Production and characterization of polyclonal Antibodies against Interferon Alpha in Mice

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Abstract

The polyclonal antibodies are used extensively for research purposes in many areas of biology, such as immunoprecipitation, histochemistry, enzyme linked immunosorbent assays (ELISA), diagnosis of disease and western blots. Typically, an animal’s immune system will generate a large group of antibodies that recognize several epitopes of a particular antigen. Interferon alpha plays an important role in immune response activation and therefore is of interest in studies related to autoimmune diseases. In this paper the production of antibodies against interferon was studied in order to quantify interferon production to analyze interferon levels in autoimmune disorders in the future. For the antibody production, one month old laboratory grade mice were injected with interferon alpha in combination with a Freund’s complete adjuvant for a course of five weeks after
which the antibodies were obtained in mouse serum. Confirmation of the production of anti-interferon alpha antibodies was carried out by the Elisa, immune dot blot and western blot analysis. An interferon alpha of approximately 20.5-21.5 KDa was detected in immunedot blot test. These antibodies may be produced in these mouse models commercially and could be used in future for treatment of autoimmune diseases by managing the interferon levels in the patients.

**Key words:** Interferon, polyclonal antibodies, mice, ELISA, Imunodot blot

1. **Introduction**

Antibodies (immunoglobulins) are glycoproteins naturally produced in response to invading foreign particles (antigens) such as micro-organisms and viruses [1]. They play a critical role in the immune system’s defense against infection and disease. Antigens recognized and bound by antibodies can be proteins, carbohydrate, bacterial or viral cell surfaces, but they may also be distinctive molecules found expressed on cancerous cells. The region of an antigen that interacts with an antibody is termed the epitope [2]. Typically, an animal’s immune system will generate a large group of antibodies that recognize several epitopes of a particular antigen. Each antibody is secreted by a different antibody-producing plasma cell. As the antibodies found in serum are collectively produced by many plasma cells (clones), they are described as polyclonal [3].

These polyclonal antibodies are used extensively for research purposes in many areas of biology, such as immunoprecipitation, histochemistry, enzyme linked immunosorbent assays (ELISA), diagnosis of disease and western blots. Polyclonal antibodies are ideally suited for use in sandwich assays as second stage antigen detectors [4].

Polyclonal antibodies are normally generated in a mammal’s body after inoculating the organism with the antigen after a specified time interval. Frequent introductions of the antigen along with suitable adjuvant initiates an immune system in the body that results in B cell activation and thus production of polyclonal antibodies [5]. Larger mammals are often preferred as the amount of serum that can be collected is greater. An antigen is injected into the mammal. This induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen the mammal’s serum is used for the purification of polyclonal antibodies [6]. The aim of production of polyclonal antibody
is to get high titer, high avidity and specificity against different epitopes of antigen [7]. Antigens with molecular weight less than 10KDa are unable to activate immune system. These antigens are conjugated with some carrier molecules (such as bovine serum albumin or keyhole limpet haemocyanin) and these molecule trigger antibody production against both antigen and carrier molecules. High titer of polyclonal antibody depends upon phylogenetic difference between the donor of antigen and the recipient of antibody production. A species with high distance will produce more specific polyclonal antibodies [8]. Antigen must be pure in order to avoid production of antibody against impurities and activation of non-specific immune response. For production of potent antibodies, it is usually necessary to use an adjuvant as part of the immunogen. These substances potentiate immune response by forming a slow release depot of antigen, by stimulating T – cell helper and antibody production [9]. Freund’s and titermax are most commonly used adjuvant for polyclonal antibody production. Two types of freund’s adjuvant are used: freund’s complete adjuvant that is used for only primary immunization and freund’s incomplete adjuvant for booster immunizations. FCA contains non metabolizeable mineral oil and heat killed mycobacterium while FIA adjuvant contains only non metabolizeable mineral oil [10]. Doses of antigen depend upon the nature of antigen and route of immunaization. Too high or too low doses can results in immunological tolerance and deviation of immunological response toward T-cell response from antibody production [11]. Antigen can be injected by different routes like subcutaneous, intramuscular, intravenous, intradermal or intraperitoneal. After primary injection and possibly further booster injections, the blood is collected and centrifuged to remove blood cells and clotting factors [12].

Polyclonal antibodies work well in many technical applications. Since they are able to recognize several different epitopes on the same target protein, because they contain a mixture of antibodies, problems with masked or denatured epitopes can be avoided [14, 10]. This kind of problem occurs during immunohistochemical staining of tissues where cross-linking of proteins often leads to antibody binding sites being inaccessible. Also, in SDS-PAGE dependent applications, such as Western blotting, most proteins are denatured, thus destroying many epitopes [13].

In this process interferon α was used as an antigen to generate anti interferon antibodies. The reason for generation of anti-interferon antibodies was to study the interferon levels of production in autoimmune diseases and find a correlation of the interferon alpha with the autoimmune diseases [14].
Normally interferons are released in the immune system as a response to detection of viral antigen in the body so that proper immune system response can be generated according to the viral entity. In autoimmune diseases, these interferons are released by detecting the body’s molecules as viral entities and thus carry out an unjustified immune response on the body’s organs by detecting them as viral bodies [13]. This study was carried out to study the production of anti-interferon alpha polyclonal antibodies that can be further used in research for treatment of autoimmune diseases to manage the elevated interferon alpha levels.

**2. Methodology**

Mice (one month old) used were laboratory grade white mice obtained from department of Zoology, university of the Punjab. Mice were kept in animal house of institute of biochemistry and biotechnology, university of the Punjab, throughout the duration of the immunization schedule [14].

**2.1 Immunization Schedule**

Interferon alpha injection having dosage of 6million IU per 3.8ml was used. As such high dose in not suitable for mice therefore 50µl of the injection suspension i.e. 78,947.36IU of antigen was used in combination with 50µl Freund’s complete adjuvant (FCA) and Freund’s incomplete adjuvant (FIA). Immunization was carried out through a period of 4 weeks in which a constant concentration of interferon alpha was injected into the subcutaneous skin of the mice. After the immunization was complete the mice were subjected to cardiac puncture to obtain blood and produce serum that contained the anti-interferon alpha antibodies [14].

The schedule and details of immunization process are as given in Table 1.

| No. on immunization | Date of immunization | Route of immunization | Amount of Antigen (Interferon Alpha) | Adjuvant | Dose |
|---------------------|----------------------|-----------------------|-------------------------------------|----------|------|
|                                    |     |                |               |                          |                       |
|------------------------------------|-----|----------------|---------------|--------------------------|-----------------------|
| 1º immunization                    | 17-10-2013 | Subcutaneous | 78,947 IU | Freund’s complete adjuvant | 50ul Ag + 50ul FCA    |
| 1º booster immunization            | 24-10-2013 | Subcutaneous | 78,947 IU | Freund’s incomplete adjuvant | 50ul Ag + 50ul FIA    |
| 2º booster immunization            | 31-10-2013 | Subcutaneous | 78,947 IU | Freund’s incomplete adjuvant | 50ul Ag + 50ul FIA    |
| 3º booster immunization            | 7-11-2013  | Subcutaneous | 78,947 IU | Freund’s incomplete adjuvant | 50ul Ag + 50ul FIA    |

2.2 Cardiac puncture

An immunized mouse was put into a beaker with a wire grid bottom under which chloroform moistened cotton was placed. The top of the beaker was closed by aluminium foil. Anaesthetized mouse was held on dissection board, chest area was cleaned with 70% alcohol. 25 G needle was attached to a 2ml syringe was inserted between left 3rd and 4th intercostal space and close to sternum. Needle was moved in a direction to the right shoulder and at angle that allowed needle to penetrate the left ventricle of the heart. Piston of syringe was withdrawn very slowly when blood appeared in barrel of syringe. Blood was withdrawn as more as possible.

2.3 Serum collection

Blood was transferred to and Eppendorf centrifuged at 8000 RPM for 10 minute. Serum was transferred to another Eppendorf and stored at -20°C.

2.4 Characterization of polyclonal antibodies

Polyclonal antibodies in serum were confirmed by different immunological techniques like ELISA, immunodot blot and westernblot.

2.4.1 ELISA
100ul of interferon α antigen (1ug/ul of carbonate buffer) was coated in wells and kept at 37°C for 45 min in humidified chamber. Wells were emptied, washed with 1X TBS and 300ul of 5% skim milk (in 1X TBS) was added to all wells. It was incubated at 37°C for 45 min in humidified chamber. Wells were washed 5 times with 1X TBS. 100ul collected serum having polyclonal antibodies against interferon α (1: 2000 dilution in carbonate buffer) was added to all wells and incubated at same conditions. Wells were emptied and washed with 1X TBS. 100ul of horseradish peroxidase conjugated antibody against polyclonal antibody (1:5000 dilution) was added to all wells and incubated at same conditions. Wells were washed 6 times with 1 X TBS and 100ul tetra methyl benzoate (substrate of HRP) was added to all wells and color change was observed.

2.4.2 Immunodot blot

Standard procedure of immunodot blot was carried out using HRPO enzyme linked antibody and hydrogen peroxide as substrate as discussed by Ahmad, Snober & Z. Samra (2014) [15]. A reddish brown spot that indicated the presence of anti-interferon alpha antibodies in mouse serum.

2.4.3 Western blot analysis

Standard western blot technique was applied to the by running SDS PAGE of the serum and then staining and destaining of the gel as discussed by Ahmad, Snober & Z. Samra (2014) [15]. The gel was then transferred to nitrocellulose membrane and then secondary enzyme linked antibody was used to detect antibody presence.

3. Results

The results are discussed below in detail.

3.1 ELISA
Figure 1: Positive ELISA test indicating presence of anti-interferon alpha antibodies in mouse serum.

Appearance of blue color on addition of TMB indicated the presence of polyclonal antibodies in the serum of mouse collected after immunization with interferon alpha (Figure 1). This result indicates that polyclonal antibodies were produced in mouse serum after immunization that have bound to the antigen providing site for binding of enzyme linked secondary body. As a result on addition of substrate colored complex was generated.

3.2 Immuno Dot blot

Figure 2: positive immune dot blot indicating presence of anti-interferon antibodies in mouse serum.
Reddish brown colored spot on membrane strip indicated presence of polyclonal antibodies in serum against antigen interferon alpha (Figure 2). Appearance of colored spot indicated presence of anti-interferon alpha antibodies in mouse serum.

3.3 Western blot analysis

Size of interferon alpha is approximately 20.5-21.5 KDa which is present as a colored band on nitrocellulose membrane (Figure 3 and Figure 4).

Figure 3: SDS page during western blot showing presence of interferon alpha in the gel in form of band along with other proteins.
4. Discussion

Antibody production is a commonly used in the field of immunology to study immunological response of the body to certain antigens and to evaluate the strength of the immune system. It is also used in the production of products such as antivenom for treatment of certain antigens and poisons [16]. Immunization is normally carried out in mice, rats, sheep, goats or rabbits depending upon the amount of antibodies that are required [15, 17]. The polyclonal antibody is commonly used for detection of multiple diseases using immunochromatographic strips. This simple and cheap test saves time and is useful for detection of diseases in areas where machinery and resources are limited [18].

Autoimmune disease is a disease that detects the body’s own organs or molecules as foreign antigen and attacks and destroys these molecules [19]. Interferon alpha is one of the main cytokine that is released as a response to an antigen in the body to initiate immune response against the foreign material. In case of autoimmune disease there would be an over production of interferon alpha that would detect bodies own cells as antigens and trigger unjust immune response [6]. This overproduction of interferon alpha can be quantified by using anti interferon alpha antibodies to carry out ELISA for the interferon and quantify the amount of antibody that binds to the molecule, for this reason production of polyclonal antibodies in necessary against interferon [12].

Previously reported studies have used different approaches to produce desired antibodies against interferon Alfa. A study attempted to produce monoclonal antibodies against human interferon Alfa by inducing normal human buffy coat cells via Sendai virus [20]. In another study, sheep polyclonal antibodies were produced against 2'-5' oligoadenylate synthetases using IFN-alpha as antigen [21]. Although animal models have been used for production of polyclonal antibodies [5], this study used a slightly different as it focused on producing antibodies specifically against IFN-Alfa in an animal model. Most of the previous research focuses on production of polyclonal antibodies against IFN Alfa in cell cultures and human peripheral blood cultures [22, 23].

In future further analysis can be carried out to quantify interferon production in autoimmune diseases. There is also the possibility of using monoclonal antibody against cytokines by the use
of hybridoma technique to more specifically quantify the cytokine production in autoimmune disease and then use this data to further find a cure for the autoimmune disorders [24].

Furthermore these antibodies can also be used in the field of nanomedicine to create nanocarriers that can bind to interferon alpha that in being over secreted in autoimmune diseases and inhibit its function, thus managing the disease by targeted therapy.

5. Conclusion

Antibody production is a growing and flourishing field. The production of polyclonal antibodies can be done easily on a cheap scale to analyze the disorders related to the immune system. The production of antibodies in mice is suitable for studies on a small scale where only small amount of antibody is required but in research related to high antibody requirement rabbit or goat is used for immunization. Local immunochromatographic strips can also be synthesized using antibodies synthesized by this method.

Conflict of Interest

The authors declare no conflict of interest.

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