

speleotherapy is almost unheard of in allopathic or alternative medicine circles. In contrast, there is a small literature on the effects of 'therapy courses' in the caves or salt mines of Eastern Europe, particularly Russia. The problems of research in speleotherapy highlight common difficulties encountered by much of the work within the field of alternative medicine. For example, the work is frequently performed by advocates of the therapy, who have little training in scientific methodology, and the therapy under investigation often relies on a constellation of activities [1], individualized according to each patient, the available facilities and the expertise of the practitioner. This latter fact makes controlled scientific investigation difficult, and comparison between trials almost impossible. Consequently, the results of such investigations are normally of very variable quality, as Y. Karakoca *et al.* point out. Despite this, remarkable claims of efficacy continue to be made for speleotherapy and other alternative therapies in the treatment of asthma. The only way to adequately rebut or confirm such claims is to conduct large controlled clinical trials in scientific centres of excellence with expertise in clinical trial methodology, as advocated by Reilly [2].

In addition, the acceptance of speleotherapy and other alternative therapies by allopathic practitioners is held back by the lack of scientific explanations, as highlighted by Y. Karakoca *et al.* However, some work has suggested that speleotherapy may have direct immunological effects [3], either increasing in the number and activity of T-lymphocytes [4], or altering complement levels [5]. Alternatively the efficacy of speleotherapy may be related to allergen avoidance, in much the same way as visits to high altitude health spas [6], which would be in keeping

with speleotherapy's synonym, underground climato-therapy. Finally, in view of the high uranium content of many Russian salt mines irradiation must not be ruled out as a potential mechanism in at least some cases.

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The major mite allergen Der p 2—a secretion of the male mite reproductive tract?

It is often stated that house dust mites spend almost all their time either eating or procreating. We are familiar with the concept that the major mite allergen Der p 1 and perhaps many others are the product of the first activity, namely digestive enzymes [1,2]. The origin of the major allergen Der p 2 which appears to be as important as Der p1 [3,4] is less certain since it is often found in higher concentrations in extracts of mite bodies than spent mite media which is rich in excrement [5]. It now appears most likely from the following sequence analysis that Der p 2 and the other group 2 allergens are a product of the

second aforementioned activity, procreation in that they are homologues of the epididymis-specific human HE1 gene product [6,7] now found in several mammalian species.

HE1 is a secretory protein produced in the epithelial cells lining most areas of the epididymis and in keeping with the high reproductive activity of the mite could be an abundant protein. Consistent with this the vas deferens and accessory gland of an unmated male are large and can fill the complete ventral part of the body between the gut and the testes [8]. The accessory gland is

Table 1. Size and cysteine residues of the group 2 allergens and the human epididymis-specific protein HE1

Molecule	Number of amino acids	Number of cysteines	Position of cysteines
Der p 2	129	6	8,21,27,73,78,119
Lep d 1	125	6	8,23,28,74,80,121
HE1	132	6	8,21,26,72,77,117

lined with epithelial cells which are rich in endoplasmic reticulum and whose secretions include electron light vesicles. The mite does not have a structure between the testis and the vas deferens so the accessory gland may have some of the epididymal functions.

The homology between the epididymal polypeptide HE1 and Der p 2 was first identified by an NCBI BLASTp search which reported a significant homology $P = 0.032$ over a 49 residue stretch. Closer inspection of the complete sequence showed further homology and importantly a similar molecular organization with almost the same number of amino acids and the same number and positioning of cysteine residues. This is also apparent for the recently published Lep d 1 sequence from *Lepidoglyphus destructor* [9] which has now been

confirmed as a group 2 allergen (Table 1). The cysteine arrangement in these molecules is distinctive because of the close spacing of C21 & 27 and C73 & 78 which form small loops by disulphide bonding [10].

The amino acid alignment shown below (Fig. 1) illustrates the sequence identities. The top line and the reference for numbering is the published sequence for Der p 2 [11], the second is the translated HE1 and the third is Lep d 1. The third line (Der*) shows different substitutions found in the Der p 2 sequence either from species variation (i.e. Der f 2) or from polymorphisms of Der p 2 (unpublished) or Der f 2 [12–15]. The residues underlined are those which are identical between the HE1 and either Lep d 1, Der p 2 or Der *. The overall identity calculated for any identity is 35% and the identity to Der p 2 alone is 28% and Lep d 1 26.4%. This is similar to the identity found for Der p 1 and other cysteine proteases of mammalian origin [16] and compares favourably with the identity between the group 2 *Lepidoglyphus* and *Dermatophagoides* sequences which is only 40%. It can be seen that many of the changes in the non-identical residues are conservative substitutions as recognized by the original NCBI search.

The sequence homology is further illustrated by runs of close sequence identity and homology which can be particularly seen in the residues 1–20 and 70–80.

Fig. 1. Alignment of Der p 2, Lep d 1 and the human epididymous-specific protein HE1. Underlined residues are those where the HE1 has identity to at least one of the mite sequences. The Der* alignment shows the residues which are found in polymorphisms of Der p 2 or Der f 2.

	10	20	30	40	50
Der p 2	DNVDV <u>KDC</u> ANHE-- <u>IKKVLV</u> -PG <u>CHG</u> SEPCIIHRGKPFQLEAVFEANQNTKTA				
HE1	EPVQFKDCGSVDGVIKEVNVSP-CPTQ-PCQLSKGQSYSVNVTFTSNIQSKSS				
Lep d 1	GKMTF <u>KDC</u> GHGE--VTELDITG-C <u>SG</u> -DTCVIHRGEKMTLEAKFAANQDTAKV				
Der2*	N.....M..D.....D.....T...L.D.....S....				
	60	70	80	90	100
Der p 2	<u>KIEIKASIDGLEVDV</u> PGIDPNACHYMK-CPLVKGQQYDIKYTNWNPKIAPK				
HE1	<u>KAVVHGILMGVPVPFPI</u> EPDGCKSGINCPIQKDKTY--SYLNKLPV---K				
Lep d 1	TIKVLAKVAGTTIQVPGLETDGCKFIK-CPVKKGEALDFIYSGTIPAITPK				
Der2*	L.....T....FV.....A.....				
	110	120			
Der p 2	<u>SENVVVTVKVMG</u> -----DDGVLA--CAIATHAKIRD				
HE1	<u>SEYP</u> --SIKLVVEWQLQDDKNQSLFCWEIPVQIVSHL				
lep d 1	VKADV-TAELIG-----DHGVMA--CG-TVHGQVE				
Der2*	LV.....N.....G...				
	I				

Another feature is that most cysteines especially the internal ones have an adjacent or next-to-adjacent proline or a glycine which could be involved in the projection of loop structures.

The overall amino acid homology and identity and molecular organization of these molecule point to a common evolutionary origin and function. This combined with the percentage of the mite body devoted to procreation makes it quite plausible that the group 2 allergens arise from secretions of the male reproductive tract. The notion is testable by immunohistochemistry or *in situ* hybridization and such secretions may be the source of other allergens.

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