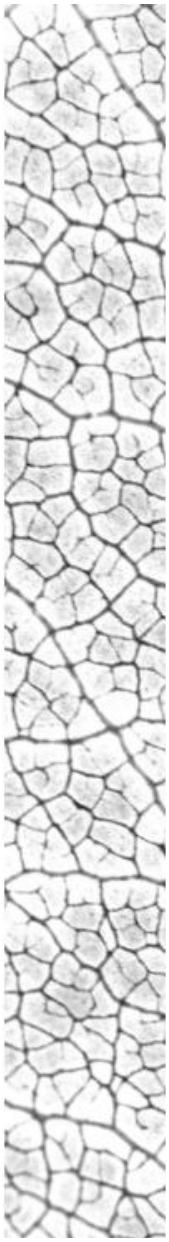

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Evaluating bioactivity of benzo[a]pyrene toward expression of cd4+cd62l+, cd8+cd62l+, and cd4+ifnÿ in the biology of measles paramyxovirus-vaccinated mice

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Abstract
Current prevention strategies suggest that immunization is still the best option against measles and can manipulate the biologic characters of these viruses. However, immunization is often ineffective in a number of children because of the biological trait of different ages. To a significant extent, this phenomenon is caused by internal and external factors that influence children's immunity. One of the external factors in this regard is the benzo[a]pyrene pollutant, both directly and indirectly. Accumulated benzo[a]pyrene can infiltrate deoxyribonucleic acid (DNA) to form benzo[a]pyrene and AhR bonds (Aryl Hydrocarbon Receptor). Such bonds affect the differentiation of immune cells, resulting in immunosuppressive activity. This study aims to analyze the bioactivity of benzo[a]pyrene with respect to the expression of $CD4^+CD62L^+$, $CD8^+CD62L^+$, and $CD4^+IFNŸ$ in a measles paramyxovirus-vaccinated mouse. Approximately 20 mg/kg BW of benzo[a]pyrene was injected using an intramuscular approach twice per week for one month. Furthermore, flow cytometric analysis was performed to determine immune cell expression. The results showed that mice under-vaccination and benzo[a]pyrene treatment suppress the expression of $CD4^+CD62L^+$ naïve T cells, increases the expression of $CD8^+CD62L^+$ naïve T cells, and reduces the expression of $CD4^+IFNŸ$. Controlling internal and external factors enhanced the effectiveness of measles immunization in children.

Keywords: Benzo[a]pyrene, measles paramyxovirus vaccine, $CD4^+CD62L^+$, $CD8^+CD62L^+$, $CD4^+IFNŸ$

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INTRODUCTION

Measles is an acute viral and contagious disease characterized by three stages: incubation, prodromal, and eruption. It is one of many infectious diseases affecting children (Rudolph 2006). The incidence of measles in Indonesia is relatively high. In 2014, the measles incidence amounted to 12,943 cases, a result which is higher than in 2013 (11,521 cases). The highest prevalence of measles considered to be at epidemic level was found in East Java Province, with 41 incidences and 187 cases. This number is followed by Banten and South Sumatra Province, which comprised 14 incidences (Untung Suseno 2015).

Based on an immunization implementation report, the achievement of immunization targets for children aged under five years was more than 90%. These data are collected from district, city, province, and national levels. This high rate of immunization target achievement should be followed by a decline in measles incidence. However, not all children in Indonesia experience the advantages of immunization (Untung Suseno 2015).

Growing children generally suffer from more than 100 different infectious diseases prior to reaching adulthood. Pathogenic microorganisms, including viruses, bacteria, and other parasites, can invade humans to begin an infection. The emergence of infectious diseases occurs more easily if the immune system fails to manage such infections. Several factors suppress human immunity, such as high rates of pollution in soil, water, and air, as well as factors associated with lifestyle (Rudolph 2006). Lifestyle is most typically related to local cultures, including eating habits.

Indonesians often consume smoked, burned, and baked foods. Such food-consumption patterns are

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increasing, particularly in children. These types of foods are harmful to children's health due to being associated with benzo[a]pyrene compounds. The levels of benzo[a]pyrene in smoked fish can reach 97.2 ppm, exceeding the Indonesia National Standard threshold (0.005 ppm) (Sarnia et al. 2018). Benzo[a]pyrene compounds are carcinogenic, immunotoxic, and immunosuppressive (Hengartner 1996). The immunosuppressive activity of benzo[a]pyrene alters the development of various immunocompetent cells, particularly T and B cells (Laupeze 2002).

In general, people have difficulty avoiding benzo[a]pyrene compounds directly or indirectly. Such compounds from the environment can enter the human body in various ways — through the digestive system, skin, and respiratory tract. Benzo[a]pyrene compounds penetrate the respiratory tract most typically due to pollution of hazardous and toxic materials in the air (Brown et al. 2009, Errayes et al. 2020, Sysa et al. 2019).

The effects of pollutants depend on exposure amount as well as host susceptibility. Exposure to air pollutants is not limited to inhalation via the respiratory tract. In addition to the active substance factors carried by these pollutants, pollutant size also determines the anatomic location of pollutant deposits and their effects on surrounding tissues. For example, the presence of fine particulate matter (PM) measuring less than 1 μm can be easily absorbed into systemic blood vessels. The presence of ultrafine PM may cause procoagulant effects that can interfere with blood circulation and facilitate pollutants spreading throughout the body (Kenrad Nelson 2005).

A high level of air pollution is associated with increasing human population and its associated requirements. In Indonesia, motor-based vehicles are the main source of air pollution. The majority of such vehicles emit high levels of pollution due to inadequate maintenance and use of poor quality fuels (such as those containing a high lead content), resulting in increased PM levels in the air. Such high PM levels lead to increased air benzo[a]pyrene concentrations. Benzo[a]pyrene is hydrophobic and is thus not easily excreted from the body; it therefore tends to accumulate in tissues. With structures that resemble nucleic bases, benzo[a]pyrene infiltrates DNA strands with relative ease, blocking the interleukin-1 (IL-1) product and causing abnormal chemical induction of cellular functions (Davila, 1996). This study aims to analyze the bioactivity of benzo[a]pyrene with respect to the expression of $CD4^+CD62L^+$, $CD8^+CD62L^+$, and $CD4^+$IFNŸ in measles paramyxovirus-vaccinated mice (*Mus musculus* L).

METHODS

This study comprises a split-plot design (separate randomized design). The research was conducted at the Laboratory of Physiology, Structure and Animal Development, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang. The research was carried out for approximately three months and has been granted ethical clearance from the Brawijaya University Animal Care and Use Committee with No: 1012-KEP-UB on February 6, 2018.

Research Procedure

In this study, approximately 40 female BALB/c mice were used. Mice were eight weeks old, with initial body weight around 20–25 g. Mice were acclimated for two weeks. The population of mice was divided by random sampling into four groups, as follows:

P1 Group: Group 1 treatment, ten mice without any exposure. Further, about five mice were evaluated for two weeks, and the others for four weeks.

P2 Group: Group 2 treatment, ten mice were treated with the measles vaccine. Further, about five mice were evaluated for two weeks, and the others for four weeks.

P3 Group: Group 3 treatment, ten mice were exposed to benzo[a]pyrene through an intramuscular approach using approximately 20 mg/kg BW twice per week. Further, about five mice were evaluated for two weeks, and the others for four weeks.

P4 Group: Group 4 treatment, ten vaccinated mice were induced by benzo[a]pyrene through an intramuscular approach of approximately 20 mg/kg BW twice per week. Further, about five mice were evaluated for two weeks, and the others for four weeks.

At the final day of treatment, each mouse was sacrificed and dissected to collect the spleen for immunological staining.

Data Analysis

A BD FACS Calibur flow cytometer was used to evaluate the expression of immune cells in treated mice (*Mus musculus* L).

RESULTS AND DISCUSSION

Expression of $CD4^+CD62L^+$

The expression of $CD4^+CD62L^+$ in the benzo[a]pyrene treated mice after week 4 was 23.72%. Differences were observed compared to standard groups. The benzo[a]pyrene treatment group demonstrated lower expression than controls. Benzo[a]pyrene treatment in the mice led to $CD4^+$ cell repression. This result is in accordance with a study conducted by Holladay regarding benzo[a]pyrene exposure in pregnant mice. Flow cytometric analysis showed that benzo[a]pyrene exposure produced a significant reduction in the presentation of $CD4^+$ fetal thymocytes. Benzo[a]pyrene is one of the PAHs that can

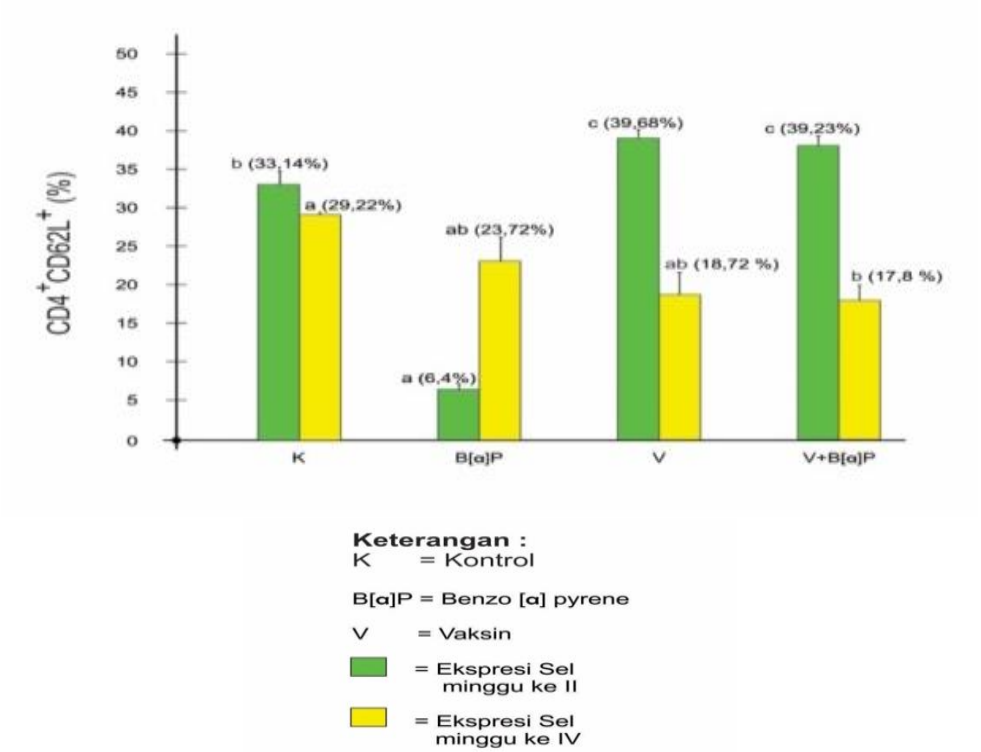

Fig. 1. Induction of benzo[a]pyrene at week 2 does not suppress naive T cell levels after vaccination. Naive T cell levels after four weeks decreased and showed no significant difference for all treatment groups. Cells were isolated from spleen BALB/C mice stained with anti- $CD4^+$ and anti-$CD62L^+$. Staining results were analyzed using flow cytometry.

destroy cells. Exposure to benza[a]pyrene suppresses cell and immune function. Based on quantitative and qualitative analysis of immune cells, antigen expression correlated with post-natal immunosuppression (Holladay SD et al. 1994, Akpan and Udoh 2017).

The expression of $CD4^+CD62L^+$ in benzo[a]pyrene group at week 4 was increased compared to week 2. This expression is due to an infection response from the second exposure to benzo[a]pyrene at week 4. Exposure to benzo[a]pyrene will induce TNF-α and IL-1β in PMN. These results indicate the involvement of [Ca (2+)]i, tyrosine kinase, inflammatory cytokines and NF-κB in the iNOS expression mediated by CYP1A1 in benzo[a]pyrene-treated mice (Kumar A et al. 2007, Platonova et al. 2019).

The level of $CD4^+CD62L^+$ in vaccinated mice was 18.72%. The expression of $CD4^+CD62L^+$ showed a difference between weeks 2 and 4. Hence, cell signal transduction activation functions as a down-regulation mechanism. In the immune system, signal transduction occurs in cell activation and suppression. In activation mechanism, the CD28 T cells will bind to B7 molecules in APC. The bond provides a transduction signal to the DNA so that appropriate transcription occurs. After becoming activated, T cells express CD152, which are important molecules with respect to down-regulation. Such down-regulation is required in the arrangement of homeostasis so that activated cells can be controlled (Rifai, 2013, Izah 2019).

In the group of vaccinated mice treated with benzo[a]pyrene, expression of $CD4^+CD62L^+$ was 17.8%. This treatment showed no significant difference between weeks 2 and 4. The $CD4^+CD62L^+$ expression decreased by 21.43% compared to week 2. This decrease illustrates the presence of cell transduction signal activation, functioning as a downregulation mechanism. Down-regulation is necessary for maintaining naive cell balance. If regulator T cells work particularly actively, naive cells fail to develop into effector cells (Rifa'i 2018, Al–Taai 2016).

Expression of $CD8^+CD62L^+$

The expression of $CD8^+CD62L^+$ after week 4 in benzo[a]pyrene-treated mice was 17.29%. This result differed to the control group and illustrated that benzo[a]pyrene is a type of pollutant that can suppress cell function. Thus, $CD8^+$ as a co-receptor on killer T cells is not able to work proportionally. This response is indicated by the expression of $CD8^+CD62L^+$ being lower than that for the control. $CD62L^+$ is a transmembrane glycoprotein and is expressed by lymphocytes, neutrophils and NK cells. $CD62L^+$ is particularly important with respect to enabling extravasation of leukocytes during inflammation (Rifa'i, 2013). Benzo[a]pyrene affects a variety of cellular functions. Therefore, the expression of $CD8^+CD62L^+$ was lower than the control group. Benzo[a]pyrene can affect the function of several organs, tissues, and cellular function;

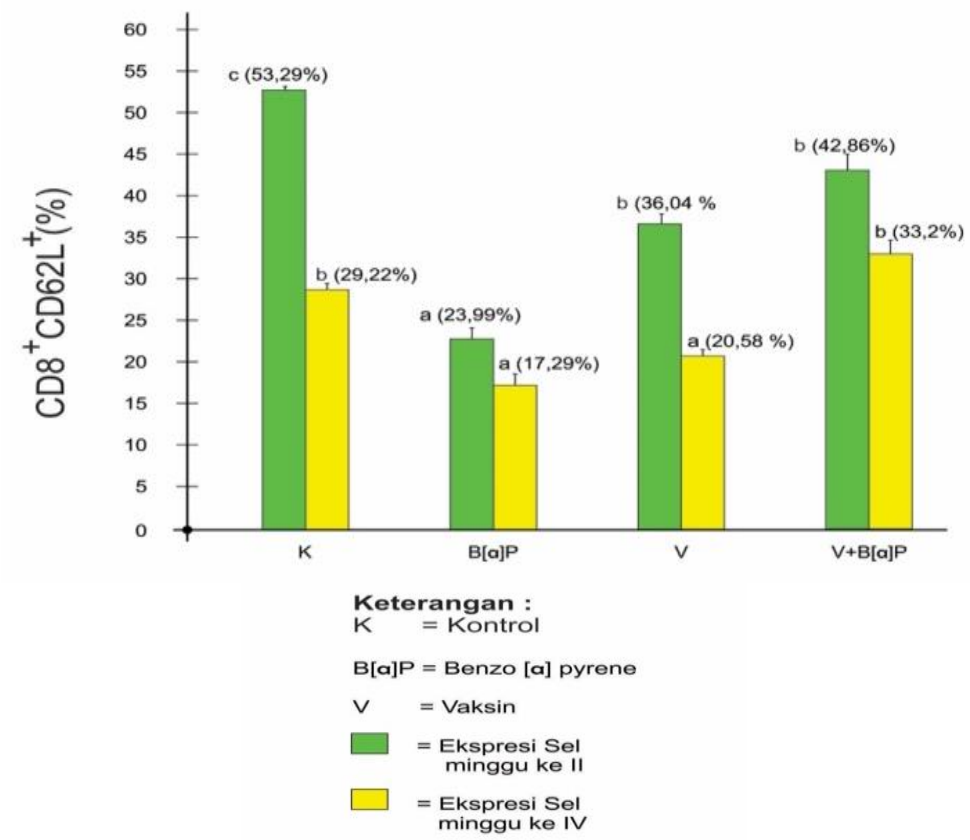

Fig. 2. Benzo[a]pyrene exposure at week 2 did not suppress naive T cell expression on $CD8^+$ as indicated by N cell expression of the vaccinated group, a result which was insignificantly different from that of vaccinated mice treated with benzo[a]pyrene. Provision of benzo[a]pyrene in mice that have been vaccinated after week 4 activates naive T cell expression on $CD8^+$ and shows differences with the vaccinated group. Cells were isolated from the spleen of BALB/C mice and stained with anti-$CD8^+$ and anti-$CD62L^+$. The staining results were analyzed using flow cytometry.

it is hepatotoxic, genotoxic, and immunotoxic (Lara Zacari et al. 2018).

In the vaccinated-mice model, different levels of $CD8^+CD62L^+$ were observed compared to the control group. The expression of $CD8^+CD62L^+$ in this group was 20.58%. This response illustrates that the paramyxovirus vaccine as a live virus is attenuated, resulting in stimulation of activation of $CD8^+$ due to killer T cell co-receptors being low. Evidence for this response is provided by research conducted by Christopher Yag, who concluded that individuals who are exposed to the measles virus for the first time may show immunosuppression and cellular immune response impairment (Christopher L. Karp 2006).

The relative number of $CD8^+CD62L^+$ in vaccinated mice treated by benzo[a]pyrene was 33.2%, a result which was different compared to the vaccinated-mice group. Exposure to benzo[a]pyrene in vaccinated mice can increase the expression of naive T cells because after week 4, the killer T cells recognize the antigen complex. This response affects the major histocompatibility complex I (MHC I); $CD8^+$ acts as a co-receptor and strengthens signal transduction so that killer T cells are activated (Rifa'i 2013).

Expression of $CD4^+IFN\text{-}\ddot{Y}^+$

Expression of $CD4^+IFN\ddot{Y}^+$ at week 4 showed no difference between control and benzo[a]pyrene-treated groups. $CD4^+IFN\ddot{Y}^+$ expression in benzo[a]pyrene-treated mice was 1.26%, illustrating a low infection response to benzo[a]pyrene. Benzo[a]pyrene is treated by the immune system as an antigen and significant cell damage can subsequently occur. An increase in interferon (IFN) expression indicates the body's response to benzo[a]pyrene. IFN is a cytokine in the form of glycoproteins produced by activated macrophages, NK cells, and various body cells containing a nucleus, released in response to viral infection. IFN has antiviral properties and can induce cells around those cells infected with the virus to become resistant to viruses. IFN can activate NK cells. Virus-infected cells become malignant and will show changes on their surface that lead to them being recognized and destroyed by NK cells. Therefore, the spread of viruses can be prevented (Baratawidjaja 2010).

The expression of $CD4^+IFN\ddot{Y}^+$ in vaccinated mice showed no difference compared to other treatment groups. The group of vaccinated mice showed a $CD4^+IFN\ddot{Y}^+$ expression of 1.51%, indicating a relatively

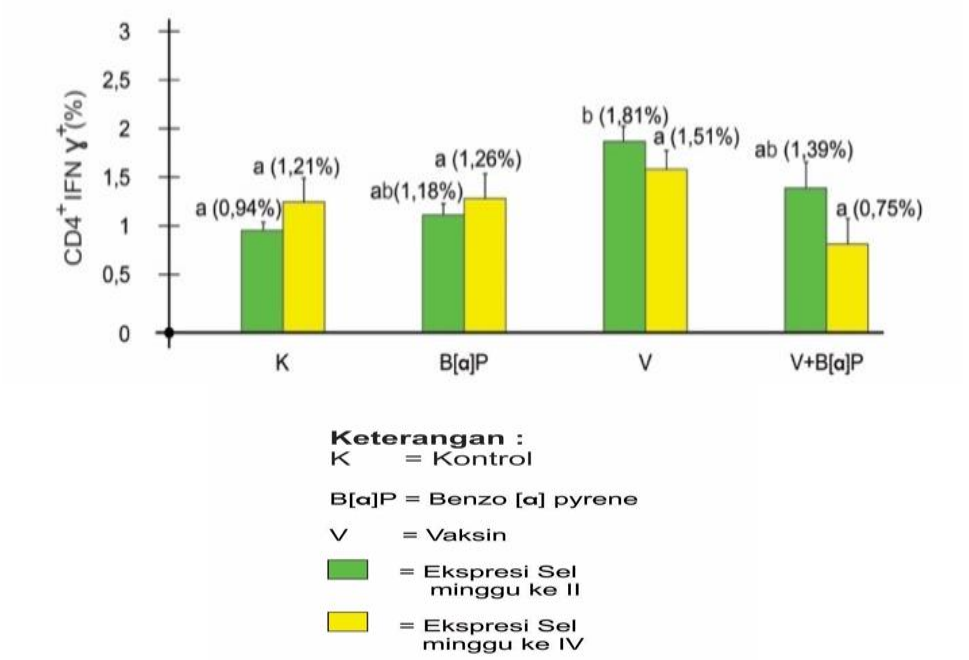

Fig. 3. Antigen exposure at week 2 increases IFNŸ$^+$ activation as an immune system mediator with insignificantly different expressions between vaccinated mice groups and the group of mice exposed to benzo[a]pyrene after being vaccinated. After week 4, IFNŸ$^+$ activation in vaccinated mice and those treated with benzo[a]pyrene decreased compared to week 2 and showed no difference between all treatment groups. Cells were isolated from spleen BALB/C mice and stained with anti-CD4$^+$ and anti-IFNŸ$^+$. The staining results were analyzed using flow cytometry.

low infection response. Interferon is a protein produced when people are infected with viruses, bacteria, or parasites. Virus-infected cells immediately produce and secrete IFN (Rifa'i 2018).

Vaccinated mice treated with benzo[a]pyrene showed no difference compared to the vaccinated-mice only group. Benzo[a]pyrene exposure decreases IFNŸ$^+$ activation. Hence, IFNŸ$^+$ activation inhibits proliferation of infected cells. IFNŸ$^+$ acts as an immunomodulator, which are agents that affect (weaken or enhance) the immune response. In immune-deficient persons, immunomodulators work by stimulating the immune system. On the other hand, in auto-reactive cases, immunomodulators function by suppressing/ normalizing the immune system. The presence of IFNŸ$^+$ allows T cells to become more sensitive to mitogens or growth factors, and therefore IFNŸ$^+$ is a modulator for the development and differentiation of T cells (Rifa'i, 2018).

CONCLUSION

The expression of CD4$^+$CD62L$^+$, CD8$^+$CD62L$^+$, and CD4$^+$IFNŸ$^+$ cells in week 4 of treatment was suppressed in paramyxovirus-vaccinated mice after treatment with benzo[a]pyrene at a dose of 20 mg/kg BB two times per week.

Finally, controlling internal and external factors enhances the effectiveness of measles immunization in children.

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