

## Mucosal Immunity Modulated by Integrative Meditation in a Dose-Dependent Fashion

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### Abstract

**Objective:** Prior research had shown that an additional training session immediately after acute stress increased release of salivary secretory immunoglobulin A (sIgA) in a group trained with 5-day Integrative Body–Mind Training (IBMT) in comparison to a control group given the same amount of relaxation training. However, 5 days of training did not influence the basal secretion of sIgA. The current study seeks to extend this finding and determine whether increasing amounts of IBMT will increase the basal sIgA level, suggesting further improvements in mucosal immune function.

**Design:** Thirty-five (35) Chinese undergraduates were randomly assigned either to an experimental group receiving 4 weeks of IBMT or a relaxation control. Salivary sIgA levels at baseline before training and three stages (i.e., rest, stress, and additional 20-minute practice) after 2 and 4 weeks training were assessed.

**Results:** The basal sIgA levels increased significantly in the experimental subjects but not in controls after 4 weeks of training. An additional IBMT practice session immediately after acute stress produced significantly higher sIgA release for the IBMT-trained group in comparison with controls at week 2 and 4. This effect was larger at week 4 than week 2.

**Conclusions:** These results indicate that the IBMT produces a change in the basal immune system and larger acute effects as the dose of training increases.

### Introduction

SECRETORY IMMUNOGLOBULIN A (sIgA), an index of mucosal immunity, plays an important role in host defense.<sup>1</sup> The secretory immune system of the upper respiratory tract's mucosal tissues is considered the body's first line of defense against pathogens. sIgA measured in saliva is a convenient and often used indicator of immune status.<sup>2</sup>

Salivary sIgA becomes a focus of interest in psychoimmunological research since it has been shown to be sensitive to variations in subjective and objective stress levels. Acute laboratory stressors, such as public speaking and mental arithmetic, appear to increase salivary sIgA.<sup>3–6</sup> Chronic psychosocial stresses are associated with reductions in sIgA.<sup>7,8</sup>

Psychologic stress is now recognized to be one of the important risk factors for upper respiratory tract infections (URTI).<sup>9–11</sup> Salivary sIgA is a major effector against pathogens causing URTI by preventing adherence of virus to the nasal and oral mucosa.<sup>12</sup> Lower salivary sIgA levels are associated with increased susceptibility to URTI.<sup>13–15</sup> Studies

suggest that when psychologic treatments increase resistance to infection of the upper respiratory tract, the salivary sIgA acutely also increases after individual sessions, but the increase of resting or basal secretion of sIgA needs longer-term training.<sup>16–18</sup>

Integrative Body–Mind Training (IBMT) integrates several key components of body–mind techniques including body relaxation, breathing adjustment, mental imagery, and mindfulness training, which can help accelerate practitioner access to meditative states.<sup>19</sup> Our previous study showed that 5 days of IBMT improves self-regulation ability and control of stress.<sup>20</sup> An additional training session immediately after acute stress induced increased release of more salivary sIgA in a group trained with 5 days of IBMT in comparison to a control group given the same amount of relaxation training (RT). However, 5 days of training did not influence the basal sIgA level. The current study set out to extend our previous finding and determine whether increasing amounts of integrative meditation training will increase the basal sIgA level, and produce further improvements in mucosal immune function in response to stress.

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## Materials and Methods

### Subjects

Thirty-five (35) healthy Chinese undergraduates (17 males and 18 females, mean age [ $\pm$ SD] = 21.31  $\pm$  0.76) without any previous training experiences participated in this study. They were excluded if they had a history of psychiatric, immune, metabolic, or respiratory disease, or had a current health problem, or reported taking any medication with known effects on the immune or endocrine systems. The human experiment was approved by the local Institutional Review Board, and informed consent was obtained from each participant.

Subjects were randomly assigned to an experimental group and a control group. Seventeen (17) experimental subjects (8 males and 9 females) continuously attended practice of IBMT for 4 weeks, whereas 18 control subjects (9 males and 9 females) received an equal period of RT.

### Experimental procedure

Before training, the IBMT and RT coaches (qualified instructors, with years of practice experience) separately gathered the experimental and control subjects to introduce the structure of the program and the training, answer the questions, and also set up the exact time, training room, and discipline for the group practice. Saliva was collected to get baseline samples.

All subjects completed group-training sessions for 4 successive weeks. Experimental and control subjects separately followed the IBMT-CD and RT-CD guided by the coaches in different rooms every night for 20 minutes. Following each training session, every subject filled out a self-report questionnaire and evaluated the practice. The coaches gave brief and immediate responses to questions from the participants as required.

All subjects performed the stress test after 2 and 4 weeks' training. After a 5-minute rest, subjects performed 3 minutes of mental arithmetic (described below) to induce stress. Following the stress induction, the experimental subjects practiced IBMT for 20 minutes, whereas the control subjects practiced 20-minute RT. Saliva samples were collected repeatedly before the stress, immediately after the stress, and after additional 20-minute practice.

### Training methods

IBMT involves several body-mind techniques including (1) body relaxation, (2) breath adjustment, (3) mental imagery, and (4) mindfulness training, accompanied with selected background music.<sup>19,20</sup> The method emphasizes no effort to control thoughts, but a state of restful alertness that allows a high degree of awareness of body, breathing, and external instructions from the CD. Thought control is achieved gradually through posture and relaxation, body-mind harmony, and balance with the help of the tutor rather than making the trainee attempt an internal struggle to control thoughts in accordance with instruction.<sup>21</sup>

RT is a form of muscle relaxation technique very popular in the West.<sup>20,22</sup> When performing RT, the practitioner focuses on the physical body. In the training session, participants follow the CD with instruction and background music, and relax each muscle group of the body starting from the

head and going down to the feet. This exercise helps participants achieve a calm state of relaxation.

### Mental arithmetic task

Mental arithmetic was used as an acute laboratory stressor.<sup>20,23</sup> Subjects performed serial subtraction of 47 from a four-digit number and responded verbally. During the 3 minutes of the mental arithmetic task, participants were prompted to be as fast and accurate as possible. If the participants did not finish the mental arithmetic in time and correctly, the computer would produce a harsh sound to remind the subjects, who were required to restart the task and do it again.

### Sampling methods and biochemical analysis

Salivary sIgA levels were assessed at baseline before training and at three stages (i.e., rest, stress, and additional 20-minute practice) after 2 and 4 weeks training.

To control for variations of sIgA levels over the circadian rhythm, saliva sample collection was performed from 2:00 PM to 6:00 PM.<sup>24</sup> About 1-mL saliva samples were collected by one-off injectors and were encased in test tubes in succession, with the tubes placed into a refrigerator under  $-20^{\circ}\text{C}$  and then thawed 24 hours later for analysis. The concentration of sIgA was analyzed by radioimmunoassay at the Dalian Medical University. Intra- and interassay coefficients of variation were below 10%. To reduce error variance caused by imprecision of the intra-assay, all samples of 1 subject were analyzed in the same run.

### Statistical analyses

All statistical analyses were performed using the Statistical Package for Social Sciences 13.0 for Windows (SPSS Inc., Chicago, IL). Data were analyzed using repeated-measures analyses of variance (ANOVAs). Pairwise comparisons were performed for each sIgA sample.

## Results

Means and standard deviations of salivary sIgA levels at baseline before training and in response to stress test after 2 and 4 weeks training are shown in Table 1.

Since assignment was random, the experimental group and control group should not differ prior to training. As expected, there was no significant difference between two groups at baseline sIgA level ( $F(1, 33) = 0.448, p > 0.05$ ).

For the sIgA levels response to stress test after 2 and 4 weeks training, repeated-measures ANOVAs were conducted with the between-group factor of Group (IBMT and RT), the within-subjects factors of Session (week 2 and week 4), and Condition (Rest, Stress, and Additional 20-minute practice). The analyses revealed significant main effects for Group ( $F(1,33) = 8.279, p = 0.007$ ), Session ( $F(1,33) = 5.768, p = 0.023$ ), and Condition ( $F(2,66) = 69.277, p < 0.001$ ), as well as significant interactions for Group  $\times$  Condition ( $F(2,66) = 9.341, p < 0.001$ ), Session  $\times$  Condition ( $F(2,66) = 8.579, p = 0.001$ ), and marginally significant interaction for Session  $\times$  Group ( $F(1,33) = 3.455, p = 0.063$ ).

*Post hoc* analysis showed that there was no significant difference between the before-training baseline and the

TABLE 1. SALIVARY SECRETORY IMMUNOGLOBIN A LEVELS ( $\mu\text{g/mL}$ ) AT BASELINE BEFORE TRAINING AND THREE STAGES AFTER 2 AND 4 WEEKS TRAINING

Time	Rest		Stress		Additional 20-min practice	
	IBMT	RT	IBMT	RT	IBMT	RT
Pre-training	262.16 $\pm$ 88.24	242.69 $\pm$ 78.83	368.71 $\pm$ 129.97 $\blacktriangle$	322.38 $\pm$ 107.89 $\blacktriangle\blacktriangle$	407.83 $\pm$ 143.45 $\blacktriangle\blacktriangle\blacktriangle$ *	306.26 $\pm$ 95.97 $\blacktriangle\blacktriangle$ *
Week 2	272.80 $\pm$ 103.49	235.52 $\pm$ 62.47	399.78 $\pm$ 149.33 $\blacktriangle$	319.84 $\pm$ 110.23 $\blacktriangle$	533.44 $\pm$ 167.52 $\blacktriangle\blacktriangle\blacktriangle\blacktriangle\blacktriangle$ ###	336.67 $\pm$ 105.16 $\blacktriangle\blacktriangle\blacktriangle$ **
Week 4	311.39 $\pm$ 114.48 $\dagger$ ,#	245.52 $\pm$ 79.15*				

Values are mean  $\pm$  SD.

\* $p < 0.05$ , \*\* $p < 0.001$  RT group vs. IBMT group.  
 $\dagger p < 0.05$  vs. Pre-training (Baseline); # $p < 0.05$ , ## $p < 0.01$  vs. Week 2.  
 $\blacktriangle p < 0.01$ ,  $\blacktriangle\blacktriangle p < 0.001$  vs. Rest;  $\blacktriangle\blacktriangle\blacktriangle p < 0.05$ ,  $\blacktriangle\blacktriangle\blacktriangle\blacktriangle p < 0.001$  vs. Stress.  
 IBMT, Integrative Body-Mind Training; RT, relaxation training.

resting sIgA levels at week 2 for either group. There was also no significant difference between two groups in the resting sIgA levels at week 2 ( $F(1, 33) = 2.048, p > 0.05$ ).

The resting sIgA level at week 4 for the experimental group (but not for the control group) was higher than week 2 ( $t(16) = -2.221, p = 0.042$ ), and baseline ( $t(16) = -2.604, p = 0.02$ ). After 4 weeks training, the resting sIgA level of the experimental group was significantly higher than that of the control group ( $F(1, 33) = 4.955, p = 0.033$ ).

Following the acute mental arithmetic challenge, the sIgA concentration significantly increased compared to the resting level for both groups at week 2 and 4 assessments. For the sIgA levels immediately after stress, no significant differences were found between two groups at each session, nor between two sessions in each group ( $p > 0.05$ ).

After stress, the experimental group received an additional 20 minutes IBMT, and the control group relaxed for 20 minutes. In comparison to the level immediately after stress, the sIgA concentration following the additional practice significantly increased in the experimental group at both week 2 ( $t(16) = -2.321, p = 0.035$ ) and week 4 ( $t(16) = -5.081, p < 0.001$ ), but no significant change was found in the control group at any sessions ( $p > 0.05$ ). The sIgA level following the additional practice at week 4 was higher than week 2 only for the experimental group ( $t(16) = -3.677, p = 0.002$ ). There was a significant difference between two groups in the sIgA level following the additional practice at both week 2 ( $F(1, 33) = 7.598, p = 0.01$ ) and week 4 ( $F(1, 33) = 22.811, p < 0.001$ ).

**Discussion**

The current study set out to determine whether 2 or 4 weeks of IBMT will increase the basal sIgA level, and produce further improvements in mucosal immune function in response to stress. The present study showed that the basal sIgA level increased significantly in the experimental subjects but not in controls after 4 weeks training; an additional IBMT practice immediately after stress rendered the experimental subjects significantly higher sIgA release than controls at week 2 and 4 stress assessments, and the effect was larger at week 4 than week 2.

sIgA is the main immunological defense of mucosal surface. Chronic psychosocial stresses are associated with reductions in sIgA.<sup>7,8</sup> A study examined the time of recovery of sIgA alterations associated with several days of academic examinations; the most striking result was that 14 days poststress basal sIgA concentrations of exam students were still significantly lower than control levels, and remained below baseline, though students recovered very soon in terms of self-reported stress.<sup>25</sup> Lower basal salivary sIgA levels are associated with increased susceptibility to URTI.<sup>13-15</sup> Reid et al.<sup>18</sup> reported that 4 weeks stress management training did not increase the resting secretion of sIgA in young adults. Hewson-Bower and Drummond<sup>16</sup> reported that the sIgA concentration increased over 13 weeks of psychologic treatment focusing on stress management and relaxation with guided imagery in children who were susceptible to recurrent respiratory infections. The present study showed that the basal sIgA level increased significantly over 4 weeks IBMT, but no effect was found over 4 weeks RT exercise. These results indicate that IBMT would be a good mucosal immune

enhancer, improving resistance to infection of the upper respiratory tract.

Acute stressors, such as public speaking and mental arithmetic, appear to increase salivary sIgA,<sup>3–6</sup> but the mechanism still remains unclear. Segerstrom and Miller<sup>26</sup> performed a meta-analysis on more than 300 empirical articles describing a relationship between psychologic stress and parameters of the immune system in human participants; acute stressors (lasting minutes) were associated with potentially adaptive up-regulation of some parameters of natural immunity, as reflected by increased number of natural killer cells and neutrophils in peripheral blood, and downregulation of some functions of specific immunity, as reflected by decreased proliferative responses; the only exception to this pattern was the increased secretion of IgA antibody, which is a product of the specific immune response. Whether this effect is part of a larger nonspecific protein release in the oral cavity in response to acute stress is an interesting question. Bosch et al.<sup>27</sup> suggested that the time frame of acute stressors is too short for the synthesis of a significant amount of new antibody; therefore, this increase is probably due to release of already-synthesized antibody from plasma cells and increased translocation of antibody across the epithelium and into saliva. The present study showed that following the acute mental arithmetic challenge, both groups significantly increased in salivary sIgA activity; for the sIgA levels immediately after stress, no significant differences were found between two groups at week 2 and 4 assessments, though the resting sIgA level at week 4 of the experimental group was higher than that of the control group.

Recently, we examined the dynamic changes in salivary sIgA response to acute stress.<sup>23</sup> The increase of sIgA response to acute stress was transient; there was a marked decrease from the level immediately after stress over the succeeding 20 minutes. In the present study, the experimental group received an additional 20 minutes of IBMT after stress, and the control group relaxed for 20 minutes. The results showed that there was no significant change in the control group from the level immediately after stress over the succeeding 20 minutes, whereas there was a marked elevation in the experimental group; there was a significant difference between two groups in the sIgA level following the additional practice at both week 2 and week 4. These results indicated that both IBMT and RT may assist in improving the mucosal immune function in response to stress, and IBMT is more effective than RT.

The present findings may indicate that the IBMT produces persistent changes in the mucosal immune system and larger acute effects as the dose of training increases. This increased responsiveness of the immune system could be important in handling stressful life events.<sup>28</sup> It is suggested that the current results provide one mechanism through which long-term practice of IBMT could achieve improved health and stress management.

There were two main limitations to this study. One was the small sample size (35 subjects) and consisted only of Chinese undergraduates. The other was the short study period (4 weeks), with no examination of whether the effects of IBMT would persist if no further practice occurred. Further studies could explore the effects of long-term training on the immune function both when practice continues and following its end and with a larger and more diverse sample.

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## Disclosure Statement

No competing financial interests exist.

## References

- Lamm ME. Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense. *Am J Physiol* 1998;274:G614–G617.
- Carins J, Booth C. Salivary immunoglobulin-A as a marker of stress during strenuous physical training. *Aviat Space Environ Med* 2002;73:1203–1207.
- Evans P, Bristow M, Hucklebridge F, et al. Stress, arousal, cortisol and secretory immunoglobulin A in students undergoing assessment. *Br J Clin Psychol* 1994;33(pt 4):575–576.
- Ring C, Drayson M, Walkey DG, et al. Secretory immunoglobulin A reactions to prolonged mental arithmetic stress: Inter-session and intra-session reliability. *Biol Psychol* 2002; 59:1–13.
- Ring C, Harrison LK, Winzer A, et al. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor, and exercise: Effects of alpha-adrenergic blockade. *Psychophysiology* 2000;37:634–643.
- Willemsen G, Ring C, McKeever S, Carroll D. Secretory immunoglobulin A and cardiovascular activity during mental arithmetic: effects of task difficulty and task order. *Biol Psychol* 2000;52:127–141.
- Gallagher S, Phillips AC, Evans P, et al. Caregiving is associated with low secretion rates of immunoglobulin A in saliva. *Brain Behav Immun* 2008;22:565–572.
- Phillips AC, Carroll D, Evans P, et al. Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. *Brain Behav Immun* 2006;20:191–197.
- Cobb JM, Steptoe A. Psychosocial influences on upper respiratory infectious illness in children. *J Psychosom Res* 1998;45:319–330.
- Cobb JM, Steptoe A. Psychosocial stress and susceptibility to upper respiratory tract illness in an adult population sample. *Psychosom Med* 1996;58:404–412.
- Cohen S, Frank E, Doyle WJ, et al. Types of stressors that increase susceptibility to the common cold in healthy adults. *Health Psychol* 1998;17:214–223.
- Mestecky J, Russell MW. Mucosal immunoglobulins and their contribution to defence mechanisms: An overview. *Biochem Soc Trans* 1997;25:457–462.
- Drummond PD, Hewson-Bower B. Increased psychosocial stress and decreased mucosal immunity in children with recurrent upper respiratory tract infections. *J Psychosom Res* 1997;43:271–278.
- Klentrou P, Cieslak T, MacNeil M, et al. Effect of moderate exercise on salivary immunoglobulin A and infection risk in humans. *Eur J Appl Physiol* 2002;87:153–158.
- Volkman ER, Weekes NY. Basal sIgA and cortisol levels predict stress-related health outcomes. *Stress Health* 2006;22: 11–23.

16. Hewson-Bower B, Drummond PD. Psychological treatment for recurrent symptoms of colds and flu in children. *J Psychosom Res* 2001;51:369–377.
17. Pawlow LA, Jones GE. The impact of abbreviated progressive muscle relaxation on salivary cortisol and salivary immunoglobulin A (sIgA). *Appl Psychophysiol Biofeedback* 2005;30:375–387.
18. Reid MR, Mackinnon LT, Drummond PD. The effects of stress management on symptoms of upper respiratory tract infection, secretory immunoglobulin A, and mood in young adults. *J Psychosom Res* 2001;51:721–728.
19. Tang YY. *Health from Brain, Wisdom from Brain*. Dalian, China: Dalian University of Technology Electronic & Audio-Video Press, 2005.
20. Tang YY, Ma YH, Wang JH, et al. Short-term meditation training improves attention and self-regulation. *Proc Natl Acad Sci U S A* 2007;104:17152–17156.
21. Tang YY, Posner MI. Attention training and attention state training. *Trends Cogn Sci* 2009;13:222–227.
22. Benson H, Greenwood MM, Klemchuk H. The relaxation response: Psychophysiological aspects and clinical applications. *Int J Psychiatry Med* 1975;6:87–98.
23. Fan YX, Tang YY, Lu QL, et al. Dynamic changes in salivary cortisol and secretory immunoglobulin A response to acute stress. *Stress Health* 2009;25:189–194.
24. Hucklebridge F, Clow A, Evans P. The relationship between salivary secretory immunoglobulin A and cortisol: Neuroendocrine response to awakening and the diurnal cycle. *Int J Psychophysiol* 1998;31:69–76.
25. Deinzer R, Kleineidam C, Stiller-Winkler R, et al. Prolonged reduction of salivary immunoglobulin A (sIgA) after a major academic exam. *Int J Psychophysiol* 2000;37:219–232.
26. Segerstrom SC, Miller GE. Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychol Bull* 2004;130:601–630.
27. Bosch JA, Ring C, de Geus EJ, et al. Stress and secretory immunity. *Int Rev Neurobiol* 2002;52:213–253.
28. Glaser R. Stress-associated immune dysregulation and its importance for human health: A personal history of psychoneuroimmunology. *Brain Behav Immun* 2005;19:3–11.

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