Induction of the immune response to periodontopathic bacteria and its role in the pathogenesis of periodontitis

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Periodontitis results from infection with subgingival plaque-forming bacteria followed by host immune responses. Recent data indicate that Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans and Bacteroides forsythus initiate and progress periodontitis (1). P. gingivalis is mostly found in deep periodontal pockets and especially in active sites (111, 212, 239). A. actinomycetemcomitans is found in pockets from patients with localized juvenile periodontitis as well as advanced adult periodontitis (72). B. forsythus seems to be related to disease activity (46, 235). Progress of the disease is arrested or slowed and the clinical condition is ameliorated by eliminating or decreasing the numbers of these bacteria (187). Bacterial ecology and immune responses in periodontal pockets are complex. Therefore, it is unlikely that only these three bacteria are the causative agents of periodontitis. However, exceeding a particular threshold or a rapid increase in the number of these three types of bacteria are closely related to the breakdown of periodontal tissues. The objective of this chapter is to summarize current understanding of the immune response induced by these bacteria and its role in the pathogenesis of periodontitis.

Critical antigens of P. gingivalis, B. forsythus and A. actinomycetemcomitans in induction of immune responses

Bacterial infection usually occurs with the following stages: attachment to host cells, proliferation of organisms, avoidance of phagocytes and damage to host cells. Host cells produce antibodies that react with bacterial components involved in these stages. We describe these components of P. gingivalis, B. forsythus and A. actinomycetemcomitans, as critical antigens.

P. gingivalis

Since Mouton et al. (154) first reported that patients with adult periodontitis had higher levels of immunoglobulin G (IgG) antibodies to P. gingivalis than control individuals, many investigators have reported that patients with periodontitis have elevated antibody levels to sonicates of P. gingivalis in serum and gingival crevicular fluid. Among the critical antigens of P. gingivalis, the following are the best understood.

Fimbriae. Fimbriae of P. gingivalis are implicated in adherence to gingival tissue surfaces. Yoshimura et al. (267) first purified fimbriae of P. gingivalis 381. Fimbriae are curled, single-stranded filaments with a diameter of 5 nm and a pitch of 33 nm. Fimbrillin is a fimbrial monomer and non-hemagglutinating protein of 43-kDa with an isoelectric point of 6.0. The antigenicity of fimbrillin differs from that of fimbriae (268). The fimA gene, which is the determinant for the major fimbrial subunit protein, has been cloned and sequenced (43). Hamada et al. (80) constructed a fimA mutant of P. gingivalis ATCC 33277 that lacked surface fimbriae and had a diminished adhesive capacity for cultured human gingival
fibA protein is essential for organism to interact with human gingival tissue cells through a function encoded by the fibA gene. Recently, a 72-kDa cell surface protein of P. gingivalis has been identified as fimbriae (Pg-II fimbriae) that differ from those (Pg-I fimbriae) composed of 43-kDa fimbrelin. Seven immunodominant regions within Pg-II fimbrial protein react with the serum of patients with periodontal diseases (166).

Sera from several periodontitis patients tend to react with fimbriae, but not with fimbrelin (266). Sera obtained from patients with adult periodontitis, rapidly progressive periodontitis and gingivitis contain high titers of P. gingivalis fimbria-specific IgG antibodies and lower levels of IgA and IgM. In contrast, sera from localized juvenile periodontitis and normal individuals exhibit much lower titers of P. gingivalis fimbria-specific IgG, IgA and IgM antibodies. Analysis of the fimbriae-specific IgG subclass response has shown that IgG3 is the major antibody subclass produced, followed by IgG1, IgG2 and IgG4 in patients with periodontitis and in normal individuals (163).

Capsular polysaccharide. Bacterial capsules have various functions: physicochemical barriers for the cell, protection against desiccation and resistance to phagocytosis by polymorphonuclear leukocytes. P. gingivalis has a dense, amorphous capsule (approximately 15 nm thick) around the outer membranes, that consists of a polysaccharide heteropolymer (255). Schifferle et al. (203) separated a polysaccharide antigen from a phenol-water extract of P. gingivalis ATCC 33277 for antigenic activity by dot immunoblotting. Most of the antibody binding activity was located in the whole cell protein fraction, with much lower amounts in the lipopolysaccharide and none in the capsular polysaccharide.

Hemagglutinin. A cell-bound hemagglutinating adhesin (HA-Ag2) of P. gingivalis was identified by crossed immunoaffinity electrophoresis to be an antigen common to the species. The non-fimbrial surface protein complex demonstrating erythrocyte-binding capacity, HA-Ag2, is composed of at least two associated polypeptides with apparent molecular masses of 33- and 49-kDa that share at least one antigenic determinant (153). A monospecific antiserum for HA-Ag2 detected a complex of 2 polypeptides with molecular masses of 43- and 49-kDa in an outer membrane preparation of P. gingivalis. All human sera from 8 patients with chronic periodontitis and 6 normal individuals, reacted with one or both polypeptides in at least one of the isotypes (IgG, IgA and IgM) tested, indicating that HA-Ag2 is an immunodominant antigen. This suggests that the mechanism of regulation of the immune response to P. gingivalis in humans is epitope-specific and that it functions via the HA-Ag2 complex (40). Recently, Chandad & Mouton (23) reported a close antigenic relationship between fimbriae and HA-Ag2. Their data indicate that epitopes belonging to fimbriae and HA-Ag2 can be found on specific domains F2 and H, specific for fimbriae and HA-Ag2, respectively, as well as common domains, and that fimbriae and the hemagglutinating adhesin HA-Ag2 may confer hemagglutinating activity upon the cells as a complex.

Lipopolysaccharide. P. gingivalis does not have the lipopolysaccharide typical of other gram-negative bacteria. The lipopolysaccharide of P. gingivalis, which lacks heptose and 2-keto-3-deoxyoctonate, shows very little endotoxic activity in classical endotoxin assays (i.e., Limulus lysate assay or Schwartzman test), although it is significantly mitogenic (135). Farida et al. (59) described serum IgG antibodies to lipopolysaccharides in various forms of periodontal disease. They found that the highest antibody titers to P. gingivalis lipopolysaccharide were associated with localized juvenile periodontitis, but they were significant only against the control group. Anti-lipopolysaccharide IgG antibodies consisted mostly of IgG2 and moderate amounts of IgG1, IgG3 prepared from P. gingivalis ATCC 33277 for antigenic activity by dot immunoblotting.
and IgG4. Periodontitis patients had significantly higher anti-lipoplysaccharide IgG1, IgG2 and IgG3 levels than individuals with a healthy periodontium (198). Lopatin & Blackburn (124) also described that patient antibody titers to P. gingivalis and P. gingivalis-lipopolysacharide were significantly elevated for IgG, IgG1 (no P. gingivalis-lipopolysacharide antibodies) and IgG2 and that IgG2 subclass was responsible for most of the antibody response to P. gingivalis and P. gingivalis-lipopolysacharide.

Enzymes. P. gingivalis produces a wide variety of enzymes which are considered as major virulence factors. Among these, collagenase and trypsin-like protease are very specific to P. gingivalis. Therefore, P. gingivalis can be easily distinguished from other black-pigmented anaerobic rods.

Mayrand et al. (136) reported that oral strains of Bacteroides asaccharolyticus that produce phenylacetic acid (later classified as P. gingivalis) differ from other Bacteroides species in collagenase activity. The collagenase produced by P. gingivalis is thiol dependent, requires metal ions and may degrade collagen fibrils as a result of mutual action with other proteases. Recently, a gene coding for a collagenase gene, prtC, has been isolated from P. gingivalis ATCC 53977 (98). This gene appears to be present in each of the three major serotypes of this organism. The molecular mass of the collagenase is estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to be 35-kDa.

Laughon et al. (117) first described a protease from P. gingivalis as a trypsin-like enzyme. This protease has a similar substrate specificity to that of mammalian trypsin, but differs in terms of thiol dependence, and it is not inhibited by soy-bean trypsin inhibitor (67).

Otagoto & Kuramitsu (173) first isolated the prtT gene, encoding a 53-kDa protease with activity against the synthetic trypsin substrate, from P. gingivalis ATCC 53977. This gene was identified downstream of the collagenase (prtC) gene on a 5.9-kb DNA fragment. The trypsin-like activity of the 53-kDa protease is thiol-dependent. Recently, they revised the sequence of the prtT gene to be larger than previously reported and suggested that the gene encompasses the region encoding hemagglutination (131). The gene product, PrtT, is a 96- to 99-kDa cysteine protease with hemagglutinin.

Gingipain was first reported as a 50-kDa cysteine protease. Three unusual features of this protease are: (a) the stimulation of amidolytic activity by glycine-containing dipeptides; (b) a narrow specificity which is limited to peptide bonds containing arginine residues; and (c) resistance to inhibition by proteinase inhibitors in human plasma (27). Pike et al. (183) isolated proteinases with arginine and lysine specificity from the high-molecular-mass fraction of the P. gingivalis culture fluid. The arginine-specific enzyme (Arg-gingipain) was a high molecular mass form of gingipain, formed by the 50-kDa gingipain noncovalently complexed with 44-kDa binding proteins, which was subsequently identified as hemagglutinins. The 60-kDa lysine-specific proteinase (Lys-gingipain) had one of these hemagglutinins complexed with it in the same manner. Lys-gingipain was a cysteine proteinase with optimal activity and stability at pH 8.0–8.5 and was extensively characterized in terms of its specificity and activation characteristics.

Yoshimura et al. (265) previously described a trypsin-like cysteine protease that also appears to act as a hemagglutinin with human erythrocytes from P. gingivalis 381. The N-terminal amino acid sequence of this 44-kDa protease has been determined not to be homologous to the sequences previously reported. Kadokawa et al. (96) also purified an arginine-specific cysteine proteinase, termed argi- gingipain, from culture supernatants of the same strain. The purified enzyme was found to be composed of a single polypeptide of 44-kDa. The proteolytic activity is thiol-dependent, but the enzyme also has in part the characteristics of both metallo and serine endopeptidases. The enzyme also degrades collagens (types I and IV) and IgG, extensively.

A distinct 64-kDa thiol protease has been cloned and sequenced from P. gingivalis W83 (15). The deduced amino acid sequence of the tpr gene revealed a domain with homology to cysteine protease from both eukaryotes and prokaryotes. The tpr gene coding this protease may be important for the growth of the organism, since the tpr-defective mutant grows very poorly in vitro.

P. gingivalis produces enzymes that degrade most serum proteins, including immunoglobulins and complement components. Fishburn et al. (62) examined the interaction between P. gingivalis culture supernatant and human serum. Hydrolysis of the major serum proteins was thiol-dependent and correlated with the trypsin-like activity of the supernatant. Serum inhibited the trypsin-like activity in a fluorimetric assay independently of the level of the IgG antibody that reacts with whole cells of P. gingivalis. Cutler et al. (37) compared the resistance of invasive and noninvasive strains to phagocytosis by human polymorphonuclear leukocytes with C3- and IgG-proteolytic activity. They found that the more re-
sistant, invasive strains accumulate less 125I-C3 than noninvasive strains. However, invasive strains degrade C3 in a dose-dependent manner, an activity that is inhibited by rabbit antiserum or adult periodontitis serum. They suggested that the C3 and IgG resistant, invasive strains accumulate less 125I-C3 than the organism by conventional culture, other methods have been investigated (144).

Listgarten et al. (123) reported that the organism most frequently found in patients with refractory periodontitis was B. forsythus. Recently, Haffajee et al. (79) examined serum antibody levels against 12 periodontopathic bacteria in 119 individuals with evidence of prior periodontal destruction. Among them, 21% had elevated antibody titers against B. forsythus.

**A. actinomycetemcomitans**

Ebersole et al. (56) reported that localized juvenile periodontitis patients have a higher frequency and level of antibodies to sonicate antigens of A. actinomycetemcomitans compared with all other diseased or normal groups. These findings indicated that antibodies to A. actinomycetemcomitans play an important role in localized juvenile periodontitis patients. Recent studies by Mombelli et al. (143) also identified the importance of these antibodies in patients with severe adult periodontitis.

**Serotype-specific carbohydrate.** A. actinomycetemcomitans is classified into serotypes a, b, c, d and e (191), and serotype-specific carbohydrates have been isolated and purified from the first three serotypes (2). The immunodominant antigen for A. actinomycetemcomitans Y4 (serotype b) is a serotype-specific carbohydrate regardless of antibody titer among reactive patients. The smeared immunodominant antigen of serotypes a and c appears to be a polysaccharide (21). Other investigators reported similar findings (177). Serotype b-specific carbohydrate was originally identified as a polymer consisting of 43.9% l-rhamnose, 49.1% p-fucose and a trace amount of fatty acid (2). A more recent study
has shown that it is an O polysaccharide consisting of trisaccharide repeats of D-fucose, L-rhamnose and D-GalNAc residues (180). Yamaguchi et al. (260) constructed a mutant strain from Y4 and showed that the mutant lacks a serotype-specific carbohydrate and induced intense chemiluminescence responses, whereas chemiluminescence responses of human polymorphonuclear leukocytes to the parent strain were very low. Adding IgG monoclonal antibodies specific for the serotype b-specific carbohydrate significantly enhanced both the chemiluminescence responses to strain Y4 and killing of the organism in the presence of complement, suggesting that the serotype-specific carbohydrate plays an important role in the resistance to host defenses elicited by polymorphonuclear leukocytes. Most of the antibody reactive with the serotype b-specific carbohydrate in patients with early-onset periodontitis is IgG2 (127). In general, IgG2 has low complement fixing ability and it interacts little with Fc receptors. However, Wilson et al. (250) reported that localized juvenile periodontitis serum contains IgG2 antibodies that opsonize A. actinomycetemcomitans, when the antibodies bound to Fc receptors expressed on the neutrophils that recognize this subclass.

**Lipopolysaccharide.** A. actinomycetemcomitans has a lipopolysaccharide that is also found in other gram-negative bacteria. The lipopolysaccharide has a broad spectrum of immunobiological and endotoxic activities, including mitogenic and polyclonal responses of mouse B lymphocytes (B cells), murine macrophage activation, interleukin 1 (IL-1) production and prostaglandin E2 release by murine macrophages, the local Schwartzman reaction and in vitro bone resorption (109). Ebersole et al. (56) determined the IgG and IgM isotypes to sonicate antigen, leukotoxin, group carbohydrate and lipopolysaccharide using an enzyme-linked immunosorbent assay (ELISA). Serum IgM antibody levels to lipopolysaccharide were increased in patients with localized juvenile periodontitis, generalized juvenile periodontitis and adult periodontitis, compared with all other groups. Farida et al. (59) reported that serum IgG antibody titers to lipopolysaccharide from two strains of A. actinomycetemcomitans were significantly elevated in localized juvenile periodontitis compared with other types of periodontal disease and with controls. Saito et al. (193) examined the prevalence of A. actinomycetemcomitans, its serotype distribution and serum immune responses against its surface antigens in 41 Japanese patients with adult periodontitis. Serum IgG titers to extracted lipopolysaccharide and fimbriab antigen of A. actinomycetemcomitans were elevated in the patient sera.

**Leukotoxin.** Baehni et al. (7) first showed that A. actinomycetemcomitans Y4 is cytotoxic for human polymorphonuclear leukocytes. The leukotoxic factor (leukotoxin) of A. actinomycetemcomitans is a protein that migrates at the position of 115 kDa on SDS-PAGE (232), and its isoelectric point is between 8.2 and 8.5 (44). Sera from localized juvenile periodontitis patients consistently (greater than 90%) contain antibodies that neutralize leukotoxin. On the other hand, sera from normal individuals or patients with other types of periodontal disease usually amplify rather than inhibit the leukotoxic reaction (231). Baker & Wilson (8) analyzed sera from localized juvenile periodontitis patients infected with A. actinomycetemcomitans and determined whether IgG antibodies expressing opsonic, bactericidal and/or leukotoxin-neutralizing activity against this organism were present in the serum. The IgG fractions obtained from serum of 3 localized juvenile periodontitis patients with elevated antibody titers to A. actinomycetemcomitans contained opsonic activity against a non-leukotoxic Y4 strain, as well as for a highly leukotoxic JP2 strain. Leukotoxin-neutralizing IgG antibodies did not influence neutrophil killing of the leukotoxic JP2 strain.

**GroEL-like protein (64 kDa).** The 64-kDa protein, to which about half of sera from patients with localized juvenile periodontitis and rapidly progressive periodontitis reacts, was purified from A. actinomycetemcomitans Y4. This protein can be significantly induced by transferring the organism grown at 30°C to a 42°C environment. Determination of the N-terminal sequence of the protein revealed that the identity between the protein and GroEL, a heat shock protein (hsp) of Escherichia coli, is 87% (160). The homology between the protein and human hsp60 is 48%. High serum IgG response to the 64-kDa protein in patients may be predominantly directed toward epitopes of the self heat shock protein as well as the mycobacterial hsp65 involved in human autoimmune arthritis (102). Kirby et al. (106) have investigated the role of the surface-associated material in a saline extract of various periodontopathic bacteria. Surface-associated material from A. actinomycetemcomitans has potent osteolytic activity in murine calvarial bone-resorbing assays. Fractionation of surface-associated material reveals that the activity is associated with a 62-kDa protein,
of which the N-terminal sequence has >95% homology to E. coli GroEL. IgG antibodies that neutralize surface-associated material are found in localized juvenile periodontitis patients (106). The 62-kDa protein appears to be same as the 64-kDa GroEL-like protein (160), since a monoclonal antibody specific for the latter also reacted with the former and inhibited its bone-resorbing activity. These results may indicate that the antibody against the GroEL-like 64-kDa protein play an important role in inhibiting the bone resorption triggered by the organism.

**Fimbriae.** Fresh A. actinomycetemcomitans isolates possess fimbriae of approximately 5 nm in diameter and several μm in length (189). Harano et al. (81) purified fimbrial protein from A. actinomycetemcomitans to obtain anti-fimbria serum. The anti-fimbria serum is an attachment inhibitor that reacts with 54-kDa protein (81). Avidity for fimbriae antigen was significantly higher in high-titer sera from patients without A. actinomycetemcomitans than with A. actinomycetemcomitans in their periodontal pockets. The elicited antibodies against the fimbriae antigen may afford protection against A. actinomycetemcomitans infection (194).

**Protein antigens.** The sonicate of A. actinomycetemcomitans contains 13 major bands (14-78 kDa). A greater proportion of sera from patients with generalized juvenile periodontitis reacts with 40- and 70-kDa antigens compared with sera from localized juvenile periodontitis and controls. In contrast, a lower ratio of sera from patients with localized juvenile periodontitis reacted with the 29-kDa antigen compared with severe periodontitis and controls. The outer membrane proteins contain major 19-, 24-, 35- and 67-kDa antigens that react with sera from all three groups (245). Wilson (249) determined serum IgG antibody levels to the 29-kDa outer membrane protein of A. actinomycetemcomitans Y4 in sera from healthy individuals and patients with localized juvenile periodontitis using ELISA. Geometric mean IgG antibody titers to the 29-kDa protein were significantly higher in sera from the localized juvenile periodontitis patients than in those obtained from periodontally healthy individuals. Twenty-two of 35 localized juvenile periodontitis sera had antibody titers greater than 2 standard deviations from the mean titer of the periodontally healthy group. Wilson & Hamilton (251) also examined the subclass distribution of IgG antibodies reactive with the 16.6- and 29-kDa outer membrane proteins in sera from localized juvenile periodontitis patients and from healthy individuals. The concentration of IgG2 antibody to the 29-kDa outer membrane protein was greater than or equal to the corresponding IgG1 concentration in 7 of 14 high-titer sera, although mean IgG1 and IgG2 concentrations were not significantly different. The concentrations of IgG1 and IgG2 antibodies to the 16.6-kDa protein were also significantly elevated in localized juvenile periodontitis sera, although to a far lesser extent than that to the 29-kDa protein. IgG antibody responses (>70% of the patients) were uniform against 65-, 38-, 29- and 17-kDa protein antigens. Both IgA and IgM specificities reflected those of IgG in each patient. The frequency of responses to outer membrane antigen of >80 kDa and to the 34-, 31- and 24-kDa antigens was positively related to the total IgG antibody levels. Antibody reactive with outer membrane antigen bands at 65-, 38-, 29-, 17-, 15- and 11-kDa antigens is present in patients who have few or several teeth infected with A. actinomycetemcomitans. Furthermore, the frequency of responses to >90-kDa antigens as well as to the 58-kDa antigen was decreased in patients who had several teeth with pockets deeper than 6 mm (50).

**Importance of carbohydrate in contrast to protein and lipid antigens**

In general, the IgG subclass response against infectious organisms is not randomly distributed among the four subclasses. Protein antigens stimulate IgG1 and IgG3 responses, whereas polysaccharide antigens result in IgG1 and IgG2 or mainly IgG2 antibodies.

Total serum IgG2 levels in periodontitis patients have been examined recently. Wilton et al. (254) estimated the levels of the four subclasses of IgG in the serum from chronic periodontitis patients and matched controls. The IgG2 levels were significantly elevated in the patients compared with the controls. Lu et al. (128) also examined distribution of IgG subclass in sera from over 700 periodontitis patients. Serum IgG2 levels increased with age, and this increase was most dramatic around puberty. The high levels of serum IgG2 in localized juvenile periodontitis may be contributing in localizing periodontal destruction. These studies indicate an important role of serum IgG2 in periodontitis patients.

Whitney et al. (248) determined the anti-P gingivalis serum IgG response and avidity and the subclass titer distributions for rapidly progressive periodontitis patients and race-matched healthy controls. The predominant antibody responses for both
patients and controls were IgG2 and IgG3, with a subclass order of IgG2 > IgG3 > IgG1 > IgG4. Similar findings were reported for anti-A. actinomycetemcomitans antibodies by Ling et al. (121). They determined the subclass IgG responses in localized juvenile periodontitis patients and periodontally healthy controls, showing that the mean value for total IgG against the serotype b strain of A. actinomycetemcomitans was more than seven-fold higher for patients and that IgG2 accounted for the major quantitative response in both patients and controls.

The importance of IgG2 antibody against bacteria has been reported in other infectious diseases. Haemophilus influenzae type b is an encapsulated gram-negative bacterium and is the most important pathogen of meningitis in young children. Serum antibody against the H. influenzae type b capsule, a linear polymer of ribosylribitolphosphate, provides protection against the organism. Children with selective deficiency of IgG2 have poor antibody response to H. influenzae type b polysaccharide vaccine, and these children are prone to develop recurrent infections caused by encapsulated bacteria (236).

The cell surface of gram-negative bacteria is usually consisted of a distinctly layered envelope around the cell. The outer membrane is composed of lipopolysaccharide, phospholipids, glycolipid and proteins. The bacteria often synthesize acidic polysaccharide capsule, which may vary in the tightness with which they are associated with the cell surface. The anatomy of the bacteria indicates that most of bacterial components located on the outer cell surface (that is, O-polysaccharide of lipopolysaccharide and capsules) are composed of polysaccharide, except proteinaceous fimbriae. Elevated serum levels of total and bacteria-specific IgG2 indicate that carbohydrate antigens locating on the surface of periodontopathic bacteria are important as a target for the host humoral response.

Local and systemic immune response in periodontal disease

Large numbers of plasma cells accumulate in localized gingival tissues with chronic inflammation, and the host immune system, which produces specific immunoglobulins, is activated by periodontopathic bacteria and their products. Studies throughout the 1970s established that ELISA can detect and measure isotype-specific antibodies to a variety of antigens, including sonicated extracts of periodontopathic microorganisms, cell-surface components and their extracellular products. Many research groups have found that periodontal disease activity or severity is associated with elevated levels of serum antibodies to periodontopathic bacteria by means of ELISA. However, the relationship between local antibody levels and disease status has not been investigated to the same extent. Recent findings regarding local and systemic immunity in periodontal diseases are summarized.

Local immune system

In general, saliva contributes to maintaining health in the oral environment. Salivary IgA is found in large quantities during salivation in the secretory form. Humoral immune responses at the mucosal level are mostly of the IgA isotype. Secretory IgA is an antibody that can traverse mucosal membranes and prevent the entry of infectious microorganisms. IgA consists of IgA1, the predominant subclass in serum, and IgA2, which predominates in the secretory form. Schenck et al. (199) reported that high levels of salivary IgA directed against bacteria in dental plaque, such as A. actinomycetemcomitans, P. gingivalis and Streptococcus mutans, might protect against the development of gingivitis. Sandholm et al. (196) measured the concentrations and levels of salivary IgG or IgA antibodies to A. actinomycetemcomitans by ELISA and found a significantly increased concentration of salivary IgG in patients with moderate or severe adult periodontitis. Although the level of salivary IgA was less influenced by periodontal conditions, the level of salivary IgG antibody to A. actinomycetemcomitans was significantly elevated in patients with juvenile periodontitis. The above results suggest that salivary IgA and IgG antibodies have potential for diagnostic or predictive aspects of disease assessment. However, several periodontopathic bacteria produce proteases that degrade IgG and IgA1 (63, 64, 103, 197). Recently, Gregory et al. (73) reported evidence of IgG, IgA and IgM proteolytic activity in A. actinomycetemcomitans, indicating an important role of immunoglobulin proteolytic enzymes in the etiology of localized juvenile periodontitis.

The level of antibody to periodontopathic bacteria in gingival crevicular fluid is not significantly correlated with periodontal conditions. Baranowska et al. (9) found no significant difference in the level of specific IgG to P. gingivalis in gingival crevicular fluid between healthy and disease sites. Furthermore, the
clinical parameters of sites with elevated or normal levels of antibody to periodontopathic bacteria, such as *A. actinomycetemcomitans* and *P. gingivalis*, do not obviously differ (228). Kinane et al. (105) measured the specific IgG, IgA and IgM antibody titers to *P. gingivalis* and *A. actinomycetemcomitans* in serum and gingival crevicular fluid by ELISA. They found that serum antibody levels to *P. gingivalis* are elevated in periodontitis patients compared with healthy individuals, whereas gingival crevicular fluid IgG levels against *A. actinomycetemcomitans* do not significantly differ among sites with several types of periodontitis (105).

Systemic immune system

To date, much has been published describing specific immune responses in periodontal diseases identified by ELISA (52). In particular, the levels of serum antibodies to *I. gingivalis* and *A. actinomycetemcomitans* have been intensively studied among patients with periodontitis and normal individuals. Tables 1 and 2 summarize some of the recent studies on serum antibody levels to *A. actinomycetemcomitans* and *P. gingivalis*. We (89, 115) studied the diversity of humoral antibody responses against *P. gingivalis* and *A. actinomycetemcomitans* in patients with various types of periodontitis. Severe adult and rapidly progressive forms of periodontitis are often related to *P. gingivalis* (239) and are associated with high serum antibody titers against this anaerobic bacterium (54, 154). We (16) recently characterized the immunodominant antigens of *P. gingivalis* 381 in high-responder patients. Although various antigenic components of *P. gingivalis* have been characterized by many investigators (110, 269), little information specifically describes patients with a high antibody response to *P. gingivalis*. In our study, the fimbrillin and the 43 kDa protein were the immunodominant antigens of *P. gingivalis* 381. On the other hand, others found that the 69, 48, 46, 43 and 41 kDa antigens are predominant (18, 41, 100, 157, 233). Further studies are needed to clarify the roles of the immunodominant antigens of *P. gingivalis* during its trans-

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<th>Table 1. Serum antibody levels against <em>A. actinomycetemcomitans</em>&lt;sup&gt;a&lt;/sup&gt;</th>
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<td><strong>Diagnosis and antigen preparation</strong></td>
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<tr>
<td>Papillon-Lefrere syndrome</td>
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<td>Prepubertal periodontitis</td>
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<td>Localized juvenile periodontitis</td>
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<td>Leukotoxin, lipopolysaccharide</td>
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<td>B-specific carbohydrate&lt;sup&gt;e&lt;/sup&gt;, lipopolysaccharide</td>
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<td>Generalized juvenile periodontitis</td>
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<td>Leukotoxin, lipopolysaccharide</td>
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<td>Rapidly progressive periodontitis</td>
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<td>B-specific carbohydrate&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>Adult periodontitis</td>
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<td>Sonicates (serotype c)</td>
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<td>Whole cells or sonicates</td>
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<td>Leukotoxin, lipopolysaccharide</td>
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<td>Mixed periodontitis groups</td>
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<td>Sonicates</td>
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<sup>a</sup> Serotype b strains were used in most of the studies.
<sup>b</sup> Serum antibody levels were compared with those of healthy individuals.
<sup>c</sup> In most studies, seropositive is defined as levels >2 standard deviations above the mean of healthy individuals.
<sup>d</sup> IgG antibody levels were elevated, and some investigators have reported elevated levels of IgA or IgM.
<sup>e</sup> Serotype b-specific carbohydrate antigen.
Table 2. Serum antibody levels against P. gingivalis

| Diagnosis and antigen preparation | Serum titer | % IgG seropositive
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<td></td>
<td>Elevated</td>
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<td>Localized juvenile periodontitis</td>
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<td>Whole-cell extract</td>
<td>IgA, M</td>
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<tr>
<td>Whole-cell extract</td>
<td>IgG, A, M</td>
<td>165</td>
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<tr>
<td>Fimbriae</td>
<td></td>
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<tr>
<td>Generalized juvenile periodontitis</td>
<td>IgG</td>
<td>IgM, A</td>
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<tr>
<td>Whole-cell extract</td>
<td>IgA</td>
<td>IgG, M</td>
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<td>Fimbriae</td>
<td>IgG, A, M</td>
<td>165</td>
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<tr>
<td>Rapidly progressive periodontitis</td>
<td>IgG (A, M)^e</td>
<td>24, 53, 154, 229, 248</td>
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<tr>
<td>Whole cells or sonicates</td>
<td>IgG, A, M</td>
<td>165</td>
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<td>Whole-cell extract</td>
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<td>Whole cells or sonicates</td>
<td>IgG (A, M)^e</td>
<td>53, 76, 229</td>
</tr>
<tr>
<td>Whole-cell extract</td>
<td>IgG</td>
<td>IgM, A</td>
</tr>
<tr>
<td>Fimbriae</td>
<td>IgG, A, M</td>
<td>154</td>
</tr>
<tr>
<td>Mixed periodontitis groups</td>
<td>IgG (A, M)^e</td>
<td>5, 39, 86, 124, 146, 158, 159</td>
</tr>
<tr>
<td>Whole cells or sonicates</td>
<td>IgG</td>
<td>IgM, A</td>
</tr>
<tr>
<td>Lipopolysaccharide Sonicates</td>
<td>IgG</td>
<td>IgG</td>
</tr>
<tr>
<td>Recurrent periodontitis</td>
<td></td>
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<tr>
<td>Sonicates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gingivitis</td>
<td>IgG, A, M</td>
<td>165</td>
</tr>
</tbody>
</table>

* Serum antibody levels were compared with those of healthy individuals.
* In most studies, seropositive is defined as levels >2 standard deviations above the mean of healthy individuals.
* IgG antibody levels were elevated, and some investigators have reported the elevated levels of IgA or IgM.

mission to elucidate the epidemiology of severe adult periodontitis. We (86) also measured serum IgG antibody titers to periodontopathic bacteria in patients with adult and rapidly progressive periodontitis at the first visit and after various types of periodontal treatment with clinically successful improvement. ELISA results showed that the mean antibody titer to P. gingivalis and Prevotella intermedia significantly decreased after treatment. In particular, the antibody titer to P. gingivalis decreased in all patients examined. These findings suggest that changes in the serum IgG titers against P. gingivalis and P. intermedia are related to suppression of the growth of such pathogens in subgingival plaque.

**Functional properties of antibodies against periodontopathic bacteria**

The first stage in any infection involves adhesion of the microorganisms to a host tissue, directly or via some intermediary microorganisms (253). Local and systemic antibodies function in antibacterial immunity through participation in several mechanisms, such as microorganism aggregation and the inhibition of adherence and colonization by microorganisms (Fig. 1). The next event in the pathogenesis of most bacterial infectious diseases is the microbial invasion of host cells. In fact, some microorganisms can invade the tissue of the periodontium after colonization by periodontopathic bacteria. Sagle et al. (192) detected antigens from A. actinomycetemcomitans in gingival tissue from patients with severe periodontitis. Many bacterial species can invade eukaryotic cells. It has been believed that bacteria enter cells through the mechanism of phagocytosis. Meyer et al. (141) and Sreenivasan et al. (214) developed an in vitro cell culture invasion model of A. actinomycetemcomitans and provided evidence that this organism invades human epithelial cells. They also demonstrated that this invasion occurs through a cytochalasin D and cycloheximide-sensitive process. Recently, Kato et al. (97) showed that the death of macrophages induced by A. actinomycetemcomitans infection occurs through
Iskiknwa et al.

Fig. 1. Functional properties of antibodies against *A. actinomycetemcomitans*. In terms of defensive function of antibody: 1. Inhibition of adhesion and invasion. 2. Complement activation. 3. Neutralization of leukotoxin. 4. Opsonization and phagocytosis. Proteases produced by *A. actinomycetemcomitans* cleave IgG, IgA and IgM (5).

Apoptosis and that this organism must gain entry into these cells to induce this mode of killing. Furthermore, serum antibody enhances phagocytosis and lyses bacteria. It is believed that this antibody helps prevent infection and promotes recovery from periodontal diseases (Fig. 1).

In the last two decades, many investigators have been involved with the immunological studies focused on a humoral immune response of patients with localized juvenile periodontitis and rapidly progressive periodontitis. Page et al. (178) reported that some patients respond by producing serum antibodies against periodontopathic bacteria during the periodontal infections, whereas others do not. It is hard to explain this phenomenon because of the complicated subgingival microflora and existence of numerous numbers of cross-reactive bacterial antigens in periodontal pockets. Under the circumstances, they speculate that upon subclinical infection with periodontopathic bacteria, subjects resistant to periodontitis may produce sufficient levels of high-avidity antibody to clear the bacteria. On the contrary, under the same conditions, susceptible subjects may mount no humoral immune response or may produce antibodies of low avidity that do not have the capacity to prevent the onset of periodontitis, allowing clinical disease to appear and to progress.

**Serum immunoglobulin subclass distribution**

The relative amounts of specific IgG subclass isotypes produced during the antibody response depend on the nature of the antigen. Bacterial protein antigens induce mostly IgG1 antibodies in humans and low levels of IgG3 and IgG4. In contrast, IgG2 subclass predominates in response to bacterial polysaccharide antigens and lipopolysaccharide (213). In general, antibodies against bacterial cell-surface components are beneficial to host defense. The four IgG subclass isotypes (IgG1, IgG2, IgG3 and IgG4) have various defensive features, including opsonic activity, complement activation and toxin inactivation (8, 185, 231). Several investigators have described the subclass distribution of serum IgG antibodies against *A. actinomycetemcomitans* in localized juvenile periodontitis patients. The IgG2 subclass predominates in response to lipopolysaccharide derived from *A. actinomyce-
Avidity of antibody to periodontopathic bacteria in periodontitis

Since microbiological and immunological studies suggested a crucial role for *Actinomyces actinomycetemcomitans* in localized juvenile periodontitis and for black-pigmented bacteria in adult periodontitis, several investigators have examined antibody levels to these microorganisms. These studies demonstrated that antibody levels were elevated in several types of periodontitis as described above, but the functional capacities of the antibodies remained indistinct. The efficacy of the immune host defense mechanism via the antibody response can be determined by measuring the avidity of the antigen-antibody interaction. Avidity, the net binding affinity between antibodies and antigens, is a useful tool with which to diagnose infectious diseases (94) and B-cell tolerance in immune responses (139). Mooney et al. (145) indicated that the avidity of antibodies to microorganisms involves many antigenic and epitopic interactions. Therefore, the avidity in this context is an overall measure of the mean affinity or overall stability of potentially extensive interactions. They compared the avidity of IgG antibodies to *Actinomyces actinomycetemcomitans* between normal individuals and patients with adult periodontitis and found no significant differences. However, Saito et al. (194) suggested that antibodies with high avidity against *Actinomyces actinomycetemcomitans* fimbriae protected patients with adult periodontitis. O’Dell & Ebersole (162) also provided evidence supporting the notion that both antibody levels and avidity contribute to the variation in host resistance to infection and disease associated with *Actinomyces actinomycetemcomitans*. Furthermore, Underwood et al. (237) reported the hypothesis that IgG antibodies against *Actinomyces actinomycetemcomitans* are important in promoting phagocytosis and killing of *Actinomyces actinomycetemcomitans* by polymorphonuclear leukocytes. The chemiluminescence assay showed that patients who developed high levels of highly avid antibodies against *Actinomyces actinomycetemcomitans* might have greater resistance to continued or repeated infection.

There is an apparent discrepancy in the antibody avidity to *P. gingivalis* in adult periodontitis. Lapatin et al. (125) showed that IgG antibody avidity to *P. gingivalis* is significantly increased in patients with periodontitis compared with healthy individuals. On the other hand, Chen et al. (24) reported that the IgG antibody avidity to *P. gingivalis* is lower in patients with rapidly progressive periodontitis, than in control individuals. Whitney et al. (248) showed that the avidity of *Actinomyces actinomycetemcomitans* serotype b (252), and IgG2 levels reactive to *A. actinomycetemcomitans* serotype b exceed the IgG1 and IgG3 levels (127). Ling et al. (121) also examined the subclass IgG responses to *A. actinomycetemcomitans* serotype b-specific antigen in 35 localized juvenile periodontitis patients and 35 periodontal healthy control individuals. They showed that both the concentration and the ratio (%) of IgG2 exceed the totals of IgG1, IgG3 and IgG4. These findings suggest that IgG, especially IgG2, is hyper-responsive to *A. actinomycetemcomitans* serotype b-specific carbohydrate antigen in patients with localized juvenile periodontitis.

The four subclasses of human IgG differ only slightly in amino acid sequence. Most of the differences are clustered in the hinge regions and give rise to unique interchain disulfide bonds among the four proteins. Recently, Ebersole & Cappelli (49) identified antibody isotypes and subclass proportions in gingival crevicular fluid and analyzed the ability of the antibodies to protect against infection with *A. actinomycetemcomitans*. Their results showed that IgG3, IgG4, IgG1 and IgG2 antibodies are elevated by 58%, 35%, 25% and 25% respectively in gingival crevicular fluid. They also examined the subclass of the IgG antibody to *A. actinomycetemcomitans* and the presence of this microorganism. The correlation between elevated IgG4 antibody and the presence of *A. actinomycetemcomitans* in subgingival plaque was positive. Furthermore, they confirmed that healthy sites contain some IgG4 antibody to *A. actinomycetemcomitans*. These findings indicated that the IgG subclass in gingival crevicular fluid affects *A. actinomycetemcomitans* colonization in periodontal pockets, suggesting that analyses of the distribution of antibody isotypes and subclass to *A. actinomycetemcomitans* in gingival crevicular fluid will help reveal the significance of the host-parasite relationship in periodontitis. However, further study is required to determine the crucial roles played by the subclass of the IgG antibody in response to other periodontopathic bacteria in several types of periodontitis.

The IgA and IgG subclass isotypes have been compared. Recently, Brown et al. (20) have identified the subclass, molecular form and the elevated level of serum IgA antibody against *A. actinomycetemcomitans*. They indicated that monomeric IgA1 antibodies to *A. actinomycetemcomitans* sonic extracts predominate in most samples before, during and after periodontal treatment, suggesting that any protective effects conferred by the IgA response to *A. actinomycetemcomitans* are compromised by proteases derived from this microorganism.

*Actinomyces* and *Prevotella* are always present in the oral microflora but one species of *Actinomyces* may dominate in most samples before, during and after periodontal treatment, suggesting that any protease effects conferred by the IgA response to *A. actinomycetemcomitans* are compromised by proteases derived from this microorganism.
the IgG response is no different between seronegative and seropositive patients with rapidly progressive periodontitis, suggesting that humoral responsiveness to infection with \textit{P. gingivalis} does not remove this organism. Further clinical studies will define the effectiveness of using antibody avidity for the diagnosis or prognosis of periodontal diseases.

\section*{Immune regulation by Th1 and Th2}

Most T lymphocytes express either CD4 or CD8 molecules on their surface. CD4$^+$ and CD8$^+$ T cells have been referred to as helper and suppressor/cytotoxic T cells, respectively. As CD4 binds class II major histocompatibility complex antigens, CD4$^+$ T cells recognize the epitopes presented by these antigens. Whereas CD8 binds class I antigens, CD8$^+$ T cells recognize those in the context of class I molecules.

The division of CD4$^+$ T cells into distinct subsets (Th1 and Th2), based on the cytokine production, provided a new framework within which to understand immune responses to infectious diseases (152). Two distinct modes of cytokine production were originally defined using mouse T-cell clones (151). Th1 cells produce IL-2, gamma-interferon (IFN-\(\gamma\)), and provide help for cell-mediated immunity and delayed-type hypersensitivity responses. Th2 cells secrete IL-4, IL-5, IL-6 and IL-10 and provide help for IgG, IgE and IgA responses. Recently, a third type of T-cell clone (Th0) was identified (10, 61, 216) that produces IFN-\(\gamma\), IL-2, IL-4 and IL-5. Analogous to the murine system, human CD4$^+$ T-cell clones can be divided into at least three distinct functional subsets (Th1, Th2 and Th0) based on their cytokine secretion patterns (77, 179).

Infectious pathogens, including bacteria, protozoa, metazoa and viruses often seem to stimulate mainly one CD4$^+$ subset (206), which is closely related to the prognosis of the infection. For instance, the outcome of a protozoan infection depends on the mouse strain infected (83). \textit{Leishmania major} induces a Th1 cell response that activates macrophages and kills parasite in C57BL/6 mice. On the other hand, a Th2 cell response is induced in susceptible BALB/c mice that cannot eliminate \textit{Leishmania} although they produce anti-parasite antibodies (83). In general, the Th1 and Th2 cell response protects the host from intracellular and extracellular pathogens and/or toxins by activating macrophages and helping to produce antibodies, respectively (92).

Th1 or Th2 cell cytokines in periodontal disease(s) have been identified by several investigators. However, various periodontal pathogens cause several diseases, and the host response can be diverse among patients or at different stages of the disease. Therefore, the reports may not be directly compared. Fujihashi et al. (66) found by means of reverse transcriptase polymer chain reaction that human gingival mononuclear cells from patients with periodontitis express IL-5 and IL-6 mRNA, but not that of IL-2 or IL-4. They suggested that lack of IL-4 prevents macrophage apoptosis, resulting in persist chronic inflammation (261). Taubman et al. (220) also examined the expression of IL-1\(\beta\), IL-2, IFN-\(\gamma\), IL-5 and IL-6 in human gingival mononuclear cells from periodontitis patients using reverse transcriptase polymerase chain reaction. They detected the predominant expression of IFN-\(\gamma\), but not of IL-4 and of little IL-5 mRNA in cells directly extracted from diseased human gingival tissues, suggesting that Th1 T cells were prominent in the lesions.

In contrast, Yamazaki et al. (263) found IL-4-producing cells in periodontal tissues by immunohistochemistry, but the mRNA was undetectable by \textit{in situ} hybridization, suggesting that inconsistent findings were partly due to the method. Aoyagi et al. (3) found IL-4- and IL-6-producing cells in peripheral blood T cells from periodontitis patients and suggested that activation of B cells by IL-4 would establish a progressive periodontitis lesion featuring B-cell infiltration, which would then induce antibody production through an immunoglobulin class switch.

Isolating T-cell clones from human inflamed gingival tissue, Wassenaar et al. (244) found CD4$^+$ T-cell clones reactive with collagen type I, and 80% of them were Th2-like phenotype (they produce high levels of IL-4 and low IFN-\(\gamma\)). They proposed that collagen-specific CD4$^+$ Th2-like T cells contribute to the chronicity of periodontitis but that their modes of activation might be controlled by Th0-like T cells specific for periodontitis-associated bacteria.

Lee et al. (118) reported that IL-2 and IL-6 were significantly greater in active sites of refractory periodontitis patients. Alveolar bone loss in active sites was correlated with increased gingival crevicular fluid levels of IL-1\(\beta\) and IL-2, suggesting that these may serve as possible indicators of disease activity in refractory periodontitis.

Evaluation of the data, however, would differ among investigators, depending on which reactions they viewed as being most important for the pathogenesis of periodontitis. Inflammatory cytokines
Induction of the immune response to periodontopathic bacteria

such as IL-1 or IL-6 might be regarded as harmful. As more than one type of cell produces cytokines, it is difficult to determine which is actively producing the inflammatory factors. For example, macrophages and B cells both produce IL-1. If the production of IL-1 by macrophages is the most relevant to periodontal destruction, macrophage activation by Th1 cells might be harmful (220), and the elimination of macrophage by IL-4 might be beneficial (261) (Fig. 2). However, if B cells were the major source of IL-1, their activation by Th2 cells might be harmful (208) (Fig. 2).

Several factors can affect the development of Th1 or Th2 responses. IFN-γ and IL-12 induce Th1 expansion, and IL-4 is required for Th2 cell maturation. IL-12 is produced by several types of antigen presenting cells, including macrophages and dendritic cells. Since IL-4 is produced by Th2 cells, the question arose as to which cells produce IL-4 to generate them in the first place. It has been suggested that evolutionary old T cells, which recognize CD1 antigens, produce IL-4 for the maturation of Th2 cells in mice (264). In this regard, cytokines produced by evolutionarily old cells are closely involved in the commitment of Th1 and Th2 cells (Fig. 3). In addition, cytokines produced by Th1 and Th2 cells regulate macrophage function (Fig. 3). Accordingly, understanding the relationship between innate and adaptive immunity will clarify the development of Th1 or Th2 responses in periodontal disease.

Role of cell-mediated immunity, hypersensitivity and mucosal immunity

Plasma cells become dominant in periodontitis, and loss of the alveolar bone and periodontal ligament are observed, while T cells are dominant in early gingivitis, and the lesion resembles delayed-type hypersensitivity (175). The factors causing the conversion from gingivitis to periodontitis have yet to be clarified. Originally, the delayed-type hypersensitivity response was variously interpreted, and it now appears to be a question of Th1 and Th2 cells.

If the cellular reactions within early gingivitis and periodontitis are regarded as beneficial and destructive, respectively, conversion from the T-cell lesion to the B cell might be destructive. Based on this notion, the delayed-type hypersensitivity response mediated by Th1 cells might be protective, and B-cell activation may be destructive (208).
odontal bone loss in rats infected with *A. actinomycetemcomitans*. Bone loss in the immunized rats was significantly elevated (224), suggesting that the delayed-type hypersensitivity response causes destruction of the periodontal tissue. These experiments were confirmed using rat Th1 and Th2 clones specific for *A. actinomycetemcomitans* (262). Yamashita et al. (262) generated the rat Th1 and Th2 cell clones specific for *A. actinomycetemcomitans* and examined the effect of adoptive transfer of T-cell clones on the periodontal disease in rats infected with *A. actinomycetemcomitans*. Adoptive transfer of the Th2 clone ameliorates the disease (262). As Th1 cells mediate delayed-type hypersensitivity responses, these findings support the previous results. Accordingly, they postulated that Th1 cells mediate delayed-type hypersensitivity and enhance periodontal disease, whereas Th2 cells abrogate the disease (220).

Available data cannot define the roles of the CD4+ T-cell subsets (Th1 and Th2) or their functions (cell-mediated immunity and help for antibody production) in periodontal disease. The oral cavity, including periodontal tissue, is the entrance to the gastrointestinal tract. The mucosal immune system is distinct from systemic immune systems in terms of lymphocyte function and differentiation.

One feature of the mucosal immune system is the regulation of secretory IgA production. The oral administration of antigens induces antigen-specific IgA responses in remote mucosal areas. Craig & Cebra (34) reported that transferring lymphoid cells isolated from rabbit Peyer's patches to irradiated allogenic recipients caused donor IgA plasma cells to appear in the intestinal lamina propria of the recipient. On the other hand, lymphocytes from systemic tissues did not migrate to these mucosal-associated effector sites. Orally administered antigens enter Peyer's patches through pinocytotic and phagocytic M cells (12). The lymphoblasts generated in Peyer's patches enter the circulation and populate remote mucosa-associated effector tissues (140). Accordingly, gut-associated lymphoid tissues including Peyer's patches are referred to as IgA-inductive sites and mucosa-associated effector tissues are called IgA effector sites. The salivary glands are major IgA effector tissues in the oral cavity (90).

The other key feature of the mucosal immune system is unique differentiation. Although most of the T cells residing in peripheral tissue develop in the thymus, some intestinal intraepithelial lymphocytes develop in the intestinal epithelium. The intestinal intraepithelial lymphocyte regulates the IgA response in the mucosal immune system.

Lundqvist & Hammarström (129) reported that intraepithelial lymphocytes express T-cell receptor γδ in normal and inflamed gingival tissues. It is notable that numerous Langerhans' cells and keratinocytes expressing major histocompatibility complex class I-like antigen, CD1, are present within normal and inflamed gingival epithelium in close proximity to T-cell receptor γδ+ T cells. They suggested that these cells constitute the first line of defense against potentially harmful microflora in the oral cavity.

The characteristics of the mucosal immune sys-
The central role in lymphocyte function and differentiation might also be defined from an evolutionary perspective. Herzenberg (84) proposed that an adaptive immune system is layered upon innate immune systems. Fig. 3 shows the evolution of the immune systems. In the innate systems, antigens (bacteria) are recognized as non-self by macrophages and eliminated by phagocytosis (Fig. 3). This reaction is initiated by recognition, followed by elimination. In the adaptive immune systems, the reaction has 3 phases: recognition, clonal expansion/differentiation and elimination (Fig. 3). The mucosal T-cell receptor γδ T cells and CD5 B cells are evolutionarily intermediate between innate and adaptive immune systems (91) (Fig. 3).

Janeway (91) proposed that receptors and response mechanisms of the innate immune system, selected throughout evolution to recognize and induce responses to infectious agents, also initiate adaptive immune responses to infectious non-self factors. T cells require costimulatory signals for clonal expansion and differentiation. Macrophages regulate T-cell functions through costimulatory factors. The benefits of this system are obvious if the autoreactive T cells reside in the peripheral tissues. Autoreactive T cells cannot be activated by self antigens expressed on peripheral tissue cells, as they lack costimulatory signals. The safety of the system has been maintained by preserving the receptors that recognize antigens during evolution.

In addition to cell-cell interactions, cytokines produced by the innate and acquired immune systems are important (Fig. 3). The contribution of the innate and adaptive immune systems and their intermediates in periodontal disease and health must be assessed.

Role of macrophages, CD4+, CD8+, natural killer cells and other immunocompetent cells

Macrophages

The central role of macrophages. Macrophages play a central role in mobilizing the host defense mechanisms against bacterial infection, because they are involved both in the initiation of responses as antigen-presenting cells and in the effector phase as inflammatory, tumoricidal and microbicidal cells, in addition to their regulatory functions. After exposure to foreign microbes, macrophages develop an increased capacity to kill bacteria and secrete a number of immune mediators that stimulate anti-bacterial responses by other cells (149).

Macrophages are actively phagocytic cells capable of ingesting and digesting exogenous antigens. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called chemotaxis. The next step in phagocytosis involves attachment of the antigen to the macrophage cell membrane. Attachment induces membrane protrusions called pseudopodia to extend around the attached material. The pseudopodia fuse the material into the cell enclosed in a membrane-bound organelle called a phagosome, which then enters the endosomal processing pathway. The lysosome fuses with the phagosome, releasing its contents into the phagolysosome. Most of the ingested material is digested and eliminated through exocytosis. Some of the digested peptides are thought to interact with the class II major histocompatibility complex molecule. The antigenic peptide-class II major histocompatibility complex then moves to the cell surface, where it can be recognized by T cells. Macrophage activity can be enhanced by certain molecules elaborated during an immune response. The macrophage membrane possesses receptors for certain classes of antibody and for certain complement components. When an antigen is coated with the appropriate antibody or complement component, it is more readily bound to the macrophage membrane and phagocytosis is enhanced. Antibody and complement serve as opsonins and the entire process is called opsonization. Although phagocytosis of antigen initially activates macrophages, their activity can be further enhanced by various activating factors. For example, IFN-γ secreted by activated T cells binds to receptors on macrophages and activates them. Because such activated macrophages develop increased phagocytic activity and higher levels of lysosomal enzymes, their ability to ingest and eliminate potential pathogens is enhanced. In addition, these activated macrophages secrete cytotoxic proteins (for example, tumor necrosis factor α) that help them eliminate a broader range of pathogens. Activated macrophages also express higher levels of class II major histocompatibility complex molecules, allowing them to function more effectively as antigen-presenting cells. Thus macrophages and T cells exhibit an interacting relationship during the immune response with each facilitating activation of the other (113).

Macrophages and keratinocytes share common surface markers at different stages of inflammatory
periodontal disease and may thus play a cooperative role in periodontal inflammation. Schlegel et al. (204) immunohistochemically investigated 29 biopsy specimens from different stages of gingivitis and periodontitis using monoclonal antibodies to determine the expression of inflammatory, resident and intermediate macrophages. Each macrophage subtype exhibited a localized pattern depending on the stage of inflammation. Furthermore, suprabasal oral gingival epithelia constantly expressed inflammatory macrophage markers, independent of the inflammation stage. In contrast, all layers of the sulcus and pocket epithelia in gingivitis and periodontitis were inflammatory macrophage marker-positive, indicating immune activation. Resident macrophage markers were expressed on basal keratinocytes independent of the stage of inflammation, whereas those for the intermediate subtype in healing tissue were constantly negative on keratinocytes. The expression of these functionally different macrophage markers on lesional macrophages and keratinocytes indicates varying differentiation and activation and suggests that these cells participate in the local immune response to periodontal infection.

Interaction between gram-negative periodontopathic bacteria and macrophages. Lipopolysaccharide is the major component of the outermost membrane of gram-negative bacteria. Lipopolysaccharide that is released from dead or living bacteria into the host tissue activates immunopathogenic reactions. Lipopolysaccharide potently stimulates immune cells, mainly monocytes and macrophages (13, 150). The interactions of lipopolysaccharide with monocytes are not fully understood. At least 6 receptors for lipopolysaccharide have been identified on host cells (130), but only the 55-kDa cell surface protein, CD14, mediates cytokine secretion (247, 257). CD14 is a glycosylphosphatidylinositol-linked glycoprotein expressed on monocytes, macrophages and neutrophils. It triggers monocyte activation and is a receptor for lipopolysaccharide. In this capacity, CD14 plays a pivotal role in endotoxin-induced monokine release during Gram-negative infections, as confirmed in *in vitro* studies with transgenic mice (60). Shapira et al. (209) reported that lipopolysaccharide derived from *P. gingivalis* elicits secretion of tumor necrosis factor α from human monocytes dependently upon CD14. CD14 can bind complexes of lipopolysaccharide and serum proteins, like lipopolysaccharide binding protein and other proteins (septins) (256) with high affinity. Monoclonal antibodies to CD14 block binding of the lipopolysaccharide complex and inhibit the cytokine secretion by lipopolysaccharide-stimulated monocytes (256, 257). These studies demonstrated that CD14 is a functional receptor for lipopolysaccharide and can mediate the responses of monocytes to lipopolysaccharide. CD14 is down-regulated by IL-4 (116) and IL-13 (32), which also inhibit cytokine secretion by lipopolysaccharide-stimulated monocytes. This suggests that down-regulated CD14 and cytokine secretion plays a major role in the anti-inflammatory effects of IL-4 and IL-13 on lipopolysaccharide-stimulated monocytes.

Antigen recognition. The manner in which macrophages present antigen to T cells is restricted by the major histocompatibility complex. Naive T cells encounter breakdown products (peptides) of antigens which bind to the major histocompatibility complex on the surface of specialized antigen-presenting cells (dendritic cells and macrophages). T cells respond to foreign antigens by producing protein effector molecules known as lymphokines and by proliferating. Major histocompatibility complex class II molecules display peptides derived from proteins internalized through the endocytic pathway and recognized predominantly by inducer T lymphocytes expressing the CD4 surface molecule. Major histocompatibility complex class I molecules display peptides derived from proteins synthesized inside the antigen-presenting cell and are largely recognized by cytotoxic T lymphocytes expressing the CD8 surface molecule (205).

Current evidence suggests that T-cell receptor recognition of antigen bound to the major histocompatibility complex is insufficient to lead to T-cell proliferation or effector function. For a helper T cell to produce sufficient IL-2 to permit autocrine-driven clonal expansion, costimulatory or accessory signals are required in addition to T-cell receptor ligation by antigen bound to the major histocompatibility complex. The costimulatory signal determines the outcome of T-cell receptor occupancy. Occupancy of the antigen-specific T-cell receptor by a peptide-major histocompatibility complex molecule complex leads to activation of tyrosine kinases and phospholipase C. These signal transduction events alone are not sufficient to activate the IL-2 gene. Instead, the calcium signal appears to activate one or more repressor genes that cause the cell to enter an anergic state. In contrast, if a simultaneous costimulatory signal is initiated by a ligand on the antigen-presenting cell, then the induction of the repressor genes is inhibited and IL-2 gene is activated, leading to a proliferative response by the T cell (205). The inter-
action of the CD28 receptor on T cells with B7 on antigen-presenting cells supplies one costimulatory signal. In the absence of this signal, T cells only partially respond and, more importantly, enter an unresponsive state known as clonal anergy in which they cannot produce their own growth hormone, IL-2, upon restimulation (95).

Macrophage-derived inflammatory mediators. The mediators in the periodontal breakdown process are probably molecules with a direct or indirect capacity to mediate tissue destruction. Currently, the most likely candidates are IL-1β, tumor necrosis factor α and prostaglandin E2 (177). IL-1β is a cytokine that is produced primarily by macrophages that have been activated by lipopolysaccharide, immune complexes or other inflammatory mediators. Several investigators found that IL-1β is significantly increased in periodontal tissues and gingival crevicular fluid from diseased, compared with healthy sites (85, 134, 177, 215). However this could be solely related to the inflammatory status of the gingiva. The main cellular source of tumor necrosis factor α is tissue macrophages. The role of tumor necrosis factor α in periodontal lesions remains obscure. Paramagnetic beads coated with a monoclonal antibody to tumor necrosis factor α detected this cytokine in gingival crevicular fluid collected directly from the gingival sulcus (190). Tumor necrosis factor α stimulates bone resorption but less potently than IL-1β (155).

Unlike the cytokines, prostaglandins are short-acting lipids, produced from arachidonic acid. It is unlikely that the small population of macrophages is the main source of prostaglandin E2 in periodontal lesions, but the production of other mediators, such as IL-1β or tumor necrosis factor α by monocytes may trigger prostaglandin E2 secretion by gingival or periodontal ligament fibroblasts (188). Using early-onset periodontitis as a model of aggressive periodontal disease, Van Dyke et al. (238) reported that monocytes from patients with early-onset periodontitis respond to lipopolysaccharide by producing elevated amounts of prostaglandin E2 and tumor necrosis factor α. The responsiveness of monocytes to lipopolysaccharide stimulation may be under genetic control. Murine models of lipopolysaccharide responders and nonresponders have been described (217, 242). Lipopolysaccharide-stimulated monocytes from the nonresponder strains secrete less mediators than lipopolysaccharide-responsive strains. The difference in the response to lipopolysaccharide by various mediators supports the notion that monocytes from individuals susceptible to periodontitis differ considerably in their inflammatory secretory response to a lipopolysaccharide challenge.

CD4+ and CD8+ T cells

Mature T cells migrate to peripheral lymphoid organs such as the spleen and lymph nodes. Monoclonal antibodies directed against surface antigens have revealed lymphocyte subsets with different functions such as T-helper (Th, CD4+) and T-suppressor/cytotoxic cells (Ts, Tc, CD8+).

Several reports have shown imbalances in peripheral blood T cells by examining the ratio of helper to suppressor T cells (CD4/CD8) or the autologous mixed lymphocyte reaction in groups of young patients with severe periodontitis. However, the CD4/CD8 ratios in patients with rapidly progressive periodontitis are inconsistent (22, 99, 104), and a suppressed autologous mixed lymphocyte reaction has been demonstrated in some individuals diagnosed with early-onset periodontitis (186, 207, 227). We (156) recently reported that the rapidly progressive periodontitis patients have significantly fewer CD8+ T cells and a higher CD4/CD8 ratio than healthy individuals. We also found that there were no significant differences in the percentages of CD4+ T cells and CD4+CD45RA+ T cells, which are responder cells in the autologous mixed lymphocyte reaction, between rapidly progressive periodontitis patients and healthy individuals. These findings suggest that rapidly progressive periodontitis patients have imbalances of peripheral blood T cells, especially a tendency towards decreased CD8+ T cells, and that cellular immune responses mediated by CD8+ T cells play a role in the pathogenesis of rapidly progressive periodontitis. On the other hand, CD4/CD8 ratios are reduced in human periodontal lesions relative to the ratios in peripheral blood and normal tissue (169, 221, 222). CD4+ populations present in diseased tissues express both CD29 and CD45R cell surface determinants, suggesting that many CD4+ lymphocytes actively differentiate into cells with memory capacity (223). The constant antigenic challenge in periodontal disease, where bacteria remain for extended periods in the periodontal pockets, induces these T cells with memory capacity, which in turn activate B cells.

CD8+ T cells can be divided into two subsets: cytotoxic cells that produce IL-10 and IFN-γ and suppressor cells that produce mainly IL-4 (195). The cytokine profile of periodontally diseased gingival tissues shows that IL-4 and IL-5 are not produced (65). IL-4 is
necessary for suppressor activity of CD8+ T cells (195). The predominance of T cells involved in cell-mediated immunity and the absence of IL-4 mediated suppressor activity might be relevant to the pathogenesis of periodontal destruction.

Natural killer cells

Natural killer cells are found in normal animals that have not apparently been exposed to relevant antigens. Natural killer cells are activated by interferons which are themselves components of the innate immune system. They also produce IFN-γ. The natural killer cells can kill a variety of transformed, virus-infected or embryo-derived cells in vitro in the absence of antibody and play a role in regulating the immune response.

Natural killer cells in periodontal diseases have been examined by immunohistochemical means. Kopp immunostained natural killer cells in inflamed gingiva using NC1 antibody and found that the number of natural killer cells decreases after oral hygiene training and/or professional therapy (112). Cobb et al. (30) investigated healthy gingiva, chronic gingivitis and adult periodontitis. They reported that the incidence of Leu-11b (CD16) positive cells increases as the clinical condition of lesions deteriorates. An immunohistochemical study by Fujita et al. (68) showed that more natural killer cells reacting with Leu-11b in the infiltrated connective tissue accumulate in severe forms of periodontal disease (severe adult periodontitis and rapidly progressive periodontitis) than in mild adult periodontitis. This evidence indicated that natural killer cells play a role in the destruction of tissues in periodontal diseases.

Other immunocompetent cells

Fibroblasts are responsible for phagocytosis of collagen and components of extracellular matrix in connective tissue remodeling (226). Cytokines can be produced by the fibroblasts themselves as well as epithelial cells, platelets and migrating phagocytes and inflammatory cells. It is postulated that once the tissue inflammation has resolved, fibroblast subtypes will return to their normal stable ratios (181).

Plasma cells and macrophages continue to predominate during the destructive phase of periodontitis. The relative contributions of each cell type during active disease remain controversial. Factors derived from macrophages and T cells are required for B cells to divide and differentiate into active plasma cells producing immunoglobulin (167, 240).

Potential for periodontitis vaccines

Vaccines have prevented several infectious diseases for many years, but they are still being investigated. It had long been recognized that individuals who recovered from a disease developed subsequent resistance to the same. In the late eighteenth century, Edward Jenner developed and established the principle of vaccination using the cross-protection conferred by cowpox virus, which is non-pathogenic in humans. Vaccination is a process that induces specific immune resistance to a bacterial or viral infectious disease. The key features of a successful vaccine are safety, effectiveness, stability, a long shelf life and relatively low cost. Vaccination against bacterial and viral infectious diseases has progressed immeasurably throughout the twentieth century. Vaccination can be accomplished by two methods: active immunization, in which an individual’s immune system is stimulated by administrating killed or live-attenuated bacteria or viruses, components or attenuated products derived from microorganisms and passive immunization, in which the antibodies formed in one individual are transferred to another (Fig. 4).

Immunization against periodontitis

The complexity of periodontopathic bacteria might be a problem in determination of antigen for vaccine against periodontal disease. A substantial number of bacteria (exceeding 300 species) appear to be involved in subgingival plaque (148). Among these, five to seven species have been implicated in the etiology of periodontitis (211), but one or two species, P gingivalis or B. forsythus, might play an important role as primary pathogen (74, 75). Furthermore, regarding antigenic epitopes in periodontopathic bacteria, some researchers have demonstrated that epitopes were shared among gram negative bacteria (6, 42, 181, 240), possibly because of the polyclonal B-cell activating properties of lipopolysaccharide (174).

Several investigations regarding the humoral immune response in periodontitis patients have been performed. Chen et al. (24) reported that 24 of 36 rapidly progressive periodontitis patients were seronegative to antigens of P gingivalis and that the median avidity of sera derived from patients was lower than that of control subjects. In other words, more than half of the patients did not mount a humoral immune response and the antibodies produced in those might not be biologically effective. Furthermore, both serum antibody titers and avidity against
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Fig. 4. Summary of three types of immunization: active, passive and DNA vaccination. Active immunization involves stimulating an individual's immune system by administering killed or live-attenuated bacteria, their components or synthetic peptides. Passive immunization involves transferring antibodies formed in one individual to another. DNA vaccination indicates that DNA plasmids encoding genes required for antigen production are transferred by intramuscular injection without adjuvant.

*P. gingivalis* antigens from 24 seronegative patients increased significantly following only scaling and root planing. Similar observations were made on groups of subjects with rapidly progressive periodontitis tested for antibodies against *A. actinomycetemcomitans* (210). Ten of 12 patients became seropositive from seronegative following scaling and root planing, and the post-treatment sera enhanced stronger capacity of phagocytosis and killing than the pretreatment ones did. Periodontal therapy, scaling and root planing, could elicit the humoral immune response in seronegative patients, resulting in seroconversion and production of effective antibodies. This might be due to bacteremia provoked by treatment (174).

These observations suggest that the development of vaccine against periodontitis might be possible and that utilization of it could be an effective method for control and prevention of periodontal disease.

**Experimental models.** Humans may not be used as experimental subjects in studies with periodontopathic bacteria. Accordingly, the prevention of colonization, adhesion and bacterial invasion have been studied in various animal models.

Nonhuman primates and humans are similar in both periodontal structure and microflora composition (176). However, ligatures must be tied around the teeth to elicit periodontitis in nonhuman primates, because it is difficult to colonize the oral cavity with *P. gingivalis* and establish periodontal lesions. McArthur et al. (137) suggested that the squirrel monkey could be a model for studying the parameters of black-pigmented anaerobic rods colonization in gingival crevices. However, the mechanisms of bacterial retention around ligated teeth are totally different from those of adhesion around the teeth or gingival tissue. Persson et al. (182) investigated the constituents of subgingival microflora and immune reactions (antibody titers and avidities against *P. gingivalis*) in experimental *Macaca fascicularis* periodontitis and concluded that *M. fascicularis* was a useful model for testing and developing vaccines for periodontitis.

There are some advantages in using rats for ad-
hension experiment. Since rats resemble humans in periodontal anatomy and bacterial composition, bone loss can be evaluated (107, 176). Furthermore, \( P. gingivalis \) quickly colonizes the rat oral cavity and induces bone loss. The invasion ability of bacteria has been investigated using the subcutaneous abscess model in mice and the subcutaneous chamber model in mice and rabbits. Kesavalu et al. (101) studied active immunization using whole cells or selected cell envelope components and suggested that the murine model would be useful for investigating the tissue-destructive components of \( P. gingivalis \).

**Active immunization**

Active immunization has been studied using whole bacterial cells, outer components or synthetic peptides as antigens. The results showed that the progression of periodontal diseases could be prevented by immunization.

**Antigen: whole cells.** Klausen et al. (108) reported that the levels of serum antibodies to both whole cells and partially purified fimbriae from \( P. gingivalis \) were elevated in rats immunized with \( P. gingivalis \) cells and that the activities of collagenase and cysteine proteinases in gingival tissues as well as periodontal tissue loss were decreased. Genco et al. (70) demonstrated that protection against invasion but not colonization by \( P. gingivalis \) was induced in the mouse chamber model by immunization with either killed heterologous invasive or heterologous noninvasive \( P. gingivalis \) strains. Kesavalu et al. (101) indicated that in the BALB/c mouse model, the immune response to whole cells or selected cell envelope components did not completely abrogate lesions but eliminated mortality. The numbers of \( P. gingivalis \) on ligatures in hamsters were reduced, and spreading infection was prevented by the subcutaneous administration of whole formalin-killed cells but not by a periordial injection (170). In squirrel monkeys (Saimiri scuireus), immunization with whole cells of monkey isolate (\( P. gingivalis \) strain 1-372) increased the level of anti-\( P. gingivalis \) IgG antibody in serum and significantly reduced colonization by the same strain in gingival crevices (137). Furthermore, Persson et al. (181) reported that immunizing \( M. fusciculatus \) with killed \( P. gingivalis \) in Syntex Adjuvant Formulation-M inhibited the progression of periodontal tissue destruction.

However, active immunization with whole cells might induce exaggerated inflammatory responses in the host. Bone density was significantly decreased in ligated teeth from nonhuman primates immunized with whole-cell antigens of \( P. gingivalis \) and \( P. intermedia \) (48). The investigators concluded that a broad-based immune response to several bacterial antigens could increase hypersensitivity reactions to the bacteria.

**Antigen: outer components.** Fimbriae from \( P. gingivalis \) play an important role in adhesion to oral tissues (172) and are also highly immunogenic (16, 108). Therefore, the effect of immunization with various fimbrial and cell surface antigen preparations upon \( P. gingivalis \)-associated periodontal diseases was investigated (58, 138). Evans et al. (58) reported that immunization with highly purified \( P. gingivalis \) fimbrial preparations (43-kDa fimbrial component), as well as with whole cells and soluble antigens of \( P. gingivalis \), protected against periodontal destruction induced by \( P. gingivalis \) in gnotobiotic rats. Gingival tissue enzymes, including collagenase and the cysteine proteases, cathepsin B and L, were markedly affected by immunization with these antigens. They suggested that fimbrial protein might serve as a model of effective vaccines against periodontitis.

Immunizing experimental animals with an outer membrane preparation (11) and polysaccharide-protein conjugate (203) isolated from \( P. gingivalis \) induces elevated levels of specific antibody and provides protection against the progression of periodontal disease. Immunization with hemagglutinin is also as efficient as that with whole cells in reducing recovery from \( P. gingivalis \) infection in ligated hamsters (170). Chen et al. (26) reported that the incidence of secondary lesions was reduced by immunization with lithium diiodosalicylate extracts of membranes from \( P. gingivalis \) in the mouse abscess model but tissue invasion was not elicited. They also demonstrated that immunization with a purified 75-kDa outer membrane protein reduces the activities of collagenase, gelatinase and cysteine proteases in gingival tissue. However, it did not prevent periodontal bone loss (58).

Okuda et al. (171) showed that the antibodies elicited against fimbriae composed of a 54-kDa protein derived from \( A. actinomyctecomitans \) 310-a protect against continued infection by this microorganism. They postulated that the IgG responses to fimbria antigen elicited by the initial contact with \( A. actinomyctecomitans \) played an important role for eliminating organisms from the periodontal pockets of patients harboring high IgG antibody avidity.

**Antigen: synthetic peptides.** Mapping the adhesion, T-cell and B-cell epitopes is essential for investigat-
ing synthetic peptide vaccines (120). T- and B-cell epitopes are recognized by T cells and B cells, respectively. Adhesion epitope mediates adherence between bacteria and host tissue through a ligand-receptor interaction. It is important to design a synthetic peptide vaccine in which antigenicity does not imply immunogenicity. Since IgG and secretory IgA may play a role in preventing bacterial adhesion to salivary glycoproteins or mucosal receptors, adhesion epitopes are also indispensable to the immune response elicited by synthetic peptide vaccines. Small antigenic peptides are normally poorly immunogenic, and it is therefore necessary for small peptides to be added a carrier molecule for inducing an immune response.

Synthetic peptides based on the protein structure of fimbrillin inhibit the adhesion of P. gingivalis to saliva-coated hydroxyapatite crystals in vitro and their binding domains are located at the carboxyl-terminal region (119). Furthermore, gingival tissue enzyme levels and horizontal bone loss were reduced by immunization with a 20 mer synthetic fimbrial peptide in the gnotobiotic rat model (57). Brant et al. (19) investigated the linear immunogenic and antigenic structure of P. gingivalis fimbrillin and identified the antigenic determinant in native fimbrillin as residues 99–110. Furthermore, they suggested that peptide immunogens would be effective as vaccines since they could adopt a more native conformation to produce effective antibodies.

**Passive immunization**

Chronic disease is not generally an indication for passive immunization by the repeated administration of a xenogeneic immunoglobulin. However, passive immunization against periodontal diseases has been attempted because of the success of active and passive immunization against P. gingivalis and S. mutans, respectively. Almost all vaccines have some side effects that range from relative local reactions to generalized, nonspecific sickness. However, passive immunization is thought to be comparatively safer than active immunization. Okuda et al. (170) reported that repeated passive immunization with rabbit antiserum to P. gingivalis hemagglutinin into the oral cavities of the hamsters reduced colonization by exogenous P. gingivalis in the periodontal region.

Furthermore, passive immunization with monoclonal antibodies against P. gingivalis effect selectively prevents recolonization by this organism in humans (14). However, the periodontal health of patients was not significantly improved in either immunized or sham-immunized patients at 6 and 12 months.

A. actinomyctemcomitans-specific T-cell clone isolated and adoptively transferred into rats, elevated serum IgG and IgM antibodies to A. actinomyctemcomitans and significantly decreased bone loss (262). Thus, T-cell regulation seems to affect periodontal disease and T helper cells apparently interfered with periodontal bone loss.

**Future directions**

Subunit vaccines have been developed based on viral and bacterial peptides or plasmid vectors. In fact, DNA vaccines that were first described less than five years ago have already progressed to phase I clinical trials in healthy adult humans. They might induce immunity to numerous agents, including periodontopathic bacteria, following confirmation of their safety (Fig. 4). DNA vaccines offer several distinct advantages. Firstly, DNA vaccines can be manufactured more easily than vaccines consisting of an attenuated pathogen, an outer or internal protein or a recombinant protein. The second advantage is that since DNA is stable by nature and resistant to extremes of temperature, storage, transport and distribution, it might be highly practical. The third advantage of vaccination with DNA is the simplicity of changing the sequences encoding antigenic proteins by means of mutagenesis and of adding heterologous epitopes by basic molecular genetics. The immunogenicity of the modified protein may be directly assessed following an injection of the DNA vaccine.

DNA plasmids encoding a gene required for antigen production are transferred by intramuscular needle injection without adjuvant. Alternatively, intradermal particle bombardment is also effective. Polynucleotides encoding pathogenic protein should not be injected. If DNA uptake is efficient, a single intramuscular injection can elicit a strong and sustained immune response. Antibodies are induced by immune responses. The immunoglobulin is ultimately an IgG, indicating a T-cell-dependent class switch (259). The responses include not only antibody induction and T-cell activation with cytokine secretion, but also the production of cytotoxic T cells.

DNA vaccination has been studied in animals. Most of the investigated pathogens have been viruses, for instance bovine herpes virus (33), hepatitis B virus (38), hepatitis C virus (114), herpes simplex vi-
ruses (133), human immunodeficiency virus-1 (243), influenza virus (234) and lymphocytic choriomeningitis virus (271). Furthermore, some pathogenic bacteria have also been investigated. Lowrie et al. (126) demonstrated that expression of the gene for a single mycobacterial antigen (Mycobacterium leprae hsp65) in adult BALB/c mice caused substantial cell-mediated protection against challenge with Mycobacterium tuberculosis. Some genes from periodontopathic bacteria has been cloned (4, 43, 69, 78, 82, 93, 142, 164, 218), and these genes could be used as a vaccine to protect against periodontitis. DNA vaccines have distinct potential for preventing various infectious diseases, including periodontal disease, in humans.

**Clinical implications of immune responses**

**Diagnostic potential of antibody against periodontopathic bacteria**

Numerous clinical and immunological studies have demonstrated the diagnostic potential of patient sera for use against several infectious diseases. The ELISA is probably the most widely applied immunological assay because a large number of samples can be rapidly evaluated. In fact, the levels of serum antibody in several types of periodontitis to bacteria including A. actinomycetemcomitans, P. gingivalis, Capnocytophaga species, Eikenella corrodens, Fusobacterium nucleatum and oral spirochetes have been examined by means of ELISA. Many investigators have reported that localized juvenile periodontitis patients have a remarkable high titer of serum antibodies to A. actinomycetemcomitans serotype b strain (54, 121, 122, 132, 185, 228, 241). A. actinomycetemcomitans serotype b strains are recovered more frequently and may exhibit greater periodontopathic potential than other serotypes (270). A serotype-specific capsular polysaccharide antigen was extracted from whole cells of A. actinomycetemcomitans Y4 (serotype b) by autoclaving, then purified by ion-exchange chromatography and gel filtration (2). These results indicated that autoclave extraction of serotype antigens is useful for serotyping A. actinomycetemcomitans strains and for detecting A. actinomycetemcomitans serotype-specific serum antibody in patients with periodontitis.

Black-pigmented bacteria such as P. gingivalis and P. intermedia are considered major pathogens in destructive adult periodontitis. Several investigators reported that fimbriae constitute an important P. gingivalis antigen in periodontal diseases. Immunization with highly purified P. gingivalis fimbriae protects against periodontal bone resorption in P. gingivalis-infected gnotobiotic rats (57). On the other hand, Chen et al. (28) have reported that immunization with P. gingivalis lipopolysaccharide neither produces detectable levels of antibody nor affords protection from a challenge infection with P. gingivalis. Thus, several experiments were performed in rodents, which are useful for assessing antibody function against P. gingivalis and for studying microbial and immune responses to periodontal diseases.

**Correlation between serum antibody level and periodontitis therapy**

To predict episodes of periodontal diseases, it is very important to provide a reliable clinical indicator for disease assessment. Ebersole et al. (55) showed that the antibody titer against P. gingivalis and A. actinomycetemcomitans increases after scaling. This result is in accord with the conclusion of Sjöström et al. (210) wherein the treatment by scaling and root planing induced a humoral immune response. These findings suggest a humoral immune response may be a major factor in the clinical improvement observed after treatment. On the other hand, some investigators have reported a significant reduction in the serum antibody titer to P. gingivalis following periodontal treatment (159, 230). We examined pre-and post-treatment serum IgG titers to periodontopathic bacteria by ELISA in samples from patients with several types of periodontitis. The antibody titer to P. gingivalis decreased in all cases, suggesting that the changes of the titer were reacted to the suppression of such pathogens in subgingival area. We proposed that the serum IgG titer to P. gingivalis can serve as a clinical indicator of periodontitis and provides an important criterion with which to evaluate periodontal treatment (86). We also measured the serum antibody titers in patients with Papillon-Lefèvre syndrome to periodontopathic bacteria, including P. gingivalis and A. actinomycetemcomitans, by ELISA (17, 88). Our results indicated a close relationship between A. actinomycetemcomitans and the pathogenesis of periodontal destruction in two patients with Papillon-Lefèvre syndrome: the percentages of A. actinomycetemcomitans in bacterial cultures were high; antibody titers to the same bacteria were high according to ELISA; immunoblots were similar; early extraction of deciduous teeth from one patient gave satisfactory results, and A.
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Actinomyetemcomitans was eradicated after clinical treatment as manifested by cultures of subgingival plaque on selective medium; serum antibody titers against A. actinomyetemcomitans were reduced after periodontal therapy. These findings suggest that serum antibody titers provide a reliable objective indicator for episodes of periodontitis, at least in patients with Papillon-Lefèvre syndrome.

Immune responses as markers of susceptibility of periodontal diseases

Haffajee et al. (79) used cluster analysis to classify 119 patients with periodontitis (3 localized juvenile periodontitis, 10 generalized juvenile periodontitis, 10 rapidly progressive periodontitis and 96 adult periodontitis) according to serum antibody levels to specific subgingival species. Ninety-two of them fell into 9 clusters with 100% similarity, and the remaining 27 could not be clustered. Cluster 1 consisted of 29 patients with elevated antibody levels to none of the test species. Seven patients in cluster 2 had elevated antibody levels to B. forsythus. Patients in the other clusters had elevated antibody levels to A. actinomyetemcomitans serotype a alone or in combination with B. forsythus, A. actinomyetemcomitans serotype b, P. intermedia or P. gingivalis. Patients with elevated antibody levels to several bacteria had significantly more active sites than those with no or one elevated response. These findings suggested that individuals at risk for further periodontal breakdown could be identified by the presence of elevated antibody levels to several bacteria. We determined antibody titers to P. gingivalis and A. actinomyetemcomitans in 50 patients with periodontitis (5 localized juvenile periodontitis, 14 rapidly progressive periodontitis and 31 adult periodontitis). Patients were classified based on antibody titers. If the antibody titer exceeded two standard deviations above the mean value of the control subjects, the serum was classified as high titer. Twenty-six patients had high titer sera to P. gingivalis, 3 patients showed high titers to both P. gingivalis and A. actinomyetemcomitans and 21 patients exhibited high titers to neither bacteria. The three patients with high P. gingivalis and A. actinomyetemcomitans antibody titers responded poorly to conventional periodontal therapy and were diagnosed as refractory periodontitis (87). These studies suggested that assessing the antibody response to periodontopathic bacteria has diagnostic potential for identifying individuals with high susceptibility to periodontal disease.

The IgG subclasses in sera from patients with peri-
odontal disease have been examined. Ebersole (47) investigated the IgG subclasses to A. actinomyetemcomitans, P. gingivalis, P. intermedia, E. corrodens and Campylobacter rectus in localized juvenile periodontitis, rapidly progressive periodontitis, adult periodontitis and normal individuals. IgG3 and IgG1 to A. actinomyetemcomitans were significantly elevated in localized juvenile periodontitis and rapidly progressive periodontitis patients, while IgG2 was similar among the disease groups. Only the rapidly progressive periodontitis patients had IgG4 to A. actinomyetemcomitans. Rapidly progressive periodontitis and adult periodontitis patients had elevated IgG2, IgG1 and IgG4 to P. gingivalis. IgG2 to P. intermedia was elevated in the diseased groups. Patients with rapidly progressive periodontitis had elevated IgG1 to P. intermedia. IgG3 to P. intermedia was not identified in any these groups. IgG4 to E. corrodens was the prominent subclass elicited in response to E. corrodens in the diseased groups. IgG2 was the primary response to C. rectus in the localized juvenile periodontitis and adult periodontitis. The IgG subclass response to these periodontopathogens substantially differed and may be associated with the risk of acquiring periodontal diseases. Lu et al. (128) determined IgG2 concentration to A. actinomyetemcomitans in sera from patients with localized juvenile periodontitis, adult periodontitis, generalized juvenile periodontitis and healthy controls. Serum IgG2 levels were elevated in localized juvenile periodontitis patients compared with healthy controls. The IgG2 antibody levels to A. actinomyetemcomitans were similar among adult periodontitis and generalized juvenile periodontitis patients and healthy controls. No other IgG subclass concentration correlated with periodontal diagnosis except for that of IgG3, which was elevated in white localized juvenile periodontitis patients. This indicated that patients who produced enough IgG2 were resistant to further periodontal destruction.

Avidity is one biological function of antibodies as described before. Mooney & Kinane (147) determined antibody avidities to P. gingivalis in adult periodontitis and rapidly progressive periodontitis patients. The results showed that IgM and IgG avidities to P. gingivalis were lower in patients with rapidly progressive periodontitis than with adult periodontitis. Chen et al. (24) examined the avidity of IgG antibodies reactive with various components from P. gingivalis in rapidly progressive periodontitis patients and normal controls before and after periodontal treatment. The avidity of IgG in rapidly progressive periodontitis sera was lower than the me-
dian avidity of control sera before treatment. However, the avidity in patients significantly increased following treatment. The authors suggested that many rapidly progressive periodontitis patients did not produce protective levels of biologically functional antibody upon natural infection by *P. gingivalis*. However, periodontal therapy stimulated the production of biologically functional antibody in patients with rapidly progressive periodontitis.

As described above, not only the levels, but also other biological functions of antibodies such as IgG subclasses and avidity are implicated in the progression of periodontal diseases. Assessment of those functional characteristics may have diagnostic value in defining susceptibility to periodontal disease.

**Conclusion**

This chapter summarizes the current understanding of the immune response induced by the bacteria involved in periodontal disease and their role in the pathogenesis of periodontitis. Critical antigens of these bacteria were reviewed. These include fimbriae, capsular polysaccharide, hemagglutinin, lipopolysaccharide, enzymes, other protein antigens from *P. gingivalis*, serotype-specific carbohydrate, lipopolysaccharide, leukotoxin, GroEL-like protein (64 kDa), fimbriae and other protein antigens from *A. actinomycetemcomitans*. The importance of carbohydrate antigens in contrast to protein and lipid ones was also discussed. Local and systemic immune responses against these antigens are elicited in periodontitis patients, and increased antibodies against these antigens have been detected in the diseased patients. The level and specificity of the antibody in saliva, gingival crevicular fluid and serum from the periodontitis patients were reviewed. In addition, the functional properties of the antibodies were discussed in terms of subclass distribution and avidity. Factors that regulate immune responses against pathogens are Th1 and Th2 responses. The immune responses in periodontitis, including cell-mediated immunity, hypersensitivity and mucosal immunity, were discussed, and the immune system was defined from an evolutionary perspective. The role of the immunocompetent cells, including macrophages, lymphocytes and natural killer cells, was also reviewed. Based on these data, the potential for a vaccine against periodontitis was discussed. Experimental models using animals and candidate antigens for the vaccine were reviewed.

Types of immunization, including active immunization, passive immunization and DNA vaccination were introduced. The clinical implications of immune responses were reviewed in terms of the diagnostic potential of antibody against periodontopathic bacteria, correlation between serum antibody level and periodontitis therapy and immune responses as markers of susceptibility.

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