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The Effect of Benzo [A] Pyrene on Changes in the Profile of CD11B + IL1 +, CD11B + IL17 +, CD4 + CD25 + in Mice (MUS MUSCULUS, L) after Getting Paramicsovirus Vaccine

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ABSTRACT
Measles is an infectious disease that attacks children. The incidence of measles in Indonesia is still quite high. The results of the immunization implementation report stated that the achievement of immunization targets for children under five reached more than 90%. However, not all children in Indonesia can enjoy the benefits of the immunization that has been given. Humans in their lives cannot avoid benzo [a] pyrene compounds, either directly or indirectly. The effect of benzo [a] pyrene on changes in immune cell profile in mice (Mus musculus L) was analyzed in vivo by giving intra-muscular injection of benzo [a] pyrene 20 mg / kg BW 2 times / week for 4 weeks. Immune cell profiles were analyzed using flowcytometry. The analysis was continued with statistical analysis using SPSS software using the one way ANOVA test with a significance level (p-value <0.05). The immune cell profile after week 4 in the group of mice that had received the vaccine and given exposure to benzo [a] pyrene was repression occurred in CD11b + IL1 +, CD11b + IL17 + activation occurred, while CD4 + CD25 + cell profiles experienced a downregulation reaction.

Keywords: Benzo [a] pyrene, measles, measles vaccine (paramiksovirus), immune cell

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INTRODUCTION
An infectious disease that is still causing problems for children in Indonesia is measles (Rudolph, 2006). The incidence of measles in Indonesia is still quite high. In 2014, there were 12,943 cases of measles reported. This figure shows an increase from the previous year, which was 11,521 cases. (Untung Suseno, 2015)

From the results of the immunization implementation report, it is stated that the achievement of the immunization target for children under five has reached more than 90%. This figure is shown from the reports starting from the district and city level, the provincial level and the national level. The high rate of achieving the immunization target should be followed by a decrease to the loss of the incidence of measles (Untung Suseno, 2015).

One of the factors causing decreased immunity in humans, especially in children, is the result of high levels of pollution and daily living patterns (Rudolp, 2006). The pattern of consuming foods that are burned and roasted too dry is indicated to contain benzo [a] pyrene compounds. Benzo [a] pyrene levels in smoked fish can reach 97.2 ppm, passing the threshold set by SNI 0.005 ppm (Sarnia, et al, 2018). Benzo [a] pyrene compounds that enter the body are carcinogenic, immunotoxic and immunosuppressive ability to suppress the immune system (Hengartner, 1996). The immunosuppressive properties of benzo [a] pyrene are carried out by inhibiting the development of various immunocompetent cells, especially the immune system, which is played by T cells and B cells (Laupeze, 2002).

Benzo [a] pyrene as one of the top-twenty of the B3 version of the Environmental Protection Agency (EPA) in 1997, (Kenrad Nelson, 2015). From the research results it is reported that in Indonesia 70% of air pollution is caused by motor vehicles. Motorized vehicles emit various hazardous substances, including: 100% black lead (Pb), 13-14% Suspended partucillary matter (SPM), 71-81% hydrocarbons, and 39-73% sodium oxide (NOX) (Kendrad Nelson, 2015).

The nature of benzo [a] pyrene is hydrophobic so that it is difficult to excrete from the body and easily accumulates in body tissues. With a structure that resembles a nucleic base, benzo [a] pyrene easily inserts itself into the DNA strands which results in blocking inter-leukin (IL-1) products which cause chemical induction abnormalities in cell function (Davila, 1996). This situation results in disturbances in the body's systems that lead to various infectious diseases, including measles

MATERIALS AND METHODS
This research is an experimental research type, and the design used is a split plot design (separate randomized design). This experimental research was carried out in a laboratory. In more detail, it is described in table 1 below:

Table 1 Research Design

Group	Duration of Exposure Type of Exposure	Second Week	Week IV
P1	No Exposure	5 repetitions	5 repetitions

P2	Measles Vaccine	5 repetitions	5 repetitions
P3	Benzo[a]pyrene 20 mg/kg BB	5 repetitions	5 repetitions
P4	Measles and Benzo [a] pyrene Vaccines 20mg / Kg BW	5 repetitions	5 repetitions

The samples used in this study were female mice of the BALB / C strain aged 8 weeks (Specific Pathogen Free / SPF), with an initial body weight of 20-25 grams, the number of samples in each group was 5 mice. The immune cell profiles in mice (Mus Musculus L) were analyzed using flow cytometry. The analysis was continued with statistical analysis using SPSS software using the one way ANOVA test. If there is significance (p-value <0.05), then a Tukey test is performed to determine significance between treatment groups.

RESULTS AND DISCUSSION

The effect of benzo [a] pyrene on changes in immune cell profile in mice (Mus musculus, L) after receiving paramiksovirus measles vaccine was analyzed in vivo by giving intra-muscular injection of benzo [a] pyrene 20 mg / kg BW for 2 weeks and 4 weeks. The experimental animals were divided into four groups, namely: mice without exposure, mice with exposure to measles vaccine, mice with exposure to benzo [a] pyrene 20 mg / kg BW 2 times / week, and mice given measles vaccine and pollutant benzo [a] pyrene. 20 mg / kg BW 2 times / week. The expression of immune cells in mice (Mus musculus, L) was analyzed using flow cytometry. The analysis was continued with statistical analysis using SPSS software using the one way ANOVA test as follows:

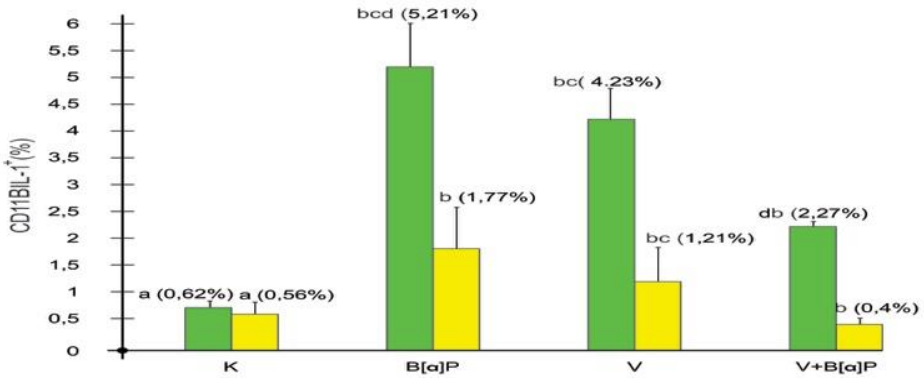

Figure 1. CD11b + IL-1 + cell profile

Figure 1 : Exposure to benzo [a] pyrene at week 2 repressed CD11b + activation as a leukocyte adhesion molecule which was shown not significantly different between the group given the vaccine and the group given exposure to benzo [a] pyrene after vaccination. After 4 weeks of exposure to benzo [a] pyrene, still repressive CD11b + activation was shown with no significant difference between the vaccinated group and the benzo [a] pyrene exposure group after vaccine administration. Cells were isolated from the spleen of BALB / C mice stained

with anti CD11b + and anti IL-1 +. The results of the staining were analyzed using flowcytometry and supported by ANOVA analysis with a significance level (□) <0.05.

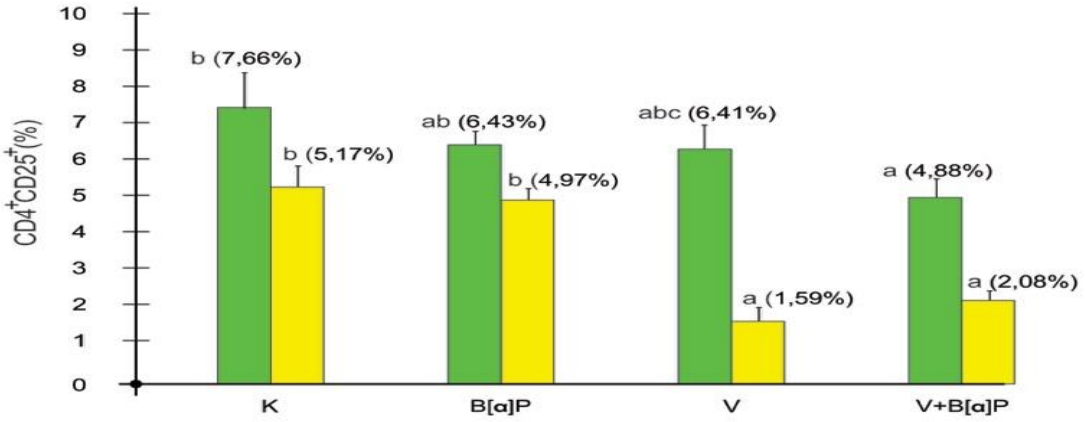

Figure 2. CD11b + IL-17 + cell profile

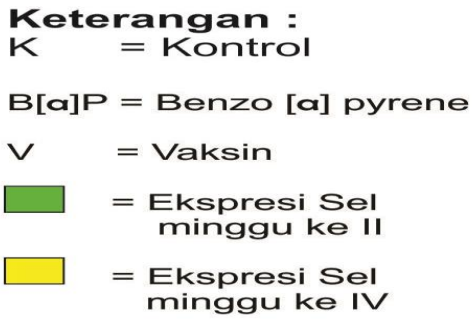

Figure 3. Administration of benzo [a] pyrene at week 2 repressed activation of regulatory T cells with the cell profile of the vaccine group was not significantly different from the group given benzo [a] pyrene after being vaccinated. After the fourth week of exposure to benzo [a] pyrene, the response was down-regulated with the cell profile of the group given the vaccine not different from the group given benzo [a] pyrene after being vaccinated. Cells were isolated from spleen of BALB / C strain mice stained with anti CD4 + and anti CD25 +. The staining results were analyzed using flowcytometry and supported by ANOVA analysis with a significance level (() <0.05.

Based on Figure 1, it can be explained that at week 2 the CD11b + IL-1 cell profile in the control group of mice was the lowest compared to the other groups of mice, namely 0.62%, so it was different from the treatment group. This statement is supported by ANOVA analysis with p <□. This cell profile occurred because there was no inflammatory response in the control group of mice. CD11b + is known as the □M integrin and forms the basis of the release of integrin receptors that recognize intercellular adhesion and extracellular components. Integrins are active signaling receptors that recruit leukocytes to sites of inflammation and increase cell activation. Integrin □ M (CD11) becomes a receptor known as macrophage antigen-1 (Mac-1) or complement type 3 (CR-3) complement (Baratawidjaja, 2010)

CD11b + IL-1 + cell profile in the group of mice exposed to benzo [a] pyrene was 5.21%. This group differs from the expression of the control group. This statement is supported by ANOVA analysis with p value (0.000) <□. This is because CD11b + functions as a leukocyte adhesion molecule against antigens. Exposure to benzo [a] pyrene as an agent, which is given to mice captured by CD11b +, is known as an integrin. Integrins are active signaling receptors that recruit leukocytes to the inflammatory site and increase cell activity. (Baratawidjaja, 2010)

The group of mice given paramiksovirus vaccine showed a CD11b + IL-1 + cell profile of 4.23%. This group showed a cell profile that was significantly different from the control group. This statement is supported by ANOVA analysis with p value (0.001) <□. This suggests that the paramiksovirus vaccine in the form of a live, attenuated virus will be captured by CD 11b and will bind leukocytes to inflammatory sites lower than exposure to benzo [a] pyrene pollutants (Baratawidjaja, 2010).

The group of mice given the measles vaccine and then exposed to the pollutant benzo [a] pyrene showed a CD11b + IL-1 + cell profile of 2.27%. This group showed a cell profile that was different from the control group. This statement is supported by ANOVA analysis with p value (0.000)> □. This cell profile suggests that given benzo [a] pyrene suppresses the inflammatory response. Giving benzo [a] pyrene will induce hypocellularity of the thymus and inhibit the normal thymocyt maturation process (Holladay SD, 1994).

The CD11b + IL-1 + cell profile after week 4 showed that between the control group and the group of mice exposed

to benzo [a] pyrene showed a difference. This statement is supported by ANOVA analysis with p value (0.004) <. CD11b + IL-1 + cell profile in mice exposed to benzo [a] pyrene was 1.77%. The exposure to benzo [a] pyrene given to the group of mice was accepted as an antigen, thereby activating CD11b + as a leukocyte adhesion molecule. This response was demonstrated by the high profile of CD11b + IL-1 + cells. The main function of IL-1 + is as an inflammatory mediator in response to infection and other stimuli. The main function of IL-1 + is the same as that of TNF + (Tumor Necrosis Factor) (Baratawidjaja, 2010). As an inflammatory mediator IL-1 + has the ability to initiate immune responses and trigger inflammation. IL-1 + is also a pro-inflammatory cytokine that acts as an endogenous pyrogen. Another effect of IL-1 + is that it affects cell proliferation, differentiation, and function of immunocompetent cells, both cells involved in the innate and specific immune systems (Rifa'i, 2013).

The CD11b + IL-1 + cell profile in the group of mice that were given exposure to the paramiksovirus vaccine showed that there was a difference with the control group. The CD11b + IL-1 + cell profile in the group of mice exposed to the paramiksovirus vaccine was 1.21%. This statement is supported by ANOVA analysis with p value (0.021) <. The paramiksovirus vaccine given is in the form of a live, attenuated vaccine, thereby stimulating an inflammatory response. This is indicated by the CD11b + IL-1 + cell profile above the control group. CD11b is known as integrin alpha M. Integrin is an active signaling receptor that recruits leukocytes to the inflammatory site and increases cell activation. Integrin alpha M (CD11b) is assembled with integrin beta-2 (CD18) into a receptor known as macrophage antigen I (Mas-1) or complement type 3 (CR3) complement (Baratawijdaja, 2010)

CD11b + IL-1 + cell profiles in the group of mice given the measles vaccine (paramiksovirus) then exposed to the pollutant benzo [a] pyrene showed no significant difference compared to the group given the vaccine. This statement is supported by ANOVA analysis with p value (0.853)> . The CD11b + IL-1 + cell profile in the treated group showed lower numbers than the group given the vaccine. This indicates that the antibodies in the mice worked well at week four of the treatment, so that the benzo [a] pyrene pollutant did not trigger the inflammatory process in the body. Measles is a serological monotypic virus, so vaccine exposure can protect against infection with surrounding viruses (Sunil R. Vaidy, et al., 2016).

Based on Figure 2 it can be explained that at week 2 the CD11b + IL-17 + cell profile in the group of mice exposed to benzo [a] pyrene was 1.74% different from the control group. This statement is supported by ANOVA analysis with p value (0.019) <. Benzo [a] pyrene given to the group of mice was accepted as an antigen so that it would activate CD11b +. CD11b + integrin receptors increase adhesion between cells and extracellular components. Integrin as an active signaling respector that recruits leukocytes to the inflammatory site and increases cell activation. Cells that bind to IL-17 + will synthesize pro-inflammatory cytokines, chemokines, and metalloproteases. IL-17 + is a cytokine that has many families. IL-17 + can become IL -17A. IL-17A is expressed by activated CD4 + TH17 cells, CD8 cells, NK cells and neutrophils. Maximum IL-17A expression will occur if during TH17 differentiation, naive T cells in humans must be exposed to IL-1, IL-6, IL-23, and TGF-. IL-17A is able to increase cytokine expression, induce adhesion molecule expression in fibroblasts, and regulate complement proteins (Baratawidjaja, 2010).

The group of mice exposed to the paramiksovirus vaccine showed a CD11b + -IL17A + cell profile of 2.83%. This level was higher when compared to the control group. So that shows the difference between the two groups. This statement is supported by ANOVA analysis with p value (0.002) <. The paramiksovirus vaccine given is a live virus that is attenuated so that it will activate the infected cells to bind to IL-17A + to synthesize pro-inflammatory cytokines, chemokines, and metalloproteases. Chemokines that are produced from the cell induction process will attract neutrophils for defense against various pathogens (Rifa'i, 2018).

Benzo [a] pyrene exposure in the group of mice that had been given the vaccine showed the highest CD11b + IL-17 + cell profile. This cell profile illustrates that the benzo [a] pyrene pollutant has been recognized as an antigen, thereby activating CD11b +. This response is strengthened by exposure to the paramiksovirus vaccine, so that the inflammatory reaction increases. This increase also indicated that naive T cells had been exposed to IL-1, IL-6 and IL-23, resulting in high IL-17 + expression (Rifa'i, 2018).

CD11 + -IL17 cell profiles at week 4 in the control group with the group of mice exposed to benzo [a] pyrene showed a difference. The CD11b + IL17 cell profile in mice exposed to benzo [a] pyree was 5.43%, whereas in normal mice it was 2.48%. This statement is supported by ANOVA analysis with p value (0.000) <. This cell profile illustrates that benzo [a] pyrene as an antigen will activate CD11b + to bind to leukocytes in an effort to fight against existing antigens. This was indicated by no difference between the CD11b + IL17 cell profiles in the group of mice exposed to benzo [a] pyrene after being given the vaccine. By inducing cells to produce chemokines, IL17 attracts neutrophils for defense against various pathogens (Rifa'i, 2018).

Prof CDl 11b + IL-17A cells in the group of mice given paramiksovirus vaccine was 1.94%. The cell profile of the mice given the paramiksovirus vaccine showed no difference when compared to the control group of mice. This statement is supported by ANOVA analysis with p value (0.991)> . This cell profile gives an idea that at week 4 the body's antibodies have been formed. This situation was indicated by the CD11b + IL-17A + cell profile under the cell profile of the control group. IL-17A + will be produced when the body's cells are exposed to pathogens from outside. These cells will produce chemokines, IL-17A + attracting neutrophils for body defense. IL-17A + will also induce the production of other proinflammatory cytokines such as TNF, IL1. In the group of mice that already have antibodies in the body, the response is not carried out (Rifa'i, 2018)

CD11b + IL-17A + cell profile showed no difference between the control group and the mice group that had been given the paramiksovirus vaccine with exposure to benzo [a] pyrene which was supported by ANOVA analysis with p value (0.991)> . This illustrates that the antibodies of mice at week four are able to fight antigens from outside, so that the inflammatory process does not occur. Antibodies formed from vaccine exposure decrease CD11b + activation as leukocyte adhesion. CD11b + which is an alpha M integrin as the basis for the release of integrin receptors that recognize adhesion between cells and extracellular components. Integrin is an active signaling

receptor that recruits leukocytes to the inflammatory site and increases cell activation (Baratawidjaja, 2010).

Based on Figure 3 it can be explained that at week 2 the profile of CD4 + CD25 + cells in the group of mice exposed to 6.43% benzo [a] pyrene. The group of mice exposed to benzo [a] pyrene showed no significant difference compared to the control group of mice. This statement is supported by ANOVA analysis with p value (0.269)> . CD4 + CD25 + are the body's regulatory T cells, which are important for maintaining the balance of homeostasis. The antigen that enters the body will stimulate a regulatory T cell reaction. The given benzo [a] pyrene is accepted by the body as an antigen that can cause infection. In the case of infection, initially the CD4 + CD25 + regulatory cells do not work. These cells remain in a naive state. This naive condition is very important to be guarded at the beginning of the infection (Rifa'i, 2013)

The mice group given the paramiksovirus vaccine showed a CD4 + CD25 + cell profile of 6.41%. The resulting cell profile was no different from the group given exposure to benzo [a] pyrene. This statement is supported by ANOVA analysis with p value (0.983)> . The paramiksovirus vaccine given is a live, attenuated virus. This vaccine can cause minor infections. In the case of infection initially CD4 + CD25 + regulatory cells do not work, these cells remain in a naive state (Rifa'i, 2013).

CD4 + CD25 + cell profiles in the vaccinated group of mice were then exposed to 4.88% exposure to benzo [a] pyrene. This group showed that the CD4 + CD25 + cell profiles were not significantly different between the groups that were given the vaccine compared to the groups that were given benzo [a] pyrene after being given the vaccine. This statement is supported by ANOVA analysis with a p value (0.249)> . This shows the absence of regulatory T cell proliferation. CD4 + CD25 + are regulatory T cells that have good regulatory power. Regulatory power is the ability to perform suppression and the ability to be a smart cell (cell intelligence in physiology). Smart cells are characterized by the ability of cells to proliferate at the right time and stop proliferating at the right time. Loss of regulatory ability can lead to susceptibility to infection. The onset of infection occurs when the regulatory T cells are over-suppressed. This excessive suppression will weaken the power of elimination against pathogenic microbes and the weak power of elimination against mutated cells (Rifa'i, 2013).

This cell profile illustrates that at week 4 benzo [a] pyrene induces a down-regulated response by CD4 + CD25 + as a body regulator. CD4 + CD25 + is a "smart cell" that is, a smart cell. This condition is characterized by the ability of cells to proliferate at the right time and stop proliferating at the right time. These cells also know when to carry out and stop suppression (Rifa'i, 2013).

The CD4 + CD25 + cell profile of the mice given the vaccine was 1.59%. The CD4 + CD25 + cell profiles in the group of mice that were exposed to the paramiksovirus vaccine showed a difference with the control group. This statement is supported by ANOVA analysis with a P value (0.005) <. This illustrates that the antibodies in the body have been formed, thus giving a down-regulated response to CD4 + CD25 +. Benzo [a] pyrene exposure in mice that had been given the vaccine showed a CD4 + CD25 + cell profile of 2.08%. This cell profile did not differ from the group that was given the vaccine. This statement is supported by ANOVA analysis with p value (0.372)> . This response shows the working of the mouse down regulation function. The down-regulatory response is needed to maintain cell homeostasis. Down regulation is carried out by TH1 and TH2 with the cytokines they produce will work in balance and become mutually down regulators and up regulators (Rifa'i, 2018).

CONCLUSIONS

Exposure to benzo [a] pyrene given to mice (Mus musculus, L) at a dose of 20 mg / kg BW 2 times / week, at week 4 affected the T cell profile of naive

that is, there is repression on CD11b + IL1 +, there is activation of CD11b + IL17 +, the profile of CD4 + CD25 + cells has a down-regulation reaction.

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