รายงานวิจัยฉบับสมบูรณ์

โครงการ โรคปริทันต์อักเสบในผู้ป่วยอายุน้อย

EARLY-ONSET PERIODONTITIS

โดย

รังสินี มหานนท์ และคณะ

ธันวาคม 2544

สัญญาเลขที่ RDG 3/05/2543

รายงานวิจัยฉบับสมบูรณ์

โครงการ โรคปริทันต์อักเสบในผู้ป่วยอายุน้อย

EARLY-ONSET PERIODONTITIS

คณะผู้วิจัย	สังกัด
อ.ทญ.ดร.รังสินี มหานนท์	ภาควิชาปริทันตวิทยา คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
อ.ทญ.อรวรรณ จรัสกุลางกูร	ภาควิชาปริทันตวิทยา คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ดร.สาธิต พิชญางกูร	ภาควิชาภูมิคุ้มกันวิทยาและการแพทย์ สถาบันวิจัยวิทยาศาสตร์สาธารณสุขทหาร

ชุดโครงการ สุขภาพช่องปาก

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย (สกว.)

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

Executive summary

TITLE : EARLY-ONSET PERIODONTITIS

ชื่อโครงการ : โรคปริทันต์อักเสบในผู้ป่วยอายุน้อย

PRINCIPLE INVESTIGATOR : Dr. Rangsini Mahanonda (อ.ทญ.ดร.รังสินี มหานนท์)

Office : Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Henry Dunant Rd, Bangkok 10330 Tel: 662 218 8850 Fax: 662 218 8851

CO-INVESTICATORS' NAMES AND ORGANIZATIONS

Dr. Orawan Charatkulangkun (อ.ทญ.อรวรรณ จรัสกุลางกูร)

Office : Department of Periodontology, Faculty of Dentistry, Chulalongkorn University Henry Dunant Rd., Bangkok 10330 Tel: (662) 218 8850 Fax: (662) 218 8851

Dr. Sathit Pichyangkul (ดร.สาธิต พิชญางกูร)

Office : Department of Immunology and Medicine, US Army Medical Component, The Armed Force Research Institute of Medical Science Tel: (662) 644 7139-41 Ext 2693

BACKGROUND OF THE STUDY

Early-onset Periodontitis (EOP) occurs in children, teenager and young adults. It is characterized by an early occurrence of periodontal attachment loss and bone resorption. The rate of disease progression is quite rapid, thus frequently resulting in early loss of teeth. Dysregulation of the immune response has been widely accepted to play role in the pathogenesis of periodontitis. Over production of immune mediators (cytokines) has been linked to tissue destruction and bone resorption. However, the precise mechanisms underlying the pathogenesis of EOP is still largely unknown.

OBJECTIVES

1. To study the mechanisms of pathogenesis of periodontal disease by focusing on the role of B cells.
2. To investigate the role of cytokine and periodontopathic bacterial products (LPS and DNA) on immune cells and gingival fibroblasts.

3. To investigate the response of EOP patients to bacterial products in terms of activation markers and cytokine production.

RESEARCH METHOLOGIES

1. Prevalence of EOP
2. Preparation of whole cell bacterial sonicates, Lipopolysaccharide, DNA (*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Actinomyces viscosus*)
3. Establish gingival fibroblast cell lines.
4. Monitor cell activation (immune cells and gingival fibroblasts) in healthy periodontal subjects and EOP patients by co-stimulatory molecule expression and inflammatory cytokine production.

EXPECTED OUTPUT

1. This study will provide the prevalence of EOP in Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Thailand.
2. To provide better understanding the mechanisms underlying the pathogenesis of EOP.
3. This work will strengthen the relationship between the three institutions, Chulalongkorn University, AFRIMS and Tokyo Medical and Dental University.

EXPECTED PUBLICATIONS

1. The role of B cells in pathogenesis of periodontal disease in journal of Clinical and Experimental Immunology.
2. The role of gingival fibroblasts in the pathogenesis of periodontal disease in Infection and Immunity.
3. Prevalence of EOP patients and immune activation profiles in Journal of Thai society of Periodontology.

แผนการดำเนินงานตลอดโครงการ 2 ปี

เดือนที่ / แผนงาน	1-6	7-12	13-18	18-24
จัดซื้อเตรียมวัสดุอุปกรณ์	3 m			
Prevalences of EOP	12 m			
เตรียมและทดสอบแบคทีเรีย	8 m			
"B cells" Study		15 m		
"Gingival fibroblast" Study			18 m	
"Immune activation in EOP" Study			18 m	
Data analysis				8 m
Report & Submission for Publication				6 m

ABSTRACT

Early-onset periodontitis (EOP) is one such syndrome and comprises a group of pathological conditions leading to loss of periodontal tissues early in life. It occurs in children, teenagers and young adults which causes considerable tissue damage over a relatively short period of time. In the case of rapid progression of the destructive disease, early loss of teeth may result. At present, the pathogenesis of EOP is not clearly understood, however, it shares a common underlying mechanisms by other forms of periodontitis. The primary etiologic agents of periodontitis are Gram negative bacteria in dental plaque, such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, and their products such as lipopolysaccharide (LPS). Host immune response to bacterial plaque pathogens, in fact, plays a crucial role in determining the outcome and severity of the disease.

Periodontitis lesions consist of a large number of infiltrating cells such as T cells, B cells and macrophages which are in activated stage. Major effort during the past decade has been focused on immunoregulatory role of T cells and their cytokines which have been well recognized for their important involvement in the pathogenesis of periodontitis. Although B cells are the majority in advanced periodontal lesions, there is no study so far able to clarify their role in the pathogenesis of the disease. It is known that B cells are recognized as one of the professional antigen presenting cells (APCs). To date, the role of APCs, which critically controls the initiation and maintenance of T cell response, has been poorly investigated in periodontitis. Langerhan's cells, keratinocytes in oral epithelium as well as tissue macrophages have been largely speculated to play role as local APCs. It is known that activation stage of APCs associated with up-regulation of co-stimulatory molecule expression (CD40, CD80, and CD86) is a key factor to trigger and maintain optimal T cell response. In this study we analyzed the expression of co-stimulatory molecules (CD80 and CD86) and CD83, a marker of mature dendritic cells, on gingival cells isolated from severe periodontitis tissues by flow cytometry. Significant up-regulation of CD86 and CD83 expression were detected in periodontitis lesions and the majority of them were observed on B cells. *In vitro* peripheral blood mononuclear cell cultures showed that stimulation with different periodontopathic bacteria including *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* , *Prevotella intermedia*, and *Actinomyces viscosus*

up-regulated both CD86 and CD83 expression on B cells. Hence, the presence of plaque bacteria may be responsible for enhanced expression on gingival B cells as seen *in vivo*. APC function by bacterial activated B cells was further investigated using allogeneic mixed leukocyte reactions (MLR). After 24h culture with either *A. actinomycetemcomitans* or *P.gingivalis*, these activated B cells performed as potent APCs in MLR and stimulated T cells to produce high levels of gamma Interferon (IFN-γ) and minimal Interleukin-5. In conclusion, periodontopathic bacterial induced B cell activation with up-regulation of CD86 and CD83 may associate with enhanced APC function. Therefore, the results of this study put forward the role of infiltrated gingival B cells as possible APCs in regulating and sustaining local T cell response in periodontitis.

Early-onset periodontitis was described by the consensus report on periodontal diagnosis presented at the 1989 World Workshop in Clinical Periodontics as the disease with the age of onset usually prior to 35 years, rapid rate of progression of tissue destruction, manifestation of defects in host defense to plaque bacteria. It was subdivided into three age-related diseases termed 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). There is, however, an area of concern in the traditional classification of EOP since we frequently encounter difficulties to classify the disease entity at the borderline which seems to overlap one another. This is for example, the differentiation between generalized juvenile periodontitis (GJP) and RPP. Therefore, a revised classification criteria which are more precise for defining the forms of EOP into several homogeneous subgroups has been recommended.

According to the Annals of Periodontology, 1996 World Workshop in Periodontics, the review of a number of studies corroborate the conclusion that EOP is more frequent in developing countries and among subjects of black race. So far there is no study in Thailand regarding the prevalence of EOP. Therefore, we carried out the cross sectional study to investigate the prevalence of the EOP patients who entered the Periodontal Clinic, Faculty of dentistry, Chulalongkorn university by using revised criteria. Among the total of 1,723 screened patients, 446 patients were periodontitis and 97 patients were under or equal the age of 40 who were then subdivided into 4 subgroups of EOP according to their clinical and

full mouth intra-oral radiographic data. The prevalence of subform I (localized JP) was 0.29%, subform II (Post localized JP) was 0.7%, subform III (localized JP-RPP) was 1.10% and subform IV (RPP) was 0.75% of periodontal patients. Taken together, EOP prevalence was 2.84% in this study, thus suggesting high prevalence of EOP. Although this study was done at the teaching dental school which does not probably reflect the true EOP of the Thai population, it is a good start not only to recognize the problem of the EOP amongst the Thais but also to stimulate future research into such specific groups of periodontitis patients. Of importance, our study would initiate the awareness among people who often misunderstand that periodontitis only occurs in adults or old-age. Particularly, awareness for dentists / general practitioners regarding early detection of EOP and early treatment which certainly save money and not so complicated.

Hyper-responsiveness of monocytes to bacterial LPS have been hypothesized to be associated with the severity of periodontal disease (Offenbacher and Salvi, 1999). Particularly, high levels of monocytic secretory mediators such as PGE2 in susceptible periodontitis subjects have been reported. In this study, we used whole blood assays to compare monocyte activation after stimulating with LPS from periodontopathic bacteria, *Porphyromonas gingivalis* between 17 pairs of EOP patients who were in a maintenance care and healthy periodontal subjects (age and sex matched). Upon stimulation with varying doses of highly purified *P.gingivalis* LPS (1, 3, 10 ng/ml), expression of co-stimulatory molecules on monocytes were analyzed by flow cytometry and production of inflammatory mediators were monitored by ELISA. In addition, expression of CD69, early activation marker, on NK and γδ T cells were evaluated. LPS stimulation resulted in the dose dependent up-regulation of co-stimulatory molecules, CD40, CD80 and CD86 on monocytes, up-regulation of CD69 on NK and γδ T cells in both EOP and healthy control groups. Generally, periodontitis patients seem to show lower expression of these molecules than healthy controls at any tested LPS concentrations, but the only significant difference ($P < 0.05$) between groups were found in CD80 expression on monocytes. Production of PGE2 and IL-1β in *P.gingivalis* LPS-stimulated whole blood cultures was quite similar between the two groups. By using the whole blood assay which probably represent the better physiological condition, our results suggest that hyper-responsiveness of monocytes to bacterial challenge may not represent determinant factor responsible for EOP.

บทคัดย่อ

โรคปริทันต์อักเสบในผู้ป่วยอายุน้อย (Early Onset Periodontitis) เป็นกลุ่มอาการของโรคที่ประกอบไปด้วยพยาธิสภาพต่าง ๆที่นำไปสู่การสูญเสียการยึดเกาะของอวัยวะปริทันต์ตั้งแต่ผู้ป่วยอยู่ในช่วงอายุยังน้อย และมีอัตราการทำลายโรคที่ค่อนข้างรวดเร็ว อันอาจทำให้สูญเสียฟันไปก่อนเวลาอันควร ถึงแม้ว่าพยาธิสภาพการเกิดโรคจะยังไม่เป็นที่เข้าใจมากนัก แต่กลไกการเกิดโรคยังคงเหมือนกับโรคปริทันต์อักเสบชนิดอื่นๆ คือมีสาเหตุหลักของโรคมาจากเชื้อแบคทีเรียในคราบจุลินทรีย์ ได้แก่ *แอคติโนเบซิลัส แอคติโนไมซิเตมคอมมิแทน* (*Actinobacillus actinomycetemcomitans*) และ *พอร์ไฟโรโมแนส จินจิวาลิส* (*Porphyromonas gingivalis*) และผลิตผลของเชื้อแบคทีเรียบางอย่างเช่น ไลโปโปลี่แซคคารายด์ (lipopolysaccharide, LPS) ส่วนการตอบสนองทางระบบภูมิคุ้มกันของร่างกายต่อเชื้อแบคทีเรีย จะเป็นตัวกำหนดลักษณะรูปแบบและความรุนแรงของโรคที่แสดงออกทางคลินิก

รอยโรคปริทันต์อักเสบ มีการสะสมของเซลล์ที่แทรกซึมมาเป็นจำนวนมากเช่น บีเซลล์ (B cells) ทีเซลล์ (T cells) และแมคโครฟาจ (macrophages) เซลล์เหล่านี้อยู่ในภาวะที่ถูกกระตุ้นแล้ว การศึกษาในช่วงเวลา 10 ปีที่ผ่านมาได้มุ่งสนใจไปยังบทบาทของทีเซลล์และไซโตคายส์ (cytokines) ในการควบคุมการตอบสนองของระบบภูมิคุ้มกัน ซึ่งเป็นส่วนสำคัญที่เกี่ยวข้องการเกิดพยาธิสภาพของโรคปริทันต์อักเสบ ถึงแม้บีเซลล์เป็นเซลล์หลักอยู่ในรอยโรคที่รุนแรง เรายังขาดการศึกษาเกี่ยวกับบทบาทของเซลล์ชนิดนี้ในขบวนการการเกิดพยาธิสภาพ อันที่จริงแล้วบีเซลล์เป็นที่รู้จักกันดีในบทบาทของเซลล์ที่เชี่ยวชาญในการเสนอแอนติเจน ซึ่งมีความสำคัญในการเริ่มและคงสภาพการตอบสนองของทีเซลล์ หากแต่ในปัจจุบันหน้าที่อันนี้ของบีเซลล์มีการศึกษากันน้อยมาก แลงเกอฮานส์เซลล์ (Langerhan's cells) เคอราติโนไซน์ (keratinocytes) ที่เยื่อบุผิวในช่องปาก รวมทั้งแมคโคฟาจ ถูกคาดว่าเป็นเซลล์ที่ทำหน้าที่นำเสนอแอนติเจนในเนื้อเยื่อปริทันต์ เป็นที่ทราบกันแล้วว่าภาวะการกระตุ้นของเซลล์ที่ทำหน้าที่นำเสนอแอนติเจนมีความเกี่ยวข้องกับการเพิ่มขึ้นของการแสดงออกของ โค-สติมูลาโทรีโมเลกุล (co-stimulatory molecules) (ซีดี40 ซีดี80 และซีดี86) และเป็นปัจจัยหลักต่อการชักนำการตอบสนองของทีเซลล์ รวมทั้งการคงสภาพการตอบสนองของทีเซลล์ในระดับสูง ในการศึกษาครั้งนี้ เราได้วิเคราะห์การแสดงออกของโค-สติมูลาโทรีโมเลกุล (ซีดี80 และซีดี86) และซีดี83 เครื่องหมายสำหรับเซลล์เดนดริทิก (dendritic cells) ที่โตเต็มที่ บนเซลล์เหงือกที่แยกออกมาจากเนื้อเยื่อปริทันต์ที่มีการอักเสบอย่างรุนแรง โดยใช้โฟลไซโตเมตรี (flow cytometry) ผลการทดลองพบว่าในรอยโรคปริทันต์อักเสบมีการเพิ่มขึ้นอย่างมีนัยสำคัญ ของการแสดงของซีดี86 และซีดี83 ซึ่งพบอยู่บนบีเซลล์เป็นหลัก เมื่อกระตุ้นโมโนนิวเคลียร์เซลล์ (mononuclaer cells) จากเลือดในหลอดทดลองด้วยแบคทีเรียที่ทำให้เกิดโรคปริทันต์อักเสบ เช่น *พอร์ไฟโรโมแนส จินจิวาลิส แอคติโนเบซิลัส แอคติโนไมซิเตมคอมิเทน, เพรบโวเทลลา อินเทอมีเดีย (Prevotella intermedia)* และ *แอคติโนไมซีส วิสโคซัส*

(Actinomyces viscosus) พบว่ามีการเพิ่มขึ้นของการแสดงของซีดี86 และซีดี83 บนบีเซลล์ ดังนั้น การปรากฏของแบคทีเรียในคราบจุลินทรีย์น่าจะชักนำการเพิ่มขึ้นของการแสดงออกบนบีเซลล์ในเหงือก หน้าที่ของบีเซลล์ในแง่ของการนำเสนอแอนติเจนสามารถตรวจสอบได้โดยใช้ allogeneic mixed leukocyte reaction (MLR) เมื่อกระตุ้นบีเซลล์ด้วยการเพาะเลี้ยงกับ*พอร์ไฟโรโมแนส จินจิวาลิส หรือ แอคติโนเบซิลัส แอคติโนไมซิเตมคอมิแทน* เป็นเวลา 24 ชั่วโมงพบว่าบีเซลล์ที่อยู่ในภาวะกระตุ้นนี้สามารถทำหน้าที่เป็นเซลล์นำเสนอแอนติเจนได้เป็นอย่างดีใน MLR และยังสามารถกระตุ้นทีเซลล์ให้ผลิต อินเทอเฟรอน แกมม่า (Interferon gamma, IFN-γ)ได้ในระดับสูง และอินเตอร์ลูคิน-5 (Interleukin-5, IL-5) ในระดับต่ำ โดยสรุป แบคทีเรียที่ทำให้เกิดพยาธิสภาพในเนื้อเยื่อปริทันต์สามารถกระตุ้นบีเซลล์ให้เกิดการเพิ่มขึ้นของการแสดงออกของซีดี86 และซีดี83 ซึ่งจะไปเชื่อมโยงกับการส่งเสริมการทำงานของบีเซลล์ในการนำเสนอแอนติเจน ผลการทดลองจากการศึกษาครั้งนี้สนับสนุนความคิดที่ว่า บีเซลล์ที่แทรกซึมอยู่ในเนื้อเยื่อปริทันต์ อาจมีบทบาทเป็นเซลล์ทำหน้าที่นำเสนอแอนติเจนในการควบคุมและคงสภาพการตอบสนองของทีเซลล์ ณ.บริเวณนั้นในโรคปริทันต์อักเสบ

โรคปริทันต์อักเสบในผู้ป่วยอายุน้อย ได้ถูกอธิบายลักษณะไว้ในรายงานการประชุมเชิงปฏิบัติการทางคลินิกปริทันตศาสตร์เพื่อการวินิจฉัยโรคปริทันต์ของโลกในปี 1989 ว่าเป็นโรคที่เกิดจากการตอบสนองของร่างกายต่อเชื้อแบคทีเรีย ที่เกิดขึ้นในผู้ป่วยอายุน้อยกว่า 35 ปี และมีอัตราการทำลายของโรครวดเร็ว นอกจากนี้โรคปริทันต์อักเสบในผู้ป่วยอายุน้อย ยังได้ถูกแบ่งออกเป็นกลุ่มโรคย่อย ๆ 3 กลุ่มตามอายุที่เกิดโรคคือ โรคปริทันต์อักเสบก่อนเริ่มวัยเจริญพันธุ์ (Prepubertal Periodontitis) ซึ่งเกิดในช่วงอายุก่อนวัยรุ่น โรคปริทันต์อักเสบในผู้เยาว์ (Juvenile Periodontitis) เกิดในช่วงวัยรุ่น และโรคปริทันต์อักเสบลุกลามรวดเร็ว (Rapidly Progressive Periodontitis) ที่เกิดในช่วงอายุ 20-30 ปี แต่อย่างไรก็ตามการแบ่งแยกโรคดังกล่าวยังมีความยุ่งยากในกรณีที่โรคอยู่คาบเกี่ยวระหว่างโรคใดโรคหนึ่ง ตัวอย่างเช่น การแยกโรคโรคปริทันต์อักเสบในผู้เยาว์ลักษณะทั่วไป (Generalized EOP) ออกจากโรคปริทันต์อักเสบลุกลามรวดเร็ว ดังนั้นการปรับเปลี่ยนการแบ่งแยกโรคโดยการใช้เกณฑ์การแบ่งที่ละเอียดมากยิ่งขึ้นจึงถูกนำมาใช้แทน

ตามที่มีการรวบรวมรายงานการศึกษาและสรุปผล ในการประชุมเชิงปฏิบัติการทางคลินิกปริทันตศาสตร์ของโลกในปี 1996 เป็นที่ทราบว่าโรคปริทันต์อักเสบในผู้ป่วยอายุน้อย พบมากในกลุ่มประเทศที่กำลังพัฒนา และในกลุ่มประชากรผิวดำมากกว่าผิวขาว แต่อย่างไรก็ตามยังไม่มีการศึกษาความชุกของโรคในประเทศไทย ดังนั้นการศึกษานี้จึงต้องการศึกษาหาความชุกของโรคปริทันต์อักเสบในผู้ป่วยอายุน้อย ในกลุ่มผู้ป่วยที่เข้ามารักษาในคลินิกของภาควิชาปริทันตวิทยา คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โดยใช้เกณฑ์การแบ่งโรคที่ละเอียดยิ่งขึ้น

จากผู้ป่วยของภาควิชาปริทันตวิทยาทั้งหมดที่ได้รับการตรวจ 1,723 คน พบว่าเป็นผู้ป่วยโรคปริทันต์อักเสบ 446 คน และในจำนวนนี้มีผู้ป่วยโรคปริทันต์อักเสบที่มีอายุน้อยกว่าหรือเท่ากับ 40 ปี

จำนวน 97 คนที่ถูกนำมาศึกษาโดยการใช้ข้อมูลที่ได้การซักประวัติ การตรวจสภาพของอวัยวะปริทันต์ทางคลินิก และการตรวจภาพเอ็กซเรย์ทั้งปาก และให้การวินิจฉัยแยกโรคผู้ป่วยออกเป็น 4 กลุ่มย่อย ตามเกณฑ์ที่ตั้งไว้ดังนี้ กลุ่มที่ 1 : โรคปริทันต์อักเสบในผู้เยาว์ลักษณะเฉพาะที่ (LJP) กลุ่มที่ 2 : โรคปริทันต์อักเสบหลังวัยเยาว์ (Post LJP) กลุ่มที่ 3 : โรคที่คาบเกี่ยวระหว่างโรคปริทันต์อักเสบในผู้เยาว์ลักษณะเฉพาะที่ และโรคปริทันต์อักเสบลุกลามรวดเร็ว (LJP-RPP) และกลุ่มที่ 4 : โรคปริทันต์อักเสบลุกลามรวดเร็ว (RPP) ผลการศึกษาพบว่าความชุกของโรคปริทันต์อักเสบในผู้ป่วยอายุน้อยแบ่งตามกลุ่มย่อย 1, 2, 3 และ 4 เท่ากับร้อยละ 0.29, 0.7, 1.10 และ 0.75 ของผู้ป่วยโรคปริทันต์อักเสบทั้งหมดตามลำดับ หรือกล่าวโดยสรุปว่า ความชุกของโรคปริทันต์อักเสบในผู้ป่วยอายุน้อย จากผู้ป่วยโรคปริทันต์ที่เข้ามารักษาในคลินิกของภาควิชาปริทันตวิทยาทั้งหมดมีจำนวนถึงร้อยละ 2.84 ซึ่งเป็นสัดส่วนที่ค่อนข้างสูง ทั้งนี้อาจเนื่องมาจากเป็นการศึกษาในสถานศึกษา ซึ่งไม่สามารถแสดงถึงความชุกของโรคในกลุ่มประชากรส่วนใหญ่ของประเทศได้ แต่อย่างไรก็ตามการศึกษานี้น่าจะเป็นจุดเริ่มต้นของการศึกษาปัญหาโรคปริทันต์อักเสบในประชากรไทยที่มีอายุน้อย และเป็นข้อมูลพื้นฐานสำหรับการวิจัยในผู้ป่วยกลุ่มนี้ต่อไป และที่สำคัญจะทำให้ทั้งทันตแพทย์ และผู้ป่วยเองได้ตระหนักถึงปัญหาของโรค และตรวจหาหรือพบโรคตั้งแต่ในระยะเริ่มต้น เพื่อสามารถแก้ไขปัญหาได้ทันท่วงที ประหยัดค่าใช้จ่ายและลดความยุ่งยากในการรักษา

การตอบสนองที่มากเกินกว่าปกติ (Hyper-responsiveness) ของโมโนไซต์ที่มีต่อแบคทีเรียไลโปโปลีแซคคาไรด์ (LPS) ได้ถูกตั้งเป็นสมมุติฐานถึงความเกี่ยวข้องกับความรุนแรงของโรคปริทันต์ (Offenbacher และ Salvi, 1999) โดยเฉพาะรายงานที่ว่ากลุ่มผู้ป่วยที่มีความไวต่อการเป็นโรคปริทันต์อักเสบ เช่น ผู้ป่วยโรคปรัทนต์อักเสบที่มีอายุน้อยจะมีสารที่หลั่งจากโมโนไซต์ในระดับสูง เช่น โพรสตาแกลนดิน (Prostaglandins) การศึกษาในครั้งนี้ whole blood assay ได้ถูกนำมาใช้ในการวิเคราะห์ภาวะการกระตุ้นของโมโนไซต์ หลังจากการกระตุ้นด้วย LPS ที่สกัดจาก*พอร์ไฟโรโมแนส จินจิวาลิส* (*P. gingivalis* LPS) ในกลุ่มผู้ป่วยโรคปริทันต์อักเสบที่มีอายุน้อย โดยเปรียบเทียบกับกลุ่มที่มีอวัยวะปริทันต์ที่แข็งแรง (อายุ และเพศตรงกันเป็นคู่) จำนวนกลุ่มละ 17 คน กลุ่มผู้ป่วยโรคปริทันต์อักเสบที่มีอายุน้อยถูกเจาะเลือดในช่วงระยะคงสภาพ (maintenance phase) ใน whole blood culture เมื่อใช้ความเข้มข้นต่างๆ กันของ *P. gingivalis* LPS (1, 3 10 ng/ml) กระตุ้น ผลการแสดงออกของโค-สติมูลาโทรีโมเลกุลบนโมโนไซต์ถูกวิเคราะห์ด้วยโฟลไซโตเมตรี และปริมาณสารที่เกี่ยวข้องกับการอักเสบที่หลั่งออกมาถูกวัดด้วยวิธีอีไลซ่า (ELISA) รวมทั้งการแสดงออกของซีดี69 เครื่องหมายภาวะกระตุ้นในช่วงแรก บนเซลล์เอ็นเค (NK cells) และแกมม่าเดลต้าทีเซลล์ (γδ T cells) ได้ถูกวิเคราะห์เช่นกัน ผลการทดลองพบว่าทั้งสองกลุ่มทดลอง การกระตุ้นด้วย *P. gingivalis* LPS นำไปสู่การเพิ่มขึ้นของการแสดงออกของโค-สติมูลาโทรีโมเลกุล ซีดี40 ซีดี80 ซีดี86 บนโมโนไซต์ การเพิ่มขึ้นของซีดี69 บนเซลล์เอ็นเค และแกมม่าเดลต้าทีเซลล์ ซึ่งการเพิ่มขึ้นนี้เป็นไปตามความเข้มข้นของ *P. gingivalis* LPS โดย

ทั่วไปพบว่า กลุ่มผู้ป่วยโรคปริทันต์อักเสบที่มีอายุน้อยมีการแสดงออกของโมเลกุลเหล่านี้น้อยกว่ากลุ่มที่มีอวัยวะปริทันต์ที่แข็งแรง และความแตกต่างอย่างมีนัยสำคัญพบที่การแสดงออกของซีดี80 บนโมโนไซด์ ส่วนการหลั่งสารโพรสตาแกลนดิน-อี2 (PGE2) และอินเทอร์ลูคิน-1 เบต้า (IL-1β) จากกลุ่มทดลองทั้งสองกลุ่ม พบว่าไม่แตกต่าง โดยสรุปผลการทดลองโดยใช้ whole blood assay ซึ่งน่าจะคล้ายคลึงกับสภาวะในร่างกาย บ่งว่า การตอบสนองที่มากเกินกว่าปกติของโมโนไซด์ ที่มีต่อความรุกรานของแบคทีเรีย ไม่น่าที่จะเป็นตัวกำหนดปัจจัยของการเกิดโรคปริทันต์อักเสบในผู้ป่วยอายุน้อย

I. B CELL STUDY

รอยโรคปริทันต์อักเสบประกอบด้วยเซลล์ที่แทรกซึมจำพวกทีเซลล์, บีเซลล์ และแมคโคฟาส จำนวนมาก ซึ่งเป็นเซลล์ที่อยู่ในภาวะถูกกระตุ้น (Seymour et al., 1993; Seymour 1987) บีเซลล์เป็นเซลล์หลักในรอยโรคปริทันต์ร่วมกับแบคทีเรียที่ทำให้เกิดโรคปริทันต์ชนิดติดสีแกรมลบจำพวก พอร์ไฟโรโมแนส จินจิวาลิส, แอคติโนบาซิลัส แอคติโนไมซิเตมคอมมิแทนส์, แบคทีรอยเดส ฟอร์ไซธัส (Gemmell and Seymour, 1991; Ishikawa et al., 1997) การกระตุ้นบีเซลล์หลายๆ กลุ่มซึ่งถูกชักนำโดยแบคทีเรียที่ทำให้เกิดโรคนั้นถือว่าเป็นกลไกที่เป็นไปได้ของการที่พบบีเซลล์เป็นเซลล์หลักในรอยโรค (Bick et al., 1981; Tew et al., 1989) การศึกษาวิจัยของกลุ่มผู้ร่วมวิจัยเมื่อเร็วๆ นี้ (Champaiboon et al., 2000) ได้แสดงให้เห็นถึงบทบาทพิเศษของไซโตคายน์ อินเตอร์ลูคิน-10 ซึ่งถูกรายงานว่ามีการพบเป็นจำนวนมากในรอยโรคปริทันต์อักเสบ (Aramaki et al., 1998; Salvi et al., 1998; Yamazaki et al., 1997) ในการส่งเสริมอย่างมีนัยสำคัญของการทำให้เกิดการเพิ่มจำนวนบีเซลล์เมื่อได้รับการเสนอพร้อมกับพอร์ไฟโรโมแนส จินจิวาลิส เท่ากับว่าการที่บีเซลล์เป็นเซลล์ส่วนใหญ่ของระบบภูมิคุ้มกันที่มีการแทรกซึมเข้ามาในรอยโรคปริทันต์อักเสบนั้น ยังไม่มีการศึกษาวิจัยถึงความเป็นไปได้อย่างแน่ชัดเกี่ยวกับบทบาทของบีเซลล์ในการดำเนินของโรค

จากการศึกษาวิจัยที่ผ่านมาส่วนมากมักมุ่งความสนใจไปที่บทบาทการควบคุมระบบภูมคุ้มกันของทีเซลล์ต่อการดำเนินของโรค ข้อมูลเกี่ยวกับการตอบสนองของกลุ่มทีเฮลเปอร์เซลล์ (ทีเฮลเปอร์-1 กับทีเฮลเปอร์-2) ในบริเวณรอยโรคที่มีการอักเสบยังคงมีความขัดแย้งกันอยู่ (Gemmell et al., 1997) หากแต่ว่าเป็นที่ยอมรับกันอย่างกว้างขวางเกี่ยวกับความไม่สมดุลย์ของการตอบสนองของทีเซลล์ที่เกี่ยวข้องกับการผลิตไซโตคายน์ แกมม่าอินเตอร์เฟียรอน, อินเตอร์ลูคิน-6, อินเตอร์ลูคิน-10 และอินเตอร์ลูคิน-13 จำนวนมาก ในการดำเนินของโรค (Aramaki et al., 1998, Fujihashi e t al., 1993; 1996; Prabhu et al., 1996) การริเริ่มและการควบคุมการตอบสนองของทีเซลล์ถูกควบคุม โดยกระบวนการเสนอแอนติเจน (Bretscher, 1992) อย่างไรก็ตามการศึกษาวิจัยเกี่ยวกับเซลล์ที่ทำหน้าที่ในการเสนอแอนติเจนในโรคปริทันต์ยังมีการสำรวจไม่เพียงพอ ในอดีตได้เชื่อว่าแลงกาฮานส์เซลล์, เคลลาติโนไซต์ในอิพิเทอเหลียมบริเวณช่องปากเช่นเดียวกับแมคโคฟาสในเนื้อเยื่อเหงือกมีบทบาทเป็นเสมือนเซลล์ที่ทำหน้าที่ในการเสนอแอนติเจนเฉพาะที่ (Crawford et al., 1989; Seymour et al., 1988; Suchett-Kaye et al., 1998; Tonetti et al., 1993; Walsh et al., 1985) ปัจจุบันภาวะที่ถูกกระตุ้นของเซลล์ที่ทำหน้าที่ในการเสนอแอนติเจนเกี่ยวข้องกับการเพิ่มขึ้นของการแสดงโค-สติมูลาโทรี่โมเลกุล (ซีดี40, ซีดี80 และซีดี86) เป็นปัจจัยหลักต่อการก่อเกิดและควบคุมการตอบสนองของที่เซลล์ที่เหมาะสม (Bretscher, 1992; Hathcock et al., 1994; Schwartz,1989) ด้วยเหตุนี้จุดมุ่งหมายของงานวิจัยนี้คือการศึกษาการแสดงออกของโค-สติมูลาโทรี่โมเลกุล และซีดี83 (เครื่องหมายสำหรับเดนได

ติดเซลล์ที่โตเต็มที่)เพื่อนำไปสู่ความเข้าใจต่อเซลล์ที่ทำหน้าที่ในการเสนอแอนติเจน ซึ่งมีบทบาทสำคัญในการกำหนดและสนับสนุนการตอบสนองของทีเซลล์ในรอยโรคปริทันต์อักเสบ

งานวิจัยชิ้นนี้กำลัง *in press* ***ใน*** *the Journal of Periodontal Research* ***และ*** *manuscript* ***รวมทั้งเอกสารอ้างอิงแนบไว้ในภาคผนวก***

II. HUMAN GINGIVAL FIBROBLAST (HGF) STUDY

1. Established 11 HGF lines (2nd report, January 2001)
2. Heterogeneity in terms of different levels of mCD14 expression on HGF were observed and some lines showed no expression of mCD14 (2nd report).
3. Plaque bacterial DNA: *Actinomyces viscosus*, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Bacteroides forsythus* were prepared (2nd report).
4. Expression of co-stimulatory molecules (CD40, CD80 and CD86) and HLA-DR on HGF was analyzed by flow cytometry after stimulation with different palque bacterial DNA. *A. viscosus*, *A. actinomycetemcomitans*, *P. gingivalis*, and *B. forsythus* and *E. coli* DNA did not significantly induce expression of co-stimulatory molecules and HLA-DR on HGF as compared with negative control (3rd report, June 2001).
5. Check the purity of bacterial DNA preparation. *A. viscosus*, *A. actinomycetemcomitans*, *P. gingivalis*, and *B. forsythus* DNA were sent to Thai Can Biotech Co., Ltd. for analysis of LPS contamination by the method of Limulus amebocyte lysate (LAL) assay. The results are shown in the table.

LPS contamination in bacterial DNA preparation

Sample	LPS contamination (EU/ml)
P. gingivalis DNA	9098
A. actinomycetemcomitans DNA	$< 10^6$
A. viscosus DNA	4.3
B. forsythus DNA	1.65

As can be seen, high concentrations of LPS in *P. gingivalis* and *A. actinomycetemcomitans* DNA preparation, the key pathogens of our study, were found. Such concentrations of LPS were too high to remove.

6. Due to the problem of LPS contamination with our bacterial DNA preparation, we decided to check if HGF lines could be activated by CpG ODN. CpG ODN 2006 is well known as potent immunostimulants for human cells.

Methods for CpG ODN 2006 preparation

Modified, Nuclease resistant, phosphorothioate ODN 2006 (TCGTCGTTTTGTCGTTTTGTCGTT) was kindly provided by Dr A.M. Krieg, the Coley Pharmaceutical Group (Wellesley, MA). The ODN 2006 contained four CpG motifs. CpG ODN 2006 was diluted in TE buffer (10 nM Tris-HCl, 1 mN EDTA, pH 8) using pyrogen-free reagents. CpG ODN 2006 preparation was tested for endotoxin using the LAL-assay (BioWhittaker, Walkersville, MD). The lower limit of detection of the LAL-assay in our laboratory was 0.03 EU/ml. Endotoxin level in CpG ODN 2006 preparation was <0.075 EU/ml.

CpG ODN 2006 stimulated HGF culture

10^5 cells/ml of HGF were cultured in 24-well culture plate with RPMI-1640 supplemented with 10% heat inactivated fetal bovine serum, 40 μg/ml gentamycin and 2.5 μg/ml fungizone at 37 °C under humidified atmosphere of 5% CO_2 in air until confluent monolayer had formed. The HGF were treated or untreated with CpG ODN 2006 for 72 h. The culture supernatants were also collected and stored at -80 °C for analysis of IL-6 production.

The results are shown in the following table

Stimulant	IL-6 (pg/ml)
Negative control	866
10 μg/ml CpG ODN2006	829
40 μg/ml CpG ODN2006	829

Due to the negative findings, we therefore consider not to further investigate our HGF study.

III. PREVALENCE OF EOP STUDY

Periodontitis is the disease that presents of gingival inflammation and destruction of the periodontium to the root surface. It has been recognized for the several years that the manifestation of periodontitis differs among patients relative to the age of onset of disease, rate of disease progression, and distribution of periodontal lesions. The consensus report on periodontal diagnosis presented at the 1989 World Workshop in Clinical Periodontics described five distinct groups: Adult periodontitis, Early-onset periodontitis, Periododntitis associated with systemic disease, Necrotizing ulcerative periodontitis and Refractory periodontitis. 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. It was subdivided into three age-related diseases termed Prepubertal periodontitis (disease initiation before puberty), Juvenile periodontitis (disease initiation between puberty and late teenage), and Rapidly progressive periodontitis (disease initiation in the early to late 20s).

Prepubertal periodontitis (PP) described the earliest attachment loss observed around deciduous or permanent teeth. It is quite rare. It occurs in localized and generalized forms, with the generalized form being most frequently associated with systemic disorder for examples Type I diabetes mellitus, Papillon-Lefvre syndrome, Leukocyte adhesion deficiency, hypophosphatasia, etc.

Juvenile periodontitis (JP) could be found in localized and generalized forms. The familial distribution is prominent. The localized form of juvenile periodontitis (LJP) has received considerable attention because of the unique clinical features of molar and incisor lesions, association with the periodontal pathogens *Actinobacillus actinomycetemcomitans* (*Aa*), and abnormalities of neutrophil function. Frequently the LJP patients present good oral hygiene, minimal plaque or gingival inflammation.

Rapidly progressive periodontitis (RPP) is commonly found associated with significant plaque and calculus. Most of the teeth in the RPP patient are affected. The familial distribution is not as prominent. *Porphyromonas gingivalis* rather than *Aa* seems to be predominant in the plaque flora in the RPP group.

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 (Ranney, 1993; Novak and Novak, 1996). 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 (Novak and Novak, 1996) 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.

In 1995, Professor JI Choi from Department of Periodontology, Faculty of Dentistry, Pusan University, Korea asked the former head of the Department of Periodontology, Faculty of Dentistry, Chulalongkorn University for collaborative research regarding epidemiological data of EOP patients in Thailand. Therefore, we carried out the project by screening periodontal patients at Chulalongkorn dental school during 1996-1997.

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. (personal communication) and Potter (1989) 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.

General rules of agreement

1. All criteria are based primarily 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 molars 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).

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

Discussion and Conclusions

As mentioned before, 1989 World workshop in Clinical Periodontics have divided EOP patients into 3 groups (The American Academy of Periodontology, 1989). 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 patients diagnosed of EOP subform I had exact clinical characteristic of LJP pattern but age range was 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 (Armitage, 1999). 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 (prevalence of LJP range from 0.1-15%) (The American Academy of Periodontology, 1996). 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 (Albander, 1997). 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 (Albander, 1997). 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 low. This might be because 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 those results were 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 results 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.

References

1. The American Academy of Periodontology. *Proceedings of the World Workshop in Clinical Periodontics*. Chicago: The American Academy of Periodontology; 1989.
2. Ranney,RR. Classification of periodontal diseases. *Periodontology 2000* 1993; 2: 13-25.
3. Novak,MJ and Novak,KF. Early onset periodontitis. *Curr Opin Periodontol* 1996; 3: 45-58.
4. Papapanou,PN. Periodontal diseases: Epidemiology. In: Annals of Periodontology, 1996 World Workshop in Periodontics. Published by the American Academy of Periodontology 1996:1-36.
5. The Forth National Oral Health Survey, Thailand, 1995 Dental Health Division. Department of Health, Ministry of Public Health published by Dental Health Division.
6. Loe,H and Brown,LJ. Early-onset periodontitis in the United States of America. *J Periodontol* 1991; 62: 608-616.
7. Choi,JI, Kim,JH, Choi,KS, and Kim,SJ. Revision of early-onset periodontitis into homogeneous phenotypic subsets. Submitted for publication.
8. Potter,RH. (1989) Etiology of periodontitis: The heterogeneity paradigm. *J Periodontol* 60:593-597.
9. Armitage,GC. Development of a classification system for periodontal diseases and conditions. *Annal Periodontol* 1999; 4: 1-6.
10. The American Academy of Periodontology. Periodontal diseases of children and adolescents. *J Periodontol* 1996; 67: 57-62.
11. Albander JM, Brown LJ and Loe H. Clinical features of early-onset periodontitis. *J Am Dent Assoc* 1997; 128: 1393-1399.

IV. IMMUNE ACTIVATION IN EOP STUDY

The difference among people in terms of the nature of the immunoinflammatory response is a potential determinant of the variability in the expression of periodontal disease observed within the population. The current concepts of pathogenesis have extended the model from a plaque-dominated process to one which the host response emerges as the dominant effect modifier in disease expression. Recent molecular and cellular data have improved our understanding of the inflammatory and immune mechanisms that result in the destruction of connective tissue, including periodontal ligament and bone. This sequence can be considered a pathway that is common to most forms of disease but is activated or inhibited to varying degrees by various components of the host response and exposure factors. Offenbacher's group (Offenbacher et al., 1993, Salvi et al.,1998, Offenbacher and Salvi, 1999) have been hypothesized that the pathogenic pathway of periodontal disease that links microbial exposure to connective tissue destruction may involve monocytic activation. Patients who develop severe periodontitis such as EOP tend to show hyper-responsiveness of monocytes to secrete inflammatory mediators e.g. Prostaglandins (PG).

Assessing immune cell function or activation *in vitro* is frequently conducted by tedious isolation of peripheral blood mononuclear cells (PBMC) that are exposed to antigens or mitogens and then assessed for blastogenesis or activation such as cytokine production (Pichyangkul at al., 2001). It is now known that such isolated cells are at some degrees of activation before adding into cultures. Early studies in EOP patients have shown the increase PGE2 production from PBMC or adherent monocyte cultures after LPS stimulation as compared with healthy periodontal subjects (Garrison and Nichols, 1989, Shapira et al., 1994, Salvi et al., 1998). At present, a far more convenient alternative to PBMC assays is the whole blood culture, prepared by diluting whole blood with culture media, exposing the culture to the substance of interest, and then conducting immune assays. Indeed, modifications of this technique in many laboratories allow for increased throughput. Here, we investigated an *in vitro* rapid and sensitive human whole blood assay, combined with flow cytometry for assessing the immune activation by *P.gingivalis* LPS on monocytes, NK cells and $\gamma\delta$ T cells and production of inflammatory mediators, IL-1β and PGE2.

Materials and Methods

Subject selection

All subjects in good general health (free of any systemic diseases), with minimum of 20 teeth, were asked to participate in the study. There were 17 pairs of EOP patients and healthy periodontal subjects (age and sex matched). Age range of both groups were 20-40 with mean age of 28. There were 5 males and 12 females in each group. Healthy periodontal group had no or mild gingival inflammation and no history of periodontitis. The EOP group were in maintenance care at the time of their blood donation and showed good clinically healthy gingiva or other wise mild gingivitis on a reduced by stable periodontium. The diseased group had history of EOP with the homogenous subform of severe generalized periodontitis. With the past periodontal illnesses, each patient revealed that at least 30% of the sites were affected with at least 5mm clinical loss of attachment and generalized loss of supporting alveolar bone were evident by periapical radiographs. These patients had already received proper periodontal treatment (hygienic phase and /or corrective phase) at periodontal clinic, Faculty of dentistry, Chulalongkorn University. All participants denied having taken any antibiotic or anti-inflammatory drugs over the last 2 months. The entire study population consisted of Thais.

Porphyromonas gingivalis LPS

Highly purified *P.gingivalis* LPS was a gift from Dr R.E. Schifferle (SUNY at Buffalo, School of Dental Medicine, Department of Oral Biology, NY). LPS was isolated from the phenol-water extract of *P.gingvalis* 381 as described by Schifferle et al. (1989) and separated from polysaccharide by Sephacryl S-400 HR chromatography. To eliminate nucleic acid contamination, the LPS preparation was treated with ribonuclease A (0.02mg/ml, 58 U/mg, Sigma) and to eliminate protein and exogenous nuclease contamination, the LPS preparation was treated with Pronase (0.05mg/ml, 45 U/mg, Calbiochem, San Diego, CA).

Monoclonal antibodies

Monoclonal antibodies (mAbs) used are listed in table below.

MAbs	Photochrome	Populations	Supplier
CD14	PE, PC5	Monocytes	Becton-Dickinson (San Jose, CA)
CD56	FITC, PE	NK cells	Becton-Dickinson (San Jose, CA)
γδ TCR	FITC, PE	T cell subset	Becton-Dickinson (San Jose, CA)
CD40	FITC	Co-stimulatory molecule	PharMingen (San Diego, CA)
CD80	FITC	Co-stimulatory molecule	PharMingen (San Diego, CA)
CD86	FITC	Co-stimulatory molecule	PharMingen (San Diego, CA)
CD69	FITC, PE	Activation antigen	PharMingen (San Diego, CA)
Mouse isotype control	FITC, PE	-	PharMingen (San Diego, CA)

Whole blood assay

10 ml heparinized peripheral blood was obtained from healthy periodontal subjects (n=17) and EOP patients (n=17) (age and sexed matched). The heparinized whole blood was diluted (1:1) with RPMI-1640 mediium containing 2mM L-glutamine, 1mM sodium pyruvate, 80 μg/ml of gentamycin (Gibco Lab, Grand Island, NY). Diluted blood was incubated with various concentration of *P. gingivalis* LPS (0, 1, 3, 10 ng/ml) in 24 wells plate at 37 ^{o}C with 5% CO_2. After 48 h culture, cells were collected for assessment of immune activation by flow cytometry and the supernatants were also collected and stored at -80 ^{o}C for further analysis of IL-1β and PGE2 production.

Flow cytometry

Whole blood cells incubated with *P. gingivalis* LPS at various concentration (0, 1, 3, 10 ng/ml) were aliquoted and then stained for 30 min at room temperature (RT) with one of six mAb combination : 1). mouse isotype control 2). anti-CD14 (PE) and anti-CD40 (FITC) 3). anti-CD14 (PE) and anti-CD80 (FITC) 4). anti-CD14 (PE) and anti-CD86 (FITC) 5). anti-CD56 (PE) and anti-CD69 (FITC) 6). anti-γδ-T cells (PE) and anti CD 69 (FITC)

The stained blood cells were treated with red blood cell lysing solution (FACS Lysing Solution, Becton-Dickinson, San Jose, CA) for 10 min at RT in the dark. The cells were washed in phosphate-buffered saline (PBS) and then reconstituted in1% paraformaldyhyde.

For monocytes and NK, 1,000 cells were analyzed using four-color flow cytometry (FACSCalibur, Becton-Dickinson, Mountain View, CA). CD14+, CD56+ cells and γδ T cellswere gated as monocytes, NK cells and γδ T cells , respectively, and then analyzed for expression of CD40, CD80, CD86 and CD69. Results were expressed as either mean fluorescence intensity (MFI) or percent positive cell.

Measurement of IL-1β and PGE2 production

Supernatant levels of IL-1β and PGE2 were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN). The sensitivity of IL-1β and PGE2 measurement were 1 and 36.2 pg/ml, respectively

Statistical Analysis

Data was analyzed using SigmaStat (Jandel Scientific, San Rafael, CA). The Student's paired *t* test was used for parametric data and the Mann-Whitney rank sum test was used for non-parametric data. P values of 0.05 or less were considered significant.

Results

1. *Dose-response assessment of P.gingivalis LPS-stimulated monocytes in whole blood culture.*

Whole blood cultures were stimulated with different concentrations of *P.gingivalis* LPS (0.32, 1.6, 8, 40, 200, 1,000 ng/ml) for 48 h.The expression of CD86 on monocytes were analyzed by flow cytometry. From the results, the concentrations of *P.gingivalis* LPS at 1, 3, 10 ng/ml were used throughout the experiments (Fig .1).

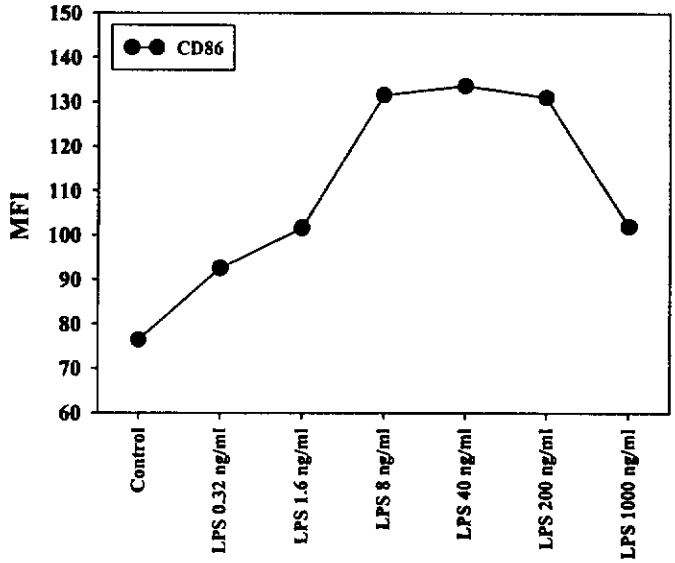


Fig.1 Dose-response assessment of P.gingivalis LPS-stimulated monocytes in whole blood culture.

2. *Comparison of whole blood and PBMC assays for assessment of P.gingivalis LPS - induced cell activation.*

Fig. 2 depicts a comparison of the whole blood versus the PBMC culture assays after incubation with *P.gingivalis*-LPS (1, 3, 10 ng/ml) for 48h. Cells were analyzed for CD40, CD86 expression on monocytes by flow cytometry. This figure shows a representative result from two experiments. Up-regulation of CD40 and CD86 expression on monocytes in whole blood assay was higher than PBVMC assay at all concentrations of *P.gingivalis* LPS used. This data suggest that whole blood assay is more sensitive than PBMC assay.

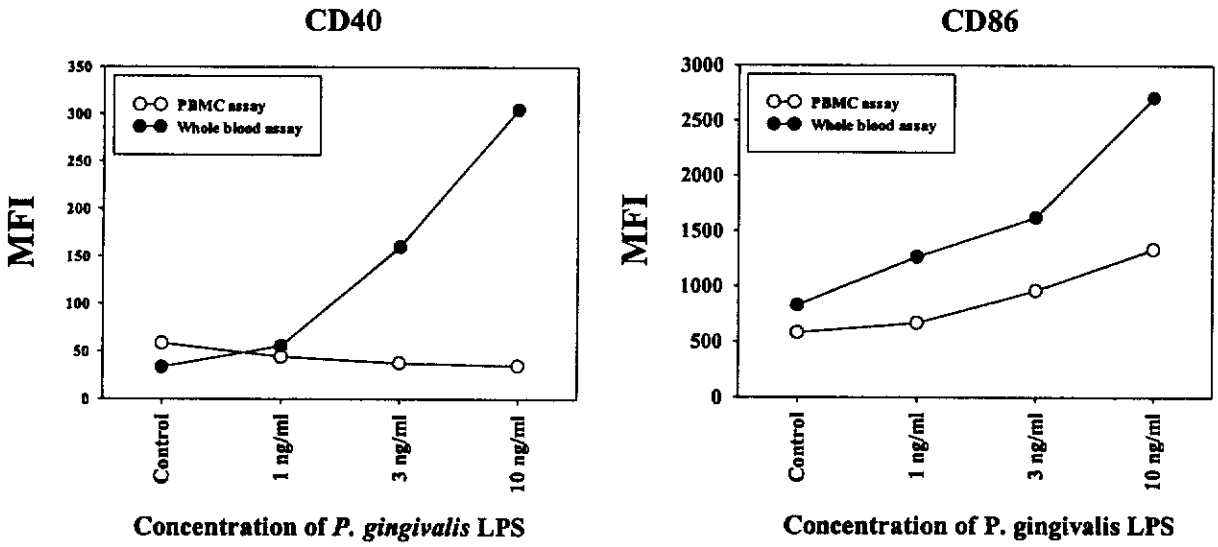


Fig.2 Comparison of whole blood and PBMC assays for assessment of P.gingivalis LPS - induced cell activation.

3. *EOP and healthy periodontal subject blood response after incubation with P.gingivalis LPS.*

Whole blood assays using EOP and healthy periodontal subjects were incubated with *P.gingivalis* LPS at 1, 3, 10 ng/ml for 48 h and then assessed for immune cell stimulation. Each symbol represents an individual blood donor. Horizontal lines are means of MFI (mean fluorescence intensity) (n=17. CD40, CD86 expression on monocytes, CD69 expression on NK cells and γδ T cells in EOP patients was lower than healthy periodontal subjects but no statistically differences was found. CD80 expression on monocytes was significantly lower at all tested *P.gingivalis* LPS concentrations in the EOP group (*$P<0.05$, Student's paired *t*-test) (Fig. 3).

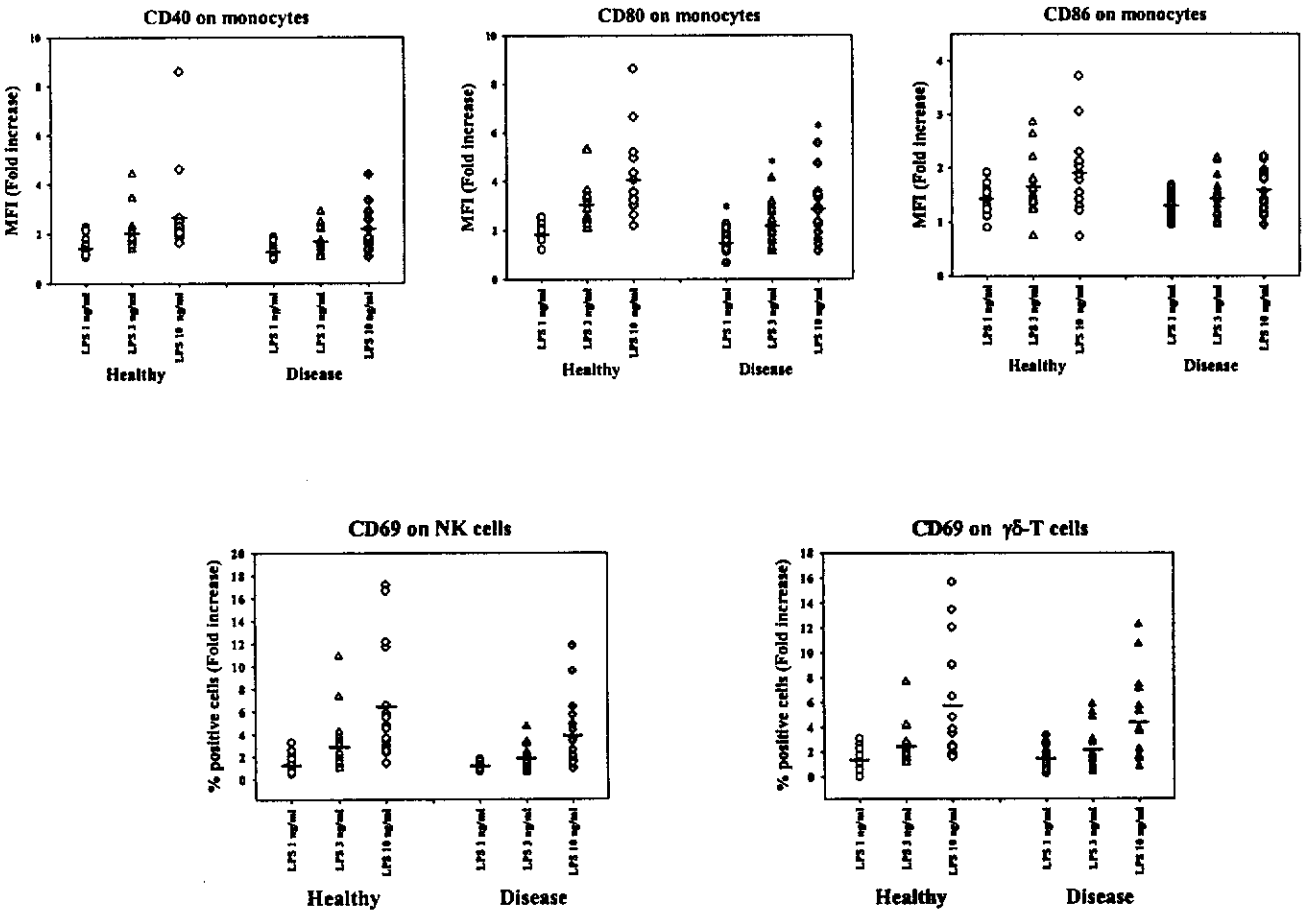


Fig. 3 EOP and healthy periodontal subject blood response after incubation with P.gingivalis LPS. Expression of co-stimulatory molecules (CD40, CD80 and CD86) on monocytes, CD 69 on NK and γδ T cells were measured by flow cytometry.

After 48 hour incubation, culture supernatants were collected and analyzed for IL-1β and PGE2 (n=17). No differences in IL-1β and PGE2 production was observed among EOP patients and healthy controls (Fig. 4).

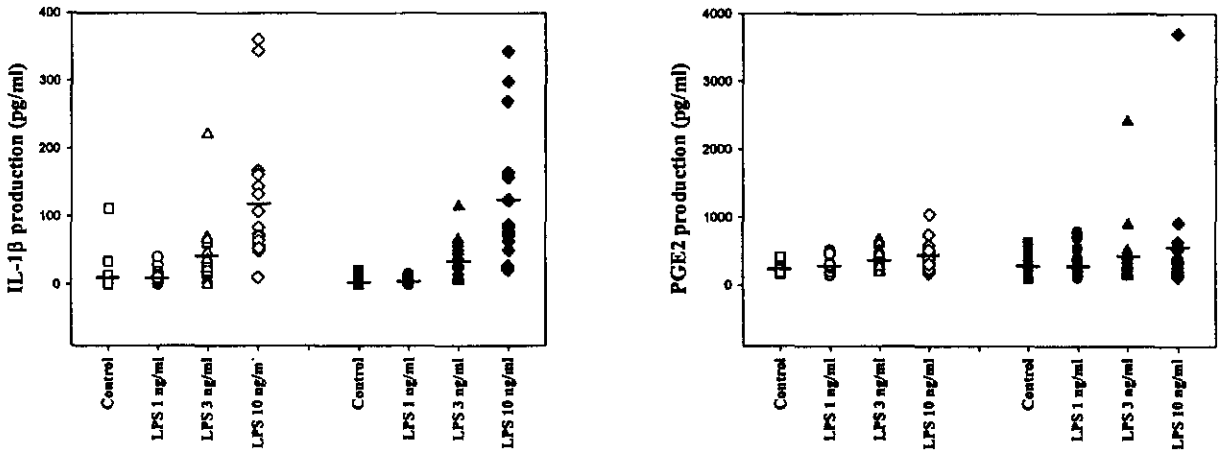


Fig. 4 EOP and healthy periodontal subject blood response after incubation with P.gingivalis LPS. IL-1β and PGE2 production was measured by ELISA.

Conclusions and discussion

Hyperresponsiveness of monocytes to bacterial LPS has been hypothesized by a well known Offenbacher's group to be associated with the pathogenic of early-onset periodontitis patients. Such hyper-responsiveness of monocytes in EOP is hypothesized to be linked with genetic trait. This group reported that monocytes of EOP secreted high levels of inflammatory mediators eg. IL-1β and PGE2 after stimulation with lipopolysaccharide. Their hypothesis has been widely accepted and referred to because it is easy to comprehend that high levels of inflammatory mediators would lead to periodontal tissue destruction.

Based on Offenbacher's hypothesis, we would like to re-evaluate. The experimental design in our study has been improved not only by taking blood from EOP patients in maintenance phase which would reflect more of genetic trait but also using whole blood assay which would represent physiological condition.

Early reports including those from Offenbacher's obtained peripheral blood from EOP for study while they were having active disease. We now know that during active disease, immune cells appear to have certain abnormality changes eg. suppression of T cells, B cells or antigen presenting cells. In order to test the hypothesis, "hyperresponsiveness of monocytes as a genetic trait of EOP", taking blood during active disease should be avoided, and thus in this study peripheral blood was taken for EOP patients during maintenance care.

Whole blood assay was used as a tool to compare and contrast the immune stimulatory effects of *P. gingivalis* LPS on monocytes, NK, γδ T cells in peripheral blood between EOP patients and healthy periodontal subjects. Early studies including those of Offenbacher's used isolated PBMC or adherent monocytes. It is now known that the process involved in PBMC or monocyte isolation (ficoll hypaque and centrifugation) is likely to pre-activate the monocytes. Hence, the old methods are not suitable for the study of monocyte activation as monitored by activation marker. We found that whole blood assays were reliable and convenient and even more sensitive to measure the expression of co-stimulatory molecules on monocytes as compared to PBMC assays for assessing *P. gingivalis* LPS activation.

Our results disagree with Offenbacher's group. We found no difference between EOP patients and healthy periodontal subjects in terms of a). IL-1β production b). PGE2 production c). CD40, CD86 expression on monocytes d). CD69 expression on NK cells e). CD69 expression on γδ T cells. However, the significant difference between the two groups was found on CD80 expression on monocytes. In conclusion we could not observe any hyperresponsiveness of monocytes in EOP patients as monitored by co-stimulatory molecule expression (except for CD80 expression) and inflammatory mediators production. The differences between patients and healthy individual in disease expression are likely to involve different players in immune system including T cells, B cells and antigen presenting cells in response to pathogens and their products. The interaction between suspected periodontal pathogens like *P. gingivalis* with immune cells in periodontal tissue should be accounted for the pathogenesis and clinical significance which remain to be investigated.

Our munuscript is in preparation for submission to international peer reviewed journal.

Reference

1. Offenbacher S, Heasman PA, and Collins JG. Modulation of host PGE_2 secretion as a determinant of periuodontal disease expression. J Periodontol 1993;64:432-44.
2. Salvi GE, Brown CE, Fujihashi K, et al. Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. J Periodontal Res 1998;33:212-25.
3. Offenbacher S, and Salvi GE. Induction of prostaglandin release from macrophages by bacterial endotoxin. Clin Infect Dis 1999;28:505-13
4. Pichyangkul S, Yongvanitchit K, Kum-arb U, et al. Whole blood cultures to assess the immunostimulatory activities of CpG oligodeoxynucleotides. J Immunol Met 2001;247:83.-94.
5. Garrison SW, and Nichols FC. LPS-elicited secretory responses in monocytes: altered release of PGE_2 but not IL-1β in patients with adult periodontitis. J Periodontol Res 1989;24:88-95.
6. Shapira L, Soskolne WA, Sela MN, et al. The secretion of PGE_2, IL-1β, Il-6 and TNF-α by monocytes from early onset periodontitis patients. J Periodontol 1994;65:139-46.
7. Schifferle RE, Reddy MS, Zambon JJ, et al. Characterization of A polysaccharide antigen from *Bacteriodes gingivalis*. J Immunol 1989;143:3035-42.

ภาคผนวก

TITLE: UP-REGULATION OF CO-STIMULATORY MOLECULE EXPRESSION AND DENDRITIC CELL MARKER (CD83) ON B CELLS IN PERIODONTAL DISEASE

Rangsini Mahanonda[1]*, Noppadol Sa-Ard-Iam[1], Kosol Yongvanitchit[2], Mahisorn Wisetchang[3], Isao Ishikawa[4], Toshiyuki Nagasawa[4], Douglas S. Walsh[2] and Sathit Pichyangkul[2]

[1]Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[2]Department of Immunology and Medicine, US Army Medical Component, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand
[3]Dental Department, Siriraj Hospital, Bangkok, Thailand
[4]Department of Periodontology, Faculty of Dentistry, Tokyo Medical and Dental University, Tokyo, Japan

Running title: enhanced co-stimulatory molecules on gingival B cells

***Address correspondence to :**

Rangsini Mahanonda, Ph.D.
Department of Periodontology, Faculty of Dentistry, Chulalongkorn University,
Henry Dunant Rd., Bangkok10330, Thailand.
Tel: 011-662-218-8850
Fax: 011-662-218-8851
E-mail: <mrangsin@chula.ac.th>

Keywords : co-stimulatory molecules, antigen presenting cells, B cells, peridontitis

ABSTRACT

T cells and their cytokines have been well recognized for their important role in the pathogenesis of periodontitis. To date, the role of antigen presenting cells (APCs), which are known to be critical for regulating T cell response, has been poorly investigated in periodontitis. In this study we analyzed the expression of co-stimulatory molecules (CD80 and CD86) and CD83, a marker of mature dendritic cells, on gingival cells isolated from severe periodontitis tissues by flow cytometry. Significant up-regulation of CD86 and CD83 expression were detected in periodontitis lesions and the majority of them were observed on B cells. *In vitro* peripheral blood mononuclear cell cultures showed that stimulation with different periodontopathic bacteria including *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* , *Prevotella intermedia*, and *Actinomyces viscosus* up-regulated both CD86 and CD83 expression on B cells. Hence, the presence of plaque bacteria may be responsible for enhanced expression on gingival B cells as seen *in vivo*. APC function by bacterial activated B cells was further investigated using allogeneic mixed leukocyte reactions (MLR). After 24h culture with either *A. actinomycetemcomitans* or *P.gingivalis*, these activated B cells performed as potent APCs in MLR and stimulated T cells to produce high levels of gamma Interferon (IFN-γ) and minimal Interleukin-5. In conclusion, periodontopathic bacterial induced B cell activation with up-regulation of CD86 and CD83 may associate with enhanced APC function. Therefore, the results of this study put forward the role of infiltrated gingival B cells as possible APCs in regulating and sustaining local T cell response in periodontitis.

INTRODUCTION

Periodontitis lesions consist of large number of infiltrating cells such as T cells, B cells and macrophages which are in an activated stage(1, 2). B cells are the majority in advanced periodontal lesions associated with Gram negative periodontopathic bacteria such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus* (3, 4). Polyclonal B cell activation induced by periodontopathic bacteria has been cited as a possible underlying mechanism of this predominantly B cell lesion (5, 6). Our recent study (7) has demonstrated an additional role of a cytokine IL-10, which has been consistently reported at high levels in periodontitis lesions (8-10), in a significant enhancement of B cell proliferative response when present together with *P. gingivalis*. Although B cells contribute the majority of infiltrated immune cells in periodontitis lesions, there is no study so far able to clarify their role in the pathogenesis of periodontitis.

Major effort during the past decade has been focused on immunoregulatory role of T cells which could be involved in the pathogenesis of periodontitis. Although the Th subset response (Th1 vs Th2) at inflamed gingival tissues is still controversial (11), it is widely accepted that imbalance of T cell response associated with high levels of cytokine production gamma interferon (IFN-γ), IL-6, IL-10, and IL-13 underlies the pathogenesis of periodontitis (8, 12-14). Initiation and maintenance of T cell response is critically controlled by a process of antigen presentation (15). However, the study of antigen presenting cells (APCs) in periodontal disease has been poorly explored. Langerhan's cells, keratinocytes in oral epithelium as well as tissue macrophages have been largely speculated to play role as local APCs (16-20). It is known that activation stage of APCs

associated with up-regulation of co-stimulatory molecule expression (CD40, CD80, and CD86) is a key factor to trigger and maintain optimal T cell response (15, 21, 22). Thus, the aim of the present study is to determine the expression of co-stimulatory molecules as well as CD83, a marker of mature dendritic cells, so as to acquire a better understanding of APCs which are obviously essential in regulating and sustaining local T cell responses in periodontitis lesions.

Our findings provide evidence that enhanced expression of CD86 and CD83 were observed on infiltrated gingival B cells in periodontitis tissues. *In vitro*, periodontopathic bacteria up-regulated expression of CD86 and CD83 on B cells and enhanced their APC function associated with predominant IFN-γ and minimal IL-5 production in allogeneic mixed leukocyte reactions (MLR). Therefore, these results suggest that periodontopathic bacterial activated B cells could serve as possible APCs in periodontitis lesion.

MATERIALS AND METHODS

Medium and monoclonal antibodies

RPMI1640 supplemented with 2 mM L-glutamine, 1mM sodium pyruvate, 80 μ g/ml of gentamycin (Gibco Laboratory, Grand Island, NY) and 10% heat inactivated autologous serum was used throughout the study.

Monoclonal antibodies (mAbs) used in this experiment were either labeled with fluorescein isothiocyanate (FITC) or with phycoerythrin (PE). mAbs against CD19 (FITC), CD14 (PE), and CD56 (PE) were obtained from Becton Dickinson (San Jose, CA). MAbs against CD80 (PE), CD86 (PE) and mouse isotype control mAbs (FITC, PE) were obtained from PharMingen (San Jose, CA). MAb against CD83 (PE) was obtained from Coulter (Miami, FL).

Bacterial preparation

Sonicated extracts of *Porphyromonas gingivalis* FDC-381, *Actinobacillus actinomycetemcomitans* Y4 (ATCC43718), *Prevotella intermedia* ATCC25261 *and Actinomyces viscosus* (clincial isolates from the Department of Microbiology, Chulalongkorn University) were used. *P.gingivalis* and *P. intermedia* were cultured in trypticase soy broth (Sigma Chemical Co., St. Louis, MO) and *A. actinomycetemcomitans* were cultured in brian heart infusion broth at 37°C under anaerobic chamber (Forma Scientific, San Jose, CA). *A.viscosus* were cultured in trypticase soy broth at 37°C in air. The bacteria were harvested by centrifugation (Beckman Instruments, Irvine, CA) at 2060 g for 15 min, washed twice in phosphate-buffered saline (PBS) (Sigma Chemical Co.). The purity was assessed by Gram staining and colony morphology on trypicase soy blood agar (Sigma Chemical Co.) for *P.gingivalis* and *P. intermedia,* and trypticase soy agar for *A. actinomycetemcomitans* and *A.viscosus*. The microorganisms were subjected to sonication with high intensity ultrasonication (High Intensity Ultrasonic Processor, microprocessor controlled 600-Watt Model, Sonic and Material Inc., Danbury, CT) at 4° C for 20 min-elapsed time, with pulse on 2.5 s and pulse off 2 s. The sonicates were examined microscopically for complete breakage of cells. Then, the protein concentration of the organism was determined by using the Bio-Rad protein assay (Bio-Rad, Hercules, CA), and the bacterial stock was aliquoted and stored at -20°C until use.

Gingival biopsies

Gingival tissue biopsies were collected from six patients with generalized severe periodontitis at Dental Department, Siriraj Hospital, Bangkok. Each patient had no history of periodontal treatment for the past 12 months. Most of their teeth showed at least 6mm probing depth with gingival inflammation. Biopsies of gingival tissues attached to the teeth with "hopeless" prognosis due to severe periodontitis were obtained. No other dental diseases such as pulpal disease were involved. Their systemic health was good and no antimicrobial drugs or anti-inflammatory drugs were taken within the past 3 months. The excised tissues were immediately placed in steriled tubes containing RPMI1640 medium, kept on ice and transferred to the laboratory within a few hours for gingival cell extraction.

Gingival cell preparation

The method of obtaining single cell suspensions from gingival tissues was modified from those described by Daly *et al.* (23) and Seymour *et al.* (24). Briefly, the tissues were washed thoroughly in RPMI1640 medium and then they were cut into small fragments (1-2 mm^3). These fragments were incubated in RPMI1640 medium containing 2 mg/ml of collagenase (Sigma Chemical Co.). The ratio of medium+collagenase to tissues was 1 ml:100 mg of tissue. After 90 min of incubation at 37°C, the residual tissue fragments were disaggregated by gentle flushing with pipette several times until single cell suspensions were obtained.

Peripheral blood mononuclear cell (PBMC) culture

Peripheral blood (50 ml) was obtained from healthy donors who presented with either normal periodontal conditions or mild gingival inflammation, but no periodontitis. PBMCs were isolated by Ficoll-Hypaque (Histopaque 1.677, Sigma Chemical Co.).

In a dose response experiment, PBMCs ($3x10^6$ cells/ml) were stimulated with varying concentrations (0, 0.016, 0.4 and 10 μg/ml) of sonicated extracts of *P.gingivalis, A. actinomycetemcomitans, P.intermedia* and *A.viscosus* for 24 h. Cells were then harvested for further flow cytometric analysis.

In kinetic studies, PBMCs ($3x10^6$ cells/ml) were cultured with or without sonicated extracts of *P.gingivalis* (10 μg/ml), or *A. actinomycetemcomitans* (10 μg/ml). The incubation periods were varied from 0, 3, 6, 12, 24 and 48 h. Cells were then harvested for further flow cytometric analysis.

T cell and B cell preparation

T cells were enriched from PBMCs by E-rosetting with neuraminidase-treated sheep red blood cells. T cell fractions were further purified by flow cytometric removal of $CD56^+$ cells (NK), $CD14^+$ cells (monocytes), and $CD19^+$ cells (B cells). B cells were purified from PBMCs by flow cytometric sorting of $CD19^+$ cells. The purity of T cell and B cell preparation were always >98% as reanalysis by flow cytometry.

Flow cytometry

Isolated gingival cell suspensions from periodontitis lesions were aliquoted in 100 μl (10^5 cells) volume and stained with 1 of 4 mAb combinations: 1) anti-CD19 (FITC) + anti-CD80 (PE); 2) anti-CD19 (FITC) + anti-CD86 (PE); 3) anti-CD19 (FITC) + anti-CD83 (PE); 4) anti-CD19 (FITC) + isotype control mAb (PE).

To determine the effect of periodontopathic bacteria on co-stimulatory molecule expression of B cells. Periodontopathic bacterial stimulated PBMCs were aliquoted in 100 μl (10^5 cells) volume and stained with 1 of 3 mAbs combinations: 1) anti-CD19 (FITC) + anti-CD86 (PE); 2) anti-CD19 (FITC) + anti-CD83 (PE); 3) anti-CD19 (FITC) + isotype control mAb (PE). The stained cells in mononuclear cell gate were then analysed using 2-color flow cytometry (FACScan, Becton Dickinson, Mountain View, CA). Results were expressed as % double positive cells.

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.

ACKNOWLEDGEMENTS

The authors thank Professor G.J. Seymour for critical review. This work was supported by Thailand Research Fund.

REFERENCES

1. Seymour GJ, Gemmell E, Reinhardt RA, Eastcott J, Taubman MA. Immunopathogenesis of chronic inflammatory periodontal disease: Cellular and molecular mechanisms. *J Periodont Res* 1993; **28**: 478-486.
2. Seymour GJ. Possible mechanisms involved in the immunoregulation of chronic inflammatory periodontal disease. *J Dent Res* 1987; **66**: 2-9.
3. Gemmell E, Seymour GJ. Phenotypic analysis of B-cells extracted from human periodontal disease tissue. *Oral Microbiol Immunol* 1991; **6**: 356-362.
4. Ishikawa I, Nakashima K, Koseki T et al. Induction of the immune response to periodontopathic bacteria and its role in the pathogenesis of periodontitis. *Periodontol 2000* 1997; **14**: 79-111.
5. Bick PH, Carpenter AB, Holdeman LV et al. Polyclonal B-cell activation induced by extracts of Gram-negative bacteria isolated from periodontal disease sites. *Infect Immun* 1981; **34**: 43-49.
6. Tew J, Engel D, Mangan D. Polyclonal B-cell activation in periodontitis. *J Periodont Res* 1989; **24**: 225-241.
7. Champaiboon C, Yongvanitchit S, Pichyangkul S, Mahanonda R. The immune modulation of B-cells responses by *Porphyromonas gingivalis* and Interleukin-10. *J Periodont* 2000; **71**: 468-475.
8. Aramaki M, Nagasawa T, Koseki T, Ishikawa I. Presence of activated B-1 cells in chronic inflamed gingival tissue. *J Clin Immunol* 1998; **18**: 421-429.
9. Salvi GE, Brown CE, Fujihashi K et al. Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. *J. Periodont Res* 1998; **33**: 212-225.
10. Yamazaki K, Nakajima T, Kubota Y, Gemmell E, Seymour GJ, Hara K. Cytokine messenger RNA expression in chronic inflammatory periodontal disease. *Oral Microbiol Immunol* 1997; **12**: 281-287.
11. Gemmell E, Marshall RI, Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontol 2000* 1997; **14**: 112-143.
12. Fujihashi K, Beagley KW, Kono Y et al. Gingival mononuclear cells from chronic inflammatory periodontal tissues produce interleukin (IL)-5 and IL-6 but not IL-2 and IL-4. *Amer J Pathol* 1993; **142**: 1239-1250.
13. Fujihashi K, Yamamoto M, Hiroi T, Bamberg TV, McGhee JR, Kiyono H. Selected Th1 and Th2 cytokine mRNA expression by CD4(+) T-cells isolated from inflamed human gingival tissues. *Clin Exp Immunol* 1996; **103**: 422-428.
14. Prabhu A, Michalowicz BS, Mathur A. Detection of local and systemic cytokines in adult periodontitis. *J Periodontol* 1996; **67**: 515-522.
15. Bretscher P. The two-signal model of lymphocyte activation twenty-one years later. *Immunol today* 1992; **13**: 74-76.
16. Crawford JM, Krisko JM, Morris GA, Chambers DA. The distribution of Langerhans cells and CD1a antigens in healthy and diseased human gingiva. *Region Immunol* 1989; **2**: 91-97.
17. Seymour GJ, Gemmell E, Walsh LJ, Powell RN. Immunohistological analysis of experimental gingivitis in humans. *Clin Exp Immunol* 1988; 71: 1-6.
18. Suchett-Kaye G, Morrier JJ, Barsotti O. Interaction between non-immune host cells and the immune system during periodontal disease: Role of the gingival keratinocyte. *Crit Rev Oral Biol Med* 1998; **9**: 292-305.

19. Tonetti MS, Schmid J, Hammerle CH, Land NP. Interepithelial antigen presenting cells in the keratinized mucosa around teeth and osseointegrated implants. *Clin Oral Impl Res* 1993; **4**: 177-186.
20. Walsh LJ, Seymour GJ, Powell RN. Gingival keratinocytes express HLA-Dr antigens in chronic gingivitis. *J Oral Pathol* 1985; **14**: 315-321.
21. Hathcock KS, Laszlo G, Pucillo C, Linsley P, Hodes RJ. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J Exp Med* 1994; **180**: 631-640.
22. Schwartz RH. Acquistion of immunologic self-tolerance. *Cell* 1989; **57**: 1073-1081.
23. Daly CG, Clancy RL, Cripps AW. Lymphocytes from chronically inflamed human gingiva. I. Cell recovery and characterization *in vitro*. *J Periodont Res* 1983; **18**: 67-74.
24. Seymour GJ, Cole KL, Powell RN. Analysis of lymphocyte populations extracted from chronically inflamed human periodntal tissues: I. Identification. *J Periodony Res* 1985; **20**: 47-57.
25. Hauschildt S, Kleine B. Bacterial stimulators of macrophages. *Int Rev Cyto* 1995; **161**: 263-331.
26. Krieg AM, Yi AK, Matson S et al. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 1995; **374**: 546-549.
27. Mattern T, Flad HD, Brade L, Rietschel ET, Ulmer AJ. Stimulation of human T lymphocytes by LPS is MHC unrestricted but strongly dependent on B7 interactions. *J Immunol* 1998; **160**: 3412-3418.
28. Kaye PM. Costimulation and the regulation of antimicrobial immunity. *Immunol Today* 1995; **16**: 423-427.
29. Orima K, Yamazaki K, Aoyagi T, Hara K. Defferential expression of costimulatory molecules in chronic inflammatory periodontal disease tissue. *Clin Exp Immunol* 1999; **115**: 153-160.
30. Cella M, Scheidegger D, Plamer-Lehmann K, Lane P, Lanzavecchia A, Alber G. Loagtion of CD40 on dendritic cells triggers production of high level of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med* 1996; **184**: 747-752.
31. Yawalkar N, Brand CU, Braathen LR. IL-12 gene expression in human skin-derived CD1a(+) dendritic lymph cells. *Arch Dermatol Res* 1996; **288**: 79-84.
32. Guery JC, Ria F, Galbiati F, Adorini L. Normal B cells fial to secrete interleukin-12. *Eur J Immunol* 1997; **27**: 1632-1639.
33. Schultze JL, Michalak S, Lowne J et al. Human non-germinal center B cell interleukin (IL)-12 production is primarily regulated by T cell signals CD40 logand, Interferon γ, and IL-10: Role of B cells in the maintenance of T cell responses. *J Exp Med* 1999; **189**: 1-11.
34. Janeway CA, Jr., Ron J, Katz ME. The B cell is the initiating antigen-presenting cell in peripheral lymph nodes. *J Immunol* 1987; **138**: 1051-1055.
35. Constant S, Schweitzer N, West J, Ranney P, Bottomly K. B lymphocytes can be competent antigen-presenting cells for priming CD4+ T cells to protein antigens in vivo. *J Immunol* 1995; **155**: 3734-3741.
36. Constant S, Sant'Angelo D, Pasqualini T et al. Peptide and protein antigens require distinct antigen-presenting cell subsets for the priming of CD4+ T cells. *J Immunol* 1995; **154**: 4915-4923.
37. Lassila O, Vainio O, Matzinger P. Can B cells turn on virgin T cells? *Nature* 1988; **334**: 253-255.

38. Krieger JI, Grammer SF, Grey HM, Chesnut RW. Antigen presentation by splenic B cells: resting B cells are ineffective, whereas activated B cells are effective accessory cells for T cell responses. *J Immunol* 1985; **135**: 2937-2945.
39. Constant SL. B lymphocytes as antigen-presenting cells for CD4+ T cell priming in vivo. *J Immunol* 1999; **162**: 5695-5703.
40. Klinman NR, Press JL. The B cell specificity repertoire: its relationship to definable subpopulations. *Transplant Rev* 1975; **24**: 41-83.
41. Mackler BF, Frostad KB, Robertson PB, Levy BM. Immunoglobulin bearing lymphocytes and plasma cells in human periodontal disease. *J Periodontal Res* 1977; **12**: 37-45.
42. Okada H, Shimabukuro Y, Kassai Y et al. The function of gingival lymphocytes on the establishment of human periodontitis. *Adv Dent Res* 1988; **2**: 364-367.
43. Ogawa T, Tarkowski A, McGhee ML et al. Analysis of human IgG and IgA subclass antibody-secreting cells from localized chronic inflammatory tissue. *J Immunol* 1989; **142**: 1150-1158.

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.

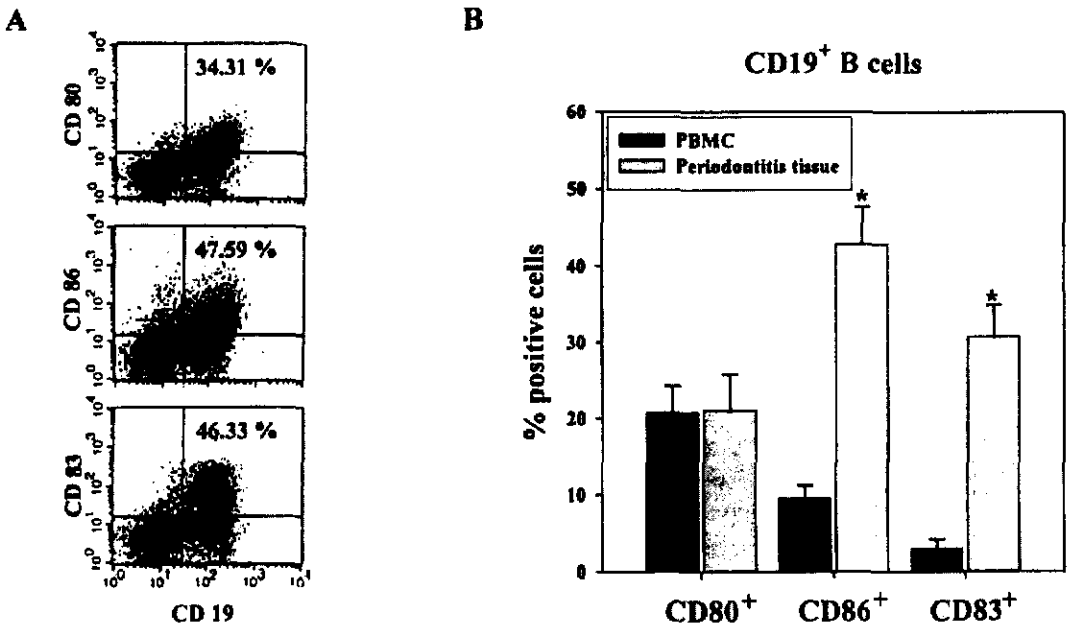

A
34.31 %
47.59 %
46.33 %
CD 80
CD 86
CD 83
CD 19
B
CD19+ B cells
PBMC
Periodontitis tissue
% positive cells
CD80+
CD86+
CD83+

Figure 2.

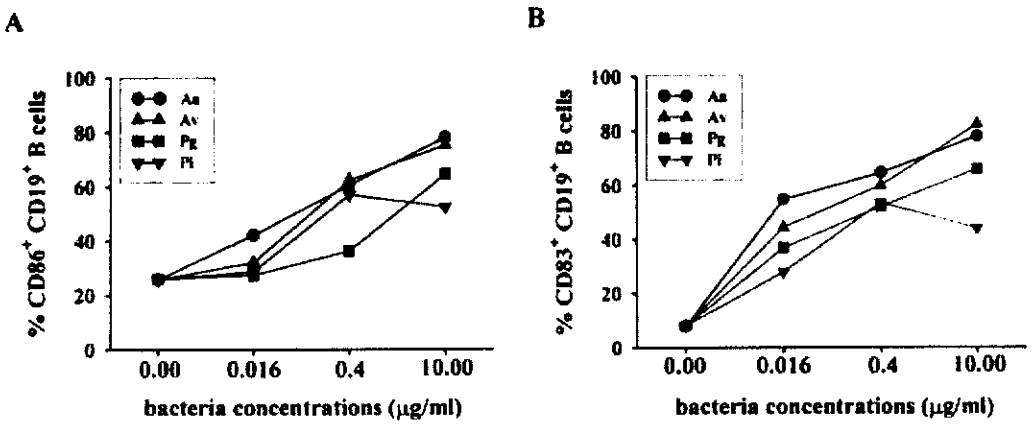

A
B
% CD86+ CD19+ B cells
% CD83+ CD19+ B cells
100
80
60
40
20
0
0.00
0.016
0.4
10.00
bacteria concentrations (μg/ml)
Aa
Av
Pg
Pi

Figure 3.

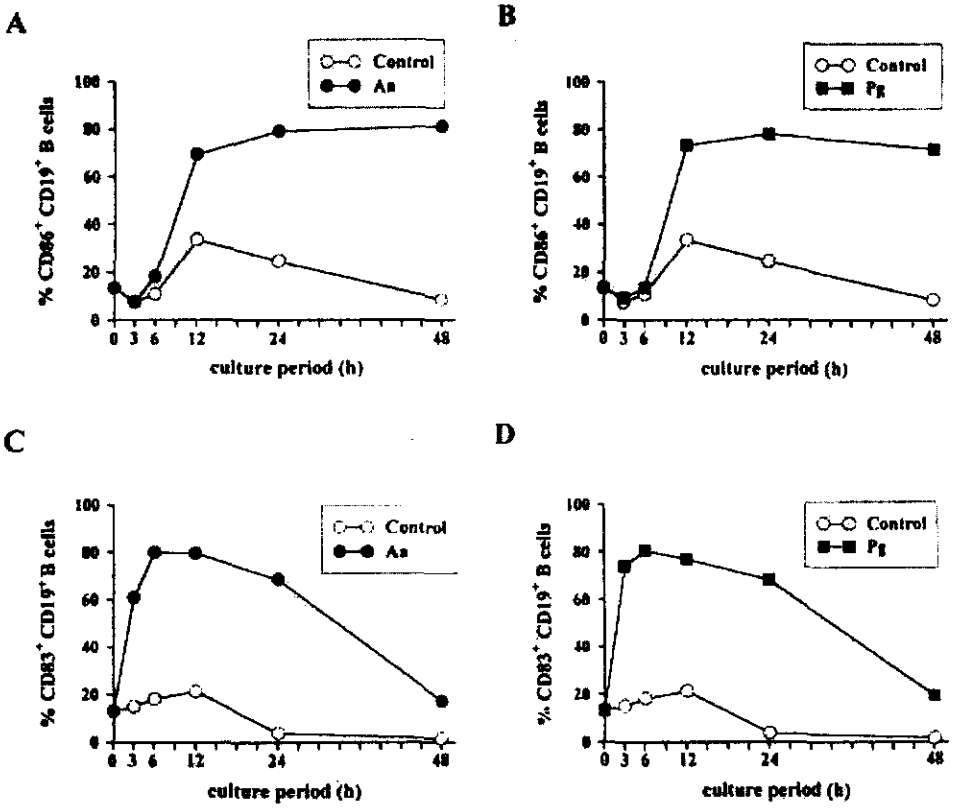
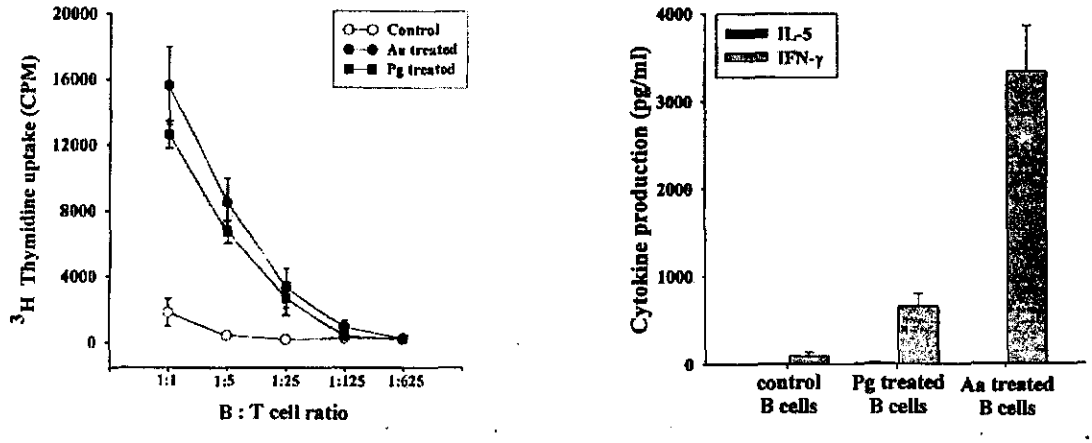

A
B
C
D
Control
Aa
Pg
% CD86+ CD19+ B cells
% CD83+ CD19+ B cells
culture period (h)
0 3 6 12 24 48
0 20 40 60 80 100

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.

O. CHARATKULANGKUN, S. TAMSAILOM and R. MAHANONDA

Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Thailand

Draft manuscript in preparation for submitting Thai Dental Association Journal

This research was supported by the Thailand Research Fund (TRF)

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.

References

1. The American Academy of Periodontology. *Proceedings of the World Workshop in Clinical Periodontics.* Chicago: The American Academy of Periodontology; 1989.
2. Ranney,RR. Classification of periodontal diseases. *Periodontology 2000* 1993; 2: 13-25.
3. Novak,MJ and Novak,KF. Early onset periodontitis. *Curr Opin Periodontol* 1996; 3: 45-58.
4. Papapanou,PN. Periodontal diseases: Epidemiology. In: Annals of Periodontology, 1996 World Workshop in Periodontics. Published by the American Academy of Periodontology 1996:1-36.
5. The Forth National Oral Health Survey, Thailand, 1995 Dental Health Division. Department of Health, Ministry of Public Health published by Dental Health Division.
6. Loe,H and Brown,LJ. Early-onset periodontitis in the United States of America. *J Periodontol* 1991; 62: 608-616.
7. Choi,JI, Kim,JH, Choi,KS, and Kim,SJ. Revision of early-onset periodontitis into homogeneous phenotypic subsets. Submitted for publication.
8. Potter,RH. (1989) Etiology of periodontitis: The heterogeneity paradigm. *J Periodontol* 60:593-597.
9. Armitage,GC. Development of a classification system for periodontal diseases and conditions. *Annal Periodontol* 1999; 4: 1-6.
10. The American Academy of Periodontology. Periodontal diseases of children and adolescents. *J Periodontol* 1996; 67: 57-62.
11. Albander JM, Brown LJ and Loe H. Clinical features of early-onset periodontitis. *J Am Dent Assoc* 1997; 128: 1393-1399.

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.รังสินี 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.