Efficacy of Pfizer vaccine on IL-12/IFN-γ pathway with related to COVID-19 infection

Ruaa Kareem Surhan[1*] and *Mayyada F.* Darweesh[2]

[1]Al-Furat Al-Awsat Technical University, College of health and medical techniques, Kufa, 31003 Al-Kufa, Iraq
[2]University of Kufa, Department of Microbiology, Faculty of Science, Kufa, Iraq

Abstract. Critical patients with pandemic COVID-19 have dysregulation in immune response, like cytokine storm, lymphopenia that led to increase mortality, vaccine is the only hope for controlling on this pandemic. However, this study design to evaluate effectiveness of Pfizer vaccine on serum level for IL-12/IFN-γ pathway with susceptibility to COVID-19. The current study included 160 participants whom separated into two groups for further analysis which included Covid-19 patients as unvaccinated and vaccinated attended from various hospitals in AL-Najaf provenance and some private clinics and healthy control groups as vaccinated and unvaccinated healthy subjects, blood samples were collected from all subjects during January - April 2022 to detect IL-12 and IFN-γ concentration by ELISA technique. Demographic study indicated the male patients was higher than female and the age distribution for unvaccinated and vaccinated were 41-50 years had the highest frequency which were 17(42.5%) and (35%) respectively. The present study observed that the concentration of INF-γ (pg/ml) were show serum level decrease with Covid-19 infection, so IFN-γ in unvaccinated lower than healthy group and higher than vaccinated patients, while IL-12(pg/ml) serum level increase with Covid-19 infection, so IL-12 in unvaccinated higher than healthy group and vaccinated patients

1 Introduction

Vaccination is one of the most powerful means to save lives and to increase the level of health of mankind [1]. COVID-19 vaccines are critical for containing the pandemic and preventing serious SARS-CoV-2 infection of the modern era, several vaccines have been developed during the past two years [2]. The mRNA-based vaccine has been developed by BioNTech /Pfizer company under the name BNT162 in December 2020 that based on messenger RNA with two proline mutations in the S protein of the lipid nanoparticle-based BNT162b2 vaccination used to prevent fusion [3].

Several immune systems activated cells are present in both pathogen-recognizing innate immune response and an adaptive immune response directed against a particular antigen , which are important in establishing the cytokine environment [4]. Dysregulated immune response to the pathogen associated with prolonged high level of cytokines (cytokine storm)

* Corresponding author: ruaa.surhan@atu.edu.iq

may develop sever complication and mediated immunopathology in sever SARS-COV2 cases [5].

IL-12 and IFN-γ play a crucial role against infectious viruses [6]. IL-12 a key player in bridging the innate and adaptive immune responses by inducing interferon (IFN) γ production by T and NK cells and thereby a TH1 type immune response against viral and bacterial infection [7]. In turn, IFN-γ markedly augments IL-12 production, thus providing an important amplifying mechanism in inflammation. This search aim to evaluate role of IL-12 /IFN-γ with related to Pfizer vaccine in Covid-19 infection.

1.1 Objectives

The study was determine the efficacy of Pfizer vaccine on IL-12/IFN-γ pathway with related to COVID-19 infection.

2 Material and methods

A case-control study was conducted on 160 participants, whom separated into two groups for further analysis which included Covid-19 patients as unvaccinated and vaccinated attended AL-Amal Center , AL-Sadar medical hospital and some private clinics in Najaf provenance and healthy control groups as vaccinated and unvaccinated during the period from January - April /2022 . 3 ml of venous blood drawn from all subjects, Serum was collected in sterile Eppendorf tubes and stored at -20 C for determination of Human INF-γ and IL-12 by ELISA assay according to (Elabscience® ELISA Kits).

2.1 Ethical approval

The study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki. It was performed with patients' consent both verbally and analytically before sampling. The research procedure, subject information, and consent form were reviewed and approved by the local ethics committee according to the document number 8356 in 9/11/2022 to get this approval.

All healthy were interviewed directly by using an anonymous questionnaire form which included (age and gender , infected withCovid-19 previously , chronic disease)

2.2 Statistical analysis

Statistical analysis was carried out by using statistical software (IBM SPSS Statistics 26). The quantitative data given as mean ± SE and the differences tested by t-test (two means) and one-way ANOVA test (more than two means). Probability of ($P \leq 0.05$) was measured to be significant statistically [8].

3 Result and discussion

According to this study, the male patients outnumbered the female patients by a margin of 26(65 %) to 14(35 %) for unvaccinated patients and 24(60%) vs 16(40%) for vaccinated compare to 21(52.5%) vs 19(47.5%) as Vaccinated healthy and 20(50%) vs 20(50%) as unvaccinated healthy. The age distribution of unvaccinated and vaccinated patients that age range between 41-50 years had the highest frequency and which were 17(42.5%) and 14(35%) respectively and so on. Table(1).

Table 1. Distribution of patients according to sex and age groups.

Sex	COVID-19 patients		Healthy group		Total (%)
	Unvaccinated	Vaccinated	Vaccinated	Unvaccinated	
Male	26(65%)	24(60%)	21(52.5%)	20(50%)	91(56.9%)
Female	14(35 %)	16(40%)	19(47.5%)	20(50%)	69(43.1%)
Mean of age	**48.25±10.6**	**43±11.8**	**42.87±14.5**	**43±9.8**	
30-21	2(5%)	5(12.5%)	4(10%)	6(15%)	17(10.6%)
31-40	5(12.5%)	9(22.5%)	14(35%)	8(20%)	36(22.5%)
41-50	17(42.5%)	14(35%)	8(20%)	15(37.5%)	54(33.8%)
51-60	8(20%)	10(25%)	11(27.5%)	8(20%)	37(23.1%)
61-70	8(20%)	2(5%)	3(7.5%)	3(7.5%)	16(10%)
Total	40(25%)	40(25%)	40(25%)	40(25%)	160(100%)

In this study the male more numerous than female and this consistent with local study - AL-Najaf city by [9] whom found that a total of 120 subjects screened for SARS-COV2 infection, among them higher rate of infectivity was in males 56%than females 44% . According to research conducted in AL-Basra/Iraq by [10], between May 31 and July 31, 2020, there were a total of 1014 admitted cases in Iraq, with men accounting for 60.4% and females for 39.6%.

This may attribute to hormonal differences related to gender that could affect the pathomechansims of infection and because of the variable immune system for both sex that is involved in COVID-19 pathogenesis which is represented by ACE2 receptor that is regulated by sex hormone [11].

In this study choose Pfizer's this consistent with previous study in Iraq such as [12] they confirmed that Pfizer's BioNTech vaccine seems to be preferred by Iraqi population as it gives highest antibody titter as well as its availability and universal usage compared to others vaccines. Also, [13] whom indicated that multiple vaccines manufactured using various techniques from several different companies have been introduced within one year of the epidemic, which was an exceptional and gigantic achievement; however, in Iraq about two - third of the participants received Pfizer vaccine followed by AstraZeneca vaccine 17% only while Sinopharm vaccine 14% and indicated that 1,000 vaccinated participants from across Iraq, 62.2% were in their 30s and 40s years and 42.5 % of the participants had a history of COVID-19 infection.

According to research by [14] the average age of a COVID-19 patient is 52.4 years old, and the disease is more deadly in the elderly. In contrast, only 2% of patients aged 19 or younger have been infected with the virus. Furthermore, if a child or adolescent does contract COVID-19, they typically experience only mild symptoms. In line with [15] they evaluate efficiency of vaccines for equaled number of participants as covid-19 infected patients with 115,326 participants and 115,326 participants healthy subjects , median age of the study participants was 31 years, approximately 69% were male and the age group between 30-39 then 40-49 represent higher aged that received P-fizer vaccine .

3.1. Immunological study

3.1.1 Evaluation of Interferon- Gama (INF-γ) in COVID-19 patients

The present study observed that INF-γ (pg/ml) concentration decrease in Covid-19 group in compare to healthy group , the results found that INF-γ level highly decreased in Covid-19 vaccinated than unvaccinated patients (30.48± 2.88 ; 11.19 ± 1.70) pg/ml, while in healthy group the Vaccinated were 65.23 ± 1.03 pg/ml and not-Vaccinated healthy were 60.05 ± 1.05 pg/ml as shown in figure (1).

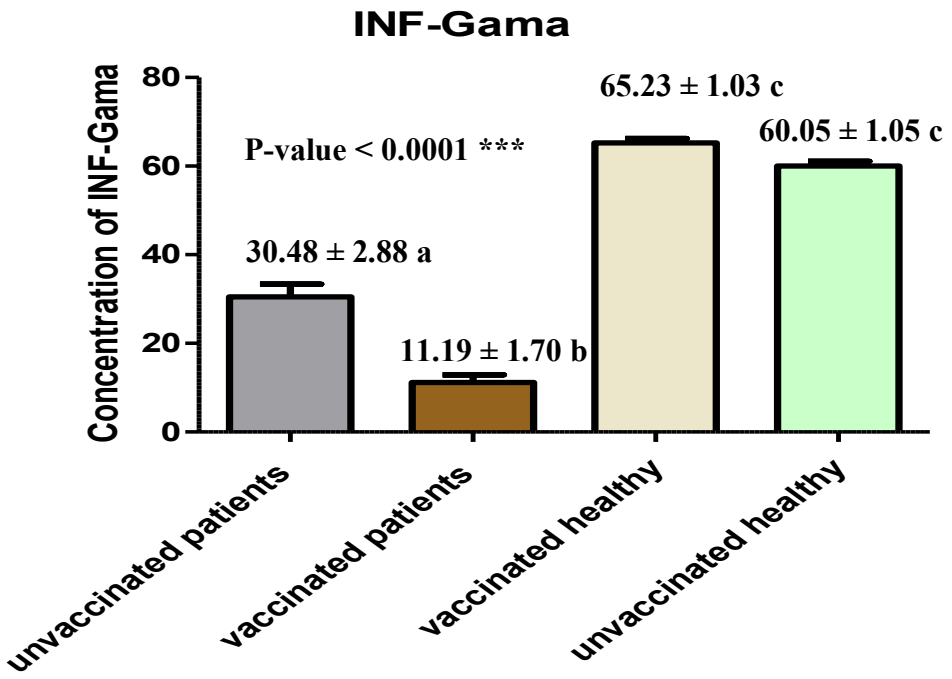


Fig. 1. Concentration of INF-γ in serum of COVID-19 unvaccinated and vaccinated , control group (vaccinated and unvaccinated). The different letters indicate the existence of significant differences

The current research found that the overall quantity of IFN-γ serum level was decrease significantly in covid-19 patients compared to healthy control at (P<0.05). The present study's findings in line with local study by [9] who confirmed that IFN-γ decreased in Covid-19 patients (26 .8 ± 5.3) compare to healthy (38.7 ± 11.4) pg/ml and highly decreased in critical cases compare to mild cases (22.3 ± 3.2 vs 30.7±16.7) . Also , [16] they demonstrated that children with COVID-19 has high level of IFN-γ compared to adults with COVID- 19, this indicates that COVID-19 infection is not severe in children and confirmed that IFN-γ serum levels has key important in state of disease .

[17] they confirmed that BNT162b2 vaccination stimulated antiviral immunity with little type I IFN response after the first dose, but a notably enhanced innate response after the secondary immunization by increased IFN-γ concentration IL-6, IL-12 . Also, [18] BNT162b2 Vaccination for healthy voleenter found robust production of neutralizing antibodies against the wild-type SARS-CoV-2 and significant increases in antigen-specific poly-functional CD4 -CD8 T cells , enhanced innate immune response as compared to unvaccinated with greater frequency of monocytes; higher concentration of plasma IFN-γ; and transcriptional signature of innate antiviral immunity.

According to Pfizer vaccine, [19] indicated that SARS-CoV-2 spike protein and specific T-cell expansion with cytokine secretion IFN-γ, IL-2 and IL-10 were observed after 2ed dose of Pfizer vaccine. [20] found that there were negative correlations between basal immunity IFN-γ, TNF-α, and IL-2 before vaccination and antibody production/neutralizing capacity after vaccination and recommended that an extreme immune response prior to vaccination may impair the production of neutralizing antibodies while vaccination regulate production of IL-2, IFN-γ, and TNF-α that lead to regulate immune response [21].

Pfizer vaccination data showed that a second dosage induced secretion of IFN-, IL-2, and IL-10 from T cells, as well as the production of SARS-CoV-2 spike protein and specific T-cell growth. Antibody production and neutralizing capacity following vaccination were found to be negatively correlated with pre-vaccination levels of IFN-, TNF-, and IL-2 by [20], suggesting that an overactive immune response before vaccination could hinder antibody production. In contrast, vaccination was found to regulate production of IFN-, TNF-, and IL-2, thereby regulating the immune response [21] who verified that 9 weeks after receiving two doses of Pfizer's vaccine, subjects developed COVID-19 S-specific neutralizing antibodies and poly-specific CD4+ and CD8+ T cells, which stimulated the production of IFN- and INF-type-1. Also, [22] they confirms a significant difference in the median IFN-γ level in vaccinated , unvaccinated patients and healthy group . Also, [23] they observed exhibited alterations in their levels of IL-18, IFN-γ, IL-12p70 and IL-10 cytokines following vaccination in immunocompetent individuals.

3.1.2. Evaluation of interlukin-12 (IL-12) in COVID-19

The present study observed that IL-12(pg/ml) concentration increase in Covid-19 group in compare to healthy group , the results found that IL-12 level highly increased in Covid-19 unvaccinated than vaccinated patients (633.3 ± 40.58 , 314.7 ± 39.62) pg/ml, while in healthy group the Vaccinated were 160.5 ± 25.62 pg/ml and not-Vaccinated healthy were 79.50 ± 4.43 pg/ml as shown in figure (2).

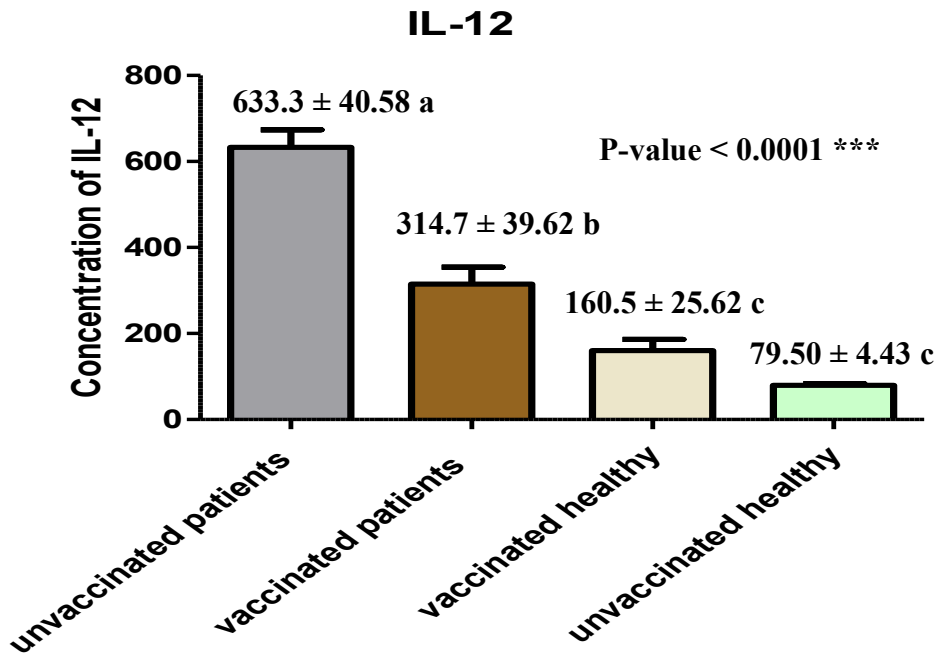


Fig. 2. Concentration of IL-12 in serum of unvaccinated vaccinated Covid patients, Vaccinated and Vaccinated healthy. The different letters indicate the existence of significant differences

When a virus enters a cell, a pathogen-associated molecular response triggers the release of the heterodimeric cytokine IL-12, which strengthens the links between innate and adaptive immune responses [24].

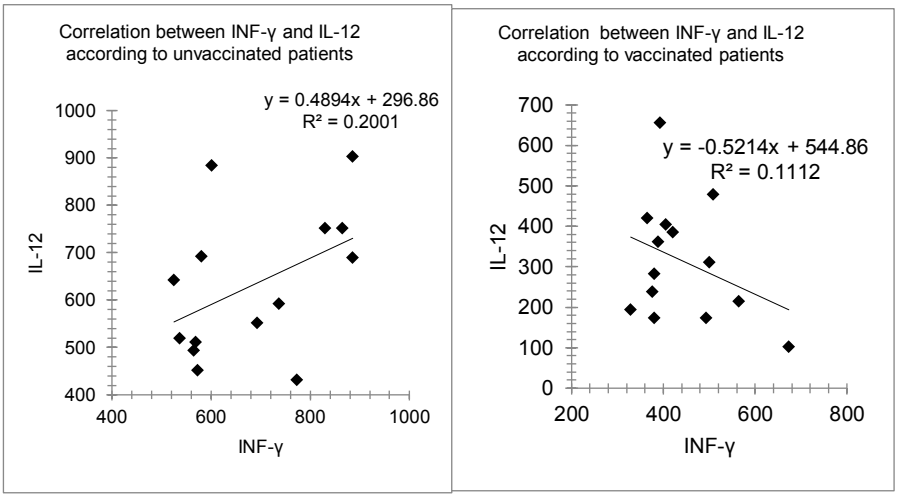
The results of the current study showed that the values of cytokines IL-12 significantly increase in SARS-CoV-2 unvaccinated patients than in healthy controls (P<0.001). This results agreement with local study in Wasit province by [25] they indicated a highly significant increase in the IL-12 (62.03%) for patient that have COVID-19 in compared with controls and showed the highest value for patients in age lowest 40 IL-12 (12.72) compared with patients that age highest than 40. In same line , [26] who found higher levels of IL-12 have been associated with COVID-19 patients compare to healthy control group .

According to Pfizer vaccine [23] they observed that immunocompetent individuals exhibited alterations in their levels of IL-12p70, IL-10, IL-18 and IFN-γ cytokines following vaccination . [27] they observed that BNT162 vaccine modulate activation of Th-1 cells to produce regular level of INF γ and IL-12 proinflammatory cytokines in Covid-19 patients.

[28] they found that, IL-18 ,TNF-α, IL-17 and IL-12 were higher elevated in the acute phase of infection while moderate elevation in Covid -19 vaccinated with two doses of Pfizer vaccine. [27] they observed that BN modulate acivation of Th-1 cells to produce regular level of INF- γ and IL-12 proinflammatory cytokines

3.1.3 Correlation between INF-γ and IL-12 serum level according to COVID-19 infection, Pfizer Vaccine and COVID-19 vaccination

The correlation coefficient as showed in Figure 4-14 which illustrated negative correlation between INF-γ with IL-12 according to COVID-19 vaccinated patients, while, according to unvaccinated patients show positive correlation.



A **B**

Fig. 3. Correlation coefficient between INF-γ and IL-12

IL-12 expression regulates the innate immune response during infection and is ultimately responsible for determining the length and type of the following adaptive immune response. Similarly, IL-12 promotes differentiation in naive CD4+ helper T cells into the Th1 phenotype that go on to produce IFN-γ and facilitate cell-mediated immunity [29].

IL-12 can drive interferon-γ (IFN-γ) production in dendritic cells (DCs), NK cells, macrophages, and T cells [30]. Also, [22] they found a significant correlation among the higher in the levels of IL-12 and IL-17A after COVID-19 antigen motivation. In similar link [31] which perform that L-12, /IFN-γ axis exposed to defend from severe vaccinia virus infection representative unique effector pathways. [32] established that IFN-γ and IL-12 absolutely control each other and type 1 inflammatory responses which are thought to cause tissue damage in autoimmune diseases

4 Conclusion

The study concluded that BNT162 vaccine modulate the activation of Th-1 and Th-17 to produce regular levels of IL-12 and IFN-γ that led to prevent cytokine storm and its complication.

References

1. Argirova R, Zlatareva A, Biot. & Biotechnol. Equipment **37(1),** 24-33 (2023)
2. Lopez SM, Sato AI, Chatterjee A. Vaccines: An overview. Viral, Parasitic, Bacterial, and Fungal Infections **1**, 699-717 (2023)
3. Arashkia A., Jalilvand S., Mohajel N. et al., Rev Med Virol. **31(3),** e2183 (2021)
4. Takeuchi O, Akira S. Immunological reviews **227(1),** 75-86 (2009)
5. Aung A, Cui A, Maiorino L, Amini AP, Gregory JR, Bukenya M, Zhang Y, Lee H, Cottrell CA, Morgan DM, Silva M. Science **379(6630),** eabn8934 (2023)
6. AL-Sherify AB, Darweesh M F.and Nima M M. Ind. J. of P. H. R. & D. **10(4),** 1589-1594 (2019)
7. Darweesh MF., Al-Obiadi AB. and Neama N. Biochem. Cell. Arch. **18(2),** 2321-2328. (2018)
8. Alsadawi AA, Alammar M, Hamid M. Al-Kufa University Journal for Biology **14(3),** 118-27 (2022)
9. Abd SA, Darweesh MF. HIV Nursing **23(2),** 912-7 (2023)
10. Ad'hiah AH, Allami RH, Mohsin RH, Abdullah MH, AL-Sa'ady AJ, Alsudani MY. Egyptian Journal of Medical Human Genetics **21(1),** 1-6 (2020)
11. Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, Agha M, Agha R. International journal of surgery **78**, 185-93 (2020)
12. Shareef LG, Al-Hussainy AF, Hameed SM. COVID-19 vaccination hesitancy among Iraqi general population between beliefs and barriers: An observational study. F1000Research **11** (2022)
13. Al Sa'ady AT, Abdulrasol ZA, Obaid AF, Alhindy HAAM, Al-Mumin AS. Journal of Emergency Medicine, Trauma & Acute Care. **(3),** 6 (2022)
14. Saghazadeh A, Rezaei N. Expert review of clinical immunology **16(5),** 465-70 (2020)
15. Chemaitelly H, Abu-Raddad LJ. The Lancet **399(10327),** 771-3 (2022)
16. Kotch C, Barrett D, Teachey DT. Expert Rev Clin Immunol. **15(8),** 813–822 (2019)
17. Liu, Y. et al. N. Engl. J. Med. **384***,* 1466–1468 (2021)
18. Arunachalam, P.S., Scott, M.K.D., Hagan, T. et al. Nature **596**, 410–416 (2021)
19. Sahin U, Muik A, Derhovanessian E et al. Nature **586**, 594– 99 (2020)

20. Song YC, Liu SJ, Lee HJ, Liao HC, Liu CT, Wu MY, Yen HR. *Humoral and cellular immunity in three different types of COVID-19 vaccines against SARS-CoV-2 variants in a real-world data analysis.* Journal of Microbiology, Immunology and Infection (2023)
21. Chung E, Subramaniam G, Dass LC. Asian J. of University Education **16(2),** 45-58 (2020)
22. Lucane, Z.; Slisere, B.; Gersone, G.; Papirte, S.; Gailite, L.; Tretjakovs, P.; Kurjane, N. Viruses **15**, 1146 (2023)
23. Tan, A.T.; Lim, J.M.; Le Bert, N.; Kunasegaran, K.; Chia, A.; Qui, M.D.; Tan, N.; Ni Chia, W.; de Alwis, R.; Ying, D.; et al. J. Clin. Investig. **131**, e152379 (2021)
24. Holder PG, Lim SA, Huang CS, Sharma P, Dagdas YS, Bulutoglu B, Sockolosky JT. Advanced drug delivery reviews **182**, 114112 (2022)
25. kadhum S.M.; Al-Askeri MA and Al-Saidi MA. Journal of Survey in Fisheries Sciences **10(3S),** 3137-42 (2023)
26. Moll-Bernardes R, De Sousa AS, Macedo AV, Lopes RD, Vera N, Maia LC, Feldman A, Arruda GD, Castro MJ, Pimentel-Coelho PM, de Albuquerque DC. Frontiers in Cardiovascular Medicine **8**, 702507 (2021)
27. Boyarsky BJ, Werbel WA, Avery RK, Tobian AA, Massie AB, Segev DL, Garonzik-Wang JM. Jama **325(21),** 2204-6 (2021)
28. Miyazato Y, Yamamoto K, Yamada G, Kubota S, Ishikane M, Sugiyama M, Ueno M, Matsunaga A, Miyoshi-Akiyama T, Ishizaka Y, Ohmagari N. Emerging Infectious Diseases **28(4),** 870 (2022)
29. A. Rahman NA, Balasubramaniam VR, Yap WB. International Journal of Molecular Sciences **24(8),** 7350 (2023)
30. Kirchhammer N, Trefny MP, Natoli M, Brücher D, Smith SN, Werner F, Koch V, Schreiner D, Bartoszek E, Buchi M, Schmid M. Science Translational Medicine **14(653),** eabm9043 (2022)
31. van Den Broek, M., M. F. Bachmann, G. Kohler, M. Barner, R. Escher, R. Zinkernagel, M. Kopf. J. Immunol. **164**, 371 (2020)
32. Michael O. Kurrer, Wolfgang Sebald, Frank Brombacher, Manfred Kopf; J. Immunol. **167(9),** 5464–5469 (2001)