Old Herborn University Seminar Monograph 2:
Interactions between the indigenous microflora and the host immune system.
Editors: Peter J. Heidt, Rodney D. Berg, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 37-47 (2004).

CYTOKINES IN ORAL DISEASE AND MUCOSAL IMMUNE RESPONSES: INTERLEUKIN 6 REGULATION OF IgG AND IgA SYNTHESIS IN NORMAL AND INFLAMED SITUATIONS

YOSHIHARU KONO[*], KENNETH W. BEAGLEY[#], KOHTARO FUJIHASHI[*], JERRY R. MCGHEE[+], MASATOMO HIRASAWA[§], MASAHARU MAKIMURA[§], SHIGEO OTAKE[§], and HIROSHI KIYONO[*]

Departments of [*]Oral Biology, [+]Microbiology and [#]Medicine, Research Center in Oral Biology, The University of Alabama at Birmingham, Birmingham, AL 35294, USA, and [§]Nihon University, School of Dentistry at Matsudo, Matsudo, Chiba 271, Japan

Lecture presented at the 2nd Old Herborn University Seminar on "Interactions between the indigenous microflora and the host immune system" on June 1, 1988

INTRODUCTION

The major antibody isotypes involved in immune responses in the oral cavity are IgG and IgA. The IgG antibodies seen in oral fluids originate from serum via the gingival crevicular fluid (GCF). Thus, the concentration of IgG antibody in GCF is very similar with those seen in serum (~12 mg/ml) (*Shillitoe* and *Lehner*, 1972). Further, some IgA antibodies, especially the monomeric form, are also derived from serum via GCF. On the other hand, predominant IgA antibody seen in oral fluids is either dimeric or larger molecular weight polymers (dIgA or pIgA, respectively) and is produced by plasma cells which reside in the salivary gland (*Mestecky* and *McGhee*, 1987; *Brandtzaeg*, 1988). This antibody isotype is extremely important in immune responses, which occur at mucosal surfaces, since most pathogens and foreign antigens enter the host through these mucous membranes. Mucosal surfaces represent over 400 square meters of surface area in humans which require immune protection (*Mestecky* and *McGhee*, 1987; *Brandtzaeg*, 1988, 1989). Both dIgA and pIgA production by plasma cells occur in mucosal associated tissues including the lamina propria regions of the gastrointestinal, upper respiratory and genitourinary tracts, and exocrine tissues. It has been estimated that greater than 10^{10} plasma cells per meter of intestine occur and approximately 80% of these are committed to the secretion of the dIgA or pIgA isotype (*Brandtzaeg*, 1989). Therefore, it is important to elucidate the exact regulatory mechanisms which modulate IgA responses at mucosal surfaces including those of the oral cavity.

Adult periodontitis (AP) is one of the most common chronic inflammatory diseases which occur in the oral tissues. AP is characterised by a local accumulation of activated B-cells and plasma cells and significant polyclonal immunoglobulin synthesis (*Brandtzaeg*, 1988). When gingival mononuclear cells (GMC) were enzymatically isolated from inflamed tissues of AP, high frequencies of plasma cells were present (*McGhee* et al., 1989a; *Ogawa* et al., 1989a). Further, when Ig producing

Table 1: Effect of cytokines on B-cells

Cytokines	Effects on B-cells
IL-2	Co-stimulation of B-cell growth and differentiation
IFN-γ	Enhances IgM and IgG2a in LPS triggered cultures Suppresses effects of IL-4 on B-cells
TGFβ	Induction of IgA isotype switching Inhibition of B-cell responses
IL-4	Induces B-cell entry into G1 Enhances IgE and IgG1 isotype switching and production in LPS and some antigen-specific B-cell systems Involvement in IgA isotype switching?
IL-5	Induces B-cell proliferation Enhances B-cell differentiation Synergises with IL-2 for B-cell proliferation Enhances IgA synthesis in $sIgA^+$ B-cells
IL-6	Induces terminal differentiation of B-cells committed to all isotypes Significantly enhances IgA synthesis in $sIgA^+$ B-cell subsets

cells were assessed by the enzyme-linked immunospot (ELISPOT) and by immunofluorescence, the GMC contained predominantly IgG followed by IgA and essentially no IgM secreting cells (*McGhee* et al., 1989a; *Ogawa* et al., 1989a). When Ig subclasses were assessed, the IgG profile was IgG1 > IgG2 > IgG3 = IgG4 and for IgA was IgA1 > IgA2 (*Ogawa* et al., 1989a). Other studies using immunofluorescence analysis of sections from gingival tissues have also shown that IgG followed by IgA are the predominant plasma cell isotypes seen in AP (*Killian* et al., 1989). Thus, it is of importance to determine the exact mechanism which regulates production of high IgG and IgA isotype responses seen in inflammatory lesions. Since recent studies with recombinant interleukins (IL) clearly showed that certain interleukins (e.g., IL-4, IL-5 and IL-6) are of particular importance in B-cell responses (*McGhee* et al., 1989b; *Kishimoto* and *Hirano*, 1988), this article summarises our recent findings on the regulation of IgA synthesis by interleukins in both the normal situation and on local production of increased IgG and IgA antibodies seen in inflamed gingiva of AP patients.

INTERLEUKIN REGULATION OF MUCOSAL IMMUNE RESPONSES

The cDNA cloning of cytokines during the past few years has led to the finding that more than one cytokine can induce B-cell responses at the stages of activation, proliferation and differentiation, and that the cytokine may also affect other immunocompetent cells including T-cells and antigen presenting cells (APCs) (*Kishimoto* and *Hirano*, 1988; *Paul* and *O'hara*, 1987). In this regard, IL-4, IL-5 and IL-6 have been shown to be key cytokines for the B-cell

activation, proliferation and differentiation (Table 1). It is generally accepted that IL-4 induces resting B-cells to enter G1, express MHC class II antigens and receptors for other cytokines (*Noelle* et al., 1984; *Rabin* et al., 1985). Activated B-cells respond to IL-5 and undergo cell division, and studies in mice have shown that this cytokine also induces B-cell differentiation and Ig synthesis (*Karasuyama* et al., 1988; *Swain* et al., 1983). However, its precise role in human Ig production remains unclear. IL-6 has been shown to induce B-cells to differentiate into plasma cells, with subsequent high rate Ig synthesis (*Hirano* et al., 1986; *Beagley* et al., 1989). In this regard, IL-6 induced high levels of IgM, IgG and IgA synthesis in mitogen-stimulated human tonsillar B-cell cultures (*Muraguchi* et al., 1988). A significant role in terminal differentiation was also suggested by the finding that anti-IL-6 antibodies, when added to pokeweed mitogen (PWM) stimulated peripheral blood mononuclear cells (PBMC), inhibited Ig synthesis of all isotypes (*Muraguchi* et al., 1988). For convenience, we will discuss major effects of IL-4, IL-5 and IL-6 on B-cell responses, especially for IgA synthesis and refer the reader to more general reviews of these cytokines for the other effects which are seen (*Kishimoto* and *Hirano*, 1988; *Paul* and *O'hara*, 1987).

Switch cytokines for IgA expression

Two important processes occur during the development of IgA producing cells which include the B-cell heavy chain switching to 3' isotypes (e.g., $\mu^+ \rightarrow \alpha^+$) and the terminal differentiation of IgA committed B-cells to high Ig secreting plasma cells. The heavy-chain gene family is present as a long sequence on a single chromosome and consists of exons (specific coding sequences) separated by introns (long stretches of non-coding DNA sequences). In the mouse, the order of heavy-chain constant-region (C_H) genes on chromosome 12 is 5'-μ-δ-γ3-γ1-γ2b-γ2a-ε-α-3'. The C_H gene order on human chromosome 14 is 5'-μ-δ-γ3-γ1-φε-α1-φγ-γ2-γ4-ε-α2-3' (*Cooper*, 1987; *Webb* et al., 1986). After the formation of V_HDJ_H gene in a developing progenitor B-cells for the antigen specificity, a V_HDJ_H-Cμ mRNA transcript is then differentially spliced for synthesis of a functional μ chain and the latter process defines the stage of a pre-B-cell (*Cooper*, 1987). Similarly, light-chain gene rearrangements (V_LJC_L) occur and, together with the V_HDJ_H exon, provide the developing B-cell with precise antigen specificity. This leads to the formation of an IgM molecule, which is inserted into the surface of cell membrane (as sIgM). Surface IgD (sIgD) of the same V_HDJ_H and V_LJC_L specificity results from differential splicing of the VDJCμ-Cδ gene complex (*Cooper*, 1987; *Webb* et al., 1986). Mature $sIgM^+$, $sIgD^+$ B-cells may be induced to switch from the production of IgM to that of any other isotype, e.g., to the expression of Cα-chain gene, a process which is broadly termed isotype switching (*Webb* et al., 1986). Generally, most agree that switching is accomplished by deletion of 5' C_H sequences to those expressed in fully differentiated plasma cells.

The best characterised interleukin which regulate 5' C_H to 3' C_H isotype switching has come from studies with IL-4 in the murine system (*Paul* and *O'hara*, 1987; *Coffman* et al., 1988). IL-4 has been shown to enhance isotype switching of $sIgG^-$ B-cells to sIgG1 bearing B-cells which lead to the enhancement of IgG1 synthesis in LPS-stimulated B-cell cultures (*Lutzker* et al., 1988). Further, IL-4 also acts directly on $sIgM^+$ B-cells for the induction

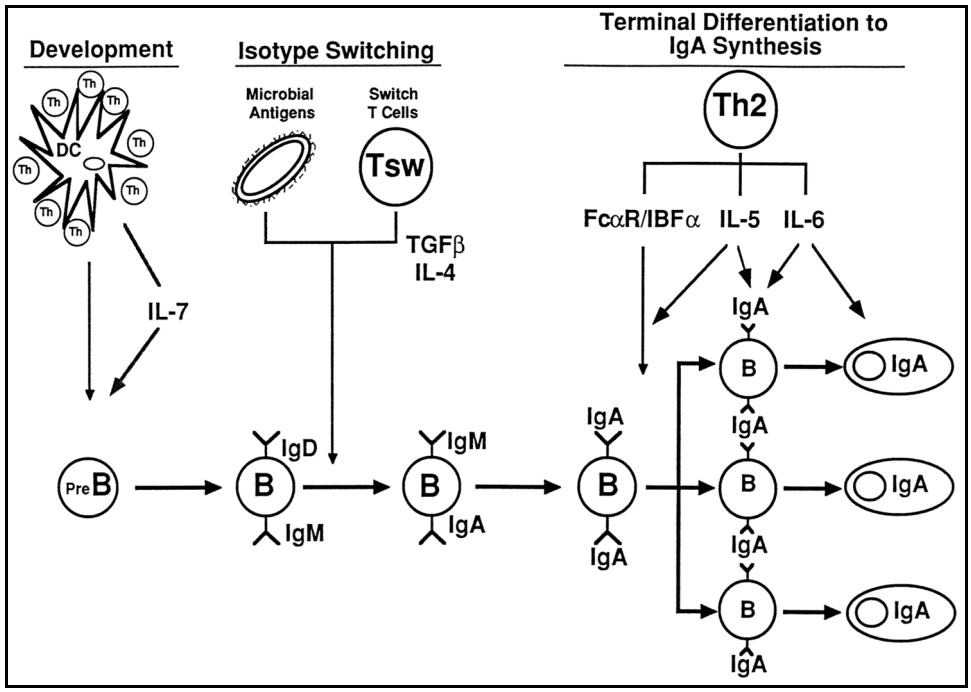

Figure 1: T-cells and cytokines involved in regulation of IgA synthesis.
DC: dendritic cells, Th: helper T-cells, Tsw: switch T-cells.

of switches to $sIgE^+$ B-cells (*Savelkoul* et al., 1988). Evidence for IL-4 involvement in IgA isotype switching has been provided by experiments using the sIgM bearing CH12LX B-cell line (*Kunimoto* et al., 1988). Incubation of CH12LX cells with IL-4 markedly enhanced numbers of $sIgA^+$ cells. On the other hand, IL-5 did not affect these cells. It was suggested that IL-4 induced the switching of $sIgM^+$ B-cells (*Kunimoto* et al., 1988). However, it is also possible that IL-4 may simply be expanding a small population of sIgA bearing cells in CH12LX cell line, since approximately 1% of these cells expresses sIgA. Therefore, it remains to be determined if IL-4 is a key interleukin for the induction of $\mu \rightarrow \alpha$ switching.

Most recent studies have provided evidence that transforming growth factor beta (TGFβ) is a switch factor for IgA (*Coffman* et al., 1989; *Sonoda* et al., 1989). Addition of TGFβ to splenic B-cells triggered with LPS gave 10-fold increases in IgA synthesis (*Coffman* et al., 1989; *Sonoda* et al., 1989). It was also shown that addition of IL-5 to TGFβ-treated cultures markedly enhanced IgA synthesis. These results have presumably suggested TGFβ induced $sIgM^+ \rightarrow sIgA^+$ commitment and IL-5 resulted in terminal differentiation. More definitive evidence for TGFβ-induced $\mu^+ \rightarrow \alpha^+$ switching was suggested by the most recent study that TGFβ induced $sIgA^+$ cells and more of the secreted form of mRNA (s-mRNA) than for the membrane form and that IL-2 enhanced these B-cells to produce more s-mRNA and total IgA synthesis (*Lebman* et al., 1990). Thus, both IL-4 and TGFβ have been shown to induce $sIgM^+$ B-cells to switch to more 3' isotypes including IgA (Figure 1).

Differentiation of IgA committed B-cells to IgA plasma cells by interleukins

It has been shown that murine IL-5 selectively enhances IgA synthesis in purified B-cell cultures (Table 1). The autoreactive T-cell lines produced both IL-4 and IL-5 after antigen stimulation and enhanced IgG1 and IgA synthesis in B-cell cultures co-stimulated with LPS (*Murray* et al., 1987). When purified IL-5 was added to LPS-driven B-cell cultures, enhanced IgA production was seen, and this effect was further augmented by IL-4 (*Murray* et al., 1987). The other studies have shown that supernatants from Th2-cell clones contained IL-5 and enhanced IgA synthesis in LPS-triggered splenic B-cell cultures (*Coffman* et al., 1987). The precise mechanisms for the IL-5 regulation of IgA synthesis was studied in more detail in Peyer's patch (PP) B-cell cultures, since PP is an important IgA inductive site which contain a high frequency of IgA committed B-cells and approximately 30-40 percent of PP B-cells are in cell cycle (*McGhee* et al., 1989b; *Beagley* et al., 1988). The addition of recombinant IL-5 (rIL-5) to PP B-cell cultures resulted in increased synthesis of only the IgA isotype (*Beagley* et al., 1988). The IL-5-induced increase in IgA synthesis was confined to the large blast B-cell subset. Further, the IL-5-induced effect was entirely restricted to the $sIgA^+$ B-cell fraction (*Beagley* et al., 1988). These studies suggest that IL-5 induces $sIgA^+$ B-cells to differentiate into cells secreting IgA. Similar findings were also noted in LPS-driven PP B-cell cultures where IL-5 induced IgA synthesis in LPS-stimulated $sIgA^+$ B-cells but not in $sIgA^-$ B-cells (*Coffman* et al., 1987; *Harriman* et al., 1988). Based on these findings, it is now clear that IL-5 is an important cytokine, which can induce IgA, committed B-cells to become IgA producing cells (Figure 1).

The major cytokine for the regulation of terminal differentiation of B-cells to plasma cells is IL-6 (Table 1), since this interleukin has been shown to induce terminal differentiation of mitogen- or antigen-activated B-cells to Ig secreting cells of all isotypes (*Kishimoto* and *Hirano*, 1988). In addition to the effect of rIL-5 on IgA, rIL-6 also induced significant increases in IgA synthesis in PP B-cell cultures (*Beagley* et al., 1989). In these studies, rIL-6 induced two- or three-fold higher levels of IgA than were seen with rIL-5 (*Beagley* et al., 1989). Further, both rIL-5 and rIL-6 induced significant increases in IgA levels in the large blast B-cell population. When PP B-cells were separated into $sIgA^+$ and $sIgA^-$ B-cell subsets by flow cytometry, removal of $sIgA^+$ B-cells abolished the effect of both rIL-5 and rIL-6 on IgA synthesis (*Beagley* et al., 1989). On the other hand, B-cells enriched for $sIgA^+$ cells and incubated with rIL5 or rIL-6 increased IgA synthesis in a dose-dependent manner; rIL-6 induced two- to fourfold higher levels of IgA synthesis than did rIL-5. Thus, both IL-5 and IL-6 induce $sIgA^+$ blast B-cell subsets to differentiate into IgA-secreting cells (*Beagley* et al., 1989). Since IL-6 induces increased numbers of B-cells which secrete IgA and in higher levels of total IgA synthesis, it is more effective for terminal differentiation than is IL-5. IL-5 and IL-6 both appear to act on B-cells already committed to IgA (Figure 1).

ROLE OF INTERLEUKINS IN ADULT PERIODONTITIS

It is well known that local infiltration of lymphoid cells, including lymphocytes, plasma cells, monocytes/macrophages and neutrophils occur in in-

Table 2: Production of IL-6 by gingival mononuclear cells isolated from patients with adult periodontitis*

Cultures contained			IL-6 activity
Samples (culture supernatants)	Con-A	Anti-IL-6	[O.D. (590-650nm) value x 1.000 ± S.D.]
GMC	-	-	411 ± 65
GMC	-	+	28 ± 13
PBMC	-	-	39 ± 20
PBMC	+	-	198 ± 29
PBMC	+	+	21 ± 14
rIL-6	-	-	395 ± 24
rIL-6	-	+	20 ± 15

*Culture supernatants from GMC or PBMC cultures were measured for IL-6 activity by biological assay using IL-6 dependent MOPC 104E kD 83 cell line. Cultures containing kD 83 cells (5 x 10^3/50 μl) and different dilution of samples (50 μl) were incubated for 48 hr. During the final 4-hr incubation, MTT was added. Isopropanol was then added for the colour development. The intensity of colour was determined by use of a Titertek Multiskan MC photometer.

flamed gingiva of AP patients (*Brandtzaeg*, 1988). These infiltrating lymphoid cells induce abnormal immunological responses in the soft tissue of gingiva, which results in the alveolar bone loss in AP. Thus, AP closely resembles the destructive reactions seen in rheumatoid arthritis (RA). In both cases, the local accumulation of activated lymphoid cells results in the increased production of both polyclonal and antigen-specific antibodies, cytokines and inflammatory mediators at the disease site which lead to the destruction of both connective tissue and bone (*Brandtzaeg*, 1988; *Snyderman* and *McCarty*, 1982). When mononuclear cells were isolated from gingiva of patients with AP, GMC contained high numbers of Ig secreting cells, especially of the IgG and IgA isotypes include their subclasses (*McGhee* et al., 1989a; *Ogawa* et al., 1989a). It was also shown that elevated local IgG and IgA subclass responses occur to the protein antigen of a major AP-associated pathogen *Porphyromonas* (*Bacteroides*) *gingivalis* (*Ogawa* et al., 1989b). Similar findings were derived from the analysis of GCF and gingival homogenates of AP patients (*Ebersole* et al., 1986; *Naito* et al., 1987; *Smith* et al., 1985). In addition, it was also shown that increased level of endogenous antigen, e.g., Type I and II collagen-specific antibody-producing cells were seen in GMC of patients with AP (*Hirsch* et al., 1988). Based upon these observations, it was important to determine the molecular mechanisms, which induce aberrant Ig responses at the local disease site. Thus, the role of cytokines, especially IL-6, in this process was reviewed in this section.

IL-6 in chronic inflammatory disease

The involvement of IL-6 in aberrant immune responses which occur in chronic inflammatory disease was originally described as a B-cell differentiation factor (BCDF) activity in the synovial fluid of RA patients (*Al-Balaghi* et al., 1984). The synovial fluid isolated from RA patients contained BCDF and induced Ig secretion in acti-

vated B-cells and in B-cell lines. Recent studies have formally proven that the synovial fluid of RA patients possesses high levels of IL-6 (*Hirano* et al., 1988). In this study, high concentrations of IL-6 were detected in synovial fluids obtained from the joints of RA patients. Further, the mononuclear cells freshly isolated from synovial fluid constitutively expressed high levels of IL-6 specific mRNA. Excess production of IL-6 in the inflamed joints of RA patients could explain the occurrence of abnormal autoantibody production by mononuclear cells in the inflamed synovia. The dysregulation of IL-6 production is also seen in other diseases. Thus, tumour cells isolated from patients with cardiac myxoma, cervical cancer or bladder carcinomas secrete abnormal amounts of IL-6 (*Kishimoto* and *Hirano*, 1988). This would contribute to the induction of high amounts of autoantibodies and autoimmune-like symptoms seen in these patients. Further, the correlation of excess production of IL-6 and induction of complement-reactive protein, an example of a non-antibody protein which appears in high quantities in serum following the onset of infection or tissue destruction has also been shown in patients with systemic lupus erythematosus (*Swaak* et al., 1989), RA (*Houssiau* et al., 1988), severe burns (*Nysten* et al., 1987) and renal transplantation (*van Oers* et al., 1988).

Involvement of IL-6 in induction of aberrant IgG and IgA synthesis at localised inflamed gingiva

Since GMC from AP patients contain high numbers of IgG and IgA antibody producing cells (*McGhee* et al., 1989a; *Ogawa* et al., 1989a,b), it was important to determine if IL-6 present in these sites account for these responses. In order to examine whether GMC from inflamed tissues produce this cytokine, GMC from AP patients were incubated for 24-72 hr and culture supernatants were then harvested for the assessment of IL-6 activity using the IL-6 dependent MOPC 104E kD 83 cell line (*Kono* et al., 1990). GMC isolated from inflamed tissues of patients with AP spontaneously produce high levels of IL-6. Thus, when freshly isolated GMC were incubated in cultures without any stimulant, high levels of biologically active IL-6 were detected within 24 hr of culture (Table 2). The addition of goat-anti-human IL-6 to GMC culture supernatants resulted in inhibition of IL-6 activity, while the same amount of normal goat serum did not affect IL-6 activity in GMC culture sups (Table 2). This formally proves that GMC spontaneously produces IL-6 (*Kono* et al., 1990). On the other hand, PBMC from AP patients did not secrete IL-6 during a 24-72 hr incubation period. In order to induce IL-6 production, it was necessary to incubate PBMC with T-cell mitogens (e.g., Con A and PHA) (Table 2). Similar findings were also seen at the mRNA level since hybridisation of total mRNA from GMC and PBMC of the same patients with a IL-6 specific cDNA probe resulted in higher levels of IL-6 specific message in GMC but not in PBMC.

Based on the finding that GMC supernatants contained high levels of IL-6, it was important to examine whether GMC produced IL-6 possesses biologically active B-cell stimulatory factor-2 (BSF-2) function. Thus, PBMC isolated from AP patients or normal subjects were incubated in the presence (or absence) of GMC supernatants for 7 days and the cultures assessed for IgM, IgG and IgA secreting cells by the ELISPOT assay. Although GMC supernatants augmented SFC of all 3 isotypes, the major increases were seen with IgG and IgA isotypes (*Kono* et al., 1990). On the other hand, stimulation of

PBMC with pokeweed mitogen (PWM) resulted in a response pattern of IgM > IgG > IgA. Addition of goat anti-IL-6 to GMC sups neutralised BSF-2 activity and completely abrogated spot forming cell (SFC) responses of all isotypes. These findings provide strong evidence that GMC isolated from inflamed gingiva of AP patients spontaneously produce high levels of IL-6 (*Kono* et al., 1990). Further, these results suggest that GMC produced other cytokines in addition to excess amounts of IL-6. One possibility is that GMC produce additional cytokines which induce switching of $sIgM^+$ B-cells to IgG or IgA bearing B-cells and trigger their responsiveness to IL-6. In this regard, it has been shown that malignant T-cells from a patient Rac with mycosis fungoides/ Sezary-like syndrome produce an uncharacterised factor(s) which induce $sIgM^+$ B-cells to switch to IgG and IgA producing cells (*Mayer* et al., 1985). Alternatively, GMC may produce cytokines which activate resting B-cells to become blasts with a $sIgD^-$ phenotype, since previous studies showed that disappearance of sIgD on the B-cell surface could lead to a stage for final maturation into Ig-producing cells (*Kuritani* and *Cooper*, 1982). Further, other studies have provided evidence that IL-6 induces Ig production in $sIgD^-$ activated B-cells (*Cooper*, 1987). Support for this latter possibility was provided by experiments where pre-incubation of normal PBMC cells or purified B-cells with GMC culture sups (even in the presence of anti-human IL-6) induced expression of IL-6R on B-cells (*Kono* et al., 1990). Further, GMC supernatant-treated PBMC were responsive to IL-6, since the addition of rhIL-6 to GMC supernatant pre-treated PBMC resulted in the enhancement of IgG and IgA SFC. These results strongly suggest that GMC produces several cytokines, which may be involved in the process of isotype switching and/or in the induction of IL6R on resting B-cells.

ACKNOWLEDGEMENTS

The authors acknowledge the excellent secretarial help of Ms. Sandra Roberts and the computer drawn figure by Mr. Masahiko Amano. The results described in this article were supported by US PHS grants DE 08228, DE 04217, DE 08182, AI 19674, AI 18958, AI 21032, and AI 28147, and by Nihon University Oota Memorial Research Fund. YK is the recipient of Nihon University Suzuki Memorial Research Fund. HK is the recipient of Research Career Development Award, DE 00237.

LITERATURE

Al-Balaghi, S., Strom, H., and Möller, E.: B cell differentiation factor in synovial fluids of patients with rheumatoid arthritis. Immunol. Rev. 78, 7-23 (1984).

Beagley, K.W., Eldridge, J.H., Kiyono, H., Everson, M.P., Koopman, W.J., Honjo, T., and McGhee, J.R.: Recombinant murine IL-5 induces high rate IgA synthesis in cycling IgA-positive Peyer's patch B cells. J. Immunol. 141, 2035-2042 (1988).

Beagley, K.W., Eldridge, J.H., Lee, F., Kiyono, H., Everson, M.P., Koopman, W.J., Hirano, T., Kishimoto, T., and McGhee, J.R.: Interleukins and IgA synthesis. Human and murine interleukin 6 induces high rate IgA secretion in IgA-committed B cells. J. Exp. Med. 169, 2133-2148 (1989).

Brandtzaeg, P.: Role of immune system - Dan-

gers of a nonholistic approach in explaining health and disease. Periodontology Today (Ed.: Guggenheim, B.). Karger, Basel, 196-208 (1988).

Brandtzaeg, P.: Overview of the mucosal immune system. Curr. Top. Microbiol. Immunol. 146,13-25 (1989).

Coffman, R.L., Shrader, B., Carty, J., Mosmann, T.R., and Bond, M.W.: A mouse T cell product that preferentially enhances IgA production. I. Biologic characterization. J. Immunol. 139, 3685-3690 (1987).

Coffman, R.L., Seymour, W.P., Lebman, P.A., Hiraki, D.D., Christainsen, J.A., Shrader, B., Cherwinski, H.M., Savelkoul, H.F.J., Finkelman, F.D., Bond, M.W., and Mosmann, T.R.: The role of helper T cell products in mouse B cell differentiation and isotype regulation. Immunol. Rev. 102, 5-28 (1988).

Coffman, R.L., Lebman, D.A., and Shrader, B.: Transforming growth factor β specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. J. Exp. Med. 170, 1039-1044 (1989).

Cooper, M.D.: Lymphocytes-B. Normal development and function. N. Engl. J. Med. 317, 1452-1456 (1987).

Ebersole, J.L., Taubman, M.A., Smith, D.J., and Frey, D.E.: Human immune responses to oral microorganisms: Patterns of systemic antibody levels of *Bacteroides* species. Infect. Immun. 51, 507-513 (1986).

Harriman, G.R., Kunimoto, D.Y., Elliott, J.F., Paetkau, V., and Strober, W.: The role of IL-5 in IgA B cell differentiation. J. Immunol. 140, 3033-3039 (1988).

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamitsu, A., Tunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T., and Kishimoto, T.: Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature 324, 73-76 (1986).

Hirano, T., Matsuda, T., Turner, M., Miyasaka, N., Buchan, G., Tang, B., Sato, K., Shimizu, M., Maini, R., Feldmann, M., and Kishimoto, T.: Excessive production of interleukin 6 / B-cell stimulatory factor-2 in rheumatoid arthritis. Eur. J. Immunol. 18:1797-1801 (1988).

Hirsch, H.Z., Tarkowski, A., Miller, E.J., Gay, S., Koopman, W.J., and Mestecky, J.: Autoimmunity to collagen in adult periodontal disease. J. Oral Pathol. 17, 456-459 (1988).

Houssiau, F.A., Devogelaer, J.P., van Damme, J., Nagant de Deuxchaisies, C., and van Snick, J.: Interleukin 6 in synovial fluids and serum of patients with rheumatoid arthritis and other inflammatory arthritis. Arthritis Rheum. 31, 784-788 (1988).

Karasuyama, H., Rolink, A., and Melchers, F.: Recombinant interleukin 2 or 5, but not 3 or 4, induces maturation of resting mouse B lymphocytes and propagates proliferation of activated B cell blasts. J. Exp. Med. 167, 1377-1390 (1988).

Killian, M., Ellegaard, B., and Mestecky, J.: Distribution isotypes including IgA subclasses in adult, juvenile and rapidly progressive periodontitis. J. Clin. Periodontol. 16, 179-184 (1989).

Kishimoto, T. and Hirano, T.: Molecular regulation of B lymphocyte response. Annu. Rev. Immunol. 6, 485-512 (1988).

Kono, Y., Beagley, K.W., Fujihashi, K., McGhee, J.R., Taga, T., Hirano, T., Kishimoto, T., and Kiyono, H.: Cytokine regulation of localized inflammation: Induction of activated B cells and IL-6 mediated polyclonal IgG and IgA synthesis in inflamed human gingiva. J. Immunol. 146, 1812-1821 (1991).

Kunimoto, D.Y., Harriman, G.R., and Strober, W.: Regulation of IgA differentiation in CH12LX B-cells by lymphokines. IL-4 induces membrane IgM-positive CH12LX cells to express membrane IgA and IL-5 induces membrane IgA-positive CH12LX cells to secrete IgA. J. Immunol. 141, 713-720 (1988).

Kuritani, T. and Cooper, M.D.: Human B cell differentiation. II. Pokeweed mitogenresponsive B cells belong to a surface immunoglobulin D-negative subpopulation. J. Exp. Med. 155, 1561-1566 (1982).

Lebman, D.A. and Coffman, R.L.: The effects of IL-4 and IL-5 on the IgA response by murine Peyer's patch B cell subpopulations. J. Immunol. 141, 2050-2056 (1988).

Lebman, D.A., Lee, F.D., and Coffman, R.L.: Mechanism for transforming growth factor β and IL-2 enhancement of IgA expression in lipopolysaccharide-stimulated B cell cul-

tures. J. Immunol. 144, 952 959 (1990).
Lutzker, S., Rothman, P., Pollock, R., Coffman, R., and Alt, F.W.: Mitogen- and IL-4-regulated expression of germ-line Igγ2b transcripts: Evidence for directed heavy chain class switching. Cell 53, 177-184 (1988).
Mayer, L., Posnett, D.N., and Kunkel, H.G.: Human-malignant T-cells capable of inducing an immunoglobulin class switch. J. Exp. Med. 161, 134-144 (1985).
McGhee, M.L., Ogawa, T., Pitts, A. M., Moldoveanu, Z., Mestecky, J., McGhee, J.R., and Kiyono, H.: Cellular analysis of functional mononuclear cells from chronically inflamed gingival tissue. Reg. Immunol. 2, 103-110 (1989a).
McGhee, J.R., Mestecky, J., Elson, C.O., and Kiyono, H.: Regulation of IgA synthesis and immune response by T cells and interleukins. J. Clin. Immunol. 2, 175-199 (1989b).
Mestecky, J. and McGhee, J.R.: Immunoglobulin A (IgA): Molecular and cellular interactions involved in IgA biosynthesis and immune response. Adv. Immunol. 40, 153-254 (1987).
Muraguchi, A., Hirano, T., Tang, B., Matsuda, T., Horii, Y., Nakajima, K., and Kishimoto, T.: The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. J. Exp. Med. 167, 332-344 (1988).
Murray, P.D., McKenzie, D.T., Swain, S.L., and Kagnoff, M.F.: Interleukin 5 and interleukin 4 produced by Peyer's patch T-cells selectively enhance immunoglobulin A expression. J. Immunol. 139, 2669-2674 (1987).
Naito, Y., Okuda, K., and Takazoe, I.: Detection of specific antibody in adult human periodontitis sera to surface antigens of *Bacteroides gingivalis*. Infect. Immun. 55, 832-834 (1987).
Noelle, R., Krammer, P.H., O'hara, J., Uhr, W., and Vitetta, E.S.: Increased expression of Ia antigens on resting B cells: an additional role for B-cell growth factor. Proc. Natl. Acad. Sci. USA 81, 6149-6153 (1984).
Nysten, M.W.N., de Groot, E.R., ten Duis, H.J., Klasen, H.J., Hack, C.E., and Aarden, L.A.: Serum levels of interleukin-6 and acute phase proteins. Lancet ii, 921 (1987).
Ogawa, T., Tarkowski, A., McGhee, M.L., Moldoveanu, Z., Mestecky, J., Hirsch, H.Z., Koopman, W.J., Hamada, S., McGhee, J.R., and Kiyono, H.: Analysis of human IgG and IgA subclass antibody-secreting cells from localized chronic inflammatory tissue. J. Immunol. 142, 1150-1158 (1989a).
Ogawa, T., McGhee, M.L., Moldoveanu, Z., Hamada, S., Mestecky, J., McGhee, J.R., and Kiyono, H.: *Bacteroides*-specific IgG and IgA subclass antibody-secreting cells isolated from chronically inflamed gingival tissues. Clin. Exp. Immunol. 76, 103-110 (1989b).
Paul, W. E. and O'hara, J.: B cell stimulatory factor 1/interleukin 4. Annu. Rev. Immunol. 5, 429-459 (1987).
Rabin, E.M., O'hara, J., and Paul, W.E.: B cell stimulatory factor (BSF)-1 activates resting B cells. Proc. Natl. Acad. Sci. USA 82, 2935-2939 (1985).
Savelkoul, H.F.J., Lebman, D.A., Benner, R., and Coffman, R.L.: Increase of precursor frequency and clonal size of murine IgE-secreting cells by IL-4. J. Immunol. 141, 749-755 (1988).
Shillitoe, E.J. and Lehner, T.: Immunoglobulins and complement in crevicular fluid, serum and saliva. Arch. Oral Biol. 17, 241-247 (1972).
Smith, D.J., Gadalla, L.M., Ebersole, J.L., and Taubman, M.A.: Gingival crevicular fluid antibody to oral microorganisms. III. Association of gingival homogenete and gingival crevicular fluid antibody levels. J. Periodontal Res. 20, 357-367 (1985).
Snyderman, R. and McCarty, G.A.: Analogous mechanisms of tissue destruction in rheumatoid arthritis and periodontal disease. In: Host-parasite interactions in periodontal diseases (Eds.: Genco, R.G. and Mergenhagen, S.E.). American Society for Microbiology, Washington, D.C., 354-361 (1982).
Sonoda, E., Matsumoto, R., Hitoshi, T., Ishi, T., Sugimoto, M., Arai, S., Tominaga, A., Yamaguchi, N., and Takatsu, K.: Transforming growth factor β induces IgA production and acts additively with interleukin 5 for IgA production. J. Exp. Med. 170, 1415-1420 (1989).
Swaak, A.J.G., van Rooyen, A., and Aarden, L.A.: Interleukin 6 (IL-6) and acute phase proteins in the disease course of patients

with systemic lupus erythematosus. Rheumatol. Int. 8, 263-268 (1989).

Swain, S.L., Howard, M., Kappler, J., Marrack, P., Watson, J., Booth, R., Wetzel, G.D., and Dutton, R.W.: Evidence for two distinct classes of murine B cell growth factors with activities in different functional assays. J. Exp. Med. 158, 822-835 (1983).

van Oers, M.H.J., van der Heyden, A.A.P.A.M., and Aarden, L.A.: Interleukin 6 (IL-6) in serum and urine of renal transplant patients. Clin. Exp. Immunol. 71, 314-319 (1988).

Webb, C.F., Burrow, P.D., Borzillo, G.V., and Cooper, M.D.: Normal and abnormal generation of isotype diversity. Monogr. Allergy 20, 1- 12 (1986).