BCG & tuberculin induced experimental lapin secondary cryoglobulinemia

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الخلاصة

جرى استخدام كل من BCG والتيوبيركلين بوصفهما منبهان مناعيان محتملان لاستثارة الاستجابة المناعية الباردة في نموذج الأرنب . وتضمنت الدراسة برامج التمنيع وفرط التمنيع ب BCG والتيوبيركلين من خلال الاستجابة المناعية الأولية والثانوية . وكانت قرائن التعرف على الاستجابة المناعية الباردة من خلال نسب الكرايوكريت في الترسيب المتعاكس بدرجة 4مْ والذوبان بدرجة 45مْ .حيث تبين ان التيوبيركلين وBCG منبهان مناعيان جيدان في استثارة الاستجابة المناعية الباردة في الأرنب .وظهرت زيادة الكلوبيولين البارد المحتثة تجريبيا في الأرنب ثانوية مختلطة مشتركة أو غير مشتركة مع عامل الرثوانية ويمكن تصنيفها من صنف II-III وبناءا على ذلك فان هذا النموذج التجريبي لزيادة الكلوبيولين البارد يوفر دعم لزيادة الكلوبيولين البارد العفوية في مرضى التدرن الرئوي .

Abstract

BCG and tuberculin were used as a possible immune stimulants for cryoresponses in a lapin model .Immunization and hyperimmunization protocols both for primary and secondary cryoimmune response were attempted .Cryocrit percentage ,protein nature through biurete reaction , ratio of cryoglobulin to total globulin of immune and hyper immune sera as well as reversible precipitable at 4c ,and solubility at 45c were identification criteria for cryoglobulin. Both BCG and tuberculin 0.05IU were found good stimulants for cryoresponse . The cryoglobulinemia was found associated and non associated with IgM anti IgG autoantibody (RF) in tuberculin case while, it was non RF associated in the case of BCG. BCG and tuberculin induce secondary mixed cryoglobulinemia of IgM-IgG-IgA type. The proposed classification was II-III class. This model stands as supportive model to the natural human mixed cryoglobulinemia associated with Pulmonary tuberculosis.

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Introduction!

Rabbits immunized with a poly valent of *St. pneumoniae* have produced in their sera large amount of cryoglobulins belonging to classes of IgM & IgG (1). While in a murine model. when mice injected with 1000μg of neutral *K. pneumoniae* polysaccharide (CPS-K).cryoglobulin was detectable in four days after injection and were most prominent after 6-10 day after injection .They were still detectable to 90 days after injection (2). Rabbits immunized with group B or group C Streptococcus show that rabbit given secondary immunization produce cryoglobulin earlier and in larger amount than rabbits given primary immunization (3). Monoclonal IgG3Rf induced cryoglobulin in vivo in murine model (4). An experimental induction of murine vasculitis induced by monoclonal antibody in which temperature sensitive immunological interaction between Fab region of cryoprotien and antigenic epitopes on the same molecules i-e an auto antibody – like reactions (5). Wolinella succinogenes membrane bond hydrogenase in concentration of 100μg were used for immunization of mice to induce IgG3 monoclonal antibody leading to cryoglobulinemia (6). Kowalewska et al 2007have been characterizing the role of cryoglobulinemia in the development of liver disease in Thymic Stromal lymphopoietin transgenic mice that mixed cryoglobulinemia and develop hepatitis .Mice implanted with hybridoma secreting 6-19 IgG3 antiIgG3 Rf with cryoglobulin activity develop a cute glomerulonephritis and vasculitis (7).The aim of the present work was at using tuberculin protein and BCG as possible immunostumilants for cryoglobulinemia in rabbits

Materials and methods

1- Stimulants :

The Purified Protein Derivative PPD the tuberculin ,the make of Sanofi Pasteur limited in a dilution of 0.05 IU and Bacill Calmett Gurien BCG of *Mycobacterium tuberculosis* , the make of Japan BCG laboratory in a dilution of 1/5 were used as stimulants for lapin cryoglobulinemia.

Lapin Animal Model :

Rabbits(*O. caniculus*) local bread brought from local market and adapted for housing condition for two weeks kept *ad. Libitum* of both food and drink .They were checked for and found to be free of pyrogenes and parasitis.

3-Experimental Design:

The experimental designe including:

i-Test for base- line cryoglobulin quantity in non treated and in saline! treated rabbits.

ii- Test for cryoresponse in primary immune response using single IM , single multisite Sc immunization protocol .

iii- Test for cryoresponse in secondary immune response using hyperimmunization programme of multisite injection and nasal applications (Inhalation)

4**- Characterization of Cryoglobulins:**

All of the scientific precusein for detection of serum cryoglobulins were in consideration as in (8). cryocrit ,cryocrit percentage ,cryoglobulin separation detection of cryoRf and determination of cryoglobulin classes were made as in(9).

Results

1- Cryoproteins:

Cryoprotein response in the sera of tuberculin immunized rabbits were found of two type . One major occurring as cryopricipitable-reverse dissolvable and one minor cryoprecipitable – no dissolvable. While these rabbits with BCG gave only the one major occurring cryoglobulins.

2- Cryoglobulin Responses :

The healthy normal Subjects were rabbit were of negative cryoglobulin responses . Like wise , two weeks adaptation with or without saline injection were of negative cryoglobulin responses .Secondary cryoresponses gave higher cryocrit percent and higher cryoglobulin concentration (Tables 1 , 2). Prolonged tuberculin or BCG exposure rabbits showed high cryocrit% and higher cryoglobulin concentration (Table(1&2)) cryoglobulin/ Globulin concentration ratio were mostly one third (Table2).

3- Cryoglobulin Nature :

The onset for first appearance of cryoglobulin in lapin fresh sera were ranged between 2-5 days .The dissolving time were ranged between 1-2.30 hrs. while dissolving temperatures were ranged 37-45c (Table 1) .This both for tuberculin and BCG immunized rabbits

4- Autoimmune and Cryoresponses:

Higher cryocrit% are associated with high IgM anti IgG autoantibody titers(Table 4). And with high IgM – IgG –IgA concentrations (Table 5) in tuberculin immunized rabbits only.

5- Immunoglobulin Immunofixation for Cryoglobulinemia

Mixed secondary cryoglobulinemia with IgM – IgG – IgA induce by tuberculin (Table 5).It is of RF and non RF association .It can be of type II –III mixed cryoglobulinemia for tuberculin immunized rabbits . While for BCG immunized rabbits were not associated with Rf (Table 5).

Table 1: Lapin Cryoglobulin dissolving time and dissolving temperture. !!

Replicate		Dissolving time single IM (4)		Dissolving time multiple Sc once (4)		Dissolving Time multiple Sc & IM(8)		Dissolving time Nasal (8)	
		15 D	**30 D**	**15 D**	**30 D**	**15 D**	**30 D**	**15 D**	**30 D**
I	R max	1.30	2	2	2.3	1.45	2	2.30	2.30
	Rmean	1.15	1.37	1.37	1.10	1.35	1.45	1.20	1.50
	Rmin	1	1	1	1	1.20	1.15	1.30	1.15
II	R max	2:0	2	3	2.3	2.30	2	2.30	2.15
	Rmean	12.5	1.23	2.4	1.55	1.55	1.42	1.48	1.41
	R min	1.30	1.15	2	1.30	1.30	1.15	1.20	1.30
Replicate		Dissolving Temp.(5)		Dissolving Temp.(5)		DissolvingTemp.(8)		Dissolving Temp.(8)	
		15 D	30 D	15 D	30 D	15 D	30 D	15 D	30 D
I	R max	45	45	45	45	45	45	45	45
	Rmean	45	45	43	45	43	45	45	45
	R min	37	45	37	45	37	45	45	45
II	R max	45	45	45	45	45	45	45	45
	Rmean	45	40.34	40.1	40.1	45	45	45	45
	R min	45	37	37	37	45	45	45	45

I = Tuberculin.
II = BCG

Table 2: The ratio of cryoglobulin to globulin in immunized and hyperimmunized rabbits.

		Cryoglobulin : Globulin!!	Ratio(%)
Single IM	Tuberculin	39 : 89	!1 : 2)
	BCG	48:44	(1: 3)
Single Multiple Sc	Tuberculin	19.48 : 48.24	(1: 3)
	BCG	23.64 : 43.48	!1 : 2)
Multiple Multisite	Tuberculin	24.32 : 49.72	(1 : 2)
	BCG	31.88 : 55.6	!1: 2)
Nasal	Tuberculin	33.84 : 58.66	(1 : 2)
	BCG	39.32 : 14.94	!1 : 3)

Table 3: Cryocrit % of lapin immune and hyperimmune sera with 0.05 tuberculin or 0.5 BCG.

Treatment groups		Single IM		Single Multisite Sc		Multiple Multisite IM& Sc		Nasal	
		15 D	30D	15D	30D	15 D	30D	15 D	30D
Tuberculin	R max	5.2	7	11.2	10	16	20.8	28	32
	Rmean	5	5.2	9.2	7	9.1	15.84	20.8	26.94
	R min	4.6	5.1	7.2	5.2	5.2	8	12	20
BCG	R max	5.2	7.2	20.4	24	16	24	16	25.2
	Rmean	4.7	5.8	15.90	14.36	12.56	21.36	13.6	17.96
	R min	4	4.8	10	14	9.2	16	8	16.8

Table 4: Cryoglobulin classes and Rf association in lapin model after tuberculin treatment .

Rout	Rf/titer	IgM conc.	IgG conc.	IgA conc.
Subcutaneous	+/ 16.666	2.065	8.189	2.455
		1.073	7.739	0.782
Nasal	/ 7.714	1.603	9.377	2.279
	_	1.471	3.748	1.138

Table 5: Immunofixation of cryoglobulinemia in hyperimmunized with Tuberculin and BCG.

Groups		IgM		IgG		IgA	
		15 D	30 D	15 D	30 D	15 D	30 D
Tuberculin Sc IM	R max	1.124	2.053	8.773	13.117	3.283	3.063
	Rmean	0.645	1.477	5.326	7.836	1.776	1.410
	R min	0.028	0.745	1.117	2.95	0.028	0.128
Tuberculin Nasal	R max	2.011	4.085	13.117	14.293	2.680	3.036
	Rmean	0.671	1.562	5.507	7.133	2.981	1.987
	R min	0.162	0.162	0.778	1.117	0.063	0.795
BCG SC, IM	R max	1.621	2.789	13.117	16.126	3.798	4.623
	Rmean	1.004	1.883	6.654	10.623	2.532	3.655
	R min	0.745	1.224	0.448	5.448	0.334	2.283
BCG Sc, IM	R max	2.011	2.011	15.506	16.126	2.564	3.798
	Rmean	1.025	0.898	9.222	10.422	1.026	1.672
	R min	0.028	0.028	4.158	5.009	1.128	0.062

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Discussion

Cryoproteins are of several types . They includes ;cryo acute phase , cryocomplement , cryofibrin , cryoglobulin and cold antibodies . Among these only cryoglobulins are of reversible perceptibility. In the present work one major reversible and one minor irreversible cryoproteins are reported (Table 1-4). The major reversible one was with following characteristics :

i- Tuberculin or BCG induciable cryoprotein.
ii- Secondary response gave higher cryocrit % and cryoconcentration both for tuberculin and BCG immunized rabbits .
iii- Reversable Cryopercipitability .
iv- Rf and non Rf associated in tuberculin while non Rf associated in BCG treated.
v- Prolonged exposure associated with high cryo% and cryoconcentration
vi- It is secondary mixed cryoglobulinemia of IgM – IgG – IgA .

Thus , it is a case of experimental mixed secondary cryoglobulinemia induced by 0.05 IU tuberculin and 1:5 BCG using different immunization protocol (Table 1-7) The proposed cryoglobuli response molecular mechanism for that RF associated is tuberculin or BCG has some epitopes are cryoglobulin active leading to monoclonal antibody including cryoglobulin in an autoantibody like mechanism (5).

While for non Rf associated cryoglobulinemia the proposed molecular mechanisms can be through thymic stromal lymphopoietin like , an interlukin 7 like cytokines with B-cells promoting properties ,produce large amount of circulating cryoglobulin of mixed Igs compositions (10). Various stimulants have been tried to produce lapin cryoglobulinemia ,such as *St. pneumoniae* (1) , Sterptococcus group B and C (3) and *K . pneumoniae* capsular poly saccharide (2) .Similarly, in murine model , the mice, bacterial membrane bond hydrogenase has been proved as stimulants for murine cryoglobulinemia (6) . Others have been used monoclonal antibody IgG3 anti IgG3 Rf for induction of cryoglobulinemia(7,10) . Let us put down in a comparative sense the characteristic features of human and rabbits cryoglobulin (Table 6).Thus there are basic similarities between human and rabbits cryoglobulins(

11) . Likewise there are basic similarities in the over all structures and functions of the rabbit and human immunoglobulins for classes IgM , IgG and IgA , bearing in minds the exception of subclass numbers, allotypes and idiotypes differences (12 , 13 ,14 ,15, 16,17 , 18, 19,20,21). Mean time there are marked reaction affinity between human and rabbits Rf (22 , 23,24). Thus , the determined immunoglobulin classes and RF association tables 1-8 are relative estimates and are of qualitative rather than quantitative issue . It appeared that the nature of this experimental cryoglobulinemia in the lapin model is secondary mixed with or without RF association and stayed as class II –III .Thus tuberculin 0.05IU and 1:5BCG are being reported for first time as stimulants for lapin cryoresponses.

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Table 6: Comparative view to human and rabbit cryoglobulins .

	Gehori	
Onset	2-7 Days	2-7 Days
Dissolving temperature	37c°	45c°
Reversible precipitation	+	
RF association	–/+	–/–
Chronicity	+	–
mixed	+	–
Secondary	+	–
Natural occurrence	ı	?
Experimental	?	–
Occurrence in serum	+	–
Occurrence in urine	ı	?

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Table 7 classes and subclasses of human and rabbit immunoglobulin !

Species	Classes and Subclasses
Man	IgG1-IgG2-IgG3-IgG4 IgM IgA1 –IgA2
Rabbit	IgG2__ _ IgG1 IgM IgA1 – IgA2

From (25).

Table 8: percent amino acid sequence homology of CH4 and Cκ

!!Human	!!γ (IgG1)	α (IgA1)	!!μ(IgM)	Cκ
Rabbit γ	67	30	31	-
Rabbit Cκ				45

From Nisonoff 1982

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