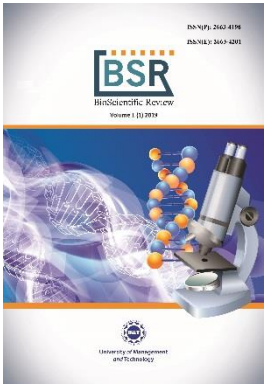

BioScientific Review (BSR)

Volume 3 Issue 4, December 2021

$ISSN_{(P)}$: 2663-4198 $ISSN_{(E)}$: 2663-4201

Journal DOI: https://doi.org/10.32350/BSR

Issue DOI: https://doi.org/10.32350/BSR.0304

Homepage: https://journals.umt.edu.pk/index.php/BSR

Journal QR Code:

Article: **Production and Characterization of Polyclonal Antibodies against Interferon Alpha in Mice**

Author(s): Mehreen Fatima[1], Azra Quraishi[2], Fatima Khalid[1]

Affiliation: [1]Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore Pakistan

[2]Department of Biochemistry, University of Agriculture University, Faisalabad, Pakistan

Article DOI: https://doi.org/10.32350/BSR.0304.03

Article QR:

Mehreen Fatima

Citation: Fatima M, Quraishi A, Khalid F. Production and characterization of polyclonal antibodies against interferon alpha in mice. *BioSci Rev*. 2021;3(4):27–36.

Indexing

Crossref

HEC

EuroPub

Internet Archive

HJRS HEC Journal Recognition System

J-Gate Indexed

LOCKSS

Estd. 1990

A publication of the
Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Production and Characterization of Polyclonal Antibodies against Interferon Alpha in Mice

Mehreen Fatima[1*], Azra Quraishi[2], Fatima Khalid[1]
[1]Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore Pakistan
[2]Department of Biochemistry, University of Agriculture University, Faisalabad, Pakistan
*Corresponding Author: mehreenfatima21@yahoo.com

Article Info

Received: June 08, 2021
Revised: November 10, 2021
Accepted: November 11, 2021

Keywords

ELISA,
immune dot blot
interferon,
mice,
polyclonal antibodies

Abstract

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 generates a large group of antibodies that recognize several epitopes of a particular antigen. Interferon alpha plays an important role in immune response activation. Therefore, it is a subject of interest for studies related to autoimmune diseases. In this paper, the production of antibodies against interferon was investigated in order to quantify interferon production with the aim to analyze interferon levels in autoimmune disorders in the future. For antibody production, one-month old laboratory grade mice were injected with interferon alpha in combination with Freund's complete adjuvant for a course of five weeks, after which antibodies were obtained in mice serum. The production of anti-interferon alpha antibodies was confirmed usingElisa, immune dot blot and western blot analysis. An interferon alpha of approximately 20.5-21.5 KDa was detected in the immune dot blot test. These antibodies may be produced in mouse models on a commercial basis and can be used in the future for the treatment of autoimmune diseases by managing the interferon levels in the patients.

1. Introduction

Antibodies (immunoglobulins) are glycoproteins naturally produced in response to invading foreign particles (antigens), such as microorganisms 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, carbohydrates, bacterial or viral cell surfaces, but they may also be distinctive molecules expressed on cancerous cells. The region of an antigen that interacts with an antibody is known as epitope [2]. Typically, an animal's immune system generates 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 the 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 it with the antigen for a specified time interval. Frequent introductions of the antigen along with the suitable adjuvant initiates an immune system in the body that results in B cell activation and thus, in the production of polyclonal antibodies [5]. Larger mammals are often preferred as the amount of serum that can be collected is bigger. An antigen is injected into the mammal. This induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen and the mammal's serum is used for the purification of polyclonal antibodies [6]. The aim of the production of polyclonal antibodies is to achieve high titer, high avidity and specificity against different epitopes of the respective antigen [7]. Antigens with a molecular weight less than 10KDa are unable to activate the immune system. These antigens are conjugated with some carrier molecules (such as bovine serum albumin or keyhole limpet haemocyanin) and these molecules trigger antibody production against both the antigens and carrier molecules. The high titer of polyclonal antibodies depends upon the phylogenetic differences between the donor of antigens and the recipient of antibody production. A species with high distance produces more specific polyclonal antibodies [8]. Antigens must be pure in order to avoid the production of antibodies against impurities and the activation of non-specific immune response. For the production of potent antibodies, it is usually necessary to use an adjuvant as a part of the immunogen. These substances potentiate immune response by forming a slow release depot of antigens by stimulating T – cell helper and antibody production [9]. Freund's and titermax are the most commonly used adjuvants for polyclonal antibody production. Two types of Freund's adjuvant are used: 1) Freund's complete adjuvant (FCA) is used for primary immunization, and 2) Freund's incomplete adjuvant (FIA) for booster immunizations. FCA contains non-metabolizeable mineral oil and heat killed mycobacterium, while FIA contains non-metabolizeable mineral oil only [10]. Doses of antigens depend upon their nature and route of immunization. Too high or too low doses can result in immunological tolerance and the deviation of immunological response from antibody production toward T-cell response [11]. Antigens can be injected by different routes such as subcutaneous, intramuscular, intravenous, intradermal or intraperitoneal routes. After primary injection followed by booster injections, blood is collected and centrifuged to remove blood cells and clotting factors [12].

Polyclonal antibodies work well in many technical applications. They are able to recognize various epitopes on the same target protein because they contain a mixture of antibodies; hence, problems

with masked or denatured epitopes can be avoided [10]. These problems occur during the immunohistochemical staining of tissues, where the 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 behind the generation of anti-interferon antibodies was to study the level of interferon production in autoimmune diseases and also to find out the correlation of interferon alpha with autoimmune diseases [14].

Normally, interferons are released in the immune system as a response to the detection of viral antigen in the body, so that a proper immune system response can be generated corresponding to the viral entity. In autoimmune diseases, interferons are released in response to the identification of the body's own molecules as viral entities. Thus, they carry out an unjustified immune response to the body's own 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 for the 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 the Department of Zoology, University of the Punjab. They were kept in the animal house of the Institute of Biochemistry and Biotechnology, University of the Punjab, throughout the duration of the immunization schedule [14].

2.1 Immunization Schedule

Interferon alpha injection with a dosage of 6 million IU per 3.8ml was used. As such a high dose in not suitable for mice; therefore, 50µl of the injection suspension, that is, 78,947.36 IU of antigen was used in combination with 50µl of FCA and FIA. Immunization was carried out over 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 to produce serum that contained the anti-interferon alpha antibodies [14].

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

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 an aluminium foil. The anaesthetized mouse was held on the dissection board and its chest area was cleaned with 70% alcohol. A 25 G needle attached to a 2ml syringe was inserted between the left 3rd and 4th intercostal space, close to sternum. The needle was moved toward the right shoulder and at an angle that allowed it to penetrate the left ventricle of the heart. The piston of the syringe was withdrawn very slowly when the blood appeared in its barrel. Blood was withdrawn as much as possible.

Table 1. Schedule and Details of the Immunization Process of Mice

No. of 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
1st Booster Immunization	24-10-2013	Subcutaneous	78,947 IU	Freund's incomplete adjuvant	50ul Ag + 50ul FIA
2nd Booster Immunization	31-10-2013	Subcutaneous	78,947 IU	Freund's incomplete adjuvant	50ul Ag + 50ul FIA
3rd Booster Immunization	7-11-2013	Subcutaneous	78,947 IU	Freund's incomplete adjuvant	50ul Ag + 50ul FIA

2.3. Serum Collection

Blood was transferred to Eppendorf and centrifuged at 8000 RPM for 10 minutes. Serum was transferred to another Eppendorf and stored at -20ºC.

2.4. Characterization of Polyclonal Antibodies

The presence of polyclonal antibodies in the serum was confirmed by different immunological techniques such as ELISA, immuno dot blot and western blot.

2.4.1. ELISA

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

2.4.2. Immuno-Dot Blot

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

2.4.3. Western Blot Analysis

Standard werstern blot technique was applied by running the SDS PAGE of the

serum and then the staining and destaining of the gel were carried out, as discussed by Ahmad [15]. The gel was then transferred to the nitrocellulose membrane. Then, secondary enzyme linked antibodies were used to detect the antibody presence.

3. Results

The results are discussed below in detail.

3.1. ELISA

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

3.2. Immuno Dot Blot

A reddish brown colored spot on the membrane strip indicated the presence of polyclonal antibodies in the serum against the antigen interferon alpha (Figure 2).

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

3.3. Western Blot Analysis

The size of interferon alpha was approximately 20.5-21.5 KDa and it was present as a colored band on the nitrocellulose membrane (Figure 3 and Figure 4).

Figure 2. Positive immuno dot blot indicating the presence of anti-interferon antibodies in mouse serum

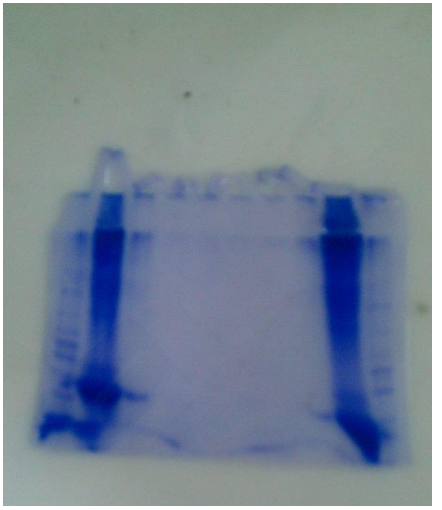

Figure 3. SDS page showing the presence of interferon alpha in the gel in the form of band during western blot along with other proteins

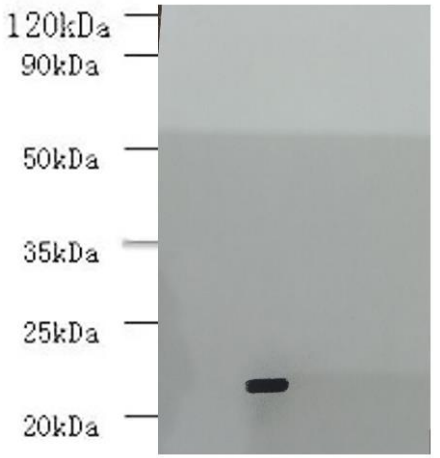

Figure 4. Western blot band showing the presence of interferon alpha antibodies in mouse serum

4. Discussion

Antibody production is commonly used in the field of immunology to study the 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 other useful substances, such as antivenom, used for the 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 required [15, 17]. Polyclonal antibodies are commonly used for the detection of multiple diseases using immunochromatographic strips. This simple and cheap test saves time and is useful for the detection of diseases in areas where machinery and resources are limited [18].

Autoimmune diseases detect the body's own organs or molecules as foreign antigens. Hence, they attack and destroy them [19]. Interferon alpha is among the main cytokines released in response to the introduction of an antigen in the body. Its release is aimed to initiate the immune response against the foreign material. In case of autoimmune diseases, there is an overproduction of interferon alpha that detects the body's own cells as antigens and triggers an unjust immune response [6]. This overproduction of interferon alpha can be quantified by using anti-interferon alpha antibodies to carry out ELISA and quantify the amount of antibodies that bind to the molecules. For this reason, the production of polyclonal antibodies is necessary against interferon [12].

Previously reported studies used different approaches to produce the desired antibodies against interferon alpha. A study attempted to produce monoclonal antibodies against human interferon alpha 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 the antigen [21]. Although animal models have been used for the production of polyclonal antibodies [5], the current study used a slightly different method as it focused on producing antibodies specifically against IFN-Alfa in an animal model. On the contrary, most of the previous researches focused on the production of polyclonal antibodies against IFN-Alfa in cell cultures and human peripheral blood cultures [22, 23].

In the future, further analysis can be carried out to quantify interferon production in autoimmune diseases. There is also the possibility of using monoclonal antibodies against cytokines through the use of hybridoma technique to more specifically

quantify cytokine production in autoimmune diseases and to find their cure [24].

Furthermore, these antibodies can also be used in the field of nanomedicine to create nanocarriers. The nanocarriers can bind to interferon alpha secreted in autoimmune diseases and inhibit their function, thus managing the disease via the targeted therapy.

5. Conclusion

Antibody production is a growing and flourishing field. The production of polyclonal antibodies is cost effective. Thus it can be carried out to analyze the disorders related to the immune system on a commercial basis. The production of antibodies in mice is suitable for studies conducted on a small scale, where only a small amount of antibodies is required. However, in research that requires the production of a high amount of antibodies, 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.

References

[1] Benjamini E, Andria ML, Estin CD, Norton FL, Leung CY. Studies on the clonality of the response to an epitope of a protein antigen. Randomness of activation of epitope-recognizing clones and the development of clonal dominance. *J Immunol*. 1988;141(1):55-63.

[2] Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. In*Antigen recognition by T cells.* 5th ed. Garland Science; 2001. https://www.ncbi.nlm.nih.gov/books/NBK27098/

[3] Cooper HM, Patterson Y. Production of polyclonal antisera. *Curr Protoc Immunol*. 2008;82(1):2-4. https://doi.org/10.1002/0471142735.im0204s82

[4] Coligan JE, Tam JP, Shao J. Production of antipeptide antisera. *Curr Protocol Neurosci.* 1997;(1):5-6. https://doi.org/10.1002/0471142301.ns0506s00

[5] Leenaars M, Hendriksen CF. Critical steps in the production of polyclonal and monoclonal antibodies: evaluation and recommendations. *Ilar J*. 2005;46(3):269-79. https://doi.org/10.1093/ilar.46.3.269

[6] Suckow MA, Stevens KA, Wilson RP, editors. The laboratory rabbit, guinea pig, hamster, and other rodents. Academic Press; 2012.

[7] Kuroiwa Y, Matsushita H, Sano A. *Complex chromosome engineering for production of human antibodies in transgenic animals*. Google Patents; 2014.

[8] Sissons JG, Oldstone MB. Killing of virus-infected cells: the role of antiviral antibody and complement in limiting virus infection. *J Infect Dis*. 1980;142(3):442-8.

[9] Kuroiwa Y, Kasinathan P, Sathiyaseelan T, et al. Antigen-specific human polyclonal antibodies from hyperimmunized cattle. *Nature Biotechnol*. 2009;27(2):173-81. https://doi.org/10.1038/nbt.1553

[10] Naimi WA, Gumpf JJ, Green RS, et al. Immunization against Anaplasma phagocytophilum adhesin binding domains confers protection against infection in the mouse model. *Infect Immun*. 2020;88(10):e00106-20.

[11] Sissons JP, Oldstone MB. Antibody-mediated destruction of virus-infected cells. *Adv Immun*. 1980;29:209-60. https://doi.org/10.1016/S0065-2776(08)60045-0

[12] Matsushita H, Sano A, Wu H, et al. Triple immunoglobulin gene knockout transchromosomic cattle: bovine lambda cluster deletion and its effect on fully human polyclonal antibody production. *PloS one*. 2014;9(3):e90383. https://doi.org/10.1371/journal.pone.0090383

[13] Benjamini E, Wan AM, Langton BC, Andria ML. Cell activation and immunogenicity. *Dev Biol Stand*. 1986;63:29-39.

[14] O'Hagan DT, Jeffery H, Davis SS. Long-term antibody responses in mice following subcutaneous immunization with ovalbumin entrapped in biodegradable microparticles. *Vaccine*. 1993;11(9): 965-9. https://doi.org/10.1016/0264-410X(93)90387-D

[15] Ahmed S, Samra ZQ. Engineering Transferrin and Epirubicin onto Magnetic Nanoparticles for Targeting Drug Delivery Through Transferrin Receptors. *Pak J Zool*. 2016;48(4):1117-1124.

[16] Bounds CE, Kwilas SA, Kuehne AI, et al. Human polyclonal antibodies produced through DNA vaccination of transchromosomal cattle provide mice with post-exposure protection against lethal Zaire and Sudan ebolaviruses. *PloS one*. 2015;10(9):e0137786. https://doi.org/10.1371/journal.pone.0137786

[17] Stills HF. Polyclonal antibody production. In*The laboratory rabbit, Guinea pig, Hamster, and other rodents* 2012 (pp. 259-274). Academic Press. https://doi.org/10.1016/B978-0-12-380920-9.00011-0

[18] Overkamp D, Mohammed-Ali S, Cartledge C, Landon J. Production of polyclonal antibodies in ascitic fluid of mice: technique and applications. *J Immun*. 1988;9(1):51-68. https://doi.org/10.1080/01971528808053210

[19] Sano A, Matsushita H, Wu H, et al. Physiological level production of antigen-specific human immunoglobulin in cloned transchromosomic cattle. *PloS one*. 2013;8(10):e78119. https://doi.org/10.1371/journal.pone.0078119

[20] Jilbert AR, Hertzog PJ, Burrell CJ, Gowans EJ, Linnane AW, Marmion BP. Production of a monoclonal antibody to human interferon-α (IFN-α) and its use to identify IFN-α-producing cells in virus infection in vivo. *Microbial Pathogen*. 1986;1(2): 159-68. https://doi.org/10.1016/0882-4010(86)90018-5

[21] Gribaudo G, Franco A, Gariglio M, Martinotti MG. Production of sheep polyclonal antibodies against the 2'-5'

oligoadenylate synthetases. *G Batteriol Virol Immunol*. 1990;83(1-12):10-16.

[22] Greenway AL, Overall ML, Sattayasai N, et al. Selective production of interferon-alpha subtypes by cultured peripheral blood mononuclear cells and lymphoblastoid cell lines. *Immunol*. 1992;75(1):182-188.

[23] Greenway AL, Hertzog PJ, Devenish RJ, Linnane AW. Constitutive and virus-induced interferon production by peripheral blood leukocytes. *Exp Hematol*. 1995;23(3):229-235.

[24] Qiu X, Audet J, Wong G, et al. Successful treatment of ebola virus-infected cynomolgus macaques with monoclonal antibodies. *Sci Translat Med*. 2012;4(138):138ra81. https://doi.org/10.1126/scitranslmed.3003876