Oral- Versus Systemic Immune Responeses Specific for *Streptococcus. anginosus* dentalvolar Infected patients

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Abstract

Streptococcus. anginosus is associated with dentoalveolar infected patient. *S. anginosus* activate B cell peripheral mucosal systems produce high titer of pathogen specific antibodies. The antibody nature at mucosal surface was of secretary type since it was 2-Mercaptoethanol (2ME) resistant and in peripheral blood was of not IgM type since it was 2MF resistant. It might be of IgG type Neutrophil N.B.T phagocytosis was found at mucosal and systemic compartments in patients higher than in normal control subjects. Significant leucocyte inhibitory factors were noted among patients in comparison to non significant in normal control subjects.

الخلاصة

الاستجابات المناعية الموضعية والبدنية المتخصصة ب S.anginosus المشتركة مع حالات Dentoalveolor infections

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الخلاصة

وجد بان *S.anginosus* تشترك مع حالات مرضى Dentoalverolar infectors و ادت هذه البكتريا لتنشيط الخلايا البائية الموضعية و الجهازية لانتاج عبارات من الاضداد المتخصصة بها في كل من السطح المخاطي من النمط الافرازي لكونه تقاوم لثاني مركابتو ايثانول و ليس من صنف IgM لكونه مقاوم لثاني مركابتو ايثانول في المصل وبين قياس البلعم بالعدلات مستويات عالية بين المرضى مقارنة بالاسوياء وان هناك مستويات معنوية من تثبيط هجرة الخلايا البيض بين المرضى مقارنة بالاسوياء

Introduction

The stomium is a reservoir for a variety of endogenous bacterial antigens and the port of entry for several exogenous bacterial antigens into the alimentary and respiratory systems. In normal state these antigens do not cause disease and swallowed away with saliva into the distal part of the gut (1)

The protection of the stomium against bacterial invasion can be confired by either one or more of the following mechanisms mucosal barrier, continual desquamation of oral epithelium secretory IgA as well as the mucus membrane action. When an imbalance do happened between oral bacteria and immune cell cells in mucosal and systemic compartments such imbalance will lead to an immune tissue injuries or to direct tissue damage induce by oral bacteria or their host specific responses mediated by local and systemic immunity (1,2). Among the known oral bacterial pathogens are streptococci which cause several dental disease conditions (2-8). *S . anginosus* was reported in peridontitis patients (9). In the present work S. anginosus is being reported in cases of peridontitis, gingivitis and chronic pulpitis together with the investigation of the immune status of the patients

Materials and methods

1- Patients and controls;

Twelve patients out of 52 were the attendance of the surgical dental clinic, college of dentistry university of Babylon during the period Juneuary 2008 to Aprial 2009.

They were diagnosed as peridontits (five cases), gingivitis (four cases) and chronic pulpitis (three cases) (2). Ten apparently healthy subjects were elected as controls)

2- Bacteriology; the dentvalveolar materials of 15 patients and 10 controls were swabbed by sterile cotton swabs into which three mls of sterile normal saline was added. Through mixing was done for these samples. Loopful inocula were taken from the swab saline mixtures and quadrate streaked onto blood agar and nutrient agar plates

then incubated under aerobic conditions. Biochemical identification of the pure isolates was made as in (10)

3- Immunoglobulin separation. The through mixed swab-saline mixtures became slightly opaque due to protein and cellular content (item two). These suspensions were centrifuged at 3000 rpm for five min. supernates were aspirated into sterile clean plastic tubes in three mls amounts. To these, three mls amounts of Polyethylen-glycol PEG-6000% solutions was added and left at 4 C for 1 hr. The protein precipitation was settled at the bottoms of the tubes(11). Then the tubes were centrifuged at 5000 rpm for 15 min., discard supernates and dissolve precipitates in 0.5% formal saline (11,12)

4: Mucosal leucocyte separations ; The deposite of the primary swab- saline suspension (item two) was washed with saline and resuspended in three mls of saline. To this washed cell suspension three mls of 3% dextrane was added the mixture was left at room temp= 25c for 20 mins. Dextrane-lecocyte upper layer was aspirated ,tubbed into plane tube and centrifuged at 300 rpm for 15 mins.The supernate was discarded and pellet saved then resuspended, washed twice with saline and reconstituted to their original volumes in sterile saline (13)

5-Blood; Blood with and without anticoagulant in three mls amounts were collected from the brachial vein of 15 patients and ten normal subjects (14).

6: Immune function tests; The standard tube agglutination test was done as in (14). Nitroblue tetrazolium reduction test on mucosal and peripheral blood leucoytes was done following the method of Park et al (15). Leucocyte lnhibitory factors LIF were done on mucosal leucocyte by capillary method. More than 30% migration inhibition was considered as significant(16).

Result:

1- Infections Agent : The dentoalveolar ,material primary plate culture revealed the growth of minute B haemolytic colonies onto blood agar plate in pure and heavy growth fashion pure culture was made and characterized as; Gram positive cocci in chains. Aerobic growth enhanced by 10% CO2 tension. Minute B-haemolytic colony morphotypes. They forms acid from lactose maltose but not from manitol and hydrolyse arginine. Negative for CAMP phenomena and for gelatine liquefaction. These characterics are consistant with S.anginosus.
2- Infection ; *S.anginosus* were diagnosed in association with peridontitis, gingivitis and chronic pulpitis. The immunologic aspects of these infections are present in the tables 1-4

3-Mucosal Immunoglobulin concentrations; The range of means of MIG contraction was 0.72- 0.74 g/L in patients and 0.22 g/L in controls.

4-Serum Globulin concentration the range of means of serum globulin concentration was 41.4-45.5 g/L and 36.22 for contro

5-Mucosal antibody:The *S.anginosus* specific mucosal antibody titers were44.8, 30 and 18.6 for peridontitis, gingivitis and chronic pulpitis as compared to normal subject these mucosal antibodies were resistant to 2ME

6: Serum antibody: The mean of the *S.anginosus* specific serum antibody titres were 448, 300 and 186.6 for peridontitic, gingivitis and chronic pulpitis as compared to 10 in normal subjects. These serum antibodies were resistant to 2ME.

7-Neutrophil phagoytosis:The mucosa Neutrophil N.B.T phayocytosis percentage of the patient were 43.2, 40.5 and 41.73 % for peridontitis , gingivitis and chronic pulpitis.

While for blood they were 36, 32.3 and 34.3% for gingivitis and chronic pulpitis as compared to in normal human subjects. Mucosal Nutrophil phagocytosis were mostely higher than that of the systemic

8-Leucoyte Inhibitory factor: The means of mucosal LIF were 52.2, 53, 5-5.3 for peridontitis gingivitis and chronic pulpity. While the mean values for systemic LIF were; 61.6, 56.25, and 67.3 for pendontitis, gingivitis and chronic pulpitis which indicate.significant inhibition percentages as compared to 99.56% in normal control subjects

Table (1) The immune status of *S.anginosns* associated peridontitis.

Number of cases	Immune paramear	Result			Mean control value
		Mean	median	range	
5	1- MIGC	0.37	0.84	0.79-0.84	0.299
	2- STPC	75.32	73.7	72-82	66.43
	3- SGC	45.5	44.3	43-47	36.22
	4- MIGT				
	2ME -	44.8	32	32-64	2
	2ME +	44.8	32	32-64	2
	5- SIGT				
	2ME -	448	320	320-640	10
	2ME +	448	320	320-640	10
	6-LIF				
	M	52.2	41.00	41-56	0.96
	S	61.6	52.00	52-65	0.95
	7- NBT				
	M	43.2	43	40-47	9.8
	S	36	33	32-39	10.8

MIGC=Mucosal Imunoglobulin concentration
STPC=Serum total protein concentration
SGC=Serum globulin concentration
MIGT=Mucosal Immunoglobulin titer.
SIGT=Serum lmmunoglobulin titer.
LIF =Leucocytes lnhibitory Factor .
M =Mucosal.
S =Systemic
N.B.T=Nitroblue tetrazolium reduction Test.

Number	Immune	Result	Mean control

		Mean median range	
4	1- MIGC 2- STPC 3- SGC	0.74 0.64 0.74-0.78 72.7 69.7 69 -81 41.7 39.5 37-48	0.299 66.43 36.22
	4- MIGT 2ME - 2ME +	 30 8 8-64 30 8 8-64	 2 2
	5- SIGT 2ME - 2ME +	 300 80 80-640 300 80 80-640	 10 10
	6-LIF M S	 53 55 43- 58 56.23 68 36-68	 0.96 0.95
	7- NBT M S	 40.25 30 30-45 32.3 27 27-38	 9.8 10.8

Table 2 : The immune status of *S.anginious* associated Gingivitis

MIGC=Mucosal Imunoglobulin concentration
STPC=Serum total protein concentration
SGC=Serum globulin concentration
MIGT=Mucosal Immunoglobulin titer.
SIGT=Serum lmmunoglobulin titer.
LIF =Leucocytes lnhibitory Factor .
M =Mucosal.
S =Systemic
N.B.T=Nitroblue tetrazolium reduction Test.

Table 3: The immune status of *S.anginosus* associated chronic pulpitis

Number of cases	Immune paramear	Result			Mcan value
		Mean	median	range	
3	1- MIGC	0.72	0.74	0.6-0. 8	0.299
	2- STPC	71.2	70.6	70 -73	66.43
	3- SGC	41.4	40.7	39-44	36.22
	4- MIGT				
	2ME -	18.6	16	8-32	2
	2ME +	18.6	16	8-32	2
	5- SIGT				
	2ME -	186	160	160-320	10
	2ME +	186	160	160-320	10
	6-LIF				
	M	55.3	52	52- 57	0.96
	S	57. 3	60	57-66	0.95
	7- NBT				
	M	41.67	46	36-43	9.8
	S	34.33	37	32-37	10.8

MIGC=Mucosal Imunoglobulin concentration
STPC=Serum total protein concentration
SGC=Serum globulin concentration
MIGT=Mucosal Immunoglobulin titer.
SIGT=Serum lmmunoglobulin titer.
LIF =Leucocytes lnhibitory Factor .
M =Mucosal.
S =Systemic
N.B.T=Nitroblue tetrazolium reduction Test.

Table 4 : The immune status of S.anginosus associated dentoalar infections

Nof cases	Immune paramear	Mean of			Mcan control value
		Peridontitis	gngivits	chronic pulpitis	
12	MIGC	0.73	0.74	0.72	0.299
	STPC	75.32	72.7	71.2	66.43
	SGC	45.4	41.7	41.4	36.22
	MIGT				
	2ME-	44.8	30	18.6	2
	2ME +	44.8	30	18.6	2
	SIGT				
	2ME -	448	300	186.6	10
	2ME +	448	300	186.6	10
	LIF				
	M	52.2	53	55.3	0.96
	S	61. 6	56.25	67.3	0.95
	NBT				
	M	43.2	40.25	71.7	9.8
	S	36	92.3	34.3	10.8

MIGC=Mucosal Imunoglobulin concentration
STPC=Serum total protein concentration
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LIF =Leucocytes lnhibitory Factor .
M =Mucosal.
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Discussion

S. anginosus is being reported in association with peridontitis gingivitis and chronic pulpitis (9,17,18,19)the rise in *S.anginiosus* specific antibody titer may indicate B cell poly clonal activiting epitope and /or Th 2 cell polyclonal activitating epitope(20). In addition to an epitope activating Toll-like receptor that reacts with pattern recognition structure on *S anginousus* as indicated by the increase in NBT in patient (21,22).As well as LIF inducing T Cell epitope(21,22) as revealed by significant migration inhibition of lecocytes in patient groups.

The 2ME resistance in mucosal antibodies means that they are of secretary type (23) While 2ME resistance in serum antibodies indicate that these antibodies were not of IgM type (22)thus, on summing up the basic immune features of the dentoalveolar infections with *S.anginosus*, one may state that

1. S. anginosus is assotiated causal with dentoalveolar infection
2. it induces an increase in mucosal globulin concentrations to levels higher than those of normal control subjects
3. it activate neutrophil phogoytosis
4. it stimulate B cell, th1 and th2 cell responses
5. it stimulate LIF cytokine production in significant level both in mucosal and peripheral blood compartments .

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