

Reviews

The Polyprotein Allergens of Nematodes

L.A. McReynolds, M.W. Kennedy and M.E. Selkirk

Symptoms of infection with a number of parasitic nematodes may be associated with IgE antibody and associated immunopathologies. This has drawn attention to parasite allergens and provoked discussion on the wisdom of their inclusion in recombinant vaccines. Here, Larry McReynolds, Malcolm Kennedy and Murray Selkirk review work on a prominent set of allergens recently characterized in several nematode species.

One of the unifying characteristics of nematode infections is their induction of high levels of IgE antibody and the resultant hypersensitivity reactions responsible for manifold pathologies^{1,2}. Such responses are now regarded as typical of those in which T helper type 2 (T_H2) cells predominate, but the nature of the stimulus that drives the immune response down this path in nematode infections is not known³. The allergens of nematodes against which these IgE responses are directed may not themselves initiate this type of response, but merely become subject to it: conjugation to nematode extracts has long been used by immunologists as a means of generating IgE to haptens^{4,5}. Nevertheless, nematode allergens are important in their own right as the direct agents of hypersensitivities, and the current effort to produce recombinant parasite proteins for immunization must be viewed in the light of the hypersensitivity reactions they might initiate or recall in previously infected individuals.

The ABA-1 allergen of *Ascaris*

Allergens have been characterized from several species of nematode, including *Ascaris suum*, *A. lumbricoides*, *Dirofilaria immitis* and *Nippostrongylus brasiliensis* (for review see Ref. 6). *Ascaris* has been regarded as the epitome of allergen-containing nematodes, and particular attention has recently been paid to one of its major allergens, ABA-1. This molecule has been characterized as the most abundant protein in the pseudocoelomic fluid of adult *Ascaris*, and it is also released by tissue-invasive larvae of the parasite⁷. Purified ABA-1 from *A. suum* was found to be potentially allergenic in experimental animals⁸, and could therefore represent an important stimulus to the potentially lethal pulmonary and intestinal hypersensitivity reactions that arise in ascariasis. Amino acid compositional analysis showed that ABA-1 was probably the same as Allergen A of *A. suum* which

had been described in the early 1970s (Ref. 9), and the Asc-1 allergen preparation provided by the National Institutes of Health (USA) has ABA-1 as the major constituent¹⁰; for Asc-1 this has now been confirmed by N-terminal sequencing (unpublished). Moreover, direct amino acid sequence analysis showed that the ABA-1 proteins from *A. suum* of pigs and *A. lumbricoides* of humans were identical over the first 41 residues from the N-terminus¹¹. ABA-1 is therefore an allergen to which infected humans will be exposed, and immunologically crossreacting antigens of similar size and abundance are found in *Toxocara canis* and *Anisakis simplex*^{12,13}, ascarids that also infect humans.

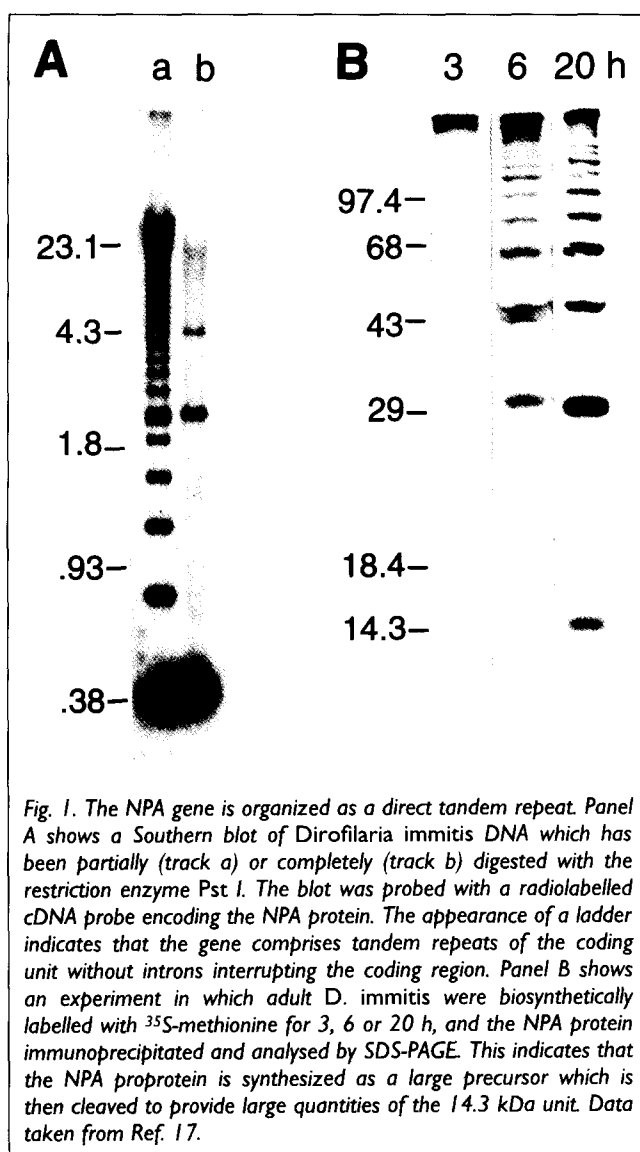
There is no consensus structure for allergenic determinants *per se*, but polypeptide allergens defined thus far from environmental sources (such as pollens and dust mites) are relatively small (generally less than 70 kDa in mass) and mostly of acidic isoelectric points (for reviews see Refs 14,15). ABA-1 has an estimated mass of 14.6 kDa in its final processed form, and an isoelectric point of 5.2 (Refs 11,13).

Homologues in filarial nematodes

While attempts to isolate the ABA-1 gene were under way, three different laboratories had independently obtained cDNAs encoding so-called 'ladder' proteins of *D. immitis*^{16,17} and *Brugia pahangi*¹⁸. This protein was originally described via radio-iodination of intact *B. malayi*, although Bolton-Hunter reagent, which penetrates the cuticle and labels the somatic tissues¹⁹, was generally required for detection²⁰. Pulse-chase biosynthetic labelling experiments in both *B. malayi*¹⁸ and *D. immitis*¹⁷ showed that the protein was synthesized as a very large (400 kDa) precursor which was then processed to a polypeptide of approximately 15 kDa (see Fig. 1). This, together with the fact that the polypeptide is glycosylated, led to the coining of the name gp15/400 for the *Brugia* protein. Analysis of cDNAs encoding fragments of the precursor protein indicated that the precursor was composed predominantly of tandemly repeated units of approximately 15 kDa, which were presumed to be excised from the proprotein via proteolytic cleavage. Partial digests of genomic DNA reinforced the tandemly repeated nature of the cDNA, and suggested that the precursor contained up to 20 repeat units (see Fig. 1). In both species, the protein is encoded by a single gene, and the mature transcripts are correspondingly large (approximately 10 kb) (Refs 16–18).

Unexpectedly, the only protein with which the ladder proteins showed any sequence similarity was ABA-1, whose N-terminal amino acid sequence had by then been obtained^{10,11}. Alignments with this sequence permitted the identification of the sites at

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which the ladder protein was presumably cleaved during post-translational processing. Adjacent to the predicted N-terminus was a cluster of four basic residues, similar to the tetrabasic sites at which many polyproteins and proproteins are cleaved by the subtilisin family of serine proteases (see Fig. 2; and Refs 21,22). The slight mismatches in the position of the tetrabasic group between parasite species could be explained by the secondary action of an exopeptidase, frequently used to modify the primary endoproteolytic cleavage products of proproteins. N-terminal sequencing of the *D. immitis* protein has subsequently confirmed the predicted site of cleavage (L.A. McReynolds *et al.*, unpublished), but this remains to be determined for *Brugia*.

Sequencing of cDNA encoding ABA-1 showed a similar pattern of repeating units and putative cleavage sites²³. Mass spectrometry of parasite-derived ABA-1 indicated that the molecular mass of the processed molecule is smaller than that encoded within a repeat unit in cDNA by approximately the mass of the four C-terminal basic residues¹³. This implies that they are trimmed off, again suggesting that an exopeptidase activity may be involved in secondary processing.

The major events in the synthesis and post-translational modification of this widespread and abundant class of nematode antigens thus became clear. Added to this are recent data that show that a recombinant protein encoded by one repeat subunit of gp15/400 from *B. malayi* is a target for strong IgE responses in lymphatic filarial infection, in particular in those individuals with elephantiasis²⁴. ABA-1 and its homologues in other nematodes may thus comprise allergens which could contribute to the pathology of many nematodiasis. Given this and the form of their synthesis, we have dubbed them 'nematode polyprotein allergens' (NPA). The term 'polyprotein' originally described polyfunctional proteins such as neuropeptide precursors²⁵. We presume that the mature products of the NPA proproteins perform the same function (see below), and the term polyprotein may therefore be somewhat misleading, but it serves as an accurate description of their mode of synthesis.

Location

In *Ascaris*, ABA-1 was originally described as a major somatic component, but it has also been found in secretions of adult worms, infective larvae and lung-stage larvae maintained *in vitro*^{7,9}. Its presence in the excretory-secretory (ES) compartment has always been treated with some caution because its internal abundance may lead to significant contamination of *in vitro*-released materials through larval moult or damage to even a small proportion of parasites. Nevertheless, the presence of homologues in the culture media of filarial nematodes maintained *in vitro*^{16,17} adds weight to the contention that NPA proteins are true secretory products of the parasites. The abundance of NPA proteins, especially ABA-1, within the parasites would suggest that they have an important internal function, although they have been detected primarily in extracellular compartments.

ABA-1 was initially isolated from the pseudocoelom of *A. suum*^{8,10,11} and immunoelectron microscopy has shown that gp15/400 is broadly distributed throughout the tissues of *B. malayi*, but it is particularly concentrated in the matrix of basal laminae bordering the hypodermis, somatic musculature and oesophagus²⁶. Lower, but significant

<i>D. immitis</i>	... Asn Glu Arg Arg Lys Arg	<u>Asn Asp His</u> ...
<i>B. malayi</i>	... Glu Asp Arg His Lys Arg Asp Asn	His Glu His ...
<i>A. suum</i>	... Thr Ser Arg Arg Arg Arg	<u>His His Phe</u> ...

Fig. 2. The amino acid sequence around the cleavage site at which the NPA polyprotein is cut into approximately 14.6 kDa units. The residues marked in bold constitute the cluster of four basic amino acids at which processing proteases of the subtilisin family are presumed to cut the proprotein. The underlined amino acids mark the beginning of the known N-terminal sequence of the *Ascaris suum* and *Dirofilaria immitis* proteins. *Brugia malayi*, unlike the other two species, has two residues intervening between the basic cluster and the position which aligns with the known N-terminus of the NPA unit in *A. suum* and *D. immitis*. The sequences flanking the cluster can vary between repeats in the NPA protein produced by any one parasite species.

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labelling in the cells overlying these extracellular matrices is consistent with multiple sites of synthesis. In adult *D. immitis*, the protein has been detected in the cuticle, another extracellular matrix¹⁷. It is apparently absent from this site in *B. malayi*²⁶ and *Ascaris* (I.M. Huxham and M.W. Kennedy, unpublished), although the reasons for this disparity are not clear.

Structure

The sequences of all the homologues show no similarity with any other protein in available databases. However, the availability of homologous sequences does allow multiple alignments for the construction of a model of the molecule with a good degree of confidence. Such an analysis, carried out by A. Brass (Manchester University, UK) predicts that the protein forms a four-helix bundle similar to carrier proteins such as cytochromes and the myohaemerythrin of marine worms²⁷. This has been tested by circular dichroism carried out by N.C. Price and S. Kelly (Stirling University, UK) on purified ABA-1, which confirmed that it is rich in α -helix to the predicted degree. Four-helix bundle proteins tend to be extremely stable, and direct testing by A. Cooper (Glasgow University, UK) using differential scanning calorimetry has shown that ABA-1 can refold following complete denaturation at 100°C. This is consistent with the observation that the allergenic activity of ABA-1 survives autoclaving¹³, although the epitopes concerned may comprise primary-sequence determinants that are less likely to be affected by heat treatment than are higher-order structures.

There is direct biochemical evidence for N-linked glycosylation of the NPA proteins of *B. malayi*, supported by the existence of a single consensus site per repeat in the amino acid sequence predicted from the cDNA¹⁸. There is no evidence from work on the respective proteins to date that the NPAs of *Ascaris* and *D. immitis* are glycosylated.

The N-termini of the NPA proproteins would be expected to bear signal peptides directing these unusual polypeptides to the endoplasmic reticulum, but no cDNAs have yet been found that encode the N-termini of the NPAs. *Ascaris suum* and *Dictyocaulus viviparus*, however, possess C-terminal extensions of 33 and 11 amino acids, respectively, to their NPAs (Ref. 23; and C. Britton and M.W. Kennedy, unpublished), but, again, the basis for this variation between species is not known.

Diversity

It was originally thought that the repeated units within the NPA array were identical for each species of nematode, but it has since become clear that this is not the case. In *Ascaris*, for example, two adjacent repeats differing by as much as 51% of their amino acid residues have been found (J. Moore, H.J. Spence and M.W. Kennedy, unpublished). A similar situation exists in *D. viviparus* but the repeats are even more diverse, such that none of seven repeat units encoded in one particular cDNA is identical to any other, and identity in predicted amino acid sequences between adjacent repeats can be as low as 31% (C. Britton and M.W. Kennedy, unpublished). Nevertheless, conserved residues are found throughout all of the units

examined so far, both within and between species, and these have proved useful in providing alignments for molecular modelling. It is remarkable that an individual parasite can produce so many different versions of the protein, and this invokes the question of whether the products of different repeat units perform the same or different functions. Molecular modelling of the various repeats in the ABA-1 array predicts that the four-helix bundle structure is conserved despite the differences, and this also applies to the NPA proteins of the filarial nematodes.

Immunogenicity and allergenic properties

In the absence of a known function for the NPA proteins, their allergenicity will remain a focus of attention. The specificity of the antibody response to ABA-1 and gp15/400 has already been examined in mice. Using H-2 congenics on BALB and B10 backgrounds, it has been determined that both the IgG and IgE response to ABA-1 is restricted to animals bearing the H-2^s haplotype of the major histocompatibility complex in mice^{8,28} and RT1^u in rats¹¹. In contrast, examination of the antibody response of H-2^d and H-2^k mouse strains to gp15/400 of *B. malayi* showed that only one of the H-2^d strains (BALB/c) produced an IgG response to the protein, and the antibody response thus appeared to be influenced by factors outside the major histocompatibility complex (MHC)²⁹.

More recently, it has been observed that ABA-1 induces a strong IgG response, but no IgE, in the context of adjuvant-assisted immunization, indicating that the protein is not intrinsically allergenic³⁰. Presumably, the T_H2-type response induced by some other characteristic of nematode infection drives the production of antigen-specific IgE, although genetic control of the immune repertoire will determine which particular antigens will be the targets of a response in any given individual. Moreover, localization of parasites in different compartments of the host (eg. gut, liver and lungs for *Ascaris*, and blood and lymphatics for *Brugia*) might affect the mode and route of antigen presentation to the immune system, which may in turn influence the nature of the consequent immune response. Analysis of antibody responses to different recombinant proteins in individuals with lymphatic filariasis suggests that the filarial NPA is indeed a preferred target of IgE antibody (M. Yazdanbakhsh *et al.*, unpublished). Now that NPA proteins are available in recombinant form, a framework exists for experimental analysis of the induction and immunoregulation of IgE-based hypersensitivity responses to nematode allergens as a whole. A particularly important aspect will be the dissection of T-cell responses, which may shed light on the inception of T_H2 predominance in nematode infections, and its relevance to immunity.

Acknowledgements

We thank Andy Brass, Alan Cooper, Nick Price, Sharon Kelly, Joyce Moore, Heather Spence, Collette Britton, Max Huxham, Rick Maizels, Maria Yazdanbakhsh, Bill Paxton, Catherine Poole and Dong Ma for valuable discussion and access to their results before publication. Our work is supported by The Wellcome Trust, Medical Research Council (UK), World Health Organization, Hoechst Animal Health and New England Biolabs.

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SCID Mice as Models for Parasitic Infections

S.L. Stanley Jr and H.W. Virgin IV

Mice with severe combined immunodeficiency (SCID mice) have become a favored model system for the study of many parasitic diseases. In this review, Samuel Stanley Jr and Herbert Virgin IV provide a brief overview of the biology of the SCID mouse, and review some examples of how the SCID mouse model has been applied to the study of the immunology of a number of different parasitic diseases.

Severe combined immunodeficiency (SCID) is the term used to describe the congenital absence of functional B- and T-cell-mediated immunity. The disease was first recognized in humans, and is characterized by recurrent devastating bacterial, fungal and viral infections in affected infants¹. The SCID syndrome is caused by a variety of autosomal recessive and X-linked genetic defects. About 15–20% of infants with SCID have a defect in adenosine deaminase (ADA), an enzyme involved in purine metabolism. In other cases, the defect appears to lie in the differentiation of T cells, with a resulting lack of mature, functional T cells in the periphery². Defects in the expression of Class I or II major histocompatibility complex (MHC) genes, interleukin 2 (IL-2) production, the IL-2 receptor, responsiveness to IL-1 and abnormalities of signal transduction have also been described in infants with SCID^{3–6}. The study of SCID has led to a number of important advances in our understanding of B- and T-cell development and function⁷.

In 1983, Bosma and colleagues reported the discovery of SCID mice⁸. These mice displayed severe lymphopenia and agammaglobulinemia and were highly

vulnerable to a variety of infectious diseases⁸ (reviewed in Ref. 9). Since their initial description, SCID mice have become an extraordinarily valuable model system for a variety of immunologic studies.

The SCID mouse

The *scid* defect was first recognized in C.B-17 mice, which are congenic with the BALB/c strain, differing only at the IgH allotype locus (IgH^b vs IgH^a in the BALB/c strain)^{8,9}. *Scid* has been mapped to chromosome 16 of the mouse, where it lies between the *md* (mahoganoid coat color) and *IgL*(λ) (lambda light chain) loci¹⁰. The defect in C.B-17/*scid/scid* mice (SCID mice) appears to lie in the recombinase activity responsible for B- and T-cell antigen receptor gene rearrangements¹¹. The failure to rearrange antigen receptor genes prevents SCID B- and T-cell precursors from differentiating into functional lymphocytes. Examination of transformed SCID bone marrow cells, long-term SCID bone marrow cell cultures and SCID mouse thymic lymphomas has demonstrated extensive deletions in rearranged Ig heavy chain (*IgH*), and T-cell receptor (*TCR*) genes, associated with failed D to J, or V to J recombination (reviewed in Ref. 9). SCID mice also exhibit an inability to repair double-stranded (ds) DNA breaks occurring after exposure to ionizing radiation or chemical agents¹². Since antigen receptor gene rearrangements may involve dsDNA breaks, this repair defect may underlie both the radiation sensitivity and the failure to rearrange antigen receptor genes seen in SCID mice.

The *scid* defect is not absolute. Occasionally, productive VDJ and VJ arrangements occur, and functional B- and/or T-cell clones can arise. SCID mice that produce functional B and T cells are called 'leaky'¹³. Leakiness is usually detected by monitoring SCID mice for serum Ig production. It is generally

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