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PROTEIN OF *Sarcoptes scabiei* var.*caprae* INDUCING RABBIT'S IMMUNE RESPONSE AND TOLL-LIKE RECEPTOR-2 (TLR-2) AS MARKER

Nunuk Dyah Retno Lastuti[1], Fedik Abdul Rantam[2], Pudji Hastutiek[1] and Dony Chrismanto[3]

[1]*Department of Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya*
[2]*Department of Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya*
[3] *Study Program of Animal Health, Faculty of Vocation, Universitas Airlangga, Surabaya*

Abstract

This research aims are to detect *S.scabiei* var.*caprae* antigenic protein which can induce cellular immune response in rabbit as acquired immunity with TLR-2 as marker. This research was performed in several stages i.e soluble protein *S.scabiei* var.*caprae* mites extraction; rabbit immunization by inoculating protein antigen *S. scabiei* var. *caprae* with dosage of 200µg, repeated five times as booster in two weeks; examination of TLR-2 expression using direct immunofluorescence technique. Cellular immune response is shown by TLR-2 expression in rabbit T limphocytes which appear yellow to green fluorescence color using fluorescence microscope. The amount of fluorescence T lymphocytes showed a significant difference ($p<0.05$) between control and various boosters, and significantly increased in 3rd booster or 42 days post immunization. The antigenic protein of *S.scabiei* var.*caprae* contains ligands, which involve in pathogen associated molecular pattern (PAMP) that can induce cellular immune response in rabbit as with TLR- 2 as marker. It shows that TLR-2 is not only involved in innate immunity but also in acquired immunity.

Keywords : *acquired immunity, goat, Sarcoptes scabiei var.caprae , TLR-2*

1. INTRODUCTION

Sarcoptes scabiei is the causal agent of the highly contagious disease sarcoptic mange (scabies) that affects animals and humans worldwide. Presently it is considered as an emerging/re-emerging parasitic disease that threatens human and animal healthy globally (1). Scabies is an endemic disease, but occasionally outbreaks can be occurred and attacks most of cattle and goats in Indonesia. Since the prevalence of the disease in man and animals is very high, the economic losses caused by the disease are enormous (2). In order to overcome those problem, it is required to develop preventive action by research for vaccine development as alternative for scabies prevention on goats in Indonesia. Vaccine development requires preliminary research through immunogenic protein exploration of *S. scabiei var caprae* which is isolated from goats. Immunogenic

protein can work maximally as vaccine if contains molecules that can stimulate T cells activation which plays role in immune system equivalent to Toll like Receptor (TLR) as innate immunity. The latest research result showed that TLR signal is capable as determiner in naive T cells toward the Th1 and Th2 response (3, 4). To investigate that TLR plays role in adaptive or acquired immunity, it requires a research towards *S. scabiei* protein immunized in rabbits to detect cellular immune response and TLR-2 as marker.

2. METHODS

Prior to immunization, five rabbits were treated as per animal welfare concept (five freedoms) and given health examination based on both clinical symptoms and laboratory tests (approved by Ethical Committee, Faculty of Veterinary Medicine, No: 630-KE). Each of experimental rabbits injected by 200 µg *S.scabiei* var.*caprae* protein. Every two weeks the injection was performed with the same protein with the dosage 200 µg each, and booster was performed 5 times in 2 weeks. For the examination of TLR- 2 was performed based on Boyum method (1968) with some modifications (5), as these following procedures, 5 ml whole blood and washed with 10 % PBS, centrifuged at 1600 rpm and temperature 10ºC for 10 minutes, next the undercoat to be put on Ficoll isopaque. The mixture contained blood and Ficoll isopaque is centrifuged at 1600 rpm and temperature 10ºC for 10 minutes. The resultant buffy coat is separated and washed with PBS. TLR-2 examination was performed as these following stages, buffy coat and 300 µl Minimum Essential Medium Eagle (MEM) incubated at 37ºC for an hour, the solution is fixated by absolute methanol, and blocked by PBS and 1% serum for 15 minutes, next the solution is washed using PBS and was added first antibody *Mouse Anti-TLR2 Monoclonal antibody*. Next, the solution was washed by PBS and Foetal Calf Serum 1% was added. Second antibody of Fluorescein isothiocyanate (FITC) conjugate was added, incubated for 45 minutes to 1 hour, and washed by PBS. The result was examined by fluoresence microscope using magnification 200x and 400x, to find out whether any yellow to green fluorescence color from T cells expressing TLR-2. If there is fluorescence light from T cells showing activated immune response, then the calculation is conducted towards the amount of fluorescent T cells.

3. RESULT

Cellular immune response is shown by the expression of TLR-2 in rabbit T cells which is marked by the yellow to green fluorescence color after rabbit immunization up to 5 times booster (Figure 1).

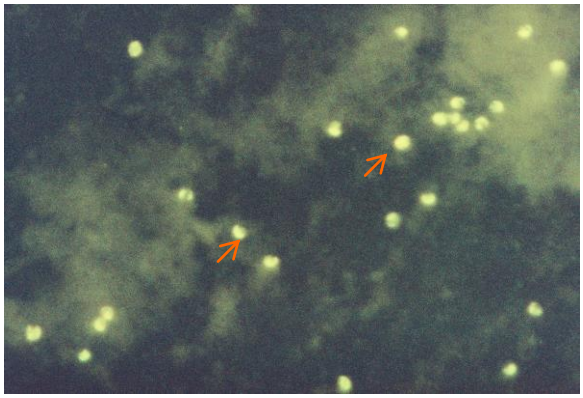

Figure 1. TLR-2 ( ) expression in rabbit T cells visualized by Fluorescein isothiocyanate (FITC) (400x).

In addition, the amount of fluorescence T cells was counted for each 20 µl buffy coat, it was shown the amount of fluorescence T cells is increased in accordance with the treatment from various boosters. The amount of T cells which express TLR-2 shows a significant difference ($p<0,05$) between control and various booster, but between booster 3 (day 42) and booster 4 (day 56) is not significantly different by statistic despite the increasing amount of fluorescence T cells, while the amount of fluorescence T cells in booster 5 (day 70) is highly increased and significantly different with booster 4 ($p<0,05$) (Figure 2).

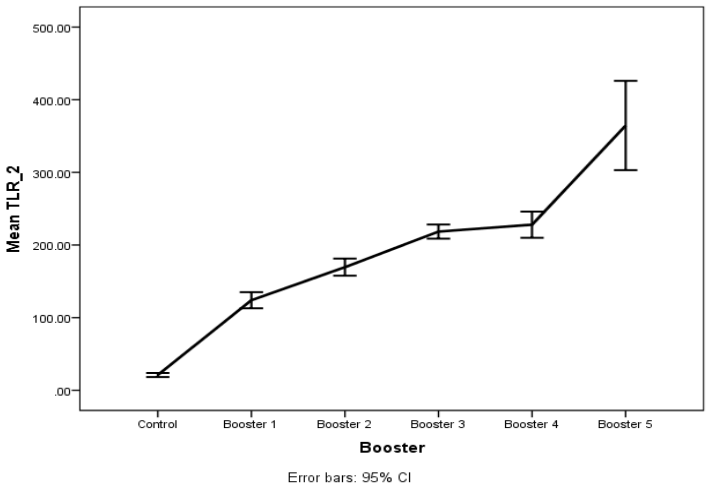

Figure 2. The amount T cells of rabbits which express TLR2 (A) resulted from immunization with soluble mite protein

DISCUSSION

According the result research, it is shown that antibody TLR-2 can recognize ligand from protein antigen *S.scabiei* var.*caprae* mites by stimulating T cells activation, marked by the presence of yellow to green fluorescence color which increased in accordance with the treatment from various boosters. Ligands that recognized by TLR-2 is consisted of lipoprotein/lipopolypeptide, flagelin, ssRNA, CpG DNA (6,7). *S.scabiei* var.*caprae* mites is an extracellular microorganism which contains antigen, when antigen

enter the body it will be catched by macrophage or dendritic cells and phagocytes cells will be activated by TLR-2 as signal transducer. The signal which produced by TLR will activate transcription factor NFκB which stimulates cytokines production. NFκB activation initiated by signal which recruits MyD88 and interacts with IL-1 receptor associated kinase (IRAK), and activating TNF receptor associated factor 6 (TRAF-6) to activate IκB kinase (IKK). Activated IKK will activate NFκB to transcript gene IL-12, IL-10, IL-4, TNF-α, IFN-γ. IL-2 roles will increase cytolytic activity from cytolytic T lymphocytes and promote Th1 cells development together with CD8 activation to produce IL-2 which stimulates proliferation and differentiation of B cells that will produce antibody. IL-4 is a cytokine which produced by subset Th2 from Th cells CD4 that functioned to induce Th2 cells differentiation and stimulate IgE production. Cellular immune response enhancement will be followed by IgG titre enhancement as humoral immune response (8). Accoring to a research, it was shown that a group of pathogen is not only recognized by one type TLR but also by another TLR such as TLR 2 and TLR 4 will recognize gram positive microbial product (6). It was predicted TLR 2 can also be activated by other various stimuli that can be developed for sub unit vaccine (9).

4. CONCLUSION

Mites of *S.scabies* var.*caprae* contains ligand which acts as receptor that involved in PAMP, this can induce cellular immune response and recognized by TLR-2 as marker. It showed that TLR-2 not only plays role in innate immunity but also in adaptive immunity.

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