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Triclosan inhibition of acute and chronic inflammatory gene pathways

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Abstract

Aim: We sought to determine whether triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether), an extensively used anti-plaque agent with broad-spectrum anti-microbial activity, with reported anti-inflammatory effects via inhibition of prostaglandin E2 and interleukin 1 (IL-1)β, could also more broadly suppress multiple inflammatory gene pathways responsible for the pathogenesis of gingivitis and periodontitis.

Materials and Methods: As an exploratory study, the effects of triclosan on the inflammatory gene expression profile were assessed ex vivo using peripheral whole blood samples from eight periodontally healthy donors. Ten-millilitres whole blood aliquots were incubated 2 h with 0.3 μg/ml *Escherichia coli* lipopolysaccharide (LPS) with or without 0.5 μg/ml triclosan. Affymetrix microarray gene expression profiles from isolated leucocytes and pathway-specific quantitative polymerase chain reaction arrays were used to investigate changes in expression of target cytokines and cell signalling molecules.

Results: Ex vivo human whole blood assays indicated that triclosan significantly down-regulated the LPS-stimulated expression of Toll-like receptor signalling molecules and other multiple inflammatory molecules including IL-1 and IL-6 and the dampening of signals that activate the T-helper type 1 acquired immune response via suppression of CD70 with concomitant up-regulation of growth factors related to bone morphogenetic protein (BMP)2 and BMP6 synthesis.

Conclusions: Anti-inflammatory effects were found in this exploratory survey, including suppression of microbial-pathogen recognition pathway molecules and the suppression of acute and chronic mediators of inflammation.

Key words: anti-inflammatory; gingivitis; inflammatory profile; lipopolysaccharide; triclosan; whole blood ex vivo

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Triclosan, an active *bis*-phenolic and non-cationic agent, has been used in oral care products due to its broad spectrum anti-microbial and anti-plaque activity (Bhargava & Leonard 1996, Jones et al. 2000, Panagakos et al. 2005, Muller et al. 2006). The anti-microbial mechanism of action of triclosan has been attributed to the specific binding and inhibition of the bacterial enzyme enoyl-acyl carrier protein reductase. This enzyme is essential for bacterial fatty acid synthesis, and when blocked, leads to incomplete bacterial membrane formation and destabilization leading to cell death (Regos et al. 1979, Heath et al. 1999). In principle, the exposure of low concentrations of triclosan and its absorption through oral mucosa may alter lipid membrane function of mammalian cells (Lygre et al. 2003, Allmyr et al. 2006) but it has been shown that there is no acute toxic effect in human cells, despite this potential mechanism (Bhargava & Leonard 1996, Sullivan et al. 2003). Dentifrices and mouthwashes containing triclosan and its metabolites are generally associated with a marked improvement in clinical periodontal status in several studies (Charles et al. 2001, Rosin et al. 2002, Xu et al. 2004). Recently, it has been suggested that local subgingival delivery of a gel containing triclosan can also exert an anti-inflammatory

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effect, in addition to anti-microbial function, by enhancing healing response in chronic periodontitis patients (Lecio et al. 2008). In vitro studies highlighting the potential anti-inflammatory effect of triclosan on monocytic and fibroblastic cell types have been conducted, demonstrating the inhibition of the pro-inflammatory mediators and cytokines including prostaglandin E2 (PGE2), leukotriene and interferon-γ (IFN-γ) pathways (Modeer et al. 1996, Mustafa et al. 2000, Elwood et al. 2007) in response to challenge to bacterial endotoxin (lipopolysaccharide, LPS). In a murine systemic infection model, triclosan also showed a significant lowering of the circulating levels of tumour necrosis factor-α (TNF-α) in the serum (Sharma et al. 2003). These findings support a potential anti-inflammatory role of triclosan in combination with the known triclosan anti-microbial activity.

The overall goal of this study was to explore the potential anti-inflammatory properties of triclosan, evaluating not only the acute mediators of inflammation but also chronic mediators of inflammation and the signalling cascades involved. Rather than examining for effects of triclosan on stimulated cell culture models, we used an ex vivo human whole blood model to examine the effects on the naturally occurring heterogeneous leucocyte populations that include T cells, neutrophils and monocytes, to assess the shift in mRNA expression levels induced by triclosan following a standardized bacterial LPS stimulation. We sought to determine the effect of triclosan on gene expression patterns that were stimulated by LPS exploring the whole transcriptome using Affymetrix arrays ($\sim$30,000 genes) and confirmed expression patterns of specific genes by quantitative polymerase chain reaction (PCR) arrays.

Materials and Methods

Subject recruitment and whole blood collection

Following approval by the UNC Institutional Review Board, eight periodontally healthy subjects were recruited and screened at UNC's General & Oral Health Clinic after written informed consent had been obtained. All donors (four males and four females, 19–44 years old) had clinically healthy periodontal tissues with probing depth (PD) <3 mm. Smokers and pregnant women were not included in the study. The participants had not received antibiotic and/or anti-inflammatory drugs, including immunoregulatory medication, at least 3 months before the study. Thirty millilitres of peripheral blood were collected by venipuncture into sterile blood collection tubes containing 150 IU sodium heparin and immediately subjected to the experiment.

Whole blood stimulation, total RNA preparation and whole transcriptome analyses

Blood samples were aliquoted into fractions for *Escherichia coli* LPS (0.3 μg/ml) stimulation in the absence (Control) or presence of 0.5 μg/ml triclosan treatment (Test). This dosage was selected based upon previous in vitro testing using THP1 cells (data not shown). In the preliminary in vitro assays cell viability was 91% after 2 h of exposure to *E. coli* LPS at 0.3 μg/ml together with triclosan at 0.5 μg/ml, as used under the same ex vivo incubation conditions, which was not significantly higher than cell viability without triclosan. The triclosan concentration of 0.5 μg/ml is close to the steady state blood level of 352 ng/ml achieved using similar triclosan dosages, as reported by Bagley & Lin (2000) and it was not toxic to cells.

After 2-h incubation by gentle shaking at room temperature, total RNA from the leucocyte population of whole blood was isolated and purified using fractionation techniques (LeukoLock; Ambion, Austin TX, USA). Briefly, anti-coagulated blood samples were filtered to collect total leucocytes. Residual red blood cells were removed by flushing with phosphate-buffered saline. Subsequently, leucocyte RNA was stabilized and released from the filter with a guanidine thiocyanate-based solution. Total RNA was quantified and measured for purity using an Agilent 2100 Bioanalyzer automated analysis system (Agilent Technologies, Palo Alto, CA, USA) at the UNC Bioinformatics Core Facility. The mRNAs purified from the captured leucocytes were depleted of globin mRNA and DNA contamination, assuring their reliability for expression profiling (data not shown) determined by gene expression profile of blood leucocytes with real-time PCR-based array – GEArray (formerly SuperArray, now SA Biosciences, Frederick, MD, USA). One microgram of RNA was reversed transcribed using Omniscript Reverse Transcriptase kit (Qiagen, Valencia CA, USA) in a total volume of 20 μl to obtain first strand cDNA by a standard protocol according to the manufacturer. We performed whole transcriptome gene expression profiling using Affymetrix human gene 1.0ST (Affymetrix Inc., Santa Clara, CA, USA). For these whole transcriptome survey studies we used seven Affymetrix gene arrays (three LPS, four LPS+triclosan) to screen for candidate pathways. Gene chip targets were synthesized from the RNA using Affymetrix target synthesis procedures. Targets were hybridized to the gene chips followed by a series of wash and stain protocols. Each sample was hybridized once and each gene chip was then scanned using photoluminescence. Affymetrix GeneChip Microarray Suite 5.0 software was used for washing, scanning and basic analysis.

Assay for common cytokine pathways gene expression profile

Whole transcriptome analyses for gene expression profiles using Affymetrix suggested that pathways involving the immune response genes (including cytokines, chemokines and chronic inflammatory genes) were significantly down-regulated by triclosan treatment. In order to confirm these preliminary findings from Affymetrix expression arrays we performed quantitative PCR analyses using 16 Superarrays (SuperArray GEArray), a pathway – focused PCR array with 96 gene panels to enable a quantitative PCR validation of mRNA levels of specific inflammatory molecules. This array included a broad panel of acute and chronic inflammatory genes and related signalling pathways [Human Inflammatory Cytokines & Receptors kit PAHS-021 (SABiosciences, Frederick, MD, USA)]. Twenty microliters of cDNA were applied into probed-coated microplates in combined with 25 µl of PCR mixture reagent per well. Relative quantification of gene expression was performed by real-time PCR procedure in an ABI prism 7000 system (formerly Applied Biosystems Inc., now Life Technologies, Carlsbad, CA, USA). The mRNA expression levels were normalized using β actin, β-2-microglobulin, GAPDH as housekeeping genes. The fold changes were calculated using the value obtained from the LPS-stimulated group as a calibrator by means of $2^{-\Delta\Delta C_T}$ method on each condition. As a

quality control, the mRNA extracted from blood leucocytes was examined using Agilent 2100 Bioanalyzer to check for purity and degradation.

Statistical analysis

Affymetrix microarray raw data were processed by the Partek® Genomics Suite™ (Partek GS) (Partek Inc., St. Louis, MO, USA) software. RMA algorithm was applied for summarization, and the normalization was performed by the quartile method. The normalized expression values were processed by the Partek batch removing tool.

The impact of triclosan treatment on LPS-induced inflammatory gene expression was evaluated by RT2 Profiler™ PCR Array, which is a quantitative PCR tool for analyzing the expression of preselected inflammatory pathway genes, in ex vivo whole blood stimulation studies. Panels of common cytokines were run in 96 well plates on an Applied Biosystems (ABI) 7000 system. Data were analysed using $\Delta\Delta C_t$-based fold-change calculations from the uploaded raw threshold cycle data. With this system we could also perform a pair-wise comparison between groups of experimental replicates to define fold-change and statistical significance thresholds, and compare all the tested groups side-by-side. The level of statistical significance was determined at $p < 0.05$. Identification of disease-specific gene expression profiles was performed using the SAS® (SAS, Cary, NC, USA) System for Windows™, Version 9.1.

Results

Effect of triclosan on gene expression profile of LPS-stimulated whole blood leucocytes

As an initial survey of the potential effects of triclosan on LPS-induced whole transcriptome gene expression, Affymetrix exon arrays were used, which enabled us to profile the expression of ~30,000 genes represented by ~45,000 probesets. The normalized expression values processed by Partek™ and a false discovery rate of <0.05 indicated that 1101 genes were found to be differentially regulated between controls and treated samples. Of these 1101 genes, 545 transcripts were identified as down-regulated and 261 up-regulated, in the treated samples compared with controls.

In order to delineate the molecular pathways modulated by triclosan treatment, the differentially regulated genes were categorized based upon functional groups classification by Gene Ontology (http://www.geneontology.org/). Since these were LPS-stimulated cells, it was not surprising to note that the majority of genes that were differentially expressed fell into the immune response category with main pathways including regulation of cell adhesion, cell–cell signalling and cell motility. However, the dominant effect was the down-regulation of Toll-like receptor (TLR) signalling, as shown in Fig. 1. Of the 54 genes associated with the TLR-signalling pathway, nine (16.7%) were shown to be significantly regulated by triclosan treatment for an overall p-value for the pathway of $p = 0.00034$. These nine gene products associated with the TLR-signalling process are illustrated in Fig. 1.

The RT^2 Profiler™ PCR Array analysis provides quantitative assessment of the expression of 86 cytokines. These data appear in Fig. 2 and Table 1.

In Fig. 2, the volcano plot indicates the differential expression of inflammatory genes in whole blood leucocytes after LPS stimulation as compared with LPS-stimulated blood samples which were simultaneously treated with triclosan, arranging genes along dimensions of magnitude of biological effect and statistical significance summarizing both by displaying fold-change (x-axis) and p-values (transformed on y-axis) by t-test criteria. The first (horizontal) dimension indicates the fold change between the two groups on a $\log_2$ scale, so that up and down-regulation are centred in the middle of the x-axis either to the right (up-regulated) or to the left as down-regulated. The second (vertical) axis represents the $-$log-transformed p-value for a t-test of differences between samples. Thus, the horizontal axis indicates the magnitude and direction of the biological impact of effect of triclosan; the vertical indicates the statistical significance, or reliability of the change moving from the origin in the middle of the plot. The ''volcano plot'' splays to the left, indicating that triclosan largely inhibits the expression of a number of cytokine genes relative to LPS alone. Seven genes are down-regulated more than twofold and 12 genes are at $p < 0.05$. As these are not corrected for multiple comparisons, care must be exercised in interpreting the significance of any single gene, as only six genes meet the stringency of Bonferroni's correction at $p < 0.0011$.

In Table 1 those genes in Fig. 2 that were significant at $p < 0.05$ are shown with fold change and p-values. Triclosan treatment significantly altered the expression of 26 (30.3%) of the 86 targeted cytokines (Table 1). Of those 26 genes, two were up-regulated and 24 (92.3%) were down-regulated. Interestingly, the two up-regulated cytokines were both anabolic growth factors [bone morphogenetic protein (BMP)2 and BMP6]. As indicated in Table 1, triclosan treatment promoted a 1.76-fold up-regulation of BMP2 and a 1.34-fold up-regulation of BMP6. The remaining cytokines were down-regulated with a range of 1.41–3.12-fold, including a few growth factors from the transforming growth factor β (TGF-β) superfamily.

Discussion

Previous studies have indicated an anti-inflammatory activity of triclosan, based upon suppression of IL-1 and PGE2 in cell culture systems. Here we demonstrate in an exploratory survey of inflammatory pathways, using a human ex vivo system, that in the presence of a potent inflammatory stimulus (*E. coli* LPS), triclosan has broad anti-inflammatory activity. The stimulation of fresh human whole blood leucocytes is an established ex vivo model that mimics the early innate immune inflammatory responses to bacterial components such as LPS, and can play a role in determining the trajectory of host responses to clinical infection. The advantage of the ex vivo whole blood model is that it involves the complex intercellular interactions that occur in vivo. Debey et al. (2004) have shown that the whole blood ex vivo LPS stimulation model also simulates the in vivo situation for the detection of phenotypic changes according to drug/environment exposures, since the cellular interaction occurs in the presence of various plasma proteins and other cellular components. This system has also been shown to be reproducible and a useful model to explore biomarker mRNA signatures using leucocyte mRNA expression (Cazalis et al. 2008) in a manner analogous to the application in this study.

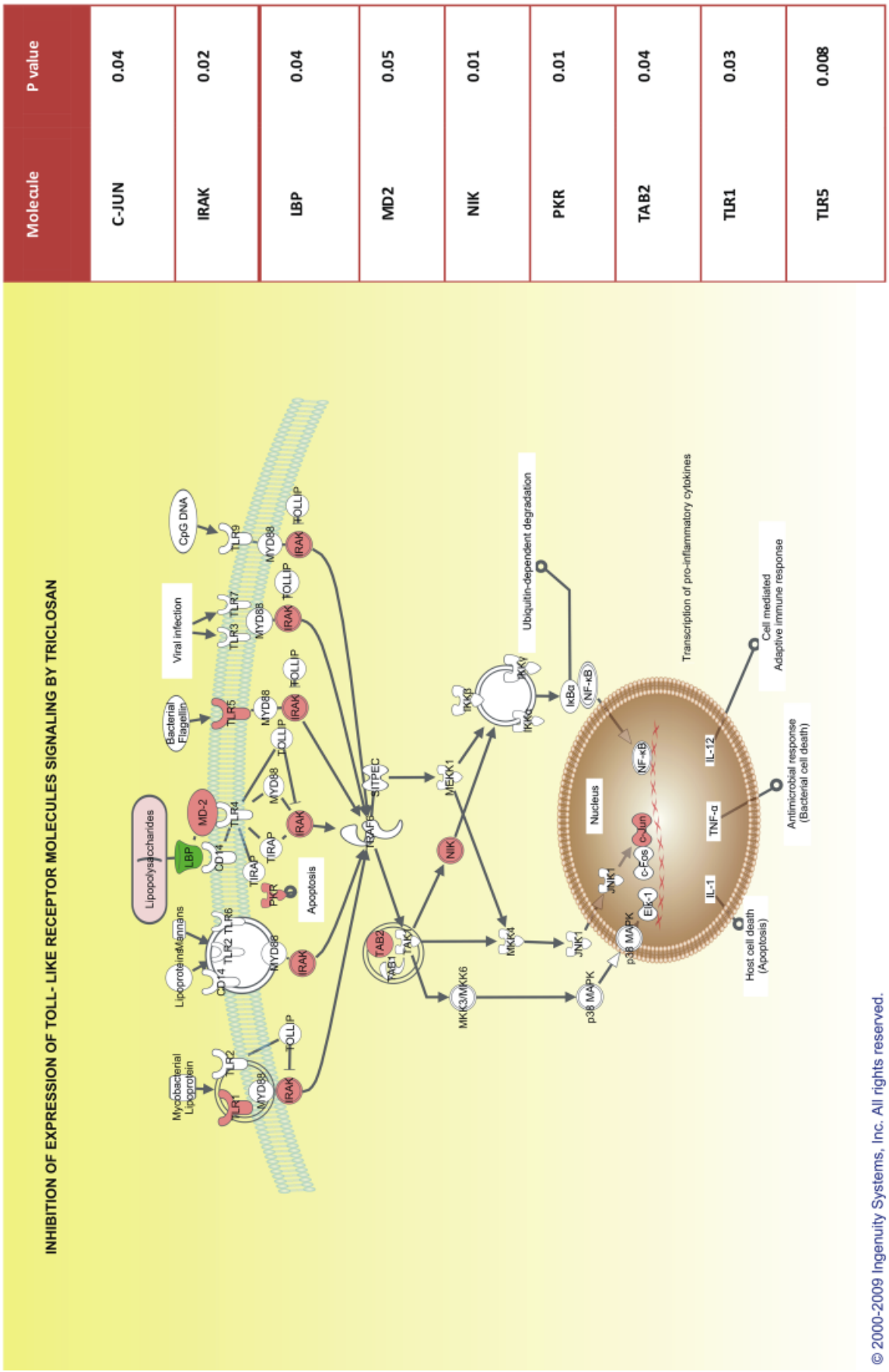

Fig. 1. The illustration depicts the localization of the gene products by cellular compartmentalization: [extracellular space, plasma membrane (PM), cytoplasm and nucleus] and indicates the down-regulated expression of Toll-like receptor (TLR) signalling molecules as an effect of triclosan treatment on lipopolysaccharide (LPS)-stimulated whole blood leucocytes with associated *p*-values. All gene products highlighted in pink are significantly down-regulated, except for LPS-binding protein (LBP) (in green) which is up-regulated.

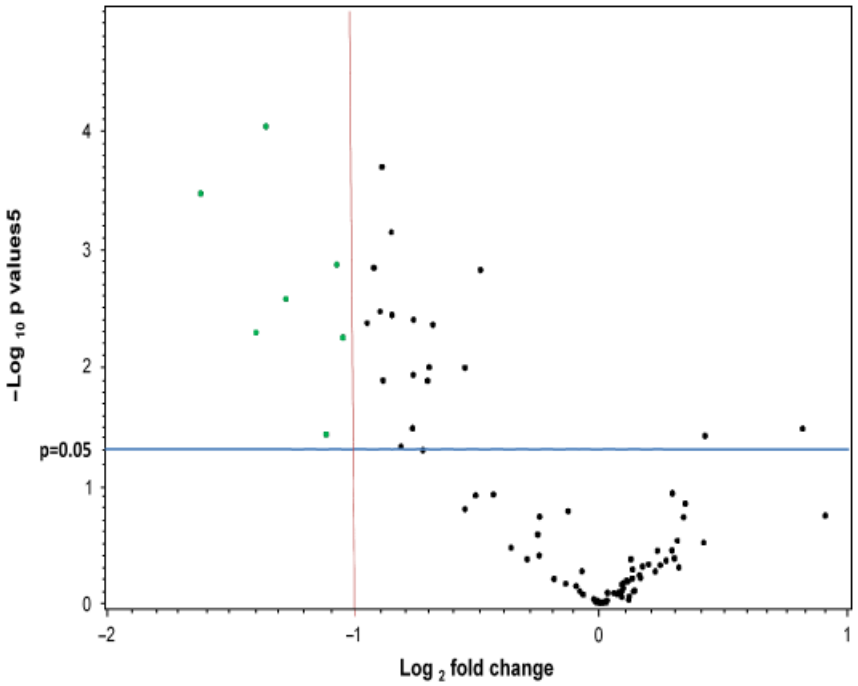

Fig. 2. Volcano plot presenting the fold change in whole blood leucocytes cytokine expression comparing the two groups [lipopolysaccharide (LPS) *versus* LPS+triclosan treated]. The vertical axis refers to the $\log_{10}$ transformed *p*-value for a *t*-test of differences between samples and the horizontal axis shows expression as $\log_2$ fold change, with -1 indicating twofold suppression and $+1$ indicating twofold up-regulation. Green dots represent the seven down-regulated genes with a more than twofold level of suppression with triclosan treatment.

Table 1. Fold changes in inflammatory markers expression levels indicating, in whole blood ex vivo LPS stimulation, the effects of triclosan treatment on the regulation of biomarker levels in leucocytes

Inflammatory molecules	Fold change (SD)	*p*-value
CSF2	0.46 (0.53)	0.04
IFN-A1	0.62 (0.31)	0.01
IFN-A2	0.39 (0.22)	<0.0001
IFN-A4	0.52 (0.28)	0.004
IFN-A8	0.54 (0.39)	0.01
IL-1F10	0.48 (0.37)	0.01
IL-1F5	0.59 (0.27)	0.004
IL-1F7	0.54 (0.30)	0.003
IL-1F8	0.56 (0.25)	0.004
IL-1F9	0.41 (0.32)	0.003
IL-6	0.57 (o.11)	0.04
IL-11	0.53 (0.22)	0.001
IL-13	0.61 (0.33)	0.01
IL-25	0.48 (0.25)	0.001
IL-19	0.59 (0.34)	0.01
IL-21	0.71 (0.16)	0.002
IL-9	0.57 (0.46)	0.047
CD70	0.55 (0.22)	0.0007
Growth/wound healing molecules		
BMP2	1.76 (0.81)	0.03
BMP6	1.34 (0.37)	0.04
GDF2	0.59 (0.44)	0.03
GDF3	0.38 (0.38)	0.005
GDF5	0.61 (0.43)	0.05
GDF9	0.68 (0.26)	0.01
INHBA	0.54 (0.15)	0.0002
LEFTY2	0.62 (0.22)	0.004
TNFRSF11B	0.32 (0.25)	0.0003

E. coli LPS (0.3 μg/ml) and concomitant triclosan treatment at 0.5 μg/ml condition was compared with *E. coli* LPS-stimulated leucocytes as control. *p*-values indicate the statistical significance of the expression levels.

LPS, lipopolysaccharide; IL, interleukin, CSF, colony-stimulating factor; BMP, bone morphogenetic protein; IFN, interferon; *E. coli*, *Escherichia coli*; GDF, growth differentiation factor; TNF, tumour necrosis factor.

The major cellular effector pathway showing differential expression by microarray analysis that was modulated by triclosan treatment of LPS-stimulate leucocytes was the TLR signalling, pathogen recognition pathway. Many molecules associated with TLR-mediated signalling are suppressed (Fig. 1, $p = 0.00034$). These include the TLR receptors for gram negative bacterial LPS [TLR4, TLR2 (for *Porphyromonas gingivalis* LPS)], Gram-positive bacterial components, including lipoteichoic acid (TLR2) and TLR5, which binds to bacterial flagella proteins and fimbria. The host recognition of bacterial LPS via TLR receptor occupancy with various bacterial ligands triggers a cascade of intracellular signalling that includes the binding of adaptor proteins, and the activation of kinases, which ultimately leads to activation of nuclear transcriptional factors nuclear factor κB (NF-κB) and C-Jun (Akira 2003). These two transcriptional regulators bind to host DNA and initiate the synthesis of multiple cytokine mRNA molecules. Triclosan seems to specifically inhibit the expression of various kinases including IRAK and PKR that are necessary for C-Jun transcriptional activation and cytokine mRNA synthesis.

The interpretation of these findings is limited, as this experiment was done for survey purposes to identify major pathways potentially modulated by triclosan. It is significant that TLR signalling emerged as the top pathway. This pathway emerged as significant using only six arrays, suggesting that using a larger sample size could have potentially resulted in many additional significantly activated pathways. However, we were focusing on inflammatory pathways since the inhibition of TLR activation would be expected to potentially suppress the expression of several cytokine genes. Although the Affymetrix array data were suggestive of the down-regulation of many cytokines, the quantitative PCR array experiments were performed using eight subjects and 16 independent assays to confirm these candidate pathways that were suggested by the Affymetrix survey. Care in interpretation must be exercised as these data are not corrected for false positives that may occur due to multiple testing. For example, using a multiple comparison correction (Bonferroni) in Table 1 with 27 independent measures a *p*-value of < 0.0018 [0.05/27] for 27 comparisons is equivalent to a *p*-value of 0.05 for a single comparison. Thus, only six mediators pass this stringency criterion.

Independent of the observed down-regulation of TLR-signalling we found significant down-regulation of many pro-inflammatory molecules that arise as a consequence of TLR-activation. Table 1 shows significant down-regulation of several IFN-α gene transcripts by Superarray (with 1.6–2.5-fold change) with a similar trend for down-regulation of the IFNs by Affymetrix microarray. IFNs regulate both innate and adaptive immunity in addition to their direct anti-viral effects (Dutzan et al. 2009). It has also been shown that TLRs selec-

tively mediate IFN gene expression in human monocyte-derived primary macrophages and that IFN-α sensitizes the cells to microbial stimulation by up-regulating the expression of TLRs and various adaptor molecules and kinases involved in TLR signalling, thus suggesting that IFN-α may significantly contribute to TLR-induced development of T helper type 1 (Th1) immune response (Siren et al. 2005). This suggests the potential for triclosan to attenuate the pro-inflammatory Th1 response that dominates in progressive periodontal lesions. The significant decrease in IFN-α expression in our ex vivo study as a result of triclosan treatment, is consistent with an anti-inflammatory activity of this agent in the presence of LPS.

IL-1, as a primary inflammatory cytokine, has been implicated in mediating both acute and chronic pathological inflammatory diseases including periodontal disease and gingivitis (Assuma et al. 1998, Graves et al. 1998, Figueredo et al. 1999, Rasmussen et al. 2000). Two functionally similar molecules, IL-1α and IL-1β, are encoded by separate genes (respectively, *IL1A* and *IL1B*). The third gene of the family (*IL1RN*) encodes IL-1 receptor antagonist (IL-1RA), an anti-inflammatory non-signalling molecule that competes for receptor binding with IL-1α and IL-1β. More recently, a novel cluster of genes encoding proteins within the IL-1 family have been identified as *IL1F5, IL1F6, IL1F7, IL1F8, IL1F9* and *IL1F10*. The IL1F5–IL1F10 genes in this cluster are widely expressed, including on activated monocytes and B cells, and signal through a range of IL-1 receptors (Smith et al. 2000).

IL-1F5 and *IL-1F9* have already been reported as relatively abundant in epithelia with a role in the regulation of epithelial inflammation (Debets et al. 2001). Our findings through PCR array analysis identified *IL1F5, IL1F7, IL1F8, IL1F9* and *IL1F10* as significantly down-regulated in the treated samples. This confirms earlier reports that triclosan inhibits IL-1β and includes in the IL1 cluster, the neighbouring gene *IL1F10*.

Another inflammatory pathway that was shown to be suppressed involved cluster of differentiation 70 (CD70) regulation. In the immune response, activated helper T cells not only secrete cytokines, but also express molecules on the surface, such as CD154 and CD70. The cell-to-cell interaction between T and B cells occurs via two signals, CD40/CD154 and CD27/CD70 that strictly regulate B-cell activation, proliferation, differentiation and cell death (Agematsu et al. 1995, Rathmell et al. 1996). Thus, the down-regulation of CD70 is consistent with a suppression of T cell/B cell interactions that are associated with initiating the acquired immune response.

IL-6, which is a critical determinant of the transition from acute to chronic inflammation (Romano et al. 1997), is down-regulated by triclosan treatment. Therefore, by blocking IL-6 expression, triclosan could potentially interfere with this transition from acute to chronic inflammation. This suggests that triclosan not only can inhibit the acute inflammatory response, but that it may also inhibit the transition from an acute to chronic inflammatory state. This would raise a question as to whether the established anti-gingivitis effect of triclosan may also have the potential to extend to preventing the transition from gingivitis to periodontal disease by means of inhibiting molecules such as IL-6 and CD70.

It is important, however, to put the anti-inflammatory effect of triclosan in perspective. TLR activation by bacterial components not only induces an inflammatory response, but it also initiates a compensatory wound healing/repair response. This is the duality associated with inflammation that simultaneously induces short-term catabolic molecular activities, while signalling for a more delayed wound healing response. For example, the effects of inflammatory cascade on the host tissues requires minutes to hours to become evident, whereas the anabolic signals, such as BMPs, require days to modulate tissue metabolism. This is an oversimplification of the signalling process, but it illustrates that anti-inflammatory actions may also suppress anabolic growth factors. This pharmacological effect is exhibited by triclosan as it generally tends to suppress the expression of many growth factors that are normally associated with the TLR cascade including growth differentiation factors (GDFs) 2, 3, 5 and 9. It is interesting that BMP2 and BMP6 are exceptions and were shown up-regulated by triclosan in this system. Notably, the gene TNFRSF11B encoding osteoprotegerin (OPG), also known as osteoclastogenesis inhibitory factor (OCIF), is a T cell produced decoy molecule that blocks bone resorption. LPS treatment would decrease T cell expression of OPG and this effect is enhanced by triclosan treatment. The net effect on osteoclast activation cannot be interpreted without understanding the effects of triclosan on other molecules associated with the immunomodulation of bone resorption (Cochran 2008). The cell–cell signalling molecules INHBA and LEFTY2, as members of the TGF-β superfamily, were also inhibited by triclosan activity.

In conclusion, triclosan has been shown to have anti-inflammatory effects in an ex vivo model that include: (1) suppression of microbial-pathogen recognition pathway molecules, (2) the suppressed synthesis of acute mediators of inflammation including IL-1, (3) the dampening of the TH1 acquired immune response activation by CD70 suppression, (4) attenuating the transition of innate immune response from acute to chronic via inhibition of IL-6 and, (5) the up-regulation of the specific growth factors BMP2 and BMP6 which pathways are involved with wound healing.

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Clinical Relevance

Scientific rationale for the study: The anti-inflammatory effect of triclosan has been demonstrated in vitro. We used an ex vivo leucocyte expression model, which mimics the early innate immune inflammatory response bacterial endotoxin to analyse the effects of triclosan on whole transcriptome expression patterns.

Principal findings: Within the limitations of this study, data indicated that triclosan dampens pathogen recognition pathways (TLRs), inhibits expression of mediators that orchestrate the transition of acute to chronic inflammation and it suppresses molecules critical to the TH1cell signalling pathways.

Practical implications: These findings suggest a broad anti-inflammatory property of triclosan that includes the suppression of many molecular signals known to be associated with the transition of gingivitis to periodontitis, as well as with the exacerbation of periodontal disease progression (e.g. IL-1 and -6). The ability of triclosan to display these effects on periodontal diseases in vivo remains to be further explored.