Different Roles of Odontoblasts and Fibroblasts in Immunity

M.-J. Staquet, S.H. Durand, E. Colomb, A. Roméas, C. Vincent, F. Bleicher, S. Lebecque and J.-C. Farges

J DENT RES 2008 87: 256
DOI: 10.1177/154405910808700304

RESEARCH REPORTS
Biological

M.-J. Staquet[1†*], S.H. Durand[1,2†], E. Colomb[3], A. Roméas[1], C. Vincent[3], F. Bleicher[1], S. Lebecque[4], and J.-C. Farges[1,2]

[1]"Odontoblasts and Regeneration of Dental Tissues" Group, Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Institut Fédératif de Recherches Biosciences Gerland Lyon Sud, Université Lyon 1, CNRS, INRA, Ecole Normale Supérieure de Lyon, INSERM ERI16, Faculté d'Odontologie, 11 rue Guillaume Paradin, F-69372 Lyon Cedex 08, France; [2]Hospices Civils de Lyon, Service de Consultations et de Traitements Dentaires, Lyon, France; [3]Université Lyon 1, EA3732, Centre Hospitalier E. Herriot, Lyon, France; and [4]Université Lyon 1, UMR5201, Centre Hospitalier Lyon Sud, Lyon, France; [†]authors contributing equally to this work; *corresponding author, marie-jeanne.staquet@recherche.univ-lyon1.fr

J Dent Res 87(3):256-261, 2008

Different Roles of Odontoblasts and Fibroblasts in Immunity

ABSTRACT

Odontoblasts and fibroblasts are suspected to influence the innate immune response triggered in the dental pulp by micro-organisms that progressively invade the human tooth during the caries process. To determine whether they differ in their responses to oral pathogens, we performed a systematic comparative analysis of odontoblast-like cell and pulp fibroblast responses to TLR2-, TLR3-, and TLR4-specific agonists (lipoteichoic acid [LTA], double-stranded RNA, and lipopolysaccharide [LPS], respectively). Cells responded to these agonists by differential up-regulation of chemokine gene expression. *CXCL2* and *CXCL10* were thus increased by LTA only in odontoblast-like cells, while LPS increased *CCL7, CCL26,* and *CXCL11* only in fibroblasts. Supernatants of stimulated cultures increased migration of immature dendritic cells compared with controls, odontoblast-like cells being more potent attractants than fibroblasts. Analysis of these data suggests that odontoblasts and pulp fibroblasts differ in their innate immune responses to oral micro-organisms that invade the pulp tissue. *Abbreviations*: TLR, Toll-like receptor; LTA, lipoteichoic acid; LPS, lipopolysaccharide; dsRNA, double-stranded ribonucleic acids; Poly(I:C), polyinosinedeoxycytidylic acid; DC, dendritic cell.

KEY WORDS: human tooth, bacteria, chemokines, Toll-like receptors, dendritic cells.

Received July 2, 2007; Last revision December 10, 2007; Accepted December 11, 2007

INTRODUCTION

Dental pulp is mainly composed of 2 types of mesenchymal cells, odontoblasts and fibroblasts, which differ in both location and function. Odontoblasts are organized as a densely packed cell layer at the pulp periphery and are responsible for dentin formation. Fibroblasts are located more centrally and carry out the synthesis and turn-over of the pulp core extracellular matrix (Mjör *et al.*, 2001; Goldberg and Smith, 2004). Both cell populations become exposed to cariogenic oral bacteria as these progressively demineralize enamel and dentin and enter the disrupted tissues to gain access to the pulp. Due to their peripheral situation, odontoblasts are the first cells encountered by oral pathogens that are represented in the carious dentin essentially by Gram-positive bacteria (*Streptococcus, Lactobacillus,* and *Actinomyces* spp.) (Love and Jenkinson, 2002). As the carious infection progresses to the pulp-dentin interface, changes in the microflora occur, characterized by a decrease of the proportion of Gram-positive aerobic bacteria and an increase of Gram-negative anaerobic ones (mainly *Fusobacterium, Prevotella* and *Tannerella* spp.) (Hamilton, 2000). The latter thus come rapidly into contact with fibroblasts present in the sub-odontoblast region. This modification of the cariogenic microflora led us to hypothesize that odontoblasts and underlying fibroblasts might possess different abilities to recognize and to help immune cells combat oral pathogens before these become deleterious for tooth pulp vitality. Both cell types might also recognize viruses present in the dental pulp (Glick *et al.*, 1991).

Successful defense against invading pathogens involves their rapid sensing through specialized pattern recognition molecules expressed by immune and non-immune cells, among which Toll-like receptors (TLRs) are key participants (Iwasaki and Medzhitov, 2004; Akira *et al.*, 2006). Ten human TLRs have been described so far. They are involved in the innate immune response and detect a wide array of molecules whose origin can be bacterial, viral, fungal, or parasitic. Among them, TLR2 is crucial for the recognition of Gram-positive bacteria components, including LTA, lipopeptides, and peptidoglycan. TLR3 is engaged by double-stranded RNA (dsRNA), which constitutes the viral genome or is generated during viral replication, and by the synthetic dsRNA analog polyinosinedeoxycytidylic acid (poly[I:C]). TLR4 is the predominant receptor for LPS, a characteristic component of the cell wall of Gram-negative bacteria. TLR activation initiates the production of pro-inflammatory cytokines and chemokines that recruit immune cells (Yoshie *et al.*, 2001). Immature antigen-presenting dendritic cells (DCs) are essential chemokine targets, especially in the inflamed dental pulp, where they are recruited early and migrate through the fibroblast-rich pulp core to accumulate in the odontoblast layer (Yoshiba *et al.*, 1996; Jontell *et al.*, 1998). We recently showed that odontoblast-like cells stimulated with LTA initiate an immune response by producing chemokines and recruiting immature DC (Durand *et al.*, 2006). LPS also up-regulated several markers of innate immunity in these cells (Veerayutthwilai

et al., 2007). Thus, odontoblasts represent, in the tooth, the first line of defense for the host. To date, the role of pulp fibroblasts in the early triggering of the innate immune response to oral pathogens remains to be specified.

In this study, we performed a systematic comparative analysis of odontoblast-like cell and pulp fibroblast responses to TLR2-, TLR3-, and TLR4-specific agonists (LTA, poly[I:C], and LPS, respectively), to determine the respective roles of these cells in dental pulp innate immunity, including the extent to which they influence immature DC migration.

MATERIALS & METHODS

Cell Culture

Twenty healthy non-erupted human third molars were collected with informed consent of the donors, in accordance with the French Public Health Code and following a protocol approved by the local ethics committee. We obtained odontoblast-like cells and fibroblasts by culturing pulp explants as previously described (Couble *et al.*, 2000). After 4 wks, cultures were stimulated for 8 hrs with 1 μg/mL highly purified *Staphylococcus aureus* lipoteichoic acid (LTA), 25 μg/mL poly(I:C), or 1 μg/mL ultrapure *Escherichia coli* LPS (strain 0111:B4) (InVivogen, San Diego, CA, USA).

Real-time PCR

RNA extraction from cultured cells, reverse transcription, and real-time PCR were performed as described (Durand *et al.*, 2006). Primer sets and annealing temperatures were: *TLR2* (forward, CCCATTGCTCTTTCACTGCT; reverse, CTTCCTTGGAGAGG CTGATG; annealing temperature, 60°C), *TLR3* (forward, TGGTTGGGCCACCTAGAAGTA; reverse, TCTCCATTCCTGG CCTGTG; 60°C), *TLR4* (forward, CTGCAATGGATCAAGG ACCA; reverse, TTATCTGAAGGTGTTGCACATTCC; 60°C), *CCL2* (forward, GATCTCAGTGCAGAGGCTCG; reverse, AAGCAATTTCCCCAAGTCTC; 68°C), *CCL7* (forward, GCACTTCTGTGTCTGCTGCT; reverse, TAGCTCTCCAGCC TCTGCTT; 66°C), *CCL26* (forward, ACCTGCTGCTTCCAA TACAGC; reverse, CATAGCTTCGCACCCAGGTC; 61°C), and cyclophilin A (forward, ATGGCACTGGTGGCAAGTCC; reverse, TTGCCATTCCTGGACCCAAA; 58°C). Results were expressed as fold-change values relative to unstimulated control odontoblast-like cell or fibroblast samples.

Flow Cytometry

Cells were obtained following trypsin/EDTA treatment of cultures and incubated for 30 min with mouse monoclonal antibodies to TLR2 (clone TL2.1, Santa Cruz Biotechnology, Santa Cruz, CA, USA), TLR3 (clone 619F7, a kind gift from the Schering-Plough Research Institute, Dardilly, France), or TLR4 (clone HTA125, Santa Cruz Biotechnology). Staining was revealed by goat anti-mouse $F(ab')_2$ IgG-FITC (Invitrogen Life Technologies, Grand Island, NY, USA). For TLR3 intracytoplasmic detection, cells were stained in Fix&Perm reagent (Invitrogen Life Technologies). Negative controls were performed with isotype-matched mouse IgGs (Sigma-Aldrich, St. Louis, MO, USA). Data were acquired on a Dako cytometer and analyzed with WinMDI 2.8 software (Scripps Institute, La Jolla, CA, USA).

Gene Arrays

Gene arrays containing cDNA fragments from human chemokines and receptors were purchased from SuperArray Bioscience Corp. (Frederick, MD, USA), and 3 independent experiments were performed as described previously (Durand *et al.*, 2006). Results were expressed as a percentage of cyclophilin A gene expression. Data are reported only for genes that were detected in the 3 tested samples. The complete array data have been deposited in NCBI's Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) (Access number GSE9560).

Antibody Arrays

Human Chemokine Antibody Array I kits were purchased from Raybiotech Inc. (Norcross, GA, USA) and used as indicated by the manufacturer. Membranes were incubated for 16 hrs at 4°C with supernatants recovered from 3 different cell cultures. They were developed with an enhanced chemiluminescence-type solution and scanned with a VersaDoc Imaging System (BioRad Laboratories, Hercules, CA, USA). Semi-quantitative analysis of the comparative intensity of the spots was performed with Quantity One 4.4.1 software (BioRad).

Generation of Immature Dendritic Cells

Immature DCs were generated from human $CD34^+$ hematopoietic progenitors isolated from human umbilical cord blood mononuclear fraction by immunomagnetic mini-MACS selection (Miltenyi Biotec, Bergisch Gladbach, Germany), as described previously (Noirey *et al.*, 2003).

Chemotaxis Assay

Cell migration was assessed by means of Costar transwell devices with 8-μm pore size. Supernatants recovered from cell cultures were added to 24-well plates. Immature DCs (1.5×10^5), suspended in RPMI supplemented with 5% fetal calf serum, were applied to transwell inserts for 4 hrs at 37°C. Inserts were then removed, and migrated cells were recovered and counted. Supernatants were analyzed in triplicate. Media alone or containing LTA, LPS, or poly(I:C) were used as controls. Results were expressed as the number of migrated immature DCs in percentage of the input cell number introduced into the insert.

Statistical Analysis

Results were expressed as mean values ± standard deviation (SD) obtained from 3 independent experiments. Statistical analysis was determined with Student's t test.

RESULTS

Using real-time PCR, we demonstrated that both odontoblast-like cells and fibroblasts expressed *TLR2, TLR3,* and *TLR4* genes (Fig. 1A). Stimulation of cell cultures with specific agonists (LTA for TLR2, dsRNA for TLR3, and LPS for TLR4) differentially regulated *TLR* genes. LTA increased *TLR2* in odontoblast-like cells, but not in fibroblasts, and failed to modify expression levels of *TLR3* and *TLR4* in any cell type. Poly(I:C) increased *TLR2, TLR3,* and *TLR4* in both odontoblast-like cells and fibroblasts. LPS increased *TLR3* in both odontoblast-like cells and fibroblasts, and *TLR4* in fibroblasts (Fig. 1A). Flow cytometry confirmed the TLR up-regulation detected by real-time PCR, except for *TLR4* and *TLR3* in odontoblast-like cells stimulated by poly(I:C) and LPS, respectively, and for *TLR2* in fibroblasts stimulated by poly(I:C), which remained unchanged (Fig. 1B).

Gene array analysis revealed that unstimulated cells expressed several chemokine genes, including *CCL2, CCL26, CXCL12*, and *CXCL14*, which were detected in both cell types,

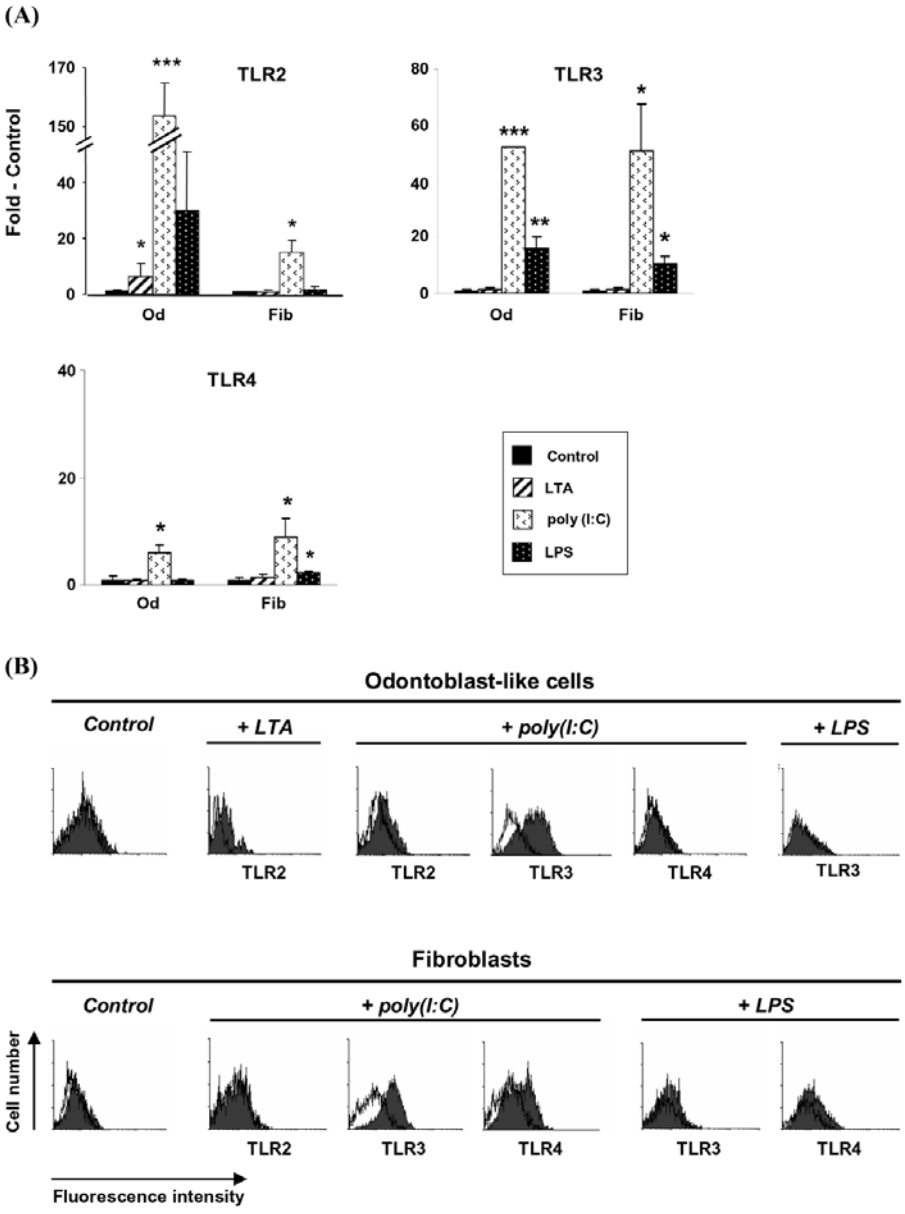

Figure 1. LTA, poly(I:C), and LPS treatment induced TLR expression changes in human odontoblast-like cells and fibroblasts *in vitro*. **(A)** Real-time PCR analysis of *TLR2, TLR3,* and *TLR4* genes. Results were normalized to cyclophilin A gene and expressed as fold-change values relative to control unstimulated cells. Both odontoblast-like cells and fibroblasts expressed *TLR2, TLR3,* and *TLR4.* Stimulation of cells for 8 hrs with specific agonists (LTA for TLR2, dsRNA for TLR3, and LPS for TLR4) differentially regulated TLR genes. LTA (1 μg/mL) significantly augmented *TLR2* expression in odontoblast-like cells, but not in fibroblasts, and failed to modify *TLR3* and *TLR4* in either cell type. Poly(I:C) (25 μg/mL) increased *TLR2, TLR3,* and *TLR4* in both odontoblast-like cells and fibroblasts. LPS (1 μg/mL) increased *TLR3* in both odontoblast-like cells and fibroblasts, and *TLR4* in fibroblasts. Data represent the mean ± SD obtained from 3 independent experiments. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$ *vs.* control cells. Od: odontoblast-like cells. Fib: fibroblasts. **(B)** Flow cytometry analysis of TLR2, TLR3, and TLR4 proteins. Isotype controls are shown as open histograms. Up-regulation was detected for all TLRs whose gene expression was increased, except for TLR4 and TLR3 in odontoblast-like cells stimulated by poly(I:C) and LPS, respectively, and for TLR2 in fibroblasts stimulated by poly(I:C) that remained unchanged. Histograms shown are representative of 3 independent experiments.

CXCL4, which was detected only in odontoblast-like cells, and *CCL7* and *CXCL2,* which were detected only in fibroblasts (Fig. 2, Table). Other genes identified on the membrane were not detected as being expressed by odontoblast-like cells and fibroblasts, or in a non-reproducible manner among the 3 tested samples.

Upon LTA stimulation, *CCL2, CCL7, CXCL2,* and *CXCL10* were significantly up-regulated in odontoblast-like cells (Table), confirming our previous results (Durand *et al.*, 2006). *CCL2* and *CCL7* were the only chemokines increased in fibroblasts. LPS significantly up-regulated *CCL2* and *CXCL10* in odontoblast-like cells, and *CCL2, CCL7, CCL26, CXCL10,* and *CXCL11* in fibroblasts. Poly(I:C) was the most potent chemokine inducer and augmented the expression of 11 and 13 chemokine genes in odontoblast-like cells and fibroblasts, respectively.

We then considered chemokines stimulated by the bacterial components LTA and LPS for verification by real-time PCR, since bacteria, and not viruses, trigger caries-dependent pulp pathogenesis (Hamilton, 2000). We focused our analysis on *CCL2, CCL7,* and *CCL26,* 3 chemokines up-regulated in infectious/inflammatory conditions and known to promote immature DC migration (Mantovani *et al.*, 2004), since the latter is an early, crucial event in the pulp immune response to intradentinal cariogenic bacteria. Results confirmed the stimulation of genes encoding these chemokines (Fig. 3A). Antibody array analysis showed CCL2 protein release by odontoblast-like cells and fibroblasts, and its increase upon LTA, poly(I:C), and LPS stimulation (Fig. 3B). CCL7 and CCL26 were not detected on the arrays (not shown). We assessed the biological relevance of the odontoblast-like cell and fibroblast chemokine responses by testing culture supernatants for their chemotactic effect on immature DCs. In transwell migration assays with supernatants from unstimulated odontoblast-like cells and fibroblasts, a mean number of 32% ± 5.0 and 27.4% ± 10.7 immature DCs migrated, respectively (Fig. 3C). Use of medium alone or medium + TLR agonist revealed a migration level similar to that of control culture supernatants (not shown). The migratory response was significantly enhanced when supernatants from LTA-, poly(I:C)-, or LPS-stimulated odontoblast-like cells or fibroblasts were added in the lower compartment. Odontoblast-like cells were more

potent attractants than fibroblasts when both cell types were stimulated by the same TLR agonist.

DISCUSSION

Intradentinal progression of bacteria during the caries process induces inflammatory and immune events in the human dental pulp, the molecular effectors of which remain largely unknown. We recently showed that odontoblast-like cells stimulated by LTA, a Gram-positive bacteria-specific component, initiate an innate immune response by producing chemokines and recruiting immature antigen-presenting DCs (Durand *et al.*, 2006). Pulp fibroblasts underlying the odontoblast layer are also at an early stage in contact with pathogens entering the peripheral dental pulp, and we hypothesized that they might also be involved in bacterial sensing, as suggested by the activation of undifferentiated dental pulp cells stimulated with LPS or LTA (Matsushita *et al.*, 1999; Telles *et al.*, 2003). We report here that pulp fibroblasts expressed TLR2, TLR3, and TLR4 *in vitro* and were able to respond to LTA from Gram-positive bacteria (through TLR2), viral dsRNA (through TLR3), and LPS from Gram-negative bacteria (through TLR4). These components differentially regulated TLR levels in odontoblast-like cells and fibroblasts. Up-regulation was detected at both gene and protein levels, except in 3 cases where the protein amount remained unchanged. The absence of TLR protein detection in these cases is probably because the protein is present at a level below the sensitivity threshold of flow cytometry (Durand *et al.*, 2006). Interestingly, LTA increased its specific receptor TLR2 only in odontoblast-like cells, suggesting that these cells are not only adapted to the recognition of Gram-positive bacteria that reach dentin tubules during the caries infection, but are also able to amplify their response to these pathogens. Conversely, LPS increased its specific receptor TLR4 only in fibroblasts. This might reflect an increased ability for fibroblasts to recognize Gram-negative bacteria that progressively replace Gram-positive ones as the caries infection reaches the sub-odontoblast dental pulp.

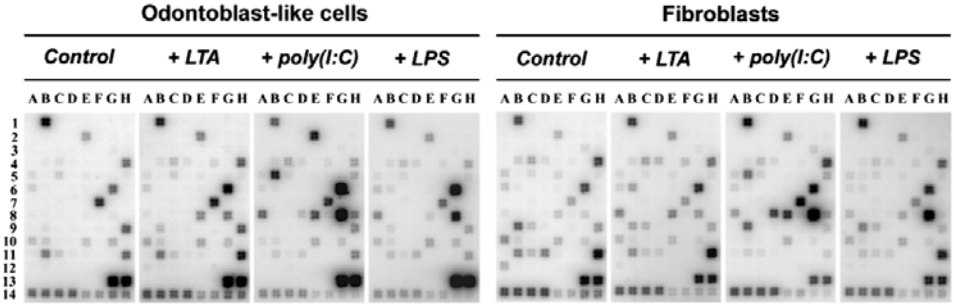

Figure 2. Odontoblast-like cell and fibroblast LTA-, poly(I:C)-, and LPS-regulated chemokine gene expression. Gene arrays showed that odontoblast-like cells expressed the chemokine genes (also listed in the Table) *CCL2* (position 6G on the membrane), *CCL26* (7F), *CXCL4* (5C), *CXCL12* (10A), and *CXCL14* (9B), and fibroblasts expressed *CCL2, CCL26, CXCL2* (4C), *CXCL12,* and *CXCL14.* Other genes spotted on the membrane were never detected or not systematically found in all 3 tested samples. Upon stimulation (8 hrs) with LTA (1 μg/mL), hybridization signals were increased in both cell types for *CCL2* and *CCL7* (8E), whereas *CXCL2* and *CXCL10* (8G) were raised in odontoblast-like cells only. Poly(I:C) (25 μg/mL) increased expression of 11 and 13 chemokine genes in odontoblast-like cells and fibroblasts, respectively. LPS (1 μg/mL) up-regulated *CCL2* and *CXCL10* in odontoblast-like cells and *CCL2, CCL7, CCL26, CXCL10,* and *CXCL11* (8H) in fibroblasts. Cyclophilin A tetraspots (position 14A-D) were used for normalization. The 3 pUC18 tetraspots used for background subtraction are in the position 13A-C. Autoradiograms shown are representative of 3 independent experiments.

Table. Quantification of Chemokine Expression Detected on Gene Arrays

		Odontoblast-like Cells				Fibroblasts			
Position	Gene	Control	+ LTA	+ poly(I:C)	+ LPS	Control	+ LTA	+ poly(I:C)	+ LPS
6G	*CCL2*	0.71 ± 0.29[a]	2.23 ± 0.43*	5.51 ± 1.67*	3.23 ± 0.19**	0.70 ± 0.20	1.27 ± 0.13*	2.23 ± 0.35*	1.71 ± 0.31*
8A	*CCL3*			1.19 ± 0.18**	0.49 ± 0.38		0.35 ± 0.30	0.75 ± 0.36*	0.15 ± 0.11
8D	*CCL5*			0.30 ± 0.09*				1.01 ± 0.25**	
8E	*CCL7*		0.42 ± 0.06**	1.69 ± 0.17**	0.18 ± 0.15	0.06 ± 0.03	0.52 ± 0.11*	2.17 ± 0.81*	0.31 ± 0.08*
8F	*CCl8*			0.92 ± 0.07**				1.15 ± 0.39*	
5H	*CCL13*			0.98 ± 0.33*	0.17 ± 0.11			0.30 ± 0.07*	0.18 ± 0.08
7E	*CCL25*			0.18 ± 0.03*				0.31 ± 0.13*	
7F	*CCL26*	1.69 ± 0.20	1.96 ± 0.25	2.44 ± 0.21	1.04 ± 0.41	0.44 ± 0.20	0.63 ± 0.13	1.61 ± 0.53*	0.93 ± 0.15*
4C	*CXCL2*		0.34 ± 0.04**	0.52 ± 0.05**		0.13 ± 0.09	0.23 ± 0.16	0.83 ± 0.40*	0.10 ± 0.05
5C	*CXCL4*	0.20 ± 0.11	0.13 ± 0.04	0.21 ± 0.04				0.19 ± 0.11*	
5B	*CXCL9*			1.99 ± 0.36**				1.38 ± 0.33*	
8G	*CXCL10*		1.00 ± 0.46*	5.33 ± 1.57*	2.29 ± 0.63*		0.42 ± 0.33	2.76 ± 0.44**	2.25 ± 0.13**
8H	*CXCL11*		0.06 ± 0.04	1.28 ± 0.18**				1.48 ± 0.49*	0.27 ± 0.11*
10A	*CXCL12*	0.19 ± 0.18	0.34 ± 0.09	0.06 ± 0.02	0.14 ± 0.1	0.28 ± 0.20	0.15 ± 0.12		0.15 ± 0.11
9B	*CXCL14*	0.06 ± 0.03	0.12 ± 0.03			1.16 ± 0.38	1.00 ± 0.36	0.66 ± 0.12	1.01 ± 0.26

[a] Values represent means ± SD of hybridization signals after background subtraction and normalization in 3 independent experiments. The cyclophilin A housekeeping gene used for normalization was assigned a value of 1. Statistical analysis showed that several genes were up-regulated in odontoblast-like cells and fibroblasts by each of the 3 agonists (see legend, Fig.2). *$p \leq 0.05$; **$p \leq 0.01$ *vs.* control cells.

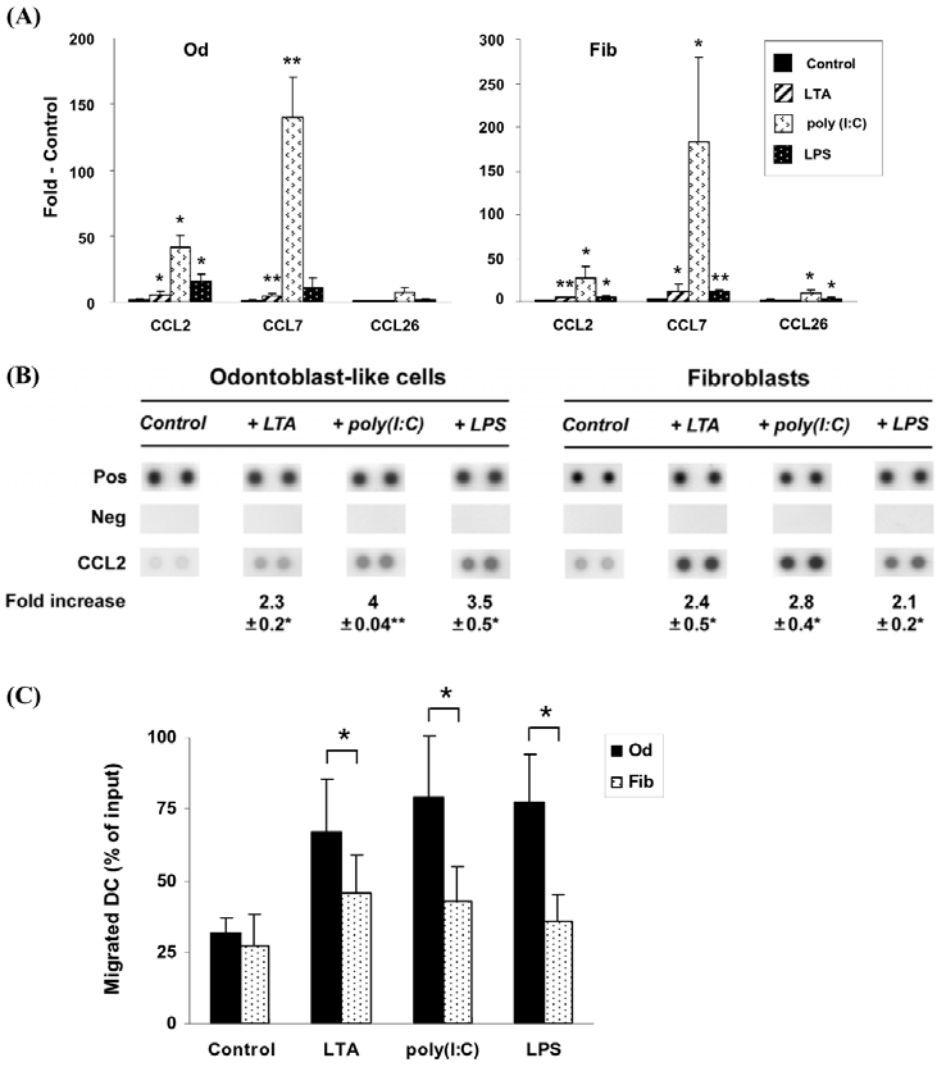

Figure 3. LTA, poly(I:C), and LPS treatment for 8 hrs up-regulated DC-attracting *CCL2, CCL7,* and *CCL26* in odontoblast-like cells and fibroblasts and stimulated immature DC migration. **(A)** Real-time PCR analysis of *CCL2, CCL7,* and *CCL26* expression. Results were normalized to cyclophilin A gene and expressed as fold-change values relative to control cells. Statistical analysis confirmed significant up-regulation of *CCL2* in odontoblast-like cells and fibroblasts by each of the 3 agonists, up-regulation of *CCL7* in odontoblast-like cells stimulated by LTA and poly(I:C), and in fibroblasts stimulated by each of the 3 agonists, and up-regulation of *CCL26* in fibroblasts stimulated by poly(I:C) and LPS. Data represent the mean ± SD obtained from 3 independent experiments. *$p \leq 0.05$; **$p \leq 0.01$ *vs.* control cells. Od: odontoblast-like cells. Fib: fibroblasts. **(B)** Antibody array analysis of CCL2, CCL7, and CCL26 release from odontoblast-like cells and pulp fibroblasts. Each anti-chemokine antibody is present on the array membrane in duplicate. After background subtraction (Neg), values were adjusted based on the intensity of control spots on the membranes (Pos). CCL2 was up-regulated in odontoblast-like cells and fibroblasts by each of the 3 agonists. CCL7 and CCL26 were not detected on the array membranes (not shown). Data represent the mean ± SD obtained from 3 independent experiments. Spots shown are representative of these experiments. *$p \leq 0.05$; **$p \leq 0.01$ *vs.* control cells. **(C)** Odontoblast-like cell and fibroblast supernatants treated for 8 hrs with LTA (1 μg/mL), poly(I:C) (25 μg/mL), or LPS (1 μg/mL) were tested for their ability to enhance immature DC migration in a transwell chamber migration assay. When supernatants from unstimulated odontoblast-like cells and fibroblasts were used, a mean number of 32% ± 5.0 and 27.4% ± 10.7 immature DCs migrated, respectively. The number of migratory immature DCs increased to 66.9% ± 18.6, 79.4% ± 22.6, and 77.6% ± 16.8 when odontoblast-like cells were stimulated with LTA, poly(I:C), and LPS, respectively. The number of migratory immature DCs increased to 45.7% ± 13.1, 42.8% ± 12.3, and 35.6% ± 9.6 when fibroblasts were stimulated with LTA, poly(I:C), and LPS, respectively. Statistical analysis revealed that odontoblast-like cells were more potent attractants than fibroblasts when both cell types were stimulated by the same TLR agonist. Results are expressed as the number of migrated cells in percentage of the input cell number, and are the mean ± SD of duplicates from 3 independent experiments. *$p \leq 0.05$ *vs.* control cells. Od: odontoblast-like cells. Fib: fibroblasts.

Odontoblast-like cells and fibroblasts responded to TLR agonists by differential up-regulation of chemokine gene expression. *CXCL2* and *CXCL10* were thus increased by LTA only in odontoblast-like cells, while *CCL7, CCL26,* and *CXCL11* were increased by LPS only in fibroblasts. This activation might induce odontoblasts and fibroblasts to mount specific immune responses by differentially influencing the various immune cell types present in the pulp tissue. Interestingly, poly(I:C) up-regulated TLR2, TLR3, TLR4, and many chemokines in both odontoblast-like cells and fibroblasts. This suggests that these cells possess the ability to mount very potent and diverse immune responses, and that viruses might also trigger an immune response in the dental pulp.

When dentin is being destroyed by caries, immature DCs are recruited early in the pulp and accumulate in the odontoblast layer close to the lesion, in a strategic location to 'sample' foreign antigens (Yoshiba *et al.*, 1996; Jontell *et al.*, 1998). We previously showed that odontoblast-like cells stimulated with LTA recruited immature DCs (Durand *et al.*, 2006). We observed, in the present study, that such recruitment also occurred when odontoblast-like cells were stimulated with poly(I:C) or LPS, and when fibroblasts were stimulated with each of these 3 agonists. While no difference was observed among the 3 agonists for each cell type, odontoblast-like cells were found to be more potent attractants than fibroblasts when stimulated by the same microbial product. This property might be necessary for odontoblasts to ensure DC movement through the fibroblast-rich pulp core to the site of pathogen invasion at the pulp-dentin interface. CCL2 was the only immature DC-attracting chemokine identified at the protein level in supernatants from stimulated cells. However, no significant difference in CCL2 production was detected between

odontoblast-like cells and fibroblasts stimulated with the same agonist. As shown in the inflamed dermis (Caux *et al.*, 2002), it is possible that this chemokine is mainly involved in the recruitment of circulating blood DCs and their migration through the endothelial barrier. The trafficking through the pulp parenchyma to the site of pathogen invasion in the odontoblast layer would thus be regulated by other chemokines and/or molecular gradients that remain to be identified.

ACKNOWLEDGMENTS

The authors acknowledge the Clinique du Parc Lyon-Bron staff for collecting tooth samples, and Pr. Schaff for expert assistance with the American English language. Real-time PCR and flow cytometry were performed at the Centre Commun d'Imagerie de Laennec (IFR62). This investigation was supported by INSERM and the Ministère de l'Education Nationale et de la Recherche.

REFERENCES

Akira S, Uematsu S, Takeuchi O (2006). Pathogen recognition and innate immunity. *Cell* 124:783-801.

Caux C, Vanbervliet B, Massacrier C, Ait-Yahia S, Vaure C, Chemin K, *et al.* (2002). Regulation of dendritic cell recruitment by chemokines. *Transplantation* 73(Suppl 1):7-11.

Couble ML, Farges JC, Bleicher F, Perrat-Mabillon B, Boudeulle M, Magloire H (2000). Odontoblast differentiation of human dental pulp cells in explant cultures. *Calcified Tissue Int* 66:129-138.

Durand SH, Flacher V, Roméas A, Carrouel F, Colomb E, Vincent C, *et al.* (2006). Lipoteichoic acid increases TLR and functional chemokine expression while reducing dentin formation in *in vitro* differentiated human odontoblasts. *J Immunol* 176:2880-2887.

Glick M, Trope M, Bagasra O, Pliskin ME (1991). Human immunodeficiency virus infection of fibroblasts of dental pulp in seropositive patients. *Oral Surg Oral Med Oral Pathol* 71:733-736.

Goldberg M, Smith AJ (2004). Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. *Crit Rev Oral Biol Med* 15:13-27.

Hamilton IR (2000). Ecological basis for dental caries. In: Oral bacterial ecology: the molecular basis. Kuramitsu HK, Ellen RP, editors. Wymondham, UK: Horizon Scientific Press, pp. 219-274.

Iwasaki A, Medzhitov R (2004). Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 5:987-995.

Jontell M, Okiji T, Dahlgren U, Bergenholtz G (1998). Immune defense mechanisms of the dental pulp. *Crit Rev Oral Biol Med* 9:179-200.

Love RM, Jenkinson HF (2002). Invasion of dentinal tubules by oral bacteria. *Crit Rev Oral Biol Med* 13:171-183.

Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 25:677-686.

Matsushita K, Motani R, Sakuta T, Nagaoka S, Matsuyama T, Abeyama K, *et al.* (1999). Lipopolysaccharide enhances the production of vascular endothelial growth factor by human pulp cells in culture. *Infect Immun* 67:1633-1639.

Mjör IA, Sveen OB, Heyeraas KJ (2001). Pulp-dentin biology in restorative dentistry. Part 1: normal structure and physiology. *Quintessence Int* 32:427-446.

Noirey N, Staquet MJ, Gariazzo MJ, Serres M, Dezutter-Dambuyant C, André C, *et al.* (2003). Withdrawal of TNF-α after the fifth day of differentiation of $CD34^+$ cord blood progenitors generates a homogeneous population of Langerhans cells and delays their maturation. *Exp Dermatol* 12:96-105.

Telles PDS, Hanks CT, Machado MAAM, Nör JE (2003). Lipoteichoic acid up-regulates VEGF expression in macrophages and pulp cells. *J Dent Res* 82:466-470.

Veerayutthwilai O, Byers MR, Pham T-TT, Darveau RP, Dale BA (2007). Differential regulation of immune responses by odontoblasts. *Oral Microbiol Immunol* 22:5-13.

Yoshiba N, Yoshiba K, Nakamura H, Iwaku M, Ozawa H (1996). Immunohistochemical localization of HLA-DR-positive cells in unerupted and erupted normal and carious human teeth. *J Dent Res* 75:1585-1589.

Yoshie O, Imai T, Nomiyama H (2001). Chemokines in immunity. *Adv Immunol* 78:57-110.