Secretory IgA in Saliva can be a Useful Stress Marker

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Abstract

To evaluate secretory immunoglobulin A (slgA) in saliva as an immunological stress marker, we reviewed the literature on slgA and its variation caused by psychosocial factors. Among the studies on the effect of academic stress on slgA secretion, we could distinguish two kinds of stress effects: the immediate stress effect which increases slgA secretion immediately after stress, and the delayed stress effect which decreases slgA secretion several days after stress. On the basis of production and secretion mechanisms of slgA, we also speculated on possible mechanisms that underlie the variations of slgA caused by stress. Eventually, we concluded that slgA in saliva can be a useful stress marker if we analyze the delayed stress effect on slgA separately from the immediate stress effect on slgA.

Key words: IgA, secretory, marker, stress, saliva

Introduction

Psychoneuroimmunological research has shown that psychosocial factors, including stress, social support, and emotion may affect susceptibility to infectious disease by influencing the immune system.

Because the immune system is a part of a complex and interactive network formed by the brain, neurotransmitters and neuropeptides, secretory glands, and various types of immune cells, no single measure of 'immune functioning' can fully express immune competence. However, for practical and ethical reasons, only a few immunological parameters can be measured in experimental research dealing with human subjects.

In several studies, secretory immunoglobulin A (slgA) has been chosen as a measure of resistance to infectious disease, because it plays an important role in the defense mechanism of mucosal membranes.

Besides, psychometric instruments (questionnaires) for measuring stress have been developed. To complement these questionnaires, immunological stress markers would be valuable objective measures.

This article reviews the literature on slgA and its variations caused by psychosocial factors, speculates on possible mechanisms that underlie the variations of slgA, and evaluates slgA, especially salivary IgA, as an immunological stress marker.

General aspects of slgA

Secretory immunoglobulin A (slgA) is found in various secretory fluids, including saliva, breast milk, and nasal, gastrointestinal, bronchial, and urogenital secretions at high levels of concentration. The secretory process and immunological functions of slgA have been extensively studied.

Briefly, the secretory process of slgA can be summarized as follows. At first, dimeric immunoglobulin A molecules, joined by a glycoprotein named J chain, are produced locally by IgA producing plasma cells in the lamina propria of mucosal membranes or in the connective tissue of glands. Some of them diffuse through basement membranes to the basolateral surface of epithelial cells, where they are taken up by the epithelial cells with polymeric immunoglobulin receptors (poly-Ig receptor), then transcytosed to the apical surface of the epithelial cells, and released into secretory fluids in the form of slgA. This secretory process is shown schematically in Fig.1.

The slgA molecule is composed of a dimeric immunoglobulin A containing the J chain, and another glycoprotein, secretory component (SC), which is a residue of the poly-Ig receptor and binds covalently to the J chain. The SC stabilizes the slgA molecule and protects it from degradation by bacterial and digestive enzymes in the secretory fluid environment.

For immunological functions, slgA antibodies prevent bacteria from forming colonies on mucosal surfaces, kill them directly or activate complements or provide synergism with innate defense mechanisms, e.g. lacto-ferrin, lacto-peroxidase, etc. They also neutralize toxins and enzymes produced by bacteria. In addition, slgA antibodies neutralize pathogenic viruses so as to inhibit their penetration into epithelial cells. Moreover, it is recently reported that even low concentrations of slgA, which can not prevent influenza A virus from penetrating into cells, inhibit...
their infectivity by damaging their activation process in the cell\(^b\). It is also reported that dimeric IgA, endocytosed by the poly-Ig receptor, can neutralize the Sendai virus which penetrates into epithelial cells\(^c\).

Besides, the sIgA antibodies prevent allergens and carcinogens\(^d\) from being absorbed through mucosal membranes into the body. Moreover, immune-complexes formed beneath the epithelial cells after antigen absorption are transported out and into secretions by the poly-Ig receptor dependent transport system (or SC dependent transport system)\(^e\).

From these sIgA functions, it may be expected that high proper sIgA secretion would prevent infections by various microorganisms in the mucosal membranes effectively. In fact, a review article concluded that the relatively high levels of sIgA in saliva is related to the lower incidence of upper respiratory tract illness, with the average effective size \(r = 0.25\)\(^f\). In addition, it is recently reported that transient salivary IgA-deficiency, which may permit various allergens to penetrate through the mucosal membranes, in the first year of life is a risk factor for the subsequent development of bronchial hyper reactivity\(^g\). Moreover, in children with a history of recurrent colds and flu, not only elevated psychosocial stress was observed but also lower sIgA/albumin ratios in saliva were detected\(^h\).

**Effect of psychosocial factors on sIgA**

Various psychosocial factors, including academic examination\(^i\)-\(^k\), daily hassles\(^l\), negative mood\(^m\), desirable and undesirable daily events\(^n\), work demand\(^o\), and various relaxing factors\(^p\)-\(^r\) were investigated as a possible sIgA modifier. Results of those studies are shown briefly in Tables 1 to 3. In all of those studies, salivary IgA was chosen as a sIgA, because it is not only

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\(^a\) The Japanese Society for Hygiene

\(^b\) NII-Electronic Library Service

\(^c\) Selective transport system of IgA into external secretions.

Psychosocial factors could affect sIgA response at the following three steps in the SC-dependent transport system: 1. number of plasma cells, 2. immunoglobulin producing activity of plasma cells, 3. expression of poly-Ig receptors or SCs.
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important for defense against upper respiratory tract infectious diseases as mentioned previously, but it also can be collected easily.

However, current literature does not show much agreement about the determinant of a slgA response, except for the relaxing factors. Studying academic stress, as shown in Table 1, a decrease in slgA level26-29, as well as no alterations30 or an increase31-35 have been reported. Studying other kinds of stressful events as shown in Table 2, the slgA response was also not simple36-39. On the other hand, all of the relaxing factors shown in Table 3 had positive effects on the slgA response.

To explain the disagreement on the stress effect mentioned above, we tried to determine some rules which control the slgA response to psychosocial factors. For simplifying the problem, we concentrated our attention on the studies on the academic stress effect as shown in Table 1. When we inspected the difference of methods of those studies, we noticed some interesting facts as follows. The slgA levels decreased when it was determined several days or a few weeks after academic stress26-29. Whereas the slgA levels increased when determined immediately after academic stress31-35. (Refer to slgA variation and sampling time in Table 1.) We may call those opposite slgA responses after stress as a delayed stress effect on the slgA response and an immediate stress effect on slgA response, respectively. The immediate stress effect seems to fade away in a short time after stress is relieved, resulting in a return of increased slgA to its initial level31-35.

As a result of this research, we have become able to understand, at least in part, the effect of other psychosocial stressors on slgA secretion, as shown in Table 2. We may say it is a case of the delayed stress effect on the slgA response that daily hassles caused a decrease in salivary IgA secretion after 4 weeks36. We may also say it is a case of immediate stress effect on the slgA response that the work demand of air traffic controllers caused an increase in salivary IgA secretion37.

However, it may not be appropriate to explain by the idea mentioned above that desirable and undesirable daily events correlate positively or negatively to salivary IgA secretion respectively38, or that a negative mood showed a positive correlation to salivary IgA levels38. We must have some other ideas to explain those emotional effects on salivary IgA secretion.

All of the relaxing factors shown in Table 3 had positive effect on the slgA response39-41. We may call those phenomena a relaxation effect on slgA response.

Table 1  Effects of academic stress on salivary IgA26-29.

<table>
<thead>
<tr>
<th>Author</th>
<th>Stress</th>
<th>Change</th>
<th>Sampling time</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jemmott</td>
<td>Exam</td>
<td>-- (p&lt;.025)</td>
<td>Five times over one year</td>
<td>Higher slgA secretion in RAS group (p&lt;.06). No recovery in IPS group.</td>
</tr>
<tr>
<td>(1983)</td>
<td></td>
<td></td>
<td>(Sept, Nov*, April*, June*, July)</td>
<td></td>
</tr>
<tr>
<td>Jemmott</td>
<td>Exam</td>
<td>-- (p&lt;.0001)</td>
<td>Three occasions (1st: 5 days before exam, 2nd*: on day of exam, 3rd: 14 days after exam.)</td>
<td>Higher slgA concentration in adequate social support group (p&lt;.05).</td>
</tr>
<tr>
<td>(1988)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouton</td>
<td>Exam</td>
<td>-- (p&lt;.01)</td>
<td>Four occasions over 2 academic years (March, April*, Sept, Oct*)</td>
<td>A weak negative correlation (between stress level and slgA secretion: r=-.25, stress level and slgA concentration: r=-.36)</td>
</tr>
<tr>
<td>(1989)</td>
<td></td>
<td>(at the most contrast, April-Sept)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deinze</td>
<td>Exam</td>
<td>-- (p&lt;.01)</td>
<td>25 days before exam, and every day for 2 weeks around exam</td>
<td>Saliva was taken every morning immediately after awakening. No relationship between slgA and URT symptoms.</td>
</tr>
<tr>
<td>(1998)</td>
<td></td>
<td>weeks around exam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiegolt-</td>
<td>Exam</td>
<td>++</td>
<td>Two occasions (1st: one month before exam, 2nd*: on the first day of exam week)</td>
<td>Plasma IgA increased.</td>
</tr>
<tr>
<td>McClelland</td>
<td>Exam</td>
<td>+ (p&lt;.06)</td>
<td>Three occasions (1st*: right after exam, 2nd*: one and 3/4 hr later, 3rd: several days later)</td>
<td>Rise of slgA right after exam was followed by a drop one and 3/4 hr later. Lower slgA, steeper drop of slgA, and greater increase of NE in stronger n power group.</td>
</tr>
<tr>
<td>(1985)</td>
<td></td>
<td>(right after exam)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evans</td>
<td>presentation</td>
<td>+ (p&lt;.1)</td>
<td>In 2 consecutive weeks during the same scheduled hours (9:30, 10:30, 11:30, 12:30), and immediately after presentation.</td>
<td>Increase in cortisol.</td>
</tr>
<tr>
<td>(1994)</td>
<td>for a science module</td>
<td>(immediately after presentation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spangler</td>
<td>Exam</td>
<td>++ (p&lt;.05)</td>
<td>15 min before and 5 and 15 min after exam.</td>
<td>SlgA also increased after the control situation.</td>
</tr>
<tr>
<td>(1997)</td>
<td></td>
<td>(5 and 15 min after exam)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: stressful period  RAS: the relaxed affiliative motive syndrome  IPS: the inhibited power motive yndrome  URT: upper respiratory tract

3
Possible mechanisms of variation of sIgA secretion caused by psychosocial factors.

As already shown in Fig.1, sIgA in saliva is produced locally by plasma cells in the salivary gland, and transported from the basolateral site of acinar cells or ductal cells of the salivary gland to their luminal site by the mechanism of SC dependent transport.

In the process of production and secretion of sIgA, we can point out three steps where psychosocial factors would affect a variation of sIgA secretion. In Fig.1, those steps are indicated by the large numerical characters, 1, 2, and 3.

The step shown by the character '1' in Fig.1 is the amount of plasma cells of the salivary gland. The number of plasma cells, expected to correlate positively with the level of IgA secretion in saliva, is in the balance with the continuous recruitment of plasma cells and the loss of them.

The plasma cells in the salivary gland will be recruited continuously to the gland in the following way. The precursor cells, B lymphocytes, are activated in mucosal associated lymphoid tissue (MALT), and induced switching to IgA. MALT includes gut associated lymphoid tissue (GALT), bronchial associated lymphoid tissue (BALT), and tonsil. The activated B lymphocytes migrate from MALT through lymphatic vessels into the blood circulation, then reach and stay in the salivary gland afterwards. Several days will pass before the B lymphocytes mature into plasma cells in the salivary gland.

At the same time, the plasma cells are lost continuously from the salivary gland, depending on the life span of the plasma cells. The average life span of plasma cells is thought to be less than 20 days, relating to the site of B cell activation, e.g. spleen, lymph nodes, lamina propria, and bone marrow.

Without the recruitment of precursor cells, continuous loss of plasma cells will result in a reduction of the number of plasma cells, and, consequently, in a decrease of sIgA secretion. In the salivary gland, rapid turnover of plasma cells was indicated in the case of chronic lymphocytic leukemia given an extracorporeal blood irradiation therapy, and showing a significant decrease of circulating lymphocytes, which means a reduction in the supply of precursor cells of plasma cells, followed by a rapid decrease in salivary IgA levels.

Favorably, in meta-analytic studies, it is concluded that psychological stress affects T and B lymphocytes in the blood so as to inhibit the number and functions of the lymphocytes. Stress hormones, cortisol and catecholamines, may be responsible for this stress effect on lymphocytes.

Since the effect of psychological stress on blood lymphocytes resembles that of extracorporeal blood irradiation, it is expected that psychological stress decreases salivary sIgA secretion in the same manner as extracorporeal blood irradiation, through a reduction in the recruitment of precursor cells of plasma cells to the salivary gland.

The decrease of sIgA secretion will happen gradually, depending on the reduction rate of the supplying precursor cells and the half life of plasma cells. It is tempting to think this speculated mechanism of a decrease in sIgA may underlie the delayed stress effect on the sIgA response, mentioned previously, meaning a decrease of sIgA secretion several days after stress.

Another step, shown by character '2' in Fig.1, is the IgA producing ability of plasma cells. There is no direct evidence that a psychological stress or relaxation modulates IgA production by plasma cells yet. However, psychological stress or relaxation may possibly modulate plasma cell activity through stimulation of nerves, cytokines, and hormones. This is considered because a histopathologic study showed that plasma cells in the lamina propria were associated with nerve fibers. Also, some studies reported that several cytokines, including interleukin-1 (IL-1), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), erythropoietin, and nerve growth factor, and hormones, including growth hormone, insulin-like growth factor-I, insulin-like growth factor-II, and insulin, influence the immunoglobulin production by plasma cells.

The remaining step which psychosocial factors might affect, shown by character '3' in Fig.1, is the SC dependent transport of IgA.
IgA by epithelial cells. The production of a secretory component by epithelial cells can be regulated by various kinds of immunological, endocrinological, and neural factors. These factors include interferon-γ, IL-4, tumor necrosis factor-α, IL-1α, IL-1β, estradiol, and androgens, cholinergic agonists, and beta-adrenergic agonists. Prostaglandin E2, vasoactive intestinal peptide, and somatostatin. However, it should be noted that the mode of effect of these factors is rather organ-specific. Cholera toxin, 8-bromoadenosine 3′,5′-cyclic monophosphate (cAMP), and 8-bromoguanosine 3′,5′-cyclic monophosphate (cGMP) significantly increased the secretory component production by submandibular acinar cells. This result implies that, in the salivary gland, IgA transport by the SC dependent system can be affected by psychological stress or relaxation through neural and/or endocrine mechanisms which vary intracellular cAMP or cGMP.

Can IgA be a useful immunological stress marker?

As shown previously, current literature showed a favorable (positive) agreement in the effect of relaxing factors, whereas it showed a disagreement in the effect of stress on the IgA response.

Stone et al. attributed the inconsistency of the IgA response to a negative correlation between IgA concentration and salivary flow and to a possible degradation of IgA by proteases in whole saliva. Moreover, they proposed their original method of measuring specific IgA antibodies in parotid saliva as a measure of immunocompetence, in place of measuring total IgA in whole saliva.

In opposition to the opinion of Stone et al., Jemmott et al. suggested that the negative correlation between the IgA concentration and salivary flow is weak and not so problematic when unstimulated saliva is used, and taking salivary flow into account when measuring IgA levels, e.g., assessing IgA secretion rate, the problem of the negative correlation between IgA concentration and salivary flow will be solved. Jemmott et al. also suggested that the measurement of IgA concentration in whole saliva is highly reproducible and stable over time. Finally, they concluded that IgA is a useful measure of resistance to infectious diseases.

It is true that Jemmott et al. have successfully showed that academic stress inhibits the IgA secretion rate. In addition, they showed personality characteristics differentiated patterns of IgA secretion rate and a positive effect of social support on IgA levels. (Refer notes in Table 1.)

However, Mouton et al. suggested that assaying salivary IgA to measure stress may not be as useful in psychophysiological

| Table 3 Effect of relaxing factors on salivary IgA. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Author          | Factor          | Change          | Sampling time                                           | Note                                |
| Dillon (1985)   | Humorous        | ++ (p=.026)     | Before and after videocassette (30 min long)            | No significant change in saliva cortisol. |
|                 | videotape       |                 |                                                       |                                     |
| Didactic        | videotape       | -               |                                                       |                                     |
| Green (1987)    | Relaxation      | ++ (p=.05)      | Before and after treatment (20 min long)                |                                     |
|                 | response        |                 |                                                       |                                     |
| Guided          | visualization   | ++ (p=.05)      |                                                       |                                     |
| Massage         | ++ (p=.01)      |                 |                                                       |                                     |
| Lying down      | ++              |                 |                                                       |                                     |
| Touching control| ++              |                 |                                                       |                                     |
| Jasnoski (1987) | Relaxation      | ++ (p=.025)     | Before and after protocol (1 hr long)                   | Negative correlation (r=-.39, p<.05) between IgA and saliva norepinephrine. |
|                 | (vs vigilance task control) |            |                                                       |                                     |
| Green (1988)    | Relaxation      | ++ (p=.001)     | Before and after 20-min relaxation practice              | Increase of serum IgA (p=.001), IgG (p<.001), IgM (p<.005) over a 3 week practice period. |
|                 |                 | ++ (p=.014)     | On the 22nd day of relaxation practice                   |                                     |
|                 | (22nd day vs 1st day) |            |                                                       |                                     |
| Olness (1989)   | Self-hypnosis   | ++              | Before and after self-hypnosis (25 min long)             | Children (6-12 yr old) were recruited. |
|                 |                 |                 |                                                       |                                     |
| Self-hypnosis   | ++ (p=.007)     |                 |                                                       |                                     |
|                 | + specific suggestions |            |                                                       |                                     |
research as expected. It was because, in their academic stress study enrolling dentistry students on four occasions over a period of 8 months, they observed a significant difference in the level of salivary IgA only for the most polarized contrast, i.e., between final exam and end of summer vacation, and they also observed a weak negative correlation between the level of salivary IgA and the stress rating only at the final exam²⁵.

In our opinion, the slgA response to psychological stress can be rather complex because of the following. The delayed stress effect, which decreases slgA secretion in saliva gradually, must be superimposed with the immediate stress effect, which increases slgA secretion in saliva immediately. Thus, an inhibition of the slgA response caused by stress experienced several days previously can be cancelled at the time of saliva sampling by an increase in the slgA response caused by another stress experienced only several hours before saliva sampling.

Therefore, when the delayed and inhibitory effect of some stress on the slgA response is concerned, it is necessary to remove immediate and increasing effect of other stresses from the slgA response. Deinzer, et al.²⁹ successfully solved the problem by sampling saliva every morning immediately after awakening and before doing anything else.

Reversely, when the immediate and increasing effects of some stress on the slgA response is concerned, the delayed and the inhibitory effect of other stresses would not affect the result, because the delayed stress effect would be almost constant during the short experimental period. From this point, slgA in saliva can be said to be a more suitable immunological marker for the immediate stress effect than for the delayed stress effect.

Complexity of slgA response to stress may be seen only in human. Interestingly, it is reported that salivary IgA can be a marker of social stress in rats³⁰. Male rats housed singly showed stable slgA levels with little variation, while those housed separately with a female showed an initial decrease in slgA followed by a steady increase. Males housed in a group (n=6) showed a steady decline in slgA levels³⁰.

In their study of air traffic controllers, Zeier, et al.³¹ suggested that positive emotional engagement is responsible for the observed slgA increase. They also suggested that measuring slgA response may be a valuable tool for differentiating between positive and negative stress effects or between successful and unsuccessful adaptation or coping with situational demands³¹.

Miletic, et al.³² reported an interesting fact that elderly persons who were excited by social events over the weekend days (friends or family member visits, worship and social events in churches, etc.) showed an increase in their slgA secretion during the weekend days.

In summary, slgA in saliva is a promising candidate as a stress marker which may be able to differentiate between positive and negative stress effects. However, some additional studies will be necessary to establish proper methods to separate the immediate stress effect and the delayed stress effect on slgA response, and to elucidate mechanisms of slgA variation caused by psychosocial factors.

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