

# LRA by ELISA/ACT® CLINICAL UPDATE # 9

## Secretory IgA

### What is secretory immunoglobulin A, and what constitutes the mucosal immune system?

Secretory IgA (sIgA) is the ‘Guard-All’ shield -- a primary defense for all mucosal surfaces when immunoreactive foreign invaders present themselves. Blood stream Immunoglobulin A (IgA) was identified three decades ago<sup>3</sup>. IgA did not become known as the antibody of mucosal secretions until 1965 when it was found in tears, saliva, and breast milk, and, subsequently in all mucosal secretions of the gastrointestinal tract, the respiratory tract, nasal secretions, bile, and genitals<sup>3,12</sup>. The IgA molecule consists of two heavy and two light immunoglobulin chains. In humans there are two subclasses of IgA (IgA<sub>1</sub> and IgA<sub>2</sub>). The distribution of these subclasses depends on where the IgA is found. Selected properties of blood and secretory IgA are shown in Table 1<sup>7,12</sup>.

Table 1. Properties of IgA in blood and mucosal secretions.

	Blood IgA	Secretory IgA
Component	$\alpha_2L_2$	$(\alpha_2L_2)_2 \cdot J \cdot SC$
Subclass	IgA <sub>1</sub> >IgA <sub>2</sub>	IgA <sub>1</sub> ≥IgA <sub>2</sub>
MW	160,000	385,000
Site of Production	bone marrow	BALT, MALT, GALT, other
Ontogeny	late	early

$\alpha$  =  $\alpha$  heavy chain, L = light chain; SC = secretory component; MW = molecular weight; BALT = bronchus-associated lymphoid tissue; MALT = mucosal-associated lymphoid tissue; GALT = gut-associated lymphoid tissue.

As indicated, sIgA differs from blood IgA in that it consists of two disulfide-linking IgA molecules, complexed with two polypeptides( a J chain and a ‘secretory component’). **For accurate testing, a sIgA procedure must analyze something unique (like the ‘secretory component’) since leaky or permeable tissues often have a mix of blood stream IgA and mucosal sIgA.** This has led to great confusion in interpreting earlier, less specific sIgA assays.

IgA is the predominant immunoglobulin in humans. Its concentration in blood is second to that of IgG, but almost all of the immunoglobulin in external secretions is sIgA. The production rate of IgA, which has a short half-life of 5 to 6 days, has been estimated to be 66 mg /kg body mass per day. In contrast, the production rate of IgG is only about 34 mg/kg/day<sup>10</sup>. More than two thirds of the IgA is produced locally in mucosal surfaces of the body and then selectively transported into body secretions; this sIgA never enters the circulatory pool (Figure 1)<sup>10</sup>. In adult humans, there are approximately 440 m<sup>2</sup> of mucosal surfaces, and these surfaces are usually the initial target for invading organisms. Thus, it might be predicted that mucosal surfaces constitute a very important component of the body’s immune defenses.

When one considers the mucosal immune system, it is sometimes helpful to look at it as two stepsclassified as :inductive and effector actions<sup>1,7,10</sup>. The action of induction occurs in organized mucosa-associated lymphoid tissue (O-MALT) at

specific locations such as the Peyer’s patches in the gut, where immune cells first confront foreign antigens. Specialized antigen-sensing cells, or M-cells, transport and release the foreign antigen into another special environment of lymphoid cells, including antigen-specific B and T lymphocytes. This step results in cell activation and subsequently maturation, differentiation, and commitment to the expression of IgA synthesis<sup>1,7,10,12</sup>.

Following antigen stimulation effector actions begin. Effector B cells leave the organized tissue and migrate to diffuse MALT sites, such as the lamina propria, interstitial tissue of mucosa and glands, and intraepithelial lymphocytes. This terminal differentiation to effector cells (plasma cells, cytotoxic T lymphocytes) brings about immunoregulation, the production of sIgA, and cytotoxic reactions to confer local immune protection<sup>1,7,10,12</sup>. Luckily, these two activities are highly organized and linked by a “homing” mechanism so that **potentially lethal pathogens are prevented from “taking over”**.

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### What role does sIgA serve in immunity?

The mucosal surfaces of the oral cavity, lungs, nasal passages, and intestinal tract are constantly exposed to a variety of foreign antigens which are mostly food and bacterial digestive remnants. In fact, the **mucosal surfaces are the major point of entry for most foreign antigens and are among the primary lines of host defense.** As such, a barrier to prevent foreign antigens from adhering and/or subsequently gaining entry to other cells is required to maintaining the integrity of mucosal surfaces. The major humoral end product in secretions that bathe mucosal surfaces is sIgA, and the IgA immunoglobulin class is preferentially utilized for its B-cell response to antigenic challenge through the mucosa. Secretory IgA prevents the attachment and absorption of foreign antigens by three separate mechanisms: antigen exclusion, antimicrobial functions, and cell-mediated immune responses<sup>12</sup>.

Antigen exclusion has been confirmed by the findings that binding of bacteria, bacterial toxins, and/or foreign antigens to cell surfaces can be prevented by sIgA (Figure 2). In particular, this sIgA-immune barrier minimizes the entry of potentially allergenic substances into the intestinal tract.

Microbial functions such as neutralization and bacteriostasis of certain viruses by sIgA have also been demonstrated. For example, it has been shown that sIgA directed against specific surface sites of selected viruses (respiratory, Sendai, influenza, etc.) are sufficient to prevent the ensuing disease<sup>12</sup>.

Cell-mediated immune responses are also one of the mechanisms of sIgA immunity as shown by many investigators. Immunologic tolerance to specific orally administered antigens, such as poliovirus vaccine, is partially, an antigen specific T-suppressor-cell-mediated reaction<sup>2</sup>. When mucosal surfaces are exposed to antigens, a sIgA response is elicited locally and at distant sites through committed IgA-producing lymphocytes homing from the Peyer's patches to other exocrine glands. Despite this ability to respond to antigenic stimulation, induction of inflammation does not appear to be a natural sequelae to IgA release, unlike T-cell and IgG-mediated immunity.

### What conditions influence the amount of sIgA in fluids, and how is the production of sIgA regulated?

To date, the sIgA in saliva has been most rigorously studied. However, it is known that concentrations of sIgA in saliva, as well as other fluids, change in response

to stimuli of both physiological and pathological origin. For example, strenuous physical exercise has been shown to lower salivary sIgA<sup>8,9,19</sup>, and has been offered as one explanation for the high incidence of upper respiratory infections after major endurance events. Levels of sIgA in saliva have also been shown to decline in persons with a high degree of psychological stress<sup>6,11</sup>. Moreover, a negative mood state has been associated with low levels of sIgA, and may reflect susceptibility to disease<sup>6</sup>. Several disease states are also associated with an under- or over-production of sIgA. Inflammatory bowel disease (IBD) is typically associated with significant increases in sIgA<sup>12</sup>. It has been postulated that a local humoral response activates sIgA and other participants of the mucosal immune system, but when sIgA is not capable of excluding the offending antigen(s) due to stimulation overload, a systemic response ensues. Under these conditions, the production of IgG increases and inflammation rather than a return to homeostasis becomes the adaptive state. In other words, IgG immune complex formation results in complement activation and epithelial damage.

Factors regulating the production of sIgA under physiologic and pathologic states are being actively investigated. The most widely accepted modulators are those linked to neuroendocrine pathways. It is well established that mucosal immunity and sIgA production are affected by selected neuropeptides and neurotransmitters<sup>12</sup>. For example, in the gut vasoactive intestinal peptide and substance P are thought to enhance the production of sIgA in Peyer's patches, whereas somatostatin appears to be inhibitory<sup>12</sup>. Recent evidence also suggests that, at least in males, androgens and estrogens may act at selected sites in the reproductive tract to increase levels of sIgA and influence its distribution<sup>18</sup>. Secretory IgA in these tissues may serve a role in controlling genital tract infections, as well as other diseases.

In addition to neuroendocrine and endocrine regulation, specific cytokines/

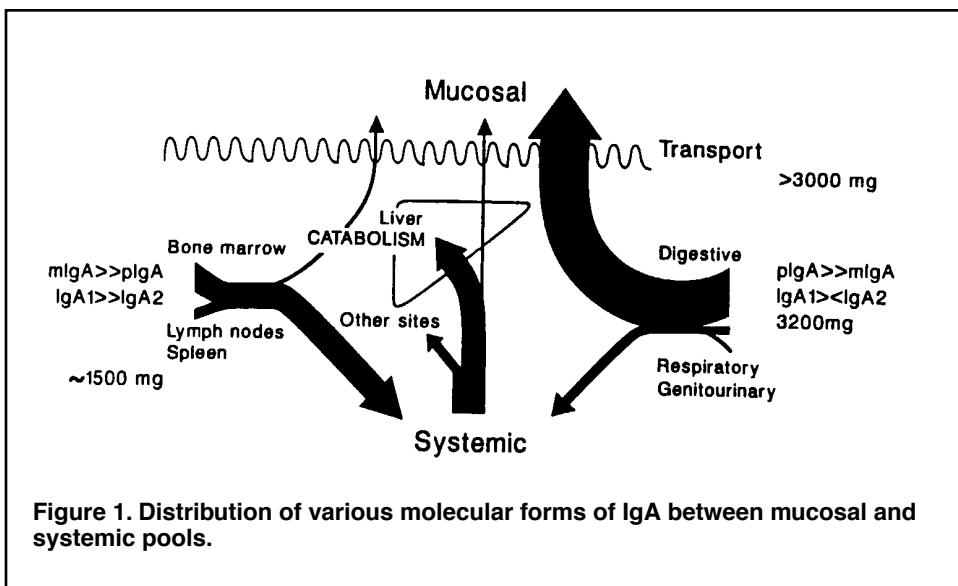
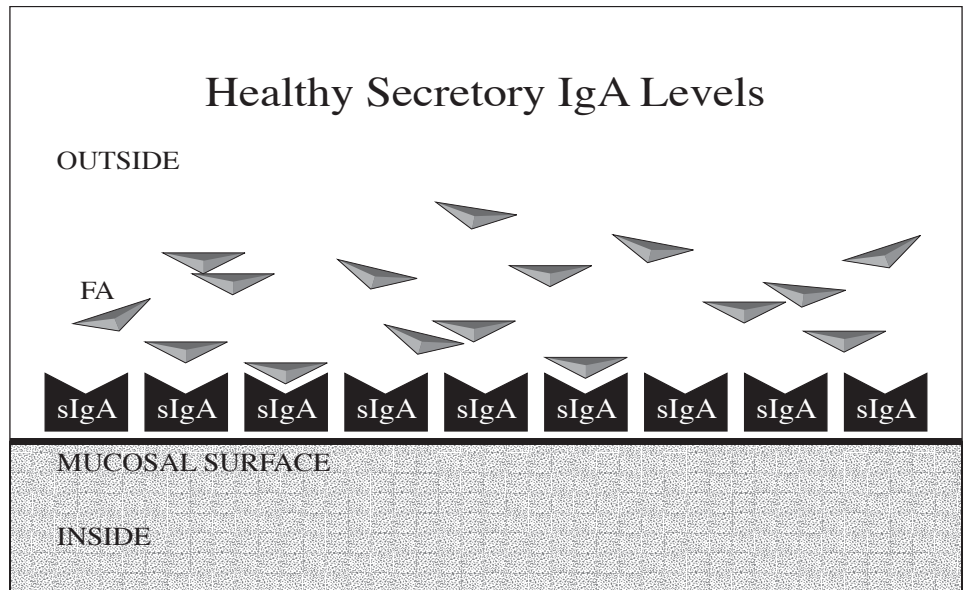


Figure 1. Distribution of various molecular forms of IgA between mucosal and systemic pools.

lymphokines, or cell products, participate in the development of an IgA response<sup>1,7,10,12</sup>. Interleukins 4, 5 and 6 (IL-4, IL-5, and IL-6) and transforming growth factor- $\beta$  (TGF- $\beta$ ) are among the cytokines that have already been identified. For example, when B cells in the O-MALT are activated, they express IL-5 receptors, and in response to IL-5, B cells proliferate<sup>1</sup>. When these proliferating B cells are exposed to IL-4-6 and TGF- $\beta$ , they are induced to express surface IgA molecules<sup>1</sup>. These IgA positive cells then leave the organized tissue and migrate to distant sites in diffuse MALT, as previously described.

**What is the relationship between sIgA and food and environmental allergies/sensitivities?**

A distinct association between the mucosal immune system and food allergies/hypersensitivities has been postulated based on a variety of clinical information. Clearly, given the large surface area of the gastrointestinal mucosal immune system, a strong association would be predicted. One of the most interesting associations relates to the role of breast feeding in the development of food allergy<sup>4,5,14-17</sup>. Breast milk contains large amounts of sIgA, some of which are specific sIgA antibodies. These are formed locally in the mammary gland by lymphocytes that had been primed in the intestines by microbial and food antigens. The ingestion of human milk containing these specific antibodies has been shown to protect against food allergies, as well as other illnesses<sup>4,5</sup>. For example, the ingestion of sIgA antibodies confers protection against the diarrheal diseases, *V. cholera* and enterotoxigenic *E.coli*<sup>4</sup>. Moreover, several investigators have shown that breast milk from mothers with infants who have cow's milk allergy (CMA) has a significantly lower total IgA content as compared to breast milk from women whose infants who do not have CMA<sup>16,17</sup>. Thus, breast milk with high levels of sIgA is most likely to protect the infant from developing food allergies and other infectious diseases.



**Figure 2. Diagram of a healthy (sIgA adequate) mucosal surface**

In addition to food allergies in neonates and infants, a number of different so-called autoimmune and inflammatory diseases have been associated with food allergies, as measured by the ELISA/ACT<sup>®</sup> Lymphocyte Response Assay (LRA). Two diseases in particular, rheumatoid arthritis (RA) and IBD, have also been associated with impairments in mucosal immunity<sup>12,13</sup>. O'Farrelly et al showed that of 44 RA patients immunologically sensitized to wheat proteins, 38 were antibody positive for IgA rheumatoid factor<sup>13</sup>. An immune response to wheat and other dietary proteins has also been associated with celiac disease and other autoimmune diseases wherein lesions of the gastrointestinal tract are common. Additionally, increased levels of immune complexes composed of dietary protein and specific IgG have been associated with gastrointestinal damage, and may be responsible for sIgA nephropathy<sup>13</sup>. These observations indicate that the mucosal immune system may be an important aspect of autoimmune disease.

Other diseases that may be associated with food allergies are fibromyalgia, systemic lupus erythematosus, myasthenia gravis, and insulin dependent diabetes. The symptoms and severity of these diseases have all been mitigated and/or removed when patients have followed the LRA by ELISA/ACT<sup>®</sup>

program, which is designed to repair and fortify/reinforce the mucosal immune system of the body. Since the **LRA by ELISA/ACT procedure specifically tests for foods and environmental antigens that are typically first encountered by the mucosal surfaces, abnormal sIgA levels show the need for LRA by ELISA/ACT testing. Moreover, monthly monitoring of improvements in sIgA would be worthwhile including in a therapeutic program.**

A healthy mucosal immune system and optimal sIgA production serve critical roles in preventing the development of and helping to repair damage from food allergies and environmental sensitivities. Thus, obtaining saliva samples for measuring sIgA may be of use in the clinical diagnosis of environmental sensitivities, autoimmune, and other chronic diseases.

**How is sIgA measured, and what are some of the clinical applications?**

sIgA has been measured by several techniques over the years. Radialimmunodiffusion (RID) was a common method of measuring sIgA in blood, but is not sensitive or specific enough for accurate sIgA measurements. The development of enzyme-linked immunosorbent assays (ELISA) has

vastly improved our ability to measure sIgA. This is currently the most common method of measuring sIgA. However, the specifics of the assay depend on the tissue to be examined. Although saliva is the most accessible tissue, sIgA has been measured in tears, gastrointestinal secretions, breast milk, and nasal fluids. The preferred tissue for obtaining diagnostic and clinically useful information is saliva, either whole saliva or parotid saliva obtained by a Kirby cup. However, several points must be considered when attempting to obtain whole saliva specimens:

- The collection should be timed and normalized for salivary flow rate.
- The sample should be collected in the morning under basal conditions to control for diurnal variations.
- The saliva should not be stimulated but rather collected under free flow conditions.
- The patient should not have participated in vigorous exercise for 6 hours prior to sample collection.
- The patient should be well hydrated.

If these steps are taken, then the results will be of use clinically, and may demonstrate potential mucosal immune system dysfunction, the presence of a compromised gastrointestinal tract, food allergies, environmental sensitivities, and/or other chronic disorders.

## References

1. Beagley KW and Elson CO. Cells and cytokines in mucosal immunity and inflammation. *Gastroenterol Clin NA* 1992; 21:347-366.
2. Carlsson B, Zaman S, Mellander L, Jalil F, and Hanson LA. Secretory and serum immunoglobulin class-specific antibodies to poliovirus after vaccination. *J Infect Dis* 1985;152:1238-1244.
3. Conley ME and Delacroix DL. Intravascular and mucosal immunoglobulin A: Two separate but related systems of immune defense? *Ann Int Med* 1987;106:892-899.
4. Cruz JR, Cano F, and Caceres P. Association of human milk sIgA antibodies with maternal intestinal exposure to microbial antigens. In: *Immunology of Milk*

*and the Neonate*; Edited by J. Mestecky et al. Plenum Press, New York, 1991:193-199.

5. Hayani KC, Guerrero ML, Morrow AL, Gomez HF, Winsor DK, Ruiz-Palacios GM, and Cleary TG. Concentration of milk secretory immunoglobulin A against *Shigella* virulence plasmid-associated antigens as a predictor of symptom status is *Shigella*-infected breast-fed infants. *J Pediatr* 1992;121:852-856.
6. Jemmott JB and McClelland DC. Secretory IgA as a measure of resistance to infectious disease: comments on Stone, Cox, Maldimarsdottir, and Neale. *Behavioral Med* 1989; 63-71.
7. Kraehenbuhl JP and Neutra MR. Molecular and cellular basis of immune protection of mucosal surfaces. *Physiol Rev* 1992; 72:853-879.
8. Mackinnon TL, Ginn E, and Seymour G. Temporal relationship between exercise-induced decreases in salivary IgA concentration and subsequent appearance of upper respiratory illness in elite athletes. *Med Sci Sports Exerc* 1991; 266:545.
9. Mackinnon LT and Jenkins D. Decreased salivary immunoglobulins after intense interval exercise before and after training. *Med Sci Sports Exerc* 1992; 25:678-683.
10. Mestecky J, Lue C, and Russell MW. Selective transport of IgA: Cellular and molecular aspects. *Gastroenterol Clin NA* 1991; 20:441-471.
11. Mouton C, Fillion L, Tawadros E, and Tessier R. Salivary IgA is a weak stress marker. *Behavioral Med* 1989;179-185.
12. Nagura H. Mucosal defense mechanism in health and disease: Role of the mucosal immune system. *Acta Pathol Jpn* 1992; 42:387-400.
13. O'Farrelly C, Melcher D, Price R, Sherwood R, Marten D, McDougall B, Goldstein AJ, and Fernandes L. Association between villous atrophy in rheumatoid arthritis and a rheumatoid factor and gliadin-specific IgG. *The Lancet* 1988; 819-822.
14. Reiger CHL, Renz H, Vestner R, Brehler C, and Horn A. Local and systemic immune response to food antigens in neonate and infants. *Allergy Proc* 1991;12:309-312.
15. Renz H, Vestner R, Petzoldt S, Brehler C, Prinz H, and Reiger CHL. Elevated

concentrations of salivary secretory immunoglobulin A anti-cow's milk protein in newborns at risk of allergy. *Int Arch Allergy Appl Immunol* 1990; 92: 247-253.

16. Saalman R, Carlsson B, Fallstrom SP, Hanson LA, and Ahlstedt S. Antibody-dependent cell-mediated cytotoxicity to  $\beta$ -lactoglobulin-coated cells with sera from children with intolerance of cow's milk protein. *Clin Exp Immunol* 1991; 85:446-452.
17. Savilahti E, Tainio V-M, Salmenpera L, Arjomaa P, Kallio M, Perheentupa J, and Siimes M-A. Low colostral IgA associated with cow's milk allergy. *Acta Paediatr Scand* 1991; 80:1207-1213.
18. Stern JE, Gardner S, Quirk D, and Wira CR. Secretory immune system of the male reproductive tract: effects of dihydrotestosterone and estradiol on IgA and secretory component levels. *J Reprod Immunol* 1992; 22:73-85.
19. Tomasi TB, Trudeau FB, Czerwinski D, and Erredge S. Immune parameters in athletes before and after strenuous exercise. *J Clin Immunol* 1982; 2:173-178.

## Contact

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Many physicians use the advanced, cost-effective sIgA measurement to determine the need for LRA by ELISA/ACT sensitivity testing.