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PROFILE OF ALLERGY HYPERPLASMA PATHOLOGIC ANTIBODY AND IMMUNOGENIC CHARACTERISTIC

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ABSTRACT

The objective of this research was to determine the allergy hyperplasma pathologic antibody and to evaluate the immunogenic characteristics. Blood and serum were collected from human suffering rhinitis, skin, eye and asthma allergies. To obtain a cloned allergen plasma protein (protein allergen that had been induced with the serum from human with allergic rhinitis, skin, eye and asthma), the goat was used as an intermediate animal. Hematological analysis showed that the leukocyte cell such as neutrophil, lymphocyte, monocyte, and eosinophil increase in allergic-suffered human. The blood smear test exhibited that the mastocyte cell was dominant which contributes to allergy activities in human body. The sodium dodecyl sulphate-polyacrylimide gel electrophoresis (SDS-PAGE) assay detected protein allergens with molecular weight of 188 kDa (IgE) and 60-62 kDa (mastocyte cell). The reactivity assay using enzyme linked immunosorbent-assay (ELISA) revealed that cloned-allergens (whole hyperplasma allergen from goat isolates) express the best reactivity at various concentrations of IgE than the leukocyte cells. This research concluded that the clones of protein allergen have better immunogenic characteristic and those proteins can be recommended as the candidate of allergen to induce the humoral immunity on host and deliver specific product of anti-allergy such as milk.

Key words: allergy, immunogenic, molecular weight

ABSTRAK

Tujuan penelitian ini adalah menentukan profil protein dan mengevaluasi sifat imunogenik allergy hyperplasma pathologic antibody. *Darah dan serum yang dikoleksi dari penderita alergi rinitis, kulit, mata, asma dipreparasi untuk ditentukan profil sel antibodi sebagai referensi* protein allergy hyperplasma antibody. *Berdasarkan uji darah lengkap, subyek dengan penderita alergi rinitis, mata, kulit, dan asma masing-masing memperlihatkan peningkatan ekspresi sel neutrofil, limfosit, monosit, dan eosinofil. Pada uji preparat darah apus ditemukan sejumlah sel mast lebih dominan. Pemeriksan profil protein dengan* sodium dodecyl sulphate-polyacrylimide gel electrophoresis *(SDS-PAGE) memperlihatkan semua serum darah terdeteksi protein dengan berat molekul 188 kDa (IgE) dan 60-62 kDa (sel mast). Pada uji reaktivitas dengan* enzyme linked immunosorbent-assay *(ELISA) memperlihatkan protein alergen satu* clone *memberikan reaktivitas yang terbaik pada berbagai konsentrasi terhadap IgE dibandingkan dengan sel neutrofil, limfosit, monosit, dan eosinofil. Dari hasil penelitian disimpulkan bahwa gabungan protein dalam bentuk sel antibodi (*protein clone*) memiliki memiliki sifat imunogenik yang lebih baik dan layak dijadikan sebagai referensi kandidat* alergen *untuk menghasilkan susu anti-alergi.*

Kata kunci: alergi, imunogenik, berat molekul

INTRODUCTION

Allergy is an abnormal reaction in the body that can cause the immune system become very active and sensitive (Kay, 2000). Allergy is caused by allergens that come into the body through respiratory tracts, food, injection or direct contact to skin (Rengganis, 2004). According to Braun-Fahrländer *et al.* (2002) chronic allergy induce inflammation in the skin, nose tract (rhinitis), eye (conjunctivitis) and allergic asthma (pulmonitis).

Allergy can be divided into two subcategories, which are atophy and anaphylaxis. Athopy is a kind of genetically allergy in which the body releases IgE antibody a respons to the allergens (Nelson, 2003). Continues exposure can cause releasing some substances such as histamine, slow reacting substance of anaphylaxis (SRS-A), and eosinophil chemotactic factor of anaphylaxis (CFC) from mast and basophil cell that can cause vasodilatation and contraction of smooth tissue/muscle. This phenomenon is facilitated by cross-linking between allergen receptor of IgE (FceR1) with high affinity to mast cell or basophile effector (Vangelista, 1999).

Pathogenesis of allergy is facilitated by antibody and the dominant antibody that has role in this process is IgE because it has a constant domain with four small unit heavy chains (Erb, 2007). It has been know that allergy happen due to the role of receptor of IgE, like Ce1-Ce4 and CeR1, with a strong binding affinity (Wang , 2003) to basophile and mast cell receptor due to the respond of IgE to antigen (Lewkowich *et al.,* 2004). FεR1 consists of 1 μ, β, chain and 2 γ chains, while in antigen presenting cell (APC) of monocyte and denderic cell do not have β chain. Extracellular part of FεR1α consists of two b chain immunoglobulin that binds to IgE with high affinity but not to β unit and γ-lg-like module (Katoh *et al*., 2000).

These informations indicate that allergy occur due to the interaction of IgE receptors, such as FcεR1, Cε1-Cε4, and α, β, γ. Those protein receptors have a role as an important intermediate for binding alergen in order to introduce to mast cell or basophil. Although allergy was dominated by genetic factor (Croner, 1992), effort to prevent this disease still investigated by the researcher, including giving anti-histamine to protect the respond of mast cell, giving corticosteroid to

protect the nose, and the application of immunotherapy in order to make T-helper cell lost the function (Gould, 2003).

Serum immunoglobulin that is a clone blood serum of allergic patient having a good properties that can be used as allergen to produce milk IgE and or IgY from chicken egg yolk that is expected can give a protection of epitope sensitivity or paratope allergen to IgE antibody receptor, mast cell, and another leucocyte as intermediate for the allergy (protein allergen), so that the anaphylaxis and hypersensitivity in the body cannot occur even though they have been exposed to the allergen (Basri, 2007; Passalacqua and Durham, 2007). The purpose of this research is to investigate the protein profile and to evaluate the immunogenicity of protein hyper plasma pathologic antibody as a candidate serum immunoglobulin for producing milk and or egg that have immunological sensitivity anti-allergy.

MATERIALS AND METHODS

This research used blood and serum from human suffering rhinitis, skin, eye and asthma allergies. To obtain a cloned allergen plasma protein (protein allergen that had been induced with the serum from allergic rhinitis, skin, eye, and asthma), the goat was used as an intermediate animal. Both proteins are then analysed with some research approach.

Analysis of Blood Cell Profile

Blood sample was analysed by haematology analyser to assess the complete blood cell profile in allergic suffered human. One drop blood from allergic human was dropped to object glass, then stroked by another object glass with the angle of 30-45° C and allowed to dry. The drying slide then was soaked into methyl alcohol for 3-5 minutes and air-dried. After drying, slide was soaked into Giemsa solution for 15-30 minutes, then washed and dried. The staining slide was observed under microscopic observation with 100x magnification. The mast cell sample was stained using Diff-quick staining. The thin blood smear in object glass was soaked into fixative solution five times before air- dried. The dried slide was soaked into solution I for five times and re-dried. Finally, the sample was soaked into solution II and dried for five minutes. The time for soaking the slide is one second for each soaking.

Analysis of Blood Serum Profile

To detect the profile of protein allergen in allergic human and intermediate animal (goat), sodium dodecyl sulphate-polyacrylimide gel electrophoresis (SDS-PAGE) that had been standardized by Bradford protein method was used (Basri, 2007). The gel composition for enzyme linked immune-sorbent assay (ELISA) consisted of separating gel and stacking/collecting gel. The stacking/collecting gel that had been prepared was put into SDS tank that was filled with reservoir running buffer stock (Bio-Rad). In the hole of separating gel was filled 20 µl of sample and 0.5 µl of standard protein (Invitrogen) in different hole as an indicator for molecule protein sample weight. Separating protein was done in two steps. Firstly, sample and protein marker in the hole was collected close to separating gel using electric power of 100 mA, 100 volt and 16 watt for 15 minutes. Secondly, Protein in separating gel was separated by electric power of 100 mA, voltage 150 volt, 15 watt, for 15 minutes.

Reactivity of Blood Serum

Reactivity protein allergen to the all allergic samples that had been explained above was tested immunologically using ELISA (Basri, 2007). The blood protein plasma was incubated in ELISA plate, and followed by reacting with serial dilution of IgE antibody. The assay was run in duplicate. After adding anti-IgE labelled horse radish peroxidase (HRP), optical density (OD) was read at wave length of 450 nm. The lowest dilution IgE showing the highest of OD which indicated the highest IgE reactivity to the plasma protein. The assay was done three times independently.

RESULTS AND DISCUSSION

The presence of IgE plasma cell from the analysed blood sample is indicated by the presence of mast cell and basophile in blood smear observation. Both cells which exposed to three of IgE induce immunological response to the allergen and secreted their product like histamine as the response of immune cell adaptation to allergen (Kay, 2000). However some researches also indicated that basophile does not response to IgE plasma cell but more active to IgG (Tsujimura *et al.*, 2008).

Beside the observation of complete blood and blood smear, blood serum in allergic rhinitis, skin, nose and asthma also confirmed with SDS-PAGE (Figure 1). The target protein in this research is to find all blood serums whose protein having molecular weight of 188 kDa and 60-62 kDa. Wigotzki *et al*. (2000) reported that the 188 kDa protein is IgE antibody while 60-62 kDa protein is mast cell and it's derivate. The increasing of mastocyte cell in blood serum as shown in this study indicates the occurrence of allergy which is clearly confirmed by profile of IgE cell protein (188 kDa) and mast cell (60-62 kDa). In addition, a cloned-allergen protein (allergic rhinitis serum, skin, nose, and asthma) was also found with molecular weight of 6, 10, 29, and 32 kDa. However, compared to each allergic blood serum, allergen protein serum showed more protein bands.

The reactivity test using ELISA was shown in Figure 2. The result showed that cloned-allergen proteins from goat (the mediator producing of cloned-allergen from whole allergen as well as human suffer of allergic rhinitis; skin, eyes, and asthma) express the best reactivity in various concentrations of IgE than blood serum of allergic rhinitis, eye, skin, and asthma. In the case of allergy, the body is sensitized by allergen which activated the body immunity response. Antibody cell plasma has a role in response of the allergen like

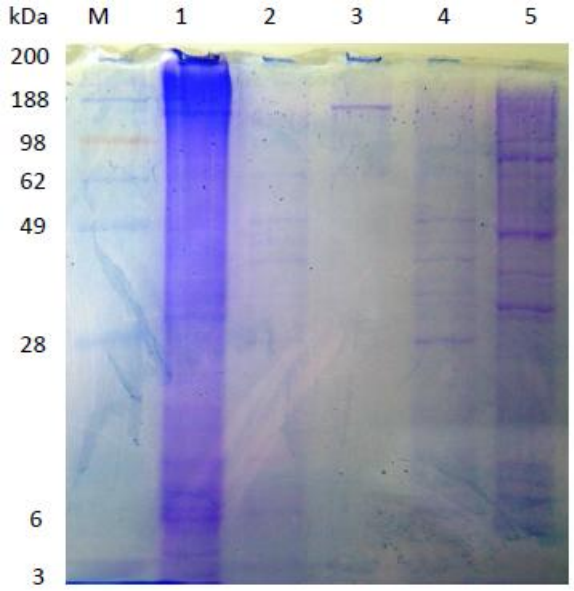

Figure 1. Blood serum protein profile of allergic patient. M= Marker, First column= Allergic rhinitis blood serum, second column= Skin allergy, third column= Eye allergy, fourth column= Allergic asthma, fifth column= Cloned-allergen protein

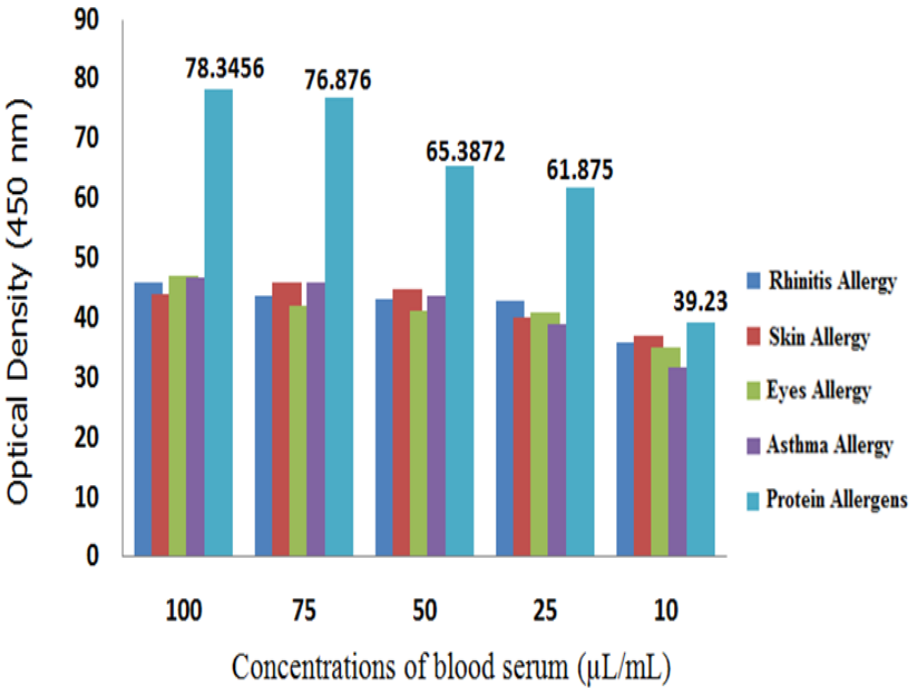

Figure 2. The score of allergic blood serum and allergen protein reactivity to the IgE. Even though in all concentration show a good reactivity, in high concentration (100 ul/ml) shows a better reactivity score. Cloned-allergen protein of goat/sheep induced by allergic blood serum show a better reactivity than rhinitis blood serum, skin, eye, and asthma

IgE and mast cell or basophile and neutrophil called allergen protein. Over sensitized can caused body physiology disorder, whether in eye, skin, nose, lung, or other body organs (Leung, 1995).

IgE is a dominant immunoglobulin class found in all allergic reaction due to having a constant domain of four small units of heavy chains. The presence of mast cell and IgE according to Horner *et al*. (1995) indicated that allergen will stimulate the production of IgE antibody through its receptor. Continues exposure can cause the releasing of some substances like histamine, SRS-A and ECF from mast cell or basophile that can cause vasodilatation and contraction of smooth tissue. This phenomenon is facilitated by cross reaction between allergen-receptor of IgE (FceR1) with a high affinity of mast and basophile cell effector (Vangelista, 1999).

It is well understood, allergy occurred due to the role of IgE receptor like Ce1-Ce4 and FceR1 with the strong affinity to mast cell and basophile receptor (Wang, 2003). FcεR1 consists of 1 µ, β, chain and 2 γ chains, while in APC from monocyte and denderic cell

do not have a chain. Extracellular part of FcεR1 consists of two chain strand of immunoglobulin which bind the high-affinity IgE unit, but not for β and γ unit (Katoh, 2000; Roopenian and Akilesh, 2007). In this case, the allergen protein has a good reactivity character to the IgE and protein serum of anti-allergen protein. Understanding the concept of antigen-antibody interaction, allergen protein of hyper plasma antibody induce the immunity through the interaction of antigen (allergen) and antibody (Zhou and Kisil, 1995) by stimulating host antibody cells and has a positive response to the allergen (Blancher *et al*., 2000).

CONCLUSION

This research concluded that the clones of protein allergen have better immunogenic characteristic and those proteins can be recommended as the candidate of allergen to induce the humoral immunity on host and deliver specific product of anti-allergy such as milk.

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