HUMAN LABORATORY lMMUNOLOGY

PREFACE

Most of the immunology laboratory manuals and handbooks tackles mainly the methodologic aspects with brief theoretical introduction.The present ; HUMAN LABORATORY IMMUNOLOGY is being informative with narrative simple language expressing bench work immunology in an essays trend.It constitutes an excerpt of 25 years immunology bench works in a recreational revisiting approaches.The text has its raw materials from works mostly done on human diseased conditions and was organized in five parts,nine sections and 27 essay chapters.Part one express essential immunology bench work,second part constitute natural innate immunity. Part three contained immune cross-roads entities.Part four constitutes humoral adaptive immunity.While part five ensembles humoral and cellular immunity.This manual was intended to serve postgraduate immunology students,immunology researchers and health professionist espacially in areas with low availability of scientific equipmental resources.

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PART ONE: ESSENTIAL IMMUNOLOGY BENCH WORK

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ESSAY - 1: ANITGEN

Abstract

Preparation and standarization of whole erythrocyte ,whole heat killed E.coli, S.typhi Vi and S.typhi protoplasmic sonicate antigens ~~were~~ are ~~outlined~~ described in an easy and striaght~~-~~forward ~~language~~manner.The ~~goal~~ purpose of the given examples was to shed a light on the importance of the antigen preparation in ~~;~~production of specific anti -sera,serologic classification of microbes~~-~~, serodiagnosis of human microbial infections andin preclinical development of microbial vaccines.

Key Words

Antigen,antisera,cell,Eschrichia, classification, infection ,microbe,serologic.

1-Introduction

Antigens are heterognous chemical compounds that have shown number of biological properties as;large molecular weight,high degree of molecular complexcity,large surfac area,own surface microstructures formed from say 10 amino acids in protein molecules and comparable number in polysacchride and lipoidal antigens these konwn as epitopes.These epitopes determine the specificity,reactivity,shared reactivity and valency of the antigen.Antigen bears an inborn property of foreigness.That is to say they are recognized by the host immune system as foriegn substances.The antigen potency was found higher in proteins followed by polysacchrides then lipids.Such antigen can initiate in responding immune system an immune responses.These immune responses can be mediated by cells or by cell mediators as humoral,cellular and /or humoral-cellular responses.As the antigen differe ,the corresponding response differe.Thus several types of immune responses can be as;Humoral,cellular,humoral-cellular, allergenic,autoimmune ,toleragenic and immunosuppressive responses.Antigen can be of, whole cell,subcellular elements, and molecular natures.

2-Preparation of Whole Cell Antigens :

2-1 :Whole Erythrocyte Antigens

The methods is being as follows

i-From a heparinized fresh whole blood collect ,draw 2 ml sample and tube it in a centrifuge tube.

ii-Centrifuge it at 2500 rpm for 5 minutes.

iii-Discard the supernate and keep pellet.

iv- Wash the pellet cells three times with saline at 2500rpm for 5 monutes each run.

v- Suspend the washed erythrocyte in saline to form 5% suspension.It is valid for use as an antigen within three days when kept at 4C in refregirator.

2-2;Preparation of whole Heat killed E.coli antigen;

The preparation way is as follows

i-prepare fresh 24hr. agar slant or petriplate culture of E.coli.

ii-Add 5 ml of sterile saline to the growth medium and sweep the growth by the loop.The resulted suspension tubed into sterile clean centrifuge tube.

iii-Centrifuge at 3000 rpm for 10 minutes and wash with saline at 3000 rpm for 10 minute for each run

iv- Incubate at 60 C for 1hr to kill the cell populations

v-Quadrate streak onto nutrient agar for purity check

vi-Count the number of cell per ml of the suspension by spectrophtometery,McFERLAND MacFerland matching tubes or WHO standard opacity tubes.

3- Counting Antigen per unit Volume:

3-1; Spectrophotometery,

i- Prepare fresh 18hr E.coli broth culture and dilute to 1:100 with sterile broth medium.
ii- 5ul of this growth transfered to Ptrauf-Hauser and let it to slip in btween the slide and cover,leave about 2 minutes at room temp in a plan equator .
iii- Count the number of bacteria in 5 squires and have mean count.Apply results to the following formula count in,one sequire x 20000 x the dilution factor.
iv- From the original fresh broth culture make serial double dilutions with broth medium as ;1:10,1:20,1:40, 1:80,1:160,1:360.
v- Read by the spectrophotometer at 560nm each of these dilutions

Table – 1; Counting while cell antigen per unit volume

Dilution	Count	Spectrophotometeric readings
1:10		
1:20		
1:40		
1:80		
1:160		
1:360		

3-2: WHO Opacity Tubes;

WHO standard laboratories gifts researchers standard opacity tube on requists.Two tubes one standard clear with opaque column that gives opacity comparable to 10 IU of antigen per unit volume.The other tube is empty clean and transparent.One ml of cell suspension tubed inthe second tube and add certain volume of slaine till reaching the opacity of the standard tube on optical matching.Caululate the dilution factor of the original suspension..

3-3:MacFerland Opacity Tube Set;

Prepare set of 1% barium chloride solution and 1% of H2SO4 solution and mix the two solutiion following the rates dipicted in,Table 2.

Table-2 :MacFerland Opacity tubes

Tube Number	1% Barium chloride volume in ml	1% H2SO4 volume in 1 ml	CFU/10 to 6
0.5	0.05	9.95	150
1	0.1	9.9	300
2	0.2	9.8	600
3	0.3	9.7	900
4	0.4	9.6	1200
5	0.5	9.5	1500
6	0.6	9.4	1800

Selecte the tube 1 for instance tube one ml. of your cell suspension and tube it in clean clear test tube similar to that used for MacFerland tubes.Add graded voliume of diluant till reaching the elected MacFerland tube on gross visual matching provid you mix well the standarized matching tube befor matching process.

4- Preparation of S.typhi Vi antigen.

The preparation procedure is as made by Shnawa and Al-Ammar 2023 as in the following stepwise way;

i-Prepare 18 hr fresh S.typhi DCA plate culture .

ii-Transfer five similar colony morphotypes to BHIB tube and incubate for 2 hrs at 37C.

iii-Transfer 0.1 ml of fresh 2hr broth of s.typhi to 250 cc conical flask containing sterile 100 ml.BHIB.

iv-Incubate at 37C in shaking water bath 60 cycle/minute for overnight to obntain dense cell population.

v-The flask growth medium distribute into 10 ml amounts to sterile centrifuge.Centrifuge at 5000 rpm for 20 minutes..The pellet is the bacterial biomass.

vi-Dry the bacterial biomas step v in incubator for 48hrs at 37C

vii-The dry bacterial biomas mixed with ethanol and centrifuged at 5000 rpm for 20 minutes.Supernate discarded ,pellet mixed with aceton and centrifuged.Keep pellet.

viii-The pellet step vii washed twice with ethanol the precipitate dried.

Iix- One gram dry weight of the dried precipitate step viii was suspended in sterile saline and shaked for 30 minutes.

ix-Suspension step iix centrifuged at 3000 rpm for 30 minutes.The precipitate mixed with ethanol to reach 0.3 M.The formed precipitate dissolved in DW and centerifuged.

x-The precipitate was redissolved inDW thev acetic acid added up to 01M.Precipitate redissolved in DW and treated with acetic acid up to 1M cocncentration.

xi- Thsolution reflexed in a reflex condesor for 24 hrs then dialysed and precipitate with ethanol.

xii-Precipitate step xi made in 3 mg per ampoule amounts

5-Preparation of S.typhi Protoplasmic Sonicate Protein;

This PSP antigen was prepared as in Shnawa and AL-Ammar 2023 as in the following steps;

i-From a fresh BHIB 18 hrs S.typhi broth culture,transfer 0.1ml. of growth to 100cc flask conaining 60 ml of sterile BHIB broth

ii- Incubate in shaking water bath 60 cycle /minute for an overnight period.

iii- Distribute the growth into sterile centrifuge tubes eac have 10 mls.

iv-Centrifuge at 5000 rpm for 10 minutes.Discard supernate and keep pellets

v-Pellet step iv washed twic with sterile saline at 3500 rpm for 10 minutes.

vi-Pellets step v reconstituted with 5 ml sterile saline and checked for purity.

vii-Pure suspension fractionated with cell sonicator at 18-20 oscillation per minute for 15 minutes under cooling condition

viii-The sonicated preparation step vii centrifuge at 3000rpm for 15 minutes.Pellet discarded ,supernate kept forming the protoplasmic mass

ix- The pure protoplasmic mass solution mixed with equal volume of 6% PEG 6000.

x- Mix and leave for 1 hr at 4C.Pellet saved and supernate discarded.Redissolve the pellet in sterile saline and dispense in 05ml, amounts in ampoules for determination of concentration in burt test.

6- Immunodiagnostic Tips

Microbial antigens in preparation are being crucial for production of immune sera,serologic classification of microbe,serodiagnosis of infectious diseases,and in preclinical development of microbial vaccines.In addition to efficacy studies using live challenge models.

7-Conclusion;

Preparation of microbial antigens are impactful for human in health and disease as a diagnostic aids and as protective tools against infections.

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ESSAY - 2 : IMMUNE COMPETENCE

Abstract

Immune cmopetence IC determine wthere the human or mammalian immune systems be able to produce immune responses to an antigen following introduction to the body.The evaluation parameters include sdtructural and functional approaches concerning phagocytosis,B cell,T cell and complement system components.Ic can be highthend by nutrition programs and would be impaired by;environmental pollution,obesity ,poor sleep

Key Words

Antigen, body, B cell, complement,competence,immune,macrophage ,T cell.

1-Introduction:

Immune competence IC is the ability to produce normal immune response following exposure to an antigen.IC is the opposite of immunedeficiency.IC actually is cellular issue that each of; B,T ,macrophage is mature and can recognize antigens and allow person to mount an immune response.IC may be impaired under cetrain environmental insults like; pollution,obesity poor sleep[Wikipedia,2023].The ingetrity of human immune system depends on a complex interplay of cells,secreted factors and serum proteins.Defects in the production or function of any of these components can results in impairment that ranges from immunedeficiency to susceptibility to infection[Lowell 2001].

2 - Indications;

There are some indications for testing immune competence,the most complicted of which is the suspicion immunodeficiency.Such suspicion is based upon the following observations; i- increased frequencey of infection , ii – failure to clear infection rapidly despite of adquate therapy iii – deissemination of local infection to distant sites , iv – *occurence of opportunistic infection and v – developments of certain cancers.*

3- Evaluations;

In order to assess the immune competence one should perform structural structure-function and functional assessments of the immune system as; i –B cell and B cell function , ii – T cell and T cell function, iii – Professional phagocytes and phaocytes functions and iv- complement and complement function.The structure ,structure-function aspects and be evaluated by immunophenotyping through the use of flow cytometery and set of mabs with aid of surface marker stainings.By which mature,immature,altered cell forms are being identified for each type of immune cell.

4- Humoral Immunity;

On suspesion of humoral immune deficiency in any person,the situation implys determination of immunoglobulin isotype and subtype levelsat serum and mucosal

compartments .Antibody function as to;tetanus,diphtheria,H.influenza,,naturally occuring iso-hemagglutinins,B cell mitogenicity and B cell flow cytometery.

5-Cellular Immunity ;

This can be done through T cell counts,T cell subtype counts through the use of mab specific for each of the surface markers concerning the subtype mature,immature,resting and altered forms.So as function is concerned;DTH test and lectin induced lymphoblast transformation ,MIF and LIF assays can be done.

6-Phagocytes:

Professional neutophil and macrophage phagocytes can be assessed through enumeration and surface marker identification through the use of mAB specific and specific stainings for mature,immature,resting and altered forms in the flow cytometeric assays,to identify CD11b/CD18 and detection of neutrophil defective morphology

7- Complement;

To assess complement function as indicated bt the reqist of the clinician,CH50 assay done and leveled as normal,subnormal and abnormal states.

Table- 1 :Structural and Functional Evaluation of immune cells involved in immune competence.

Feature	B cell	T cell	NK cells	Professional phagovcytes
Structure by flow cytometery	i-B cell,B cell total count ii-CD19,CD19 absolute	i-T cell, T cell total count ii-T cell CD4,Cd4 absolute iii-CD3,Cd3 absolute iv-CD8,CD8 absolute v-CD4/CD8 ratio	i-NK ,NK total ii-CD16+ CD56,CD16+CD56 absolute	i-CD11b/CD18 ii-Alteration in neutrophil morphology
Function	i-Immunoglobulin isotype levels ii-antibodies to tetanus,dipheria iii-iso-hemagglutinins B cell mitogenicity	i-DTH test ii-ly,mphoblast transformation assay iii-MIF,LIF cytokines		i-NBT ii- Microbial phagocytosis iii- NET formation

	assay			

8- Immune homeostasis:

Infections cause ~~do change~~disruptions in immune homeostasis~~,~~ in both ~~in~~ human an mammalian bodies. In most cases, ~~Vaccination~~ vaccination ~~in most cases restors~~restore these homeostatic changes to the normal state through resilience mechanisms, as shown in Table 2~~through the resilience mechanisms to the normal state,Table 2~~.

Table – 2 :Immune competence during infection and vaccination.

Inducer	Immune competence Iclevel	Immune homeostatic change	Immune resilience IR	Restoration to the normal
Infection				
Stope	High IC	+	+	-valid IR, restoration
Proceed	Low IC	+	-/w+	-Invalid IR, no restoration
Vaccination				
Protect	High IC	+	+	-Valid IR restoration
Failure	Low IC	+	-/w+	-invalid IR, no restoration

9-Population Immune Competence PIC;

PIC can be expressed in any population contracting infection or undergoes vaccination programs.The PIC levels expressed as;low,moderate and high immune competence.The immunized portion of the population may lend immunity to the rest un-immunized fraction of the population.

10- Immunodiagnostic Tips :

i- Structure,structure-function and functional assessments of patients immune cells in case of suspection with immunodeficiency.
ii- Immunophenotypes is the corner stone in the evaluation process.
iii- Good immune resilience means good immune competence
iv- Population immun competence appeasr at most in three levels as;low moderate and high

11- Conclusions;

Immune competence inform us about the extent of how healthy is the immune status and well being of man and mammals.

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ESSAY – 3 : IMMUNE RESILIENCE*

ABSTRACT

The immune resilience IR and the allied concepts are being expressed in this opinion paper.IR is the capacity to preserve or restore immune competence,control inflammation and stress.IR is suggested as an individual immune trait.It can be quantified through;measuring CD8+/CD4+ T cell,gene expression signature of the effector immune cells in infection,inflammation and stress; effector like T cells in cancer patients;minimal inflammatory reaction in allergy and cytokine in stress patients.Currently workers have been proposed a grading system to IR activities.It was in four grades.Grade I is the optimist activity and grade IV is the worest activity.The molecular mechanisms behind IR is cell – cell communication through signaling pathways.IR is operable in;infection,vaccination,allergy, maternal immune activation ,stress and cancer.A program for an animal model and human case study of vaccine resilience was suggested.

KEY WORDS

Allergy,asthma,cancer,competence,infection,inflammation,immune,resilience.

1-Introduction

The primordial contribution to Immune resilience[IR] has been traced to around a decade ago and being in the current mode of research was in the latest three years[1-24].IR is the capacity to preserve or restore immune copetence[IR].IR impacts human health walfare and life span.High IR is strongly correlated with improve health outcomes and confere resistance to viral infections and skin cancer[1-9].The objecive of the present overview paper ensembels as an at glance insight to the concept of IR and related concepts[10,11,12].

2-Concept

The IR concept can be defined as the capacity to preserve or restore immune competence and controls inflammation in face of infection or antigenic stimulation[6].Or it can be the capacity to preserve and/or rapidly restore immune functions that promotes disease resistence and control inflammation in infectious and stress associated diseases[5].It is of multifactorial etiogenesis.Among which the immune factors.A third definition is the ability to deal with microbial invading pathogens[12]

*Re-edited from;

Shnawa I M S.2023.An At Glance insight to the immune resilience.British Journal of Healthcare and Medical Reaserch.10(4):109-114.

3-Allied Concept

Herd or population immunity [HI] means vaccination of a part of the herd individuals will confere immunity to the other part.It is also multifactorial nature,among which immune causes. HI is mainly due to;past infection,past vaccination,pre-immunity.It is genetically encoded by major histocompatibility system [MHS],and of three major immune fractions.The low,moderate and high responders[10].Herd immune plots are either gaussian distribution or skwed types[11].Both IR and HI are;multifactorial and mostly based on the individual variations,Table- 1,depicted a comparative view.

Table – 1 :Immune resilience and herd immunity a comparative view.

Features	Immune Resilience[1-9]	Herd Immunity[10,11]
Based on and Concered with	Individual variation,concered with individuals	Individual variation,concerend with population
Nature of immunity	Individual concerns	Immunizationof apart lend immunity to the other par
Grades/fractions	Four grades	Three fractions
Gene encodement	Immune trait genes	MHS
Induction	Natural	Inducive
Function in	Infectious diseases,vaccination, stress and cancer	Infection,vaccination
Quantification	CD8+/CD4+,gene expression signature,cytokines	Antibody,cytokine,immune cells counts

Not only herd immunity can be of relation to immune resilience,also immune tolerance related to immune resilience IR.Immune tolerance IT is the ablity of the immune system to sutibley respond to environment.Proper immune tolerance means that the immune system under risk of exposure it does not over-react or under react to normal every day food inborn and environmental immunogens , allergens and even your body tissue antigens.In such a case one donot have food intolerance,chemical sensetivities or autoimmune condition.Immune resilience is the ability of individual immune system to deal with a biological pathogens.IR define whether or not pathogen infect and cause significant and ongoing immune responses.Loss of IT is going to impair IR.Improving IR to improve IT .To recover impaired IT is through improving gut microbiome diversity[12,13,14,15],Table- 2.

Table – 2 : Comparative View to Immune resilience and immune tolerance.

Features	Immune resilience IR [5,13,14]	Immune Tolerance IT[13,14,15]
Genetics	Gene encoded in T cell genome	Gene encoded in T and B cell genome
Concerne	Mainly T cells	Mainly T and B cells
Immune function	Restore immune protection	Dampen autoimmune reactions
Gut microbiome	Infleunced IR	Influenced the IT
Detection	Quantitation of CD8+/CD4+ Tcells,gene expression signature	Detection of tolerant T and Tolerant B cells

4-Observational Attitude

Biodiversity has been rised up due to;geographic,genome variation,gene pool variation,migration patterns and nutrional factors.Biodiversity in human being can best be represented by the individual variation in biological responses to both external and internal stimuli[10,16].Why do individuals express differences in life span,health status, and infectious disease susceptibility across the age.One logical possibility is that variation in immune trait contribute to these differences[5].

5-Immune Trait Variation

The molecular genetic make up,the infections ,nutritional status and the other environmental insults are the most impactful factors that shape the individual immune trait variations , which in turn impacts the host immune responses to infectious agents.The mechanisms for IR may ensemble high immune competence and prevention of infection or inflammation .These infection resistence mechanisms may confere advantages for a lower co-morbidity.High immune trait functioning is more prominant in femals than in males[5].

6-Grades Of IR

Four immune health grades have been described as it starts with grade I and ends up with grade grade IV.Such grading system is based on two basic parameters as i- the balance betweenCD8+/CD4+ T cells and the immune cell gene expression signature.An immune heath grade I express best immune preventive function and grade IV showed the worist immune preventive function[4,5].The optimal immune resilience is associated with; i- lower HIV risk , ii – survival during covid-19 and sepsis and iii – longivity [5].

7-Molecular Mechanisms For IR

Every individual good immune competence reflects ; i – high immune resilience , ii- high functional immune cross talk [17] , iii – good neronal cell-immune cell unit functions[18] , iv - well functional organ-organ axis [19,20].Conversly, low IR activity reflects either low or abolished these molecular mechanisms[17-20].

8-IR In Maternal Immune Activation

Maternal immune activation MIA has been implicated in the etiology of psychiatric illness.Not all progeny exposed to MIA developed psychiatric illness,but some are susceptible others are resielient to MIA.In an experimental animal settings of laboratory mice,it has been tempted to elucidate reslielence in MIA.Poly(I:C) based MIA was induced in C57BL61X mice on gestation day 12.Control dams were subjected to comprehensive test battery when reached adulthood 12 weeks of age onwards.Behavioral characterization showed that the progeny were stratified in to susceptible and resilient to MIA.This means that there were variations both in the genetic and immune response homogenity in these exerimental mice[7,8].

9-IR In Allergy

The factors operable in predicting risks for allergy and asthma are complex.There is a paradigm whereby a balance between allergy and asthma risk and protective factors encounter them.Interaction of human being with environment,microbes and biologics in addition to the epigenetic adaptations contribute in immyne homeostasis.Hence, allergic diseases are good indicators to the twisted relation to environment.In various non-communicable infectious diseases,the protective IR role of the immune system indicate low grade inflammation without an apparent cause[4]

10-IR In Stress

Depression and anexiety have been linked with inflammation.Though it is not clear whether inflammation predate the onset of or it contribute to dpression and anexiet y .It was reported that there is a pre-existing individual differences in the peripheral immune system that predict and promote stress susceptibility.Dpression and anexiety are associated with increase of release of cytokines from the peripheral immune cells.IL6 was found regulated by stress both in man and mice.Thus it is a must for IR high functional status to be associate with stress releave ,normalization of cytokine levels and inhibition of inflammation[1].

11-IR In Tumors

IN human tumor microenvironment,there found to be an effector-like T cells.These cells are characterised by ; i – High cytotoxic activity , ii- marked proliferative activity iii - high migrative capacity in peripheral blood , iv- able to withstand the pressure of chronic tumor antigen stimulation without being exhausted and v- express qick expansion with ful cytotoxic function upon the therapy with immune check point inhibitor ICI.Thus ,such T cells holds the position of resilient T cells and the case as immune reslilience and the situation found logic to explain why some human tumor patients responds well ICI and others not.[21].

12-IR In Microbial Pathogen-Host Interactions

When the total sum of the virulence factors of a pathgenic invador balanced by highly competitive host immune system,disease will not be manifested.Conversely

,when the total sum of the immune system defense mechanisms failed to evads the vagor of the pathogen virulence disease will appears[22,23].

13-Vaccine IR

In an experimental animal settings,animal group vaccinated using vaccine adjuvanated with TH1 promoting adjuvant mounts a memory immune response that is baised twards TH1.While this vaccine primed group encounter subsequent infection with Th2 immune response in oreder to escape the immune attack.If the immune memory induced during vaccination is resilient, the pathogen will be unsuccessful at redirecting the immune response and the group will be protected.If the vaccination in this vaccine primed animal group did not induce a resilient immune response,the pathogen will succeed in redirecting the recall response and survive,so that the primed animal group is not protected[24]

14-Proposal For IR Animal Model

The proposed model immune system can be the rabbit,the IR quantitation system may be inflammatory and anti-inflammatory cytokine measurements.The experimental rabbit settings will be as ;A – Normal rabbit group , B – bacterial infected skin group , C – Bacterin primed group and D - bacterin primed chellenged with skin infecting bacteria.Blood collected , sera separeted and cytokines be determined via elisa kets to determine IR status

15-Proposal For IR in Human Case Study

In an experimental human case study proposal.Election of BCG vaccinated normal human individual with age range of 40-50 years and BCG vaccinted pulmonar tubeculus patients of matching age ranges.Then , detect CD8+/CD4+ Tcell through flow cytometery, one anti-inflammatory cytokine one inflammatory cytokines in their peripheral blood through eliza lets to determine the IR status.

16-Conclusions

Immune resilience IR gains several inpractice applications like;infection ,vaccination,stress and cancer.The quantification parameters are different in different IR aimings,Table – 3.

Table – 3 : Quantitation parameters in various IR conditions.

Condition	Immune	Quantitation	Reference

	Resilience		
Infection,vaccination,inflammation	Operable	CD8+/CD4+ Cell balance,gene expression signature	Ahuja et al.[5]
Allergy	Operable	Low grade inflammation	Haahtella et al [4]
Stress	Operable	Cytokine detection	Hode et al.[1]
Cancer	Operable	Effector like T cells	Gicobi et al.[21]
Infection	Operable	Natural and specific antibody	Bergof et al. [9]

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ESSAY - 4: IMMUNISATION PROTOCOLS

Abstract

Small mammalian labortory animals form the connerstone in the field of experimental immunology as they mimic the.They simulate the biochemistry,physiology and immunology of human beings.It is a state of similarity but not identity.Laboratory animals served an array of biological uses among which is the immunization protocols[IP].Immunization protools in turn furnish as answer about antigenicity, immunogenicity and immune efficacy of immunogens and /or vaccines.IP is not fixed to one trend.It is didderent as the purpose behind the application of that IP.Ten tends for immunization protocols are being outlined in the present essay.

Key Words

Animal, antigenicity, efficacy,identity,immunogenicity, laboratory mammals,vaccine.

1-Introduction;

The nature of the IP depends onto the purpose behind the application of IP.They differ in fine details,but should have basic denomotor for all.These shared points are;fixed shedule,leave period due date,test bleed,final bleed due date post to the booster dose.Though there are some determinal precautions[item-2].

2-Precautions;

i- Animals should be acclimatized to the animal house climate and kept at an add libitum during the experimentation period.
ii- Lipoidal adjuvant should not be applied Intrvenous route.Smaller immunizing doses always advised for IV route.
iii- Pregnant animals should be drawn out of the experiment.
iv- Immunizing dose should be carfully adjusted within the limits betwen low dose and high dose tolerance.
v- During the IP shedule if any animal got disease signs and /or symptome should be away from the plane of the experiment

3- Varieties;

There are variety of IP trends known in experimental immunology.The 25 years local experience in this theme ten or slightly more of IP trends were applied and was briefly mentioned in table 1.

4- Individual Immunisation Protocols

In the following tables ten examples for a variety of immunisation protocols Tables 2-11;

Table – 1 : Lapin Immunization Protocls.

Sequence	Immune Priming Purpose	Reference
1-	Nonspecific cellular immune response	Mishell an Shiigi 1980
2-	Study primary immune rsponse to Egg albumin	Shnawa and Algebori 2003
3-	Study primary immune response To EA-FIA	Shnawa Algebori ,2003
4-	Bacterin specific Primary	
5-	Immune response Skin DTH,by FCA	
6-	Secodary Immune response to EA or bacterin	
7-	Secodary Immune response to attenuated bacterin	
8-	Secodary Immune response to Vi+IFA for vaccine evaluation	Shnawa and Mahdi 2023
9-	Secodary Immune response to PSP for vaccine evzluation	Shnawa and Mahdi 2023
10-	Secondary Immune response for Bacterin immunogenicity through IV	Shnawa,2003

Table – 2 :Nonspecific cellular Immune response,Macrophages.

1-Priming Purpose:Study the non-specific cellular Immune response
2-Antigen/stimulant: Thyogylcolate 3% 5 mls.
3-Adjuvant: Nil.
4- Test Animals, 3 rabbits
5-Dose frequency;once
6-Injection route/site;IP

7-Priming Nature;Single injection single site injection protocol 8- Leave duration:4-7 days 9-Cellular collection,inject 5 ml.PBS IP the aspirate cell suspension 10-Notes:Leukocyte collection for MIF.

Table – 3: Egg albumin Immunogenicity in rabbits

1-Priming Purpose:Study the immunogenicity of EA in primary immune response. 2-Antigen/stimulant:EA 2%.solution. 3-Adjuvant;nil. 4-Test Immune system;Rabbits 5 for test,5 for controls 5-Dose frequency;Once. 6-Injection route/site; IM,SC,2 mls 7-Priming Nature:Multisite multi-injection protocol.1ml,IM,1ml SC at four sites 8-Leave duration;Ten days 9-Test bleed ; at the end of the day 10. 10-Notes;nil.

Table 4 :Immunogenicity of EA incorporated with IFA.

1- Priming Purpose;Immunogenicity of EA-IFA 2- Antigen/stimulant;2% EA solution. 3- Adjuvant; IFA 4- Test animals; five rabbits for test and five for controls. 5- Dose frequency; once. 6- Injection route/site; 1 ml IM, 1ml SC in four sites 7- Priming Nature: multisite multi-injection protocol,hyperimmunization. 8- Leave duration;Two weeks. 9- Test bleed;At the end of the day 14. 10- Notes;Nil.

Table – 5: immunogenicity of an attenuated bacterin.

1-Priming Purpose:Immunogenicity of an attenuate bacterinthrough primary immune response. 2-Antigen/stimulant;Attenuated bacterin,1x 10 to 6 CFU /ml. 3-Adjuvant;Nile. 4- Test immune system: Rabbits five for the antigen and five for the control. 6-Injection route/sites;1ml IM ,1ml Sc in four sites 7-Priming Nature:Multisite, multi-injection protocol. 8-Leave duration:One week up to 10 days 9- Notes: Nile.

Table- 6 : Skin DTH test.

1- Priming Purpose: Primary Immune response for DTH test 2- Antigen/Stimulant: FCA 3- Adjuvant;Intrinsic adjuvanicity

4- Test immune System:Rabbit five for test and five for controls.
5- Dose frequency: Once
6- Injection route/site: One ml IM
7- Priming nature: Intramuscular single injection.
8- Leave duration: 3-4 weeks
9- Test DTH;,observation vfor 4 hrs till 48 hrs.

Table -7 : Immunogenicity of egg albumin with IFA or attenuate bacterin –IFA in secondary Immune response.

1-Priming Purpose:EA immunogenicity,attenuated bacterin Immunogenicity in secodary immunr respose.
2-Antigen/Stimulants:EA 2%-IFA, attenuated bacterin 1 x10 to 6.
3-Adjuvant;IFA mixed with the antigens prepriminy one;one
4-Test Immune system:Rabbit five for test and five for controls.
5-Dose frequency:one week apart for two weeks.
6-Injection route/site: first week 2ml antigen-IFA mixture injected as 1lml IM,One ml SC at four sites. Second week one ml SC to four different site.
7-Priming nature:Multisite multinjection protocol/hyperimmunization.
8-Leave duration: one week
9-Test bleed: at the day 21
10 final bleed post to theend of poster dose due time.

Table -8 :Immunogenicity of attenuated bacterin during secondary immune response.

1-Priming Purpose;Study the immunogenicity of attenuated bacterin during secondary immune rsponse
2-Antigen/stimulant;attenuated bacterin 1 x10 to 6 /ml.
3-Adjuvant: nil.
4-Test immune system: five Rabbits for the antige and five for control.
5-Dose frequency; first week 2ml ,second week 1ml.
6-Injection route/sites;first week,one ml IM,one ml SC in four sites
Second week one ml SC four different sites
7-Priming nature:multisite multi-injection protocol.
8-Leave duration: one week post to last injection
9-Test bleed:at the day 21
10-Final test bleed: a week after the booster dose.

Table- 9 : Immunogenicity of S.typhi Vi during secondary immune response.

1-Priming Purpose:immunogenicity of Vi dring secodary immune response for vaccine evaluation
2-Antigen;Vi antigen
3-Adjuvant:IFA
4-Test Immune system: five rabbits for Vi-IFA and five for control.

5-Dose frequency:3mg/ml completed to five ml by saline in five weeks apart. 6-Injection route:oral 7-Priming nature: Per Os 8-leave duration: one week 9-Test bleed; at the day 4 10-final bleed;one week post to booster dose

Table – 10 : immunogenicity of protoplasmic sonicate prtein for vaccine evaluation.

1-Priming Purpose: PSP immunogenicity during secodary immune responses for vaccine evaluation. 2-Antigen:PSP 3 mg/ml completed to five ml. 3- adjuvant: IFA 4- Test Immune system: rabbits five for PSP Rabbits five for IFA,five for control. 5-Dose frequency: five weeks in a week a part,IFA one week one ml 6-Injection route/sites ;PSP oral,IFA SC four sites. 7-Priming Nature:Oral mucosal,PSP;IFA ,SC 8-Leave duration: one week for PSP,sixth week for IFA 9-Test Bleed: at the day 35 10-Final bleed : one week sfter the booster dose

Table – 11 : Immunogenicity of a bacterin during secodary immune response.

1-Priming Purpose: study the immunogenicity of attenuated bacterin during secodary immune response. 2-Antigen: attenuated bacterin 1x10 to 6 CFU/ml. 3-Adjuvant nil. 4-Test immune system: rabbits five for the antigen, five for the control. 5-Dose sequence: Three doses one week apart as ;1 ,0.75,0.5 ml. 6-Injection route/site:IV in marginal ear vien 7-Priming nature IV. 8-Leave Period: one week 9-Test bleed; at the day 28th 10-Final bleed: one week post to the booster dose.

5-Immunodiagnostic Tips:

Immunogenicity of bacterin in lab animal inflict a light on the immune potentials in human being as both are mammals with marked genetic relatedness as preparation of immune sera, evaluation of vaccine ,toxin and virus neutralization.

6-Conclusions;

Lapin animals are valid immune models for a variety of immune potentials of the in test possible immunogens and vaccines

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ESSAY – 5 : HUMAN_ANIMAL CORRELATES

Abstracts

Human-Animal form a unified commun in any ecologic niche.Recently,workers advocated the concept of unified health system for man and animals.The correlates of mammals to man seems to be the closist mong other animal ranks.These correlates are based upon the similarty but not identity of small mammals to man.This theme formed the basis of the use of these animals as an immune models for human immunity.Small mammalian animal models helps in;production of immune sera,development of vaccines,dource of normal immunoglobulin and fresh complemet.

Key Words

Animal,correlates, human, immune, laboratory ,man, mammals,normal,rank,sera.

1-Introduction

Human-Animal correlates are evident in the current theme of unified health system that ensembles both in one health system.Traditionally known that man can cause animal disease known as anthropozooic infections.In comparison,animals can cause human diseases termed as zooanthrpophilic infections.Allergens of animal origin can induces allergenic human diseases .Human activities induced allergens may pose health problems to their house hold or contactinig animals.The allergenicity in both of these cases may be through direct or indirect ways.Finally,house hold animals may pose some health problemes to the harbouring human family.Occupation, as;farmers,butchers, wool soters, pigeon fancers got number of diseases of animal origin through skin cotact,inhalation,food and/or drink consumption or water born and/or droplete induced diseases.So man and animals are being more close that they differ.

2-Human Being ;

Human being is of a higher mammalian rank gains distictive upward walking on two feets.He is;thinker,creative,speaker, and giver.logicians considered human as speaking laughing animals.

3-Human Immune System:

Human is being a higher mammalian rank own well developed closed immune system type.Human immune system composed of ;hemopoietic,lymphoid,mononuclear cell component as well as complement andkinin component.The distinictive function of human immune system is recognition,reaction and distruction of the whatsoever nature invador

4-Mammalian Immune System;

Basically the mammalian immune systems are similar but not identical to human immune system.There are rankwise differences in some components of mammalian immune system in between ranks and between ranks and man.MHC genetic system bears gene differences among mammals and man.Fetus-mother correlations are somewhat different inman than in mammals in characteristic protein molecules and the encoding genes.Mucosal reproductive compartment humoral immune responses as isotype are different from that of man.Leukocyte antigens and erythrocyte antigens are different inmammals than in man. GVHR are basically same principles of rejection but transplantation,lymphocyte antigens are different.

5-Humn-Small Mamalian Immune System Correlates;

Researchers allover the world had and have been demonstrated that mice,rabbit and nohuman primates are gentically related to hman beings.Similarity but not identity are quitely different,Table-1.

Table- 1 :Ma-Rabbit-Mouse Immune system Correlates

Immune Entity	Human	Rabbit	mice
MHC I II III	 MHCI MHCII MHCIII	 I II	 H2(KD) H2i(1) H2-1(s)
Leukocyte Antigens	HLA1 HLA2 HLA3	RLA RLAD	MLK ML2i ML2-1s
Lymphocyte Markers CD1 CD2 CD3 CD4 CD8 CD19 CD20 CD5	+ + + + + + + +	+ - + + + - - +	+ + + + + + + +
Immunoglobulin IgM IgG IgA IgD IgE	 + + + + +	 + + + - +	 + + + + +
Mucosal Inductive Site Nose Ear Trachea	 + + +	 + + +	 + + +
Mammary Gland	+	+	+

GIT	+	+	+
Cecum	+	+	+
Appendix	+	-	+
Peyre's Patchs	+	+	+

6- Small Mammalian Animals As Laboratory Animals;

6-1: Principles:

Mammals are rather similar but not identical in tissue and organ responses to human beings.Based on this theme there is a similarity in physiologic,immunologic,serologic and pathologic processes among man and small mammalian animals.Though,there some exception to this theme that some immune entities which are confined to either man or animal.

6-2:Purpose:

Small mammalian animal served an array of goals in immunology laboratory and can be outlined as in the followings;

i-Source of normal and immune sera.

ii-Source of complement components.

iii- Live challenge models

iv-Perclinical development of vaccines.

v-In-vivo toxin and virus immune neutralization models.

vii-Valid source model for uncultivable microbial immunogens.

6-3:Types of Laboratory Animals;

There are a spectrum for laboratory animals found valid for teaching and research in most of biology including immunology laboratories such as;Rabbits,mice,gunia pig,rat,golden hamster and chimpanzi .As well as the specific pathogen freeSPF and the gnotobiotic animals.Laboratory animals are of use inseveral practical issues as mentioned in the item 6-2 through either injection of a substance or drawing a sample.Injection can be through;intraocular IO,Intraperitoneal IP,intramuscular IM,Intravenous IV,intradermal ID,oral or chathetrization through vien or trachea.Collection of blood canbe performed through;bleeding,cardiac puncture,exanguated draining from vien or through dissection.

6-4:Handling and Manuplations:

When any worker planned to use laboratory animals,it is a must to bear in mind a set of properties,precautions and behavioral issues as follows;

i-The new commer introduced to the housing environment should be examined for natural infections,carrier state,daziness, canabolic potentials, pregnancy,and/orsluggishness.Any of these issues lead to exlude animal from being of use

ii-The newly introduced animals to the housing conditions,should be acclimatized at least for two weeks and have the add libitum conditions of food and drinks.There are general international regulations and guides for housing and handling laboratory animals.

iii-Susceptibility of the animal to theto be used biologics.

iv-Handling easyness and and hardness.Any person went to handle laboratory animal shoud be aquinted and gained a practice of proper handling of animals befor coming to handel the animal.

v-Possibility ofzoonotic transfer of the disease to human.

vi- infection outbreaks within the laboratry animal herd be a possible source of total distruction of the herd

vii-Special measures should be taken when there is a plan to use SPF or gonobiotic animals.

viii-On finishing from the adopted labratory animal in accordance with the experiment due date .Animals can be incinerated,autocalved or buried in soil.

6-5:Assignments:

One tempting to use animals in groups for short or long term experimentation,one should assign them.Assignment can be performed as;i-tagging with an ear tags,ear cut patterns,tail cut patterns and /or heat made finger print and in a replicate runs.

7-Immunodiagnostic Tips:

Laboratory animals holds as impactful postionin immunology bench works as in ; i-immunodiagnosis oftoxin induced human microbial infections , ii –Preclinical development of microbial vaccines , iii-source of complement and normal as well as immune sera and iv- Source of live immunogens of incultivable human microbes

8-Conclusions:

Mammals and man are holding common slimilarities.Laboratory small animal models are valuable for human in health and disease.

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SECTION TWO;IMMUNE RESPONSES

Essay Six;Blood,Plasma,and Mucus

Essay Seven: Cell separation

ESSAY - 6 : HUMAN AND MAMMALIAN BLOOD AND MUCOSAL SECRETIONS

Abstract

Human and mammalian blood are of a tissue of fluid nature.Blood is formed from cellular contents and fluid content.The fluid content is plasma/serum contaning proteins.Cellular contents includes leukocytes,erythrocytes and platlets.Functionally leukocytes are forming the connerstone of natural innate,immune cross-roads and adaptive immunity in both of human and mammalian beings.This basic informations are being of crucial importance in immunodiagnosis and seroepidemology of human microbial infections.

Key Words

Adaptive,blood,cross-road, erythrocytes,innate,leukocytes,natural,plasma,serum.

1-Introduction

In immunlogy bench work,blood,body fluids, mucous scrap and mucosal aspirates were theclinical sample noted day by day work.Though the most common of which is the blood.The present essay covers the processing of blood from collection till performing the immunologic test then preservation of the to be preserved materials[1-7].

2- Collection

Blood collection procedures vary depending on the subject and the required blood volume:

- In adults, blood is typically collected from the brachial vein, but the carpus vein or jugular vein can be used if necessary.
- In children, blood is collected from the jugular, inguinal, or ankle veins.
- In small mammals, small amounts of blood can be drawn from the tail vein in mice. For larger volumes, methods like cervical dislocation and head decapitation are employed to drain nearly all blood.
- In rabbits, small amounts can be collected from the marginal ear vein, while approximately 10 mL can be drawn via cardiac puncture. For larger volumes, blood collection involves decapitation and draining the entire blood content.

In all cases, the collected blood should be stored appropriately:

- Heparinized tubes for preserving plasma and cells.
- Non-heparinized tubes for collecting serum

~~.The collection of blood from adult human beings should be basically from brachial vien ,but it can be done from the carpus of the hand,if not from the jagular vein. Children blood collection be from the jagular ,inguinal or ankel veins.Small~~

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~~mammalian can be from the cut of tail vein in mouse for small blood amounts.Im case iof need for large amounts can be made by cervical dis-location and head cut to drain allmost all blood contents from the mouse.In rabbits,for small amounts from cut in the marginal ear vein , while for about 10 ml. it can be from heart by cardiac puncture.Larger amounts can be by head cut,collection by drainig whole animal blood.In all of the mentioned cases the collected blood should be tubed into either heparinized tubes or tubes without heparin.Heparinized for plasma and cells savings.While nonheparinized for serum saving.~~

3-Whole Blood Dilution,

It is to be noted,that in some immunologic tests whole blood is recommonded to be diluted.Blood can be diluted by PBS,Alsover's medium or sterile broth medium.Dilution rate can be 1:10,1:20,and or 1 :100 in accordance with the method instruction.

4-Saving Plasma:

Plasma is the whey colored fluid supernate layer onto the cellular content of the centrifuged heparinized blood.Plasma can be of use as fresh or revived cryopreserved at -18C,or chemically preserved by benzilchonium cholrid solution,or merthiolate solution.For short term preservation one to one volume mix of plasma with alsover's solution at 4C to one week only.Tubes containing plasma for preservation should be labelled by sample name,code,researcher name ,date of presevation and date of collection.

5-Saving Sera;

The supernate layer of the centrifuged non-heparinized clotted blood is the serum.Serum tubbed aliqoutes can be preserved at -18C in the chest freezer of refregirator.Or it can be preserved by chemical preservatives like bezelchonium chlorid or merthiolate and kept at 4C at referegirator.Tubes containing sera should be labelled with;name of the sample-code,name of researcher,date of preserrvation and date of collecion.

6- Dilution Theme:

Patients and control sera after revived from preservation and attening the recommonded temperature for test performing,will be diluted as the special test recommond but most common types of serum dilution are;

i-Double;

AS;

Tubes 1 2 3 4 5 6 7 8 9 10

Diluant 0.5 ml ----------

Serum 0.5 ml----------

Dilution 2 4 8 16 32 64 128 256 512, 1024

ii-Decimal Double

Tubes 1 2 3 4 5 6 7 8 9 10

Diluant 0.9ml 05 ml.----------

Serum 0.1ml 0.5ml----------

Dilution 10 20 40 80 160 320 640 1280 2048 5128

iii- Decimal;

Tubes 1 2 3 4 5 6 7 8 9 10

Diluant 0.9ml----------

Serum 01ml----------

Dilution 10 100 1000 10000 100000 1000000...

7- Titre Concept;

The in use concept of antibody titre can be explained in to ways as;

i- The reciprocal of the highest dilution that showed clear positive test results
ii- The reciprocal of the dilution that showed 50% clear positive test results.

Though the outstanding trend is the first trend.titre is important in;

i-Determinaton of type of the immune sera.

ii-Reaction intensity, antibody reaction affinity.

iii-Deterction of thev phase of the infection in the infection cycle

iv-Determination of basline,immune conversion,cutoff limit,clinical level.

v-Determination of immune protectivity in some microbial infections.

vi-Seroepidemiologic probe for screening the infection cycle and infection state in the cycle.

8- Determination of Standard Protein Concentrations

Protein spectrophometery by the goal of determination of concentration needs the construction of standard protein concentration curve, through preparing series of double dilution of fixed concentration of albumin and determine the spectrophometeric color value for each concentration in the series.Raw results subjected to simple linear regression analysis to find up the linear regression equation usable for estimation of concentration of any color readings ithin the limits of curve readings to corresponding concentration. As in the followings

i-Disolve 60 grams of albumin in one liter of distilled water.

ii-Make double dilutions as;60,30,15,7.5,3.75,1.875,0.687,and 0.328 g/L.

iii-Take a 0.1 ml from each of the prepared test dilutions and add them to series of tube each containing 5 ml.Biurt test.

iv-Incubate at dark for 15 minutes

v-set up the eight test tube fro 1 to eight

set up the one reagent blank

set up the one standard blank

vi- Read at 560 nm

vii-A sample experiment done for construction of the standard curve was;

Y- = -3.512+443.9 xi

Xi represent the unknown color reading of the patient serum sample.

9-Determination of Protein concentration in serum ,serum globulin,and mucosal globulins

9-1: Determination of Total Serum Proteins

i- The to be test serum for the total protein revived to attain 37C or to the ambiant room temperature.
ii- Set up 5 ml biurt reagent to each of test blank and standard tubes
iii- Add 0.1 ml serum to the biurt reagent in the test tube,0.1ml standard to standard tube and nothing to the blank tube respectively
iv- Incubate at bthe dark for 15 minutes at room temperature.
v- Set up blank,standard and test tubes.
vi- Read at 560 nm.
vii- Record the spectrophotometeric color value
viii- Apply the readings to the linear regression equation.

9-2:Determination of serum globulin concentration;

i-Tube 5 ml serum in a clean sterile test tube and add 0.9 gm of powder anhydrus ammonum sulfate

ii-Incubate at room temperature for 30 minutes.

iii-Centrifuge at 3500rpm for ten minutes.discard supernate and keep precipitate

iv-Add 2.5 ml of distilled water for solubilization,the add 0.7 gm of anhydrous amonum sulfate powder.

v- Incubate for 15 minutes at room temperature

vi- Centrifuge at 3500 rpm for 15 minutes

vii- Discard supernate and keep pellete.

viii-Suspend the pellete in 5 ml PBS

ix-Transfer 0.1ml to a tube containing 5 ml biurt reagent and incubate in dark forv 15 minutes

x-Read the color value at 560 nm, apply it to the linear regression equation.Find out the concentration of serum globulin.

9-3:Separation and determination of mucosal Globulin Concentration From Fecal Sanmple[2].;

A

i-Collect fecal sample from patient or normal subject.

ii-Mix well the fecal material.

iii-Weigh 2 gms from the mixed feces.Put into sterile flask then add 10ml 0.5% saline the homoginize.

iv-Centrifuge at 3500 for 1 /2 hr.

v- Collect the supernate and recentrifuge at 4000 rpm for 15 minutes.Save the supernate and discard the pellet.

vi-Mix 5 ml from the supernate step v with 5 ml 40% saturate amonium sulfate solution and let it stand for 1/2 hr at room temperature.

vii-Centrifuge at 3500 rpm for 1./2 hr.Discard supernate,keep pellete and disolve it with 1ml.saline in atemperature of 4C.

viii-Mix 0.2 solution step vii with 5 ml biurt reagent,0.1 standard to 5ml biurt reagen and nothing to blank ,incubate in dark for 15 minutes

ix-Read the sopectrophometeric value and apply it to the linear reggression equation to calculate the concentration of the fecal globulin concentration in health andisease.

9-3-B;Fecal globulin separation by PEG;

The steps from 1-4 in 9-3-2A are the same

v-Mix 5 ml fro step 4in 9-2-3A with 5ml PEG 556% 6000 in tris buffer the vortex it let it o stand at room temperature for 1/2 hr

vi- Centrifuge at 3500 rpm for 1/2 hr,discard supernate and keep pellete.the pellete disolve in 1ml tris buffer and dialyse against PBS then keep at 4 C.

vii-Add 0.2 ml from dialysed pellet suspesion step 6 to 5 ml of biurt reagent and incubate in dark for 15 minutes.

viii-Read at 560nm in spectrophometer and estimate concentration by application of the color reading to the regression formula to calculate the concentration.

9-4:Mucosal Globulin Separation From Urine[6,7]:

i- From a urine collect draw 5 ml and tube it in centrifuge tube.
ii- Centrifuge at 3000 rpm for 5 minutes.Save supernate and discard pellet.
iii- Four ml from the supernate urine mixed with 4ml PGE 6% 6000 in tris buffer let to stand at refrigrator for 1/2 hr. At 4 C
iv- Centerrfuge at 5000 for 30 minutes
v- Discard supernate and keep pellet then disolve in 1ml normal saline
vi- Add 0.2 ml from the solutio step v to 5ml biurt reagent and incubate in dark for 15 minutes
vii- Read in spectrophotometer at 560 nm ,the reading color value apply it as xi in the regression formula.

10- Immunodiagnostic Tips:

The serum and mucosal globulin concentration and titre with the corresponding pathogen antigen be important in determination of baseline concentration and titre, immune conversion titre and concentration and concentration and titre of clinical indications.These informations are of crucial importance both in immunodiagnosis and seroepidemiology.

11- Conclusions;

Dilution theme is of importance in determination of concentration and titre of globulin versus antibody in patients and control heplful in disgnosis and epidemiology of human microbial infections.

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ESSAY – 7 ; CELL SEPARATION

Abstract

The theme of cell separation is a usefull in biological teaching and research.Adherance density and antibody binding are the outstanding known methodologies .Density gradient based methods find their way widely immunological in-vitro bench work immunology. separation methods from blood,body fulids,tissues and mucous aspirates are beig discussed for the assessment of immune cell functions in human beings.

Key words

Antibody,adherance, cell, density,function,gradient,immune,separation.

1-Introduction

Cell separation is powerful tool widely used in biological ,immunological and biomedical teaching and researchs.For research the ability to sort cells in to distinct populations enables the study of individual cell types separated from heterougnous cells starting population without contamination from other cells.Cell separation methods principally based onto; adherance,density and antibody binding.During cell separation one have to be awar of; purity,recovery and viability. Of targeted test cells.Density based methods seemed to be gaining wide application in biology and immunology teaching and research laboratories[1-5].

2-Separation of White Blood Cells[1];

The method is based upon density gradient centrifugation as in the following sequence,

i-Whole blood is diluted with PBS

ii-The diluted blood is carefully layered onto centerifgation medium in a conical tube avoiding any mixing

iii-Centerifuge at the appropriate velocity without breaking,distinct phases can be obsereved

ontaining mononuclear cells ,c- centerifugation medium , d-erythrocyte and granulocyte,can be aspirated from the intephase.

Separation of blood cells can be done by other way using dextran solution as in the following steps[2]

i-Collect 8 mls. Blood from a donar brachial vien.

ii-Prepare 5% dextran 264000MW in PBS.

iii-Mix the 8mls. Of the blood with 2mls dextran solution in a tube.

iv-Incubate in a standing upright position ant 37C for 45minutes.

v-Plasma on upper surface phase contains leukocytes,aspirate the leukocyte and tube it in centrifuge tube.

vi- Centrifuge at 400g for 30 minutes

vii-Pellets resuspend in PBS

iix-wash the pellete[leukocytes] in PBS three times at 200g for 15 minutes.

3- Separation of Leukocytes from mucous materials and body fluids aspirates[1-2].

Aspirates from vagina anduterus ranging 2-5 mls in size be diluted in PBS till reaching * mls in size. Then process them in the following sequence,

i-Centrifuge at 200 g for 15 minutes.

ii-Discard supernate and keep pellete.

iii-Resuspend pellete in 8 mls.PBS and mix.

iv-Carfully layer the cell suspension onto 2ml dextran 5% in PBS.

v- incubate at standing upright position at 37C for 45 minutes.

vi-discard supernate,collect pellete

vii-Resuspend into 4ml PBS.

Iix-Centrifuge at 200g for 5 minutes and was withPBS three times

ix-Resuspend the pelletein PBS,be the target cells.

4- Separation from solid tissues[1].

Solid tissue in some instances the target cells.Target cells can be separated as in the following way;

i-Macerate and disociate tissue,cells diffuse into the bathing medium

ii-The resulting cell populations filtered so that

iii-Obtain single cell suspension.

iv- The supension carefully layered onto centerfuging medium avoiding mixing to give

v-a cell rich interphase between the centerifugation medium and the suspension buffer.

Note;

It is possible to isolate different cells by removing from supernate or interphase and then recentrifuge at different contrifugation media and angular velocities untill distinct cell fractions are observed.

5- Separation of lymphocytes from mouse lymph nodes[4].

This can farely be done as a mouse injected in its food pad by 0.25ml.of Freund complet asdjuvant and leave for 3 to 4 weeks.,then process in the following steps,

i- Kill the mouse by CO2 inhalation or cervical dislocation.
ii- With scissors and forceps,reflect skin from the body and pin the skin to the board.
iii- Use the forceps to serch for mesentric lymph node found in the mesentry of ascending colon,largest mesentric lymph node.
iv- Transfer the lymph node with forceps to a fresh tissue culture dish containig PBS
v- Tease the node with forceps providing not breaking it to small fragments
vi- Collect cells inPBS.
vii- Centrifuge at 5000rpm for 5 minutes ,was three times with PBS

6-Immunodiagnostic Tips[1-5]

Cell separation technology is being promising for;

i- cell based immunotherapy of cancer.
ii- Immune cell function assessments
iii- Tissue regenerative research.

7-Conclusions

For immunology invitro bench works and immunology researchs,density gradient centrifugation technology is being the outstanding trend.

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PART TWO ; NATURAL INNATE IMMUNITY

Section Three;Humoral Innate Immunity

Essay Eight :Acute Phase Protein C

Section Four:Cellular Innate Immunity

Essay Nine: Pyuria

Essay Ten Pyospermia

ESSAY – 8 : ACUTE PHASE PROTEIN C

Abstract

Acute Phase Protein C served as an indicator for the immune and inflammatory responses .They are of positive and negative CRP types.Acute phase responses may be of low and high level reactants.Local experience had been showing that CRP were elevated in chronic ;metabolic,viral and bacterial diseases.Local prepared CRP was rhumboid,translucent ambur colored crystal forming colloidal solution and of proteinecious nature.It had been found as equating the standard commercial makes in its detection ability.

Key Words

Acute,CRP,high,low,level,PHA.

Introduction

In acute state,human and vertebrate bodies induced by infections,inflammations tissue injuries,burns,trauma and advanced cancer[1].The rise up of levels acute phase proteins is inline with with an acute disease conditions where the liver temporarly increase the synthesis of more than 30 different acute phase proteins[2].The APP synthesis occur when hepatocytes are exposed to IL6,IL1 and TNF alpha released locally from other adjacent cells [3].Among these 30 acute phase proteins,the acute phase protein C[CRP] is considered as an archetypal acute phase proteins[2].The steriochemical structure of CRP as revealed by electon microscopy is composed of five identical non-covalent linked monomere arranged in a planner ring with pentameric symmetery [4].CRP bind specifically to the phosphocholine group and polysaccharide on microbial surfaces in a calicum dependent nmanner[5].CRP can act as an opsonin activating classical complement pathway and promote phagocytosis[3]. CRP amplify immune responses[6] and ihibits spread of the infectious

agents[7].Acute phase protein C are of positive as CRP and negative type as prealbumin[8].The adverse effects of acute phase protein C includes;fever,anemia of chronic diseases,anarorxia,lethargy,amyloids and csachexia[8,9].Vaccination induces homeostatic changes which in turn initiate inflammatory responses with severity classifid into major,moderate,or minor[10].CRP level can monitors the clinical prescribing of antibiotics[11].

Inducers

Infection,inflammations,tissue injury,burns,trauma and advanced cancer[12].

Immune Responses

CRP promotes an amplification of immune responses.As such it may induce specific immune responses in mammalian experimental animals.

Role In Immunopathogenesis

When an inducer of what so ever nature insults onto the hepatic cells to synthesize ,produce and release IL6 ,IL1,TNF alpha to the visicinity of hepatocyt,hepatocytes exposed to these cytokines will synthesis,produce and release of increasin amounts of CRP

Clinical Sampling

Blood sera and Blood plasma

Processing

Program 1: Commercial CRP make.

i-Collect 5 ml.blood from brachial vein and tubbed it in to blood collecting tubes without anticoagulant.

ii- Let it clotted at room temperature for at least half hour.

iii- Centrifuge at 5000 rpm for 5 minutes

iv- Aspirate supernatent fluid the serum and kep in aliqouts in appendrouph tubes

v- Run serial dilutions of serum as recommoded by manufacturing company

vi-Run a drop of whole patient serum and admix with latex CRP reagent,if positive the run the serial dilutions with late to determine the concentration and/or titre.

Program 2; Local CRP make/Preparation of CRP

i-One liter from pleural fluid was collected from a case of St.pneumoniae pneumonia and defatinized

ii-50% and 70% amonium sulfate saturation were temepted for initial fractionation in a stepwise manner.Fibrin accumilated

iii-The mixture of defatinated plueral fluid-amonium sulfate was subjeted to the removal of fibrin

iv-The defatinated defibrinated plueral fluid –amonium sulfate mixture allowed to stand at room temperature overnight.The incubated mixture form a precipitate.Then ,mixture was centrifuged,supernate discard and keep precipitate

v-Precipitates washed twice with normal saline.Washed precipitate resuspended in 200 ml.DW. it appeared as ambur colored colloid,the CRP

vii-The ambur colord CRP colloid be characterized following the belowmentioned criteria;

a-Fix up the crystal morphology, and texture .

b-Fix up the color.

c-check up the precipitability in protein precipitants

d-Check the reactability with protein identification reagents[13]

e-Check up the reactability with commercial Latex CRP reagent,Table-1.

Program 3:Preparation of lapin anti-CRP immune sera

A-Specific Immune Priming

Equal volumes of the prepared CRP,standard CRP and FCA mixed and used for specific immune priming of two test rabbit groups each of five via muisite multi-injection protocol [14].

B-Specific Immune Sera

i- after the leave week elapsed blood collected from heart by cardiac puncture and tubed in blood collecting tube without anticoagulant.
ii- Centerifuge at 3000rpm for 5 minuts
iii- Aspirate the serum fro each group,determine concentration[] and dispense in appendroph tube in alliqouts ,keep at -18 till use.

C- Settings

1-Prepare tanned sheep RBC and coat it with standard commercial CRP

2-Prepare tanned sheep Red cell and coa it with the local make of CRP

3-Supply commercial make of latex antiCRP kit.

4-Sera from diabetis,brucellosis and Hpatitis B &C were collected,dispensed in alliqoutes of 0.5 ml and kept at -18 till use

5-Bring the supplied and prepared immune reagent to 37C

6-Run single drop of each sera and single drop of each antigens,mix and wait for formation of aggltination clups.

7-Positive cases sera were diluted as recommended by the working methods. And equal amounts of antigen were added,incubated for 45 minutes at 37C

8-Read concentration and titre.

Findings

The local make of anti-CRP parallels with that of standared commercial CRP tempted in pateints with diabetes,brucellosis and hepatitis B&C.Tables 1 -4 .

Table – 1 :Biological characteristics of local CRP[16].

Characteristics
i-Well contoured,flat,rhumboid plates and translucent crystal in majority,minority were of tiny needle morphology ii-form colloidal solution iii-umber color iv-reactogenic with protein testing agents v-Reactogenic with standard anti-CRP reagents

Table – 2 : Acute phase Protein C levelings.

Condition	Concentration levels mg./L	Reference
Baseline Acute infections	2 – 10 100 up to 1000 folds	8 8
Baseline Uncomplicated infections	2 50 – 100	11
Metabolic inflammation, uremia,cardiac ischemia	Low levels 2 – 10	11

Table – 3 : Lapin anti-CRP antibody titres[16].

CRP make	Standard anti CRP	Local anti-CRP
standard	3200	1600
Local prepared	800	3200

Table 4 : CRP in Chronic hepatitis,Brucellosis and Diabetes meletis[16].

Patient sera	Latex CRP titre	Latex CRP Concentration mg/L	Number of fold increase than basline	PHA titre mean Standard CRP	PHA titre mean ,local CRP make
Chronic Hepatitis B	16.7	218	100	12.8	11.4
Chronic Hepatitis C	16.7	218	100	12.8	11.4
Chronic Brucellosis	14	436.8	200	13.6	12.6
DM1	29.8	432.8	200	29.6	29.6
DM2	27.2	436	200	27.7	22.6
Control		2.57			

Interpretation

The increase of CRP concentration levels indicate acute and chronic inflammation.Low concentration levels indicate mild inflammatory process mostly associated with chronicity.Chronic bacterial viral, and metabolic diseases associated with high levels of CRP as local past experience was showing[16].

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ESSAY - 9: PYURIA

Abstract

Natural cellular immune responses in human urine known as pyuria.low grade pyuria is non-sense .Moderate to high grade pyuria were evident among symptomatic uropathy patients.Such findings is attributed either to infection or inflammation.There were shifts in :nature,quantity and quality of urinary microbiome during the uropathic disease.

Key Words

Infection,injury, grade,inflammation, microbiome,pyuria

1-Introduction

Neutrophils and monocytes are holding the first and second line defenders and stands as the cornerstone of immune surviellance in urinary tract.They represent the innate immune response and the acute and/or sub-acute inflammatory responses.In urinary system such responses are known as"pyuria"[1].Pyuria is the presence of significant numbers of phagocytes in urine flow due to inflammatory or infectious insults[2].Pyuria response can be the sole response or in conjugation with hematuria(The presence of erythrocytes in urine flow),hematuria also can be classified as low,moderate and high grades(More than 10 leukocyte/ul).There was instances were only hematuria existed in the urine flow voide[3,4,5]

2-Inducer

Injury,infection,stone,renal disease,Table- 1[6].

Table – 1 : Urine sediment interpretations.

Feature	Significance
Erythrocytes	Tumor,stone,glomerulonephritis
Leukocytes	Infection,inflammation
Cast	Kigney disease
Crystals	Stone
Epithelial cells	

3-immune Responses

Innate cellular immune respones orchestrating adaptive immune responses.

4-Immunopathogenesis

Instant injury induces acute infinflammatory responses.Persistent injury may mediated sub-acute and/or chronic inflammatory responses. Infectious agents reach urinary tract through either of the following routes as;Ascending from the perineum to urethra, then up to bladder.Descending from the renal blood filtrating metabolic byproduct together with urine.As well as hematogenous of lymphogenous infection routes.Neutrophils or monocytes facing the invadors take up the germs phagocytize them and process them into epitopes.This may be followed by local or systemic humoral and/or cellular immune responses.Failure of immune surviellance cells to combates the invadors lend the invador the chance for producing uropathies[7].

4 – Clinical Samplings

Clean cath midstream urine samples after previous dis-infection of area with apropriate di-infectants.

5- Processing

A - Program One ; Pyuria gradings.

Midstream voided urine collects from 1173 uropathy in and out patients from both sexes and age groups of 18 to 65 years old.urine sediment wet mounts onto a glass slides were made and examined at 40x magnification as in [2-6].

B – Program Two : Uropathy Microbiome.

Urine within the urinary tract befor voiding is sterile and no evident microbiome population noted so far.But during voiding inthe fore male urethra and female urethra harbours coliforms,diphthroids,S. Epidermidis,S.saprophyticus and non-hemolytic streptococci.While ,inuropathy patients the urinary microbiome undergoen shifts in nature,quantity and quality of their components[8-11].The collected urine samples wer e processed for culturable microbiome onto blood and McConkeys agars and identified as in[12,13,14].

6 – Findings :

Program One : Pyuria gradings

Moderate to highgrade pyuria or pyuria-hematuria were markedly observerd,Table – 2 A & B.

Table -2 : Pyuria gradings in uropathy patients.

Natural Cellular responses	Findings
A/ Moderate to High Grasde pyuria = = Pyuria – hematuria = = Hematuria	949;1173,80.9% 186: 1173,15.8% 38:1173,3.2%
B/ Collective Moderate to high grade Pyuria Hematuria	 1135: 1173,96.8 % 224: 1173,19.1 %

Program Two;Urinary Microbiome

E.coli , K. Pneumoniae,P aurogenosa ,Proteus.spp.,S.aureus and St.pyogenes were the evident cultivable uropathogens,they gave at most pure and havey growth

Table – 3 : Urinary Microbiome.

Microbiome/cultivable.	Components
E.coli	153:285,53.6%
K.pneumoniae	22 : 283,6.6%
P.aeruginosa	23:285,8.0%
Proteus.spp.	19:285,6.6%
S.aureus	52;285,18.2%
St.pyogenes	1 : 285,0.35%

7- Interpretation

Moderate to high grade pyuria in uropathy patients constitute an indicator for natural cellular immune response for inflammation and/or infection[2,3,4].Normal versus inflammatory microbiome have shown an evident shift from normal state of urethral microbiome from both quality ,quantity and nature of the forming components of the microbiome,Table – 4 [10,11].

Table – 4 : Urinary Microbiome shift in uropathy patients[14].

Normal urinary microbiome	Uropathy urinary microbiome
Coliforms	E.coli
Diphthroids	K.pneumoniae

S.saprophyticus	P aeruginosa
S.epidermidis	Proteus spp.
Non-hemolytic streptococcus	S.aureus
	St.pyogenes

8- Immunodiagnostic Tips

i-Strict following the grading limits as nonsignificant(low) and significant grade(moderate,High)[2].

ii- Moderate and High grades of the infectious pyuria linked with urinary microbiome shifting from major positive to major negative gram phentypes[7,9,10 11].

9-Conclusions

Moderate to high grades pyuria stands as indicator of infection and inflammation.

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ESSAY - 10 : PYOSPERMIA

Abstract

When suspects of infertitlity have neither genital abnormality ,nor got accident to thier genital tract,free of drug and on absteine.Pyospermia of less than one million leukocyte per ml is nonsignificant.Leukocyte counts got levels of more than one million leukocytes per ml. is significant and have a clinical bearings as a sign of infection and/or inflammation. Pyospermia is implicated in semen pathology,subfertitlity and infertility.

Key Words

Absteine,inflammation,infection,infertility,pyospermia,subfertility.

1-Introduction

Immunesurviellance mechanisms ISM are everpresent allover human body compartments .ISMs are mostly performed by the innate immune cells,the neutrophils and monocytes [1].The recognition of these cells in human semen can either be normal, subnormal or abnormal pathologic[2].The presence of leukocytes in semen is known as pyospermia,PS.PS is graded as clinical significantSPS and non-significant NPS.NPS grade is below one million per ml.SPS is at most more than one million per mlof semen.PS is natural cellular immune responses to an infectious events in male genital tract or it mere acute or sub-acute inflammatory response nature.It may interply with fertitlity state,sperm pathology and reproductve oucomes[2-6].

2- Inducers

Injury,infection

3- Immune Responses

PS persay it can be an innate cellular immune responses[1].

4- Immunopathogenesis[1]

Sperm antigens reaching human blood stream recognized as non-self and taken up by innate immune cells then processed and presented to either B or T cells or to both.This presentation intiate an autoimmune responses finalized by an autoimmune infertility.

5-Diseases

Subfertility,infertility.

6-Patients and Samplings

The suspect sub or infertile males were evaluated by consultant urologist and sergeons for; variocele,hydrocele,cryptochidisms,inguinal hernia operation,congenital abnormalities .veneral,chronic and febrile diseases[3].Semen was collected from these suspects provide that they are on absteine and free of drug directly into aclean disposable petri-plates by masturbation in a room near the laboratory.The containers labelled with name,age,absteine period and time of collection[6].

7- Processings

A-Program One:Semen Analysis

The specimens were incubated at 37C for 30 minutes to allow liquification.Then wet mounts were made to have a clue about the possible agglutination[7],and stained filmes for uncovering of the possible other sperm morphologic pathotypes.Neubar and Thoma slides were used for sperm counts[8-9].

B-Program Two ; Pyospermia gradings

From the liquified semen specimens micropippet drop of ul size was applied into Neubar and Thoma slides for counting white cells ,count levels of white cells;

Counts less than one million are non-significant

Counts more than one million are significant

8- Findings

A- Program One;Spermatology

Seventy nine were infertile male patients,the study group.They were subgrouped in accordance with their spermatologic nature into three subgroups as; asthenospermia,oligospermia and azoospermia,Table – 1.

Table -1 : Sperm counts abnormalities.

Group	Findings
Asthenospermia	48:79,48.79%
Oligospermia	18:79,22.78 %
Azoospermia	13:79,16.45%
Total	79:79,100%

B/ Pyospermia Gradings

The means of leukocyte counts in the semen samples were; 5.683,6.166 and 2.38 millionn per ml for astheno,oligo and azoospermia cases.Hence,pyospermia were evident in all of the groups to a variable degrees,Table – 2.

Table – 2: Pyospermia levels among infertile human subjects

Infertile groups	Leukocyte levels Mean-/+SE, in milliom per ml	Incidence	Pyospermia ranges In Million per ml.
Asthenospermia	5.6383,0.56	45:48	2-25
Oligospermia	6.166, 1.11	18:18	4-20
Azoospermia	2.3,0.924	8:13	2-14

9- Interpretations[11-14]

Pyospermia may be in association with defects in ; semen profile,ejaculate volume,sperm counts,sperm motility as well as sperm function.As a result of oxidative stress,cytokine secretions,reactive oxygen intermediats by leukocytes can induces also reduction in capacity of sperm-oocysts penetration.Pyospermia stands as an indication for;

i- Genital infection.
ii- Influx of tissue harbouring leukocytes
iii- Inflammatory response

iv- Part of the normal non-specific cellular immune surveillance of human immune system in the genital compartment[15].

10-Immunodiagnostic Tips

i- Suspects should be on abstiene and free of drug before lending semen samples
ii- Collect by masturbation with willing of the patient.
iii- Pyospermia stands as as natural cellular immune indicator of inflammation in male genitalia
iv- Cutoffs are being below one million per ml of semen

11- Conclusions

Pyospermia was evident in the three test groups to variable degrees.Such findings hold more than one immune and pathologic bearings.

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PART THREE : IMMUNE CROSS-ROADS

Section five ; Complement

Essay Eleven:Complement Bactericidal Power

Essay Twelve: hypercomplementemia

ESSAY - 11 : COMPLEMENT BACTERICIDAL POWER

Abstract

Human fresh blood express bactericidal power BP.BP reside in the fresh plamsa portion,the complement.Mixing of diluted blood with diluted E.coli fresh culture and incubation at37C for 0,10,20 and 40 minutes followed by quadrate streak culture onto nutrient agar plates.The inoculated plates were incubated overnight at 37C.Reduction in growth was the end point of bactericidal action.Diluted blood from a healthy adults 20-40 years old have shown reduction in growth got increased as the time of incubation increased.Likewise,the normal aged subjects showed same action profile.Though the aged with cardiomyopathes have shown nulified to reduced bactercidal activity.In a lapin immune model antibody in primary and secondary immune responses supports the complement bactericidal action.

Key Words

Bactericidal power,blood,complement,growth reduction,plasma.

1-Introduction

Complement system in healthy state have a number of immunobiologic functions as;immune cell lysis,opsonization,and immune complex formation.The activation of complement system performed in three pathways,the classical ,the properidin and the lectin pathways.The activation events statrts with initiation,amplification, and membrane attack that terminates the immune cell lysis[1-9].

2-Inducers:

Infection,inflammation[1,2].

3- Complement Response And immunopathogenesis;

The catalytic metabolism of complement parallels with the involvment in immune tissue injuries.While excessive anabolic metabolism also involved with immunopathology.The immune complex formation is linked with immune complex deposition diseases like that of type III hypersensitivity ,autoimmune glomerulonephritis,and autoimmune thyroditis[1].

4-Diseases

Hypo,hyper and extreme hypercomplementemia as well as immune complex deposition diseases[1].

5- Clinical Samplings

Fresh blood, fresh blood plasma and fresh blood sera.

6-Processings

A-Program One:Normal adult and aged[8-9].

i-Collect five mls. Blood samples from five normal adults of 20-40 years old,and five mls blood from norma aged subjects 60-80 years old in a blood collecting tubes with anticoaggulants then mix.

ii-The bloodsamples were diluted with strile saline 1:10.

iii-Dilute 1:100 fresh 6 hrs fresh broth culture with sterile broth.

iv- Transfer inocula from diluted fresh broth culture and inoculate them with the diluted blood samples.

v- Incubate the inoculated blood samples at 37C for;0,10,20 ,and 40 minutes.

vi-At the assigned time of incubations transfer inocula and quadrate streak them onto nutient agar plates and incubate at 37C for an overnight.

vii-read the growth reduction at 0,10,20,and 40 minutes.

B- Program Two [8-9];

i- In a group of 38 aged patients from both sexs with an age range of60-80 years old.Collect blood samples into blood collecting tubes with anticogulant and mix them
ii- Perform the test as in Program One.

C-Program Three[4]:Synergy of lapin antibody with complement.

1-Two rabbit test groups each of three and a third group of three as control they were immune primed as in the following ,Table – 1.

2- Saline 0.85 in 0.9 ml amounts was delivered into each of the three capped 12x 100mm blood collecting tubes.0.1 ml of blood from primed and control rabbits and mixed.

3- Six hrs fresh broth culture of E.coli was diluted 1:100 with sterile nutrient broth.

4- 0.05 ml. of the diluted E.coli broth culture were added to the diluted rabbits blood.

5-Blood-E.coli mixtures were incubated at 37C for 0 ,10,20 ,40 and 60 minutes.

6-Aspirate 0.1 ml volume from each tube anddelivered to the surface onf nutrient agar plate and spreaded ,made induplicate.Incubate at 37C for an overnight period

7- Score the remaining viable count and percent inhibition

Table – 1 : Immunopriming Protocol[4].

Immune response time curve	Dose	Number of rabbits	routes	Dosing frequency/week	Leave peroid in weeks	Blood collection time	notes
Primary	0.2 IFA 1ml EA-IFA 1ml	3	IM IM SC	Five doses in a daily frequency In paranodular areas pelvic and subclavian	One Day Three weeks	No Post to the leave time	For conditioning
Secondary	1ml EA-IFA 1ml EA-IFA	3	IM SC	First dose,first week In paranodular areas pelvic and subclavian	Two weeks later Second dose	 One week	Post to the leave time
Control	2ml	3	IM,Sc			Three weeks	

7-Findings

A-Complement bactericidal ower were found active at 10 minutes and highly active 20&40 minutes in adults of 20-40 years old,normal aged have shown reduction of graowth at 20 and 40 minutes.

B-Complement batericidal power were nil or reduced at 10 and 20 and fairly active at 40 minutes in aged with cardiomyopathies.

C- The growth inhibition by the complement action was higher in secondary than in primary immune response time curve.Growth inhibition both in primary and secondary responses were higher than that of controls.There were indications for a linear relatioship between the time period of incubation and the percentage growth inhibition by diluted blood action.These difference in growth inhibition between the test and control groups were statistically significant and regression analysis was of simple linear type.,Table- 2.

Table – 2 :lapin Complement bactericidal action synergistic with antibody responses.

Time in minutes	Control Remaining %10000	% inhibion	Primary Response, remaining% x 10000	% inhibition	Secondary respose, remaining% x10000/inhibition %
0	100	0	100	0	100 /0%
20	8.9	31	4.4	46	1.6 /84%
40	5.1	45	3.6	64	0.8 /92%
60	2.01	67	2.1	79	0.5 /95%

8- Interpretation

A-In normal adults,the growth reduction onto streak plates increased as time of incugation increase[8].

B-Ageing associated with reduced or hpoactive complement .Ageing with cardiomyopathies associated with nil or reduced bactericidal power[8].

C- Antibody of rabbit to EA in primary and secodary immune responses supports the bactericidal action of the complement to a variable degrees.Such findings are inline with other workers findings,Table -2 .The bactericidal test in program two was semiquantitative while in program three was quantitative and stands as modification from the the method of Wardlaw 1982[4],Table – 3 dipicted the comparison

Table – 3 ; Complement bactericidal semi and quantitative methods

Parameter	Fresh human blood [9,8]	Fresh primed rabbits blood[4]
Essay nature	Semiquantitative	Quantitative
Test bacteria	E.coli Lilly	Local E .coli
Fresh 2hrs broth culture dilution	1:100	1:100
Fresh blood dilution	1:10	1:10
Incubation time at 37C for	0,10,20,40 minute	0,20, 40 minute
End point	Growth reduction around streak line	Remaining colony count ,% groth iinhibition
Growth inhibtion curve	Not applicable	Linear correlation betwen incubation time and growth inhibition %

9- immunodiagnostic Tips

The essay is emi-quantitative,easey,demonstrable for detection of batericidal activity of complement with whole diluted blood used.Current studies confirmed the importance of complement bactercidal power in presence of antibodies for vaccine efficacy assessments[5-7].

10- Conclusions

The indicator cell is bacterial,the diluted blood served as source of fresh plasma,the end point was either reduction or abolishing of colonial growth.Complement bactercidal power newly introduced to the area of vaccine efficacy assessments[5-7].

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ESSAY -12 : HYPERCOMPLEMENTEMIA

Abstract

Hypercomplementemia HRC was defined.Diagnostic protocols and indications were stated.Local research experience have been showing that hypercomplementemia found in form of single or combined C3,C4 together with extreme hypercomplementemia EHRC.HRC,EHRC were characterized as secondary,non-genetic,infection or inflammation induced.They were reported in; periodontitis,gengivitis,multigravada women on contraceptive with or without misscariage.

Key Words

Combined, C3 ,C4, extreme,hyercomplementemia,single.

Aberrasion, Complement,Combined,hyper and extreme hyper coplementemia.

Introduction

The complement system is formed from fresh vertebrate plasma proteins in agroup of components ranging from 30 up to 50 with their own receptors.These components were appearing in human plasma electroporogram in alpha1 ,alpha 2 ,beta and gamma globulins.Such colocation of complement with immunoglobulin does not mean similarity,it mean differences in biological and immunological characteristics for antibodies.The protein nature of such components was heat labile at

56C for half hour.The activation of complement system established in sequential manner of their own components with marked subnit assembley and functional units.Complement play a role in; phagocytosis,cell lysis, and has fluid and memberane phases and activated through three steps;initiation,amplification and membrane attack.Three activation pathways are known as; classical,properdin and lectin pathways.Properidin work in innate immunity.Classical and lectin in adaptive immunity.Complement action spectrum covers both innate and adaptive immunity.The levels of complement components can be normal,subnormal and abnormal.HIC is being the abnormal leveling in human plasma[1-10]

.

Causal Entities

Infection,inflammation,autoimmune disease,allogenic and xenogenic transplants.

Immune Potentials

The plasma complement components may have an array of immune potentials including; immune cell lysis,opsonization,regulation of immune responses,regulation of inflammatory responses and as a recognition system[2]..

Role In immunopathogenesis

Hypercomplementemia state is inline with the level of disease activity.Complement deposited in immune injured tissue.Complement anabolic and catabolic are associated with certain disease state.Complement induced tissue lesions are rather similar in man and laboratory animals.HRC and HC were noted among number of human pathologies as in ,Table 1

Table – 1 : Complement system aberrasions in huma diseases[2].

Hypocomplementemia[2]	Hyper complementemia [2]
SLE	Nephropathy[2]
Renal disease	Periodontitis[9]
Rheumatoid disease	Gengivitis[8]
Recurrent infection	Multigravada women on contraceptives[10]

Clinical Samplings

Fresh human plasma,fresh tissue scrap,pyogenic materials.

Processings

Program 1 :Blood[6-10]

I -Collect 5ml blood fro the human brachal vein in a blood collecting tube with anticoagulant.

Ii – Centrifuge at 3000 rpm for five minutes..Discard pellete and keep supernate the plasma.

Iii – Load 5ul amounts in to the wells of anti-C3 and anti C4 partigens

iv- incubate at 37C or 25 C for an overnight then read the precipitation zone.

v- Compare the sizes of the ppt zones with the standard reding table of the manufacturer company

vi-determine the normal,subnormal and abnormal levels.

Program 2 : Pyogenic materials

Aspirtes from gum,peridondium and uterine materials and tubbed them individually into centrifuge tubes then;

i-Centerifuge at 5000 rpm for five minutes.

ii-Discard pellets and save supernates

iii- Process the supernates as in blood plasma program.

Findings

C3 and C4 levels in fresh human plasma both in health and disease can be as;normal,subnormal or abnormal levels.Local experience have shown;single and combined HRC of C3 and C4 in gengivitis ,periodontitis, multigravada woment on contraceptives.

Interpretation

Subnormal concentration values of complement C3 and C4 means hpocmoplementemia while abnormal concentration values to 2 folds and more than the normal values means hyercomplementemia.

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PART FOUR : ADAPTIVE IMMUNITY

Section Six: Humoral adaptive Immunity

Essay Thirteen ; Normoglobulinemia

Essay Fourteen; Cryoglobulinemia

Essay Fifteen :Pyroglobulinemia

ESSAY -13 : NORMOGLOBULIN

Abstract

The electrophorogram of fresh human serum showed;prealbumin,albumin and glubulin fractions.Globulin fraction expressed as alpha,beta and gama globulins.Gamma globuline behaves to teperatue as;normoglobulins,cryoglobulins and pyroglobulins.The normoglobulnes concetration levels were of ;subnormal,base line,immune conversion and abnormal limits.Clinically, each of these limits needs an established gain wine test and clear cut end ponit.Tests that are well established in this contex are; agglutination ,precipitation, neutralization,complement fixation[now becomes old fashioned],color formation , color change and radioactive emmision estemation.All these tests and end points holds the position of normoglobulin functions.

Key Words

Abnormal,agglutination,baselins,cryoglobulin,globulin,normoglobulin,pyroglobulin.

1 -Introduction

The serology science depends mainly on the function of; normoglobulin,cryoglobulin and pyroglobulin in health and disease.Hypo-normoglobulin indicated immune defieicency .Hypernormoglobulin will indicate active infection in acute sub-acute and /or chronic state in human beings.The nature of the isotype may gave a clue about the infection state.IgM indicate acute state,IgM-IgGis inline with sub-acute while IgG,IgA may reflects chronic infective state.IgD and IgE may be involved solely or impart with allergy[ ]

2- Inducers

Infection

3- Immune Response:

Human infection in general are either inducing humoral,cellular or both.These responses are acutually a results of the immune conversion state where the ab titre and concentration increased levels than that of the baseline in healthy state controls.Antibodies leveling are being as follows;

I – subnormal;hypoactivity

ii-basline;normal

iii-cutoff ;suspects.

iv-Clinical ; abnormal,hyperactivity.

4-Immunopathogenesis

During human microbial infections shifts from the immune homeostatic state.Virulence associated antigens can cause immune tissue injuries if the host immune system fails to combats the vajor of the pathogen through direct tissue toxicity or moleular mimicry of the microbial antigens with the host self antigens.Since at most the tissue injuries of the host are either caused by the microbe itselve or its toxins or due to both.Hypersensitivity and /or autoimmune as well as granuloma responses might be the expected sequalae to infections[ ].

5- Samplings

Blood ,mucosal materials,dammaged local tissues.

6- Philosophy of Serology

The philosophy of serology reactions laid on the following holdings;

i-The use of standarized antigen to screen unknown antibodies

ii-the use of standarized antibodies to detect an unknown antigen

iii- foundation of negative and positive controls for the test reactions.

7- Common Features of Serology Reactions;

In the functional and experimental senes the reaction between antigen and antibody is known as serologic reaction or antigen-antibody reaction.serologic reaction can be performed in-vitro and in-vivo.All serologic reactions are ensembeled in general manifestation as;

i-Assurance for optimal proportion between the number of antigen units per unite volume with the antibody molecules per unite volume.Disproportion in this balance llead to zonation phenomenon.

ii-Assurance for establishment of an optimal propotion of electrolyte ion concentration in the reaction environment.

iii-The increase in reaction temperature leads to increase in reaction rate within the limits of protein denaturation of the reactants.On running the reaction on the laboratory bench bring the reactants to the to the advised temperature for the test performance.

iv- The nature of the antigen- antibody reaction dynamic through the union of ag-ab can be separated on dilution of any reactants.

v-There is a proportion between the valencey of ab and the valancey of ag.

vi- The extent to which the reactants being sensitive to complement.

vii-Sequence of the steps,precision, and aquarcey of the applied procedure.

8-Serologic reactions End ponits

All of the known chemical,biological,serological and/or immunological reactions manifest an evidence based end ponits

i-Visible clumps.

ii-visible precipitate

iii-color reaction appearance

iv-Change in the reactants color

v- cell lysis.

9- Serology Results Scoring Parameters

There are three basic serologic results scoring parameters as;

i-Titre

ii-Concentration

iii-Complex precipitation

10- Limitations of the serological reactions

Both of the invitro and in-vivo serologic reactions are vulnerable to one or more of the following limitations;

i-Sensitivity limits

ii-Specificty limits

iii-Cross reactivity limits

iv- Presence or abscence of blockers

v-Reactants concentration unbalance."zonation".

vi-Presence or abscence of artifacts.

11-Quality Controls:

In general,quality control means collective scientific means used to detect the source of errors in serology tests used in diagnostic purposes.

11—1;Glossery,

i-True positive;It is the sucess of immune reagent in use for detecting the presence of immune molecules in test system.

ii-True Negative;

It is the abscence of the to be traced immune molecules from the reaction system.

iii- False Positive

It is the presence of the exrernal effectors like the nature of the method used or internal like the presence of cross-reacting antigen in the reaction system.

iv- False Negative

it means the failure of an immune reagent to detect the to be traced immune molecules in the reaction system

11-2;Mathematical Models;

i-Index of Sensitivity

It is the smallest amount or concentration of immune molecules possible to be detected by the standard test antigen in the test patients serum and sxpressed as;

Sen Index =(True positive-false negative)/true positive.

ii-Index of Specificity

It isthe estimation % of an antigen reaction affinity with its own specific antibody

Sp.Index =(true negative- false positive)/ true negative

iii-Diagnostic Specificity index

It represents the number of normal individuals whom expressed abnormal results when tested with the assigned testv system,

D.sp.= true negative/(true negative +false positive).

iv-Diagnostic Sensitivity index

It represents the abnormal results of patients undiscovered by the immune test system.

It is evident that both of Se.I and D.sp. indicies discovers impart the possible interpretation of the results before you know patient case.

D sen.= True positive/(true positive+false negative)

12-Agglutination Tests

Agglutination described as secondary serodiagnostic tool.It is the reaction of a partculate antigen with its on specific antibody under standarised reaction conditions and following the common features of serologic reactions.There some serologic phenomena that may accompanyed this reaction as;cross-agglutination,masking effect, autoagglutination ,non- specific agglutination and zonation .The types of agglutination tests are; direct microbial agglutination, microbial hem-agglutuination,passive hem-agglutination,iso-hemagglutination,latex agglutination and cold agglutination.The possible applications of these agglutination types are; microbial serodiagnosis,microbial infection serodiagnosis ,blood grouping,pregnancy test,C-reactive protein,infectious mononucleosis and atypical pneumonia.The agglutination test can be performed in qualitative, direct slide test,semi-quantitative, microtitration method and quantitative,the standard tube method. In direct slide agglutination,adrop of serum dropped onto clean glass slide,the adrop of standard antigen dropped on to the slide near the serum drop , mix up the two drops and wait for 1-2 minutes to watch the appearance of clumps size and naure.In semi-quantittaive slide agglutination;a slide of several wells are to be tempted,various size drops made of the patients serum,then fixed volume of the standard antigen added to these drops,mixed and wait for up to two minutes for the appearance of clumps,record at which size of serum, clumping happened.In tube agglutination test,adecimal double dilution method used for serum dilution in tubes then fixed equavilant size of a standard anitgen added.Incubate for overnight at 37C ,then read the titre"the reciprocal of the highest dilution that gave positive results.In tube agglutination test watch for;incubation temperature and time,dilution trend and percentage of the reactants .The results profile may be;positive ,negative,false positive and false negative results

13- Precipitation Test

It is described as secodary serologic reaction.It is a reaction between soluble antigen with its specific antibody under standard conditions of the reaction and following the common features of the serology reactions.Two forms of precipitation reaction are known as;

i-The reactants diffuse in aliquid or gelly reaction medium and when optimal proportion of reactants reached precipitation happened.

ii- One of the reactants diffuse in an electric field the other reactant added and allowed to diffuse in same medium under the effect of the electric field.When the optimal proportion between the reactants reached precipitation hapened as an archs

Precipitation own wide range of application profile in immunology laboratory as;

i-Study the antibody levels and antibody isotype nature in patients in question.

ii-Epidemiologic investigation to an unknown pathogen specific antibodies using standard pathogen antigen.

iii-Epidemiologic investigation to an unknown pathogen specific antigen using stadarized pathogen specific antibodies.

iv- Determination of microbial toxin identity.

v- Determination of number of antigen-antibody system in the reaction medium

vi-Microbial immune classification systems.

There are a variety of precipitation tests known and avialable in practice as; ring test,immunodiffusion in gel,capsular swelling test and immunoelectrophoriesis.The end point is a ring form at the interface between the antigen antibody system.Precipitation zone in radial immunodiffusion in gel read as zone size and arch size,location area and thickness in immunoelectrophoriesis tests.

14- Neutralization Test

Itb is a serologic test betwen at most cases a soluble antigen with its specific antibody of a toxin or a virus in-vivo.Or bioactivity of toxin or virus in-vivo.The mechanisms of neutralization test are larglly unclear but interpretated as follows

I-Antibody unite with the catalytic pathway of the antigen.

ii-Unite with toxic site of the antigen molecule.

iii-Antibody unite with other sites of antigen molecule causing steric hindrance which in turn impeds reaction with the substrate

iv-Antibiody rune the antigen structure which lead toimpairment to its reaction with target tissue.

v-Antibody unite with the antigen facilitate its phagocytosis.

The application of neutralization tests in

a-Identification of toxin in toxin induced human microbial infection both in-vitro and in-vivo.

b-Identification of virus in an ex-vivo and in-vivo test systems

The invitro neutralization tests are ;Nagler's reaction,Elek's test,hemolysis immune inhibition,ASOT,virus neutralization and quantitative plaque reduction.While the invivo are;mouse protection test,Schick's test,Dick's test and Schultz-Charlton reaction.

14-1: Mouse Protection Test

This test is an in-vivo application of neutralization test.It is of use in toxin identification in Clostrida,diphtheria and plague in mice.The cell free culture filterate of the toxin with antisera and incubated for half an hour at room temperature then, the mixture injected into mice as in the following schdeule;

Group One;Cell free culture filterate alon

Group Two: The incubate Cell free culture filterate-toxin mixture

Group Three;Saline control

Score:

Death of mice in the groups one and two means the toxin in th filterate is not specific to the anti-toxine used.Death only in group one and live in group two and three means tha t the antitoxin is specific with toxin in the flterate

14-2: Nagler's Tests

On egg yolk agar plate;anti-toxin spread onto half of the plate and the other half left as such.Make two streaks one on the toxin applied half and the other on that without anti-toxin.Incubate the plate at 37C for an over-night.Score of the test be;abscence of opalescence in the toxin applied half means that the anti-toxin neutralize the toxin,and it is specific to it.

15- Complement Fixation Test

The formation of immune complexes[antigen-antibody complexes] in solution can be monitored by the ability of these complexes to consume complement

proteins.Complement fixation test CF assay are widly used to detect immune responses to infectious agents.This test is so sensitive and depends on; antigen-antibody complex standard complement added and sheep antisheep red cell indicator system.T he test occur in two steps as;

i- Equavilant amounts of ab-ag mixed and incubated then standarized volume of fresh complement added and incubated.
ii- Addition of antibody sensitized sheep red cells and incubated

Tube	Antigen	Antibody	complement	Sensitized sheep red cells	Results
1	+	+	+	-	+
2	-	+	+	+	-
3	+	-	+	+	-

Interpretation

1-The complement attachs to the antigen –antibody complex and get fixed and no more avilable free to reacts with indicator system and the score is positive CFT

2-The complement is free avialable so it is not consumed by the complexes and reacts with the indicator system.The score is negative CFT

3- Antigen is alone and no complex formed ,hence complement is free to combine with the indicator system.The score is negative CFT

4-Addtion of more complement leads to erronus CFT results.

Note: Currently in practice CFT is replaced by Eliza test.

16- Eliza Test:

When an antigen...any antigen or antibody is covalently coupled to an enzyme,it may be quantified by determining the color reaction with spectrophotometery that at which the enzyme convert a clear substrate to colored product.The most commonly used technique is the sandawich essay uses two different epitopes on the same antigen whose concentration need to be determined.A fixed quantity of one antibody is attached to a series of replicate solid support such as a plastic microtitration wells.The test solution containing the antigen at an unknown concentration.Or aseries of standard solutions with known concentration of an antigen are added to the well and allowed to bind.The remaining unbound antigen removed by washing and a second antibody which enzyme linked is allowed to bind.The antigen serve as bridge,so the more antigen in the test or standard solution are used ,the enzyme liked

to second antibody will bind.The results from the standard solution are used to construct a binding curve for the second antibody as a function of antigen concentration from which the quantities of antigen in the test solution may be infered.The bsic steps of ALIZA test are as in the followings;

i-Bind first antibody to the wells of microtitration plate.

ii-Add a varying concentration of the antigen,let it to bind.

iii-Remove the unbound antigen by washing.

iv-Add labeled second antibody specific to the non-overlapping epitope of the antigen,and let it to bind.

v-Revove the unbound labeled by second wash.

vi-Determine the amount of bound second antibody as a function of the antigen added.

vii-Read the color change.

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ESSAY - 14 : CRYOGLOBULINEMIA

[ICD-10-CM Diagnostic Code D89.9 cryoglobulin]

Abstract

Cryoglobulinemia can be classified as primary (genetic) or secondary (acquired), with the latter being the focus of this discussion. Cryoglobulins are categorized into systemic and mucosal types. Immunofixation studies have revealed that systemic cryoglobulins can exhibit either a single isotype or a mixed variant isotype. However, mucosal cryoglobulins were found to be non-fixable using commercial antisystemic cryoglobulin reagents, likely due to the presence of a secretory component unique to mucosal cryoglobulins.

Patients with cryoglobulinemia showed significantly higher cryoglobulin concentrations compared to control subjects. This finding suggests an immune conversion, where baseline cryoglobulin levels escalate to clinically significant concentrations, consistent with an immune response characteristic of cryoglobulinemia.~~Cryoglobulinemia is of primary genetic and secondary aquired.secondary cryoglobulinemia is being tackeled.Cryoglobulin was of systemic and mucosal type.Immunofixation studies has shown that systemic types showed single or mixed variant isotype.Though mucosal type was being non fixable by commercial antisystemic cryoglobulin.This may be attributable to the presence of secretory piece of the mucosal cryoglobulin.The concentration of cryoglobulin in patients were higher than that of control subjects.A finding indicating initiation of an immune conversion from base line concentration to clinical significant concentration in line with an immune response of cryoglobulin type~~[1].

Introduction

Exposure to coldness may leave an un-wanted immune consequences among some vulnerable persons.This may be due to the presence of clod types of immunoproteins known as cryoglubulin which may be precipitated in vessels in skin,joints, peripheral nerves and kidneys.Cryoglobulin exhibits pathogenic potentials[Kolopp-Sarada And Mossee 2018[2].

Inducer

Presumption 1:

Patient's effector B cells synthesize and produce normoglobulin but such secreted normoglobulin undergoes steric modifications of the molecule with expostion of non-polar residua reduction of solubility parallels with non-covalent interactions leading to evolvment of cryoglobulin precipitation.

Presumption 2 :

There are cryo potent effector B cells that can be triggered by a cryopotent antigenic epitope to mount a cryoglobulin humoral responses[1].

Presumption 3 :

There was an abnormal effector B cells associated with cryoglobulin response, but such association does not indicate a causal relationship to the disease state[3].

Immune Responses

It is rather a stepwise events in which cryopotent antigenic epitopes builtin in an antigen[infection,vaccine] may be taken up by macrophages ,processd,expressed onto macrophage membrane in association with-MHC molecules then presented to Th2 cells.Th2 cells in turn activate naive B cells to be an effector B cell[supposed to be abnormal] to produce cryoglobulin[1].

Immunopathogenesis:

Such cryoglobuiln humoral responses may initiate an immune tissue injuries following formation of complexes with complement fractions in blood vessel bedding in one or more of the following tissue prediliction sites as; skin,joints,peripheral nerves and increased in kidneys.Hence, the cryopotent immunogenic epitopes may have;pneumogenic ,nephritogenic ,lymphogenic and /or granulomatogenic responses[4]leading to an immune mediated immune tissue injuries in these organ compartments.The suggested immunopathogenic mechanisms of cryoglobulins is associated with B cell lymphoproliferation,autoantibody production,rheumatoid factor activity,cryoglobulin-complement complexes beside the ineffective cryoglobulin clearance by macrophages or monocytes[5].There were evidences that B cells circulating in HCV infected patients associated with mixed cryoglobulin are profoundly abnormal with low expression of CD21 but such abnormal B cells were not correlated with neither cryoglobulin nor with vasulitis relapse[3].

Diseases

Cryoglobulins are associated with human immunoproliferative diseases,immune mediated diseases and intracellular microbial infections both as a pathogen and as a marker as well as postvaccination and as amarker of ageing[6].Secondary mixed cryoglobulinemia were traced in;pulmonary tuberculosis ,brucellosis ,typhoid, tonsillititis ,periodontitis and gengivitis[1].Cryoglobulin in these cases may precipitate in blood vessels causing vasculitis and /or occlusion of the affected blood vessels[7].Experience in laboratory animals cryoglobulins proved to be pathogenic in murine model causing pneumogenic ,nephritiogenic and granulomatogenic effects with an induction of cytokine network[8,9,10].While in lapin model,cryoglobulin using typhoid antigens as inducer,they cause;pneumogenic,nephritogenic and lymphogenic effects[11].Secodary cryoglobulin in rabbits modes post to BCG vaccination[12] and post to brucella vaccination[13].Cryoglobulin classification, pathogenesis and disease were presented in Table – 1.infectious and vaccine induced secondaery cryoglobulinemia are presented in Table – 2.

Table – 1 : Classification of Human Cryoglobulinemias[3].

Cryoglobulin Type	Clinical signs	Disease
Type I,monoclonal;IgM, IgG	Cutaneous ;purura,acrocyanosis,necrosis,ulcer ,livido reticularis Extracutaneous;peripheral neuropathy,renal and joint involvment\ Systemic vasulitis	MGUS,SM,Waldinstrom macroglobulinemia Multiple myeloma
Type II,monoclonal and polyclonal	Monoclonal,cutaneous,Meltzers Triad Polyclonal, Extracutaneous;renal disease,glomeulonephritis Neuropathy,intestinal ischemia,alveolar hemrrhage, CNS involvment, myocardial failure	Infection Multiple myeloma Non-infection;RA,LES ,B cell lymphoma NHL Soild tumors
Type III,polyclonal	Cutaneous Extracutaneous;peripheral neuropathy,renal and joint involvment	Infectious Endocarditis,spirochetes,fungal infections,parasitosis Non-infectious;LES,Ra,intestin

		al disease,biliary cirrhosis ,solid tumors

Table – 2 : Infections and Vaccine induced secondary cryoglobulinemia

Infectious Diseases	Test subjects	References
Tuberculosis	Human &lapin	[14,12]
Typhoid	Human& Lapin	[11,15]
Brucella	Human & lapin	[16,13]
Tonsillititis	Human	[17]
Peridontitis	Human	[18]

Clinical Samplings

Blood ,urine, periodontal,gum, and tonsiler materials.

Processing

Note ;

Stepes in the programes 1 , 2 & 3 should take in consideration the 14 standards recommonded by Lynch [19].Temperature in;tubes,reagents,manuplations should be made upon reaching 37C,then processing statrts.

Program - 1.

i-Collect human blood from brachial vein and let it coagulated and save sera.

ii- Load 2 mL of patient and control sera into cryoglobulin tubes and store them in an upright position in a tube rack~~Load 2ml amounts of patients and control sera to cryoglobulin tube and keep them up right position in a tube rack.~~

iii-Incubate at 4C for 1-5 days

iv- Watch the appearance of precipitate in the loaded tubes,describe the texture of the precipitate

v-Centrifuge at for in cooling centerfuge discard supernate and keep the precipitate,wash twice the ppt with cold sterile saline.

vi- Complete the pellete with formal normal saline 0.5% at 37C to the original volume of the sera.

vii-Divide the volume into two tubes one incubate at 37C and the other at 45C for up to 15 minutes

iix-Make biurt test for identification and concentration

ix-Supply low level partigen of IgM,IgG,IgA and looad them with 5ul for each plate and incubate at 4C for overnight.Determine the isotype.

Program – 2.Urine Sample

i-Collect human urine under aseptic condition

ii-Allocate and centerfuge 5ml. at 5000rpm for 10 minutes.Discard pellet and keep supernate

iii-Aspirate 4 ml urine superante and add 4ml PEG 6000 6% solution.

iv- Incubate for 1 hr up to overnight at 4C at refregerator.

v-Centerifuge at 5000rpm for 15 minutes.Discard supernate and keep pellet.

vi-Suspend the pellete with 4ml formal normal saline 0.5% and dissolve at 37C

vii-Load 2 ml of the urine globulin solution in cryoglobulin tubes in an upright position in a tube rack and incubate atv 4C for 1 t0 5 days.Watch the texture of the ppt. And calculate cryocrit%.

iix-Centerifuge in cooling centrifuge at 3000 rpm for 5 minutes.Discard supernate and keep pellete.Wash twice with 0.5% sterile formal normal saline.

ix-Suspend the washed pellet with 4 ml saline.Dissolve at 37C for 15 minutes

x-Make biurt test for protein identity and concentration

xi-Load 5 ul of this cryog. Into low level IgM,IgG,IgA partigens and incubate at 4 C for fixation of isotype

xii- React with RF,CRP and complement.

Program –3 Pyogenic Materials[20]

i - Pyogenic Materials from gum peridontium, and tonsiles were completed up to 2ml. by sterile saline,vortox mixed and cennterifyged at 5000rpm for 10 minutes.

ii-Discard the ppt and keep the supernates,the process them as in urine program.

Findings

Cryoglobulin characteristics;texture, cryocrit %, concentration ,isotype will be affixed as;

i-Precipitation at 4C

ii-Dissolution at 37 & 45C

iii-Texture nature

iv- Biurt Result,concentration levels

v- Single or mixed variant isotype

vii- Systemic or mucosal cryoglobulin

viii-Typable or untypable

Interpretation

Cryoglobulin concentrations in patients be higher than in normal subjects.It may be due; i - to the fact of immune conversion , ii- may indicate a pathologic role interplayed by the cryoglobulin.Untypability of mucosal cryoglobulin can be attributed to the presence of secretory pecies in the mucosal variant of cryoglobulin ,iii- it can be due to minimal concentrations below the sensitivity limits of the commercial antiglobulin plates since they are suited for serum cryoglobulins[1].

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ESSAY - 15 : PYROGLOBULINEMIA

Abstract

Single or combined monoclonal IgM,IgG,IgA ,IgD pyroglobulin isotypes are associated with lymphoproliferative diseases,leukemia,macroglobulinemia,ageing and arthropathy.

Introduction

The human gamma globulin ir ramified in to three variants as;normoglobulin,cryogolbulin and pyroglobulin.Normoglobulin concentration levels determines its role in pathogenesis of human diseases.Cryoglobulin is an abnormal globulin associate with pathogensis of human diseases.Pyroglobulin is rarely involved in in human pathology.

Inducer

There were two presumptions as;

1-During synthesis and secretion of normoglobulin noncovenlent intermolecular reactions happened[1].

2- There were either exogenus or endogenus pyropotent antigenic epitope capable for inducing an immune response[2].

Immune Response

Humoral immune responses specific to the pyropotent antigenic epitope.

Immunopathogeneses

Association or coexistence of pyroglobulin may or may not have any pathologic bearing. The suggested immunopathogenesis of pyroglobulinemia may be as;the antigen containig the pyropotent epitope processed and presented to Th2 lymphocyte.The activated Th2 activate neive B cell to grow,proliferate,expand and diferentiated to either effector pyropotent B cell or memory pyro active B cells.The effector pyropotent B cell scerete,pyroglobulin that might be of the potential for inducing immune tissue injuries.

Diseases

It is an atypical gamma globulin characteized by their an irreversable denaturation at 56C.PH below 3 or above 9 inhibits pyroprecipitation.The presence of pyroprotein is the cause of pyroglobulinemia.Pyroglobulin precipitate bind complemet,reacts with rheumatoid factor,produce cutaneous anaphylaxis ,generalized passive anaphylaxis and passive arthus phenomena[Umass Profile].Pyroglobulin isotypes were IgM,IgG,IgA,IgD.It was reported in lymphoproliferative disease,autoimmune diseases,beta thalasemia and ageing,Table- 1.

Table-1 : pyroglobuline pathology timleline.

Acheivement	Immune Features	References
Coining the term pyroglobulin	Blood serum contians heat coagulable globulin,have high degree of correlation with multiple myeloma	[2]
IgA pyroglobulinemia	Coaggulation abnormality,hyperviscosity in multiple myeloma	[1]
IgG(L) pyroglobulin	Plasma cell leukemia with large amounts of IgA and IgG myeloma protein.Two	[3]

	pyroglobuli variants	
IgMlambda yroglobulin	Waldenstrom syndrome,heavy and light chain IgM	[4]
Single monoclonal IgM,IgG,IgA pyroglobulin	Cause no clinical symptome	[5]
Monoclonal IgM pyroglobulinemia	Wadenstrom macroglobulinemia	[7]
IgD Plasma cell leukemia Pyroglobulin coexisted with cryoglobulin	Urine and serum two varaints of IgD pyroglobulins Pyroglobulinemic arthopathy	[8] [2]

Processing

The processing of clinical samples can be as fallows;

Program – 1:

i-Collect blood from the pateint,let it to clot and save serum

ii – The test serum divided into two portions one incubated at 56C for 30 minute.The other portion incubated at 60C for 30 minutes and watch the appearance of precipitates in both cases

iii-Adjust the PH of the sera one at 3 and the other at 9 and incubated for a while the watch the dissolution of the precipitates at both of the cases.

iv-React solubilized pyroglobulin portions of the serum with complement, Acute phase proteins, and rheumatoid factors and report the reaction profiles

.

Program – 2:

I - Pyroglobulin fractionation with sephadex G200 column

ii-Ultracentfugation

iii- Turbidity study of heated pyroglobulin with spectrophotometer at 400nm .

iv – Watch the number of peaks.[3].

V – Determine the viscosity{6,8].

Program – 3

1 - Perform bone marrow and the affected lymph node biopsies for the patient.

Ii – Supply a select of lymphocyte CD markers

iii-Set the flow cytometery tool to uncover the nature of the lymphocyte abnormality[6].

Findings

Single or combined monoclonal IgM,IgG,IgA,IgD pyroglobulin isotypes may be expectable defined the lymphocyte abnormality from the flow cytometery studies..

Interpretation

Pyroacting B cell clones may present within the components of patients immune system that can be stimulated to produce either /or IgM,IgG,IgA,IgD pyroglobulin isotypes. Pyroglobulin pathological responses have been reported in plasma cell leukemia ,betathalasemia ,oteoclast myeloma,ageing and arthropathy.

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PART FIVE : ADAPTIVE IMMUNE RESPONSES

Section Seven : Humoral And Cellular Immunity

Essay Sixteen : Immune Cells

Essay Seventeen :Cytokines

ESSAY - 16 : IMMUNE CELL

Abstract

Structural and functional aspects of the immune cells are important in the biology of immune responses.Macrophage,B and T cells are the basic players in the cellular events of the immune responses.Neutrophils and macrophages performed the phagocytic function.Macrophages are mainly take up,process and present antigens .B cells, however,may act as an antigen processing and presenting antigens to the helper cells.helper cells in turn activates either naive B or naive T to be either effector or memory lymphocytes.Activated effector B cells produce antibodies and cytokines.Activated effector T cells produce cytkines.Programs for ;NBT pahagcytosis,e-rosette T cell counting,leukocyte inhibitory factors for T cell cytokines and skine delayed type hypersensitivity are being texted.

Key Words: Activated, antigen, B cells, effector ,DTH,immune,memory,response.

1-Introduction

Natural innate and adaptive immune cells are developed within the bone marrow from the differentiation of stem cells.Stem cells are differentiated by the action of cytokines into lymphoid progenitor that developed into primary lymphoid cells ,myeloid progenitors that developed into granulocytes and lymphomeyloid progenitors that developed into monococyte series of cells.Granulocytes take part in the process of phagocytosis which is actually innate immune response.Monocyte series,the macrophages are taking part in immune ross-road responses,e.i.function both in innate and adaptive immune responses.B and T lymphocytes are the main players in the adaptive immune responses.Antigenic stimulation may activate naive B to be an effector antibody and cytokine producing or memory cells.Naive T cells when, activated to be either effector T cells producing cytokines or memory T cells.Memory B and memory T cells are functioning in recall responses on second exposure to an antigen and helpful in boosting vaccination programs.

2-Phagocytosis

2-1:introduction

Phagocytes functioonally can be subdivided into professional and non-professional phagocytes.The professional are;Neutrophil, macrophages and dendritic cells.While the non-professional are epithelial cells and lymphocytes.Phagocytes perfom the act of phagocytosis and stands as the first and second linears of the immune defense mechanisms. They engulf ,endocytose and digest the invadors.Phagocyte recognition be TLR mediated as it recognized the pathogen associated molecular patterns on the microbial surfaces. phagocytosis is a step wise process starts with chemotaxis, opsonization, phagosome and phagoclysosome formation and digestion.It can be demonstrated practically by either dye uptake test or by microbial phagocytosis.The inducer is either infectious invasion or inflammatory injury.It constitute non-specific cellular immune responses.The clinical samples are;blood,mucosal scrap,body fluid aspirates.The dey uptake test is as follows

i-From blood with anticoagulant,transfer 0.05 ml. into centrifuge tube

ii-Add 0.05 ml.of NBT dey solution to the blood and mix.

iii-Incubate at 37C for 15 minutes

iv-Mix well and transfer a drop onto a surface of clean glass slide.

v- by the end of theother slide spread the blood-dye mixture allover the surface of the slide,dry in air and fix with ethanol for 3 minutes.

vi- Flood the film with Geimsa stain for 1hr the wash with buffer saline for 30 sec. Dry in air and examine under100x oil immersion lense.

vii-Score 100 leukocytes and claculate those having the Formazan stipplings as a percntage to 100

% of dye up take phagocytosis = (Number of cells with stipplings)/(Number of nonstippling and number of stippling cells) x 100

Levelings

Normal = 6-7 %

Subnormal below 6%

Abnormal more than 7%

3-Lymphocyte Viable Counts

One of the cellular immune functional levels of lymphocyte is by the determination of viable lymphocyte counts.Since the more viable the lmphocyte the more immune potent individuals.The test layed down on the fact that viable lymphocyte does not take vital dyes.Whil dead lymphocytes take up vital dyes like trypan blue,eosine,and negrosine.To establish this count on should count 200 lymphocytes covering both stained and non-stained cells.Transparentsmaal cells are the viable while large colord cells are the dead.The program is as follows;

i-Bring animal lymph node,defatinate it in a bathing nutritive solution in a petri-plate.

ii-Macerate by scalpel and forcep the lymph node tissue so that the nutritive solution become turbed by the dueling cells.

iii-Collect the swimming cells as suspended form within the nutritive medium in a centrifuge tube.

iv-Centrifge at 3000rpm for 5 minutes,descard the supernate and keep the pellet.Wash twice at sam speed and time in sterile normal saline

v-In other test tube mix one drop of cells with one drop of the dye.

vii-Transfer a drop from cell-dey mixture to Neuber and Thoma slide

iix-Calculate the transparent small cells in 200 lymphocytes.

ix- Apply the following mathematical formula;

Number of Viable lymphocyte =(Number of unstaied cell)/ 200 conted cells x 100.

4- T cell E-rosette Counts

The presence of CD2 in T cells is forming the basic principle of E –rosette counts.The test is performed as in the followings

i-Add 3 ml of lymphoprep solution in to centrifuge tube.On the lateral side of the tube slide gently 1.5 to 5 mls of heparinized blood without mixing.

ii-Centrifuge at 400 g for 30 minutes at room temperature.Buffy coat layer formed at the cell-plasma interface.

iii-By Pasteur pippet aspirate carfully the buffy coat layer of the cells into a centrifuge tube.

iv-Centrifuge at 400g for 30 minutes at room temp.Keep th pellet and discard the supernate.

v-Resupend the pellets with 0.5ml. sterile saline and add 0.05 autologus plasma to the supension.

vi-Mix 0.1ml of lymphocyte-autologus plasma mixture with 0.1 of washed sheep red cells in a test tube.

vii-Incubate at 37C for 15 minutes ,then centrifuge at 200 g for 15 minutes.

Iix-Re-incubate the mixture at 4C for 1 hr with supernate still on pellet.

ix-Resuspend tube gently forward and backward up to 2 to 3 times

x- Make blood smears,fix it with ethanol for 10m minutes , dry in air and stain with Geimsa.

xi- Calculate 100 lymphocytes and measure y those forming roses with speep red cells.

5-Leukocyte Inhibitory Factor LIF;

The white blood cells that are taken from patients suffering from intracellular microbial infection do contained macrophages bearing epitopes that can stimulate the cell mediated immunity and delayed type hypersensitivity reactions assesited by cytokines .Th apparent clinical gross reaction may be delayed type hypersensitivity and cellular reaction levels as leukocyte migration inhibitory factors.The test made by two methods as i- A drop onto agarose gel surface and ii- capillary method.Clinical samplings are either blood and /or mucosal scrap or body fluid aspirates.The method was as follows;

i-By heparinated capillary tube draw blood from blood collecting tube containing patients blood.

Ii-Close the cellular part end with plasticine

iii-Centrifuge in hematocrit centrifuge for minutes

iv-Two layers are formed, the upper plasma and lower cellular contents,the interface the buffy whit cell layer,cut the capillary at the buffy coat layer.

v- In an agar gel petri-plates make 15mm diameter wells for test and control ,the affix the cutted capillaries in the well so that the buffy coat are free to lumen of the wells.

vii- add 50 ul of the nutritive medium and 50ul of sensitizor and keep the plate in upright positions in a jar with humid moisture.

Iix-Put the jar containg the test plates in the incubator for 24 hrs at 37C.

ix-Read the LIF% as in the following formula

LIF% =(migration diameter with sensituizor)/ (migration diameter without sensitizor)x100

Inhibition of 30% or more is significant.

6-Skin DTH Test;

The skin constitute complete system covering human and mammalian body internal organs and it functions as;mechanical barriers against infection and injuries,ii-secret unwanted body secretions,iii- forms the nonspecific immune barrier and iv- serves as a model for testing allergy and microbial permeability factors.The criteria made skin suitable for test materials includes; i- large surface area ,i-devoid from keratin,iii-clear after hair deplition or the under side of the tail or sheep tailing flap.Skin allergy tests served several applications as diagnosis of intracelular microbial nfection inducing DTH lile tuberculin test.The principle of immune skin tests are;neutralization,immediate type hypersensitivity as arthus reaction and passive cutanious anaphylaxis and DTH.The skin delayed type hypersensitivity tests can be as follows;

i-Inject 0.5 ml of freund complete adjuvant in thigh muscle of gunia pig

ii-leave for three weeks.

iii-Inject 0.1 ml tuberculin PPD intradermal i skin hair deplited area

iv-observe the skin injected area at; 4,8,12,18,24 and 48 hrs post injection for; erythema,induration,necrosis,swelling and edema.

v-Score the size of induration area to the nearest mm.Areas below 6 mm negative, 10 mm and more are positive.

7- Immune Diagnostic Tip

Classical capillary and agar drop technique and intraperitoneal injection method for MIF are recently substituted by Eliza MIF determination and the classical e - rosset testing substituted by flow cytometric analysis which gave more idea about nature of the cellular identity and extent of inviolvment in the immune status of the case.

8-Conclusion

Both invitro and invivo cellular immune tests are being presented.Up dates also were mentioned.

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ESSAY - 17 : CYTOKINES

Abstract

Cytokines are low molecular weight secretory regulator proteins stands a chemical signal language.They are produced by nucleated cells.The profiles of cytokine responses were found as valid biomarkers both for infection and vaccination.Macrophage migration inhibitory factor,IL2,IL4 desicriminate betwen padiatric BCG vaccinee and non- vaccinee controls.IL12P40 delineate between primary and and chronic oldpulmonary tuberculosis in adulthood.There were and ge dependent variation in IL12P40 levels in old pulmonary tuberculosis but not in primary pulmonary tuberculosis.

Key Words:Cytokine,infection, primary,pulmonary,response,tuberculosis,vaccinee.

1-Introduction;

Cytokines are secreted regulator proteins ,Table -1 ,that ghave effects on hemopioetic non-hemopoietic cells.They are synthesized and secreted by nucleated cells like leukocytes, epithelial and adpose cells.Cytokines are including;interleukines,chemkines,tumor necrosis factors,growth factors and interferons.The boarder of cytokine world are ill defined and rather unstable.The basic functional features of cytokine actions is the signal language fashion.Cytokines can be classified in accordance with,structure,function,capacity to interact with different classes of receptors,Table -2,&3[1].

Table – 1 : Regulatory secretd proteins

Features	Cytokines	Growth factors	hormones
Chemical nature	Protein	Protein	Protein
Function	Regulator	Regulator	Regulator
Source	All nucleated cells	Special cell and tissue	Special cells and tissues
Mode of secretion	Constitutive and inductive	constitutive	constitutive
Site of action	Short distant,short duration time	Distant site of action	Distant site of action
Reaction nature	Pleotropic	Restricted to certain cell type	Restricted to certain cell type
Transmission way	Signal transduction	Signal transduction	Through out blood

Table – 2:Functional classification of cytokines

Class	Example
Hemopoietic	IL5,GM-SCF,SCF
Natural innate immunity	INFalpha,INF beta,IL1,IL8
Specific adaptive immunity	IL2,IL4,IL15
Inflammatory	IL1,IL8,IL6
Anti-inflammatory/immunosuppressive	IL1ra,IL10,TGFB

Table – 3 :Shared or boarder-line cytokine functions

Cytokines	Shared function
INFgamma,IL18,IL12	Innate and adaptive immunity
MIF alpha	Growth factoe and inhibitory
IL6	Innate,and Growth factor
IL13	Adaptive and inhibitory

2- Inducers:

Regulatory constitutive,infection, vaccination.

3-Cytokine Responses :

Natural innate,cross-road,and adptive cytokine responses.Macrophages,B ,T,epithelial and adipose cells on stimulation they may synthesize and produce cytokines.

4- Immunopathogenesis;

Excessive cytkine production during inflammatory or immune responses are implicated in cytokine storm formation,and DTH immunopathology in the form of immune tissue injury.

5- Diseases

Microbial and metabolic diseases

6- Clinical Sampling;

Blood

7-Processings;

7-1 :Program One : infection

7-1-1: Patients

Twenty four primary pulmonary tuberculosis patients,22 chronic old pulmonary tuberculosis pateints and ten normal control subjects were the test and control groups.Blood samples were collected from the test vand controls into blood collecting tubes wsithout anticoagulant.Sera were saved at -18 till use.These patients sera were processed with IL12P40 cytokine following the instruction of the manufacturing company[2].

7-2;Program Two;Vaccination

7-2 -1: Vaccinee Subjects,

A total of 30 BCG vaccinee childs with age range of 1up to 10 years old and 30 normal non-vaccinee child subjects were the test and control groups.Blood wer collected into blood collecting tubes with out anticoagulant.Sera were saved at -18C till use.These sera were processed for MIF,IL2, IL4 cytokines assessment by eliza kets following the manufacturer instruction.

7-3; Eliza Technique

The basic steps of eliza test are similar in all makes, using sandwech tecnique but the details are texted throughly by the manfacturing company and be birefed as follows;

i-Add pateints,vaccinne subject and control sera that may contain the specific cytokine to the well containing precoated anticytokine onto the well surface.

ii-Add biotinylated anticytokine to the reaction mixture in the well, the second added antibody bind by the addition of strepto varidinHRP after incubation unbound strepto varidinHRP washed with washing solution

iii-Substrate solution added,color change happened in accordance with concentration of the cytokine in the test sera.

iv- The acidic stop solution added to stop the reaction .

v- Use the microtitration plate reader to match the absorbance at 450 nm.

80-Findings;

8-1:Infection

Primary pulmonary tuberculosis has shown higher concentration than old pulmonary tuberculosis.Both of the disease forms and the overall were higher than the controls.Old pulmonary tuberculosis was slight higher than the controls.The age range has shown influence on the IL12P40 cytokine levels.Chronicity affect decrease in this cytokine levels.In primary pulmonary tuberculosis there were neither stage dependent nor age dependent variation in this cytokine levels.,Table- 4.

Table- 4 :IL12P40 cytokine concentration mean among different forms of pulmonary tuberculosis

Disease Entity	IL12P40 concentration means in pg/ml.[2]
PPTB	475.95
OPTB	232.34
Overall PTB	395.4
Old sputum shedder	341.28
Old sputum non-shedder	201.86
Controls	224

8-2; BCG Vaccinee childs

BCG vaccinee childs of 1-10 years old have shown increase in IL4,IL2 and MIF cytokine concentration means than the controls.T cell cytokine MIF ,IL2 were higher than B cell cytokine IL4 as compared to normal concentration values.

Table – 5 ; Cytoiknes profile of chidhood BCG vaccinee.

Test Subjects	IL4 conc. Mean pg/ml.	IL2 conc.mean,pg/ml.	MIF conc.meas ,pg/ml.[3]

BCG vaccinee	356.727	931.801	11.062
Controls	224.281	548.884	6.459

9- Interpretations

9-1 : Infection

The IL12P40 assessment can be useful as a biomarker for the disease delination bewen primary and old pulmonary tuberculosis,and bewteen old shedder and old non-shedders.

9-2; BCG vaccinee;

BCG vaccination of chidren induce an increase in concentration means of IL4,IL2 and MIF cytokines than in non-vaccinee controls.this indicates thast humoral ,cellular and DTH responses are involved in pos-BCG vaccination responses[3].

10-Immunodiagnostic Tips;

The concentration means of IL4,IL2 and MIF delineate vaccinee from nonvaccinee childs.IL12P40 cytoikine delineate between primary and chronic old pulmonary tuberculosis.These cytokine are of use as biomarkers of infection and vaccination of tuberculosis.The same results trend may be expected in cytokine responses of other intracellular infectious microbial diseases[4].

11- Conclusions

Cytokine responses are valid in diagnosis of infection state and and postvaccination state.They are suggested in other intracellular microbial disease as infection and as vaccination.

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PART FOUR ; ADAPTIVE IMMUNITY

Section Eight : Bacteriosis

Essay Eighteen : Typhoid

Essay Ninteen :Tuberculosis

Essay Twenty :Brucellosis

ESSAY 18 : TYPHOID

Abstract

Mucosal globulin both in the gut and urinary tractwere tempted todiagnose typhoid.Cellular immunity was matched by gut mucosal and systemic leukocyte inhibitory cytokines.Urinary and gut mucosal globulin as well as leukocyte inhibitory factors were tempted as a battery and found to be clinically meaningful in typhoid patients.Thus S .typhi initiate mucosal and systemic humoral and cellular immune responses.outer membrane protein antigenOMP was better for diagnostic purpose than anti-0 and anti-H of Salmonella typhi.

Key Words:

Antibody,antigen,gut , leukocyte , LIF,mucosal, OMP.

1-Introduction;

Typhoid is febrile communicable bacterial infectious disease presented clinical manifestation that overlape with;brucellosis,paratyhoid,malaria,influenza and dengue fever[1-4].The laboratory diagnosis of typhoid feverbased onto two assays.First, bacteriological for blood,feces,bone marrow and urine culture.Second the immunoassays including anti-IgM,IgG specific for S.typhi and widal tests which in the current use[5,6]

2-Inducers:

Somatic,flagellar and outer membrane protein antigens of S.typhi

3-Immune Responses:

Humoral mucosal and systemic as well as cellular immune responses.

4-Immunopathogenesis:

Since S.typhi is an intracellular parasite it will intiate cellular immune responses and delayed type hypersensitivity mediated by LIF and MIF cytokine responses.Excessive cytokine production explain the immune tissue injury accompanyed the typhodin skin reaction and over-reactions.

5-Disease: Typhoid,Enteric fever

6-Samplings:

Heparinized and non-heparinized blood,feces and urine.

7- Processings;

7-1: Program One :

Lateral Immunochromatographic assay[6].

7-1-1:patients:

Sixty patients with suspected typhoid disease were the test group and 30 normal subjects.Blood collected from brachial vein non-heparinized blood collecting tubes .Sera were saved and kept under -18C till use.

Program two:Serum and Mucosal Agglutinins[6]:

7-2-1:Patients;

The 60 patiets and 30 controls program one were sero-reacted with S.typhiO,H and OMP antigens by agglutination and hemagglutination respectively.

7-3 : Program Three:Cellular Immune responses [7]:

7-3-1:Patients

Blood were collected from brachial vein of 80 clinically proven typhoid patients and 20 normal control subjects in heparinized collecting tubes.Leukocyte Inhibitory factor was done for the patients and controls.

8- Findings:

8-1:Program One:Typhoid Specific Immunoglobulins

Thirty out of 60 patients were showing S.typhi specific IgM antibodies,23 out of 60 were with IgM-IgG and 7 out of 60 gave IgG antibodies,Table-1.

Table- 1:S.typhi Specific Antibodies

Antibody Isotype	Number to Total [6]
IgM	30:60 (50%)
IgM-IgG	23:60 (38.33%)
IgG	7:60(11.66%)

8-2: S.typhi Agglutinins;

Systemic humoral immune responses to OMP,O and H antigens are higher than that of mucosal immune responses.OMPH antibody titres were higher than that of antiO and antiH.OMP-PHA was better than anti-O and anti-H in diagnosis of typhoid patients.Mucosal responses found to be helpful for diagnosis when systemic responses were abscent.

Table -2 : S.typhi Specific Agglutinins[6]

Antibody type	Urine mean titre	Urine Specificty index	Urine sensitivity index	Serum mean titres	Serum specificity index	Serum sensitivity index
Anti-OMP	49.3	96.7	71.7	1484	96.7	98.3

Anti-O	52.9	90	68.3	1404.5	76.7	88.3
Anti-H	40.3	93.93	80	1438	83.3	98.33

The immune response patterns in typhoid patients were found as;Systemic-Mucosal and systemic for OMP.Systemic-Mucosal,mucosal and systemic,Table-3.

Table – 3 : Immune Response Patters in Typhoid Patients

Antigens	Response patterns	Ratios[6]
OMP-PHA	Systemic-Mucosal Systemic	57:60(95%) 3:60(5%)
O-H widal	Systemic-mucosal Mucosal Systemic	54;60(90%) 5:60(8.3%) 1:60(1.7 %)

8-3 ; Cellular Immune Responses:

Typhoid patients have shown significant LIF both in systemic 76:80(95%) and mucosal 80:80 (100%)responses,Table-4.

Table 4:Leukocyte Inhibitory Factors In typhoid Patients

LIF test	Significant	Non-Significant [7]
Patients systemic	20-60%,76;80( )	70 % up,4:80()
Patients mucosal	20-60%,80:80(100%).	70%up ,0:80(%)
Control systemic		99.55%
Control mucosal		98.55%

9- Interpretation

S.typhi antigens induce gut and urinay mucosal humoral immune responses as well as systemic humoral immune responses.Sytemic response levels were mostly higher than mucosal.Urinary mucosal globulin is valid issue in diagnosis of typhoid in case of abscence of systemic responses.Leukocyte inhibitory cytokines were found significant both in systemic and mucosal response levels to variable degrees.

10-Immunodiagnostic Tips

i-Qualitative Ig determination

ii-Anti-OMP detemination by PHA both at systemic and mucosal compartments

iii-Leukocyte inhibitory cytokines both at systemic and mucosal compartments.

iv-Salmonella typhi O H agglutinins stands as an adjunct criteria

The tips i-iv are forming battery for diagnosis of typhoid.

11-Conclusions

Anti-OMP antibodies were more valid than antiO and Anti-H in diagnosis of typhoid.Urinary mucosal antibodies are valid for typhoid diagnosis when systemic response were negative.

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ESSAY 19 : TUBERCULOSIS

Abstract

Non-specific and spesific cellular immune responses demonstrated by NBT phagocytosis,E rostte T cell count and leukocyte inhibitory cytokine responses.NBT and E rosette count in patients were lower than that of control subjects.Leukocyte inhibitory cytokine responses were significant in patients and non-significant in controls.The cytokine profile of padiatric BCG vaccinee as MIF,IL2 and IL4 were of higher concentration means than controls non-vaccinee.Immunoglobulin isotypes of chronic pulmonary tuberculosis were within the normal range of normal control humam subjects.IgA concentration means were higher in chronic PTB patients those with sputum AFB shedders and those with tuberculin positive skin test.IL12+P40 cytokine concentration means can delineate chronic from active pulmonary TB and delineate chronic old PTB shedder from nonshedders.Acute phase protein C was of higher concentration means in active PTB than in chronic forms.

Key Words

Active ,chronic,old ,primary pulmonary tuberculosis.

1-Introduction;

The tuberculus disease both in man and animals holds the position of challenge to the clinicians,epideiologist and immunologists.So far the immune diagnosis of tuberculosis is concerend,several immune assays were being documented like; Tuberculus agent have composites of multiple antigenic epitopes as; B ,T,allergenic epitopes as well as autoreactive epitopes were identified.Protective immunity is being T cell mediated[1-11].

2- Inducers;

M.tuberculosis epitopes[1,2].

3-Immune Responses:

A spectrum of immune responses may mounted in M.tuberculosis human and animal diseases like;Cellular,humoral,allergenic, autoimmune and granulomatogenic responses[1-2].

4-Immunopathogenesis:

In primary active tuberculosis,cellular T cell responses dominates the sceen of the immune response,in addition to immediate type IgE mediated responses accompanyed by eosinophilia and basophilia to lesser extent.Though in chronic tuberculosis delayed hypersensitivity and granulomatogenic responses were noted as casien and chon lesions[1,2].

5- Disease:

Tuberculosis,pulmpnary tuberculosis with acute and chronic forms.

6- Disease;

Heparinized and non-heparinized blood samples.

7-Processings

7-1:Cellular Immunity of Infections

7-1-1:Patients

A total of 125 pulmonary tuberculus patients as proved by their clinical presentation and 23 normal control subjects were the test and ollected fromcontrol groups.Blood were collected from the brachial vein of the patients and controls into

heparinized collecting tubes.Blood samples were processed for; NBT,E-rosette T cell count and Leukocyte inhibitory factors[3].

7-2 :Cytokine profile for BCG vaccinee childs

A total of 30 BCG vaccinee 15 non-vaccinee childs were the test and control groups of 1-10 yearsc old. Blood was collected in blood collecting non-heparinized tubes.sera were saved at -18C till use.On revival to the room temperature they were processed for ;MIF,IL2 and IL4 cytokines by eliza assays following the manufacturor instructions[4].

7-3: Immunoglobulin Isotype;

A total of 51 PTB patients and 12 controls were the test and control materials.Blood were collected from the brachial vein of both of the test and controls in non-heparinized collecting tubes.sera were saved and kept at -18 C till use.On revival to the room temp,sera were applied as 5 ul amounts to the wells of radial immunodeffusion plates for determination ofIgM ,IgG and IgA[5].

7-4:Program Four: Acute Phase Protein C

A total of 50 pulmonary TB patients brcahial and 25 controls were the study groups.blood were collected from the brachial vein in to non-heparinized blood collecting tubes.sera were saved at -18C till use.on revival to room temp they were tested for CRP[6].

7-5: IL12+p40 cytokine:

24 active PTB and 22 old chronic PTB were the study group.Blood were collected from the brachial vein of the test and controls in non-heparinized blood collecting tubes.Sera were save at -18C till use.On revival to the room temp.they were processed for IL12+P40 cytokine determination[1].

8-Findings

8-1: Nitroblue tetrazolium reduction phagocytosis were showing 6.213% as compared to normal 11.695%. T lymphocyte e-rosette formation count have shown17.525% as compared to the normal value 25.782%.Leukocyte inhibitory factor cytokines was 44.214% significance as compared to 85.64% non-siginificant LIF%.PTB patients showed depressed phagocytic activity,depressed e-rosette count and significant LIF cytokine %.,Table -1.[3].

Table- 1 :Cellular Immunity in Tuberculus Patients

Groups	NBT	E-rosette count	LIF%

PTB%	6.2130	17.525	44.214
controls	11.695	25.782	85.640

8-2: Cytokine Profile[4]

BCG vaccinee childs have shown higher MIF,IL2,IL4 concentration means than controls.Thus BCG vaccinee initiated humoral, cellular and hypersensitivity reactions as invitro by MIF ,IL2,IL4,Table -2.

Table –2; BCG vaccinee cytokine profile.

Groups	MIF ng/ml	IL2 ng /ml.	IgMng/ml.
BCG vaccinee	11.2+-1.67	952.27+_114.37	356.72+-44.199
Non-vaccinee control	6.454+-97.33	562.61+_97.33	224.28+_41.45

8-3: Ig istypes inPTB patients.

IgA,IgG & IgM concentration levels of Pulmonary Tuberculosis, patients were at most within the normal value range .Tuberculin positive group and AFB shedders have shown higher IgA values than controls, Table 3 & 4.

Table-3:Serum Ig isotypes in pulmonary tuberculus Patients[5]

Groups	IgA	IgG	IgM
PTB	5022_+2.9	17.94+_13.56	3.28+-1.01
controls	4.11+-6.32	19.505+-4.205	2.38+-0.89

Table -4 :IgA in AFB and tuberculin positive PTB.

Groups	IgA concentration means
AFB+ Control	5.5+-2.68 2.35+_0.93
Tuberculin + Controls	5.48+_2.125 2.91 +-0.89

8-4 :Acute Phase Protein C:

PTB patients have shown higher concentration means 45.1 mg/ml as compared to less than 6 mg/ml. in controls,Table- .5.

Table – 5: Acute phase protein C in tuberculus patients

Groups	CRP concentration mean	CRP titre means
PTB patients	45.1	6.87

Controls	Less than 6	

8-5: IL12+P40 in PTB patients;

IL12+P40 cytokine levels in tuberculus patients,it delineate old chronic and primary active.Aswell as old shedders from non-shedders

Table 6: IL12+P40 levels in pulmonary tuberculus patients.

Groups	IL12+P40 pg/ml.
Acute Active PTB	425.95
Overall patients	395.4
Old PTB	232.34
Old PTB,shedders	341.28
Old PTB,non-shedders	201.86
Controls	

9- Interpretation;

IL12P40 descriminates between active PTB and chronic old.And between AFB shedders and nonshedders[1].Acute phase protein C was rised up in active primary PTB[6].The cytokine MIF,IL2 and IL4 have shown increasing concentration means than controls.This can be attributed to the induction of humoral ,cellular and hypersensitivity in BCG vaccinee[4].NBT and E rosette lower results than control indicate immunosuppressive responses.ILF assay have shown significant inhibition% which indicate production ILF cytokines[3].

10-Immunodiagnostic Tips;

i- CRP levels indicate active PTB[11]
ii- IL12+P40 concentration levels delineate active from old PTB and old shedders from non-shedders[1].
iii- MIF,IL2, and IL4 concentration mean levels were higher than control[4].
iv- NBT,E rosette counts have been lower than controls,[3]..
v- LIF have been shown significant% than the non –significant of controls[3].

11- Conclusions:

MIF,LIF and IL12+p40 seemed to be valid as cytokine battey of diagnosis of Pulmonary PTB.Acute Phase protein C discriminate between active primary tuberculosis and the cold chronic tuberculosis.

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ESSAY 20 : BRUCELLOSIS

Abstract

Brucella meletensis possess multiple epitope composition as B cell epitope,autoreactive epitopes and cryoacting epitopes.The humoral agglutinins have been identified in 6.3% of PUO patients and suspects.Auto-immune response the RF,anti-mitochondrial,anti-cardiolipin and anti-nuclear factor autoantibodies to an-overall percentage of25% of the tested brucellosis patients.Mixed variant cryoglobulin responses of IgM-IgG and IgM-IgG-IgA responses have been documented.Brucella meletensis vaccine induced cryoglobulin responses in rabbits.

Key Words :

Agglutinins,Auto-antibodies,Brucella,cryoglobulin,epitope,immunoglobulin,mixed,variant.

1-Introduction:

Brucella is an intracellular bacterial infection of zoonotic origin.Human are infected by direct contact with animals or indirect from consumption of raw milk and its products.The disease is of world wide distribution.It expresses an endemic infection pattern,whenever farm animals are raised in large numbers.Incidence of brucellosis varies from counttry to other.Composites antinicity have been noted in brucella,some epitope induce B cells,other induce T cells and a third induce autoimmune responses.The fourth types may be allergenic inducing DTH.Chronic brucellosis may initiate granulomatous responses[1-6].The spectrum of the immune functions of a host contracting brucella infection is as stated in the following Table-1.

Table – 1 : The immune functions of human immune system in brucellosis[5].

Immune cells	Acute	chronic	Recovery	Normal
CD4+ T cells	Low	low	Low	
CD8+ T cells	High	high	High	
TH1 cells	High	high		low
Th2 cells	High			high
Th17 cells	High	low		low
TLR 4 lymphocytes	High	high	Hogh	high

2-Inducers:

Various types of antigenic epitopes of Brucella abortus and Brucella meletensis[1-6].

3- Immune Responses:

Humoral diagnostic,cellular diagnostic and protective. As well as delayed type hypersensensitivity and granulomatous responses[5].

4-Immunopathology:

Brucella at chronic infection state have an immune evasion mechanisms to avoid the innate and adaptive immunity in order to establish the intracellular infection niche for long persistence.There were a TH1 cell distubances noted in chronic brucellosis[1-3].Interferon gamma was found to be the principle cytokine active against brcella infections.Helper cells,TH1,Th2,TH17 were associated with both immunity and immune protection against live challenge[5].

5- Disease:

Human PUO and arthritis.Animals at most causing abortion.

6-Clinical Samples:

Heparinized and non-heparinized blood samples collecte from the brachial vein.

7- Processing:

7-1: Brucella Circulating Agglutinins:

A total of 812 PUO patients suspoected with brucellosis were tested for brucella agglutinins in the peripheral blood in hilla city main hospitals[1].

7-2:Autoantibodies:

A total of 32 clinically proven brucellosis patients interviewed in hilla main hospitals.Blood were collected,sera saved and kept at -18C till testing.On revival to room temperature they were tested with an array of auto antibodies[6].

7-3 : Secodary Cryglobulinemia:

A total of 50 clinically proven brucellosis patients and 10 normal subjects as a control.Blood were collected ,saved and kept at -18 C till assay.On revival to room temperature they were pricessed for cryoglobulin response[2].

7-4: Vaccine induced Cryoglobulinemia:

Brucella meletensis approved vaccine were tempted for specific immune priming in rabbits using nasal , IM-SC,and IM-IV specific priming protocols in rabbit groups each of three rabbits[7].cryoglobulin response were matched as in[2].

8- Findings:

8-1:Program One: Circulating Agglutinins;

From 812 tested PUO suspects for Brucella agglutinins 51:812(6.3%) were seropositive for brucellosis.

8-2:Program Two:Autoantibodies[7];

When the 32 proven brucellosis patients with an array of autoantibodies they were presenting as;antimitchondrial antibodies 1:32 ,anticardiolipin antibodies 2:32 ,rheumatoid factor 3:32 , and antinuclear factor 2:32 with atotal of 8:32, Table-2.

Table – 2 : autoantibodies in Human Chronic Brucellosis.

Autoantibody type	Seropositivity %

Rheumatoid factor	3;32
Anti-mitochondrial	1:32
Anticardiolipin	2:32
Antinuclear factor	2:32
Overall	8:32

8-3: Program Three: Secodary Cryoglobulinemia[2].:

Secondary mixed variant cryoglobulinemia were determined in human brucellosis patiients,Table – 3.

Table- 3 : Immune features of cryoglobulin resoponse among human brucellosis patintes

Criteria	Immune features
Cryoprecipitating Appearance	4C 1-5 days
Cryocrit % Protein concentration	7.28% 20-47 mg/ml.
Cryoglobulin response %	27:50(54%)
IgM-IgG response IgM-IgG-IgA response	10:27(37.1%) 17:27(62.9%)
IgM concentration mean	1.255 mg/ml.
IgG concentration mean	10.224mg/ml.
IgA concentration means	2.915mg/ml.

8-4 : Program Four: Vaccine Induced Cryoglobulinemia[7];

There were an intragroup individual variation in cryoglobulin concentration in all of the primed roups.The IM-IV protocol immune response of higher competence than IM-SC group.Both of IM-IV and IM-SC were of higher competence than nasal.Secodary immune responses at the fourth week were higher than primary first week cryoglobulin response in the three protocols. The overall of the secondary 6th week showed comparable results to the 4th week with slight noted waning in the immune response.

Rabbit replicates	Primary 1st	Seondary 4th week	Primary Im-SC	Secodary IM-SC 4th	Primar y IM-IV,1st	Secodary IM-IV,4th

	week Nasal	nasal	1st week	week	week	week
R1	3.8	8.1	5.2	15.6	8.3	23.0
R2	2.7	7.2	6.2	18.6	11.5	26.5
R3	5.3	9.98	9.7	23.8	13.6	21.3
R mean	3.93	8.36	7.66	19.366	11.8	28.6
SE	1.028	2.387	1.028	2.387	1.703	10673

9- Interpretation:

Human humoral immune responses in brucellosis cases and PUO suspects through agglutinins,cryoglobulins and immunoglobulin isotypes as well as vaccine induced cryoglobulinemia responses in which secodary responses at 4th week gave potent immune response than primary response.The more the exposure to vaccine within the priming limits the more potent cryoglobulin response.Findings were matching those reported by other workers in this field[1-7].

10-Immunodiagnostic Tips

i-Doing immune cell landscape by flow cytometery in human brucellosis and vaccinee is advisable.

ii-Cryoglobulin responses are evident both in infection and vaccination

iii-Mixed vaiant isotypes in human clinical brucellosis was reported.

iv-interferon gamma and T cell immunity are the protective for brucellosis in human beings

v-Chronic brucellosis in human beings associated with auto immune responses of multiple types.

vi- Rabbit vaccine induced cryoglobulin response model was proved.

11- Conclusions;

Immune agglutinin and cryoglobulin and autoimmune RF,antimitocondrial,anticardiolipin and antinuclear factors were evident in human brucellosis.

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PART FIVE ;NATURAL INNATE AND ADSAPTIVE IMMUNITY

Section Nine : Human Disease Conditions

Essay 21 ; Persistent Pyuria

Essay 22 ;Vaginosis

Essay 23 :Infertility

Essay 24 : Burns

Essay 25 :Allergy

Essay 26 :Diabetes

Essay 27 : Anergy

ESSAY 21 : PERSISTENT PYURIA

Abstract

A total of 105 persistent pyuria patients and ten normal control subjects were the investigated microbiota composition,microbiota dysbiosis,humoral and cellular immune responses both at mucosal and systemic compartments.Different uropathogen induced different immune responses.The major immune features were;dysbiosis to urinary microbiota,facultative and obligate intracellular uropathogens associated with humoral and cellular immune responses both at mucosa and peripheral blood.Extracellular uropathogens were linked with mucosal and systemic humoral immune responses.

Key Words

Cellular,humoral,dysbiosis,facultative,obligate,microbiota.

1-Introduction

One of the most common human urinary tract problems is the presence of leukocytes in the voided human urine samples.These leukocytes are loaded with the engulfed external microbial invadors and are known as pus cells,and the case was described as pyuria P.When ,the state of pyuria was recurrent and paients complain expressed over long time the state was designated as Persistent pyuria PP[1,2].The immunology of human urinary tract during infection and/or inflammation holds the forefront position in the current relevent literature[3-6].The present chapter aimed to map;dysbiosis of urnay microbiota,humoral and cellular mucosal and systemic immune responses of bacterial persistent pyuria.

2- Inducers:

Lymphogenic,hematogenic,nigbourhood extension,dscending and/or ascending invading microbes.

3-Immune Resposes:

Local natural innate humoral and cellular responses.Inaddition to mucosal , systemic humoral and cellular adaptive immune responses.Such responses mainly depending on the nature os antigenic make up of the invading microbes[3-6].

4- Immunopathogenesis;

Local mcosal or systemic antibodies within the niche of urinary tract can be of an unwanted immune potentials.Antibody-Complement complexes may form a stricture or blockade in the urethral passages.Local cytokine excessive release might lead to immune tissue injuries to the urinary tract.The microbial acute infection in case of uncontrol may proceed to subacute then to chronic in chronic state may associate with autoimmunity whereby might terminated with neoplastic transformations.

5-Disease:

Persistent Urinary Tract Infections.

6- Samplings;

Clean catch midstrean urine samples under aseptic conditions.Heparinised and nonheparinized blood samples.

7-Processings;

7-1: Program One:Urinary microbiome Composition,

7-1-1: Patients And Controls;

A total of 105 patients complained signs and symptomes of pyuria and they were diagnosed as persistent pyuria and ten normal control subjects.The applicable cutoff of the PP was as the growth of 10 to five cell/ml, on urine plate count assay.Primary plate culture study followed by isolation and purfication of the causals

will give account about the composition of urine microbiota both in health[biosis] and disease[dysbiosis].

7-2;Program Two:Urinary Tract Immunity;

Patint and control urine samples were centrifuged at 5000rpm for five minutes.Pellets cells surved for LIF assay.Supernates in a rate of 5ml admixed with equal amounts of PEG 6% 6000. Let for one hour at 4 C and centrifuge a t 5000 rpm for ten minutes.Pellets represents mucosal Ig and antibody studies.Can be dissolved in sterile formal normal saline .Heparinized blood samples were centrifuged at 5000rpm for 5 minutes.Cell washed twic with saline and surved as a source for LIF capillary assay.Non-heparinized blood samples were clotted and sera saved for antibody studies[8-11].

8- Findings;

8-1; Urinary Microbiota

Urinary microbiota in PP has shown dysbiosis to most frequent uropathogens E.coli,S.aureus the Klebsiella,Table -7.

Healthy Controls	Persistent Pyuria
Coliform	E.coli 40:105
Coryneform	S.aueus 20:105
S.epidermidis	K.pneumoniae 15:105
	Proteus mirabilis 12;105
	Proteus vulgaris 10;105
	P.aeruginosa 8:105

8-2: Urinary Tract Immunity in PP;

Bacterial persistent pyuria increase;TSP,SG,UMG,mucosal and systemic ab in comparison to normal control values.E.coli,S.aureus,P.aeruginosa induced humoral and cellular immune responses to clinically significant levels..Klensiella.pneumoniae, Proteus species induced humoral mucosal and systemic antibody responses to significant titre levels.Serum IgM,IgG and IgA increased in patient than in controls.

Table 2 :Urinary tract immunity during human persistent pyuria.

uropathogen	number	TSP g/l	SMG g/l	UMG g/l	Mucosal/sytemic antibody titre	Mucosal/systemic LIF	IgG	IgA	IgM
E.coli	40	72.74	43.82	0.72	40/360	0.5/0.57	23.39	3.09	2.23
S.aureus	20	71.78	40.81	0.72	40/360	0.5/0.65	15.77	2.82	2.06

K.pneumoniae	15	75.42	42.28	0.72	36/360		20.49	3.9	5.18
P.mirabils	12	73.44	42.28	0.67	36/360		15.89	3.14	2.60
P.vulgaris	10	73.24	0.62	0.62	40/400		12.71	2.96	2.04
P.aeruginosa	8	74.68	0.67	0.67	40/400	0.52/0.65	13.51	2.23	2.12
Control	10	70.27	34.42	0.2		0.98/0.95	11.97	2.44	1.95

9-Interpretation;

9-1;Program One:Microbiota dysbosis;

The predominance of uropathogens has shown that E .coli the Klebsiella pneumoniae from the gram negative and S.aureus from the gram positive hence the majr uropathogen was E.coli[12].This finding is inline with this study and that of others abroad[6,13,14].Hence the dysbiosis was represented by the dominance of the uropathogen E.coli.

9-2 :Program Two:Persistent Pyuria Immunity:

The current information about urinary tract immunity has been documenting the pivotal role of the innate cellular immune responses paving the way towards the role of TLR4 acivity.Beside rising the theme that cystitis mostly associated with an inhibition of immune responses with lack of antibody responses[3,6].Different uropathogen has shown different immune responses[4].The present results was showng that P.aeruginosa and E.coli uropathogens induces different immune responses than that of K.pneumoniae which was inline with that of[4]. The battery of agglutination,haeagglutination,Ig isotypes and LIF were forming the basis of suggestive for immune classification of persistent pyuria[SPPIC] as in the followings;

SPPIC –I: Microbiota dysbiosis,intracellular,induces humoral and cellular immune responses both at mucosa and peripheral blood

SPPIC- II : Microbiota dysbiosis,extracellular induces humoral immune responses in both mucosal and systemic compartments

SPPIC-III:Microbiota dysbiosis, deprived immune state covers, humoral and/or cellular immune responses.Diabetic,pregnancy , ageing,under cytotoxic drug regmins[Immune compromy].

SPPIC – IV : Microbiota dysbiosis, variable imune responses and uncertain causals.

10- Immunodiagnostic Tips:

i-PP indicate UTI and microbiota dysbiosis.

ii-Facultative and obligate intracellular uropathogens associated with both humoral and cellular immune responses both at mucosal and systemic compartments

iii- Extracellular uropathogens associtaed with humoral immune responses both at mucosal and systemic compartments

iv- urinary mucosal anitbody was valid for diagnosis of infectious PP as that of systemic immune responses.

v-Mucosal and sytemic immune responses associated with infectious PP.

vi-PP immune classification was suggested.

11- Conclusions:

Major immune features of infectious PP can be as; Microbiota dysbiosis,mucosal and systemic immune responses intiated.Different uropathogens initiate different immune responses.

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ESSAY 22 :VAGINOSIS/VAGINITIS

Abstract

One of the most common health problems in married women life is the case of vaginosis. It impacts the health state and well being of women.The microbiome of normal vagina have been reported.Bacterial vaginosis cause dysbiosis in the compostion of vaginal microbiome both in qualitative and quantittative manner.immunopathology of bacterial vaginitis ensembles derangment of mucus membrane,degradatiion of IgA and /or immunosuppression.The dysbiosis reveals dominance of E.coli K1 bacteria.This genitopathogen induced neutophil NBT phagocytosis,humoral systemic and mucosal,cellular systemic and mucosal immune responses to a higher limits than in controls.

Key Words

Bacteria,microbiome,mucosa,vagina,vaginosis/vaginitis.

1-Introduction:

The female reproductive tract stands as an unique mucosal compartment in which the local antibody isotype is IgG and much of which derived from circulation.As an immune compartment it constitute a hormone dependent lymphoid aggregates

consisting of CD8+ T cells,B cells and macrophages in the uterine stratum basalis with different composition and probabley have different function[1-5].The immune cells habouring subepitelial tissue of female genital tract particularly in the endocervix and to lesser extent in the fallopian tubes and uterus are plasma cells secreding polymeric IgA had been well documented and the polymeric IgA receptor,the membranous precursor form of the secretory component SC had been documented in the overlyng epithelium.Thus in these locations SIgA is assembeled and transported in to the lumen,the mechanism whereby IgG from circulation or produced by the resistant IgG secreting plasma cells is transfered to lumen remain unclear.Mucosal cell mediated immune mechanisms are known and operative[6].

2-Inducers:

A way from the existing reproductive microbiome,E.coli was the dominant genitopathogen associated with vginitis.

3-Immune responses:

Female reproductive tract mucosal compartment initiated mucosal humoral and cellular immune responses as a counterpart to the systemic humoral and cellular immune responses.These responses might be operable in protection within the limits toleragenic and immunosuppressive immune reactions.

4-Immunopathogenesis:

Th17 cells/Th17 cytokine axis,neutrophil mediated killing and oxidative stess during the infection events.Genitopathogens have five mechanisms for subverting or manipulation of the host immune responses[7].The vaginal pathogens may suppress and circumvent host microbial defense mechanisms and propagate their survival/dominance without overt inflammnation.Mucosal barrier and IgA degraded.IL8 cytokine response inhibited by neutrophil infiltration[8].Though the C. albicans immunopathogenesis attributed to neutrophil anergy state[9].

5- Immune Features:

Bacterial vaginosis/vaginitis have a characteristic immune features dipicted in ,Table -1.

Table -1 : Immune features of married women bacterial vaginitis

Features
1-Dysbiosis of reproductive microbiome 2-Involvment of Th1,Th2 and Th17 heper cells 3-Degradation of mucosal barrier and IgA.

4-CD3 and CD4 T cell immunodeficiency
5-Inhibition of IL8 cytokine responses
6-Incapcitation of neutrophil and monocyte chemotaxis
7- Initiation of inflammatory immune responses.

6-Disease :

Bacterial vaginitis

7- Clinical Samplings:

Heparinized and non-heparinized blood samples and vaginal swabs from patients and controls.

8-Processings:

8-1;Program One;Vaginal microbiome:

8-1-1: Patients And Controls:

A total of 352 women with vaginitis were elected as test group.Thirty normal women subjects were the control group.Vaginal swabs were done by an expert clinician.Standard culture methodology were applied.Colony morophotypes,pure isolates were obtained and characterized biochemically and serologically for the genitopathogen E coli.K1.[10].

8-2:Program Two:Humoral Immune Responses;

Non-heparinized blood samples were collected from both of patients and controls.Sera saved at -18 C till use.On revival to room teperature sera and vaginal mucosal globulin were tested with E.coli K1 capsule antigen and cell free culture filterate of E coli.k1[11]

8-3:Program Three ; Cellular Immune Responses:

Heparinized blood samples and vaginal mucosal cells of patients and controls were processed for NBT phagocytosis [ ]and capillary LIF[ ].

9- Findings:

9-1:Program One : Vaginal Microbiome

There were qulaitative and quantitative shift in the compostion of normal women and bacterial vaginitis women.Lactobacillus spp. ,Corynebacterium spp.,Staph.epidermidis were abolished from the microbiome and E.coli K1 dominated the sceen of the microbiome,Table-3.

Table-3 : Vaginal Microbiome in test and control women.

Normal micrbiome		Microbial Vaginosis dysbiotic micrbiome	
C.abican	3:30	C.albicans	108:352
Corynebaterium spp	14:30	E.coli	52:80
Lactobacillus spp	6:30	Klesiella spp	23;80
Staph epidermidis	3:30	Enterobacter spp	3:80
E.coli	2:30	Proteus spp	3:80
Coliforms	2 :30	S.aureus	4:80
		St.pyogenes A	5:80

9-2: Program Two: humoral immune Responses

Both of humoral agglutinin ad heamagglutinin titres in systemic and nucosal responses were higher than the controls,Table-4

Table -4: Anti-capsular E.coli K1 and Anti-CFCF responses among vaginitis and control women.

Groups	Systemic response	Mucosal response	Systemic/mucosal ratio
Patients Agglutination Passive hemagglutination	 320-5120 320-2560	 32-512 32-256	 5/1-20/1 5/1 – 20/1
Controls Agglutination Passive hemagglutination	 10-40 20-40	 2-4 2-4	 20/1 10/1

9-3 : Program Three;Cellular Immune Responses;

The Leukocyte inhibitory cytokine responses were high significant at sytemic and significant at mucosal responses as copared to non-significant in control women.Both systemic and mucosal NBT% phagocytosis in vaginitis patients were higher than control,Table -4.

Table-4:Cellular immune responses of vaginitis women

Groups	Systemic LIF %	Mucosal LIF%	Systemic NBT %	Mucosal NBT%
Patients Capsule CFCF	 58.5 46.5	 60.7 48.34	 61.04	 42.14

Control Capsule CFCF	100 89.7	85.6 80.0	46.6	20.3

IL 1-alpha cytokine concentration means were higher than that of controls.While,IL8 cyokine concentration mean were lower than that of controls,Table-5.

Table -5 : Cytokine Responses among vaginitis women

Cytokine response type	Concentration means in pg/ml.
IL1-alph Patients Control	 107.702 81.845
IL8 Patients Control	 97.529 204.16

10-Interpretations:

Bacterial vaginosis caused dysbiosis in the microbial composition as compared to the normal vaginal microbiome.It was expressed as quantitative and qualitative of the vagina[11].E.coili K1 was the dominant pathogen arised by the induction of bacterial vaginosis[12].E.coli K1 initiated systemic and local agglutinin and hemagglutinin response higher than those of control[13,14].Systemic NBT phagocytosis were showing high NBT reduction percentages than mucosal.Systemic and nucosal have shown higher percentages than controls[15] .Leukocyte inhibitory factor cytokine responses were showing highly significant inhibition at mucosal compartment as compared to high systemic levels [16,17].IL1-alpha express high concentration means than controls.while,IL8 were showing lower concentration means than controls [17,18].

11- Immunodiagnostic tips;

i- Vaginal microbiome dysbiosis was pathognomic with bacterial vaginosis.
ii- E.coli K1 vaginosis induced humoral and cellular specific and non-specific responses both at mucosal and systemic levels.
iii- Anticapsular antibody,NBT%,IL1-alpha,&IL8 were found valid immuno diagnostc battery for bacterial vaginosis
iv- Oversecretion of LIF,IL1-alpha accounts for the immunopathogenesis of bacterial vaginitis.

12-Conclusions:

Phagocytic activity,humoral and celliular as well as cellular mediators are involoved in the immunopathogenesis and immunity of E.coli K1vaginosis.

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ESSAY- 23 : INFERTILITY

Abstract

Infertility in human is a complex and of multiple etiology.Immune infertility may be attributed to humoral,cellular and/or both.Clinical anti-sperm agglutinins and hemagglutinin were frankly noted among infertile couples.Leukocyte inhibitory factor cytokines were found both in peripheral blood and mucosal leukocytes.Though the systemic was reaching the significant levels while at the mucosal surface were reaching border line signinificance.

Key Words:

1-:introduction:

Etiology,infertlity, ranged from 10-30% of the population in swetizerland .unexplained infertility ranges up to 15% .Antisperm antibody in male and 13 up to 80 % in females.immune infertility can be explained as sperm antigen tolerance becomes down leading to pathogenesis of infertility.Antigen tolerance in male implys a balance between orchitogenetic T cells and regulatory T cells.Sperm antigen face the blood-testis barrier and this well down regulate cell mediated immunity and humoral mediators.While sperm antigen tolerance in female in which ovrarian antigen make balance between oophoritogenic T cells and suppressor T cells.Sperm

flush ends with minority of sperms that able to reach fallopian tubeinsitu undergoes sperm phagocytosis,immunosuppressive factors,integrity of mucosal epithelium as well as genetic factors effects.Such immune events in both male and female reproductive factors may lead to occurrence of immune disease such as orchitis,necrosis,atrophy,oophoritis and oocystitis.Thus there were an imbalance between autoreactive T cells and regulatory T cells.Anti-sperm antibody responses occur when there is testicular pathology.While in females there were a distruption in the mucosal layer,lymphocyte in semen,antibody coated sperm,abnormal senescence sperm,and sperm in gastro intestinal tract,sperm is within the peritoneal cavity.The testicular atrophy explain pathogenesis of infertility.Anti-sperm antibody induced infertility of unclear mechanisms and may be due to sperm autoagglutination,sperm cytotoxicity, sperm phagocytosis,and antibody coated sperms[1-10]

2- Inducers:

Anti-sperm antibody,testicular antigen,ovarian antigen,sperm cytotoxicity and the imbalance beween reproductive T cells and regulatory T cells[1,9].

3- Immune Responses:

Both in male and female mates humoral and cellular immune responses occures within their reporoductive tracts in the postmating periods.

4- Immunopathogenesis:

At the humoral arm anti-sperm antibody production,orchitis,atrophy, antibody coated sperm may pose to immune infertility.While ,at the cellular level imbalance between testiculogenetic and oophoritogenic T cells and the regulatory T cells pose to an immune tissue injury followed by immune infertility[1,3,5].

5- Disease;

Immune infertility in male mate ,female mate or both[1-10].

6-Samplings:

Semen,overian aspirates.

7-Processing:

7-1:Patients And Controls:

A total of 125 infertile couples were the test group and 25 normal control subjects.

7-2:program One : Antisperm antibody[9];

Non-heparinized blood samples were collected fro both of test and control groups.Sera were saved at -18C till use.On revival to room temperature.Test and control sera were reacted with sperm antigen and sperm sonicated antigens through standard tubt agglutination and passive hemagglutination tests.Likewise, globulins were separated from semen plasm and ovarian aspirtes and processed by agglutination and hemagglutination.

7-2 : Program Two:Cellular Immune Responses

Heparinized patients and control blood samples were processed for leukocyte inhibitory factor by capillary method[Sobeg,1968].

8- Finidings:

8-1: Sperm Specific antibodies:

Both of the systemic and mucosal humoral antisperm antibodies were proved reaching the limits of clinical titres,Table -1.

Table – 1 :humoral systemic and mucosal anti-sperm antibody among infecterile couples

Response nature	Agglutinins	Hemagglutinins
Systemic	320-3200	320-10240
Mucosal	40-320	32-2048

8-2 :Program Two : Cellular Immune Responses:

Significant leikocyte inhibitory factor cytokines response at the systemic response levels and boarder line inhibition at the levels of mucosal response,Table- 2

Table – 2 : leukocyte inhibitory factor among infertile subjects.

Response nature	Male	Female
Systemic	0.64%	0.63%
Mucosal	0.68 %	0.69 %

9- Interpretations:

Both of humoral,cellular and delayed type hypersensitivity responses were noted among infertile couples as compared to normal status among controls.The antigenic epitopes involved in the causation of immune infertility among infertile couples may

be;T independent,T dependent with an allergenic potentials or combination of any of them[1-10].

9- Immunodiagnostic Tips;

i-High antisperm agglutinins and hemagglutinins are pathognomic with infertility.

ii-Significant Systemic and boarderline significant at mucosal surface are pathognomic with infertility

iii- Tips i and ii can be of use a test battery for duiagnosis of immune infertlity

10-Conclusions;

Mucosal humoral and cellular as well as systemic humoral and cellular immune responses are involved in the causation of human infertile couples.

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ESSAY- 24 : BURN:

Abstract

The skin burn infections are complex in their immunology.To start with,it is essential to inlight the situation by uncovering the nature of microbiota of normal skin and skin burn infections.At most gram negative bacteria dominate gram positive bacteria.Skin burn infection induce dysbiosis in skin microbiota.To this end,P aeruginosa and klebsiella spp. Were dominating the sceen in a survy of 100 skin burn patients.P.aeruginosa initiate humoral systemic , mucosal and cellular mucosal as well as systemic responses.While Klebsella spp.induced humoral systemic and mucosal responses only.

Key Words;

Burn,dysbiosis,infection,immune,Klebsiella,microbiota,mucosa,Pseudomonas.

1-Introduction:

Human skin in health and disease stands as a peripheral immune system as compared to systemic immune system whereby it contained skin associated lymphoid tissue SALT.SALT composed of;lymphocyte,dendritic cells,hols the position of regulatory lymphoid tissue facing the invadors and intrudors.The skin lymphoid tissue able to opsonize different antigens and present them to lymphocytes.The topography

of skin lymphoid tissue be able to receive immune signals from skin.Skin T cells posess distinctive affinity for skin associated lymphoid tissue.Skin immune surveillance established by Langerhans cells,keratinocytes,T cells against microbial invasion[1-5].

2- Inducers:

Predisposing factors,friction and abrrations. Burn preconditioned the skin for microbial invasions.

3-Immune Responses:

Skin professional phagocytes engulf the invading microbes and kills them.Then they process their antigens.The intracellular processed antigen within the phagocytes will bind MHC molecules and surface appeared on the phagocytes.The antigen surface bearing phagocytes present them to T herlper 1 or 2 which in turn induced naive cells to grow,proliferate and expand as either effector or memory B or T cells.Keratinocytes starts to synthesize and produce IL1-alpha and TNF gamma and migrate to the paracortical areas of the draining lymph nodes.In the draining lymph nodes skin dendritic cells activate naive resting T cells leading to clonal proliferation.The proliferated clones bears skin homing receptors and be in contact with fine blood vesseles in infected skin epidermis.Such clones inters tissue and face profound cells expressing the potentials of elimination of invadors [5,6].Hence,both humoral and cellular elements participate actively in skin immune response to various microbial invadors,Table-1.

Table-1 : Skin Burn Immunity.

Innate	Adaptive
1-low activity of phagocyte,NK,neutrophil epithelial adhesion resptors	1-low total T cell counts in first week
2-High phagocyte prostglanidin,increase of IL10 and IL4	2-Decrease of monocyte IL2 and INF gamma
3-inhibition of B and T cell activity,decrease of IL-1 B and alternative complement cacade	3-lower of IgG prouction,lower TH1/Th2 ratio
	4- Imbalance between CD4 helper and CD8 suppressor T cells
	5-Increase of IL10 and IL4

4- Immunopathology

The pathology of skin burn involved three squential stages;zone of coaggulation includes protein coagulation,zone of stasis with low tissue perfusion and zone of hyperremia due to vascular dilitation.While immunopathology stats as the cytokine and growth factors stimulated by inflammation enable burn patients to overcome

infection events.Persistent activity of complement,acute phase proteins and production of anti-inlammatory cytokines leads to change in lymphocyte activity,activation of stress response and migration of immune cells to the site of injury are related to postburn local and systemic immunopathology[10].Burn injury induced early and profound upregulation of adaptive immunity and activation biomarkers and down regulation of innate immunity and stress inflammation biomarkers[2,3].

5- Disease:

Burn infections

6- Clinical Samplings

Burn swab,burn scrap,heparinized and noheparinized blood samples

7- Processing[9]:

7-1: Program One Skin burn microbiome

7-1-1:Patients And Controls:

A reterospective survey were made to skin burn infection covers 120 cases in addition to a prcatical laboratory working survey ensembeled in 100 patients ,besides 200 skine burn infected patients in the burn hospital ward.

7-1-2: Manipulation

Skin burn swabs from 200 skin burn infected patients and 25 control subjects were processed for standard culture methods.Colony morphotypes,pure culture as well as biochemical tests to the skin associated microbes.[10].

7-2; Program Two;Post skine burn infection immunity;

The skin burn infected microbiome in same Babylon province showed three dysbiosis patterns ;First, on reterospective as dominance of Pseudomonas and E.coli, second in the prospective in the general laboratory routine as the dominance of S.aureus and P.aeruginosa and the third in the study of skin burn ward as the dominance of P.aerugnosa and Klebsiella,Table -2.dimicrobic dysbiosis dominates that of monomicrobic[5,6,7,8].

Table -2 :Dysbiosis of human skine burn infection microbiome[9].

Normal skin microbiome	Reterospective routine survey	Routine laboratory survey	Field burn ward study

	P.aeruginosa 18;120 E.coli 18:120 Klebsiella pneumoniae 16: 120 S.aureus 10:120 Proteus sp 10:120 Streptococcus sp 5:120 Enterobacter sp 1:120 E.coli& Proteus 1:120	S.aureus 20:100 P.aeruginosa 8;100 Klebsiella pneumoniae 7;10 E.coli 7:100 Proteus sp 5:100 S.epidermidis 3:100 E.coli & Proteus 2:100 P.aeruginosa & Klebsiella 2:100 Enterobacter sp 1:100 Acintobacter sp 1:100	P.aeruginosa 50:200 Klebsiella pneumoniae 38:200 Klebsiella pneumoniae& P.aeruginosa 7:200 E.coli 5:200

8-2 : Program Two ;Post skin burn infection immunity

Both of the P.aeruginosa and Klebsiella pneumoniae induced increase in NBT % than normal subjects both at systemic and mucosal compartments.Significant LIF cytokine inhibition was noted both at local and systemic levels in P.aeruginosa infected patients.While non-significant in K.pneumoniae skin burn infected patients.IL6 cytokine levels both at systemic and local responses were higher than normal in both of P.aeruginosa and Klebsiella pneumoniae skin burn infected patients.Agglutinin levels were significant and less significant in P.aeruginosa and Klebsiella pneumoniae skin burn infected patients both at systemic and cellular compartments respectively.P.aeruginosa stimulated humoral and cellular immune responses.While,K.pneumoniae induce humoral immune responses both at systemic and local responses.Table- 3.

Table – 3 :Immune Features of human Skin Burn infection

Pathogen	NBT systemic	NBT local	IL6 systemic	IL6 local	LIF systemic	LIF local	Agglutinin systemi	Agglutinin local
P.aeruginosa	40.24	32.24	110.4	96.3	55.16	52.36	640 2ME res	128
K.pneumoniae	36.73	29.24	119.56	231.16	86.72	87.44	160 2 ME 133.3	21.33 2ME 10.66

Normal	15.72		12.45		88.52		23.33	

9- Interpretation

9-1 : Skin post burn infection microbiomes

The noted microbiome dysbiosis in skin post burn infection was inline with that of other workers,but it had been in contradiction with others .The comparison of these results with other workers abroad was inline with[5,7,8] .

9-2: Post burn infection Immunity:

Humoral mucosal and systemic immune responses of skin burn infection patients were showing higher titres than that of the controls.NBT phagocytosis were higher in patients than in controls.The results of LIF cytokines in peripheral blood and local skin were inline with[9]

10 - :Immunodiagnostic Tips:

i- P.aeruginosa stimulate humoral and cellular immune responses while K.pneumoniae induce humoral immune responses

ii- Both systemic and mucosal immune responses were higher in patients than in controls

iii- IL6 cytokine responses had been high in systemicand mucosal in patients than in controls

iv- Tips i-iii can be of use as an immune diagnostic battery for human post burn skin infections.

11-Conclusions:

The anitgenic structure of the skin burn P.aeruginosa can be of T cell independent and T cell dependent epitopes.While that of Klebsiella pneumoniae may composed B dependent and T and B dependent epitopes.

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ESSAY - 25- I : IGE VERSUS ALLERGY

Abstract

Currently IgE elevations of concentration in human peripheral blood stream have more than one mind bearing,classically it is pathognomic with immediate type hypersensitivity. Since it appears that it stands as multipotent antibody molecules.It expresses an array of immunologic functions as;disease association,disease marker,protective and regulatory roles.BCG vaccinee have shown elevated concentration levels than non-vaccinee.

Key Words

Introduction

In immune homeostasis state of human beings there were with low level of free IgE,low number of IgE producing plasma cells and short lived memory B cells.IgE functions in; fluctuation of the vents of antigen presentation by macrophage to T cells,enhances the cellular and humoral immune responses against autoantigens and against neoantigens , facilitating tumor cell lysis,associated with allograft rejection and atheromatus cardiovascular diseases.IgE in combination with mast cells,basophils and eosinophils constitute th hallmark of type 2 immunity which may be dysregulated in neumerous allergic conditions[1].IgE has dominating role in host protection from parasitic infestations espacially helminth[2,3]

Inducers;

Tuberculus allergens,fungal allergens

Immune Responses;

IgE mediated allergenic responses.

Immunopathology

The events of allergic reactions consists of the prduction of IgE in response to either out or indoor allergens,binding of IgE to Fc receptors of mast cells,cross-linking of bond IgE by allergen upon re-exposure,and release of mast cells mediators,such as;histamine, leads to rapid increase in vascular permeability and smooth muscle contraction resulting in many of the symptomes of allergy.

Disease

TB preventive mass vaccination in childhood.

Samplings

Blood samples from BCG vaccinee childs.

Processing Program;

A total of 30 BCG vaccinee and 30 non-vaccinee childs were subjected to blood samplings the sera were saved.IgE estimation in serum samples of both vaccinee and non-vaccinee were determined by specific IgE ELIZA ket in accordance with the manufacturer instructions[4] .

Findings

The BCG vaccinee were showing Higher IgE concentrations than non-vaccinee.

Table – 1 : IgE levels among BCG vaccinee and non-vaccinee childs.

Subjects	Number of subjects	Mean ofIgE concentration in pg/ml.
BCG vaccinee	30	578.35
BCG non-vaccinee controls	30	355.733

The minimum IgE levels of BCG vaccinee were lower than the minimum levels of normal control childs

Interpretation

Normal non-BCG vaccinee IgE levels give a clue to the limits of exposure to envirnmental allergen .BCG vaccinee expressed higher IgE levels than controls which

accounts for the role of the allegenic M.tuberculosis specific epitopes.Lower limits of vaccinee IgE than that of controls may be attributed to either low limits tolergens or to the presence of an immunosoppressive epitopes within the BCG vaccine[5,6,7]

Immunodiagnostic Tips

1-IgE suggested to be multipotent antibody molecules.

2-IgE higher levels among BCG vaccinee childs than in controls

3-BCG might includes toleragenic and/or immunosuppressive epitopes in its antigenic make up.

Conclusion

Current essay showed an IgE association BCG vaccinee childs with possible inclusion of toleragenic and/or immunosuppressive epitope in BCG antigen make up leading to low limits IgE concentrations in some vaccinee childs.

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ESSAY - 25-II: EOSINOPHILIA

Abstract

Eosinophilia are of primary and secodary cellular immune responses.In a group of tuberulus patients and controls.Blood and sputum were collected.Blood and sputum films were made in accordance with standard methodologies.Dry blood and sputum films were stained by Leishman stain.In patients the mean numbers of eosinophils were;7.5850% and2.2600% in mucosal and systemic compartment respectively.Mucosal and systemic eosiniphilia were higher in PTB patient than that of controls.Immediate and secondary eosinophilia is being suggested inPTB patients.

Key Words

Eosinophil,eosinophilia,mucosal , primary, secondary ,systemic.

Introduction

Eosinophilia is a state of an increase of eosinophils in peripheral blood and at mucosal compartment.It is associated with wide range of;allerigic,rheumatic, infestation, infection, neoplastic and idiopathic disorders[1].Trichnella spiralis invokes a primary relatively low levels of eosinophilia,whereas repeated exposure to the invading agent results in an augmented or secondary eosiophilic response.It was graded as ;mild,moderate and severe eosinophilia[2,3].Guidelines fordiagnosis and management of eosinophilia were solicited by an expert committe of hematologists[4]

Inducers;

Allergens,parasite,microbial and neoplastic allergenic fractions.

Immune Responses

Natural cellular immune responses of eosinophilic nature[2]

Immunopathogenesis;

On exposure and re-exposure to allergen,IgE cross-link IgE receptors on the surface of mast cells and basophils,degranulation and histamin,leukotrins B4 release from mast cells and basophils.These vasoactive amins,eosinophils and IL5 lead to killing of heminth parasite by the activity eosinophil extracellular killing events .These events associated with type I hypersensitivity reactions[1]

Disease;

Allergy,atopia

Sampling;

Blood and sputa

Processing Program;Differential leukocyte counts for blood and sputa.

In an experimental setting,125 PTB patients and 25 normal human subjrcts.Standard methodologies were applid for collection of blood and sputa samples.Leishman stained blood and sputa films were subjected to differential leukocyte counts[5]

Findings;

The mean count values for blood and sputa for PTB patients were;5.7750 and 7.5850 eosinophils percentages for blood and sputa respectively.Eosinophils mean counts for normal controls blood was 2.2600,Table-2.Mucosal and systemic eosinophils wee noted in PTB patients but not in controls.

Table-2;Eosinophilia in PTB and controls

Patients and control samples	Eosinophila mean counts in patints	Eosinophilia mean counts in controls
Blood	5.7750	2.2600
Sputa	7.5850	-

Interpretations

Mucosal and systemic eosinophilia were noted in Pulmonary tuberculosis patients but not in controls.Mucosal eosinophilia were higher than systemic eosinophilia in PTB pateints.It was suggested that secretion of IL5 in patients blood implicated in the rise of mucosal eosinophilia[5,7,8].

Immunodiagnostic Tips;

1- Absolute eosinophils count is gold standard diagnostic criteria in PTB patients.
2- Balance between eosinphils production and trafficing from bone marrow to blood and other body compartments is essential for determinaions both for mucosal and systemic eosinophilia.
3- Eosinophilia ,IgE and vasoactive amins and IL5 determinations is rather acceptable battery for laboratory diagnosis of atopia.

Conclusions

Mucosal and systemic eosinophilia was determined in Pulmonary tuberculus patients.Mucosal eosinophilia were higher than systemic eosinophilia.

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ESSAY- 26 : DIABETES

Abstract

The immune state of diabetic patients were investigted via two programs.One for presence of autoantibodies which were showing the dominance of RF followed by thyroid and anti-cardiololipin auto antibodies both in DM1 and DM 2.A finding that indicate a risk for thyroditis and cardiovacular diseases among diabetic patients.Reduced non-specfic and specific cellular immune responses represented by; NBT phagocytosis and leukocyte inhibitory factor cytokines as well as reduction of the pathogen specific antibodies among diabetic as compared to non-diabetic patients.Diabetic suffers from reduced immunune and enhanced autoimmune responses.

Key Words

Antibody,autoantibody,cell ,diabetic, immune ,pathgen,specific.

1-Introduction

The diabetic syndrome is basically of two main types.The insulin dependent IDM and insulin independent diabetes IIDM.The characteristic immune features are; abnormalities in distribution of B cells,cytokine involved in B cell distribution,autoreactive T and B cells distribution as well as the insulin receptor on the surface of the cells.The immunopathogenesis of IDM can be summed up as; vuiral infection or inflammatory stimuli that activate APCs to secrete mediators that attract lymphocyte to migrate and enters pancreastic tissue.Cytokine produced by lymphocytes or APCs lead to distribution of beta cells.Islet cell antibodies,insuline auto-antibodies and insulin-anti-insulin antibodies.In IIDM,the insulin resistant DM,the disease effects is caused by the interference of antibody binding to its receptor leading to insulin resistant with the appearance of hyperglycemias and keto-acidosis[1-4].DM as systemic disease induce pathologic effects on various organ/systems of human body including vulnerability to infection and immunosuppression.

2-Inducers;

Family history,gentic predisposition,viral and inflmmatory diseases.

3-Immune responses;

Depressed humoral and cellular immune responses.Potentiation of autoreactive cells and auto-antibodies.

4-Immunopathogenesis;

Maldistribution of B cells,T cells,auto-reactive B and autoreactive T cells.As well as inhibtion of insulin receptors on the surface of the cells as in ,Table- 1.

Table 1 : Comparative Immune Features of Diabetic Types.

IDM immune features	IIDM immune features
Viral or inflammatory stimuli	Maldistribution of immune cells
Stimuli activate APCs	Interference of insulin receptors by antibodies
Activation of lymphocyte and APCs to produce cytokine excess	Antibody inhibits insulin binging to the receptors
Beta cells distribution	Insulin resistance
Auto antibody to cells and insulin	

5- Disease ;

Diabetes mellitis

6- Sampling;

i-Heparinised and non-heparinised blood samples

ii-Periodotal samples

iii- Urine sample

7-Processings:

7 – 1 ; Program One : Diabetic Auto-antibodies

In a group of 53 IDM and 50 IIDM patients and 106 controls.Blood samples were collected from brachial vein in blood collecting tubes free of heparin both from patients and controls.The samples were left to clot and then centrifuge at 2500 rpm for 5 minutes.Supernates the sera were collected and saved at – 18C till use.Sera revived to room temperature to test for a spectrum of auto-antibodies following manufacturer instructions,Table- 2.

7- 2 : Program Two : Diabetic Immune Status

Ten diabetic and ten non-diabetic dento-alveolar infected patients[1-3]. For both sexs were the attendence of teaching dental clinic during the period of Jan-2009 to Dec.2009,college of dentistry unviersity of Babylon.These patients were elected as

a test groups.Besides other ten apparently normal healthy subjects as a control group matching the age group of the patients.Aerobic microbiota of the dentolalveolar materials were mapped as in [5,6].Samples were submitted as sterile cotton swabs and emersed in three mls sterile saline in plane tubes,through mixing was done for these samples and designated as swab-salin mixtures SSM.SSM becomes lightly opaque due to the cellular and protein contents.This SSM suspensions were centrifuged at 3000 rpm for five minutes.Supernates were aspirated and tubbed in sterile clear plane tubes and designated as supernate tubes ST.Three mls of PEG 6000 6% solutions were added to these STs then left for one hr at 4C then proceeds for globulin separation as in[7,8].Tubes containing SSM deposits designated as DTs.DTs were washed once wsith sterile saline and resuspend pellets in three mls sterile saline.The deposite washed cell suspensions were mixed with three mls of dextrane solution and left for 20 minutes at room temperature[approximately 20 C].The dextrane-leukocyte upper layer was aspirated and tubbed in to plan tubes then proceeds for leukocyte sepration[9].

Heparinised and non-heparinised blood samples were collected from patients and controls[10].Standard tube agglutination test was done on patients sera and SIgs with their respective antigens[10].NBT phagocytosis was carried out as in[11].Leukocyte inhibitory factor was done both on mucosal and peripheral blood leukocytes[12].

8- Findings :

8-1:Program One;Autoantibodies

Rf autoantibodies recorded of higher percentages among diabetic patients I &II folowed by ATPoAb,AT ag Ab AcAb and ANA.The occurence percentages were ranging from 2 to 35%.

Table- 1: Autoantibodies among diabetic and controls.

Types of auto antibodies	Healthy control	DM I	DM II	Total
RF	3:106[2.83%]	19:23[35.85%]	11:50[22%]	30:103[29.13]
AMAb	0:106[0.0%]	0:53[0%]	0:50[0.0%]	0:103 [0.0%]
ATPoAb	1:106[0.94 %]	6:53[11.32]	2:50[4%]	8:103[7.77 %]
ATGAb	1:106 [0.94%]	6:53[11.33%]	2:50[4%]	8:103[7.77%]
AcAb	1:106[0.94%]	5:53[9.43%]	2:50[4 %]	7:103[6.8%]
ANA	1:106[0.94]	5:53[9.34 %]	1:50[2.0 %]	6:103[5.83%]

RF=Rheumatoid factor ATGAb=antithyroglobulin antibody

AMAb=Antimitochondrial antobody ACAb=Anticardiolipin antibody

ATPoAb= Antithyroid peroxidase ANA= Antinuclear antibodies

8-2 : Program Two: Diabetic immune state.

There were reduced phagocyte functions,reduced leukocyte inhibitory factor cytokine production and reduced anitibodies specific for bacterial pathogens as compared to non-diabetic controls,Table -2 & 3 .

Table -2: Humoral immune Status of Diabetic and controls.

Associated causals	Number of patients	Mucosal Ig concentrations ,mg/ml	Serum total protein concentration Mg/ml.	Serum globulin concentration Mg/ml.	Mucosal antibody titre	Systemic antibody titres
Diabetic S.aureus St.sanginosus	6 4	0.55 0.61	68.63 70.78	37.76 38.75	32 40	160 200
Nondiabetic S.aureus St.sanginosus	6 4	0.85 0.77	72.33 71.23	41.82 43.75	28 26	266.6 260
Control	10	0.249	66.43	36.22	2	10

Table – 3 : Cellular Immune Status of diabetic , non-diabetic and controls.

Associated causals	Number of patients	Mucosal NBT%	Systemic NBT%	Mucosal LIF	Systemi LIF	
Diabetic S.aureus St.sanginosus	6 4	27.3% 26.25%	38.33% 28.5%	0.77 0.76	0.77 0.82	
Nondiabetic S.aureus St.sanginosus	6 4	43.66 41.5	38.5 33.25	0.535 0.525	0.66 0.62	
Controls	10	9.8	10.8	0.946	0.945	

9-Interpretations;

9.1-Program One autoantibodies

Pathologic levels of autoantibodies were noted among DM I and DMII patients rated from 2 to 35% as compared to0.94% in control subjects.The most dominant autoantibody was RF followed by antithyroid,antithyroglobulin and then antinuclear factors.These findings may stands as a morker for estimating risk factors for thyroditis,cardiovascular diseases among dibetic patients[AlFahar 2000,sherwin 2000,Lindberg 1999,Hassan 2003].

9-2 :Program Two:diabetic Immune State;

It was noted that both humoral and cellular immune functions were reduced in diabetic as compared to nondiabetic and controls.Such findings was inline with other workers[20,21].And can be interpretated on the bases of one or more of the followings;i –humoral fluctuations in insulin levels , ii – impaired antibody production by B cells[20]and iii – baise or counter regulation of Th1/Th2 cells[21,22].

10- immunodiagnostic Tips;

1-Reduction of immune state due to homonal -immune function imbalance.

2-During the infection in-vivo events bacterial pathogens mounts reduced humoral and cellular immune responses.

3-Such 2-35% of autoantibodies constitute a risk factors for thryroditis and cardiovascular diseases.

11- Conclusions;

In diabetic patients,humoral and cellular arms of the immune responses expressed forms of reduced functions renders patients vulnerable to thyroid and cardiovascular diseases.

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ESSAY - 27 : ANERGY

Abstract

Anergy is either essential,genetic or secondory,aquired state.The genetic seemed to be linked to the gene GRAIL overexpression.The aquired appeared to be linked to HIV infection or other immune comporomized state like that of chronic tuberculosis.In chronic tuberculosis there is an excessive persistent antigenic stimulation leading to reduced or abolished costimulatory signal for T cells.Local past experience have shown that in adult tuberculus patients gross clinical anergy expressed as negative or reduced but, at the cellular level as reduced e rosette T cell formation,reduced leukocyte inhibition and reduced NBT phagocytosis.While in childhood,Gross clinical anergy associated with 40% of cellular anergy since the immune responses in childhood is divergant.

Key Words

Anergy ,Clinical, cellular, costimulatory ,compromized signal.

Introduction

A- Phenomemnon

When an ID tuberculin test done to BCG vaccinee human subjects,48 hr watching to the vaccinee,may showed reduced or no reaction at all.This case is known as anergy[1].

B-Conceptuations

The in-vivo interaction between an antigen and its specific cellular receptors ,the reaction may results either activation and differentiation of cell and the state is known as positive selection.Piositive selection mostly induce cell to leave the interpase state GO and inters the cell cycle,giving rise to a clone of differentiated effector cells,the" clonal selection".Or to a negative selection as the inactivation or death of cells happened.Such events is being known as "clonal deletion".In clonal deletion single

cell is destroyed,the host has lost the potentials to develope the clone of cells which recognize the epitope in quesion.Clonal selection is considered as an effective mechanism to maintain the balance of self/nonself recognition.During these selection events,when the cell is not killed but made functionally in-active,the outcome is termed as anergy[1,2].

B- The Sene of Anergy

i-When T cells recognize an antigen in a non-stimulating manner ,they becomes in-active producing a state of immune tolerance,which is specific only affecting TH cells that respond onyly to a particular antigen.Persistent tolerance without cell death leading to a state known as clonal anergy[3].

ii- Anergy is clinically defined as the abscence or reduction of DTH skin test reactivity to a commonly encountered antigens or it is a lessen reactivity of a previously positive skin DTH test[4].

iii-Anergy is the immune hypoactivity in an expected immune reactive sensitized individuals.It includes both failure to express DTH and hypoactive anergic B cells[5].Hence clinical anergy is a gross organ tissue reaction,and cellular anergy covering both T and B cells[5,6].

iv- T cell anergy is one of other mechanisms thought to act in the prephery to ensure tolerance to self.Anergy is being a T cell clones rendered unresponsive to subsequent re-stimulation by the first antigen activating them through TCR signal 1 without apropriate co-stimulation[7].

v-Characteristics of Anergic T cells

Anegic T cells express an array of biological characteristics as

a-Defective in the capacity of TCR to couple to protein kinase

b-Defective coupling capacity of TCR to protein kinase reduces transport factor-activating protein AP-1 expression[8].

c-Defetcive AP-1 lead to defective IL2 secretion which lead to anergic cells

d-Overexpression of the gene GRAIL reduces IL2 which in turn induce T cell anergy[7]

e-IL10 secretion induce anergy by down regulatory molecules on APC.

vi-Characteristics of Anergic B cells[9]

Anergic B cells have the following characteristics

a-Negative in surface IgM expression

b- Attenuated function

c-Naturally foun on induced autoreactivity

d-Tolerant B cells

e-Irresponding to scond co-stimulatory signal.

D-Anergy In Chronic Tuberculosis

In anergic pulmonary btuberculus patients,there may be a sustained M.tuberculosis derived antigenic stimulation of the naive T cells which results in IL10 but not INF gamma production that mediated the generation of anergic Mtb.specific T cells with an immunosuppressive properties.In the presence of IL10,the Mtb infected host becomes tolerant to Mtb antigens[10].The development of Mtb specific T cell anergy due to the stimulation in the abscence INFgmma and presence of IL10[11].

Inducer

Chronic intracellular bacterial antigen stimulation to the immune cells and their sequalae

Immune responses

T cell mediated immune responses with DTH and granuloma responses.

Immunopathogenesis

Droplet or inhalation M.tb.infection lead the microbe to find port of entry to the upper respiratory then lower respratory tract,gain foot hold through multiplication.Mtb.taken up by macrophage processed through Cd1 pathway for lipoidal antigen processing mechanisms and presented to naive T cells to be activated to cytokine producing TH1 and /or Th2 T cells.Over cytokine production and production of reactive O intermediates leads to DTH tissue injury and in late course of the responses to granuloma formation characteristics of tuberculus pneumonia through theChronic antigenic stimulation finalized by anergic tuberculosis[4].

Disease

Pulmonary and miliary tuberculosis.

Clinical samplings

Blood, and sputum

Processings

Program 1: Adulthood Tuberculus Anergy

From a 82 anergic ,43 allergic tuberculus patients and 23 control,sputum and blood samples were collected .Sputum samples were processed for leukocyte

differential count an AFB.Tuberculin DTH and BCG scar were performed[4].Blood samples were collected in anti-coagulant containing blood collecting tubes for NBT[12],Erostte [13],and LIF[15].

A-NBT[12]

i-Dissolve 0.2 gm NBt in 100 ml sterile saline,stor in dark bottol at 4C

ii-Mix 0.1 ml fresh blood with 0.1 ml NBT,incubate at 37C in room temp,then for additional15 mint,then make blood film

iii- Wash the remaining,dry

iv-Examine in oil immersion lense and count the formazan stippling leukocyte among100 leukocyte .Recod the percentages.

C- E-rosette[13]

i-Separate the lymphocyte by adding 1-2 ml. ml blood carefuly layerd to 3 ml lymphoprep solution in a tube without mixing.

ii- Centerfuge at 400 g for 30 minutes at room temp ,buffy coat found at the interface

iii-using Pateure pippete collect carefully the buffy coat white cells then add to other tube

iv-Re-centerifuge at 400 g for 30 minutes,the wash three times with PBS at 400g for 15 mnit each wash.

v-The pellet was resuspended in 0.5 ml. saline to which 0.05 ml autologous plasma was added

vi- Mix gentley 0.1 ml of plama-lymphoprep mix with 0.1 ml sheep RBC,incubate at at 27C for 15 minutes the centrifuge at 200g for 15 minutes.

vii- Re-incubate the mixture at 4C for 1 hr. With supernate still on pellet.

viii-Re-suspend the mixture in the tubes gently back and forward up to 2 to 3 times,the make blood smears, fix with ethanol for 10 minutes and dry in air.

ix- Stain with Geimsa stain,by flooding the slide with stain for 10 minutes,wash the stain and dry

x- Examine in oil immersion lense and calculate the e-rosette forming lymphocytes among 100 lymphocytes and calculate the percentages.

D- LIF[15].

i-Using haparinized capillary tubes,boold was drawn in duplicate from the blood collecting tubes.

ii-Close one of the ends with plasticine

iii- Centerifuge with hematocrit centrifuge for 5 minutes.

iv-Brok the capillary at the buffy coat interface.

v- Cut a 15 mm diameter wells in an agar –agar petri-plates.

vi- Apply the cutted capillaries in to agar gell wells.

vii-Add 50 ul of Eagle's medium and 50 ul sensitizer antigen,tuberculin, and 50ul saline to the control well

iix- In a humid jar,the plates were incubated in an upright position overnight at 37C

ix-read the migration with and without sensitizor by optical reader.

$$\text{x- calculate Lif\%} = \frac{\text{Distance with sensitizor}}{\text{Distance without sensitizor}} \times 100$$

Inhibition more than 30 % is significant.

Program 2: Childhood Anergy

i- In a population of 90 childs containg ;30 anergic,30 allergic and 30 controls
ii- Using standard simple randum sampling,15 from each group was selected
iii- Collect blood from brachial vein in an anticoagulant containing blood collecting tubes
iv- Save sera at -18C in an appendroph tubes till use
v- Process for eliza Anti Mif and anti-IL2 for each of the groups
vi- Record color change and compare it with that of control as in the manfacturer instruction
vii- Calculate the MIF and IL2 concentrations in each case.

Findings

Program 1;

Anergic pulmonary tuberculs adult patient group have shown lower NBT,E rosette and moderate LIF percetages[ ].,Table- 1.

Table – 1;Anergy parameters in adulthood.

Patient groups	NBT%	E rosette %	LIF%
Anergic**	5.41*	14.260	52.27
Allergic	7.11	20.29	36.16
control	11.69	25.78	85.34

*Mean values

**The intergroup significance of the t 1+1 statistics between;Anergic-Control,Allergic-cntrol, and Anergic-Allergic were ranging beween high signifcant to Significant at P value of 0.001 to 0.05.

Program 2

The childhood tuberculus clinical gross anergy in BCG vaccinee were associated with 6:15,40% cellular anergy and 9:15,60% cellular allergy.Clinical allergic BCG vaccinee childs have shown 8:15 53.33% cellular anergy and 7:15,46.66% cellular allergy.Anergy state to BCG vaccine have divergent cellular immune responses[ ],Table-2.

Table – 2 : Anergy Parameter in childhood.

Subjects	MIFng/ml.	Reduced reaction	IL2 ng/ml.	Reduced reaction
anergic	12.341	6:15,40%	988.4	5:15,33.33%
Allergic	9.783	8:15,53.33%	887.2	5:15,33.33%
Control	6.459		548.8	

Interpretation;

Program one; It was intended to investigate anergy state in tuberculus patients in an adult population.Program-2, undertook anergy in childhood BCG vaccinee .The results in adulthood have shown homogeneity among anergic and allergic patients.While in childhood vaccinee there were a heterogeneity and divergence in the immune responses to the BCG vaccine.Sine only 405 of clinically anergic wer showing cellular anergic expressions and 60% were showing allergic cellular reactions[Yan et al.2013].,Comparative insight of the findings in both of the programs with other worker dipicted in Table - 3 .

Table – 4: Anergy Criteria in tuberculosis.

Patients groups	Test battery	Conclusion	references
Adulthood tuberculosis anergy	Tuberculin,LIF,e-rosette,NBT	Negative,reduced Lif,e-rosette and NBT%	AL-Sadi et al2007
Childhood BCG anergy	BCG scar,MIF,IL2	Negative,MIF and IL2 lower than control	Karim and Shnawa 2022
Adulthood tuberculosis anergy	Tuberculin,inhibitory cytokines,T cells and apoptosis	Negative tuberculin,enhanced inhibitory cytokines ,reduced V gamma2	Yan et al 2013.

		T cells,ehnahced apoptosis	

Immune Diagnostic Suggested Tips

From the fore going paragraphs one can make an excerpt of an eight immunodiagnostic tips.

Table-5 : The Immunodiagnostic Tips for tuberculus anergy

Immundiagnostic Criteria suggeste for Anergy
i-Skine test,BCG scar,tuberculin
ii-NBT %
iii-E-rosette T Cell counts
iv-Flow cytometery for CD4+T cells, gamma delt T cells
v- Classical LIF and MIF
vi-IL2,Il10,INFgamma,MIF cytokine eliza determinations
vii-Determination AP-1 levels
iix-determination of gene expression profile of the gene GRAIL.

Conclusion

Anergy is a state of clinical reduction or abolising of gross skin reaction as well as reduction or abolishing of cellular reactivity to the antigen in quesition.It is attributed to;failure of secod co-stimulatory singnal in T or B cell , and or over expression of the gene GRAIL.

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