

## Cryoglobulin Induction in Rabbits by Endotoxin Injection Experimentally

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**Abstract :** Cryoglobulin was induced by injection of endotoxin (lipopolysaccharide) into four groups of rabbits by Intramuscular (I.M.) Intravenous (I.V.), I.M. & I.V. routes. This experiment revealed induction of acute phase protein in range mean (33.0-45.4) and cryoprotein concentration mean (60.5-63.2) and cryocrit percentage mean (7.8-13.2) and increasing of Rheumatoid Factor in all rabbits except in rabbits control and also succeeded in fixation the kind of cryoglobulin to reveal the type secondary mixed cryoglobulinemia of IgM-IgG-IgA. So this is leading to probability used cryoglobulin as marker for bacterial diseases.

### Introduction

Cryoglobulin composes of immunoglobulins and complement components and precipitates upon refrigeration of serum and plasma, the first description of the cryoprecipitation phenomenon belongs to Wintrobe and Buell in 1933 when they could describe a patient with signs and symptoms of hyperviscosity associated with multiple myeloma<sup>1</sup>. Cryoglobulinemia is characterized by the presence of cryoglobulins in the serum which precipitate at temperatures below 37°C and redissolve on rewarming<sup>2</sup>. It may refer to a clinical syndrome of systemic inflammation which associated with HCV infection, skin purpuric lesions, also in non-healing vasculitic leg ulcers, and in the peripheral neuropathy, these effects caused by cryoglobulin-containing immune complexes<sup>3</sup>.

Cryoglobulinemia may be classified based on cryoglobulin composition with the Brouet classification into the follows: Type I cryoglobulinemia, or simple cryoglobulinemia, is the result of a monoclonal immunoglobulin, usually immunoglobulin M (IgM) or, less frequently, immunoglobulin G (IgG), immunoglobulin A (IgA), or light chains. Types II and III cryoglobulinemia (mixed cryoglobulinemia) contain rheumatoid factors (RFs), which are usually IgM and, rarely, IgG or IgA. These RFs form complexes with the fragment, crystallizable (Fc) portion of polyclonal IgG. The actual RF may be monoclonal (in type II cryoglobulinemia) or polyclonal (in type III cryoglobulinemia) immunoglobulin. Types II and III cryoglobulinemia represent 80% of all cryoglobulins<sup>4</sup>.

Monoclonal cryoglobulinemia is almost invariably associated with a well known haematological disorder and is frequently asymptomatic. Similarly, circulating mixed cryoglobulins are often detected in many infectious and systemic disorders<sup>5-7</sup>. There was a relationship between cryoglobulinemia and chronic infection due to gram negative bacteria such as brucellosis and typhoid<sup>8-9</sup>. Endotoxin represented by lipopolysaccharide (LPS) has the ability to induce cryoglobulins in mice<sup>10</sup>.

The aim of this study proving the ability of LPS extracted from E.coli to induce cryoglobulin in rabbits as animal model and to relate between this result and some bacterial infection and ability to use it in clinical test and to open the door for further studies in this field.

## Materials and Methods

### Animal models

In this study 12 local rabbits (*Oryctolagus cuniculus*) males were used in immunological experiment, with age between ( 8-9) months and weighing between (2-3.75) kg were kept in animal house of biology department, Babylon university, Iraq.

### Experimental Protocol

12 rabbits were tested for cryoglobulin response by injection LPS. These rabbits were divided into 4 groups 3 rabbits in each group, 1<sup>st</sup> group was injected Intramuscular (I.M.) the 2<sup>nd</sup> group was injected Intravenous (I.V.) and the 3<sup>rd</sup> group was injected I.M. and I.V., while the 4<sup>th</sup> group which was representing the control was injected with distilled water.

### LPS source

The LPS was extracted from *Escherichia coli* which was isolated from urinary tract infection<sup>11</sup>.

### Sera Collection and Diagnosis

5 ml of blood sample were collected from each rabbit in the 4<sup>th</sup> group, clotted then sera were separated and stored at -18°C. Then Sera were tested for cryoprecipitation, acute phase protein and immunofixation<sup>12</sup>.

### Serum Cryoglobulin Responses

The 6<sup>th</sup> standards suggested by Lunch<sup>13</sup> were done and followed by determination of cryoglobulin. The essential inclusion criteria were precipitated in (1-7 days) at 4°C dissolved at 37°C or 45°C within 1.5-2.5 hours and reprecipitated at 4°C.

### Biuret Positive

The texture was gelatinous and crystalline as well as determination of cryoglobulin isotype<sup>14</sup>.

### Immunofixation

Immunodiffusion plate by using radial (from LAT.s.r.l. Milano) and the cryoresponse showing mixed cryoglobulin of IgM, IgG, IgA<sup>15</sup>.

### Count of Rheumatoid Factor (RF)

This count was done by using RF-latex kit. (Spinreact S.A. contra Santa Coloma 7E 17176 SANT)<sup>16</sup>.

## Results

**Table 1 Acute phase protein in serum of injected rabbits**

Injection route	Mean of concentration	Titer
Intramuscular (I.M.)	45.4	7.6
Intravenous (I.V.)	43.4	7.4
I.M. & I.V.	33.0	5.7

**Table 2 Cryoprotein in serum.**

Injection route	mean	Low limit	High limit
(I.M.)	63.2	53.2	68.9
(I.V.)	62.6	50.7	64.5
I.M. & I.V.	60.5	55.6	69.2

**Table 3: Cryocrite percentage**

Injection route	mean	Low limit	High limit
(I.M.)	7.8	4.2	8.5
(I.V.)	9.4	6.0	10.2
I.M. & I.V.	13.2	11.3	14.2

**Table 4: Rheumatoid Factor (RF)**

Groups of rabbits	Dillutions	Titer	Concentration
R1	1/8	8	64
R2	1/8	8	64
R3	1/16	16	128
R1	1/8	8	64
R2	1/4	4	32
R3	1/8	8	64
R1	1/2	2	16
R2	1/8	8	64
R3	1/8	8	64

**Table5 Fixation the kind of cryoglobulin in serum of rabbits immunized by LPS.**

Concentration of Ig.(mg/ml)	After 7 days	After 15 days	Range of concentration
IgM	1.88	1.00	1.44
IgG	10.42	9.22	9.82
IgA	2.53	3.74	3.14
IgM	1.02	0.90	0.96
IgG	6.64	12.05	9.35
IgA	1.63	1.67	1.35

## Discussion

In this study we try to asses the ability of LPS to induce cryoglobulin experimentally in serum of rabbits as in other studies worked on this field<sup>17-18</sup>. Immunoglobulin as well as cryoglobulinare proteins from glycoproteins<sup>19</sup>. and the cryoprotein is globular<sup>20</sup>. and the cryoprotein concentration is marker(probe)for the ability of LPS to induce cryoglobulinemia as in other work <sup>21</sup>. The cryocrit percentage is probe of cryoglobulinemia in immuized rabbits<sup>22</sup>. The increasing of cryoglobulin referes to ability of LPS to increase the production of cryoglobulin by B cells and memory T- cells <sup>23</sup>.So this is agree with other studies which proved the increasing of cryoglobulin in Lapin animals<sup>24-25</sup>.the increasing of Rheumatoid Factor (RF) is proved of depletion of antigen(LPS)<sup>26</sup>.and all the cryoglobulin are consisting of immune complexes containing RF called mixed cryoglobulin<sup>27-28</sup>.so this study presents more than kind of Immunoglobulin (IgM-IgG-IgA)revealed that the experimental cryoglobulinemia was of secodary mixed cryoglobulinemia of IgM-IgG –IgA type in accordance with other study<sup>29</sup>.

## Conclusion

The ability of LPS to induce secodary mixed cryoglobulinemia of IgM-IgG –IgA type in animal model so this may lead to use such type of cryoglobulinemia in the clinical test .

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