Identification of amino acids that equip lipocalins with allergenic potential

D. Klaver¹, B. Posch¹, A. Geisler¹, B. Redl², N. Reider¹, C. Heufler¹

¹ Department of Dermatology, Medical University Innsbruck, Austria; ² Division of Molecular Biology, Biocenter, Medical University Innsbruck, Austria

The lipocalin family of proteins comprises the majority of mammal derived respiratory allergens and some endogenous proteins. Previously, we could show that DCs treated with two different pairs of homologous lipocalins, each consisting of an exogenous allergenic (Can f 1/Fel d 4) and an endogenous non-allergenic lipocalin (Lcn-1/MUP), induce divergent immune responses. To determine the allergenic potential of lipocalin allergens we compared the amino acid (AA) sequence of allergenic and non-allergenic lipocalins and identified AAs only present in allergens. Whether these conserved AAs harbour the potential to prime DCs towards the induction of an allergic Th2 immune response was tested with hybrid-lipocalins consisting of the non-allergenic Lcn-1 sequence substituted with the conserved allergenic AAs.

1. Experimental Setup

2. Crosstalk of lipocalins and dendritic cells direct the type of immune response according to their allergenic potential

3. Amino acid sequences of Lcn-1, Hybrid-lipocalin 2 (HL2) and Hybrid-lipocalin 2 control (HL2Ko)

Lcn-1	HHLLASDEEIQDVSGTWYKAMTVDREFPEMNLESVTPMTLTLEGGNLEAKVTMLISGRCQEVKAVLEKTDEPGKYTADGGKHVAYIIRSHVKDHYIFYCEGELHGKPVRGVKLVG
HL2	HHLLASDEEIQDVSGTWYKAMTVDREFPEMNLESVTPMTLTLEGGNLEAKVTMLTSGRCQEVKAVLEKTDEPGKYTADGGKHVAYIIRSHVKDHYIFYCEGELHGKPVRGVKLVG
HL2Ko	HHLLASDEEIQDVSGTWYKAMTVDREFPEMNLESVTPMTLTLEGGNLEAKVTMLTSGRCQEVKAVLEKTDEPGKYTADGGKHVAYIIRSHVKDHYTFYCEGELHGKPVRGVKLVG
	125 150
	RDPKNNLEALEDFEKAAGARGLS ^T ESILIPRQSETCSPGSD
	RDPKNNLEALEDFEKAAGARGLS ^Q ESILIPRQSETCSPGSD
	RDPKNNLEALEDFEKAAGARGLSQESILIPRQSE ^Q CSPGSD

Fig. 1: Insertion of conserved allergenic amino acids into the Lcn-1 sequence (upper row) at corresponding sites to obtain Hybrid-lipocalin 2 (HL2) (middle row) and Hybrid-lipocalin 2 control (HL2Ko) (lower row). To identify conserved allergenic amino acids, Can f 1 and Lcn-1 as well as Fel d 4 and MUP sequences were aligned to exclude any overlapping amino acids. Next, sequences of Can f 1 and Fel d 4 were aligned to find eleven amino acids which were only present in Can f 1 and Fel d 4. Further, sequences of the cat allergen Fel d 7 and the horse allergen Equ c 1 were added to the alignment to find 5 conserved amino acids within the 4 allergenic sequences. As a last step, the amino acid sequence of the cow allergen Bos d 2 was added whereupon 2 amino acids could be identified which were present in Can f 1, Fel d 4, Fel d 7, Equ c 1 and Bos d 2, but not in non-allergenic Lcn-1 or MUP to obtain Hybrid-lipocalin 2 (HL2). To investigate if also the position of the amino acids matters, a control hybrid-lipocalin 2 (HL2Ko) was designed by inserting the 2 identified amino acids at the next possible position nearby.

4. HL2 induces less maturation marker expression in dendritic cells than non-allergenic Lcn-1 and HL2 control

Fig. 2: Hybrid-lipocalin 2 (HL2) exhibits divergent maturation marker expression in comparison to non-allergenic Lcn-1 and hybrid-lipocalin 2 control (HL2Ko). iDC were incubated with either Can f 1, Lcn-1, Fel d 4, MUP, HL2 or HL2Ko for 48h. Maturation marker expression was determined via FACS. iDC: immature dendritic cells; mDC: mature dendritic cells; MFI: mean fluorescence intensity. *p < 0.05; **p < 0.01; ***p < 0.001.

5. HL2 induces less expression of IL-12p70 in dendritic cells than non-allergenic Lcn-1 and HL2 control

Fig. 3: Hybrid-lipocalin 2 (HL2) differentially promotes IL12p70 expression compared to non-allergenic Lcn-1 and hybrid-lipocalin 2 control (HL2Ko). iDC were incubated with either Can f 1, Lcn-1, Fel d 4, MUP, HL2 or HL2Ko and IL12 p70 content in the supernatants was analyzed by ELISA. *p < 0.05; **p < 0.01; ***p < 0.001.

Conclusion

The obtained results indicate that the allergenic potential of lipocalins to induce an allergic Th2 immune response might be restricted to 2 conserved allergenic AAs. Moreover, not only the identity but also the position of the AAs within lipocalins seems to account for their allergenic potential.