

immunotherapy of infectious disease. Some viruses appear to have adapted to this environment such that little or no effective immunity results, whereas other pathogens elicit immunity. For intrahepatic immunity to be delivered, there must be some kind of 'functional switch', in which lymphocytes with NK markers may be important. In summing up, J. Hoofnagel (Bethesda, MD) and H. Johnson (Gainesville, FL) asked the conference some provocative questions

about human disease. Here are two: In chronic hepatitis C, how and why does interferon therapy work? And in measles and rubella, where there is no tropism of the virus for the liver, why is there a 'benign hepatitis' with transient abnormality of liver enzymes? Not long ago, these would have been rhetorical questions. After this conference, we could synthesize some new hypotheses. Maybe next time, we'll have some answers.

Cliona O'Farrelly is at St Vincent's University Hospital, Dublin, Ireland; Nick Crispe (nick.crispe@yale.edu) is in the Immunobiology Section and the Liver Center, Yale University School of Medicine, New Haven, CT 06520-8011, USA.

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Allergy to lipocalins: a consequence of misguided T-cell recognition of self and nonself?

Tuomas Virtanen, Thomas Zeiler, Jaakko Rautiainen and Rauno Mäntyjärvi

To date, all major mammalian allergens cloned and sequenced, with the exception of cat Fel d 1, are members of the lipocalin family of proteins. The lipocalin allergens include Mus m 1 (mouse), Rat n 1 (rat), Can f 1 and Can f 2 (dog), Equ c 1 and Equ c 2 (horse), and Bos d 2 (cow) (<http://www.expasy.ch/cgi-bin/get-prosite-entry?PS00213>). Isoforms of these proteins are synthesized in the liver and in several secretorial tissues; they are found in dander, hair, urine and saliva, and are spread as aeroallergens. An important food allergen, Bos d 5 (β -lactoglobulin of cow's milk), and a cockroach allergen Bla g 4 are also lipocalins.

The name 'lipocalins' was proposed in 1987 for a group of functionally and structurally related proteins (see Ref. 1). Since then, the number of proteins fulfilling the criteria for lipocalins has increased continuously; the group currently includes more than 150 proteins (<http://www.expasy.ch/cgi-bin/get-prosite-entry?PS00213>). Numerous lipocalins function as carriers of small lipophilic molecules such as vitamins, pheromones and odorant molecules. Examples of human lipocalins include retinol binding protein (RBP), apolipoprotein D

The molecular mimicry between lipocalin allergens and endogenous lipocalins at the T-cell level may explain why the immune response against lipocalins is Th2-dominated and results in allergy. This view is supported by recent studies of autoimmune and parasitic diseases and peptide analogues.

(APD), von Ebner's gland protein (VEGP) and PP14 (Fig. 1).

The overall sequence identity between lipocalins is usually less than 20%. The so-called 'kernel lipocalins' share three conserved sequence motifs, as opposed to the 'outlier lipocalins', which have only one or two. The sequence similarities are reflected in the three-dimensional structural similarity of lipocalins^{1,2}.

Allergy and autoimmunity as deviated immune reactions

Allergy and autoimmunity can be viewed as opposite poles of detrimental immune reactions. For the immediate type of allergy, a characteristic feature is the aberrant production of IgE antibodies against innocuous

environmental compounds in persons with an atopic genetic background. At the cellular level, T helper 2 (Th2) cells play a crucial role because they are an important source of interleukin 4 (IL-4) and can induce the Ig class switch to IgE (Ref. 3). In this context, it is appropriate to recall the similarities between immune responses in allergy and in parasitic diseases. Th2 dominance, IgE production and eosinophilia are characteristic features in both conditions^{3,4}.

By contrast, the progression of autoimmune disorders in animal models is often associated with a Th1 response. Deviation of the immune response towards Th2 dominance appears to result in prevention or improvement of the autoimmune pathology⁵.

T cell meets lipocalin

Very little information is available regarding human T-cell responses to lipocalin allergens. Bos d 2, the major bovine lipocalin allergen, induces only a weak stimulation of peripheral blood mononuclear cells (PBMCs) from highly allergic persons with strong skin-prick test reactivity to Bos d 2 (Ref. 6). By contrast, Bos d 2-specific CD4⁺ T-cell clones, once established, responded strongly

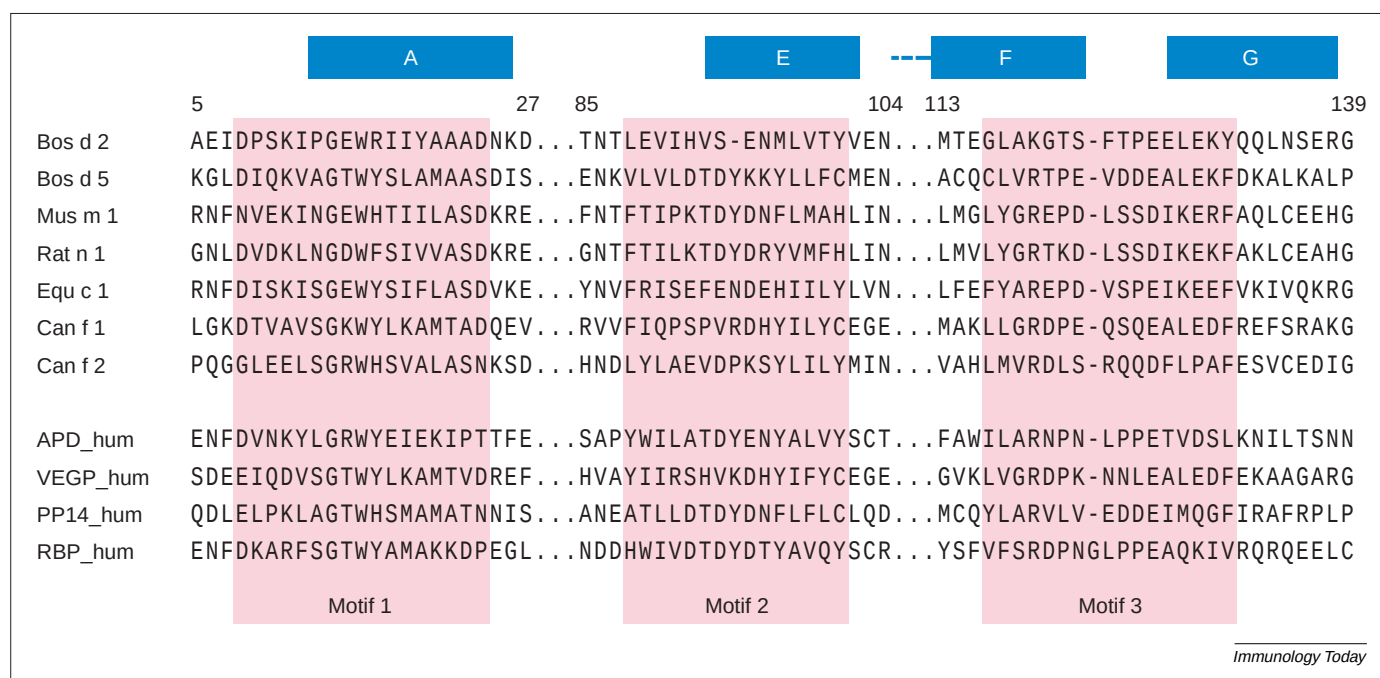


Fig. 1. Alignment of three segments of the mammalian lipocalin allergens and representative human lipocalins. Segments containing sequences corresponding to the three motifs conserved in the kernel lipocalins are shown in pink boxes. The amino acid numbers refer to the sequence of Bos d 2. The blue bars A, E, F and G indicate T-cell epitopes in Bos d 2 (modified figure reproduced, with permission, from Ref. 6).

to the allergen. The mapping of T-cell epitopes revealed that Bos d 2 contains a limited number of epitopes. One of these, epitope G (Fig. 1), located in the C-terminal α -helix, was recognized by all T-cell lines and by the great majority of isolated T-cell clones. Clones responsive to epitope G were of the Th0-like (7/17 clones) or Th2-like (10/17) phenotype, whereas 3/6 of the clones responsive to other epitopes exhibited the Th1-like phenotype. Interestingly, epitope G as well as two other epitopes overlapped with the conserved regions of lipocalins. Furthermore, computer predictions suggested that the animal allergens and human lipocalins included in the analysis would have an epitope in the area corresponding to epitope G in Bos d 2.

Why do lipocalins cause allergy?

Based on the observations described above, we propose that the allergenicity of lipocalins is associated with the state of the immune system adapted to the presence of endogenous lipocalins.

The proposal implies that the allergenicity of lipocalins manifests itself as a weak, Th2-dominated cellular response, as observed with Bos d 2. To explain the observation three interrelated concepts should be considered: (1) the precursor frequency of

Bos d 2-specific T cells may be low in the periphery; (2) the regulatory mechanisms of peripheral tolerance may suppress and/or cause a deviation in the response; or (3) the Bos d 2-specific T cells recognize the allergen inefficiently.

A low precursor frequency of Bos d 2-specific T cells in the periphery is conceivable because, during thymic maturation, the numerous endogenous lipocalins supposedly lead to the elimination of the majority of T cells that could potentially react against exogenous lipocalins. In this regard, one of the human lipocalins, PP14, is interesting. PP14 is produced by endometrial glands but is also found in high concentrations in the amniotic fluid between the eighth and tenth weeks of pregnancy and in the maternal serum⁷. PP14 has 43% amino acid identity with Bos d 5 (bovine β -lactoglobulin). The identity includes the sequence TDY, which is shared by several lipocalin allergens in motif 2 (Fig. 1).

One factor explaining the weak proliferative response of PBMCs against Bos d 2 could be the relatively low number of epitopes recognized in the allergen. A recent study indicated that the total number of epitopes found in Bos d 2 was seven, and that the highest number of epitopes an individual's T cells could recognize was five⁶. Interestingly, there are previous data alluding that

animal allergens in general might contain fewer T-cell epitopes than other allergens⁸.

The second point, the contribution of peripheral tolerance in regulating responses against Bos d 2, is a rational possibility because exposure of the immune system to exogenous allergens resembling self-antigens might represent a danger. A strong Th1-dominated response against allergens could result in autoimmune reactions against endogenous lipocalins. To avoid their activation, potentially lipocalin-reactive T cells are likely to be under the control of peripheral tolerance. As a consequence, the cellular response to an exogenous self-mimicking lipocalin allergen would also be weak. It has been shown that anergic T cells can suppress T-cell responses *in vitro* when the epitope recognized by the anergic cells is present in the culture⁹. Furthermore, an endogenous peptide analogue has been observed to antagonize the proliferative response of T cells to the exogenous wild-type peptide *in vivo*¹⁰. The considerations presented above do not, however, explain the Th2-dominance of the response against Bos d 2.

One possible way to limit autoimmune destruction could be a spreading of the response towards less harmful, protective epitopes in the exogenous antigen, as seen in a mouse model of adjuvant-induced arthritis¹¹. Furthermore, Moudgil *et al.*¹¹ suggest

that the response against a self-protein homologous to the exogenous antigen represents a regulatory mechanism that limits the immune reaction. Interestingly, this kind of regulatory response is considered to be Th2-like¹². Another possibility for limiting tissue destruction could be that the immune response is raised early towards the less harmful epitopes of the antigen, as seen in another animal model of arthritis¹¹. In allergy to Bos d 2, epitope G could represent a protective or regulatory epitope because the response against it is predominantly Th2/Th0-like.

The third possibility related to the previous topic and explaining Th2 deviation could be inefficient recognition of the allergenic epitopes. It seems that the flexibility of T-cell epitope recognition can result in different phenotypes of T cells¹³. There are several studies showing that stimulation with peptide analogues (altered peptide ligands) induces changes in the cytokine profiles secreted by T cells^{14–16}. Studies on epitope recognition suggest that it is the low affinity of a peptide for an MHC class II molecule and/or a T-cell receptor that favours a Th2-type response¹⁷. It is therefore possible that the lipocalin-specific T cells could recognize the crossreacting, mimicking epitopes in exogenous lipocalin allergens, most probably in an inefficient way, and this would lead to suboptimal activation and secretion of Th2-type cytokines.

Recent studies have revealed that the IgE-binding epitopes of a *Schistosoma japonicum* protein exhibit similarities to the IgE-binding epitopes of known allergens¹⁸. The five proteins showing top similarity scores with one of the epitopes of a Schistosomal protein were all lipocalins, and four of them were important animal allergens (Bla g 4, Mus m 1, Rat n 1, Bos d 5). In Bos d 5, the common motif is located in the predominant IgE-binding peptide¹⁹. As an extension of the concepts presented above, the common denominator of the quality of immune responses against parasites and against allergens might be the tendency to avoid Th1-dominance in order to minimize deleterious effects to the host. This view is supported by the findings that in helminth infections the antigen-specific proliferative responses of PBMCs can become downregulated^{4,20},

whereas IgE responses are elevated²¹, resembling the situation with Bos d 2 (Ref. 6).

Concluding remarks

Because only some of the proteins of an allergen source are allergenic, it is conceivable that the allergen itself could have a role in deviating the immune response in favour of Th2 dominance and IgE production. We suggest that one factor explaining the allergenicity of lipocalins could be the molecular mimicry between endogenous lipocalins and exogenous lipocalin allergens. Because a significant number of allergens are evolutionarily conserved proteins²², the mechanism of allergenicity proposed here for lipocalins might be an indication of a more general phenomenon. It would be interesting to know whether the characteristics of cellular responses against other allergens with conserved human homologues^{23,24} are similar to those observed with Bos d 2.

Tuomas Virtanen (tuomas.virtanen@uku.fi), **Thomas Zeiler**, **Jaakko Rautiainen** and **Rauno Mäntyjärvi** are at the Dept of Clinical Microbiology, University of Kuopio, POB 1627, FIN-70211 Kuopio, Finland.

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