Chapter 9
Salivary Bioscience, Immunity, and Inflammation

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Abstract The study of immune and inflammatory markers in saliva has gained increased attention in recent years with the advancements in assay technology and a heightened focus on cross-systems biology and psychoneuroimmunology. Salivary immune markers are important for the study of both oral and systemic health. Salivary inflammation, in particular, has been widely examined across many fields as both an area of interest and a source of confounding variance. In this chapter, we discuss the opportunities and challenges of studying immune markers in saliva and review the current state of knowledge regarding the study of salivary immune biomeasures, including salivary cytokines, C-reactive protein, and immunoglobulins. Analysis and interpretation issues particularly important for studying immune-related analytes, such as the impact of oral and systemic health, the interpretation of the serum–saliva correlation, and multisystem measurement and analysis techniques, are discussed. Finally, we discuss future directions for the study of salivary immune markers and applications of this research to clinical care and health monitoring and surveillance programs.

Keywords Inflammation · Salivary immunoglobulins · Cytokines · C-reactive protein · MMP

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9.1 Introduction

The ability to measure immune markers in saliva provides unique opportunities in the study of inflammation, health, development, and disease risk. Using saliva as a biospecimen in health-related research and clinical programs may help broaden participation in research, increase our ability to monitor and track disease risk, progression, and treatment, and improve the ecological validity of assessments of immune function. The promise of salivary immune markers to advance our understanding of the biopsychosocial factors affecting health and development has garnered the attention of researchers from various fields, including public health, psychology, and medicine. Despite exciting advances in our understanding of the correlates of salivary immune markers, there remain critical validity, reliability, and conceptual questions that need to be addressed regarding the interpretation and implications of our findings.

Salivary immune markers have potential value for measuring oral, mucosal, and systemic immune function, though limitations in our ability to isolate these sources of variability contributes to uncertainty in interpreting salivary immune marker findings. The immune system in the oral cavity includes the functioning of resident immune cells in the mouth (e.g., in the salivary glands), migrating immune cells from systemic circulation, and the mucosal immune system (Bergmeier, 2018). With bidirectional connections to both the gut mucosal and the systemic immune systems, oral immune processes may provide exciting opportunities to use salivary immune markers as indices of immune function (Bergmeier, 2018). The value of salivary immune markers for informing health, however, has not been fully examined in the literature. Researchers in the fields of oral biology, dentistry, periodontology, and oral cancer examine immune markers in saliva to study risk of oral health problems such as dental caries, periodontitis, gingivitis, and oral cancer (Belstrøm, Damgaard, Könönen, Gürsoy, & Gürsoy, 2017; Rhodus, Ho, Miller, Myers, & Ondrey, 2005; St. John et al., 2004; Teles, Likhari, Socransky, & Haffajee, 2009; Zhang et al., 2016) (see Chaps. 8 and 18 for discussions of salivary biomeasures and oral cancers and periodontal medicine). Researchers in fields such as psychoneuroendocrinology and public health typically use salivary immune markers to gain insight into how specific exposures, such as psychosocial stress and environmental toxins, are related to systemic health and well-being. While converging evidence from across these fields point to the value of salivary immune markers in the study and understanding of oral, physical, and mental health, there has been little cross-disciplinary work linking traditional oral biology studies with studies that use salivary immune markers to make inferences about systemic health.

In this chapter, we will discuss the current state of knowledge regarding salivary immune markers in the study of overall health and development. To provide context concerning the study of salivary immune markers in biobehavioral and health research, we discuss the history of the field of psychoneuroimmunology (PNI) and its adoption of salivary bioscience methods to study the biopsychosocial processes underlying health. We also provide recommendations for the investigation and

interpretation of salivary immune markers in biobehavioral and health studies and discuss exciting research and clinical opportunities afforded by advancing the study of salivary immune markers.

9.2 History of Psychoneuroimmunology and the Integration with Salivary Bioscience

While the origins of PNI date back to 1964 (Solomon & Moos, 1964), the field truly blossomed in the 1970s and 1980s (Ader, 2000) through research on brain–immune connections and their implications for adaptive immunity—the slow-acting but highly specific arm of the immune system that hinges on T- and B-cell activation. Throughout the 1980s and 1990s, psychological stress was widely viewed as immunosuppressive (Segerstrom & Miller, 2004). Toward the end of the 1990s and early 2000s, research on immune-to-brain communication (Maier & Watkins, 1998), age-related increases in innate immunity (Papanicolaou, Wilder, Manolagas, & Chrousos, 1998), and depression-related alterations in immune function (Miller & Raison, 2016) steered the field toward studying innate immune inflammation (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). Since that time, the body's rapid response to infection and injury has dominated research in PNI—from the impact of stress on inflammation (Marsland, Walsh, Lockwood, & John-Henderson, 2017; Rohleder, 2014), to immune system contributors to neural development and function (Bilbo & Schwarz, 2012; DiSabato, Quan, & Godbout, 2016), and the clinical implications of all of the above. Throughout these research areas, plasma and serum markers of innate immune inflammation, including pro-inflammatory cytokines (interleukin(IL)-1β, IL-6, and tumor necrosis factor(TNF)-α) and the acute phase C-reactive protein (CRP), dominated inflammation assessments from the measurement of stimulated cytokine levels in vitro to large-scale epidemiologic research.

The salivary compartment may be favorable for studying links between psychosocial factors and inflammation for several reasons, many of which were articulated in a systematic review by Slavish and colleagues on salivary inflammation and stress (2015b). First, saliva allows us to collect biospecimens in ecologically valid contexts with minimal disruption to normal, everyday activities. We can also collect multiple saliva samples across time and with a high sampling frequency while minimizing participant burden. In addition, many inflammatory markers, such as IL-1β and IL-8, are present at high concentrations in saliva and are highly detectable (Byrne et al., 2013).

Interestingly, the history of using salivary immune markers in human psychosocial research has unfolded in a manner similar to the broader field of PNI. In the mid-1990s and early 2000s much of the emphasis was on demonstrating changes in immune markers during stress exposures in the laboratory (Segerstrom & Miller, 2004). However, as the clinical relevance of short-term immune changes in the

laboratory began to receive more scrutiny (Kiecolt-Glaser, Cacioppo, Malarkey, & Glaser, 1992), the emphasis shifted to using salivary markers that are more ecologically valid. The earliest reports of using salivary assays of cytokines in the context of psychological factors emerged in the late 1990s across independent groups (Dugue (1996) included IL-2; Nishanian, Aziz, Chung, Detels, and Fahey (1998) studied soluble TNFα). Studies in early 2000s focused on changes in salivary immune markers as a function of stress exposures, including social (Dickerson, Kemeny, Aziz, Kim, & Fahey, 2004) and physical stressors [exercise—(Ilardo et al., 2001; Minetto et al., 2007); sauna—(Dugue, 1996)]. As the decade advanced, research interests expanded to include associations between salivary immune markers and naturalistic stress exposures [police officers' shifts—(Zefferino et al., 2006); exams—(Lester, Brown, Aycock, Grubbs, & Johnson, 2010)]; neural activity (Master et al., 2009; Slavich, Way, Eisenberger, & Taylor, 2010); emotional and interpersonal experiences (Chiang, Eisenberger, Seeman, & Taylor, 2012; Moons, Eisenberger, & Taylor, 2010); and development (Riis, Granger, Dipietro, Bandeen-Roche, & Johnson, 2015).

Modern PNI research has increasingly focused on how signals from the brain are transmitted to the immune system via neuroendocrine pathways, as well as the impact of immune system signals on neural functioning. For example, previous studies have shown peripheral inflammation is correlated with task-related activation of neural regions associated social-affective processes (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008; Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009; Muscatell et al., 2015; Prossin et al., 2011). Some research suggests that similar associations can be found with salivary inflammatory markers (O'Connor, Irwin, & Wellisch, 2009; Slavich et al., 2010). This may be partly due to a process whereby the brain receives information from the local trigeminal nerve during acute oral inflammation (Navarro, Iyomasa, Leite-Panissi, Almeida, & Branco, 2006). Integrating salivary immune processes with neural activity involves assessing multisystem functioning, such as examining whether changes in autonomic and hypothalamic–pituitary–adrenal (HPA) axis measures mediate links between psychosocial factors and immune changes; honing in on intracellular mechanisms linking neuroendocrine signals and changes in immune cells; and examining how immune signals influence critical cellular processes such as cell metabolism and repair. Developments in multiplex technology are now allowing PNI researchers to examine such cross-system relations with more analytes available for assay from single biospecimens. The clinical relevance of these neuroendocrine–immune studies for the health of individuals and groups is of ever-increasing interest.

9.3 Current State of Knowledge

This section synthesizes the current research examining salivary immune markers in humans including the study of cytokines, CRP, matrix metalloproteinases, and markers of humoral immune processes. These biomeasures are either

directly produced by or act synergistically with the immune system in the oral compartment. While not an exhaustive list of salivary immune-related analytes, the analytes discussed below represent the most commonly examined immune markers in saliva.

9.3.1 Functions of Salivary Immune Markers

Salivary Cytokines Cytokines are key signaling molecules of the immune system. While many types of cells may secrete cytokines, the primary sources of cytokine secretion are lymphoid cells that are involved in the initiation, amplification, or attenuation of immune activity. Cytokines are categorized into different families such as interleukins, interferons, growth factors, and tumor necrosis factors, and they differ in their sources of secretion and biologic activities [for review, see Granger, Granger, and Granger (2006)]. Although they are often grouped into pro-inflammatory (e.g., IL-1, TNF-α) and anti-inflammatory (e.g., IL-4, IL-10) categories, many cytokines can have both pro- and anti-inflammatory effects (e.g., IL-6) (Cavaillon, 2001). Cytokine receptors have been identified on central nervous and endocrine system cells [e.g., Besedovsky et al. (1983) and Miyake (2012)], meaning that cytokine concentrations may effect multiple biological systems, the brain, and human behavior (Maier & Watkins, 1998).

Cytokines in saliva have varying origins; they may be expressed by the salivary glands or resident immune cells in the mouth (e.g., in the gingiva), secreted from lymphoid cells that have migrated into the oral mucosa, or come from serum constituents that pass into the oral fluid from the general circulation (Brennan & Fox, 2010; Gröschl, 2009; Moutsopoulos & Konkel, 2018). Salivary cytokines secreted from cells in the oral compartment are thought to be involved in the coordination and mobilization of local oral immune processes (Brennan & Fox, 2010; Moutsopoulos & Konkel, 2018). Whereas cytokines from serum constituents that are found in saliva may, to some extent, reflect systemic immune processes.

Salivary C-Reactive Protein CRP is an acute phase protein primarily synthesized by the liver in response to inflammatory cytokines (Sproston & Ashworth, 2018). CRP activates the complement system, which assists the immune system in killing and clearing pathogens from the body (Sproston & Ashworth, 2018). As such, it is part of the body's systemic inflammatory response and increases quickly after tissue damage or an infection (Sproston & Ashworth, 2018). Both pro- and anti-inflammatory effects of CRP have been described (Sproston & Ashworth, 2018).

CRP in saliva is assumed to be an overflow from the blood compartment, perhaps entering saliva through the inflammatory exudate of gingival tissues (Giannobile et al., 2009), i.e., gingival crevicular fluid (Megson et al., 2010). CRP and other acute phase proteins can also pass from blood to saliva via diffusion through the porous capillaries around the salivary glands, or through a process called ultrafiltration, which is filtration through the spaces between salivary gland cells (Pfaffe, Cooper-

white, Beyerlein, Kostner, & Punyadeera, 2011). Recent reports also find evidence of CRP being produced locally by the gingiva (Lu & Jin, 2010; Maekawa et al., 2011). Presumably, CRP has a similar role in saliva as it does in blood—to trigger an immune response. However, no studies to date have examined this explicitly; rather most research has focused on salivary CRP as a biomarker of systemic inflammation.

Matrix Metalloproteinases Matrix metalloproteinases (MMPs) are a family of enzymes that activate leukocytes and help regulate the immune and inflammatory response in the oral cavity (Hannas, Pereira, Granjeiro, & Tjäderhane, 2007; Smigiel & Parks, 2017). MMPs are involved in tissue degradation and restructuring, as well as cell proliferation, migration, and apoptosis (Hannas et al., 2007). The study of salivary MMPs has primarily focused on their ability to index oral health problems. Salivary MMP-8 may be a central biomarker for periodontal disease (Hannas et al., 2007; Zhang, Li, Yan, & Huang, 2018) and has been associated with oral HPV infection (Haukioja, Tervahartiala, Sorsa, & Syrjänen, 2017). In addition, MMP-9 may be a biomarker of malignant disorders (Venugopal & Maheswari, 2016). MMP inhibitors (e.g., TIMP-1) have also been examined in conjunction with salivary MMPs as a measure of MMP regulation [e.g., with HPV; (Haukioja et al., 2017)].

Humoral Immune Markers Immunoglobulins, also known as antibodies, are secreted by B cells and are key aspects of humoral immunity. Immunoglobulins bind to and neutralize specific pathogens, including viruses, toxins, bacteria, and fungi. In salivary research, virus-specific immunoglobulins (e.g., for the Epstein–Barr virus and cytomegalovirus) are often examined as indices of viral exposure or viral load. Chapter 13 reviews salivary antibodies as indicies of pathogen exposure and infection. In this chapter, we focus on total concentrations of select salivary immunoglobulins.

Total salivary concentrations of immunoglobulins A and G are commonly examined in salivary bioscience research and provide important information about humoral immunity in the oral cavity. Secretory IgA (SIgA) and IgG are the two most common immunoglobulins in saliva (Brandtzaeg, 2013). IgG enters saliva primarily from circulation through crevicular fluid (Brandtzaeg, 2013), and some IgG is produced within the oral cavity by the salivary glands and gums (Brandtzaeg, 2013). IgG protects the body from infection by a variety of viral, bacterial, and fungal pathogens. For these reasons, whole saliva total IgG may be a valuable indicator of oral mucosal inflammatory disease, such as periodontitis (Taubman & Smith, 1993).

SIgA, the secreted form of the IgA antibody, is integral to the immune function of mucous membranes and plays major role in gut immunity and mucosal homeostasis (Mantis, Rol, & Corthésy, 2011). Salivary SIgA is produced by plasma cells in the salivary glands and is secreted via exocytosis (Brandtzaeg, 2013). As part of the adaptive immune system, salivary SIgA is more dynamic than other salivary proteins and its secretion from the parotid gland is reportedly more stable than its oral concentration (Brandtzaeg, 2013). SIgA in the mouth plays an important role in protecting epithelial cells and teeth from bacteria, toxins, and viruses (Teeuw, Bosch, Veerman, & Nieuw Amerongen, 2004).

9.3.2 *Do Salivary Immune Markers Reflect Systemic Immune Processes?*

The strength of the associations between immune and inflammatory markers measured in blood and whole saliva differ depending on the marker. Below we summarize the current understanding of the extent to which key salivary immune markers represent systemic immune functioning, and we offer suggestions regarding the interpretation of salivary immune marker concentrations.

Salivary Cytokines There is limited evidence supporting significant associations between concentrations of cytokines measured in saliva and blood. Importantly, these cross-specimen relations also vary considerably by cytokine. For example, Williamson and colleagues (2012) examined 27 cytokines in saliva and plasma samples from healthy adults and found that only three, interferon (IFN)-ɣ, IL-6, and macrophage inflammatory protein-1β, showed significant associations across biospecimen (at medium effect sizes—correlation coefficients = 0.31 − 0.34). A significant serum–saliva correlation for IFN-ɣ (as well as IL-2 and IL-12p70) was also found in a study of adolescents (Byrne et al., 2013). However, these relations were only significant when non-detectable cytokine concentrations (>80% of serum concentrations) were replaced with zero, rather than excluding them (Byrne et al., 2013). A significant and moderate saliva to blood correlation was also reported for IL-6 in a sample of young men (Nam, Kim, Chang, & Kho, 2019), and Fernandez-Botran and colleagues (2011) also reported a modest plasma–saliva correlation ($r = 0.29$, $p < 0.05$) for IL-6 in a sample of post-menopausal women. However, this correlation was only significant for biospecimens collected on one of two study visits; the correlation between plasma and salivary IL-6 collected during the second visit was only 0.10 ($p = 0.41$) (Fernandez-Botran et al., 2011). Other studies have also failed to find an association between salivary and plasma levels of IL-6 (e.g., Cullen et al. 2015) including another study of post-menopausal women which found that GM-CSF and IL-5 demonstrated significant serum–saliva correlations, but levels of IFN-ɣ, IL-10, IL-1β, IL-2, IL-4, IL-6, IL-8, and TNF-α did not (Browne et al., 2013). Furthermore, Riis and colleagues (2014) measured GM-CSF, IFN-ɣ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, and TNF-α in serum and saliva samples from adolescent girls and found that only IL-1β showed a significant serum–saliva correlation. However, this association was weak and not consistent across time (Riis et al., 2014). It is important to note that the latter study statistically corrected for multiple comparisons, which is not yet standard in salivary bioscience studies but may be appropriate in studies examining multiple immune markers simultaneously. Given the current evidence, salivary cytokine concentrations should generally be interpreted as measures of oral immune processes, rather than systemic immune function.

C-Reactive Protein Of all the immune markers discussed in this chapter, salivary CRP has the strongest evidence supporting a significant and meaningful correlation between systemic and salivary concentrations. Several studies have shown

significant correlations between blood and salivary concentrations of CRP (Browne et al., 2013; Byrne et al., 2013; La Fratta et al., 2018; Ouellet-Morin, Danese, Williams, & Arseneault, 2011; Out, Hall, Granger, Page, & Woods, 2012; Pay & Shaw, 2019; Punyadeera, Dimeski, Kostner, Beyerlein, & Cooper-White, 2011). These studies find medium to large effect sizes for correlations between serum or plasma and salivary CRP (correlation coefficients = 0.38 − 0.92). Significant serum–saliva correlations for CRP are not, however, a universal finding [e.g., Dillon et al. (2010) and Pay and Shaw (2019)]. Although additional research is needed in healthy and clinical populations, the extant literature suggests that CRP may be a salivary immune marker that has significant associations with systemic inflammatory processes and measures of physical and psychological health.

Salivary MMPs and Immunoglobulins Salivary MMPs and immunoglobulins are typically used to index oral mucosal immune processes (Lahdentausta et al., 2018; Rathnayake et al., 2013; Salminen et al., 2014) and are not generally examined in relation to serum levels. SIgA is secreted from the mucous membranes, so associations with systemic IgA are not generally examined. While most salivary IgG leaks into the oral compartment from the blood, salivary IgG concentrations are thought to correlate with oral health and periodontal problems (Brandtzaeg, 2013; Taubman & Smith, 1993). Therefore, the primary utility of these indices is likely as measures of local oral and/or mucosal, rather than systemic health.

Recommendations The current literature does not support the use of most salivary immune markers as indices of systemic health. Most of these studies, however, examined relations within healthy samples and some used assay kits not validated for use with saliva. It is also important to note that the strength and nature of associations between immune markers in saliva and blood may depend on the oral and/or physical health of the population studied. Acute illness or inflammation of either the oral compartment (e.g., periodontitis) or systemically (e.g., infection) may affect the extent to which salivary and serum immune markers are correlated. For example, in a sample of healthy adolescents with no signs of oral disease, the serum–saliva correlation for CRP varied by level of serum CRP (Byrne et al., 2013). Among adolescents with higher levels of systemic inflammation (higher serum CRP), there were similarly high levels of salivary CRP, and CRP was significantly associated across biospecimen (serum–saliva correlation: $r = 0.62$, $p < 0.01$) (Byrne et al., 2013). In contrast, among adolescents with lower levels of systemic inflammation (lower serum CRP), salivary CRP did not correlate with serum CRP (serum–saliva correlation: $r = 0.11$, $p = 0.79$) (Byrne et al., 2013). Similar findings have been found in other studies of salivary and serum CRP (Pay & Shaw, 2019).

In addition to oral and physical health, age and developmental stage may also influence the strength and nature of serum–saliva correlations for salivary immune markers. For example, very early in development, before the emergence of teeth, and during old age, when there is typically an increase in medication use and/or disease pathologies, may represent periods of life when salivary immune markers are more or less related to systemic immune function. Variation in serum–saliva correlations

by age and health/disease status, however, has not yet been fully examined in the literature.

It is also important to consider the coordinated and synergistic nature of immune processes in the oral cavity when interpreting serum–saliva associations. Salivary immune markers tend to be highly intercorrelated and more variable than immune markers in serum (Riis et al., 2014). The dynamic local immune environment of the oral cavity therefore likely influences salivary concentrations of immunosensitive markers more so than serum concentrations. For example, adiponectin, an immunosensitive biomeasure of metabolic function, exhibits a stronger serum–saliva correlation after accounting for variance related to oral inflammation (salivary cytokines and MMP-8) (Riis et al., 2017). Similar processes likely affect the serum–saliva associations of immune markers such as CRP and cytokines. Recent findings that CRP is produced within the oral compartment (Lu & Jin, 2010; Maekawa et al., 2011) highlight the importance of controlling for local oral immune processes even when significant serum–saliva associations are found. Future studies that are able to parse the variance in salivary immune markers due to oral and systemic health will provide important information about the extent to which immune measures in saliva may reflect systemic immune processes. Furthermore, rigorous examinations of salivary immune markers that consider oral and systemic disease states and include diverse samples are needed to fully understand the utility of salivary immune markers as indices or oral and systemic health.

9.3.3 Are Salivary Immune Markers Sensitive to Stress?

The opportunity to examine neuroendocrine–immune relations with salivary biomeasures has been an exciting advancement for biobehavioral and health researchers, especially those in the field of PNI. Encouraging findings from salivary immune marker studies illustrate the bidirectional relationship between stress, stress-related pathologies, and immunity. For example, salivary measures of immunity (i.e., salivary SIgA, SIgA1, SIgA2, and secretory component) and early-life inflammatory events (indexed by salivary SIgA) have been correlated with various pathologies including anxiety and post-traumatic stress disorder (Ulmer-Yaniv et al., 2018). Others have used salivary immune markers to show a link between immunocompetence and psychological stress [e.g., Engeland et al. (2016)].

Despite a relatively deep and growing literature examining neuroendocrine–immune relations in saliva and the stress sensitivity of salivary immune markers, the interpretation of these findings is complicated by the study of a wide range of populations and stressor paradigms in a variety of laboratory and real-world settings. It is also important to note that the sensitivity of an individual's immune and inflammatory responses to stress are also influenced by several factors, including age and developmental stage, history of chronic and acute stress exposure, the nature

of their neuroendocrine response, and the nature and intensity of the stressor (acute vs. chronic). Methodological oversights, such as inadequate adjustment for oral health and salivary flow rate, also make cross-study comparisons difficult. With these caveats in mind, we present the current findings of stress-related change in salivary cytokines, CRP, MMPs, and immunoglobulins below.

Cytokines Although studies are limited, acute laboratory stress has been somewhat consistently associated with increased levels of inflammatory cytokines in saliva including IL-1β, TNF-α, and IL-6 [for review, see Slavish, Graham-Engeland, Smyth, and Engeland (2015a)]. Furthermore, there is evidence from one study that salivary inflammatory reactivity to acute social-evaluative stress is positively associated with neural activity during a social rejection task (Slavich et al., 2010).

In assessing the strength and nature of salivary cytokine stress responses, it is important to consider how individual and environmental factors may influence the immune response. For example, there is some evidence that age and sex may moderate the salivary cytokine response to acute stress. One study of adults found greater increases in salivary cytokine (IL-6, IL-8, IL-10, and IL-4) concentrations after a pain stressor task among older, compared to younger, adults (mean age of older adults = 68.3; mean age of younger adults = 21.4 years) (Sorenson et al., 2017). The use of a pain stressor task in this study is important to note, as physical stressor tasks may not elicit the same type of neuroendocrine stress response as social-evaluative, emotional, and/or socio-cognitive stressor tasks employed by other studies. However, consistent with a pattern of age-related increases in salivary cytokine stress reactivity, two studies of preschool-aged children found stable and/or decreasing pro-inflammatory salivary cytokine trajectories across a series of emotional and cognitive lab stressor tasks (Riis et al., 2015; Tyrka, Parade, Valentine, Eslinger, & Seifer, 2015). While a study of elementary school-aged children (mean age = 8.75) found mixed stress-related trajectories for salivary IL-6 with 55% decreasing and 45% increasing in IL-6 concentrations across the study tasks (El-Sheikh, Buckhalt, Granger, Erath, & Acebo, 2007). Two of these studies of cytokine stress reactivity among children also found stress-related pro-inflammatory salivary cytokine trajectories varied by sex (El-Sheikh et al., 2007; Riis et al., 2015).

Beyond sample demographics, participant characteristics and their subjective approach to the lab stressor tasks may further moderate stress-related salivary cytokine concentrations. For example, individual affect, cognitive control, and attention to the task, as well as history of trauma and level of perceived discrimination, have all been shown to moderate pro-inflammatory cytokine stress responses in the lab (Maydych, Claus, Watz, & Kleinsorge, 2018; Newton et al., 2017; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016; Szabo, Fernandez-Botran, & Newton, 2019)

Outside the laboratory, several studies find associations between real-world stressors and levels of pro-inflammatory cytokines. Early-life adversity, fear of deportation, and chronic family stress have been associated with elevated baseline concentrations of inflammatory cytokines in saliva (Martínez, Ruelas, & Granger, 2018; Tyrka et al., 2015). Perceived discrimination stress has also been associated

with baseline salivary IL-6 concentrations among homosexual adults (Doyle & Molix, 2016). However, echoing the importance of accounting for individual differences, these relations varied by sex and individual expression of identity (Doyle & Molix, 2016). Associations between real-life acute stress and salivary markers of inflammation are similarly complex. La Fratta and colleagues found that salivary IL-1β and IL-8 were higher in anticipation of an academic exam and decreased after, while IL-6 increased from pre- to post-exam (2018). Importantly, these patterns of stress-related change in salivary cytokines were mirrored in plasma cytokine levels (La Fratta et al., 2018). Similar findings were reported from a study of undergraduate students performing a real-life public speaking task; salivary IL-1β decreased from pre- to post-task (Auer, Calvi, Jordan, Schrader, & Byrd-Craven, 2018). Despite an overall declining IL-1β trajectory, participants with higher levels of social anxiety had greater increases in IL-1β concentrations across the task (Auer et al., 2018). These real-world examinations of acute stress and inflammation in the oral compartment may seem contradictory to lab-based study findings of increases in pro-inflammatory cytokines in response to stressor tasks. However, pre-stressor levels in real-world studies likely reflect anticipatory stress levels, rather than true baseline levels (as measured in the lab).

CRP There are only a few studies examining acute stress-related changes in salivary CRP, and these studies point to a complex stress response model for salivary CRP. In a series of studies of African American adults, Lucas and colleagues found that changes in salivary CRP across the Trier Social Stress Test (TSST) were related to self-reported subjective stress, stress appraisal, affect, and level of perceived discrimination during the task (Laurent, Lucas, Pierce, Goetz, & Granger, 2016; Lucas et al., 2016, 2017). Furthermore, relations between stress-related levels of salivary CRP and cortisol also varied by individual stress appraisals during the task (Laurent et al., 2016). Given the complexities demonstrated in the Lucas studies, it is not surprising that other studies have failed to find significant changes in salivary CRP across the TSST [e.g., Campisi, Bravo, Cole, and Gobeil (2012)]. Outside of the laboratory, a study of acute socio-cognitive exam stress also found no significant stress-related changes in salivary nor serum CPR among young adult men (La Fratta et al., 2018).

Studies of chronic stress and adversity, however, report relatively consistent associations between life stressors and salivary CRP. For example, poor parental monitoring and fewer positive parenting behaviors have been associated with higher levels of salivary CRP in children and adolescents (Byrne, Badcock, et al., 2017; Byrne, Horne, et al., 2017). Similarly, early-life adversity has been linked to higher levels of salivary CRP among infants (David, Measelle, Ostlund, & Ablow, 2017; Measelle, David, & Ablow, 2017). However, similar relations were not found when examined among young children [e.g., Tyrka et al. (2015)]. Underscoring the complexity of the salivary CRP stress relations and their implications for health and well-being, Maldonado and colleagues found that salivary CRP moderated the relation between acculturative stress and anxiety in a sample of adult Latino Americans (2018).

Immunoglobulins SIgA secretion is under neuroendocrine control and salivary concentrations are sensitive to acute and chronic stress (Brandtzaeg, 2013; Teeuw et al., 2004). There is evidence that SIgA increases in response to acute stress and is suppressed during chronic stress (Birkett, Johnson, & Gelety, 2017; Brandtzaeg, 2013; Laurent, Stroud, Brush, D'Angelo, & Granger, 2015; Ohira, 2005; Phillips et al., 2006). Chronic psychological stressors have been associated with lower concentrations of the IgA1 subtype in particular (Engeland et al., 2016). However, neuroendocrine stress responses, affect, externalizing problems, and emotional support experienced during the acute stressor may moderate SIgA changes in response to acute stress (Laurent et al., 2015; Ohira, 2005).

Results from studies of real-life stressors are mixed. A large study of adults found that SIgA secretion rate was inversely associated with life stress load (Phillips et al., 2006). However, results from a study of mother–child dyads found higher SIgA concentrations among war-exposed women and adolescents compared to controls, even a decade post trauma (Ulmer-Yaniv, Djalovski, Priel, Zagoory-Sharon, & Feldman, 2018; Yirmiya, Djalovski, Motsan, Zagoory-Sharon, & Feldman, 2018). In the same sample, SIgA concentrations were positively associated with symptoms of depression among mothers, and internalizing, externalizing, and anxiety symptoms among their children (Ulmer-Yaniv, Djalovski, Priel, et al., 2018; Yirmiya et al., 2018). Byrne and colleagues found complementary results with higher child salivary SIgA concentrations associated with poor parental monitoring (2017).

Exercise Stress Both immune (e.g., SIgA and IgG) and inflammatory (e.g., CRP) markers are implicated in the healthy adaptation to exercise stress and training. Inflammation is a necessary component of muscle regeneration, and it has been shown that use of non-steroidal anti-inflammatory drugs can attenuate muscle protein synthesis (Bondesen, Mills, Kegley, & Pavlath, 2004). Findings in salivary immune markers are mixed. Some studies of salivary SIgA in response to acute exercise find no change in analyte concentrations and secretion rates, while others find increases or decreases across the exercise and recovery period (Campbell & Turner, 2018; Keaney, Kilding, Merien, & Dulson, 2018). The effects of longer physical training periods among athletes have found reductions in SIgA during high training periods (Keaney et al., 2018). Although, these findings are not universal, and the implications for health are unclear (Campbell & Turner, 2018).

Reports on salivary CRP responses to exercise stress are limited. A pilot study of men who completed a maximal effort exercise test found no changes in salivary CRP across the task (Hernandez, Fuller, Stone, Carpenter, & Taylor, 2016). In ultra-endurance athletes, there was an increase in salivary CRP after a long-distance run, but not after an open water swim (Tauler, Martinez, Moreno, Martínez, & Aguilo, 2014). Exercise-related increases in salivary cytokines, including IL-1β, IL-6, TNF-α, and IL-8, have been found among adults (Hayashida, 2016; Slavish et al., 2015b). However, these effects are not universal. Exercise-related changes in IL-6 are especially varied and may depend on the type of exercise and the sample

collection protocol (e.g., swab vs. passive drool) (Cullen et al., 2015; Hayashida, 2016; Slavish et al., 2015b).

Recommendations Several studies have shown that salivary immune markers differ with respect to the sensitivity and directionality of changes in response to stress and these responses vary by the type of stress exposure (Slavish et al., 2015b). Therefore, while many of the salivary immune markers of interest exhibit synergistic physiologic effects, hypotheses about salivary immune marker stress reactivity should consider each analyte individually as well as part of a coordinated immune response in the oral cavity.

Relations between the immune markers and biomeasures of other physiologic systems, such as the HPA and autonomic nervous system, are also particularly important to consider in the context of the stress response. The neuroendocrine stress response may be associated with the magnitude and/or timing of the immune response. There is very little research examining the timing of immune and inflammatory stress responses in the oral compartment and how these stress response profiles vary by individual and stressor characteristics. For example, salivary cortisol research has shown that basal and stress-related hormone profiles may be confounded by personal history of stress exposure, so it is important to control for these confounds or focus on within-person changes when evaluating these hormone levels (Lucas et al., 2016; Slavish et al., 2015a). Similar confounds may affect salivary immune marker concentrations and dynamics. Future studies should directly address these sources of variability.

9.3.4 Do Salivary Immune Markers Exhibit Developmental Trajectories?

The immune system begins developing in utero and continues throughout early childhood (Veru, Laplante, Luheshi, & King, 2014). While adult levels of immunocompetence are typically achieved around age five (Veru et al., 2014), the immune system continues to adapt throughout life to support tissue repair and growth, as well as protect the body against toxic exposures, pathogens, and disease. The flexibility of the immune system in the oral cavity may be particularly important for protecting and maintaining health in the context of a dynamic and variable antigen environment. There are also developmental changes specific to the oral cavity, such as the growth and loss of teeth and the progression of oral diseases, which may influence oral immune function.

Age-related changes in resting concentrations of many salivary immune markers, including cytokines and MMPs, have not been rigorously examined. Many immunoglobulin concentrations in saliva vary across age group. Salivary immunoglobulin levels may be particularly variable during infancy when salivary gland development, breastfeeding, and immunization exposures likely influence their measured concentrations (Brandtzaeg, 2013). SIgA is detectable in early infancy and may increase

with age; although this is not a universal finding [e.g. Evans et al. (2000) and Ewing et al. (2010)] and age trajectories may vary by SIgA subtype (Jafarzadeh, Sadeghi, Karam, & Vazirinejad, 2010; Weemaes et al., 2003).

Salivary CRP concentrations may also increase with age. Looking at salivary CRP concentrations across studies, CRP in saliva tends to be higher in adults compared to children and adolescents. Average or median salivary CRP concentrations in studies of adults typically range from approximately 100–8500 pg/ml (Browne et al., 2013; Laurent et al., 2016; Mohamed, Campbell, Cooper-White, Dimeski, & Punyadeera, 2012; Ouellet-Morin et al., 2011; Out et al., 2012), while levels in adolescents and children are generally lower with mean or median levels in these studies ranging from approximately 7–2030 pg/ml (Byrne, Badcock, et al., 2017; Byrne, Horne, et al., 2017; Byrne et al., 2013; Cullen et al., 2017; Goodson et al., 2014; Naidoo, Konkol, Biccard, Dubose, & McKune, 2012; Shields, Slavich, Perlman, Klein, & Kotov, 2019). Importantly, these summary statistics are drawn from studies of samples across a wide range of ages and body compositions with varying saliva sample collection times, and some measurements were adjusted for flow rate while others were not. Other factors, such as health status and smoking behaviors, may also be confounding the observed age differences in salivary CRP. Additional research is needed to understand the underlying causes and correlates of developmental differences in salivary CRP.

Recommendations Our current understanding of age-related changes in salivary immune markers is largely based on cross-sectional studies and cohort effects. Rigorous, longitudinal examinations of developmental changes in salivary immune markers are needed to further our understanding of age-related changes in the oral immune environment and to help us interpret age-related differences in salivary immune markers. These studies should consider oral and systemic health factors, such as oral health and disease, obesity, diet, and chronic stress exposure, which may influence immune marker concentrations and contribute to age-related differences in analyte concentrations.

9.4 Methodological Issues

In some ways, the widespread incorporation of salivary immune markers in biobehavioral and health research has pushed the study of salivary immune analytes out beyond our methodological understanding of these biomeasures. In this section, we provide our recommendations for current and future research to advance our understanding of salivary immune markers in studies of health and well-being.

9.4.1 Saliva Collection and Processing Methods

In the absence of rigorous research examining the impact of collection technique on each salivary immune marker, the collection of whole unstimulated saliva via passive drool is the recommended "universal" method for saliva collection. Collecting whole saliva allows for the examination of the widest range of salivary analytes and avoids inconsistencies in concentrations of some immune markers that may depend on the idiosyncrasies in oral fluids produced by different salivary glands (Ruhl et al., 2004). SIgA may be particularly sensitive to sample collection and processing procedures (Brandtzaeg, 2007). Investigators should review the existing literature on their specific analyte and consult with their assay manufacturer and laboratory staff when determining sample collection and processing protocols.

With approximately 30,000 neutrophils entering the gingival crevice from the blood every minute and cytokine expression across various oral glands and tissues (Gröschl, 2009; Moutsopoulos & Konkel, 2018), differences in immune marker concentrations across biospecimen type (e.g., whole saliva vs. crevicular fluid) is expected. For example, IgG concentrations will be higher in crevicular fluid than in whole saliva as IgG enters the oral compartment primarily via crevicular fluid and may be diluted in whole saliva (Brandtzaeg, 2013). Biospecimen type may also influence the strength of the association between oral immune markers and those measured in serum (Nishanian et al., 1998).

Salivary flow rate is another important factor to consider when examining salivary immune marker concentrations. Analytes that are produced locally, such as SIgA, and those that are brought into saliva from circulation via the crevicular fluid, such as salivary CRP and total IgG, have known associations with flow rate (Brandtzaeg, 2013; Pay & Shaw, 2019). The impact of flow rate on other salivary immune markers, such as salivary cytokines, is less clear. Given the lack of rigorous investigation of flow rate and immune markers in saliva, investigators should record saliva collection duration and sample weight in studies that will examine salivary immune markers. This will allow researchers to assess the impact of flow rate on analyte concentrations. Chapter 4 discusses flow rate calculations and statistical adjustments for flow rate.

9.4.2 Statistical Considerations and Cautions

Salivary immune markers typically display a strong positive skew with a long tail of very high concentrations. Some markers, like IL-10, are typically present at very low concentrations and may even be undetectable with current assay technology (Browne et al., 2013; Byrne et al., 2013; Riis et al., 2014; Shields et al., 2019). Others, like IL-8 and IL-1β, are generally present at very high concentrations in saliva (Browne et al., 2013; Byrne et al., 2013; Riis et al., 2014, 2015, 2017; Shields et al., 2019). The interpretation and statistical handling of such varying levels of

salivary immune markers is complicated by a lack of established norms and cutoffs. We refer investigators to Chap. 4 for a review of how to address kurtotic and skewed distributions. The pre-analytic processing of salivary immune marker data is especially important given their unique distribution and ranges which may preclude the use of raw analyte data in parametric statistical models. We recommend that researchers report their data processing steps and the results from both raw and cleaned (e.g., extrapolated, replaced, or transformed) data in their manuscripts.

The immunoassay and multiplex assay technology used to measure immune markers in saliva is very advanced. Commercially available tests consistently report detection limits in the very low pg/mL range, and modern multiplexing assays have log-scale dynamic ranges. These advances in technology allow for the simultaneous testing of a large number of analytes. While this provides a wealth of information, highly correlated determinations of salivary immune markers from each individual may require advanced statistical modeling to parse the variance and individual effects of each immune marker alone and as part of a coordinated immune response. Investigators should also be particularly aware of issues related to collinearity among salivary immune markers. Chapter 4 reviews statistical approaches and methods used in salivary biomeasure studies. We also present code for an interactive case study with mock salivary immune marker data here (https://github.com/michellebyrne1/immune-multiplex). This web link allows the reader to explore issues related to salivary immune marker multiplexing, collinearity, and data cleaning, and how differences in data processing steps may affect study results. It is important to note that the data cleaning procedures included in our web link serve as simplified examples of steps investigators have traditionally taken to help visualize and process their salivary immune marker data. Some of these approaches are now outdated, and we recommend researchers read Chap. 4 for a detailed review of and guidelines for salivary biomeasure data cleaning and analysis.

While there are generally high correlations among salivary inflammatory markers, analytes should be examined both together, as an overall index of inflammation, and as separate analytes with potentially unique mechanisms of action and associations with other analytes and health conditions. The production of salivary immune markers and their individual roles in both local and systemic immune processes may be unique. For example, IL-8 is released by neutrophils, the most common immune cell entering the mouth, and is involved in neutrophil migration and inflammation in the oral compartment (Hasturk, Kantarci, & Van Dyke, 2012; Moutsopoulos & Konkel, 2018). With concentrations of IL-8 in saliva generally higher than many other cytokines [e.g., Browne et al. (2013), Byrne et al. (2013), La Fratta et al. (2018), Riis et al. (2014, 2015, 2017), and Shields et al. (2019)], salivary IL-8 may play an important and specific role in oral health and disease [e.g., Belstrøm et al. (2017), Finoti et al. (2017), Sahibzada et al. (2017), and St. John et al. (2004)]. Despite robust correlations between IL-8 and other inflammatory cytokines, IL-8 may have unique physiologic effects worthy of independent investigation.

Finally, rapid technological advancements in our ability to study salivary immune markers should be paired with rigorous and disciplined scientific study designs and

analytic testing. Unless research studies are explicitly exploratory, specific hypotheses for each immune marker and research question should be stated a priori. Researchers can preregister these hypotheses on a platform such as the Open Science Framework. Even when hypotheses are exploratory, researchers should correct for multiple comparisons by using a Bonferroni correction, or similar method for conducting multiple statistical tests, in order to reduce Type I error [e.g., Riis et al. (2014)].

9.4.3 Confounding Factors and Covariates

Smoking Behavior and Tobacco Exposure Smoking is an important confounder in the measurement of salivary immune markers. Smoking may have acute and chronic effects on the oral immune environment. Levels of salivary cytokines, CRP, immunoglobulins, and MMPs have been associated with smoking and environmental tobacco smoke exposure (Azar & Richard, 2011; Evans et al., 2000; Ewing et al., 2010; Haukioja et al., 2017; Lira-Junior, Åkerman, Gustafsson, Klinge, & Boström, 2017; Riis et al., 2014, 2015). Oral health and disease are also strongly associated with smoking behavior and environmental tobacco smoke exposure (Hanioka et al., 2019; Hasmun et al., 2017; Javed, Ahmed, & Romanos, 2014). In the absence of self-reported smoking data, salivary cotinine can be assessed to test for exposure to nicotine. However, cotinine levels will only reflect recent exposure (approximately exposure in the past 16 h) and will not provide information about the duration of smoking behavior which may be important for understanding the impact of smoking on the oral immune system (Jarvis, Russell, Benowitz, & Feyerabend, 1988).

Blood Leakage into Saliva Given their role in maintaining oral health and their potential transport into saliva via crevicular fluid, many of the salivary immune markers discussed in this chapter may be influenced by blood leakage into saliva. Associations between immune makers and blood leakage may be due to increased passage of serum immune markers into saliva and/or increased local production of salivary immune markers in response to injury in the oral compartment. As such, blood leakage may affect both salivary immune maker levels and the observed serum–saliva correlation.

Saliva samples should be visually examined for contamination by blood and flagged if there is evidence of contamination [see Kivlighan et al. (2004) for details]. Blood leakage in the oral compartment can also be assessed with salivary transferrin and used as a statistical control.

Oral Health In dentistry, periodontology, oral cancer, and public oral health research, oral immune markers, such as MMPs, immunoglobulins, and cytokines, have been associated with oral inflammation, disease, and health (Rangbulla, Nirola, Gupta, & Batra, 2017; Rathnayake et al., 2013; Salminen et al., 2014; Taba, Kinney, Kim, & Giannobile, 2005; Taubman & Smith, 1993). Nearly half of all adults in the USA have periodontitis and rates of periodontitis vary by racial/ethnic group (Eke

et al., 2018). Biobehavioral and health researchers, however, often fail to appropriately assess and adjust for oral health in their studies of salivary immune markers. Unexamined associations between oral health and salivary immune marker concentrations may have considerable impacts on study findings that aim to use salivary immune markers as indices of systemic health. In conceptualizing the role of oral health in these studies, it is important to consider the many ways in which oral immune function may be related to systemic health. For example, oral immune function may: (1) initiate a systemic immune response and/or influence systemic health; (2) be initiated and/or influenced by systemic immune function; and (3) reflect a number of health and immune-related factors that are related to both systemic and mucosal health. Building our understanding of the complex, bidirectional relations among oral, mucosal, and systemic health and immune function requires a thoughtful, interdisciplinary approach to study planning and hypothesis generation, as well as careful measurement and adjustment for potential confounding factors.

The study of cardiovascular disease provides an interesting case study in our developing understanding of oral–systemic immune relations. Atherosclerosis is the primary contributor to cardiovascular disease (CVD). The early phase of atherosclerosis includes the induction of an inflammatory response which eventually leads to arterial plaque development (Berliner et al., 1995). Periodontal disease (PD) is a bacteria-induced infection which also elicits an inflammatory response (Basconés-Martínez et al., 2009). An initial study in 1989 reported that oral health status was associated with myocardial infarctions (MI), commonly known as heart attacks (Mattila et al., 1989). While a causal relation between PD and atherosclerotic vascular disease has not been established, the evidence to date supports a modest association between these two diseases (Lockhart et al., 2012; Scannapieco, Bush, & Paju, 2003). These relations may hint at important cross-system connections between oral disease and other physical and mental health disorders as dysregulated inflammation is considered a pathophysiologic mechanism for a number of health conditions, including diabetes, depression, and Alzheimer's Disease (Dai, Golembiewska, Lindholm, & Stenvinkel, 2017; Manthiram, Zhou, Aksentijevich, & Kastner, 2017; Saltiel & Olefsky, 2017).

Covariation among oral health problems and systemic health and disease risk is an interesting and important area of research to which salivary bioscience investigators can make substantial contributions via the study of salivary immune markers. While aiming to disentangle the effects of oral, systemic, and mucosal immune function on health, this line of research will also need to examine covariation in oral and systemic health from a broader, social perspective. In the USA, there are vast overlapping disparities in health and access to care for both oral and systemic health; in many cases, individuals at higher risk for oral health problems are also at higher risk for physical and mental health problems (U.S. Department of Health and Human Services, 2000). This covariation is especially important in studies that use salivary immune markers to study the impact of environmental and psychosocial exposures on health. Failing to adjust for oral health in these studies may considerably affect the interpretation and implications of the findings.

The most common approach to assessing oral health in biobehavioral and health studies is via self-report questionnaires. There are several self-report tools and approaches available to assess participants' oral health [e.g., Fisher-Owens et al. (2007), Liu et al. (2016), Nirmal, Ramachandiran, Anand, Sathiamurthy, and Sekaran (2005), and World Health Organization (2013)]. Associations between responses on these questionnaires and levels of salivary analytes related to oral inflammation and disease (cytokines, MMPs, immunoglobulins) have not been fully examined, and, in some cases, no relations have been found [e.g., Riis et al. (2017)]. Concentrations of salivary immune markers, such as MMPs, cytokines, and immunoglobulins may also be used to statistically control for variance associated with oral health (Riis et al., 2017); however, these approaches have also not yet been validated and may not be appropriate when the focus of investigation is inflammation. With marked disparities in oral health and access to oral health care, conceptual overlap among health behaviors for oral and physical health, and known associations between oral, mucosal, physical, and mental health, investigators should carefully consider the role of oral health and inflammation in their research questions and statistical models.

Sex Sexual differentiation of the immune system begins in utero and differences in inflammatory and immune responses by sex are seen across the lifespan (Bouman, Jan Heineman, & Faas, 2005; Klein & Flanagan, 2016; Taneja, 2018). Sex hormones, such as estradiol, may also influence immune marker levels in saliva (Teeuw et al., 2004), and secretion rates of salivary SIgA may vary by sex (Rutherfurd-Markwick, Starck, Dulson, & Ali, 2017). Sex-related differences in salivary immune markers can be seen in children as young as 5 years old (El-Sheikh et al., 2007; Riis et al., 2015, 2016). Sex may influence the levels of immune markers in saliva, as well as their stress sensitivity and associations with other biomeasures (Riis et al., 2015, 2016; Teeuw et al., 2004). Sex may also interact with other important covariates, such as age and developmental stage. For example, the developmental trajectories and the effects of pubertal status and timing on salivary acute phase proteins and cytokines are not yet known. Investigations using salivary immune markers should examine the effects of sex, and sample size calculations may need to anticipate sex-stratified analyses.

Other Health-Related Variables Other factors including mood, psychosocial stressors, sleep, diet, medication use, body mass index, menstrual phase, pregnancy, current physical health, and physical activity [for review, see O'Connor et al. (2009)] should be considered as possible covariates in studies examining salivary immune markers. Factors related to systemic health and functioning have the potential to confound salivary immune measures as well as their relations with health conditions of interest. For example, a meta-analysis examining the methodological rigor in studies of serum CRP and its relations with depression found that only a small number of studies controlled for age, sex, obesity, medical conditions, substance/medication use, and psychosocial factors (Horn et al., 2018). Importantly, the effect size of the association between serum CRP and depression was small in the studies

that controlled for these confounders (Horn et al., 2018). Similar examinations are needed for the appropriate interpretation of salivary immune marker study findings.

Time Since Waking and Diurnal Patterns Circadian and diurnal rhythms are essential for health and wellness. Salivary immune markers may provide insight into these patterns in ways that would not be possible with traditional blood-based assessments of immune function. Unfortunately, to date, few studies have examined the variability in salivary immune markers across the day.

Diurnal patterns have been reported for SIgA (Hucklebridge, Clow, & Evans, 1998; Li & Gleeson, 2004; Pritchard, Stanton, Lord, Petocz, & Pepping, 2017), salivary IL-6 (Izawa, Miki, Liu, & Ogawa, 2013; Reinhardt, Fernandes, Markus, & Fischer, 2019), and salivary CRP (Hernandez & Taylor, 2017; Out et al., 2012; Rudnicka, Rumley, Lowe, & Strachan, 2007). These markers share a common general pattern with higher levels in the morning and lower levels in the afternoon. Specifically, the diurnal cycle for SIgA begins with a peak upon awakening, and then an immediate decline to stable concentrations across the rest of the day (Hucklebridge et al., 1998; Pritchard et al., 2017). Salivary CRP levels are also elevated upon awakening and decline thereafter (Hernandez & Taylor, 2017; Izawa et al., 2013). Interestingly, the diurnal pattern for CRP may be specific to the oral compartment as studies of serum CRP have failed to find a significant pattern in diurnal serum CRP concentrations and it has been reported to be stable over a 24-h period (Meier-Ewert et al., 2001; Rudnicka et al., 2007). Compared to SIgA and CRP, the decline of salivary IL-6 after awakening is muted. There may also be an increase in concentrations proximal to bedtime (Izawa et al., 2013; Sjögren, Leanderson, Kristenson, & Ernerudh, 2006). This pattern is different than those observed in serum and plasma IL-6 which have been found to exhibit a "morning trough" without a morning or evening peak (Nilsonne, Lekander, Åkerstedt, Axelsson, & Ingre, 2016).

There is limited research examining the diurnal pattern of other immune markers in saliva. However, given the overlapping functions of many of the immune analytes of interest, investigators should consider time of waking and time of day when examining immune markers in saliva. It is also important to note that the physiologic mechanisms underlying the diurnal patterns in salivary immune markers may reflect processes specific to the oral environment, and this may be especially true for analytes with diurnal patterns that are unique to salivary concentrations and not observed in blood measurements. On the other hand, diurnal patterns in salivary immune markers may also reflect coordinated action across multiple physiologic systems within the body. For example, marked diurnal changes in HPA axis activity, reflected in salivary cortisol concentrations, may influence concentrations of immune markers in saliva across the day, and, given cortisol's potent anti-inflammatory properties, these effects may vary by analyte.

Beyond a methodologic confound, the physiological significance of a biomeasure's diurnal pattern has potential implications for health and disease outcomes. For example, sleep quality, duration, and disorders have been associated with variation in some salivary immune markers [e.g., El-Sheikh et al. (2007), Nizam,

Basoglu, Tasbakan, Nalbantsoy, and Buduneli (2014), and Reinhardt, Fernandes, Markus, and Fischer (2016)]. Given sleep's crucial role in repair and restorative processes, studies of the diurnal patterns of salivary immune markers may provide important information about health and disease risk. Closely monitoring changes in immune marker values across the day and understanding the relationships between these patterns and biobehavioral correlates (e.g., activity, diet, and blood pressure) may provide insight into optimizing health and reducing disease risk.

9.4.4 Salivary Immune Marker Stability and Reliability

Establishing measurement reliability is a key component of validating new biomeasures for the study of health and development. Measurement reliability for salivary immune markers at the level of the assay is generally strong and has improved considerably with the development of new assay technology. Coefficients of variation for these assays are typically low and within the accepted range for salivary bioscience studies (<5% for intra-assay CV; <15% for inter-assay CV). In contrast, the within-person short- and long-term stability of most salivary immune markers and acute phase proteins is largely unknown.

Given their diurnal patterns, the stability of some salivary immune marker concentrations likely varies by sampling time. For example, in a study of healthy young adults, concentrations of salivary IL-6 across two consecutive days showed weak to strong correlations depending on the sample timing (r's = 0.21 − 0.90) (Izawa et al., 2013). The strongest associations in salivary IL-6 concentrations across the two days were found at night and early in the morning (Izawa et al., 2013). In the same study, salivary CRP concentrations were correlated across two consecutive days of sampling (r's = 0.23 − 0.96), and, as seen with IL-6, the strength of the associations varied by sampling time (Izawa et al., 2013). The weakest across-day associations for salivary CRP were found in the afternoon and early evening (Izawa et al., 2013). For saliva samples collected on the same day, one study of adolescent girls found salivary IL-1β, IL-6, IL-8, TNF-α, IL-18, and CRP displayed moderate/strong correlations (r's = 0.38 − 0.81; p's < 0.05) across a 120 min waiting period (Shields et al., 2019).

Few studies have examined the long-term stability of salivary immune markers. One study examining salivary cytokines in healthy adolescent girls across a 3-year study period found that across-year correlations varied by cytokine and were generally weak to moderate in strength (r's = 0.02 − 0.46) (Riis et al., 2014). These findings were largely confirmed by another study of salivary IL-1β, IL-6, IL-8, TNF-α, IL-18, and CRP in adolescent girls which found similarly weak correlations across an 18-month period (r's = 0.04 − 0.31) (Shields et al., 2019). In this study, salivary CRP stood out as one of the more stable immune markers with a correlation of 0.31 ($p < 0.001$) across the 18-month study period (Shields et al., 2019). More robust long-term stability in salivary CRP was also found in a study of adult women

with salivary CRP showing moderate stability across 3 years of assessments (r's = 0.46 − 0.61, p <0.01) (Out et al., 2012).

The oral compartment is exposed to the outside world and therefore vulnerable to a wide range of threats from which immune processes in the blood are protected. The oral cavity is also home to extremely dense and diverse communities of microbes which influence oral, and potentially systemic, health (see Chap. 7 for a discussion of the oral microbiome). Oral and physical health may therefore rely on a local, oral immune response that is inherently more variable than that observed in blood. Baseline concentrations and changes in immune markers in saliva may represent immune processes responding to extrinsic factors, such as exposure to environmental pollutants and antigens (e.g., tobacco smoke and pollen), intrinsic factors related to oral health (e.g., dental caries and bacterial load), as well as intrinsic factors related to systemic health (e.g., infection or fever). Researchers should take these factors into consideration when interpreting between and within-person differences in salivary immune marker concentrations.

9.5 Future Directions and Opportunities

The integration of salivary immune markers into biobehavioral studies of health has provided insight into the complex mechanisms underlying health and development. These studies have allowed us to measure relations between salivary immune markers and the family environment (Byrne, Badcock, et al., 2017; Byrne, Horne, et al., 2017), social stress (Lucas et al., 2016, 2017; Slavich et al., 2010; Slavish et al., 2015a), sleep (El-Sheikh et al., 2007; Zheng et al., 2014), and depression and other mental health symptomatology (Cicchetti, Handley, & Rogosch, 2015; Delany et al., 2016; Keller, El-Sheikh, Vaughn, & Granger, 2010; Riis et al., 2016), as well as neuroendocrine–immune variation in response to environmental and psychosocial stressors (O'Connor, Irwin, & Wellisch, 2009; Riis et al., 2015; Slavish et al., 2015b). Despite this progress, we believe that furthering the study of salivary immune markers requires rigorous methodological investigations as well as an interdisciplinary, cross-systems approach to the study of health and development. Through this deliberate advancement of salivary immune marker research, we hope to gain a deeper, more nuanced, and meaningful understanding of the processes influencing health.

Multisystem Assessments The oral immune environment is complex with multiple analytes being secreted, expressed, and transported into the oral fluid from different areas of the mouth and body. Therefore, while many immune markers may work synergistically in the oral compartment and salivary concentrations often strongly covary within individuals, single marker assessments of immune function may not be useful for understanding oral or systemic immune processes as a whole and should not be interpreted as such. Instead, models that measure multiple analytes can highlight the synergistic effects that occur within the immune system, such as ratios

between pro- and anti-inflammatory cytokines, as well as associations between inflammatory markers and other markers of immune activation such as SIgA. A critical aspect of examining oral immune processes may also lie in the oral microbial environment. While a relatively new area of study, advancing understanding of how the oral immune system interacts with these microbial communities, and how these interactions effect oral and physical health is a growing and important area of research (see Chap. 7 for a review of the oral microbiome). Finally, multisystem studies that use salivary biomeasures to examine how the immune system interacts with other biological processes, such as gonadal and neuroendocrine function, will be especially important for advancing our understanding of overall health and development.

It is also vital to measure immune system functioning along other analytes that are immune sensitive, such as cortisol. Several salivary bioscience studies have reported neuroendocrine–immune associations in behavioral science (Laurent et al., 2016; Riis et al., 2015, 2016). Neuroendocrine–immune relations are also implicated in the pathophysiology of many diseases. For example, chronic inflammation may trigger a cycle of glucocorticoid resistance or negative feedback associated with depression (Pariante & Miller, 2001). One study using serum biomeasures found that a lower cortisol to CRP ratio, suggesting an overproduction of inflammatory markers in relation to glucocorticoid release, was associated with depression, specifically for women (Suarez, Sundy, & Erkanli, 2015). Salivary bioscience is poised to contribute to this growing field of multisystem health and pathophysiology. Saliva is generally easier and cheaper to collect than other biospecimens and modern multiplex assay technologies, adapted for use with saliva, allow for the measurement of several analytes from a single aliquot.

Clinical Populations and Applications Of all the immune markers discussed in this chapter, based on the current state of knowledge, salivary SIgA and CRP have the most promise for clinical applications for systemic health. SIgA has the potential to help identify individualized alternative therapies and targeted interventions for mood disorders (Kreutz, Bongard, Rohrmann, Hodapp, & Grebe, 2004) and anxiety (Ma, Serbin, & Stack, 2018). With respect to precision medicine, SIgA is now a proposed marker of patient status in a variety of diseases such as oral cancer (Zhang et al., 2017) and multiple sclerosis (Kaplan et al., 2018). Such discoveries may provide noninvasive, complementary tools for providers to help improve patient outcomes.

In the last few decades, SIgA has also been used as a marker of over-training and impending upper respiratory infections (URI) in athletic populations who experience high levels of physical stress. However, the recent discovery of SIgA's diurnal pattern may impact the findings of historical studies in which SIgA may have been sampled at sub-optimal or inconsistent times (Pritchard et al., 2017). Still, URI are the most common, noninjury-related reason that athletes seek medical attention, and reductions in SIgA values may correlate with URI onset (Gleeson & Pyne, 2016). SIgA levels are also highly associated with training load (Engels, Kendall, Fahlman, Gothe, & Bourbeau, 2018) which could help coaches and athletic trainers mitigate

decrements in sports performance due to over-training or nonfunctional overreaching. These studies point to great promise for using salivary SIgA to easily monitor a variety of clinical patients (Kaplan et al., 2018; Zhang et al., 2017), athletes, and at-risk populations like children (Ma et al., 2018) and older adults (Jiang, Yin, Li, Chen, & Gu, 2018).

Salivary CRP is the inflammatory maker with the most consistent serum–saliva correlation, making it a candidate for potential systemic health applications. Studies have shown that salivary levels of CRP are associated with measures of physical (Goodson et al., 2014; Naidoo et al., 2012) and psychological (Cicchetti et al., 2015) health. Salivary CRP has also been related to pediatric health conditions with higher levels associated with pediatric obesity, poor cardiorespiratory health (Naidoo et al., 2012), and allergic asthma (Krasteva et al., 2010). Higher levels of salivary CRP have also been linked with active and passive smoking (Azar & Richard, 2011), subacute thyroiditis (Rao et al., 2010), and reduced cognitive function in childhood (Cullen et al., 2017).

The American Heart Association and the US Centers for Disease Control and Prevention have established thresholds for high sensitivity CRP in blood and cardiovascular disease risk (Grundy et al., 2000). Unfortunately, clinically relevant reference ranges for salivary CRP have not yet been identified. Given generally low concentrations of CRP in saliva, more sensitive detection techniques may be warranted. However, a preliminary study by Out and colleagues found that salivary CRP concentrations reliably differentiated participants with high vs. low plasma CRP levels suggesting that salivary CRP could be a potential indicator of CVD risk (2012). With additional research and technological advances in detection and collection methods, salivary CRP has potential clinical applications for heart disease and a range of other inflammation-related conditions (Pay & Shaw, 2019).

Large-Scale Monitoring, Assessment, and Treatment As potential windows into immune functioning, salivary immune markers hold great potential for use in large-scale health programs and interventions. Point-of-care testing for salivary immunoglobulins, cytokines, CRP, and MMPs have been developed and are being tested to determine their utility in oral and physical health screening and treatment programs (Herr et al., 2007; Khan, Khurshid, & Yahya Ibrahim Asiri, 2017; Rathnayake, Gieselmann, Heikkinen, Tervahartiala, & Sorsa, 2017). The large-scale application of these easy-to-use and rapid tests of immune function would create new opportunities to assess, treat, and track community health using objective, biologic measures in the field. For example, salivary immunoglobulins may be useful indices of infection exposure and response to vaccination (Heaney, Phillips, Carroll, & Drayson, 2018; Lim, Garssen, & Sandalova, 2016; Pisanic et al., 2017, 2018). Chapter 30 provides additional discussion of the potential for salivary biomeasures to influence community and public health.

9.6 Concluding Comments

There is vast untapped potential for the study of salivary immune markers to advance our understanding of health and development. Realizing this potential will require both basic methodological studies, as well as studies that inform the reshaping of our conceptual understanding of salivary immune markers. Future investigators should also work toward establishing a set of reporting standards for the collection and processing of saliva samples, assay results (e.g., percent CVs, percent undetectable), data cleaning techniques, and study paradigms (e.g., TSST vs. physical stressor, timing of the sample collections). Adding this level of transparency into salivary immune marker studies will help us understand the differences observed across studies and move the field toward a set of standard protocols.

Methodologic studies that examine the validity, reliability, stability, correlates, and confounders of salivary immune markers in healthy and clinical samples are needed to provide the foundation for future salivary immune marker research. Basic methodologic studies examining the influence of typical salivary bioscience confounders, such as individual characteristics, and sample timing, collection, and processing methods, are needed to improve our ability to attribute changes in salivary immune markers to changes in functioning rather than unaccounted for biases and/or confounding in the data. Additionally, we recommend experimental research that includes the induction of inflammatory states through therapeutic drug administration, vaccines, and oral inflammatory activation to further our understanding of the within-person interactions among oral, mucosal, and systemic inflammation, and how changes in one compartment influences function across the body. Finally, to develop our understanding of between-person differences in salivary immune markers, we recommend expanding research into varying populations across sexes, developmental stages, races, ethnicities, and health and disease states.

Beyond methodologic studies, we believe interdisciplinary research that bridges across fields such as PNI, oral health and periodontology, public health, human performance, and medicine, and recognizes the importance of understanding complex, multisystem biologic processes will be key to furthering salivary bioscience and health research. Rather than viewing the associations between salivary immune markers and oral, mucosal, and systemic health as a confound to be measured and parceled out in study designs or statistical models, we believe this complexity may make salivary immune markers uniquely valuable biomarkers of health and well-being.

References

Ader, R. (2000). On the development of psychoneuroimmunology Robert. *European Journal of Pharmacology, 405*, 167–176.

Auer, B. J., Calvi, J. L., Jordan, N. M., Schrader, D., & Byrd-Craven, J. (2018). Communication and social interaction anxiety enhance interleukin-1 beta and cortisol reactivity during high-

stakes public speaking. *Psychoneuroendocrinology, 94*, 83–90. https://doi.org/10.1016/j.psyneuen.2018.05.011

Azar, R., & Richard, A. (2011). Elevated salivary C-reactive protein levels are associated with active and passive smoking in healthy youth: A pilot study. *Journal of Inflammation, 8*, 37. https://doi.org/10.1186/1476-9255-8-37

Bascones-Martínez, A., Muñoz-Corcuera, M., Noronha, S., Mota, P., Bascones-Ilundain, C., & Campo-Trapero, J. (2009). Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. *Medicina Oral, Patología Oral y Cirugía Bucal, 14*, 10–15. https://doi.org/10.4317/medoral.14.e680

Belstrøm, D., Damgaard, C., Könönen, E., Gürsoy, M., & Gürsoy, U. K. (2017). Salivary cytokine levels in early gingival inflammation. *Journal of Oral Microbiology, 9*, 1364101. https://doi.org/10.1080/20002297.2017.1364101

Bergmeier, L. A. (2018). Immunology of the oral mucosa. In L. Bergmeier (Ed.), *Oral Mucosa in Health and Disesae. A Concise Handbook* (pp. 53–67). Cham: Springer. https://doi.org/10.1007/978-3-319-56065-6_4

Berliner, J., Navab, M., Fogelman, A., Frank, J., Demer, L., Edwards, P., … Lusis, A. (1995). Atherosclerosis: Basic mechanisms. Oxidation, inflammation, and genetics. *Circulation, 91*, 2488–2496.

Besedovsky, H., del Rey, A., Sorkin, E., Da Prada, M., Burri, R., & Honegger, C. (1983). The immune response evokes changes in brain noradrenergic neurons. *Science, 221*, 564–566.

Bilbo, S., & Schwarz, J. (2012). The immune system and developmental programming of brain and behavior. *Frontiers in Neurendocrinology, 33*, 267–286. https://doi.org/10.1016/j.yfrne.2012.08.006

Birkett, M., Johnson, L., & Gelety, C. (2017). Investigation of sex differences in sIgA response to the trier social stress test. *Stress and Health, 33*, 158–163. https://doi.org/10.1002/smi.2680

Bondesen, B. A., Mills, S. T., Kegley, K. M., & Pavlath, G. K. (2004). The COX-2 pathway is essential during early stages of skeletal muscle regeneration. *American Journal of Physiology. Cell Physiology, 287*, C475–C483. https://doi.org/10.1152/ajpcell.00088.2004

Bouman, A., Jan Heineman, M., & Faas, M. M. (2005). Sex hormones and the immune response in humans. *Human Reproduction Update, 11*, 411–423. https://doi.org/10.1093/humupd/dmi008

Brandtzaeg, P. (2007). Do salivary antibodies reliably reflect both mucosal and systemic immunity? *Annals of the New York Academy of Sciences, 1098*, 288–311. https://doi.org/10.1196/annals.1384.012

Brandtzaeg, P. (2013). Secretory immunity with special reference to the oral cavity. *Journal of Oral Microbiology, 5*, 1–24. https://doi.org/10.3402/jom.v5i0.20401

Brennan, M., & Fox, P. (2010). Cytokine mRNA expression in the labial salivary glands of healthy volunteers. *Oral Diseases, 6*, 222–226. https://doi.org/10.1111/j.1601-0825.2000.tb00117.x

Browne, R. W., Kantarci, A., LaMonte, M. J., Andrews, C. A., Hovey, K. M., Falkner, K. L., … Wactawski-Wende, J. (2013). Performance of multiplex cytokine assays in serum and saliva among community-dwelling postmenopausal women. *PLoS One, 8*, e59498. https://doi.org/10.1371/journal.pone.0059498

Brydon, L., Harrison, N. A., Walker, C., Steptoe, A., & Critchley, H. D. (2008). Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biological Psychiatry, 63*, 1022–1029. https://doi.org/10.1016/j.biopsych.2007.12.007

Byrne, M., Badcock, P., Simmons, J., Whittle, S., Pettitt, A., Olsson, C., … Allen, N. (2017). Self-reported parenting style is associated with children's inflammation and immune activation. *Journal of Family Psychology, 31*, 374.

Byrne, M., Horne, S., O'Brien-Simpson, N., Walsh, K., Reynolds, E., Schwartz, O., … Allen, N. (2017). Associations between observed parenting behavior and adolescent inflammation two and a half years later in a community sample. *Health Psychology, 36*, 641.

Byrne, M. L., O'Brien-Simpson, N. M., Reynolds, E. C., Walsh, K. A., Laughton, K., Waloszek, J. M., … Allen, N. B. (2013). Acute phase protein and cytokine levels in serum and saliva: A

comparison of detectable levels and correlations in a depressed and healthy adolescent sample. *Brain, Behavior, and Immunity, 34*, 164–175. https://doi.org/10.1016/j.bbi.2013.08.010

Campbell, J. P., & Turner, J. E. (2018). Debunking the myth of exercise-induced immune suppression: Redefining the impact of exercise on immunological health across the lifespan. *Frontiers in Immunology, 9*, 1–21. https://doi.org/10.3389/fimmu.2018.00648

Campisi, J., Bravo, Y., Cole, J., & Gobeil, K. (2012). Acute psychosocial stress differentially influences salivayr endocrine and immune measures in undergraduate students. *Physiology and Behavior, 107*, 317–321. https://doi.org/10.1016/j.physbeh.2012.09.003

Cavaillon, J. (2001). Pro- versus anti-inflammatory cytokines: myth or reality. *Cellular and Molecular Biology, 47*, 695–702.

Chiang, J. J., Eisenberger, N. I., Seeman, T. E., & Taylor, S. E. (2012). Negative and competitive social interactions are related to heightened proinflammatory cytokine activity. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 1878–1882. https://doi.org/10.1073/pnas.1120972109

Cicchetti, D., Handley, E. D., & Rogosch, F. A. (2015). Child maltreatment, inflammation, and internalizing symptoms: Investigating the roles of C-reactive protein, gene variation, and neuroendocrine regulation. *Development and Psychopathology, 27*, 553–566. https://doi.org/10.1017/S0954579415000152

Cullen, A. E., Tappin, B. M., Zunszain, P. A., Dickson, H., Roberts, R. E., Nikkheslat, N., … Laurens, K. R. (2017). The relationship between salivary C-reactive protein and cognitive function in children aged 11–14 years: Does psychopathology have a moderating effect? *Brain, Behavior, and Immunity, 66*, 221–229. https://doi.org/10.1016/j.bbi.2017.07.002

Cullen, T., Thomas, A. W. A., Web, R., Hughes, M. G., Webb, R., & Hughes, M. G. (2015). The relationship between interleukin-6 in saliva, venous and capillary plasma, at rest and in response to exercise. *Cytokine, 71*(2), 397–400. https://doi.org/10.1016/j.cyto.2014.10.011

Dai, L., Golembiewska, E., Lindholm, B., & Stenvinkel, P. (2017). End-stage renal disease, inflammation and cardiovascular outcomes. *Contributions to Nephrology, 191*, 32–43. https://doi.org/10.1159/000479254

David, J., Measelle, J., Ostlund, B., & Ablow, J. (2017). Association between early life adversity and inflammation during infancy. *Developmental Psychobiology, 59*, 696–702.

Delany, F. M., Byrne, M. L., Whittle, S., Simmons, J. G., Olsson, C., Mundy, L. K., … Allen, N. B. (2016). Depression, immune function, and early adrenarche in children. *Psychoneuroendocrinology, 63*, 228–234. https://doi.org/10.1016/j.psyneuen.2015.10.003

Dickerson, S. S., Kemeny, M. E., Aziz, N., Kim, K. H., & Fahey, J. L. (2004). Immunological effects of induced shame and guilt. *Psychosomatic Medicine, 66*, 124–131. https://doi.org/10.1097/01.PSY.0000097338.75454.29

Dillon, M., Opris, D., Kopanczyk, R., Lickliter, J., Cornwell, H., Bridges, E., … Bridges, K. (2010). Detection of homocysteine and C-reactive protein in the saliva of healthy adults: Comparison with blood levels. *Biomarker Insights, 5*, 57–61.

DiSabato, D., Quan, N., & Godbout, J. (2016). Neuroinflammation: The devil is in the details. *Journal of Neurochemistry, 139*, 136–153. https://doi.org/10.1111/jnc.13607

Doyle, D., & Molix, L. (2016). Minority stress and inflammatory mediators: Covering moderates associations between perceived discrimination and salivary interleukin-6 in gay men. *Journal of Behavioral Medicine, 39*, 782–792. https://doi.org/10.1097/CCM.0b013e31823da96d. Hydrogen

Dugue, B. (1996). Cytokines in saliva. Basal concentrations and the effect of high ambient heat (sauna). *Stress Medicine, 12*, 193–197. https://doi.org/10.1002/(SICI)1099-1700(199607)12:3<193::AID-SMI701>3.0.CO;2-Z

Eisenberger, N. I., Inagaki, T. K., Rameson, L. T., Mashal, N. M., & Irwin, M. R. (2009). An fMRI study of cytokine-induced depressed mood and social pain: The role of sex differences. *NeuroImage, 47*, 881–890. https://doi.org/10.1016/j.neuroimage.2009.04.040

Eke, P. I., Thornton-Evans, G. O., Wei, L., Borgnakke, W. S., Dye, B. A., & Genco, R. J. (2018). Periodontitis in US adults: National Health and Nutrition Examination Survey 2009–2014.

Journal of the American Dental Association, 149, 576–588.e6. https://doi.org/10.1016/j.adaj.2018.04.023

El-Sheikh, M., Buckhalt, J. A., Granger, D. A., Erath, S. A., & Acebo, C. (2007). The association between children's sleep disruption and salivary interleukin-6. *Journal of Sleep Research, 16*, 188–197. https://doi.org/10.1111/j.1365-2869.2007.00593.x

Engeland, C. G., Hugo, F. N., Hilgert, J. B., Nascimento, G. G., Junges, R., Lim, H. J., … Bosch, J. A. (2016). Psychological distress and salivary secretory immunity. *Brain, Behavior, and Immunity, 52*, 11–17. https://doi.org/10.1016/j.bbi.2015.08.017

Engels, H., Kendall, B., Fahlman, M., Gothe, N., & Bourbeau, K. (2018). Salivary immunoglobulin A in healthy adolescent females: Effects of maximal exercise, physical activity, body composition and diet. *The Journal of Sports Medicine and Physical Fitness, 58*, 1096–1101.

Evans, P., Der, G., Ford, G., Hucklebridge, F., Hunt, K., & Lambert, S. (2000). Social class, sex, and age differences in mucosal immunity in a large community sample. *Brain, Behavior, and Immunity, 14*, 41–48. https://doi.org/10.1006/brbi.1999.0571

Ewing, P., Otczyk, D., Occhipinti, S., Kyd, J., Gleeson, M., & Cripps, A. (2010). Developmental profiles of mucosal immunity in pre-school children. *Clinical and Developmental Immunology, 2010*, 196785. https://doi.org/10.1155/2010/196785

Fernandez-Botran, R., Miller, J. J., Burns, V. E., & Newton, T. L. (2011). Correlations among inflammatory markers in plasma, saliva and oral mucosal transudate in post-menopausal women with past intimate partner violence. *Brain, Behavior, and Immunity, 25*, 314–321. https://doi.org/10.1016/j.bbi.2010.09.023

Finoti, L. S., Nepomuceno, R., Pigossi, S. C., Corbi, S. C., Secolin, R., & Scarel-Caminaga, R. M. (2017). Association between interleukin-8 levels and chronic periodontal disease. *Medicine (Baltimore), 96*, e6932. https://doi.org/10.1097/MD.0000000000006932

Fisher-Owens, S. A., Gansky, S. A., Platt, L. J., Weintraub, J. A., Soobader, M.-J., Bramlett, M. D., & Newacheck, P. W. (2007). Influences on children's oral health: A conceptual model. *Pediatrics, 120*, e510–e520. https://doi.org/10.1542/peds.2006-3084

Giannobile, W., Beikler, T., Kinney, J., Ramseier, C., Morelli, T., & Wong, D. (2009). Saliva as a diagnostic tool for periodontal disease: Current state and future directions. *Periodontology 2000, 2000*(50), 52–64. https://doi.org/10.4103/2468-6360.186487

Gleeson, M., & Pyne, D. B. (2016). Respiratory inflammation and infections in high-performance athletes. *Immunology and Cell Biology, 94*, 124–131. https://doi.org/10.1038/icb.2015.100

Goodson, J. M., Kantarci, A., Hartman, M. L., Denis, G. V., Stephens, D., Hasturk, H., … Welty, F. (2014). Metabolic disease risk in children by salivary biomarker analysis. *PLoS One, 9*, e98799. https://doi.org/10.1371/journal.pone.0098799

Granger, D. A., Granger, G. A., & Granger, S. W. (2006). Immunology and developmental psychopathology. In D. Cicchetti & D. Cohen (Eds.), *Developmental neuroscience* (pp. 677–709). New York, NY: Wiley.

Gröschl, M. (2009). The physiological role of hormones in saliva. *Bioessays, 31*, 843–852. https://doi.org/10.1002/bies.200900013

Grundy, S., Bazzarre, T., Cleeman, J., D'Agostino, R., Hill, M., Houston-Miller, N., … Wood, D. (2000). Prevention conference V: Beyond secondary prevention: Identifying the high-risk patient for primary prevention: Medical office assessment: Writing Group I. *Circulation, 101*, E3–E11.

Hanioka, T., Morita, M., Yamamoto, T., Inagaki, K., Wang, P.-L., Ito, H., … Ogawa, H. (2019). Smoking and periodontal microorganisms. *Japanese Dental Science Review, 55*, 88–94. https://doi.org/10.1016/j.jdsr.2019.03.002

Hannas, A. R., Pereira, J. C., Granjeiro, J. M., & Tjäderhane, L. (2007). The role of matrix metalloproteinases in the oral environment. *Acta Odontologica Scandinavica, 65*, 1–13. https://doi.org/10.1080/00016350600963640

Hasmun, N., Drummand, B., Milne, T., Cullinan, M., Meldrum, A., & Coates, D. (2017). Effects of environmental tobacco smoke on the oral health of preschool children. *European Archives of Paediatric Dentistry, 18*, 393–398.

Hasturk, H., Kantarci, A., & Van Dyke, T. E. (2012). Oral inflammatory diseases and systemic inflammation: Role of the macrophage. *Frontiers in Immunology, 3*, 1–17. https://doi.org/10.3389/fimmu.2012.00118

Haukioja, A., Tervahartiala, T., Sorsa, T., & Syrjänen, S. (2017). Persistent oral human papillomavirus (HPV) infection is associated with low salivary levels of matrix metalloproteinase 8 (MMP-8). *Journal of Clinical Virology, 97*, 4–9. https://doi.org/10.1016/j.jcv.2017.10.011

Hayashida, H. (2016). Effects of the menstrual cycle and acute aerobic exercise on cytokine levels. *Journal of Sports Medicine and Doping Studies, 06*, 6–10. https://doi.org/10.4172/2161-0673.1000173

Heaney, J. L. J., Phillips, A. C., Carroll, D., & Drayson, M. T. (2018). The utility of saliva for the assessment of anti-pneumococcal antibodies: investigation of saliva as a marker of antibody status in serum. *Biomarkers, 23*, 115–122. https://doi.org/10.1080/1354750X.2016.1265009

Hernandez, L., Fuller, S., Stone, M., Carpenter, J., & Taylor, M. (2016). Catabolic/anabolic hormone and inflammatory marker responses to a graded exercise test in military men. *Medicine and Science in Sports and Exercise, 48*, 273–274.

Hernandez, L., & Taylor, M. (2017). Diurnal pattern of salivary C-reactive protein and associations with biobehavioral correlates in military men. In *Medicine and science in sports and exercise*. Philadelphia, PA: Lippincott Williams & Wilkins.

Herr, A. E., Hatch, A. V., Throckmorton, D. J., Tran, H. M., Brennan, J. S., Giannobile, W. V., & Singh, A. K. (2007). Microfluidic immunoassays as rapid saliva-based clinical diagnostics. *Proceedings of the National Academy of Sciences, 104*, 5268–5273. https://doi.org/10.1073/pnas.0607254104

Horn, S., Long, M., Nelson, B., Allen, N., Fisher, P., & Byrne, M. (2018). Replication and reproducibility issues in the relationship between C-reactive protein and depression: A systematic review and focused meta-analysis. *Brain, Behavior, and Immunity, 73*, 85–114.

Hucklebridge, F., Clow, A., & Evans, P. (1998). The relationship between salivary secretory immunoglobulin A and cortisol: Neuroendocrine response to awakening and the diurnal cycle. *International Journal of Psychophysiology, 31*, 69–76. https://doi.org/10.1016/S0167-8760(98)00042-7

Ilardo, C., Toniolo, A., Aimone-gastin, I., Abdelmouttaleb, I., Gueant, J., & Desor, D. (2001). Effects of psycho-physical stress (competitive rafting) on saliva interleukin-1 beta. *Stress and Health, 17*, 9–15.

Izawa, S., Miki, K., Liu, X., & Ogawa, N. (2013). The diurnal patterns of salivary interleukin-6 and C-reactive protein in healthy young adults. *Brain, Behavior, and Immunity, 27*, 38–41. https://doi.org/10.1016/j.bbi.2012.07.001

Jafarzadeh, A., Sadeghi, M., Karam, G. A., & Vazirinejad, R. (2010). Salivary IgA and IgE levels in healthy subjects: Relation to age and gender. *Brazilian Oral Research, 24*, 21–27. https://doi.org/10.1590/S1806-83242010000100004

Jarvis, M., Russell, M., Benowitz, N., & Feyerabend, C. (1988). Elimination of cotinine from body fluids: Implications for noninvasive measurement of tobacco smoke exposure. *American Journal of Public Health, 78*, 696–698. https://doi.org/10.2105/AJPH.78.6.696

Javed, F., Ahmed, H., & Romanos, G. (2014). Association between environmental tobacco smoke and periodontal disease: A systematic review. *Environmental Research, 133*, 117–122.

Jiang, M., Yin, Z., Li, S., Chen, X., & Gu, J. (2018). Effect of positive psychological intervention on well-being, 2-week illness prevalence, and salivary immunoglobulin A. *Western Journal of Nursing Research, 40*, 889–906. https://doi.org/10.1177/0193945917690733

Kaplan, B., Golderman, S., Ganelin-Cohen, E., Miniovitch, A., Korf, E., Ben-Zvi, I., . . . Flechter, S. (2018). Immunoglobulin free light chains in saliva: A potential marker for disease activity in multiple sclerosis. *Clinical and Experimental Immunology, 192*, 7–17. https://doi.org/10.1111/cei.13086

Keaney, L. C., Kilding, A. E., Merien, F., & Dulson, D. K. (2018). The impact of sport related stressors on immunity and illness risk in team-sport athletes. *Journal of Science and Medicine in Sport, 21*, 1192–1199. https://doi.org/10.1016/j.jsams.2018.05.014

Keller, P. S., El-Sheikh, M., Vaughn, B., & Granger, D. A. (2010). Relations between mucosal immunity and children's mental health: The role of child sex. *Physiology and Behavior, 101*, 705–712. https://doi.org/10.1016/j.physbeh.2010.08.012

Khan, R., Khurshid, Z., & Yahya Ibrahim Asiri, F. (2017). Advancing point-of-care (PoC) testing using human saliva as liquid biopsy. *Diagnostics (Basel), 7*, E39. https://doi.org/10.3390/diagnostics7030039

Kiecolt-Glaser, J., Cacioppo, J., Malarkey, W., & Glaser, R. (1992). Acute psychological stressors and short-term immune changes: What, why, for whom, and to what extent? *Psychosomatic Medicine, 54*, 680–685.

Kiecolt-Glaser, J., McGuire, L., Robles, T., & Glaser, R. (2002). Emotions, morbidity, and mortality: New perspectives from psychoneuroimmunology. *Annual Review of Psychology, 53*, 83–107.

Kivlighan, K., Granger, D. A., Schwartz, E., Nelson, V., Curran, M., & Shirtcliff, E. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva Katie. *Hormones and Behavior, 46*, 39–46. https://doi.org/10.1016/j.yhbeh.2005.10.005

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews. Immunology, 16*, 626–638. https://doi.org/10.1038/nri.2016.90

Krasteva, A., Perenovska, P., Ivanova, A., Altankova, I., Bocheva, T., & Kisselova, A. (2010). Alteration in salivary components of children with allergic asthma. *Biotechnology and Biotechnological Equipment, 24*, 1866–1869. https://doi.org/10.2478/V10133-010-0050-2

Kreutz, G., Bongard, S., Rohrmann, S., Hodapp, V., & Grebe, D. (2004). Effects of choir singing or listening on secretory immunoglobulin A, cortisol, and emotional state. *Journal of Behavioral Medicine, 27*, 623–635.

La Fratta, I., Tatangelo, R., Campagna, G., Rizzuto, A., Franceschelli, S., Ferrone, A., … Pesce, M. (2018). The plasmatic and salivary levels of IL-1β, IL-18 and IL-6 are associated to emotional difference during stress in young male. *Scientific Reports, 8*, 1–10. https://doi.org/10.1038/s41598-018-21474-y

Lahdentausta, L., Paju, S., Mäntylä, P., Buhlin, K., Tervahartiala, T., Pietiäinen, M., … Pussinen, P. J. (2018). Saliva and serum biomarkers in periodontitis and coronary artery disease. *Journal of Clinical Periodontology, 45*(9), 1045–1055. https://doi.org/10.1111/jcpe.12976

Laurent, H. K., Stroud, L. R., Brush, B., D'Angelo, C., & Granger, D. A. (2015). Secretory IgA reactivity to social threat in youth: Relations with HPA, ANS, and behavior. *Psychoneuroendocrinology, 59*, 81–90. https://doi.org/10.1016/j.psyneuen.2015.04.021

Laurent, H. K., Lucas, T., Pierce, J., Goetz, S., & Granger, D. A. (2016). Coordination of cortisol response to social evaluative threat with autonomic and inflammatory responses is moderated by stress appraisals and affect. *Biological Psychology, 118*, 17–24. https://doi.org/10.1016/j.biopsycho.2016.04.066.Coordination

Lester, S., Brown, J., Aycock, J., Grubbs, S., & Johnson, R. (2010). Use of saliva for assessment of stress and its effect on the immune system prior to gross anatomy practical examinations. *Anatomical Sciences Education, 3*, 160–167. https://doi.org/10.1002/ase.161

Li, T., & Gleeson, M. (2004). The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and alpha-amylase responses. *Journal of Sports Sciences, 22*, 1015–1024.

Lim, P. W., Garssen, J., & Sandalova, E. (2016). Potential use of salivary markers for longitudinal monitoring of inflammatory immune responses to vaccination. *Mediators of Inflammation, 2016*, 6958293. https://doi.org/10.1155/2016/6958293

Lira-Junior, R., Åkerman, S., Gustafsson, A., Klinge, B., & Boström, E. A. (2017). Colony stimulating factor-1 in saliva in relation to age, smoking, and oral and systemic diseases. *Scientific Reports, 7*, 1–8. https://doi.org/10.1038/s41598-017-07698-4

Liu, H., Hays, R. D., Marcus, M., Coulter, I., Maida, C., Ramos-gomez, F., … Crall, J. (2016). Patient-Reported oral health outcome measurement for children and adolescents. *BMC Oral Health, 16*. https://doi.org/10.1186/s12903-016-0293-x

Lockhart, P. P. B., Bolger, A. A. F., Papapanou, P. P. N., Osinbowale, O., Trevisan, M., Levison, M. M. E., … Baddour, L. M. L. (2012). Periodontal disease and atherosclerotic vascular disease: Does the evidence support an independent association? A scientific statement from the American heart association. *Circulation, 125*, 2520–2544. https://doi.org/10.1161/CIR.0b013e31825719f3

Lu, Q., & Jin, L. (2010). Human gingiva is another site of C-reactive protein formation. *Journal of Clinical Periodontology, 37*, 789–796. https://doi.org/10.1111/j.1600-051X.2010.01600.x

Lucas, T., Lumley, M., Flack, J., Wegner, R., Pierce, J., & Goetz, S. (2016). A preliminary experimental examination of worldview verification, perceived racism, and stress reactivity in African Americans. *Health Psychology, 35*, 366–375. https://doi.org/10.1037/hea0000284

Lucas, T., Wegner, R., Pierce, J., Lumley, M. A., Laurent, H. H. K. H. H. K., & Granger, D. D. A. D. A. (2017). Perceived discrimination, racial identity, and multisystem stress response to social evaluative threat among African American men and women. *Psychosomatic Medicine, 79*, 293–305. https://doi.org/10.1097/PSY.0000000000000406

Ma, D., Serbin, L. A., & Stack, D. M. (2018). Children's anxiety symptoms and salivary immunoglobulin A: A mutual regulatory system? *Developmental Psychobiology, 60*, 202–215. https://doi.org/10.1002/dev.21590

Maekawa, T., Tabeta, K., Kajita-Okui, K., Nakajima, T., Yamazaki, K., Tomoki, M., … Kazuhisa, Y. (2011). Increased expression of C-reactive protein gene in inflamed gingival tissues could be derived from endothelial cells stimulated with interleukin-6. *Archives of Oral Biology, 56*, 1312–1318. https://doi.org/10.1016/j.archoralbio.2011.04.010

Maier, S., & Watkins, L. (1998). Cytokines for psychologists: Implications of bidirectional immune-to- brain communication for understanding behavior, mood, and cognition. *Psychological Review, 105*, 83–107.

Maldonado, A., Preciado, A., Buchanan, M., Pulvers, K., Romero, D., & D'Anna-Hernandez, K. (2018). Acculturative stress, mental health symptoms, and the role of salivary inflammatory markers among a Latino sample. *Cultural Diversity and Ethnic Minority Psychology, 24*, 277–283.

Manthiram, K., Zhou, Q., Aksentijevich, I., & Kastner, D. L. (2017). The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. *Nature Immunology, 18*, 832–842. https://doi.org/10.1038/ni.3777

Mantis, N. J., Rol, N., & Corthésy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology, 4*, 603–611. https://doi.org/10.1038/mi.2011.41

Marsland, A., Walsh, C., Lockwood, K., & John-Henderson, N. (2017). The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain, Behavior, and Immunity, 64*, 208–219. https://doi.org/10.1016/j.bbi.2017.01.011

Martínez, A. D., Ruelas, L., & Granger, D. A. (2018). Household fear of deportation in relation to chronic stressors and salivary proinflammatory cytokines in Mexican-origin families post-SB 1070. *SSM-Population Health, 5*, 188–200.

Master, S., Amodio, D., Stanon, A., Yee, C., Hilmert, C., & Taylor, S. (2009). Neurobiogical correlates of coping through emotional approach. *Brain, Behavior, and Immunity, 23*, 27–35.

Mattila, K. J., Nieminen, M. S., Valtonen, V. V., Rasi, V. P., Kesäniemi, Y. A., Syrjälä, S. L., … Jokinen, M. J. (1989). Association between dental health and acute myocardial infarction. *BMJ, 298*, 779–781.

Maydych, V., Claus, M., Watz, C., & Kleinsorge, T. (2018). Attention to emotional information is associated with cytokine responses to psychological stress. *Frontiers in Neuroscience, 12*, 687. https://doi.org/10.3389/fnins.2018.00687

Measelle, J. R., David, J., & Ablow, J. C. (2017). Increased levels of inflammation among infants with disorganized histories of attachment. *Behavioural Brain Research, 325*, 260–267. https://doi.org/10.1016/j.bbr.2016.12.001

Megson, E., Fitzsimmons, T., Charmapatni, K., Bartold, P., Dharmapatni, K., & Bartold, P. (2010). C-reactive protein in gingival crevicular fluid may be indicative of systemic inflammation. *Journal of Clinical Periodontology, 37*, 797–804.

Meier-Ewert, H. K., Ridker, P. M., Rifai, N., Price, N., Dinges, D. F., & Mullington, J. M. (2001). Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clinical Chemistry, 47*, 426–430.

Miller, A., & Raison, C. (2016). The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nature Reviews. Immunology, 16*, 22–34. https://doi.org/10.1038/nri.2015.5

Minetto, M. A., Gazzoni, M., Lanfranco, F., Baldi, M., Saba, L., Pedrola, R., … Rainoldi, A. (2007). Influence of the sample collection method on salivary interleukin-6 levels in resting and post-exercise conditions. *European Journal of Applied Physiology, 101*, 249–256. https://doi.org/10.1007/s00421-007-0484-x

Miyake, S. (2012). Mind over cytokines: Crosstalk and regulation between the neuroendocrine and immune systems. *Clinical Experimental Neuroimmunology, 3*, 1–15. https://doi.org/10.1111/j.1759-1961.2011.00023.x

Mohamed, R., Campbell, J.-L., Cooper-White, J., Dimeski, G., & Punyadeera, C. (2012). The impact of saliva collection and processing methods on CRP, IgE, and Myoglobin immunoassays. *Clinical and Translational Medicine, 1*, 19. https://doi.org/10.1186/2001-1326-1-19

Moons, W. G., Eisenberger, N. I., & Taylor, S. E. (2010). Anger and fear responses to stress have different biological profiles. *Brain, Behavior, and Immunity, 24*, 215–219. https://doi.org/10.1016/j.bbi.2009.08.009

Moutsopoulos, N. M., & Konkel, J. E. (2018). Tissue-specific immunity at the oral mucosal barrier. *Trends in Immunology, 39*, 276–287. https://doi.org/10.1016/j.it.2017.08.005

Muscatell, K. A., Dedovic, K., Slavich, G. M., Jarcho, M. R., Breen, E. C., Bower, J. E., … Eisenberger, N. I. (2015). Greater amygdala activity and dorsomedial prefrontal—Amygdala coupling are associated with enhanced inflammatory responses to stress. *Brain, Behavior, and Immunity, 43*, 46–53. https://doi.org/10.1016/j.bbi.2014.06.201

Naidoo, T., Konkol, K., Biccard, B., Dubose, K., & McKune, A. (2012). Elevated salivary C-reactive protein predicted by low cardio-respiratory fitness and being overweight in African children: Cardiovascular topic. *Cardiovascular Journal of Africa, 23*, 501–506. https://doi.org/10.5830/CVJA-2012-058

Nam, Y., Kim, Y. Y., Chang, J. Y., & Kho, H. S. (2019). Salivary biomarkers of inflammation and oxidative stress in healthy adults. *Archives of Oral Biology, 97*, 215–222. https://doi.org/10.1016/j.archoralbio.2018.10.026

Navarro, V. P., Iyomasa, M. M., Leite-Panissi, C. R., Almeida, M. C., & Branco, L. G. (2006). New role of the trigeminal nerve as a neuronal pathway signaling brain in acute periodontitis: Participation of local prostaglandins. *Pflugers Archiv: European Journal of Physiology, 453*, 73–82.

Newton, T., Fernandez-Botran, R., Lyle, K., Szabo, Y., Miller, J., & Warnecke, A. (2017). Salivary cytokine response in the aftermath of stress: An emotion regulation perspective. *Emotion, 17*, 1007–1020.

Nilsonne, G., Lekander, M., Åkerstedt, T., Axelsson, J., & Ingre, M. (2016). Diurnal variation of circulating interleukin-6 in humans: A meta-analysis. *PLoS One, 11*, 1–17. https://doi.org/10.1371/journal.pone.0165799

Nirmal, B., Ramachandiran, H., Anand, V., Sathiamurthy, A., & Sekaran, P. (2005). Tools for evaluating oral health and quality of life. *Journal of Pharmacy and Bioallied Sciences, 7*, S414–S419.

Nishanian, P., Aziz, N., Chung, J., Detels, R., & Fahey, J. L. (1998). Oral fluids as an alternative to serum for measurement of markers of immune activation. *Clinical and Diagnostic Laboratory Immunology, 5*, 507–512.

Nizam, N., Basoglu, O. K., Tasbakan, M. S., Nalbantsoy, A., & Buduneli, N. (2014). Salivary cytokines and the association between obstructive sleep apnea syndrome and periodontal disease. *Journal of Periodontology, 85*, e251–e258. https://doi.org/10.1902/jop.2014.130579

O'Connor, M., Bower, J., Cho, H., Creswell, J., Dimitrov, S., Hamby, M., . . . Irwin, M. (2009). To assess, to control, to exclude: Effects of biobehavioral factors on circulating inflammatory markers. *Brain, Behavior, and Immunity, 23*, 887–897.

O'Connor, M. F., Irwin, M. R., & Wellisch, D. K. (2009). When grief heats up: Pro-inflammatory cytokines predict regional brain activation. *NeuroImage, 47*, 891–896. https://doi.org/10.1016/j.neuroimage.2009.05.049

Ohira, H. (2005). Social support and salivary secretory immunoglobulin A response in women to stress of making a public speech. *Perceptual and Motor Skills, 98*, 1241. https://doi.org/10.2466/pms.98.4.1241-1250

Ouellet-Morin, I., Danese, A., Williams, B., & Arseneault, L. (2011). Validation of a high-sensitivity assay for C-reactive protein in human saliva. *Brain, Behavior, and Immunity, 25*, 640–646. https://doi.org/10.1016/j.bbi.2010.12.020

Out, D., Hall, R. J., Granger, D. A., Page, G. G., & Woods, S. J. (2012). Assessing salivary C-reactive protein: Longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. *Brain, Behavior, and Immunity, 26*, 543–551. https://doi.org/10.1021/nl061786n.Core-Shell

Papanicolaou, D., Wilder, R., Manolagas, S., & Chrousos, G. (1998). The pathophysiologic roles of interleukin-6 in human disease. *Annals of Internal Medicine, 128*, 127–137.

Pariante, C. M. C., & Miller, A. A. H. (2001). Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. *Biological Psychiatry, 49*, 391–404. https://doi.org/10.1016/S0006-3223(00)01088-X

Pay, J. B., & Shaw, A. M. (2019). Towards salivary C-reactive protein as a viable biomarker of systemic inflammation. *Clinical Biochemistry, 68*, 1–8. https://doi.org/10.1016/j.clinbiochem.2019.04.006

Pfaffe, T., Cooper-white, J., Beyerlein, P., Kostner, K., & Punyadeera, C. (2011). Diagnostic potential of saliva: Current state and future applications. *Clinical Chemistry, 57*, 675–687. https://doi.org/10.1373/clinchem.2010.153767

Phillips, A. C., Carroll, D., Evans, P., Bosch, J. A., Clow, A., Hucklebridge, F., & Der, G. (2006). Stressful life events are associated with low secretion rates of immunoglobulin a in saliva in the middle aged and elderly. *Brain, Behavior, and Immunity, 20*, 191–197. https://doi.org/10.1016/j.bbi.2005.06.006

Pisanic, N., Ballard, S.-B., Colquechagua, F. D., François, R., Exum, N., Yori, P. P., . . . Kosek, M. N. (2018). Minimally invasive saliva testing to monitor norovirus infection in community settings. *The Journal of Infectious Diseases, 219*(8), 1234–1242. https://doi.org/10.1093/infdis/jiy638

Pisanic, N., Rahman, A., Saha, S. K., Labrique, A. B., Nelson, K. E., Granger, D. A., . . . Heaney, C. D. (2017). Development of an oral fluid immunoassay to assess past and recent hepatitis E virus (HEV) infection. *Journal of Immunological Methods, 448*, 1–8. https://doi.org/10.1016/j.jim.2017.04.012

Pritchard, B. T., Stanton, W., Lord, R., Petocz, P., & Pepping, G. J. (2017). Factors affecting measurement of salivary cortisol and secretory immunoglobulin a in field studies of athletes. *Frontiers in Endocrinology (Lausanne), 8*, 1–7. https://doi.org/10.3389/fendo.2017.00168

Prossin, A. R., Koch, A. E., Campbell, P. L., McInnis, M. G., Zalcman, S. S., & Zubieta, J. K. (2011). Association of plasma interleukin-18 levels with emotion regulation and mu-opioid neurotransmitter function in major depression and healthy volunteers. *Biological Psychiatry, 69*, 808–812. https://doi.org/10.1016/j.biopsych.2010.10.014

Punyadeera, C., Dimeski, G., Kostner, K., Beyerlein, P., & Cooper-White, J. (2011). One-step homogeneous C-reactive protein assay for saliva. *Journal of Immunological Methods, 373*, 19–25. https://doi.org/10.1016/j.jim.2011.07.013

Rangbulla, V., Nirola, A., Gupta, M., & Batra, P. (2017). Salivary IgA, interleukin-1b and MMP-8 as salivary biomarkers in chronic periodontitis patients. *The Chinese Journal of Dental Research, 20*, 43–51.

Rao, N. L., Shetty, S., Upadhyaya, K., Prasad, R. M., Lobo, E. C., Kedilaya, H. P., & Prasad, G. (2010). Salivary C-reactive protein in Hashimoto's thyroiditis and subacute thyroiditis. *International Journal of Inflammation, 2010*, 1–5. https://doi.org/10.4061/2010/514659

Rathnayake, N., Åkerman, S., Klinge, B., Lundegren, N., Jansson, H., Tryselius, Y., . . . Gustafsson, A. (2013). Salivary biomarkers of oral health—A cross-sectional study. *Journal of Clinical Periodontology, 40*, 140–147. https://doi.org/10.1111/jcpe.12038

Rathnayake, N., Gieselmann, D.-R., Heikkinen, A., Tervahartiala, T., & Sorsa, T. (2017). Salivary diagnostics—Point-of-care diagnostics of MMP-8 in dentistry and medicine. *Diagnostics, 7*, 7. https://doi.org/10.3390/diagnostics7010007

Reinhardt, É. L., Fernandes, P. A. C. M., Markus, R. P., & Fischer, F. M. (2016). Short sleep duration increases salivary IL-6 production. *Chronobiology International, 33*, 780–782. https://doi.org/10.3109/07420528.2016.1167710

Reinhardt, E., Fernandes, P., Markus, R., & Fischer, F. (2019). Night work effects on salivary cytokines TNF, IL-1β and IL-6. *Chronobiology International, 36*, 11–26.

Rhodus, N., Ho, V., Miller, C., Myers, S., & Ondrey, F. (2005). NF-κB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. *Cancer Detection and Prevention, 29*, 42–45.

Riis, J. L., Bryce, C. I., Ha, T., Hand, T., Stebbins, J. L., Matin, M., . . . Granger, D. A. (2017). Adiponectin: Serum-saliva associations and relations with oral and systemic markers of inflammation. *Peptides, 91*, 58–64. https://doi.org/10.1016/j.peptides.2017.03.006

Riis, J. L., Granger, D. A., Dipietro, J. A., Bandeen-Roche, K., & Johnson, S. B. (2015). Salivary cytokines as a minimally-invasive measure of immune functioning in young children: Correlates of individual differences and sensitivity to laboratory stress. *Developmental Psychobiology, 57*, 153–167. https://doi.org/10.1002/cncr.27633.Percutaneous

Riis, J. L., Granger, D. A., Minkovitz, C. S., Bandeen-Roche, K., DiPietro, J. A., & Johnson, S. B. (2016). Maternal distress and child neuroendocrine and immune regulation. *Social Science and Medicine (1982), 151*, 206–214. https://doi.org/10.1016/j.socscimed.2015.12.043

Riis, J., Out, D., Dorn, L., Beal, S., Denson, L., Pabst, S., . . . Granger, D.A. (2014). Salivary cytokines in healthy adolescent girls: Intercorrelations, stability, and associations with serum cytokines, age, and pubertal stage. *Developmental Psychobiology, 56*, 797–811. https://doi.org/10.1002/dev.21149

Rohleder, N. (2014). Stimulation of systemic low-grade inflammation by psychosocial stress. *Psychosomatic Medicine, 76*, 181–189. https://doi.org/10.1097/PSY.0000000000000049

Rudnicka, A., Rumley, A., Lowe, G., & Strachan, D. (2007). Clinical perspective. *Circulation, 115*, 996–1003.

Ruhl, S., Homberaer, S., Betz, R., Sukkar, T., Schmalz, G., Seymour, R. A., . . . Thomason, J. M. (2004). Salivary proteins and cytokines in drug-induced gingival overgrowth. *Journal of Dental Research, 83*, 322–326. https://doi.org/10.1177/154405910408300410

Rutherfurd-Markwick, K., Starck, C., Dulson, D., & Ali, A. (2017). Salivary diagnostic markers in males and females during rest and exercise. *Jounal of the International Society of Sports Nutrition, 14*, 27. https://doi.org/10.1186/s12970-017-0185-8

Sahibzada, H. A., Khurshid, Z., Khan, R. S., Naseem, M., Siddique, K. M., Mali, M., & Zafar, M. S. (2017). Salivary IL-8, IL-6 and TNF-α as potential diagnostic biomarkers for oral cancer. *Diagnostics, 7*, 21. https://doi.org/10.3390/diagnostics7020021

Salminen, A., Gursoy, U. K., Paju, S., Hyvärinen, K., Mäntylä, P., Könönen, E., . . . Pussinen, P. J. (2014). Salivary biomarkers of bacterial burden, inflammatory response, and tissue destruction in periodontitis. *Journal of Clinical Periodontology, 41*, 442–450. https://doi.org/10.1111/jcpe.12234

Saltiel, A. R., & Olefsky, J. M. (2017). Inflammarory linking obesity and metabolic disease and metabolic disease. *The Journal of Clinical Investigation, 127*, 1–4. https://doi.org/10.1172/JCI92035

Scannapieco, F., Bush, R., & Paju, S. (2003). Associations between periodontal disease and risk for atherosclerosis, cardiovascular disease, and stroke. A systematic review. *Annals of Periodontology, 8*, 38–53.

Segerstrom, S., & Miller, G. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin, 130*, 601–630. https://doi.org/10.1038/mp.2011.182

Shields, G. S., Kuchenbecker, S. Y., Pressman, S., Sumida, K. D., & Slavich, G. M. (2016). Better cognitive control of emotional information is associated with reduced pro-inflammatory cytokine reactivity to emotional stress. *Stress, 19*, 63–68. https://doi.org/10.3109/10253890.2015.1121983

Shields, G. S., Slavich, G. M., Perlman, G., Klein, D. N., & Kotov, R. (2019). The short-term reliability and long-term stability of salivary immune markers. *Brain, Behavior, and Immunity, 81*, 650–654. https://doi.org/10.1016/j.bbi.2019.06.007

Sjögren, E., Leanderson, P., Kristenson, M., & Ernerudh, J. (2006). Interleukin-6 levels in relation to psychosocial factors: Studies on serum, saliva, and in vitro production by blood mononuclear cells. *Brain, Behavior, and Immunity, 20*, 270–278. https://doi.org/10.1016/j.bbi.2005.08.001

Slavich, G. M., Way, B. M., Eisenberger, N. I., & Taylor, S. E. (2010). Neural sensitivity to social rejection is associated with inflammatory responses to social stress. *Proceedings of the National Academy of Sciences, 107*, 14817–14822. https://doi.org/10.1073/pnas.1009164107

Slavish, D., Graham-Engeland, J., Smyth, J., & Engeland, C. (2015a). Salivary markers of inflammation in response to acute stress. *Brain, Behavior, and Immunity, 44*, 253–269.

Slavish, D. C., Graham-Engeland, J. E., Smyth, J. M., & Engeland, C. G. (2015b). Salivary markers of inflammation in response to acute stress. *Brain, Behavior, and Immunity, 44*, 253–269. https://doi.org/10.1016/j.bbi.2014.08.008

Smigiel, K., & Parks, W. (2017). Chapter five—Matrix metalloproteinases and leukocyte activation. *Science, 147*, 167–195.

Solomon, G., & Moos, R. (1964). Emotions, immunity, and disease. *Archives of General Psychiatry, 11*, 657–674. https://doi.org/10.1016/j.humimm.2009.07.015

Sorenson, H., Cruz-Almeida, Y., Tighe, P., Wallet, S. M., Riley, J. L., Aguirre, M., . . . Riley, J. L. (2017). Age differences in salivary markers of inflammation in response to experimental pain: Does venipuncture matter? *Journal of Pain Research, 10*, 2365–2372. https://doi.org/10.2147/jpr.s138460

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-reactive protein at sites of inflammation and infection. *Frontiers in Immunology, 9*, 1–11. https://doi.org/10.3389/fimmu.2018.00754

St. John, M., Li, Y., Zhou, X., Denny, P., Ho, C.-M., Montemagno, C., . . . Wong, D. (2004). Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. *Archives of Otolaryngology—Head and Neck Surgery, 130*, 929–935. https://doi.org/10.1001/archotol.130.8.929

Suarez, E. C., Sundy, J. S., & Erkanli, A. (2015). Depressogenic vulnerability and gender-specific patterns of neuro-immune dysregulation: What the ratio of cortisol to C-reactive protein can tell us about loss of normal regulatory control. *Brain, Behavior, and Immunity, 44*, 137–147. https://doi.org/10.1016/j.bbi.2014.09.008

Szabo, Y. Z., Fernandez-Botran, R., & Newton, T. L. (2019). Cumulative trauma, emotion reactivity and salivary cytokine levels following acute stress in healthy women. *Anxiety, Stress and Coping, 32*, 82–94. https://doi.org/10.1080/10615806.2018.1524377

Taba, M., Kinney, J., Kim, A. S., & Giannobile, W. V. (2005). Diagnostic biomarkers for oral and periodontal diseases. *Dental Clinics of North America, 49*, 551–571. https://doi.org/10.1016/j.cden.2005.03.009

Taneja, V. (2018). Sex hormones determine immune response. *Frontiers in Immunology, 9*, 1–5. https://doi.org/10.3389/fimmu.2018.01931

Taubman, M., & Smith, D. (1993). Significance of salivary antibody in dental diseases. *Annals of the New York Academy of Sciences, 694*, 202–215. https://doi.org/10.1196/annals.1340.018

Tauler, P., Martinez, S., Moreno, C., Martínez, P., & Aguilo, A. (2014). Changes in salivary hormones, immunoglobulin A, and C-reactive protein in response to ultra-endurance exercises. *Applied Physiology, Nutrition, and Metabolism, 39*, 560–565. https://doi.org/10.1139/apnm-2013-0466

Teeuw, W., Bosch, J. A., Veerman, E. C. I., & Nieuw Amerongen, A. V. (2004). Neuroendocrine regulation of salivary IgA synthesis and secretion: Implication for oral health. *Biological Chemistry, 385*, 1137–1146. https://doi.org/10.1515/BC.2004.147

Teles, R., Likhari, V., Socransky, S., & Haffajee, A. (2009). Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. *Journal of Periodontal Research, 44*, 411–417.

Tyrka, A. R., Parade, S. H., Valentine, T., Eslinger, N. M., & Seifer, R. (2015). Adversity in preschool-aged children: Effects on salivary interleukin-1β. *Development and Psychopathology, 27*, 567–576. https://doi.org/10.1017/S0954579415000164

U.S. Department of Health and Human Services. (2000). *Oral health in America: A report of the surgeon general* (Vol. 44, pp. Rockville, MD, 16–Rockville, 28). National Institute of Dental and Craniofacial Research.. https://doi.org/10.1089/pop.2013.0038

Ulmer-Yaniv, A., Djalovski, A., Priel, A., Zagoory-Sharon, O., & Feldman, R. (2018). Maternal depression alters stress and immune biomarkers in mother and child. *Depression and Anxiety, 35*, 1145–1157. https://doi.org/10.1002/da.22818

Ulmer-Yaniv, A., Djalovski, A., Yirmiya, K., Halevi, G., Zagoory-Sharon, O., & Feldman, R. (2018). Maternal immune and affiliative biomarkers and sensitive parenting mediate the effects of chronic early trauma on child anxiety. *Psychological Medicine, 48*, 1020–1033. https://doi.org/10.1017/S0033291717002550

Venugopal, A., & Maheswari, T. (2016). Expression of matrix metalloproteinase-9 in oral potentially malignant disorders: A systematic review. *Journal of Oral and Maxillofacial Pathology, 20*, 474–479.

Veru, F., Laplante, D. P., Luheshi, G., & King, S. (2014). Prenatal maternal stress exposure and immune function in the offspring. *Stress, 17*, 133–148. https://doi.org/10.3109/10253890.2013.876404

Weemaes, C., Klasen, I., Göertz, J., Beldhuis-Valkis, M., Olafsson, O., & Haraldsson, A. (2003). Development of immunoglobulin a in infancy and childhood. *Scandinavian Journal of Immunology, 58*, 642–648. https://doi.org/10.1111/j.1365-3083.2003.01344.x

Williamson, S., Munro, C., Pickler, R., Grap, M. J., & Elswick, R. K. (2012). Comparison of biomarkers in blood and saliva in healthy adults. *Nursing Research and Practice, 2012*, 1–4. https://doi.org/10.1155/2012/246178

World Health Organization. (2013). *Oral health surveys: Basic methods* (5th ed.). Geneva, World Health Organization.

Yirmiya, K., Djalovski, A., Motsan, S., Zagoory-Sharon, O., & Feldman, R. (2018). Stress and immune biomarkers interact with parenting behavior to shape anxiety symptoms in trauma-exposed youth. *Psychoneuroendocrinology, 98*, 153–160. https://doi.org/10.1016/j.psyneuen.2018.08.016

Zefferino, R., Facciorusso, A., Lasalvia, M., Narciso, M., Nuzzaco, A., Lucchini, R., & L'Abbate, N. (2006). Salivary markers of work stress in an emergency team of urban police (1 degree step). *Giornale Italiano Medicina del Lavoro ed Ergonomia, 28*, 472–477.

Zhang, C., Cheng, X., Li, J., Zhang, P., Yi, P., Xu, X., & Zhou, X. (2016). Saliva in the diagnosis of diseases. *International Journal of Oral Science, 8*, 133–137. https://doi.org/10.1038/ijos.2016.38

Zhang, L., Li, X., Yan, H., & Huang, L. (2018). Salivary matrix metalloproteinase (MMP)-8 as a biomarker for periodontitis. *Medicine (Baltimore), 97*, 1–6. https://doi.org/10.1097/MD.0000000000009642

Zhang, S., Zhang, X., Yin, K. E., Li, T., Bao, Y., & Chen, Z. (2017). Variation and significance of secretory immunoglobulin A, interleukin 6 and dendritic cells in oral cancer. *Oncology Letters, 13*, 2297–2303. https://doi.org/10.3892/ol.2017.5703

Zheng, H., Li, R., Zhang, J., Zhou, S., Ma, Q., Zhou, Y., . . . Lin, J. (2014). Salivary biomarkers indicate obstructive sleep apnea patients with cardiovascular diseases. *Scientific Reports, 4*, 1–7. https://doi.org/10.1038/srep07046