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Correlation Between Influenza Virus Replication and ATP6V0C of Trivalent Influenza (TIV) Vaccination in Ferret Ira Humairah1,2, Reviany V. Nidom3, Setyarina Indrasari3,4, Ema Qurnianingsih2, Arif Nur Muhammad Ansori3,4, Irine Normalina1,3, Chairul Anwar Nidom3,6 1Doctoral Student, Faculty of Medicine, Universitas Airlangga, Surabaya, 60286, Indonesia, 2Lecturer, Department of Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, 60286, Indonesia, 3Researcher, Professor Nidom Foundation, Surabaya, 60115, Indonesia, 4Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia, 6Professor, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia Abstract Influenza virus infections are a major public health threat. Influenza viruses are associated with high mortality and morbidity worldwide through seasonal epidemics. Vaccination programs are available, but unpredictable antigenic changes in circulating strains require annual modification of seasonal influenza vaccines. Influenza vaccines are given, one of which is a trivalent influenza vaccine (TIV) aimed at the formation of antibodies that are specific to influenza viruses by B cells. In this study TIV vaccination has an effect in the form of decreasing viral replication in ferret that have been tested with H1N1 and H3N2 viruses. The results of this study are data about viral replication obtained from hemagglutination tests (HA test) and ATP6V0C expression with SDS-Page. Keywords: ATP6V0C expression, ferret, TIV vaccination, viral replication Introduction Influenza virus infections are a major public health threat. Influenza viruses are associated with high mortality and morbidity worldwide through seasonal epidemics. Recent studies estimate that 290,000–645,000 deaths occur worldwide due to seasonal influenza annually1. Influenza is an acute respiratory infection caused by influenza viruses that circulate in all parts of the world. Viruses multiply through the process of replication. Measurement of the amount of virus in the body can be done after the incubation period, which is around days 1 to 3 after infection occurs. Viruses can be obtained from several specimens taken from the respiratory tract, which are recommended to detect the virus in patients with respiratory infections, for example from nasal swabs, swabs and nasopharyngeal aspirations, endotracheal swabs, and bronkoalveolar tissue2,3. Corresponding Author: Ira Humairah Email: ira-h@fk.unair.ac.id, canidom58@gmail.com Infection starts when a virus enters a cell, the virus uses many cellular functions of the host cell in each stage of its life cycle, so the identification of the function and role of the host cell in virus replication is an important key to understanding the life cycle mechanism of the virus4. The RNA replication process of influenza viruses occurs in the host cell nucleus, the ribonucleoprotein (RNP) complex of viruses released from the virus must be moved into the nucleus through the nucleus pore5. The transport process triggers the host cell to express the vATPase complex gene, one of which is the ATP6VOC gene. This complex gene is a gene that will encode the vacuolar ATPase component, a component in the vATPase complex that is needed in the process of acidification of intracellular organelles in the RNP transport process into the host cell nucleus6,7. Based on the recommendations of the WHO, the influenza vaccine is currently a trivalent inactivated vaccine (TIV)/trivalent whole virus vaccine made from a live attenuated virus, consisting of A/California/7/2009 (H1N1) pdm09-like virus, A/Switzerland/9715293/2013 (H3N2)-like virus and B/Phuket/3073/2013-like virus8. Materials and Method Object of research Subjects in this study were Ferret, the gold standard laboratory animals in influenza virus infections and aerosol infections compared to other animals, the reasons include the similarity in clinical symptoms if ferrets were infected by influenza viruses with clinical symptoms that occur in humans, there is a distribution of similar receptors in the channel breathing in ferrets and humans and the ability to be infected with human isolates without the need for adaptation9. This study used 25 ferrets that were divided into 5 groups, with the treatment of one shot vaccination with different doses. Research Methods The administration of intramusculatr TIV vaccine in this study used an one shot method with dosage variation of 3.8 µg; 7.5 µg; 15µg; and 30µg. The vaccine and viruses used in this study came

from PT. Biofarma. Blood samples were taken at the time of termination which is the 36th day. Whereas nasal wash and oral swab sampling was performed on days 3, 4, 5, 7, 9, and 14 after the challenge test9,10. Challenge tests using H1N1 and H3N2 viruses in the form of mixed infections. The presence and linkages of ATP6V0C host cell expression and post-vaccination virus replication can be taken into consideration in regard to the effectiveness of various vaccine doses and the vaccine delivery methods that have been given. Samples used for examination of viral replication by hemagglutination test are samples from nasal wash and oral swab collection, while for the examination of ATP6V0C expression ferret blood serum is used, which is done by SDS-Page (Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis). The SDS-Page results were followed by reading the expression of ATP6V0C protein using the DocTM EZ Gel (Biorad). Result and Discussion All groups showed positive results of viral replication with HA test since day 3 after the challenge test, indicating that the challenge test was successful so that positive HA values were obtained for all groups. This is in accordance with the standard pattern of influenza virus growth in the infected human body, virus titers will experience exponential growth within 2-3 days after the challenge test, then experience an exponential decline until undetectable on days 6 to 8 after the challenge test11. Table 1. Mean replication of the virus with the HA test after the challenge test. Group n Viral load titre (in HAU units), day after challenging test 3rd 4th 5th 7th 9th 14th A1 5 23.2 ± 25.9 2 ± 3.5 3.2 ± 7.2 0. 4 ± 0.9 0 0 B1 5 9.6 ± 8.8 16 0 0 0 0 C1 5 6.8 ± 6.3 4.8 ± 7.2 0 0 0 0 D1 5 10.4 ± 13.7 4.8 ± 7.2 0 0 0 0 E1 5 11.2 ± 7.2 3.2 ± 4.4 3.2 ± 7.2 0 0 0 TIV vaccine is expected to be able to increase the production of antibodies, antibodies that are formed are expected to be able to recognize HA proteins from viruses, so they cannot be attached to sialic acid receptors on host cells 12. Viruses that cannot bind to the host cell receptor are blocked from entering the cell, so they cannot use the replication machine in the host cell to multiply themselves. This mechanism can reduce the number of virus replications detected in the HA test in this study. One of the virus strains used in the TIV vaccine used in this study was the H1N1 A/California/7/2009 (H1N1) pdm09 virus, this strain corresponds to the virus used in the challenge test, the A/California/7/2009 (H1N1 virus) pdm09-like virus. The degree of vaccine protection depends on the compatibility of the strain in the vaccine with the circulating virus12. The H3N2 virus used in the challenge test is different from the H3N2 virus strain used in the vaccine, but this is expected to cause cross protection so that the antibodies produced are still able to provide protection13. Research conducted by Skowronski and the team in 2014, which carried out a vaccination test with a dose of 30 µg/mL, showed no significant difference in growth of viral titers between groups, and peak viral titer growth on days 1 to 2 then decreased from day to day 3rd after challenge testing14. In another study using variations in vaccine dosage and type of vaccine administration through aerosols, it turned out that the results of growth of virus titers did not show significant differences between groups, on the 3rd day after the challenge test11. Normal clinical dose, which usually does not exceed 15 µg HA protein, WIV (Whole Inactivated Virus) induces adequate neutralizing antibody titers, but generally fails to induce any cellular response regardless of the route of administration15. Research by Budimir shows that some high doses of WIV, are able to induce significant amounts of CTL (cytotoxic T lymphocyte) specific inactivated viruses in mice16–18. This is in accordance with this study which also uses a vaccine with a dose twice the usual which is 30µg. It is possible that high doses of vaccine in this study could induce CTL thus providing a better protective role, but unfortunately this study did not measure the immune response of host cells. However, from the level of virus replication in the D1 group (dose 30 µg) there was indeed a significant decrease in the average viral replication from day of 3rd to 4th. The important role of membrane fusion activity and the presence of viral ssRNA for CTL induction are established16–18. This role is also in accordance with this study which suggests the role of ATP6V0C in endosome membrane fusion. Intramuscular administration of WIV has proven to be more effective in inducing CTL compared to intranasal16,17. This was confirmed by Takeda who found that intranasal vaccination with WIV failed to induce T cell responses19. This may cause viral replication to be detected several days after the challenge test in almost all vaccinated groups. It is thought that the WIV inactivation method can have an effect on its immunogenicity. In addition to increasing doses, the cellular response induced by WIV can also be increased by the addition of adjuvants20. Similar to studies with WIV, the addition of alum into the virus has been shown to damage cellular responses in mice26, because it further increases the tendency for TH responses to TH2. Fig. 1 Results of SDS-Page serum ferret. The blue ribbon and the pink line emphasized show protein expression in the area approaching the marker. Note: M: Marker 10-250 kDa; 1-9 serum ferret samples before treatment and after vaccination and challenge test. 1 = A1.3 pre vaccination; 2 = B1.3 pre vaccination; 3 = B1.3 post vaccination; 4 = C1.2 pre vaccination; 5 = C1.3 post vaccination; 6 = D1.2 prev. 7 = D1.2 post vaccination; 8 = E1.2 pre vaccination; 9 = E1.3 post vaccination. The humoral immune response generated by inactivated virus vaccines currently does not provide adequate cross-protection against inactivated virus vaccines that do not match the type of infecting virus. Specific inactivated virus vaccine T cells must recognize the epitope that they are conserving and therefore must have the potential for cross-protection21. be expressed by the group that gets the vaccination but it will be expressed by the control group. ATP6V0C is a protein with a molecular weight of 16 kDa, with SDS-Page examination its expression can be seen at the bottom approaching the marker region in the 15 kDa region. The ribbon that appears positive in the area is located at well number 9 filled by the control ATP6V0C is one of the factors expressed by host cells, which have a role in the process of replicating influenza virus cells. Vaccination is expected to prevent the virus from entering the cell, so ATP6V0C will not group. The ATP6V0C band was not expressed in all vaccination treatment groups, but was expressed in the control group. Table 2 Results of analysis of SDS-Page 1 protein expression dimensions of one shot serum ferret samples Molecular Weight (kDa) Marker well 1 well 2 well 3 well 4 well 5 well 6 well 7 well 8 well 9 1 250 180 155.6 147.8 147.8 150.0 161.4 139.1 161.4 232.4 2 150 143.4 137.1 131.0 131.0 135.0 135.0 68.6 54.7 173.6 3 100 64.5 87.6 49.4 112.8 116.2 118.0 55.3 48.9 56.0 4 75 51.5 50.6 38.8 47.7 47.7 51.2 49.4 37.2 49.1 5 50 41.1 39.4 27.2 38.8 38.5 27.2 25.6 33.6 34.1 6 37 36.1 35.4 25.4 31.9 27.0 26.3 13.7 31.4 27.1 7 25 27.0 26.9 23.7 26.2 25.2 24.2 26.7 15.5 8 20 26.0 25.0 10.5 23.2 23.4 12.8 14.7 9 15 23.7 23.5 11.2 11.4 10 10 10.9 10.2 Table 2 shows that well number 9 containing the control group obtained a ribbon filled in kDa 15.5. This is close to the size of ATP6V0C which is 16 kDA. Whereas in other wells namely the group that got the vaccination, the band that was close to the 15 kDA marker was almost non-existent. Research conducted by Pavelin et al. proved that ATP6V0C produced almost complete blocks in cytomegalovirus production. This shows that endosome acidification is very important for HCMV replication. Cell viability tests show that the reduction in viral replication is not due to cellular toxicity caused by ATP6V0C knockdown and small transfection of RNA does not induce interferon responses22. Targeting ATP6V0C can represent a blocking mechanism in the assembly and release of virions during latent infection. Acidification has been proven to be necessary for efficient signaling by toll endosome residents such as receptors and for efficient class II MHC presentations23,24. Blocking endosome acidification by targeting ATP6V0C may be an effective way for viruses to interfere with both innate and adaptive immune responses27. Another study on flavivirus conducted by Barrows et al. revealed that the endoplasmic reticulum membrane protein complex facilitated the biogenesis and / or assembly of receptors needed in the plasma or endosomal membrane which had an indirect effect on viral infection, namely the mechanism of virus entry in cells25. This shows that ATP6V0C plays an important role in virus replication, so that when ATP6V0C is not expressed due to vaccines, the virus cannot replicate and ultimately the results of virus replication will go down. Conclusion In this study TIV vaccination has an effect in the form of decreasing viral replication in ferret that have been tested with H1N1 and H3N2 viruses. The results of this study are data about viral replication obtained from hemagglutination tests (HA test) and ATP6V0C expression with SDS-Page. Conflict of Interest : The authors declare that they have no conflict of interest. Source of Funding: This study supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia. Acknowledgement: We thank Arif Nur Muhammad Ansori for editing the manuscript. Ethical Approval: This study was approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. References 1. Iuliano, A. D. et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet. 2018; 391: 1285–1300. 2. WHO. WHO information for laboratory diagnosis of pandemic (H1N1) 2009 virus in humans - revised. Pathology. 2009: 1–49. 3. WHO. Preliminary review of D222G amino acid substitution in the haemagglutinin of pandemic influenza A (H1N1) 2009 viruses; 2009. 4. Watanabe, T., Watanabe, S. & Kawaoka, Y. Cellular networks involved in the influenza virus life cycle. Cell Host Microbe. 2010; 7: 427–439. 5. Dou, D., Revol, R., Östbye, H., Wang, H. & Daniels, R. Influenza A virus cell entry, replication, virion assembly and movement. Front. Immunol. 2018. 9: 1–17. 6. König, R. et al. Human host factors required for influenza

virus replication. Nature. 2010; 463: 7. 8. 9. 813–817. Cheng, H. et al. A parallel genome-wide RNAi screening strategy to identify host proteins important for entry of Marburg virus and H5N1 influenza virus Emerging viruses. Virol. J. 2015; 12: 1–9. WHO. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness September. Wkly. Epidemiol. Rec. 2015; 81: 445–452. Ryan, K. A. et al. Cellular immune response to human influenza viruses differs between H1N1 and H3N2 subtypes in the ferret lung. PLoS One. 2018; 13: 1–15. 10. Marriott, A. C. et al. Low dose influenza virus challenge in the ferret leads to increased virus shedding and greater sensitivity to oseltamivir. PLoS One. 2014; 9. 11. Smith, W., Andrewes, C. & Laidlaw, P. A Virus Obtained From Influenza Patients. in Animal Influenza 2016 (ed. Swayne, D. E.) 66–68. Lancet; 1933. 12. Cox, R. J., Brokstad, K. A. & Ogra, P. Influenza Virus: Immunity and Vaccination Strategies. Comparison of the Immune Response to Inactivated and Live, Attenuated Influenza Vaccines. Scand. J. Immunol. 2004; 59: 1–15. 13. Wong, S. S. & Webby, R. J. Traditional and new influenza vaccines. Clin.Microbiol.Rev. 2013; 26: 476–492. 14. Skowronski, D. M. et al. Low 2012-13 influenza vaccine effectiveness associated with mutation in the egg-adapted H3N2 vaccine strain not antigenic drift in circulating viruses. PLoS One. 2014; 9. 15. Okamoto, S. et al. Intranasal immunization with a formalin-inactivated human influenza A virus whole-virion vaccine alone and intranasal immunization with a split-virion vaccine with mucosal adjuvants show similar levels of cross- protection. Clin. Vaccine Immunol. 2012; 19: 979– 990. 16. Budimir, N. et al. Heterosubtypic cross-protection induced by whole inactivated influenza virus vaccine in mice: Influence of the route of vaccine administration. Influenza Other Respi. Viruses. 2013; 7: 1202–1209. 17. Budimir, N. et al. Critical Role of TLR7 Signaling in the Priming of Cross-Protective Cytotoxic T Lymphocyte Responses by a Whole Inactivated Influenza Virus Vaccine. PLoS One. 2013; 8. 18. Budimir, N. et al. Induction of heterosubtypic cross-protection against influenza by a whole inactivated virus vaccine: The role of viral membrane fusion activity. PLoS One. 2012; 7. 19. Takeda, M., Leser, G. P., Russell, C. J. & Lamb, R. A. Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. Proc. Natl. Acad. Sci. U. S. A. 2003; 100: 14610– 14617. 20. Hong, D. K. et al. Cationic Lipid/DNA Complex- Adjuvanted Influenza A Virus Vaccination Induces Robust Cross-Protective Immunity. J. Virol. 2010; 84: 12691–12702. 21. Soema, P. C., Van Riet, E., Kersten, G. & Amorij, J. P. Development of cross-protective influenza A vaccines based on cellular responses. Front. Immunol. 2015; 6: 1–9. 22. Pavelin, J. et al. Systematic MicroRNA Analysis Identifies ATP6V0C as an Essential Host Factor for Human Cytomegalovirus Replication. PLoS Pathog. 2013; 9: 1–13. 23. Bénaroch, P. et al. How MHC class II molecules reach the endocytic pathway. EMBO J. 1995; 14: 37–49. 24. Macfarlane, D. E. & Manzel, L. Antagonism of immunostimulatory CpG-oligodeoxynucleotides by quinacrine, chloroquine, and structurally related compounds. J. Immunol. 1998; 160: 1122–31. 25. Barrows, N. J. et al. Dual roles for the ER membrane protein complex in flavivirus infection: viral entry and protein biogenesis. Sci. Rep. 2019; 9: 1–16. 26. Bungener, L. et al. Alum boosts TH2-type antibody responses to whole-inactivated virus influenza vaccine in mice but does not confer superior protection. Vaccine. 2008; 26: 2350–9. 27. Pavelin, J. et al. Systematic MicroRNA Analysis Identifies ATP6V0C as an Essential Host Factor for Human Cytomegalovirus Replication. PLoS Pathog. 2013; 9(12): e1003820. Indian Journal of Forensic Medicine & Toxicology, July-September 2020, Vol. 14, No. 3 1957 1958 Indian Journal of Forensic Medicine & Toxicology, July-September 2020, Vol. 14, No. 3 Indian Journal of Forensic Medicine & Toxicology, July-September 2020, Vol. 14, No. 3 1959 1960 Indian Journal of Forensic Medicine & Toxicology, July-September 2020, Vol. 14, No. 3 Indian Journal of Forensic Medicine & Toxicology, July-September 2020, Vol. 14, No. 3 1961 1962 Indian Journal of Forensic Medicine & Toxicology, July-September 2020, Vol. 14, No. 3