Mixed leukocyte reactions (MLR)

Purified B cells ($1x10^6$ cells/ml) were cultured in the presence or absence of *A. actinomycetemcomitans* or *P.gingivalis* sonicated extracted (10 μg/ml) for 24h. Cell cultures were then treated with mitomycin C (30 μg/ml, Sigma Chemical Co.) for another 30 min at 37°C and then washed 5 times in PBS. *A. actinomycetemcomitans* or *P.gingivalis* stimulated B cells at various concentrations were cultured with $1x10^5$ purified allogeneic T cells in 96-well U bottomed plates (Costar, Cambridge, MA). ^3H thymidine (0.5 μCi/200 μl) was added on day3 and cultures were incubated for another 18 h. Cells were then harvested and radioactivity was measured using a liquid scintillation counter (Beta Plate, Wallace, Turku, Finland).

Cytokine determination

IFN-γ and IL-5 were measured by ELISA (R&D system, Minneapolis, MN) in supernatants of MLR cultures of allogeneic T cells stimulated with either *P.gingivalis*-B cells, *A. actinomycetemcomitans*-B cells or control (unstimulated) B cells. Supernatants were collected at day4 to determine the levels of cytokine production.

Statistical analysis

The data were analyzed using the computer program SigmaStat for DOS (Jandel Scientific, San Rafael, CA). Results were expressed as mean ± S.E. Statistical analysis was performed using Student's t test. *P* values of 0.05 or less were regarded as significant.

RESULTS

Expression of co-stimulatory molecules, CD80 and CD86, and CD83 on infiltrated B cells isolated from periodontitis tissues

In this study we analysed the expression of co-stimulatory molecules, CD80 and CD86 as well as CD83, a marker associated with dendritic cell maturation on gingival cells isolated from severe periodontitis tissues using 2-color flow cytometry. Fig. 1A. illustrates that gingival cells in the mononuclear cell gate that expressed CD80, CD86 and CD83 were mainly $CD19^+$ B cells. The percentage of B cells that expressed CD80, CD86 or CD83 in periodontitis lesions (n=6) was compared with those in normal PBMCs (n=5). Results in Fig. 1B demonstrate that the percentage of resting peripheral blood $CD19^+$ B cells expressing CD86 or CD83 was very minimal (mean $CD86^+CD19^+$ = 9.85%±1.68; mean $CD83^+CD19^+$ = 2.96% ±1.28), whereas a significant increase of the percentage of B cells expressing CD86 or CD83 was clearly observed in gingival cell preparation (P <0.05) (mean $CD86^+CD19^+$ 42.85% ±4.13; mean $CD83^+CD19^+$ 30.81% ±4.51). We did not observe any significantly change in the percentage of B cells expressing CD80 between gingival cell preparation and normal PBMCs.

Periodontopathic bacteria induced up-regulation of CD86 and CD83 expression on B cells in PBMC cultures

Bacterial products; lipopolysaccharide (LPS) and DNA have been reported to activate B cells associated with the enhanced expression of co-stimulatory molecules (25-27). It is possible that the observed gingival B cell expression of CD86 and CD83 could be induced by local plaque bacteria. In this experiment we *in vitro* assessed 4 well-known plaque bacteria *P.gingivalis*, *A. actinomycetemcomitans*, *P.intermedia* and *A.viscosus* for their ability to induce expression of CD86 and CD83 on peripheral blood B cells. Fig. 2A and Fig. 2B. illustrate that all four bacteria enhance the percentage of $CD19^+$ B cells expressing $CD86^+$ and $CD83^+$ in a dose dependent manner. *A. actinomycetemcomitans* and *A.viscosus* seemed to be potent inducers of CD86 and CD83 expression of B cells.

We further examined the kinetics of CD86 and CD83 expression on B cells induced by *A. actinomycetemcomitans* or *P.gingivalis*. The time course study indicates that the *A. actinomycetemcomitans* or *P.gingivalis* induced expression of CD86 (as indicated by % $CD19^+CD86^+$) was detected at 12 h after stimulation and then sustained during 48h of culture period (Fig. 3A; Fig. 3B.). Whereas the induced expression of CD83 by *A. actinomycetemcomitans* or *P.gingivalis* was detected as early as 3 h after stimulation declined after 24 h and reached the base line by 48 h (Fig. 3C; Fig. 3D.).

Augmentation of APC function by periodontopathic bacteria stimulated B cells

To correlate the up-regulation of CD86 and CD83 with the functional activity, the antigen presenting capacity of *A. actinomycetemcomitans* and *P.gingivalis* treated B cells to allogeneic T cells was tested in MLR using different B:T cell ratios (1:1, 1:5, 1:25, 1:125, 1:625). Results in Fig. 4A. show that treatment of purified $CD19^+$ B cells for 24 h with 10 μg/ml *A. actinomycetemcomitans* and 10 μg/ml *P.gingivalis* led to enhancement of T cell stimulatory activity in MLR as compared with unstimulated control B cells. The highest T cell proliferative response to either *A. actinomycetemcomitans* or *P.gingivalis*

stimulated B cell was observed in the culture where equal numbers of B and T cells were used (B:T ratio = 1:1). At this optimal ratio, B cells stimulated with *A. actinomycetemcomitans* led to a 10 fold increase in ^{3}H-thymidine uptake of T cells as compared to unstimulated B cells and to a lesser degree of a 7 fold increase when B cells were stimulated in *P.gingivalis.*

In addition to T cell proliferative response, we also demonstrated the ability of bacterial stimulated B cells to induce cytokine production from allogeneic T cells in MLR where the B:T ratio of 1:1 was used. Fig. 4B illustrates that both *A. actinomycetemcomitans* or *P.gingivalis* stimulated B cells induced predominantly IFN-γ, and minimally IL-5 production in MLR. *A. actinomycetemcomitans* stimulated B cells consistantly induced higher levels of IFN-γ production than *P.gingivalis* stimulated B cells; in all 4 experiments.

DISCUSSION

It is now known that the interaction between host defense mechanisms and plaque bacteria is unquestionably important in the pathogenesis of periodontitis. Large number of gingival lymphocyte infiltrates as well as locally produced cytokines involve in immune activation which is initiated and perpetuated by periodontopathic bacteria (1, 4). However, the activation process is complex and largely undefined. Antigen presentation has been recognized as being critical in a successful immune response, in particular the initiation and maintenance of a T cell response to antigens. Activation stage of APCs associated with enhanced expression of co-stimulatory molecules is a key factor for optimal T cell activation (28). The study of APCs in periodontitis has been obviously overlooked in the past. In our current study, we investigated the expression of co-stimulatory molecules, CD80 and CD86, and CD83, a specific marker of mature dendritic cells, on gingival cells isolated from severe periodontitis tissues. It was evident that there were a number of cells expressed these molecules locally in inflamed gingival tissues and of interest the expression of the co-stimulatory molecule and CD83 were predominantly found on gingival B cells.

A significant increased in number of B cells expressing CD86 and CD83 was identified in periodontitis lesion as compared with those in PBMCs. These findings agree with the recent report by Orima *et al.* (29). They described very high proportions of co-stimulatory molecule (CD80, CD86 and CD40) -expressing B cells in gingival tissues of periodontitis patients (range 65-100%). It is possible that local plaque bacteria or products may responsible for the induced expression of CD86 or CD83 on gingival B cells. Our results from bacterial-stimulated peripheral blood B cells *in vitro* seem to support this notion. Well known plaque bacteria, *P.gingivalis,, A. actinomycetemcomitans, P.intermedia* and *A.viscosus* were able to induce CD86 and CD83 expression of B cells in PBMC cultures. The expression kinetic of CD86 and CD83 on B cells was quite different. CD83 exprression was detected very early, at 3 h after stimulation peaked at 6 h and progressively decreased to baseline within 48 h. Whereas CD86 expression was detected later at 12 h after stimulation and sustained during 48 h of culture period. The ability of *A. actinomycetemcomitans* and *P.gingivalis* to activate B cells to up-regulate co-stimulatory molecules was shown to associated with their ability to enhance APC function of B cells in MLR.

Types of APCs may influence Th subset response such as myeloid dendritic cells which express high levels of co-stimulatory molecules and produce IL-12 promote the production of Th1 response (30, 31). Whereas B cells do not produce IL-12 thus induce Th2 response (32). Our study, however shows that *P. gingivalis* stimulated B cells could drive a Th1 response in MLR associated with large amount of IFN-γ production and minimal IL-5 production. Schultze *et al.* (33) reported similar observation in which CD40-activated B cells induce large amount of IFN-γ but not IL-10 in MLR. We did not detect IL-12 production from either *A. actinomycetemcomitans* and *P.gingivalis* stimilated B cells (data not shown), hence suggesting the observed IFN-γ production in MLR is IL-12 independent. *A. actinomycetemcomitans* stimulated B cells were consistently better APCs than *P.gingivalis* stimulated B cells with regards to induction of allogeneic T cell proliferation and IFN-γ production.

Little is known about the role of B cells as APCs in periodontitis. Recent study has shed some light by showing cognate T-B cell interactions in the periodontitis lesion

(29), thus suggesting a possiblility that B cells may be engaged in antigen presentation to T cells. The ability of B cells to function on APCs for priming T cells is not new and has been a controversial subject (34-37). Activation of B cells associated with up-regulation of co-stimulatory molecule expression is critically required for optimal T cell priming (38, 39). B cells uptake antigen through antigen-specific surface immunoglobulin (Ig) receptor much more efficient than other APCs, however, the low frequency of the antigen-specific B cells has limit the ability to function as primary APCs *in vivo*. The frequency of B cell of any one specificity appears to be in the order of 2.5- $4x10^4$ for the murine lymphoid organs (40).

In periodontitis lesion, the number of IgG^+ B cells which characterize as memory B cells are known to be elevated (41-43). Thus, we hypothesize that the increased frequency of these antigen-specific memory b cells which are in activation stage (high expression of co-stimulatory molecules) would allow them to serve as important APCs to amplify local T cell response in periodontitis tissues. Further study is required to support this hypothesis.

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FIGURE LEGENDS

FIG. 1. Flow cytometric analysis of CD80, CD86 and CD83 expression on B cells. A). a representative result (n=6) of gingival cells isolated from severe periodontitis lesion. Gingival cells were double stained with a) FITC conjugated anti-CD19 + PE conjugated anti-CD80 b) FITC conjugated anti-CD19 + PE conjugated anti-CD86 c) FITC conjugated anti-CD19 + PE conjugated anti-CD83 as described in methods. Gates were set with isotype control antibodies conjugated with FITC and PE (data not shown). Up-regulation of CD80, CD86 and CD83 on gingival B cells were shown as % double positive cells in upper right corner of each dot plot. B). Mean percentage of $CD80^+$, $CD86^+$ and $CD83^+$ $CD19^+$ B cells were compared between gingival cells from periodontitis lesions (n=6) and PBMCs from healthy donors (n=5). Results are expressed as mean ± SE. *, $P < 0.05$ compared with PBMCs.

FIG. 2. Dose response of different periodontopathic bacterial effect on CD86 and CD83 expression by B cells in PBMC cultures. PBMCs from healthy donors were stimulated with varying concentrations (0, 0.016, 0.4 and 10 μg/ml) of sonicated extracts of *P.gingivalis* (Pg), *Actinobacillus actinomycetemcomitans* (Aa), *P.intermedia* (Pi) and *A.viscosus* (Av) for 24 h. A). A representative result (n=2) of CD86 expression on B cells was analyzed by flow cytometry and depicted as % $CD86^+CD19^+$ B cells. B). A representative result (n=2) of CD83 expression on B cells was analyzed by flow cytometry and depicted as % $CD83^+CD19^+$ B cells.

FIG. 3. Kinetic study of CD86 and CD83 expression on B cells in periodontopathic bacterial stimulated PBMC cutlures. PBMCs were cultured without or with sonicated extracts of *P.gingivalis* (Pg) (10 μg/ml) , or *A. actinomycetemcomitans* (Aa) (10 μg/ml). The incubation periods were varied from 0, 3,6 ,12, 24 and 48 h. A). A representative result (n=2) of CD86 expression on B cells in PBMC culture stimulated with *A. actinomycetemcomitans*. B). A representative result (n=2) of CD86 expression on B cells in PBMC culture stimulated with *P.gingivalis*. C). A representative result (n=2) of CD83 expression on B cells in PBMC culture stimulated with *A. actinomycetemcomitans*. D). A representative result (n=2) of CD83 expression on B cells in PBMC culture stimulated with *P.gingivalis*. Results are expressed as % double positive cells.

FIG. 4. Periodontopathic bacterial treated B cells are efficient APCs in MLR. Purified B cells were stimulated for 24 h with 10 μg/ml *A. actinomycetemcomitans* (Aa) or 10 μg/ml *P.gingivalis* (Pg), processed as described in Materials and Methods, and then used as stimulator cells. A) Allogeneic purified T cells were co-cultured with varying concentrations of purified B cells (1:1, 1:5, 1:25, 1:125, 1:625) that had been stimulated for 24 h with *A. actinomycetemcomitans* or *P.gingivalis* or medium control. ^3H thymidine (0.5 μCi/well) was added at day3 and cultures were incubated for another 18 h. Cells were harvested, and ^3H thymidine incorporation was measured. Results are expressed as mean ± SE (n=3). B). Equal numbers of allogeneic purified T cells were co-cultured with purified B cells that had been stimulated for 24 h with *A. actinomycetemcomitans* or *P.gingivalis* or medium control. Supernatant were harvested at day 3 and assessed for IFN-γ and IL-5 production by ELISA. Results are expressed as mean ± SE (n=4).

Figure 1.

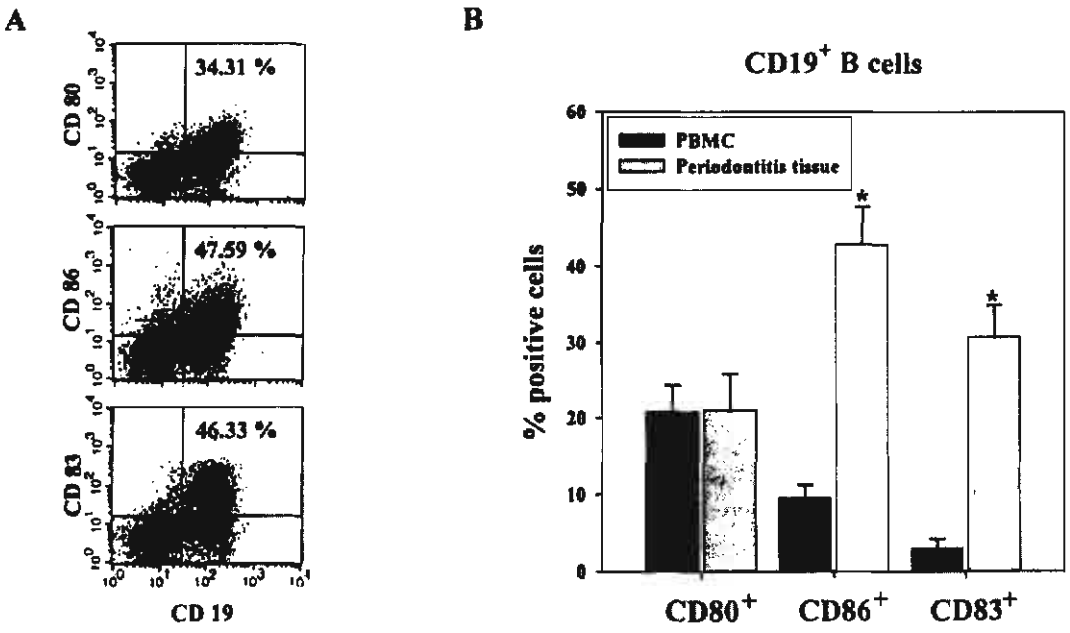

A
34.31 %
47.59 %
46.33 %
CD 80
CD 86
CD 83
CD 19
B
CD19+ B cells
PBMC
Periodontitis tissue
% positive cells
CD80+
CD86+
CD83+

Figure 2.

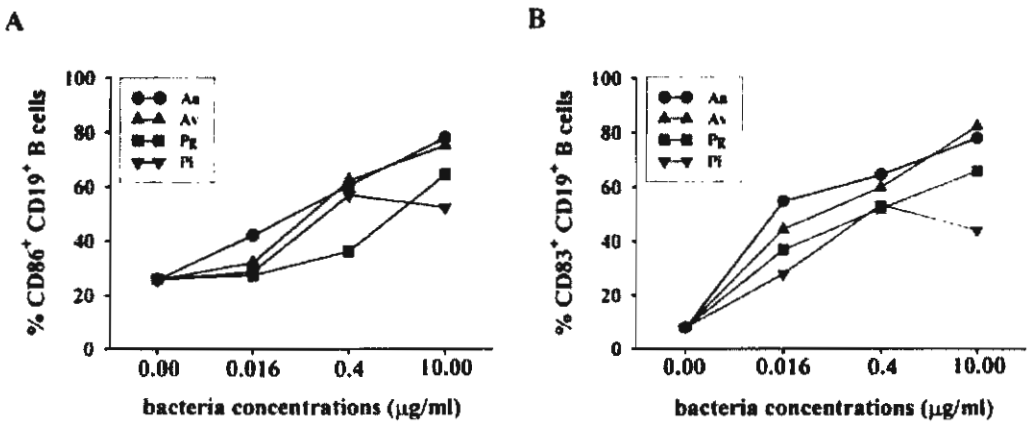

A
B
% CD86+ CD19+ B cells
% CD83+ CD19+ B cells
100
80
60
40
20
0
0.00
0.016
0.4
10.00
bacteria concentrations (μg/ml)

Figure 3.

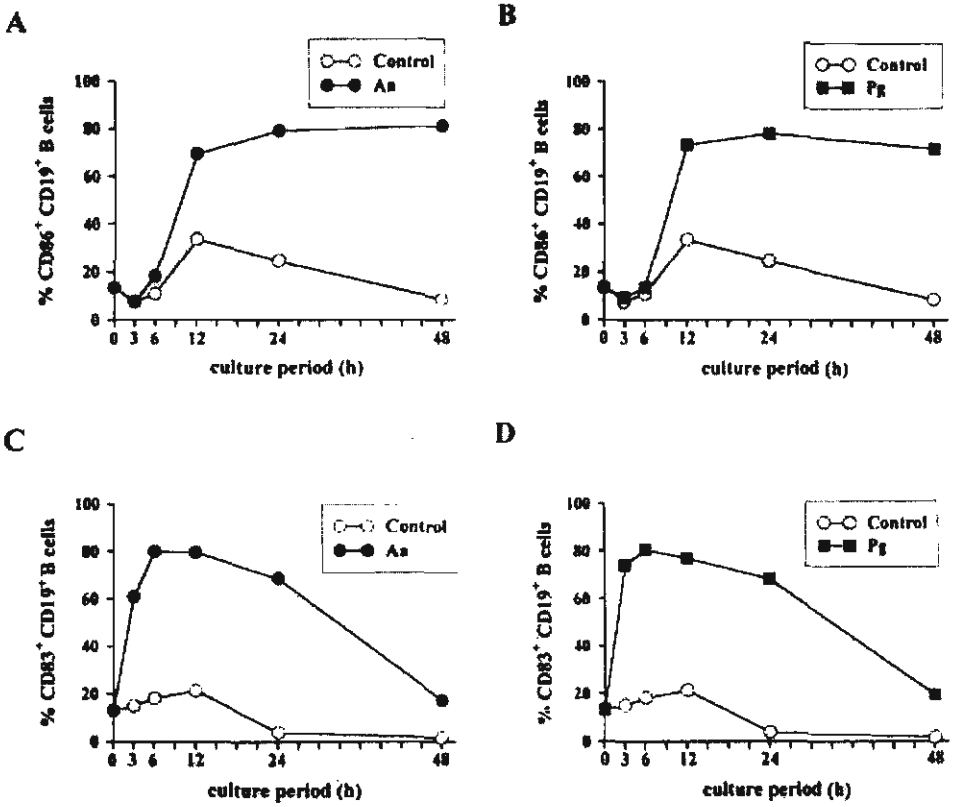
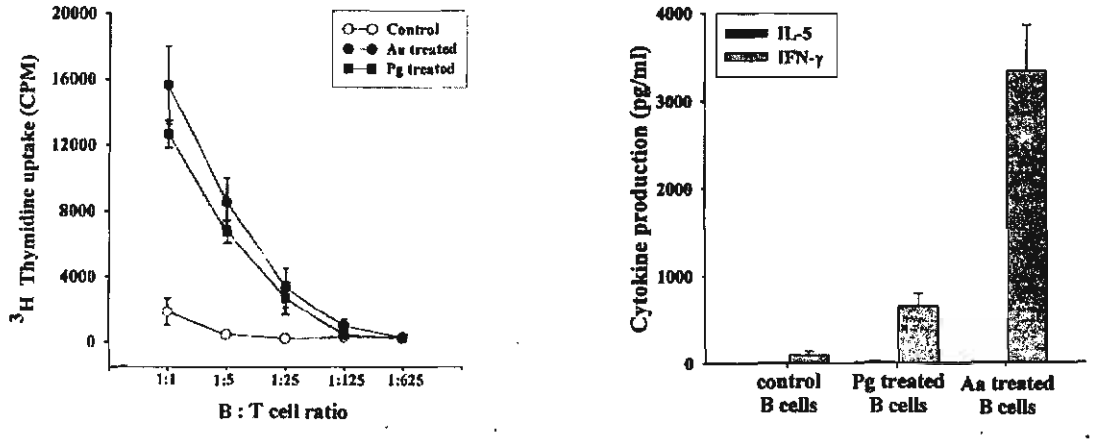

A
B
C
D
Control
Aa
Pg
% CD86+ CD19+ B cells
% CD83+ CD19+ B cells
culture period (h)
0 3 6 12 24 48
0 20 40 60 80 100

Figure 4.


20000
16000
12000
8000
4000
0
^{3}H Thymidine uptake (CPM)
Control
Aa treated
Pg treated
1:1
1:5
1:25
1:125
1:625
B : T cell ratio
4000
3000
2000
1000
0
Cytokine production (pg/ml)
IL-5
IFN-γ
control B cells
Pg treated B cells
Aa treated B cells

Prevalence of Early-onset Periodontitis in Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Thailand.

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Abstract

Evidence from a few studies suggested high prevalence of Early-onset Periodontitis (EOP) patient in developing countries and among Black race. So far, in Thailand, the EOP data is still lacking. In this study the prevalence of EOP was evaluated from the patients entering in Periodontal clinic; Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Thailand during the school year of 1996. Among total 1723 periodontal patients, 466 were periodontitis patients. The 97 periodontitis patients under or equal to 40 years of age were clinically examined for full mouth attachment level and radiographic bone loss to investigate distribution and pattern of diseases. Here we used revised criteria which was modified to obtain homogeneous subgroup. The subjects were divided into 4 subforms of EOP; Distinct localized juvenile periodontitis (subform I), Slightly and slowly disseminating pattern (subform II), Generalized and rapidly disseminating pattern (subform III) and Distinct rapidly progressive periodontitis pattern (subformIV). Patients whose bone destructive pattern did not fall into these 4 subforms had further been designated as having early or moderate adult periodontitis. Prevalence of EOP patients in subform I, II, III and IV were 0.29, 0.70, 1.10 and 0.75 % of periodontal patients, respectively. Taken together, prevalence of EOP patients was 2.84% in this study. Even the results of this study did not represent the prevalence of EOP in the whole population, it is the first EOP report providing baseline data for future study.

Introduction

According to the consensus report on periodontal diagnosis presented at the 1989 World Workshop in Clinical Periodontics, Early-onset periodontitis (EOP) was defined of age of onset usually prior to 35 years, rapid rate of progression of tissue destruction, manifestation of defects in host defense and composition of the associated flora different from that of Adult periodontitis (1). It was subdivided into three age-related diseases, Prepubertal periodontitis (PP) (disease initiation before puberty) , Juvenile periodontitis (JP) (disease initiation between puberty and late teenage years) and Rapidly progressive periodontitis (RPP) (disease initiation in the early to late 20s). However, there is an area of concern in the classification of EOP. It lies in the differentiation of generalized juvenile periodontitis (GJP) and RPP. Such difficulties to distinguish between these two groups have often been reported (2,3). For examples, some cases of EOP start with a localized pattern of destruction and apparently progress to generalized involvement with time. Yet other cases seem to start with a generalized pattern. Among the generalized cases, the American Academy of Periodontology recognizes a distinction between GJP and RPP based in part on age of onset. However, efforts to identify age of onset in the studies of EOP have not been easy to do so, considering that age at diagnosis can differ substantially from age of onset. Furthermore, some studies even considered the possibility that GJP and RPP may represent similar diseases manifesting at different ages (3) Therefore, a revised criteria of EOP classification would now be required. The criteria age of 40 instead of 35 years old was used in this study because the patients in Thailand came to see the dentist quite late or only when they thought of having diseases.

According to the Annals of Periodontology, 1996 World Workshop in Periodontics, the review of a number of studies corroborate the conclusion that Early-onset periodontitis is more frequent in developing countries and among subjects of black race (4). In Thailand, the only data available of this age group comes from the Forth National Oral Health Survey (1995) with a prevalence of periodontitis of 5.8% in the age group of 17 to 19 years old (5). This percentage is considered to be quite high when compared with a prevalence of Juvenile Periodontitis of 0.66% (0.53% of LJP and 0.13% of GJP) in the age group of 14 to 17 years old in the National Survey of the United States (6). A further extensive clinical study into other subgroups of Early-onset periodontitis such as RPP among the Thai population is obviously necessary to support the previous report of the high disease prevalence.

Materials and Methods

Subjects

Prospective screening of periodontal status had been performed on 1799 periodontal patients who had entered the Periodontal Clinic, Faculty of Dentistry, Chulalongkorn University during the school year of 1996 (May 1996 – March 1997). 76 of these patients could not participate the study and were excluded from the data. Finally, data from total 1723 periodontal patients was analyzed. The patients diagnosed of periodontitis and under or equal 40 years of age were taken for full mouth clinical examination and radiographic interproximal bone levels. Then the patients were subdivided into one of four subgroups by using revised clinical scales depending on disease destruction patterns according to criteria the modification of Choi et al. (submitted for publication) (7) and Potter;1989 (8) as follow. Patients whose bone destructive pattern did not fall into any of these subgroup categories had further been designated as having either early or moderate adult periodontitis.

Clinical criteria

1. All criteria is based upon the clinical examination and radiographic interproximal bone level measurements of all the teeth present.
2. The first molar should be present in at least one quadrant and the total number of remaining teeth should be greater than 25.
3. Patients of the same ethnic origin should be screened.

Subform I: Distinct LJP Pattern (LJP Pattern)

1. 12-25 years of age
2. maintained localized fashion of classical LJP: first molars and/or incisors
3. at least one first molar should be involved with attachment loss more than or equal to 5 mm.
4. Amounts of plaque and calculus, bleeding on probing (BOP), and gingival redness may be variable

Subform II: Slightly and slowly disseminating Pattern (Post-LJP Pattern)

1. starting age may be higher than LJP, extending up to the late 30's (20-40 years old)
2. very slow disseminating into neighbouring teeth (from first molars/incisors to second molars and canines/premolars)
3. number of involved adjacent teeth limited to 1 or 2 per quadrant, and the attachment loss of involved sites should be less than 5 mm.
4. Strongly maintained characteristic LJP pattern of bone destruction
5. Calculus, plaque, BOP, redness variable

Subform III: Generalized and Rapidly Disseminating Pattern (LJP-RPP Pattern)

1. Strongly maintained characteristic LJP pattern of bone destruction
2. Dissemination into more than two neighboring teeth per quadrant with attachment loss more than or equal to 5 mm.
3. Progression severe and rapid resulting in the generalized pattern of bone destruction and attachment loss
4. Age may be higher than subform I extending up to the late 30's (20-40 years)
5. Differences from a distinct RPP pattern (subform IV);
   a) clearly demonstrated LJP pattern of involvement (first molar/incisor pattern)
   b) high possibility of this form being a rapid deterioration of localized forms (subform I and II)
6. Calculus, plaque, BOP, redness variable

Subform IV: Distinct RPP Pattern

1. Generalized severe bone destruction
2. Localized first molar/incisor pattern not easily recognized
3. Either the result of rapid aggravation of subforms I,II,III or may be completely different in the nature of progression
4. Clearly demonstrated rapidity of progression by involvement pattern
5. Missing canines/premolars demonstrated with age (up to the late 30's)
6. Calculus, plaque, BOP, redness variable

Results

Among the total of 1,723 screened patients of the same ethnic, 446 patients were diagnosed of having periodontitis. Within total periodontitis patients, 97 patients (48 male and 49 female) were under or equal the age of 40 (age range 20-40) and were 32.97 mean of age. They were fully accessible for their clinical and full mouth intraoral radiographic data. Of these 97 patients, 20 (20.62%) patients had lost more than three first molar and hence could

not be classified into any subgroups. Consequently, the radiographic data of 77 patients were examined to confirm the diagnosis from clinical data. When the clinical data was not compatible with the age criteria, the diagnosis would be depended upon clinical characteristic. 5 patients (5.15%) were classified into subform I (LJP pattern), 12 patients (12.37%) were classified into subform II (Post-LJP pattern), 19 patients (19.59%) were classified into subform III (LJP-RPP pattern) and 13 patients (13.40%) were classified into subform IV (RPP pattern), respectively. The remaining 28 patients (28.87%) did not fall into any of the four disease categories and then were diagnosed as having either early or moderate Adult periodontitis (AP) (Table1). The prevalence of each subform I, II, III and IV of EOP comprised 1.12, 2.69, 4.26 and 2.91% of total periodontitis patients (446) and 0.29, 0.70, 1.10 and 0.75% of total periodontal patients (1723), respectively (Table 1). EOP, taken as a whole, comprised 10.98% of total periodontitis patients and 2.84% of total periodontal patients. The distribution of age and gender of each subform of EOP was also shown (Table 2).

Discussion and conclusion

As mentioned before, 1989 World workshop in Clinical Periodontics have divided EOP patients into 3 groups (1). This seems to be quite crude to classify the disease manifestation into extreme ends, in particular LJP and RPP. Thus we could frequently overlook the borderline of diseases that overlap one another. In this study, revised clinical criteria was used to classified the early onset periodontitis patients into more precise form with homogenous subgroups and might enable us to look into how these subgroups may develop from one to another. This criteria used older age of onset than usual due to patient's late seeking for treatment in developing country. As shown in Table 2, all 5 patient diagnosed of EOP subform I had exact clinical characteristic of LJP pattern but age range were 25-34 years old which were older than the age criteria of this subform. This result showed that using only age-based criteria to classify the disease is not acceptable and this was confirmed by recent change of classification of periodontal diseases by the American Academy of Periodontology 1999 (9). To compare the prevalence of EOP with other studies is difficult since prevalence of Juvenile periodontitis was usually studied instead of EOP because of disease homogeneity (range from 0.1-15%) (10). Furthermore, several authors have examined the distribution of EOP in various population, race, different age range by a number of different methods and criteria. As evidences shown, the prevalence of EOP is different in race for example, African-American adolescents have higher prevalence of EOP than Hispanic and relative low prevalence in white adolescents (11). Even the prevalence of EOP patients found in this study was 2.84% which was quite consistent with prevalence of the United States national survey during the 1986-1987 school year of 2.7-4.0% prevalence of EOP (11). Nevertheless, when comparing to Thailand national oral health survey of year 1995 of 5.8% of periodontitis in the age group of 17 to 19 years old, the prevalence of EOP in this study was quite little. This might be the result of differences in criteria and methods of disease assessment. In the national oral health survey, CPITN system and having probing depth more than 3 mm. were used while full mouth attachment level with radiographic data and having attachment loss more than or equal to 5 mm. were required for this study. When this result was compared to study of Choi which used the same criteria, the prevalence of EOP from total periodontitis patients were quite similar (10.98% and 14.5% respectively). Due to using full mouth clinical and radiographic examination required in this study, survey in large population could not be possible. So the result from this study could not represent the prevalence of EOP in whole Thai population, however, it might be useful to give baseline data for further study in this specific patient group especially in Thailand.

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Table 1 : Numbers and proportions of patients classified into each EOP subform and AP, respectively.

Total periodontal patient number who could be determined				1723
Total periodontitis patient number				446 (25.88%)
Aged ≤ 40 years		97 (21.75%)		
Aged > 40 years		349 (78.25%)		
Total ≤ 40 year-old periodontitis patient number			97	
Subform I (LJP)	5	(5.15%)*	1.12%**	0.29%***
Subform II (Post-LJP)	12	(12.37%)	2.69%	0.70%
Subform III (LJP-RPP)	19	(19.59%)	4.26%	1.10%
Subform IV (RPP)	13	(13.40%)	2.91%	0.75%
AP	28	(28.87%)	6.28%	1.60%
Unclassify#	20	(20.62%)	4.48%	1.16%

* proportions (%) relative to ≤ 40 year-old periodontitis patients (97)
** proportions (%) relative to total periodontitis patients (446)
*** proportions (%) relative to total periodontal patients (1723)
\# patients who could not be classified due to loss of first molar more than three

Table 2 : Distribution of age and gender of four subforms EOP, Adult Periodontitis (AP) and unclassify group

Subform	Number of patient	Gender		Age range (year)	Mean of age (year)
		Male	Female		
I	5	3 (60%)	2 (40%)	25 – 34*	28.4
II	12	7 (58.33%)	5 (41.67%)	20 - 38	30
III	19	11 (57.89%)	8 (42.11%)	21 – 40	34
IV	13	7 (53.85 %)	6 (46.15%)	24 – 39	34.69
AP	28	9 (32.14%)	19 (67.86%)	21 – 40	33.21
Unclassify	20	11 (55.0%)	9 (45.0%)	21 – 40	33.45

* Discussed in conclusion

IMMUNE ACTIVATION IN EOP

RESULTS

1.Up-regulation of co-stimulatory molecules on monocytes in *P.gingivalis*-stimulated whole blood cultures

Table 1. Mean fluorescence intensity (MFI) of whole blood cultures of healthy and EOP patients incubated with *P. gingivalis* LPS with various concentration (0, 1, 3, 10 ng/ml) for 48 h and then assessed for CD40 expression on monocytes (n = 17).

Monocytes	Healthy #1	EOP #1
CD40	MFI	MFI
Isotype control	57.94	55.43
Control	84.57	170.38
LPS PG 381 1 ng/ml	151.54	190.55
LPS PG 381 3 ng/ml	179.97	183.68
LPS PG 381 10 ng/ml	218.72	234.69

Monocytes	Healthy #2	EOP #2
CD40	MFI	MFI
Isotype control	72.12	53.38
Control	107.54	91.06
LPS PG 381 1 ng/ml	125.66	87.11
LPS PG 381 3 ng/ml	166.97	99.83
LPS PG 381 10 ng/ml	215.89	119.55

Monocytes	Healthy #3	EOP #3
CD40	MFI	MFI
Isotype control	116.33	122.56
Control	652.18	303.41
LPS PG 381 1 ng/ml	776.35	471.65
LPS PG 381 3 ng/ml	1072.77	878.62
LPS PG 381 10 ng/ml	1361.11	1334.74

Monocytes	Healthy #4	EOP #4
CD40	MFI	MFI
Isotype control	21.81	27.15
Control	13.91	22.85
LPS PG 381 1 ng/ml	31.67	34.41
LPS PG 381 3 ng/ml	47.76	37.65
LPS PG 381 10 ng/ml	64.09	36.80

Monocytes	Healthy #5	EOP #5
CD40	MFI	MFI
Isotype control	16.66	15.57
Control	21.71	16.15
LPS PG 381 1 ng/ml	22.95	20.38
LPS PG 381 3 ng/ml	37.49	26.51
LPS PG 381 10 ng/ml	50.89	35.52

Monocytes	Healthy #6	EOP #6
CD40	MFI	MFI
Isotype control	20.04	14.81
Control	23.22	18.38
LPS PG 381 1 ng/ml	34.66	20.59
LPS PG 381 3 ng/ml	42.68	31.68
LPS PG 381 10 ng/ml	45.65	47.44

Monocytes	Healthy #7	EOP #7
CD40	MFI	MFI
Isotype control	17.62	21.55
Control	21.40	32.15
LPS PG 381 1 ng/ml	31.85	31.15
LPS PG 381 3 ng/ml	41.12	39.92
LPS PG 381 10 ng/ml	38.86	52.21

Monocytes	Healthy #8	EOP #8
CD40	MFI	MFI
Isotype control	15.19	16.22
Control	19.37	14.67
LPS PG 381 1 ng/ml	22.20	20.18
LPS PG 381 3 ng/ml	34.97	35.35
LPS PG 381 10 ng/ml	51.62	49.11

Monocytes	Healthy #9	EOP #9
CD40	MFI	MFI
Isotype control	26.47	14.38
Control	29.04	14.87
LPS PG 381 1 ng/ml	33.70	16.16
LPS PG 381 3 ng/ml	48.79	21.14
LPS PG 381 10 ng/ml	56.28	23.17

Monocytes	Healthy #10	EOP #10
CD40	MFI	MFI
Isotype control	17.82	14.59
Control	14.25	10.71
LPS PG 381 1 ng/ml	22.58	15.07
LPS PG 381 3 ng/ml	32.73	23.49
LPS PG 381 10 ng/ml	35.30	27.59

Monocytes	Healthy #11	EOP #11
CD40	MFI	MFI
Isotype control	14.48	14.72
Control	13.00	13.42
LPS PG 381 1 ng/ml	27.76	14.55
LPS PG 381 3 ng/ml	57.39	18.99
LPS PG 381 10 ng/ml	111.79	23.25

Monocytes	Healthy #12	EOP #12
CD40	MFI	MFI
Isotype control	15.85	19.37
Control	18.58	15.18
LPS PG 381 1 ng/ml	27.63	28.18
LPS PG 381 3 ng/ml	38.27	34.21
LPS PG 381 10 ng/ml	43.03	44.06

Monocytes	Healthy #13	EOP #13
CD40	MFI	MFI
Isotype control	21.44	13.71
Control	22.49	19.15
LPS PG 381 1 ng/ml	34.07	22.80
LPS PG 381 3 ng/ml	37.40	23.49
LPS PG 381 10 ng/ml	49.05	25.87

Monocytes	Healthy #14	EOP #14
CD40	MFI	MFI
Isotype control	18.37	15.95
Control	21.03	13.67
LPS PG 381 1 ng/ml	28.64	17.87
LPS PG 381 3 ng/ml	43.66	21.14
LPS PG 381 10 ng/ml	48.01	25.48

Monocytes	Healthy #15	EOP #15
CD40	MFI	MFI
Isotype control	16.18	18.87
Control	14.43	29.78
LPS PG 381 1 ng/ml	16.27	30.61
LPS PG 381 3 ng/ml	19.85	31.32
LPS PG 381 10 ng/ml	23.73	31.77

Monocytes	Healthy #16	EOP #16
CD40	MFI	MFI
Isotype control	15.47	18.39
Control	24.02	23.16
LPS PG 381 1 ng/ml	33.01	40.63
LPS PG 381 3 ng/ml	44.39	57.17
LPS PG 381 10 ng/ml	50.99	65.93

Monocytes	Healthy #17	EOP #17
CD40	MFI	MFI
Isotype control	14.39	16.08
Control	13.01	12.19
LPS PG 381 1 ng/ml	15.28	15.47
LPS PG 381 3 ng/ml	19.90	19.93
LPS PG 381 10 ng/ml	26.63	22.26

Table 2. Mean fluorescence intensity (MFI) of whole blood cultures of healthy and EOP patients incubated with *P. gingivalis* LPS with various concentration (0, 1, 3, 10 ng/ml) for 48 h and then assessed for CD80 expression on monocytes (n = 17).

Monocytes	Healthy #1	EOP #1
CD80	MFI	MFI
Isotype control	57.94	55.43
Control	44.91	46.71
LPS PG 381 1 ng/ml	84.35	51.19
LPS PG 381 3 ng/ml	153.40	71.34
LPS PG 381 10 ng/ml	194.50	102.34

Monocytes	Healthy #2	EOP #2
CD80	MFI	MFI
Isotype control	72.12	53.38
Control	71.06	58.60
LPS PG 381 1 ng/ml	120.99	64.53
LPS PG 381 3 ng/ml	145.64	116.74
LPS PG 381 10 ng/ml	186.80	172.31

Monocytes	Healthy #3	EOP #3
CD80	MFI	MFI
Isotype control	61.14	54.31
Control	42.12	34.02
LPS PG 381 1 ng/ml	70.63	45.18
LPS PG 381 3 ng/ml	101.04	93.27
LPS PG 381 10 ng/ml	138.58	111.94

Monocytes	Healthy #4	EOP #4
CD80	MFI	MFI
Isotype control	21.81	27.15
Control	7.43	16.05
LPS PG 381 1 ng/ml	17.76	28.32
LPS PG 381 3 ng/ml	39.57	37.56
LPS PG 381 10 ng/ml	49.44	44.59

Monocytes	Healthy #5	EOP #5
CD80	MFI	MFI
Isotype control	16.66	15.57
Control	12.01	7.04
LPS PG 381 1 ng/ml	14.52	9.59
LPS PG 381 3 ng/ml	30.35	17.23
LPS PG 381 10 ng/ml	38.68	24.07

Monocytes	Healthy #6	EOP #6
CD80	MFI	MFI
Isotype control	20.04	14.81
Control	16.46	10.26
LPS PG 381 1 ng/ml	28.84	18.43
LPS PG 381 3 ng/ml	34.43	30.45
LPS PG 381 10 ng/ml	36.23	36.61

Monocytes	Healthy #7	EOP #7
CD80	MFI	MFI
Isotype control	17.62	21.55
Control	10.91	17.23
LPS PG 381 1 ng/ml	20.36	20.23
LPS PG 381 3 ng/ml	27.77	26.80
LPS PG 381 10 ng/ml	32.70	32.84

Monocytes	Healthy #8	EOP #8
CD80	MFI	MFI
Isotype control	15.19	14.67
Control	8.54	8.48
LPS PG 381 1 ng/ml	15.40	16.14
LPS PG 381 3 ng/ml	27.79	34.89
LPS PG 381 10 ng/ml	42.31	47.19

Monocytes	Healthy #9	EOP #9
CD80	MFI	MFI
Isotype control	26.47	14.38
Control	16.16	9.00
LPS PG 381 1 ng/ml	26.38	13.87
LPS PG 381 3 ng/ml	46.13	18.59
LPS PG 381 10 ng/ml	57.75	30.57

Monocytes	Healthy #10	EOP #10
CD80	MFI	MFI
Isotype control	17.82	14.59
Control	11.24	8.72
LPS PG 381 1 ng/ml	20.97	15.13
LPS PG 381 3 ng/ml	26.75	24.54
LPS PG 381 10 ng/ml	34.30	30.30

Monocytes	Healthy #11	EOP #11
CD80	MFI	MFI
Isotype control	14.48	14.72
Control	6.53	9.00
LPS PG 381 1 ng/ml	16.49	10.89
LPS PG 381 3 ng/ml	34.35	15.06
LPS PG 381 10 ng/ml	56.40	20.54

Monocytes	Healthy #12	EOP #12
CD80	MFI	MFI
Isotype control	15.85	19.37
Control	9.67	12.88
LPS PG 381 1 ng/ml	17.45	28.92
LPS PG 381 3 ng/ml	29.70	40.57
LPS PG 381 10 ng/ml	39.20	60.81

Monocytes	Healthy #13	EOP #13
CD80	MFI	MFI
Isotype control	21.44	13.71
Control	13.52	9.32
LPS PG 381 1 ng/ml	25.79	14.73
LPS PG 381 3 ng/ml	32.75	17.41
LPS PG 381 10 ng/ml	47.83	21.86

Monocytes	Healthy #14	EOP #14
CD80	MFI	MFI
Isotype control	18.37	15.95
Control	11.02	24.28
LPS PG 381 1 ng/ml	25.11	16.27
LPS PG 381 3 ng/ml	40.09	27.13
LPS PG 381 10 ng/ml	47.93	27.89

Monocytes	Healthy #15	EOP #15
CD80	MFI	MFI
Isotype control	16.18	18.87
Control	8.46	15.41
LPS PG 381 1 ng/ml	13.67	19.59
LPS PG 381 3 ng/ml	18.88	19.21
LPS PG 381 10 ng/ml	27.31	25.01

Monocytes	Healthy #16	EOP #16
CD80	MFI	MFI
Isotype control	15.47	18.39
Control	9.46	12.25
LPS PG 381 1 ng/ml	19.76	25.64
LPS PG 381 3 ng/ml	27.05	29.52
LPS PG 381 10 ng/ml	30.54	34.35

Monocytes	Healthy #17	EOP #17
CD80	MFI	MFI
Isotype control	14.39	16.08
Control	7.70	16.15
LPS PG 381 1 ng/ml	15.59	19.30
LPS PG 381 3 ng/ml	25.94	21.19
LPS PG 381 10 ng/ml	40.06	23.17

Table 3. Mean fluorescence intensity (MFI) of whole blood cultures of healthy and EOP patients incubated with *P. gingivalis* LPS with various concentration (0, 1, 3, 10 ng/ml) for 48 h and then assessed for CD86 expression on monocytes (n = 17).

Monocytes	Healthy #1	EOP #1
CD86	MFI	MFI
Isotype control	14.84	18.63
Control	127.10	118.80
LPS PG 381 1 ng/ml	140.44	127.68
LPS PG 381 3 ng/ml	211.48	123.49
LPS PG 381 10 ng/ml	226.13	131.93

Monocytes	Healthy #2	EOP #2
CD86	MFI	MFI
Isotype control	211.30	133.70
Control	2765.28	1278.67
LPS PG 381 1 ng/ml	2490.14	1643.86
LPS PG 381 3 ng/ml	2032.98	1671.10
LPS PG 381 10 ng/ml	2042.91	1487.83

Monocytes	Healthy #3	EOP #3
CD86	MFI	MFI
Isotype control	61.43	54.57
Control	300.72	230.12
LPS PG 381 1 ng/ml	425.76	306.43
LPS PG 381 3 ng/ml	421.45	378.37
LPS PG 381 10 ng/ml	386.72	511.49

Monocytes	Healthy #4	EOP #4
CD86	MFI	MFI
Isotype control	21.81	27.15
Control	49.97	95.45
LPS PG 381 1 ng/ml	95.11	118.88
LPS PG 381 3 ng/ml	142.45	113.88
LPS PG 381 10 ng/ml	152.99	128.82

Monocytes	Healthy #5	EOP #5
CD86	MFI	MFI
Isotype control	16.53	15.53
Control	46.97	49.53
LPS PG 381 1 ng/ml	61.06	77.79
LPS PG 381 3 ng/ml	70.29	106.07
LPS PG 381 10 ng/ml	88.96	106.90

Monocytes	Healthy #6	EOP #6
CD86	MFI	MFI
Isotype control	20.04	14.81
Control	77.46	54.31
LPS PG 381 1 ng/ml	87.60	69.68
LPS PG 381 3 ng/ml	111.18	79.97
LPS PG 381 10 ng/ml	148.05	84.63

Monocytes	Healthy #7	EOP #7
CD86	MFI	MFI
Isotype control	17.62	21.55
Control	73.96	120.73
LPS PG 381 1 ng/ml	104.37	135.67
LPS PG 381 3 ng/ml	93.61	118.49
LPS PG 381 10 ng/ml	113.82	114.42

Monocytes	Healthy #8	EOP #8
CD86	MFI	MFI
Isotype control	15.19	14.67
Control	59.64	56.90
LPS PG 381 1 ng/ml	102.62	95.66
LPS PG 381 3 ng/ml	99.74	124.61
LPS PG 381 10 ng/ml	113.72	111.49

Monocytes	Healthy #9	EOP #9
CD86	MFI	MFI
Isotype control	26.47	14.38
Control	71.89	53.96
LPS PG 381 1 ng/ml	80.40	67.85
LPS PG 381 3 ng/ml	97.78	83.61
LPS PG 381 10 ng/ml	101.75	76.54

Monocytes	Healthy #10	EOP #10
CD86	MFI	MFI
Isotype control	17.82	14.59
Control	71.45	59.97
LPS PG 381 1 ng/ml	114.26	91.79
LPS PG 381 3 ng/ml	87.34	89.91
LPS PG 381 10 ng/ml	86.26	112.62

Monocytes	Healthy #11	EOP #11
CD86	MFI	MFI
Isotype control	14.48	14.72
Control	37.27	38.81
LPS PG 381 1 ng/ml	71.48	56.20
LPS PG 381 3 ng/ml	98.07	72.35
LPS PG 381 10 ng/ml	138.79	85.63

Monocytes	Healthy #12	EOP #12
CD86	MFI	MFI
Isotype control	15.85	19.37
Control	57.35	99.43
LPS PG 381 1 ng/ml	83.53	140.89
LPS PG 381 3 ng/ml	95.14	112.30
LPS PG 381 10 ng/ml	120.36	135.30

Monocytes	Healthy #13	EOP #13
CD86	MFI	MFI
Isotype control	21.44	13.71
Control	62.36	34.90
LPS PG 381 1 ng/ml	99.85	48.95
LPS PG 381 3 ng/ml	112.09	52.91
LPS PG 381 10 ng/ml	117.32	62.28

Monocytes	Healthy #14	EOP #14
CD86	MFI	MFI
Isotype control	18.37	15.95
Control	74.33	81.48
LPS PG 381 1 ng/ml	91.33	80.79
LPS PG 381 3 ng/ml	101.88	93.94
LPS PG 381 10 ng/ml	115.00	94.02

Monocytes	Healthy #15	EOP #15
CD86	MFI	MFI
Isotype control	16.54	19.30
Control	60.52	90.56
LPS PG 381 1 ng/ml	89.19	86.05
LPS PG 381 3 ng/ml	96.80	87.09
LPS PG 381 10 ng/ml	122.95	113.31

Monocytes	Healthy #16	EOP #16
CD86	MFI	MFI
Isotype control	15.47	18.82
Control	39.13	64.66
LPS PG 381 1 ng/ml	61.14	88.09
LPS PG 381 3 ng/ml	85.94	91.34
LPS PG 381 10 ng/ml	90.16	116.02

Monocytes	Healthy #17	EOP #17
CD86	MFI	MFI
Isotype control	14.39	16.78
Control	45.97	64.31
LPS PG 381 1 ng/ml	70.74	78.44
LPS PG 381 3 ng/ml	81.94	93.22
LPS PG 381 10 ng/ml	97.76	116.52

2. Up-regulation of CD69 expression on NK cells in P.gingivalis-stimulated whole blood cultures

Table 4. Mean fluorescence intensity (MFI) and % positive cells of whole blood cultures of healthy and EOP patients incubated with *P. gingivalis* LPS with various concentration (0, 1, 3, 10 ng/ml) for 48 h and then assessed for CD69 expression on NK cells (n = 17).

NK Cells	Healthy #1		EOP #1	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	5.01	0.09	5.65	0.39
Control	9.33	8.43	8.26	5.36
LPS PG 381 1 ng/ml	13.14	13.29	8.55	5.66
LPS PG 381 3 ng/ml	28.24	31.98	9.12	6.48
LPS PG 381 10 ng/ml	46.93	48.32	10.32	8.47

NK Cells	Healthy #2		EOP #2	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	5.51	0.10	5.12	0.00
Control	9.48	8.57	11.94	8.71
LPS PG 381 1 ng/ml	8.51	6.49	8.49	5.71
LPS PG 381 3 ng/ml	17.96	18.16	8.18	5.30
LPS PG 381 10 ng/ml	38.19	40.78	12.80	8.91

NK Cells	Healthy #3		EOP #3	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	6.31	0.25	5.16	0.00
Control	8.02	3.19	7.17	2.41
LPS PG 381 1 ng/ml	8.98	2.07	8.00	3.40
LPS PG 381 3 ng/ml	10.96	7.32	8.84	5.71
LPS PG 381 10 ng/ml	20.65	19.59	26.14	28.51

NK Cells	Healthy #4		EOP #4	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	6.54	0.88	6.81	1.61
Control	7.34	1.45	14.56	11.41
LPS PG 381 1 ng/ml	8.95	2.07	15.16	12.33
LPS PG 381 3 ng/ml	8.27	3.29	14.27	13.34
LPS PG 381 10 ng/ml	12.17	7.56	21.03	20.66

NK Cells	Healthy #5		EOP #5	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	6.66	0.30	5.26	0.31
Control	14.50	14.08	6.38	3.36
LPS PG 381 1 ng/ml	13.78	15.28	7.39	5.41
LPS PG 381 3 ng/ml	14.36	14.98	7.94	5.36
LPS PG 381 10 ng/ml	22.98	20.55	8.36	6.86

NK Cells	Healthy #6		EOP #6	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	5.46	0.11	5.58	0.00
Control	8.39	3.65	14.29	13.26
LPS PG 381 1 ng/ml	7.85	4.57	11.98	11.60
LPS PG 381 3 ng/ml	12.24	12.03	16.52	18.51
LPS PG 381 10 ng/ml	36.92	43.20	45.64	47.58

NK Cells	Healthy #7		EOP #7	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	5.41	0.88	4.71	0.40
Control	8.14	6.86	7.76	5.93
LPS PG 381 1 ng/ml	12.47	11.76	8.08	7.91
LPS PG 381 3 ng/ml	10.38	9.30	8.88	9.59
LPS PG 381 10 ng/ml	15.78	18.83	20.18	27.33

NK Cells	Healthy #8		EOP #8	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	7.66	0.54	4.17	0.94
Control	17.85	19.12	6.60	6.58
LPS PG 381 1 ng/ml	17.96	18.40	7.68	8.46
LPS PG 381 3 ng/ml	19.44	20.28	24.20	27.18
LPS PG 381 10 ng/ml	59.32	48.20	68.29	55.15

NK Cells	Healthy #9		EOP #9	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	4.45	0.94	3.63	0.45
Control	10.86	12.10	8.00	7.35
LPS PG 381 1 ng/ml	12.31	15.62	9.36	11.19
LPS PG 381 3 ng/ml	16.61	21.92	7.91	8.53
LPS PG 381 10 ng/ml	44.12	39.41	7.00	7.26

NK Cells	Healthy #10		EOP #10	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	3.17	0.53	3.27	0.21
Control	6.51	4.59	5.65	4.13
LPS PG 381 1 ng/ml	4.58	3.17	5.89	4.57
LPS PG 381 3 ng/ml	5.37	5.88	7.77	7.50
LPS PG 381 10 ng/ml	21.80	24.26	21.52	22.61

NK Cells	Healthy #11		EOP #11	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	3.63	0.29	3.78	1.14
Control	5.75	4.28	7.29	6.89
LPS PG 381 1 ng/ml	12.53	13.32	4.98	5.00
LPS PG 381 3 ng/ml	49.19	43.70	8.41	7.38
LPS PG 381 10 ng/ml	103.89	68.92	6.45	6.53

NK Cells	Healthy #12		EOP #12	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	3.30	0.33	3.88	0.32
Control	4.78	4.03	6.88	6.82
LPS PG 381 1 ng/ml	4.63	4.20	11.89	9.87
LPS PG 381 3 ng/ml	6.25	4.26	27.56	20.79
LPS PG 381 10 ng/ml	11.99	9.11	73.11	42.18

NK Cells	Healthy #13		EOP #13	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	4.26	3.80	3.17	0.76
Control	5.85	6.21	5.93	5.48
LPS PG 381 1 ng/ml	8.36	9.96	6.17	6.71
LPS PG 381 3 ng/ml	19.23	21.42	8.72	7.92
LPS PG 381 10 ng/ml	42.95	44.01	12.31	13.09

NK cells	Healthy #14		EOP #14	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	3.28	0.75	3.67	1.23
Control	4.87	6.00	21.17	18.73
LPS PG 381 1 ng/ml	4.21	3.20	20.84	22.38
LPS PG 381 3 ng/ml	10.71	16.90	45.50	40.34
LPS PG 381 10 ng/ml	17.11	29.63	100.29	64.99

NK cells	Healthy #15		EOP #15	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	2.98	0.84	3.59	2.81
Control	8.41	9.41	12.51	20.29
LPS PG 381 1 ng/ml	9.14	9.15	9.38	17.18
LPS PG 381 3 ng/ml	13.37	14.18	12.61	17.65
LPS PG 381 10 ng/ml	35.83	32.28	26.31	28.33

NK cells	Healthy #16		EOP #16	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	2.81	1.15	3.04	1.11
Control	8.14	14.47	9.47	13.40
LPS PG 381 1 ng/ml	9.22	18.16	21.42	20.55
LPS PG 381 3 ng/ml	27.55	33.03	61.07	41.92
LPS PG 381 10 ng/ml	78.31	61.16	88.51	55.05

NK cells	Healthy #17		EOP #17	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	3.32	1.54	2.82	1.20
Control	12.17	14.29	4.49	7.00
LPS PG 381 1 ng/ml	8.61	9.54	6.47	11.58
LPS PG 381 3 ng/ml	18.15	13.86	7.12	10.10
LPS PG 381 10 ng/ml	48.11	33.96	16.43	20.88

3. Up-regulation of CD69 expression on γδ T cells in P.gingivalis-stimulated whole blood cultures.

Table 5. Mean fluorescence intensity (MFI) and % positive cells of whole blood cultures of healthy and EOP patients incubated with *P. gingivalis* LPS with various concentration (0, 1, 3, 10 ng/ml) for 48 h and then assessed for CD69 expression on γδ T cells (n = 17).

γδ T cells	Healthy #1		EOP #1	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	28.95	0.71	5.15	0.00
Control	11.05	6.95	13.28	8.53
LPS PG 381 1 ng/ml	9.17	4.51	9.67	7.23
LPS PG 381 3 ng/ml	13.20	9.53	10.90	8.52
LPS PG 381 10 ng/ml	23.53	15.38	13.72	13.16

γδ T cells	Healthy #2		EOP #2	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	8.01	0.20	7.26	0.42
Control	7.03	0.40	7.57	2.29
LPS PG 381 1 ng/ml	6.87	0.20	10.39	3.70
LPS PG 381 3 ng/ml	7.25	0.50	9.42	3.46
LPS PG 381 10 ng/ml	8.47	2.00	9.46	4.53

γδ T cells	Healthy #3		EOP #3	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	5.85	0.10	6.08	0.41
Control	8.07	2.36	6.97	1.75
LPS PG 381 1 ng/ml	9.27	4.24	7.92	2.91
LPS PG 381 3 ng/ml	9.67	4.75	9.23	4.09
LPS PG 381 10 ng/ml	8.79	3.92	17.71	5.43

γδ T cells	Healthy #4		EOP #4	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	6.07	0.00	5.21	0.10
Control	7.85	2.10	6.17	1.81
LPS PG 381 1 ng/ml	7.59	2.00	7.33	2.51
LPS PG 381 3 ng/ml	8.88	3.22	6.68	2.40
LPS PG 381 10 ng/ml	18.34	7.41	7.21	2.91

γδ T cells	Healthy #5		EOP #5	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	5.78	0.42	5.46	0.30
Control	7.25	2.17	5.73	0.81
LPS PG 381 1 ng/ml	7.42	2.59	6.20	1.12
LPS PG 381 3 ng/ml	8.55	3.94	8.55	2.94
LPS PG 381 10 ng/ml	13.39	7.21	15.64	5.75

γδ T cells	Healthy #6		EOP #6	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	5.06	0.10	5.49	0.00
Control	6.13	1.56	7.85	2.56
LPS PG 381 1 ng/ml	7.13	2.71	7.37	1.96
LPS PG 381 3 ng/ml	7.03	2.59	8.65	3.52
LPS PG 381 10 ng/ml	7.53	2.47	12.00	9.27

γδ T cells	Healthy #7		EOP #7	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	6.44	0.22	2.91	0.00
Control	5.15	1.69	4.11	1.27
LPS PG 381 1 ng/ml	5.11	1.77	3.81	1.26
LPS PG 381 3 ng/ml	6.72	3.66	11.25	7.46
LPS PG 381 10 ng/ml	21.29	13.58	26.85	15.58

γδ T cells	Healthy #8		EOP #8	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	6.61	0.42	3.12	0.21
Control	18.55	17.70	3.41	0.52
LPS PG 381 1 ng/ml	24.96	22.81	4.20	1.25
LPS PG 381 3 ng/ml	33.36	30.25	3.97	1.15
LPS PG 381 10 ng/ml	42.65	33.79	4.88	2.51

γδ T cells	Healthy #9		EOP #9	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	2.68	0.35	3.09	0.79
Control	2.65	0.23	3.59	1.60
LPS PG 381 1 ng/ml	2.84	0.71	3.22	1.17
LPS PG 381 3 ng/ml	3.13	0.94	4.58	1.07
LPS PG 381 10 ng/ml	5.66	3.61	8.52	5.40

γδ T cells	Healthy #10		EOP #10	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	1.96	0.00	4.10	0.52
Control	2.88	0.73	4.64	1.79
LPS PG 381 1 ng/ml	3.27	1.88	2.88	0.84
LPS PG 381 3 ng/ml	11.86	5.60	2.94	1.47
LPS PG 381 10 ng/ml	20.91	9.85	3.49	2.34

γδ T cells	Healthy #11		EOP #11	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	2.68	0.11	2.78	0.10
Control	2.89	1.40	5.24	1.87
LPS PG 381 1 ng/ml	3.26	1.10	6.92	2.36
LPS PG 381 3 ng/ml	3.74	1.53	8.78	3.18
LPS PG 381 10 ng/ml	3.91	2.19	18.12	9.38

γδ T cells	Healthy #12		EOP #12	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	4.12	1.21	4.72	0.62
Control	4.33	1.89	3.95	1.77
LPS PG 381 1 ng/ml	3.85	2.75	6.01	2.88
LPS PG 381 3 ng/ml	5.67	3.14	4.45	2.77
LPS PG 381 10 ng/ml	14.83	9.41	5.78	3.15

γδ T cells	Healthy #13		EOP #13	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	4.62	1.09	2.77	0.50
Control	5.06	3.31	4.85	3.02
LPS PG 381 1 ng/ml	16.11	2.86	8.93	6.90
LPS PG 381 3 ng/ml	10.90	10.40	9.07	5.09
LPS PG 381 10 ng/ml	.18.79	15.52	12.89	10.20

γδ T cells	Healthy #14		EOP #14	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	11.414	2.13	2.61	0.39
Control	8.49	5.62	13.65	14.11
LPS PG 381 1 ng/ml	10.46	8.06	7.89	7.59
LPS PG 381 3 ng/ml	17.10	11.96	7.95	6.29
LPS PG 381 10 ng/ml	32.58	18.94	12.27	11.07

γδ T cells	Healthy #15		EOP #15	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	2.46	0.32	8.74	3.10
Control	7.84	10.36	7.66	7.32
LPS PG 381 1 ng/ml	13.84	12.29	20.36	14.71
LPS PG 381 3 ng/ml	19.84	18.76	51.14	23.50
LPS PG 381 10 ng/ml	35.16	26.08	71.86	33.06

γδ T cells	Healthy #16		EOP #16	
CD69	MFI	% Positive	MFI	% Positive
Isotype control	3.09	0.82	3.57	1.29
Control	8.82	4.94	6.12	5.42
LPS PG 381 1 ng/ml	5.89	3.10	5.46	5.57
LPS PG 381 3 ng/ml	7.73	5.50	7.88	7.15
LPS PG 381 10 ng/ml	23.53	10.82	9.13	8.19

4. Cytokine production of *P.gingivalis*-stimulated whole blood cultures

Table 6. IL-1β production (pg/ml) of whole blood cultures of healthy and EOP patients incubated with *P. gingivalis* LPS with various concentration (0, 1, 3, 10 ng/ml) for 48 h (n = 17).

	Healthy #1	EOP #1
Control	2.215	5.156
LPS PG 381 1 ng/ml	28.045	1.812
LPS PG 381 3 ng/ml	69.654	28.536
LPS PG 381 10 ng/ml	167.258	63.228

	Healthy #2	EOP #2
Control	5.156	0.000
LPS PG 381 1 ng/ml	5.541	0.000
LPS PG 381 3 ng/ml	14.814	10.467
LPS PG 381 10 ng/ml	54.031	71.948

	Healthy #3	EOP #3
Control	0.000	0.000
LPS PG 381 1 ng/ml	0.994	1.812
LPS PG 381 3 ng/ml	6.535	6.037
LPS PG 381 10 ng/ml	48.962	87.713

	Healthy #4	EOP #4
Control	0.000	0.000
LPS PG 381 1 ng/ml	0.000	3.208
LPS PG 381 3 ng/ml	0.000	6.037
LPS PG 381 10 ng/ml	10.467	49.884

	Healthy #5	EOP #5
Control	12.536	12.536
LPS PG 381 1 ng/ml	11.976	12.536
LPS PG 381 3 ng/ml	47.577	48.962
LPS PG 381 10 ng/ml	73.783	122.419

	Healthy #6	EOP #6
Control	6.079	0.210
LPS PG 381 1 ng/ml	5.886	12.012
LPS PG 381 3 ng/ml	37.135	66.388
LPS PG 381 10 ng/ml	144.125	269.505

	Healthy #7	EOP #7
Control	1.584	7.832
LPS PG 381 1 ng/ml	3.415	9.411
LPS PG 381 3 ng/ml	22.041	27.015
LPS PG 381 10 ng/ml	57.385	84.120

	Healthy #8	EOP #8
Control	1.763	20.855
LPS PG 381 1 ng/ml	4.167	4.926
LPS PG 381 3 ng/ml	28.018	31.543
LPS PG 381 10 ng/ml	132.809	298.266

	Healthy #9	EOP #9
Control	1.584	7.049
LPS PG 381 1 ng/ml	14.854	9.014
LPS PG 381 3 ng/ml	24.023	12.794
LPS PG 381 10 ng/ml	84.120	23.527

	Healthy #10	EOP #10
Control	1.763	0.372
LPS PG 381 1 ng/ml	4.926	4.546
LPS PG 381 3 ng/ml	17.623	41.755
LPS PG 381 10 ng/ml	70.656	124.940

	Healthy #11	EOP #11
Control	1.054	0.000
LPS PG 381 1 ng/ml	17.734	1.944
LPS PG 381 3 ng/ml	60.023	8.539
LPS PG 381 10 ng/ml	360.688	27.015

	Healthy #12	EOP #12
Control	4.167	2.858
LPS PG 381 1 ng/ml	8.422	14.854
LPS PG 381 3 ng/ml	25.018	115.444
LPS PG 381 10 ng/ml	64.261	343.044

	Healthy #13	EOP #134.167
Control	33.865	9.411
LPS PG 381 1 ng/ml	39.762	10.406
LPS PG 381 3 ng/ml	66.388	19.089
LPS PG 381 10 ng/ml	161.258	77.096

	Healthy #14	EOP #14
Control	111.489	0.000
LPS PG 381 1 ng/ml	111.489	0.000
LPS PG 381 3 ng/ml	221.185	40.355
LPS PG 381 10 ng/ml	344.008	164.399

	Healthy #15	EOP #15
Control	2.674	2.125
LPS PG 381 1 ng/ml	9.808	12.415
LPS PG 381 3 ng/ml	23.527	54.232
LPS PG 381 10 ng/ml	53.183	162.981

	Healthy #16	EOP #16
Control	13.061	8.277
LPS PG 381 1 ng/ml	13.271	8.277
LPS PG 381 3 ng/ml	31.136	61.113
LPS PG 381 10 ng/ml	107.807	21.175

	Healthy #17	EOP #17
Control	1.586	1.306
LPS PG 381 1 ng/ml	9.503	5.609
LPS PG 381 3 ng/ml	36.802	41.595
LPS PG 381 10 ng/ml	107.270	156.999

Table 7 PGE2 production (pg/ml) of whole blood cultures of healthy and EOP patients incubated with *P. gingivalis* LPS with various concentration (0, 1, 3, 10 ng/ml) for 48 h (n = 17).

	Healthy #1	EOP #1
Control	328.253	341.020
LPS PG 381 1 ng/ml	315.906	262.325
LPS PG 381 3 ng/ml	412.137	306.908
LPS PG 381 10 ng/ml	428.043	337.787

	Healthy #2	EOP #2
Control	303.956	286.729
LPS PG 381 1 ng/ml	275.679	283.935
LPS PG 381 3 ng/ml	289.544	251.986
LPS PG 381 10 ng/ml	295.241	334.582

	Healthy #3	EOP #3
Control	204.348	164.306
LPS PG 381 1 ng/ml	144.879	128.214
LPS PG 381 3 ng/ml	191.214	148.684
LPS PG 381 10 ng/ml	176.476	146.776

	Healthy #4	EOP #4
Control	213.380	195.539
LPS PG 381 1 ng/ml	262.325	239.468
LPS PG 381 3 ng/ml	267.607	364.454
LPS PG 381 10 ng/ml	374.952	549.547

	Healthy #5	EOP #5
Control	264.956	275.679
LPS PG 381 1 ng/ml	275.679	232.162
LPS PG 381 3 ng/ml	295.241	206.858
LPS PG 381 10 ng/ml	404.414	262.325

	Healthy #6	EOP #6
Control	165.619	138.240
LPS PG 381 1 ng/ml	165.619	123.499
LPS PG 381 3 ng/ml	604.655	178.038
LPS PG 381 10 ng/ml	1040.445	193.098

	Healthy #7	EOP #7
Control	263.222	204.260
LPS PG 381 1 ng/ml	308.448	197.521
LPS PG 381 3 ng/ml	430.073	299.551
LPS PG 381 10 ng/ml	467.947	342.915

	Healthy #8	EOP #8
Control	163.592	314.495
LPS PG 381 1 ng/ml	151.664	287.999
LPS PG 381 3 ng/ml	213.447	282.352
LPS PG 381 10 ng/ml	230.109	326.875

	Healthy #9	EOP #9
Control	204.260	414.237
LPS PG 381 1 ng/ml	220.494	391.530
LPS PG 381 3 ng/ml	186.565	373.501
LPS PG 381 10 ng/ml	232.553	398.965

	Healthy #10	EOP #10
Control	173.849	215.781
LPS PG 381 1 ng/ml	199.754	193.098
LPS PG 381 3 ng/ml	265.896	199.754
LPS PG 381 10 ng/ml	326.875	202.000

	Healthy #11	EOP #11
Control	235.014	265.896
LPS PG 381 1 ng/ml	282.352	208.824
LPS PG 381 3 ng/ml	323.743	199.754
LPS PG 381 10 ng/ml	395.231	349.516

	Healthy #12	EOP #12
Control	199.754	145.848
LPS PG 381 1 ng/ml	260.567	118.125
LPS PG 381 3 ng/ml	422.083	138.240
LPS PG 381 10 ng/ml	744.284	119.907

	Healthy #13	EOP #134.167
Control	276.786	610.532
LPS PG 381 1 ng/ml	459.247	544.191
LPS PG 381 3 ng/ml	660.114	514.124
LPS PG 381 10 ng/ml	587.423	634.734

	Healthy #14	EOP #14
Control	243.086	271.826
LPS PG 381 1 ng/ml	508.923	361.047
LPS PG 381 3 ng/ml	615.069	424.208
LPS PG 381 10 ng/ml	514.841	520.796

	Healthy #15	EOP #15
Control	220.494	570.763
LPS PG 381 1 ng/ml	314.495	707.642
LPS PG 381 3 ng/ml	265.896	2409.960
LPS PG 381 10 ng/ml	308.448	3701.599

	Healthy #16	EOP #16
Control	410.514	630.385
LPS PG 381 1 ng/ml	465.218	785.776
LPS PG 381 3 ng/ml	508.907	891.197
LPS PG 381 10 ng/ml	583.231	918.263

	Healthy #17	EOP #17
Control	186.385	103.835
LPS PG 381 1 ng/ml	293.753	172.408
LPS PG 381 3 ng/ml	456.210	430.527
LPS PG 381 10 ng/ml	514.113	519.397

กิจกรรมที่เกี่ยวข้องกับการนำผลจากโครงการไปใช้ประโยชน์

1. ได้รับเชิญประชุมและเสนอผลงานวิจัย เรื่อง "Up-regulation of co-stimulatory molecule expression and dendritic cell marker (CD83) on B cells in Periodontal disease" ในงานประชุม American Society of Microbiology 100th General Meeting ณ. เมือง Los Angeles ประเทศสหรัฐอเมริกา มีนาคม 2543 โดย ผศ.ทญ.ดร.รังสินี มหานนท์
2. วิทยากรบรรยาย เรื่อง "The forgotten in infiltrated B-cells in Periodontal disease" ในการจัดอบรม เรื่อง "The pathogenesis of Periodontitis (Immunology)" โดยความร่วมมือของคณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล, Tokyo Medical and Dental University, ชมรมปริทันตวิทยาแห่งประเทศไทย และชมรมปริทันตวิทยาแห่งประเทศญี่ปุ่น วันที่ 23-25 สิงหาคม 2543 โดย ผศ.ทญ.ดร.รังสินี มหานนท์
3. สัมภาษณ์ทางโทรศัพท์ ในหัวข้อเรื่อง "โรคร้ามะนาด" ทางสถานีวิทยุ จส.100โดย อทญ.อรวรรณ จรัสกุลางกูร
4. สัมภาษณ์ลงหนังสือพิมพ์มติชน ในหัวข้อเรื่อง "เร่งวิจัยโรคปริทันต์อักเสบวัยเด็กพุ่ง" ลงวันที่ 22 พฤศจิกายน 2544 โดย ผศ.ทญ.ดร.รังสินี มหานนท์
5. สัมภาษณ์ทางโทรศัพท์ ในหัวข้อเรื่อง "การป้องกันโรคปริทันต์" ในรายการคุ้มครองสุขภาพทางสถานีวิทยุ FM 98.5 วันที่ 2 ธันวาคม 2544โดย ผศ.ทญ.ดร.รังลินี มหานนท์
6. ได้รับเชิญประชุมและเสนอผลงาน เรื่อง "Prevalence of Early-onset Periodontitis in Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Thailand" ณ. Tokyo Medical and Dental University, ประเทศญี่ปุ่น วันที่ 15-16 มีนาคม 2545 โดย อทญ.อรวรรณ จรัสกุลางกูร

วัตถุประสงค์	กิจกรรมที่วางแผนไว้	กิจกรรมที่ดำเนินงานมา	ผลที่ได้รับ
1.To study the mechanisms of the pathogenesis of periodontal disease by focusing on the role of B cells	a.Extraction of gingival cells from tissue biopsies of periodontitis patients. Analysis of cell surface markers on gingival infiltrated B cells. b. Compare the ability of different plaque bacteria to stimulate B cells to express CD86 and CD83 c. Investigate role of plaque bacteria activated B cells as antigen presenting cells	a. Expression of co-stimulatory molecules on B cells by flow cytometry. *b.* - Prepared sonicated extracts of *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia* and *Actinomyces viscosus* - Used flow cytometry to measure CD86 and CD83 expression c. - Mixed leukocyte reaction was used as an *in vitro* model to investigate antigen presenting cell function of B cells. - Cytokines released from T cells in allogeneic mixed leukocyte reactions were monitored by ELISA	In press in the Journal of Periodontal Research, titled "Up-regulation of co-stimulatory molecule expression and dendritic cell marker (CD83) on B cells in periodontal disease". The work was presented in The American Society for Microbiology, 100th General meeting at Los Angeles, CA, US. May 21-May 25, 2000.
2. To investigate the role of cytokines and periodontopathic bacterial products on immune cells and gingival fibroblasts	a. Establish human gingival fibroblast (HGF) lines b. Analysis of mCD14 expression on HGF lines c. Investigate activation of HGF by bacterial lysates, bacterial DNA and chemically synthesized CpG ODN DNA	a. 11 HGF lines were established b. Anaylsis of mCD14 expression on HGF by flow cytometry c. - Preparation for *P. gingivalis, A. actinomycetemcomitans, P. intermedia* and *A. viscosus* DNA -Measured expression of co-stimulatory molecules (CD40, CD80, CD86), and HLA-DR on HGF by flow cytometry after stimulation with bacterial DNA. -Check purity of bacterial DNA (LAL assay) -Obtained chemically synthesized CpG ODN from Dr AM. Krieg (Department of Internal Medicine, University of Iowa College of Medicine, Coley Pharmaceutical Group. US) -Analysis of IL-6 production after stimulation with CpG ODN 2006 by ELISA	- HGF lines were heterogeneous with different levels of mCD14 expression. - Some of them show undetectable mCD14 expression. - High contamination of LPS in DNA preparation which were unable to remove - No significant HGF stimulation by CpG ODN was observed. CpG ODN 2006 (potent for human cells) was used. - Due to heterogeneity of HGF (mCD14 expression), LPS contamination in DNA preparation and the negative results obtained from CpG ODN experiment, we decided not to further investigated.
3. To investigate the response of early-onset periodontitis (EOP) patients to bacterial products in terms of activation markers and cytokine production This is to test hypothesis of Offenbacher's group : hyper-responsive monocytic trait of EOP	a. Study of prevalence of EOP in Periodontal clinic, Chulalongkorn University b. - Recruit EOP patients and healthy periodontal subjects (age, sex matched) - Investigate *P.gingivalis* LPS stimulation on monocytes in whole blood cultures.	a. Cross sectional study of EOP. Use revised clinical criteria for a precised form. Examination of total 1,799 periodontal subjects includes clinical periodontal exam., history taking and full mouth X-ray. b. - n=17 in each group (EOP vs healthy periodontal subjects) - Obtained *P.gingivalis* LPS from Dr R.E Schifferle (Depart. of Oral Biology, SUNY at Baffalo, US). - Analysis of co-stimulatory molecule (CD80,CD86, CD40) expression on monocytes (CD14), CD69 expression on NK cells and γδ T cells by flow cytometry. - Measurement for IL-1β and PGE2 production by ELISA	a. This is the first report to investigate the specific early-onset group of periodontitis patients in Thailand. Considerably high prevalence of EOP (10.9% of total periodontitis subjects) was evident in this study. The data is of value as based line for future investigation. The manuscript in preparation for submission to the Journal of Thai Dental Association . b. No differences was found between EOP patients and healthy periodontal subjects in terms of 1). IL-1β production 2). PGE2 production 3). CD40, CD86 expression on monocytes 4). CD69 expression on NK cells 5). CD69 expression on γδT cells However, the significant difference between the two groups was found on CD80 expression on monocytes. Hyper-responsiveness of monocytes as monitored by up-regulation of CD40, CD80 and CD86 and the production of IL-1β and PGE2 was not observed in EOP group. This data disagree with previous reports by Offenbacher group (1986, 1998, 1999). Our manuscript is in preparation for submission to international peer reviewed journal.