EBV binding is expressed in tonsil epithelium *in vivo*. This raises the clear possibility that CD21 is used for entry into epithelial cells at this site.

The historical disparities in antibody staining of sections of oral epithelium are easy to understand given the differential staining of different subclones of Caco2 cells with a panel of monoclonal antibodies to CD21 described here, although the reasons behind the disparities are unclear. In the case of the Caco2 clones at least they cannot be attributed to allelic differences since the genetic background of the cells is similar. Each of the B cell lines tested reacted well with all of the monoclonal antibodies, as did the only epithelial cell line engineered to express CD21 from a transfected cDNA copy and, reassuringly, no CD21 mRNA was detected in an epithelial cell line that failed to react with any of the CD21 antibodies. One possibility is that CD21 expressed at lower levels in unengineered epithelial cells interacts with other epithelial cell proteins that alter its conformation. Perhaps this in turn changes with subtle alterations in the differentiation status of individual clones.

The demonstration of CD21 transcripts in tonsil epithelium does not unequivocally prove that protein capable of binding EBV gp350/220 is expressed on the cell surface. However, the coincidence of expression on epithelial cells in the lymphoid tissue thought to be a major site of EBV persistence in a healthy carrier and a primary site of replication in the newly infected individual, is striking. Infection of an epithelial cell is not exclusively dependent on expression of CD21 as both cell free virus (14,36) and virus associated with B cells (50) are capable of entry. CD21 can however, play a significant role in increasing the efficiency of the process (13).

It also seems possible to put to rest the question of whether most other normal healthy oral epithelium in the oral cavity expresses CD21. Perhaps the most surprising negative finding was that there was no CD21 mRNA detectable in tongue epithelium. Oral hairy leukoplakia, an EBV-driven lesion typically seen on the lateral tongue when it occurs in end-stage AIDS patients, has been reported to express CD21 transcripts (51). However, it was also suggested in the same study that EBV gene products may themselves be responsible for upregulation of CD21 and it certainly seems possible that expression levels may change with disease state. In this respect it is perhaps notable that preliminary analyses of small numbers of dysplastic lesions from the tongue of individuals not infected with the human immunodeficiency virus indicate that CD21 transcripts are present under these circumstances as well (authors' unpublished data). This raises the potential concern that susceptibility to infection changes with physiologic status and is consistent with the findings of molecular alterations in epithelia of populations at high risk for development of nasopharyngeal carcinoma (52,53).

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Figure 1.

Flow cytometric analysis of the reactivity of monoclonal antibodies to CD21. a) EBV-negative Akata cells, b) Raji, c) parental Caco2 cells, d) Caco2 subclone 1, e) Caco2 subclone 2, f) Caco2 subclone 3, g) Caco2 subclone 4, h) SVKCR2 cells, i) AGS cells. Antibodies used were HB5 (blue), 6F7 (green), OKB7 (red) and 1F8 (black) or an isotype control (black dotted line).

Figure 2. Validation of the $2^{-\Delta\Delta C_t}$ method for determination of relative levels of expression of CD21. The target gene, CD21, and the internal control, GAPDH, were amplified from serial dilutions of cDNA and the ΔC_t ($C_{tCD21} - C_{tGAPDH}$) was calculated for each. The slope of the curve fitted using least square linear regression analysis was 0.086.

Table 1

Primers and probes used for nested and real-time PCR

Gene	Sense and anti-sense primers and labeled probes	GeneBank ID	Exon	Location	Amplicon size
CD21 forward	5'-GGGTTTCTTGGCTCTCGTC-3'	NM_001877	1,2	117-136	90 bp
CD21 reverse	5'-AATAATAAATAATCCGGCAATTAGG-3'		1,2	181-206	
CD21 probe	5'-/56-FAM/TCCTCGGATTCTTGTGGCTCTCCT/36-TAMSp/-3'		1,2	147-172	
CK19 forward	5'-TACAGCCACTACTACAGGAC-3'	NM_002267	1	425-444	76 bp
CK19 reverse	5'-CAATCCTGGAGTTCTCAATGG-3'		2	480-500	
CK19 probe	5'-56-FAM/AACTTTGTCCCGCAGGT/36-TAMSp/-3'		1 & 2	453-469	
CD20- outer forward	5'-TATCAGCGATTTCATCTTCAGG-3'	NM_152886	1	2-25	211
CD20-outer reverse	5'ATTGCTTATTGTCTAGTCTCTTCC-3'	NM_152866	2	191-214	
CD20 forward	5'-CAGTGTGTGTGAGAAACAAC-3'		1	50-70	
CD20 reverse	5'-CAGGATCTGAGTCTCCAAGG-3'	NM_00073	2	124-143	94 bp
CD20 probe	5'-/56-FAM/CACCACTGAAGTCCGC/36-TAMSp/-3'		1 & 2	73-89	
CD3 forward	5'-GGTCTACTTCAATTGCTGGACAGG-3'		4	428-451	
CD3 reverse	5'-TGGCAACAGAGTCTGCTTG-3'	NM_002046	5	480-499	71 bp
CD3 probe	5'-56-FAM/TGGAGTTTCCAGTCGAGAGCTTCAG/36-TAMSp/-3'		4 & 5	453-478	
GAPDH forward	5'-GGGAAAGCTTGTCAATCAATGGA-3'		4	268-288	
GAPDH reverse	5'-CGCCCCACTTGAATTGG-3'	NM_002046	5	323-340	72 bp
GAPDH probe	5'-VIC-CCATCACCATCTTCCAGGAGGAGA-TAMRA 3'		4 & 5	293-317	

Table 2
Expression of CD21 in unfixed cells freshly isolated or cultured *in vitro*.

Cells/cell lines	GAPDH (Ct)	CD21 (Ct)	CD20 (Ct)	CK19 (Ct)	ΔCt (CD21-GAPDH)	$\Delta\Delta Ct$	$2^{-\Delta\Delta Ct}$
Peripheral B lymphocytes	28.96±2.57*	31.31±2.60	28.88±2.55	-	2.35±1.02	-5.90	59.714
Akata Burkitts Lymphoma B cell line	18.88±0.18	27.13±0.14	23.33±0.20	-	8.25±0.76	0	1
Raji Burkitts Lymphoma B cell line	25.85±0.53	31.57±0.21	33.74±0.16	-	5.72±0.09	-2.53	5.776
Lymphoblastoid B cell line	33.06±0.43	37.25±0.52	39.09±0.27	-	4.19±0.89	-4.06	16.679
AGS gastric carcinoma cell line	16.00±0.08	-	-	21.70±0.25	-	-	-
Caco2 colon carcinoma cell line	17.33±0.69	29.64±0.11	-	26.79±0.20	12.31±0.16	4.06	0.0599
Caco2 subclone 1	18.03±0.42	27.55±0.71	-	28.31±0.72	9.52±0.55	1.27	0.415
Caco2 subclone 2	17.02±0.18	26.46±0.08	-	27.59±0.39	9.44±0.16	1.19	0.438
Caco2 subclone 3	17.79±0.88	29.29±0.13	-	28.40±0.23	11.5±0.12	3.25	0.105
Caco2 subclone 4	17.78±1.19	28.66±0.04	-	26.92±0.75	10.88±0.31	2.63	0.162
SVKCR2 keratinocyte cell line	17.23±0.13	15.99±0.09	-	35.77±0.43	-1.24±0.46	-9.49	719.076

* Standard deviation of the mean of 3-4 separate amplifications.

Table 3
Expression of CD21 in cells captured from formalin fixed, paraffin embedded, hematoxylin/eosin-stained sections of tissue from different areas of the oral cavity and from lymph node germinal centers.

Tissue source	Cell type	GAPDH (Ct)	CD21 (Ct)	CD20 (Ct)	CK19 (Ct)	ΔCt (CD21-GAPDH)	ΔΔCt	2 ^{-ΔΔCt}
Tonsil (n=5)	epithelial	29.50±1.71 *	37.43±0.78	-	35.66±1.39	7.93±1.87	-0.32	1.248
Uvula (n=6)	epithelial	27.76±2.39	-	-	33.26±2.24	-	-	-
Soft palate (n=4)	epithelial	27.70±2.53	-	-	33.47±2.14	-	-	-
Buccal mucosa (n=1)	epithelial	27.99±1.34	-	-	38.72±0.99	-	-	-
Tongue (n=4)	epithelial	29.10±1.42	-	-	35.81±1.71	-	-	-
Germinal Center (n=5)	lymphocyte/ follicular dendritic cell	26.77±0.63	26.59±0.32	29.18±0.55	-	-0.18±0.42	-8.43	344.892

* Standard deviation of three separate RNA isolations from each tissue sample.