

) Determine SIgA in some clinical samples by passive haemagglutination

Dr. Azhar .A. L. Al -Thahab

Department of Biology ,College of Science ,University of Babylon

Abstract :

Immunoglobulins were isolated from tonsillar tissue ,appendix ,cerebrospinal fluid ,and vaginal secretion from different patients by polyethylene glycol 6% . Anti SIgA was prepared by injection the rabbit with standard SIgA and adjuvant (sun flower oil) .Detection of SIgA in isolating immunoglobulins by passive haemagglutination . We found that higher titers with appendix ,vaginal secretion and low titers with tonsillar tissue and cerebrospinal fluid .

Introduction

More than two decades ago ,the major immunoglobulin (Ig) present in exocrine secretion was shown to be a dimeric IgA covalently bound to an epithelial glycoprotein of about 80 KDa (Tomasi *et al* 1965). The secretory immune system is the best defined aspect of mucosal immunity ,this adaptive humoral defense mechanism depends on a fascinating cooperation between local B cell system and the secretory epithelia (Brandtzaeg ,1994) .The mucosal surface area of an adult individual amounts to some 400 m² the main humoral mediator of specific mucosal immunity are secretory IgA and IgM (SIgA & SIgM) antibodies , although the most active SIgA system occurs in the gut ,secretary immunity is also operates in the female genital tract ,with considerable SIgA production in the cervical mucosa and fallopian tubes (Brandtzaeg ,1994 ; Brandtzaeg ,1996). High proportion of B cells enzymatic ally dissociated from human appendix ,a gut associated lymphoreticular tissue (GALT) expresses the IL-6R ,and that recombination human IL-6 induces significant increases in the number of Ig producing cells are restricted to the IgA isotype (Parslow *et al* ,2001). Further IgA2 the major sub class , however ,significant number of IgA 1 producing cells are also seen . In contrast human tonsillar and peripheral blood B cells express low levels of IL- 6 R receptor (Fujihashi *et al* ,1991) . The aim of this study was to determine the type of Ig that present in different clinical samples from patients .

Materials and methods :

1- Rabbits

Two Newzealand white rabbits (2 kilogram) were used to prepare SIgA

2- Samples

10 tonsillar tissue(tonsillectomy patients) , 10 cerebrospinal fluid (CSF meningitides patients) ,10 vaginal secretion(women had vaginitis) , 10 appendix (appendicectomy patients)

3 – Polyethylene glycol 6%

About six Polyethylene glycol (HO (C₂ H₄O)_nH) BDH , M.W,6000 was dissolved in small amount of tris solution (12 grams tris material NH₂C(CH₂OH)₂ Taab company) in small amount of distilled water(D.W) , and completed to 1 liter with D.W, pH was adjusted to 6 by using HCl (0.1) (Johanston ,1982).

4- Formal saline

This solution was prepared at 0.5% concentration by adding 0.5 formaldehyde (H-CHO) BDH to 99.5 ml normal saline .

5- Standard SIgA

Standard SIgA was obtained from Dr .Mestecky ,the University of Alabama at Birmingham ,UAB station. SIgA 1.3 gm was dissolved in 1 liter of phosphate buffer solution

6- Separation of immunoglobulin

Separation of Igs were according to (Johanston ,1982 ;Shnawa and Thwaini ,2002)

7- Immunization schedule of 6 rabbits

A total of 0.5 ml SIgA solution and 0.5 ml sunflower oil was mixed and injected rabbit subcutaneous in different site and after 6 days another dose was injected subcutaneously and intracellular at different sites , leave for one week and then blood was drawn by heart puncture and .blood was collected by clean tubes, serum was separated by centrifuged at 2500 rpm for 5 min .Serum was tanned with tanned red blood cells .

8-Passive haemagglutination test

It was done according to (Bokman ,1983) coat the tanned sheep red blood cells with antigen (antiSIgA).4ml saline ,1ml of antiSIgA and 1ml of tanned sheep red blood cell were mixed in test tube and let for 10 minutes at room temperature. Tube was centrifuged for 5 min at 2500 rpm the supernatant was detected and the packed cells were washed three times and resuspended to 1ml saline. Microtitration plate was used to dilute Ig extract .50 µl of normal saline was added to each wells of plate including ten well and eleventh well was considered as control for each plates.50 µl of Ig extract was added to first well and mixed and pipette by micropipette to second well until 10 well and incubated at 36 C° for 45 min and then titer was recorded (Bokman ,1983).

The Results :

1- Vaginal and cerebrospinal fluid secretion

The results of haemoagglutination test as follow, samples taken from patients having vaginitis due to B-streptococcal infection and candidiasis showed high titers 16 , 32 , while those with G⁻ infection showed low titers 4 , 8 table (1) .The results of haemoagglutination test for samples CSF taken from patients with meningitis due to *Haemophilus influenza* showed low titers table (1)

Table (1) The titers of heamagglutination between anti SIgA and different vaginal secretion and CSF

TITERS OF VAGINAL SECRETION	TITERS OF CEREBROSPINAL FLUID
4	2
4	2
4	8
8	2
32	2
32	2
32	4

16	4
16	8
16	4

2 -Tonsillar tissue and appendices immunoglobulin extractions -

Low titers were showed with immunoglobulin extract from tonsillitis 2,4 while higher titers were showed with appendix 32,64 as table 2

Table (2) The titers of heamagglutination between anti SIgA & immunoglobulin extractions from tonsillar and appendix

TITERS OF TONSILLITIS TISSUE	TITERS OF APPENDIX EXTRACTION
8	32
8	32
16	64
16	64
16	64
8	32
8	32
16	64
16	64

Discussion :

In this study we used different secretions (vaginal secretion ,tonsillar tissue ,cerebrospinal fluid and appendix tissue) .These secretions were obtained from different patients. Standard SIgA was used to determine which of these secretion was SIgA .from the result the appendix was showed high titer with antiSIgA .This agreed with other studies that appendix has been shown to exhibit features of gut associated lymphoid tissue and this includes dome region covered by a unique epithelium containing follicle associated or microfold cells that function in the uptake of gut antigens for induction of mucosal immune responses ,thus the appendix has anatomical and functional characteristics in common with Peyers patch which have been well characterized to be major IgA induction sites (Bokman ,1983; McGhee *et al* ,1989) .Tonsillar tissue appear low titer 8 and some 16 this due to that tonsillar B

cells are mainly IgA1 subclass while the distribution of surface Ig positive (SIgA) B cells in appendix B cells population is SIgA>SIgG>SIgM, and the SIgA B cells express higher level of IL-6R. (Johanston, 1982, Beagley *et al*, 1989). Some vaginal secretions appear low titer (4, 8 titers) this agreed with other study that showing general predominance of specific IgG in cervical secretions in infection with gram negative bacteria and in normal secretion (Kutteh *et al* 1996; Russell and Mestecky, 2002), other cases appear (16,32 titers) this due to infection with Streptococcal vaginatis infection (Hordnes *et al*, 1996). Cerebrospinal fluid secretion appear low titer because the presence of blood-CSF barrier in normal and pathologic condition.

References

- Beagley, H.; Lee, F.; Kiyono, H.; Everson, M.; Hirano, T.; Kishimoto, T. and McGhee, J.R.** (1989) Interleukins and Koopman
- Bokman D.E.** (1983) Functional Histology of appendix. Arch. Histol. JPN. 96:261-292
- Boyden S.V.** (1951). The adsorption of protein on erythrocytes treated with tannic acid and subsequent haemagglutination by anti-protein serum. J. Exp. Med. 93: 106.
- Fujihashi K., McGhee J.R., Lue C., Beagley K.W., Taga T., Hirano T., Kishimoto Mestecky J. and Kiyono H.** (1991). Human appendix B cells naturally express receptors for and respond to interleukin 6 with selective IgA1 and IgA2 synthesis. J. Clin. Invest. 88:248-252.
- Hordnes, K.; Tynning, T.; Kvan, A.I.; Jonsson, R. and Haneberg, B.** (1996). Colonization in the rectum and uterine cervix with group B Streptococci may induce specific antibody response in cervical secretions of pregnant women. Infect. Immun. 64: 1643-1652.
- Johanstone, A. and Thorpe, R.** (1982). Immunochemistry in Practice; pp1-65. Blackwell Scientific Publications, London.
- Kutteh, W.H.; Prince, S. J.; Hammond, K.R.; Kutteh, C.C. and Mestecky, J.** (1996). Variations in immunoglobulins and IgA subclasses of human uterine cervical secretions around the time of ovulation. Clin. Exp. Immunol. 104(3):538-542.
- McGhee, J.R.; Mestecky, J.; Elson, C.O. and Kiyono, H.; Everson, W.** (1989). Regulation of IgA synthesis and immune response by T cells and interleukins. J. Clin. Immunol. 9:165-199
- Parslow, T.G.; Stites, D.P.; Terr, A.I.; and Imboden, J.B.** (2001) Medical immunology, Tenth edition, McGraw-Hill, USA
- Russell, M.W. and Mestecky, J.** (2002). Humoral immune responses to microbial infections in the genital tract. Review. Microbes and Infection 4: 666-666.
- Shnawa, I. M. S. and Thwaini, Q. N.** (2002). Lapin Mucosal humoral versus EDDC3 systemic humoral and cellular Immune response post to intracellular administration of heat killed *C.fetus*. J. Baby Univ. 7(3): 538-543.

Tomasi ,T.B.,Tan E.M.,Solomon, A.and Predergast, R.A.Journal of
Experimental medical Biology(1965) 121:101-121 .

.