Large scale expression of two potential scabies mite allergens

M Descoleaux, S Cash-Deans, K Mounsey, and SF Walton

Allergy and Immunobiology Laboratory, Inflammation and Research Cluster

University of the Sunshine Coast, Queensland, Australia

ABSTRACT

In remote scabies endemic Australian Aboriginal communities, rates of asthma are low despite high rates of house dust mite sensitisation. Notably locals have extremely high levels of reactivity to different house dust mite allergens than the general population. Our hypothesis is that cross-reactive IgE antibodies generated as part of the immune response in scabies accounts for the high prevalence rates of house dust mite sensitisation and low rates of asthma observed in these communities. To characterise this cross allergy two purified recombinant scabies mite homologues to major house dust mite allergens, tropomyosin (Sar s 10) and arginine kinase (Sar s 20) have now been identified, cloned, expressed and purified. These purified proteins will enable cross protective antibody responses to be evaluated in people with both scabies and asthma. Outcomes from this study will potentially aid future diagnostic and preventative measures for asthma in Aboriginal populations.

INTRODUCTION

Scabies

• Scabies is a disease of the skin caused by the burrowing 'itch' mite Sarcoptes scabiei
• Scabies is a particular problem in many remote Aboriginal communities in central and northern Australia, relating primarily to levels of poverty and overcrowding
• Studies by us and others have demonstrated that patients with sensitisation to HDM exhibit circulating antibodies that recognize unknown antigenic determinants on scabies mites (SM)[2,3] and vice versa antisera to SMs react to antigenic determinants on HDM
• Der p 10 (tropomyosin) and Der p 20 (arginine kinase) HDM allergens
• A study investigating the allergenic profile of scabies Homarid antigens in an Australian Indigenous community in Western Australia revealed exceptionally high titres of IgE antibody binding directed at the conserved proteins Der p 10 (tropomyosin) and Der p 20 (arginine kinase) and not with the expected Der p 1 and Der p 2 allergens[4].

METHODS

Amplification of Sar s 10 and Sar s 20 cDNAs
• Both Sar s 10 and Sar s 20 cDNAs were amplified by PCR using AmpliTag Gold mix (Intronogen) from S. scabiei cDNA library

Expression of recombinant Sar s 10 and Sar s 20
• Both Sar s 10 and Sar s 20 cDNAs were sub cloned into pGEM T-easy vector and sequenced with M13 primers (AGRF- Brisbane)

To visualize protein expression, cell extracts at each time point were resolved using SDS-PAGE and visualized after Coomassie blue staining.

Purification of His-tagged Sar s 10 and 20 using Ni-NTA agarose beads
• For all fractions, proteins were separated by SDS-PAGE and visualized by Coomassie blue staining.

RESULTS and DISCUSSION

Cloning of Sar s 10 and Sar s 20 cDNAs
• The Sar s 10 and Sar s 20 cDNAs were cloned into an open reading frame of 855bp encoding a predicted protein of 285aa with 95.08 identity to the HDM homologous protein Der p 10 (fig 2, top panel).
• The Sar s 10 predicted protein exhibited the characteristic secondary structure elements found in the tropomyosin protein family.
• The Sar s 20 PCR product contained a 771bp sequence corresponding to a protein of 257aa with approximately 100 N-terminal amino acids missing (fig 2, lower panel). Sar s 20 showed 87.16% identity to the homologous Der p 20 protein.
• Secondary structural elements found in all arginine kinase proteins were also present in the Sar s 20 (fig 3A).

Purification of His-Sar s 10 recombinant protein
• To determine the best E. coli host for protein expression, His-sar s 10 protein was expressed in different BL21 bacterial hosts: BL21(DE3), BL21(DE3)pLysE and BL21(DE3)pLysS.
• Compared to non-induced samples, a protein of MW = 40 kDa appeared overexpressed in the BL21(DE3) strain after 4 hours of induction (fig 3A).
• Western blot analysis of the protein extracts with anti-histidine antibody showed a strong signal at 40 kDa suggesting that the overexpressed protein corresponds to recombinant His-Sar s 10 protein (fig 3B).
• To determine whether His-Sar s10 protein could be purified under denaturing or non-denaturing condition, BL21(DE3) E. coli cells expressing His-Sar s 10 were analysed for native and non-native sensitisation (fig 4A). Maximum protein amount was retrieved in the supernatant suggesting that His-Sar s 10 is soluble (fig 4A, lane C), and further purification steps confirmed that it can be bound to Ni-NTA agarose beads under native conditions (fig 4A, lanes E, F). Maximum protein amount was retrieved in the supernatant suggesting that His-Sar s 10 is soluble (fig 4A, lane C).
• Further purification steps confirmed that it can be bound to Ni-NTA agarose beads under native conditions (fig 4A, lanes E, F).
• Western blot analysis of the purification steps up to binding of the protein to Ni-NTA agarose beads showed a strong signal for the beads extract (fig 4B, lane G), confirming binding of His-Sar s 10 to the purification beads.

Purification of His-Sar s 20 recombinant protein
• BL21(DE3) E. coli was found to be the most suitable strain for expression of Sar s 20.
• Time course analysis showed overexpression of a protein at 30 kDa, corresponding to the expected molecular weight of His-Sar s 20 (fig 5A) and confirmed by western blot, with the anti-histidine antibody recognizing His-Sar s 10 at 30 kDa (fig 5B).
• Bacterial lysis showed that His-Sar s 20 is soluble and can thus be purified under native conditions (fig 5C, His-Sar s 20 in the clarified fractions).
• The Sar s 20 recombinant protein was expressed in three different BL21 bacteria hosts: BL21(DE3), BL21(DE3)pLysE (fig 2, lower panel). Sar s 20 showed 87.16% identity to the HDM arginine kinase (fig 3B).

FUTURE DIRECTIONS

• To determine immunogenicity, each protein will be assessed by indirect Enzyme-Linked Immunoassay (ELISA) with blood from scabies infected subjects (fig 6).
• To determine cross reactivity both proteins will be assessed by competitive Enzyme-Linked Immunoassay (ELISA) with blood from scabies infected subjects (fig 6).
• Purification of both Sar s 10 and 20 under native conditions allows preservation of the tertiary structure of the proteins allowing for antibody binding to non linear epitopes. However, for Sar s 20, allowing the missing portion of the protein will be essential to generate the correct 3D structure of the full length protein.

REFERENCES

3. E. coli was found to be the most suitable strain for expression of Sar s 20.
4. Time course analysis showed overexpression of a protein at 30 kDa, corresponding to the expected molecular weight of His-Sar s 20 (fig 5A) and confirmed by western blot, with the anti-histidine antibody recognizing His-Sar s 10 at 30 kDa (fig 5B).
5. Bacterial lysis showed that His-Sar s 20 is soluble and can thus be purified under native conditions (fig 5C, His-Sar s 20 in the clarified fractions).
6. The Sar s 20 recombinant protein was expressed in three different BL21 bacteria hosts: BL21(DE3), BL21(DE3)pLysE (fig 2, lower panel). Sar s 20 showed 87.16% identity to the HDM arginine kinase (fig 3B).