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RNA viruses alter house dust mite physiology and allergen production with no detected consequences for allergenicity

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Abstract

RNA viruses have recently been detected in association with house dust mites, including laboratory cultures, dust samples, and mite-derived pharmaceuticals used for allergy diagnosis. This study aimed to assess the incidence of viral infection on *Dermatophagoides pteronyssinus* physiology and on the allergenic performance of extracts derived from its culture. Transcriptional changes between genetically identical control and virus-infected mite colonies were analysed by RNAseq with the support of a new *D. pteronyssinus* high-quality annotated genome (56.8 Mb, 108 scaffolds, N50 = 2.73 Mb, 96.7% BUSCO-completeness). Extracts of cultures and bodies from both colonies were compared by inspecting major allergen accumulation by enzyme-linked immunosorbent assay (ELISA), allergen-related enzymatic activities by specific assays, airway inflammation in a mouse model of allergic asthma, and binding to allergic patient's sera IgE by ImmunoCAP. Viral infection induced a significant transcriptional response, including several immunity and stress-response genes, and affected the expression of seven allergens, putative isoallergens and allergen orthologs. Major allergens were unaffected except for Der p 23 that was upregulated, increasing ELISA titers up to 29% in infected-mite extracts. By contrast, serine protease allergens Der p 3, 6 and 9 were downregulated, being trypsin and chymotrypsin enzymatic activities reduced up to 21% in extracts. None of the parameters analysed in our mouse model, nor binding to human IgE were significantly different when comparing control and infected-mite extracts. Despite the described physiological impact of viral infection on the mites, no significant consequences for the allergenicity of derived extracts or their practical use in allergy diagnosis have been detected.

KEYWORDS
allergen, *Dermatophagoides*, house dust mites, physiology, RNA virus

INTRODUCTION

House dust mites (HDM) of the family Pyroglyphidae (Acari: Astigmata) are the principal elicitors of indoors allergy globally (Sánchez-Borges et al., 2017). HDM-allergy is diagnosed and treated by allergen immunotherapy (AIT) using pharmaceutical products derived from extracts of industrially reared HDM. Mite culture conditions are key determinants of the final potency and consistency of these products, which depend on a complex repertoire of allergens produced by HDM (www.allergen.org) (Vidal-Quist et al., 2017). The physiological status of the mites can thus have an impact over standardization, a primary concern of the industry (Casset et al., 2012; Zimmer et al., 2021).

Viruses infect almost every living species on Earth, establishing relationships with the host that vary from pathogenic to mutualistic, and being essential for evolution (Harris & Hill, 2021). Recent studies have detected the presence of RNA viruses in different allergy-eliciting HDM, including one of the most medically important species, *Dermatophagoides pteronyssinus* (Trouessart) (Guo et al., 2021; Vidal-Quist et al., 2021) (*Dp*). Most of these viruses show relationship with genotypes infecting invertebrates, not mammals, thus their pathogenicity to humans is unlikely. Viral RNA has been detected in house dust samples and HDM laboratory cultures, and proof was provided that viruses can also be found (viable or not) in commercial *Dp* extracts used for allergy diagnosis and in reference standards from the United States Food and Drug Administration (FDA) (Vidal-Quist, Vidal, et al., 2021).

The possible incidence of these viruses on both mites and atopic human individuals, after exposure to dust particles or commercial extracts from infected mites, is mostly unknown. From the HDM perspective, the identified RNA viruses seem to establish persistent chronic infections that do not induce pathogenic effects compromising survival or development (Vidal-Quist, Vidal, et al., 2021); yet it remains to be explored to what extent they can alter the mite's physiology. From the human's viewpoint, such viruses may have the potential to (positively or negatively) influence the sensitization process and/or the development of the allergic reaction, either by the direct effect of viral components (Iwasaki, 2012), or by altering the HDM allergenic profile or immunogenicity, as it has been suggested for other allergenic mites harbouring different microbiomes (Hubert et al., 2019).

Arthropods have evolved a myriad of mechanisms to counter pathogen infections, from behavioural strategies or physical barriers to sophisticated cellular and humoral innate immunity responses (Evans et al., 2006; Liu et al., 2017; Möllmann & Colgan, 2022), yet our knowledge of mite's immune system remains scarce. Comparative genomics has shown that components of the canonic immune system in *Drosophila melanogaster* (Meigen) are not always conserved in mites (Palmer & Jiggins, 2015). Of note, viral infection in *Dp* appears to be located in digestive epithelia (Vidal-Quist, Vidal, et al., 2021), which is precisely the site where many of the allergens are expressed, including the three major allergens Der p 1, 2, and 23 (Herman et al., 2014; Park et al., 2000; Weghofer et al., 2013). It is therefore possible that viral infection might affect the production of allergens or other immunoactive factors such as digestive proteases, among others (Jacquet, 2021; Reithofer & Jahn-Schmid, 2017).

The present work takes advantage of two genetically identical *Dp* colonies generated in a previous work from the same inbreeding line (Vidal-Quist, Vidal, et al., 2021), one virtually free of viruses (as per RT-PCR) and one artificially infected with five different RNA viruses, to study the impact of viral infection over the mite's physiology and allergenicity. Specifically, using a new *Dp* genome assembly and annotation, we inspect mite transcriptional changes that result from viral infection, with emphasis on viral response and allergen-related genes. We also compare major allergen accumulation and allergen-related enzymatic activities in whole cultures and purified mite bodies from both colonies. Finally, we investigate whether viral infection alters the allergenicity of mite extracts by assessing airway inflammation in a mouse model of HDM-induced asthma, as well as by detecting IgE levels on sera from *Dp*-sensitized subjects in ImmunoCAP IgE-binding assays.

EXPERIMENTAL PROCEDURES

Mite cultures

Control and virus-infected colonies were established in our laboratory in a previous investigation. RNA viruses (DerpV1, 3, 4, 5 and 6 as detected by RT-PCR; NCBI Bioproject PRJNA684040) were transmitted to cultures of the inbreeding line J-1-1-1-1 (Vidal-Quist et al., 2019) (control colony) by inoculating virus-containing faecal pellets from an in-house stock colony (Vidal-Quist, Vidal, et al., 2021). Both colonies have been maintained in laboratory standard growth conditions as described elsewhere (Vidal-Quist et al., 2015). Prior to the experiments, the expected viral load in both colonies was confirmed by RT-PCR following published methods (Vidal-Quist, Vidal, et al., 2021) (Figure S1). The head-to-head comparison of control and virus-infected colonies throughout different stages of culture maturation was achieved by seeding test cultures at a 1:14 ratio with cultures of both colonies synchronized at the late exponential growth phase (140 mg of a 39 days-old seed culture onto 2 g of fresh growth medium, using cotton-plugged 50 ml Erlenmeyer containers). Replicated cultures were inspected periodically to confirm synchronicity between and within colonies. Whole mite culture (WMC) samples (40–60 mg; exact weights recorded) were pulled after thoroughly mixing the culture and stored at −20°C until extraction. Mixed-stages mite bodies were separated from WMC at the exponential growth phase by the paper disk method (Vidal-Quist et al., 2015), and sampled using a sterile needle for RNA or aqueous extraction. All the activities were compliant with the rules of the Bioethics and Biosecurity Committee of Consejo Superior de Investigaciones Científicas (CSIC, Spain).

Genome assembly and annotation

A genome assembly was obtained by Oxford Nanopore Technologies (ONT) sequencing, using high molecular weight (HMW) DNA isolated from eggs of the inbred colony J-1-1-1-1 (obtained in a previous work (Vidal-Quist et al., 2019)) as template. Details on methods for

bioinformatic assembly and gene annotation and curation are described in text S1 (section A), together with NCBI accession numbers for raw sequencing data. Briefly, ONT reads (1,145,352 reads, N50 = 27,051 nt, ~360x coverage) were assembled followed by several consecutive rounds of polishing using ONT, Illumina gDNA, and RNA sequences (in that order). Then, haplotigs and repeated contigs were identified and purged to obtain the final assembly (NCBI database accession JAMYKS000000000; Bioproject PRJNA843460). Protein coding genes were first automatically predicted, followed by manual review and curation of gene names and/or models after visualizing mapped RNA sequences using GenomeView (https://genomeview.org). Finally, the completeness of genome assembly was assessed using the annotated proteome and the lineage dataset arthropoda_odb10 or arachnida_odb10 as reference.

RNA extraction, RNAseq and RT-qPCR

Total RNA was extracted from purified mixed-stages mite body samples with the TRIzol Reagent (Life Technologies, Carlsbad, USA) following manufacturer instructions, adding glycogen as carrier and diluting in ultrapure RNAse-free water. RNAseq procedure together with in silico analysis, including differential expression, GO enrichment and KEGG pathway analyses, as well as RT-qPCR methodological details are described in text S1 (section B). Table S1 indicates the set of primer pairs used for RT-qPCR.

ELISA and enzymatic assays

Extracts from mite bodies or whole mite cultures (WMC) were obtained by homogenization in ice-cold 0.15 M NaCl followed by estimation of soluble protein using the Lowry method with the DC Protein Assay kit (Bio-Rad, Hercules, CA, USA) and BSA as standard. Der p 1 and Der p 23 allergens were quantified by Sandwich ELISA using the Der p 1 ELISA kit (EL-DP1A) and the Der p 23 ELISA kit 2.0 (EPC-DP23-1), respectively (Indoor Biotechnologies, Charlottesville, USA). Der p 2 allergen was quantified using the ALK's in-house Der 2 ELISA kit (Barber et al., 2012). It is to be noted that preliminary assays showed that Indoor Biotechnologies Mite Group 2 ELISA kit (EL-D2) was not compatible with the Der p 2 variant produced by the inbred colonies used in this study (variant Der p 2.0101 in the J-1-1-1-1 colony; Vidal-Quist et al., 2019). Six enzymatic activities were estimated on mite extracts following previously described methods (Vidal-Quist, Ortego, Castañera, & Hernández-Crespo, 2017). Briefly, activities related to allergens Der p 1 (cysteine protease), Der p 3 (trypsin), Der p 6 (chymotrypsin), and Cathepsin D (body-associated aspartic protease used as marker of exponential growth) were analysed using fluorometric assays; whereas Der p 4 (alpha-amylase) and Der p 8 (glutathione S-transferase, GST) were estimated by colorimetric assays. Reaction conditions, including substrates, additives (such as protease inhibitors or activators), and buffers are summarized in Table S2; detailed protocols are available in the cited reference.

Murine asthma model with mite-derived extracts

Mice of the strain C57BL/6 were exposed to *Dp* extracts to induce experimental allergic asthma, as previously described (Debeuf et al., 2016). In brief, mice were sensitized intratracheally on day 1 with either 1 μg *Dp* extract or with PBS as control, followed by 10 μg intranasal challenges from day 6 to 10. Four types of extracts were assayed: extracts from control and virus-infected bodies (pool of extracts of bodies separated from four replicated cultures per mite line); and extracts from control and virus-infected WMC (pool of extracts from six replicated cultures, sampled close to the population peak at day 39). Mice were euthanized on day 14 by an overdose of pentobarbital. The readouts of the experiment included: immune cell infiltration in the bronchoalveolar space assessed by flow cytometry; in vitro restimulation of the mediastinal lymph nodes with commercial HDM extract to study cytokine production by ELISA; and assessment of immunoglobulin levels in serum. All animal procedures were approved by the Ethical Committee of Ghent University (Belgium).

IgE-binding assay

Binding of IgE from sera of *Dp*-sensitized patients was quantified by Fluoroenzyme Immunoassay in the Phadia ImmunoCAP system using the four types of mite extract described above as allergen source. Informed consent was obtained from all patients to conduct the analysis, which was approved by the Institutional Ethics Committee of Complejo Hospitalario Universitario de Santiago (Spain) (Code 2018/434) and complied with the recommendations of the Declaration of Helsinki. Briefly, mite extracts were biotinylated in a 20-fold molar excess using Biotin Labeling kit (Roche, Basel, Switzerland) following manufacturer instructions. After biotinylation, mite extracts were covalently linked to Streptavidin-ImmunoCAPs (o212; ThermoFisher Scientific, Waltham, MA, USA) during 1 h at room temperature and IgE-binding assays were performed in a Phadia 250 ImmunoCAP system using undiluted patient's sera.

Data analysis

Specific enzymatic activity, allergen content, RT-qPCR gene transcription data (Cq' values, obtained by log2 transformation of normalized relative quantities, as described in Vidal-Quist et al., 2015), and human serum IgE concentration were compared using unpaired *t*-test or 2-way ANOVA coupled with post-tests, as indicated for each assay. Prior to parametric tests, normality was confirmed by the Shapiro–Wilk test. Murine lung inflammation, cytokine production and mouse serum immunoglobulin concentrations were compared by Mann–Whitney nonparametric test. Statistical analyses and graphs were generated using GraphPad PRISM v9.01 (GraphPad Software, La Jolla, USA).

TABLE 1 Differential expression of allergen-related genes in *D. pteronyssinus* upon viral infection

Locus ID	Allergy significance[a]	Related allergen group	Description	Short name	RNAseq			RT-qPCR	
					Fold-change[b]	*p* value	TPM[c]	Fold-change[d]	*p* value
derpt01g07660	Allergen	28	Heat shock protein 70-like Hsp70 − 1; Der p 28 allergen	Der p 28	**4,47**	**<0,001**	60	**5,12**	**<0,001 (6, 27.3)**
derpt06g03860	Related function	28	Small heat shock protein-like sHsp−1	sHsp−1	**2,29**	**<0,001**	336	**2,88**	**<0,001 (6, 11.9)**
derpt11g02040	Related function	28	Small heat shock protein-like sHsp-7_1	sHsp-7_1	**1,47**	**<0,001**	150	**1,44**	**0,013 (6, 3.5)**
derpt10g00650	Allergen	23	Chitin binding Peritrophin-A domain-containing protein PMP − 15; Der p 23 allergen	Der p 23	**1,32**	**<0,001**	529	**1,50**	**0,004 (6, 4.6)**
derpt06g01930	Potential isoallergen	30	Ferritin-like protein FERR-2; Der p 30 allergen-like	Der p 30-like	**1,20**	**0,009**	753	n.a.	n.a.
derpt15g00030	Allergen	1	Peptidase C1A; Cathepsin L-like; Der p 1 allergen	Der p 1	−1,07	0,388	9112	1,07	0,497 (6, 0.7)
derpt25g00850	Allergen ortholog	34	RidA family protein; Der f 34 allergen-like	Der f 34-like.2	**−1,16**	**0,032**	259	n.a.	n.a.
derpt01g05430	Allergen ortholog	35	ML domain protein MLDP-2; Der f 35 allergen-like; LOW QUALITY PROTEIN	Der f 35-like	**−1,17**	**0,005**	6984	n.a.	n.a.
derpt07g03760	Allergen	15	Chitinase; Der p 15 allergen	Der p 15–2	**−1,17**	**0,036**	1117	**−1,14**	**0,019 (6, 3.2)**
derpt07g03780	Allergen	15	Chitinase; Der p 15 allergen	Der p 15–1	**−1,18**	**0,021**	935	**−1,14**	**0,019 (6, 3.2)**
derpt11g00110	Allergen	14	Apolipophorin-like lipid binding protein; Der p 14 allergen	Der p 14	**−1,19**	**0,024**	1733	**−1,31**	**0,059 (6, 2.3)**
derpt17g00050	Allergen	8	Glutathione S-transferase GST-1; Der p 8 allergen	Der p 8	**−1,20**	**0,021**	1261	−1,16	0,173 (6, 1.5)
derpt06g02040	Allergen	2	ML domain protein MLDP-1; Der p 2 allergen	Der p 2	**−1,21**	**0,004**	18,434	−1,10	0,381 (6, 0.9)
derpt17g00060	Potential isoallergen	8	Glutathione S-transferase GST-2; Der p 8 allergen-like	Der p 8-like	**−1,27**	**<0,001**	3768	n.a.	n.a.
derpt21g00080	Related function	1	Peptidase C1A, Cathepsin L-like	DpPap-4_1	**−1,36**	**<0,001**	513	**−1,54**	**0,046 (6, 2.5)**
derpt08g01390	Allergen	3	Peptidase S1A; trypsin-like; Der p 3 allergen	Der p 3	**−1,40**	**<0,001**	1367	**−1,29**	**0,030 (6, 2.8)**
derpt48g00130	Allergen	6	Peptidase S1A; chymotrypsin-like; Der p 6 allergen	Der p 6	**−1,41**	**<0,001**	1122	**−1,38**	**0,011 (6, 3.6)**
derpt07g02770	Allergen	9	Peptidase S1A; Der p 9 allergen	Der p 9	**−1,68**	**<0,001**	1245	**−1,53**	**0,004 (6, 4.6)**

[a]'Allergen' indicates official recognition by the WHO/IUIS as *D. pteronyssinus* (*Dp*) allergen (www.allergen.org); 'isoallergen' indicates an encoded protein showing >67% sequence identity to an officially recognized *Dp* allergen; 'allergen ortholog' indicates an encoded protein showing same function, structure and high sequence identity to an officially recognized *D. farinae* allergen; 'related function' denotes a protein of similar function to other *Dp* allergen.

[b]Transcription fold-change as assessed by RNAseq comparing the virus-infected *Dp* colony and the control colony ($n = 4$); negative values indicate lower expression in the viral-infected colony. *p* values were adjusted for multiple testing with the Benjamini-Hochberg procedure which controls false discovery rate (FDR); *p* values ≤0.05 are highlighted in bold digits.

[c]TPM (Transcript Per Million; average from four control replicate libraries).

[d]Transcription fold-change as assessed by reverse transcription and real time quantitative PCR (RT-qPCR) comparing the virus-infected *Dp* colony and the control colony ($n = 4$); negative values indicate lower expression in the viral-infected colony. RPL13a and α-tubulin were used as reference genes for normalization. Der p 15–1 and Der p 15–2 were amplified together using the same qPCR primer pair. Statistical differences between Cq' values (log2 transformation of normalized relative quantities) were assessed by unpaired *t*-test; *p* values are indicated together with degrees of freedom and *t*-statistic level (in brackets), $p \leq 0.05$ are highlighted in bold digits; 'n.a.' denotes not analysed by RT-qPCR.

RESULTS

Genome assembly

A high-quality assembly was obtained by nanopore sequencing of HMW DNA from eggs of the previously obtained inbred colony J-1-1-1-1 (Vidal-Quist et al., 2019). Quality parameters were on par with the best assemblies obtained so far on allergy-eliciting Astigmata mites (Table S3). The assembled genome size was 56.8 Mb in 108 scaffolds, with N50 of 2.73 Mb, and the largest contig being 5.93 Mb. More than 3600 genes were manually curated, paying special attention to genes encoding allergens and related proteins, peptidases, heat shock proteins and immunity/stress-related proteins; as well as those corresponding to transcripts showing significant differences in expression (>1.3-fold) between control and virus-infected colonies (see below). Finally, 11,180 genes were annotated, 58.3% of which were associated to gene ontology (GO) terms. BUSCO analysis on the annotated proteome revealed 92.6% completeness and 0.9% duplication (lineage arthropoda_odb10 as reference), and 96.7% completeness and 1.8% duplication (arachnida_odb10), confirming that our assembly and annotation represent well this genome. For comparison, it is worth to note that a lower 91.1% completeness was estimated in the same conditions (using arthropoda_odb10) for the annotated proteome of the most recent NCBI's *Dp* assembly, GCA_003076615.3. Our genome assembly is publicly available at NCBI (PRJNA843460) and ORCAE, where the annotation will be open under request for further curation (https://bioinformatics.psb.ugent.be/orcae/).

Overall impact of viral infection on gene transcription

The alignment of RNAseq libraries to the *Dp* genome assembly resulted in an overall alignment rate of 95.0% for libraries originated from the control colony. By contrast, it was reduced to 70.7% in libraries from the virus-infected colony as a result of the presence of raw reads of viral origin (24.5% of reads aligned to a database of viral genomes identified in our stock colony (Vidal-Quist, Vidal, et al., 2021); Table S4). Five viral genotypes were confirmed in the infected colony (DerpV1, 3, 4, 5, and 6), being DerpV3 and 1 the most abundant, whereas only DerpV3 could be fully covered by alignment with reads of the control colony, and at a much lower rate (>4400-fold less abundant, representing less than 0.002% of the reads; Tables S4 and S5). No virus was detectable by RT-PCR in the control colony (Figure S1).

Differential expression analysis revealed 1059 genes being significantly regulated as a result of infection (9.5%; adjusted *p*-value for false discovery rate, FDR ≤0.05). Fold-change estimates varied from maximum 4.47-fold upregulation to 1.68-fold downregulation in the infected colony (results for the complete transcriptome are available in Table S6). GO enrichment analysis was conducted separately on both upregulated and downregulated gene sets to reveal general

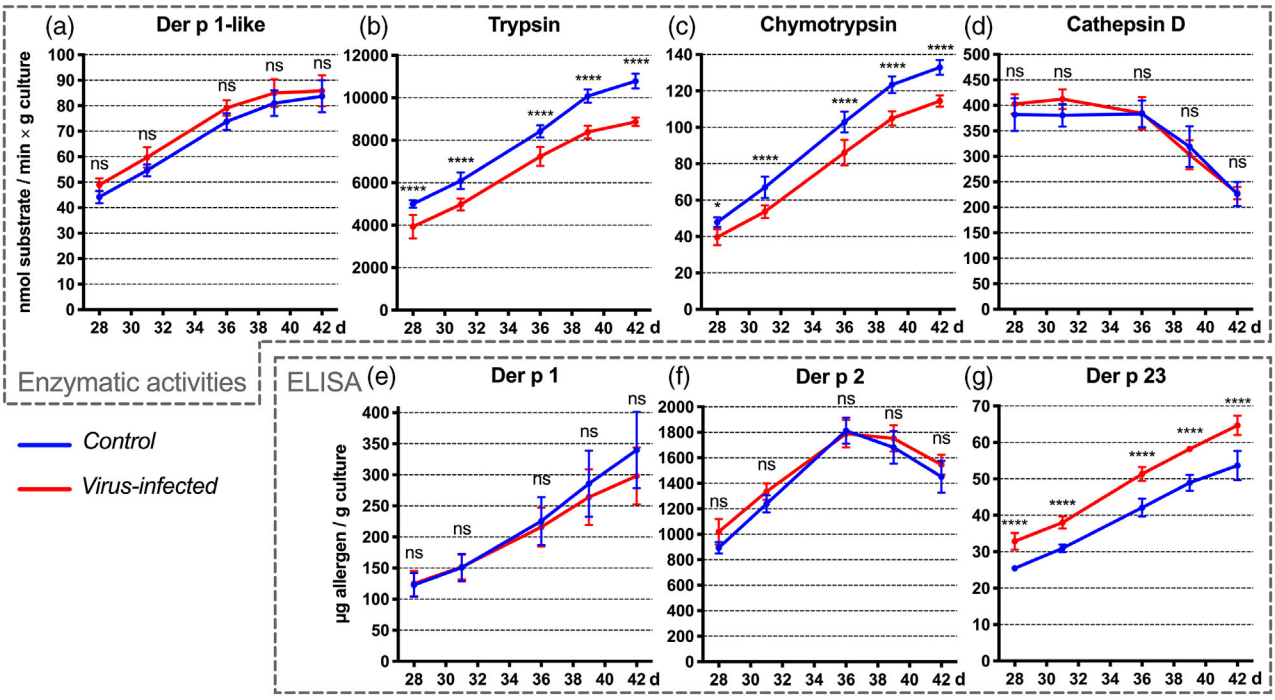

FIGURE 1 Comparison of enzymatic activity and allergen profiles during different phases of culture growth of the control and virus-infected *D. pteronyssinus* colonies. Whole culture samples were extracted at days 28, 31, 36, 29 and 42, and analysed for specific enzymatic activities (panels a–d) and major allergen content by ELISA (panels e–g). Data are means and SE of six biological replicates. Estimates (dependent variable) were compared by two-way ANOVA (virus content and culture age as independent variables) considering repeated measures (for culture age factor), followed by Sidak's post-test for comparisons at each time point. 'ns' denotes 'not significant'; * $p < 0.05$; **** $p < 0.0001$.

TABLE 2 Differential expression of antiviral immunity-related genes upon viral infection

Gene family[a]	Number of genes[b]	Differentially expressed genes (fold-change)[c]	Nondifferentially expressed genes
RNAi pathway			
Canonic Ago	2	–	derpt02g04930; derpt12g00670
Divergent Ago	10	derpt11g01240 (1.16); derpt45g00250 (1.14); derpt11g00350 (−1.13); derpt18g01040 (1.31); derpt18g01060 (1.40)	derpt08g01700; derpt08g00990; derpt18g01030; derpt18g01050; derpt18g293350
Dicer	3	derpt02g06500 (1.24)	derpt15g02300; derpt42g00130
RdRP	4	derpt05g03620 (1.30)	derpt15g00500; derpt18g01450; derpt19g00970
Recognition receptors			
TEP	2	derpt11g00970 (1.20)	derpt01g03490; derpt02g06210; derpt33g00780
Toll pathway			
Spätzle	6	derpt13g00370 (1.18)	derpt03g04090; derpt06g00520; derpt10g00760; derpt15g00210; derpt16g01200
Pelle	2	derpt05g03380 (1.32); derpt24g00360 (1.18)	–
Sarm	1	derpt13g00870 (1.27)	–
JAK/STAT pathway			
STAT	3	derpt01g04790 (1.26)	derpt03g05410; derpt07g01350
Diedel	3	derpt17g00840 (1.24)	derpt01g292620; derpt23g01060
Imd pathway			
IKKb	2	derpt16g00690 (1.23)	derpt04g00260
Nup98-antiviral response			
Forkhead domain TF-like	14	derpt02g00260 (1.51); derpt18g00820 (1.18)	derpt02g00250; derpt02g06040; derpt03g05110; derpt05g01200; derpt06g01900; derpt06g03570; derpt07g03560; derpt12g01310; derpt12g01700; derpt19g00060; derpt19g00800; derpt34g00310
Putative antimicrobial peptides			
Defensin-like	6	derpt66g00210 (1.64); derpt66g00200 (−1.23); derpt52g00130 (−1.30);	derpt52g00140; derpt52g03885; derpt01g06150
Ixodidin-like	6	derpt01g09820 (1.56); derpt02g409250 (1.24); derpt09g02230 (−1.25); derpt07g01980 (−1,0.25)	derpt04g04270; derpt22g00650
Other Cysteine-rich peptides	23	derpt02g03790 (1.28); derpt02g409200 (1.27); derpt22g00450 (1.26); derpt50g00250 (−1.15); derpt22g00430 (−1.16); derpt50g199880 (−1.18); derpt21g01100 (−1.26); derpt09g17460 (−1.29); derpt09g17470 (−1.29)	derpt50g199870; derpt50g199860; derpt50g00230; derpt02g01690; derpt50g00240; derpt07g04900; derpt50g00245; derpt57g00020; derpt50g199850; derpt09g17510; derpt09g02130; derpt09g17520; derpt01g03260; derpt02g409010
Glycine/Histidine rich peptides	1	derpt02g03400 (1.53)	–

[a]Ago (argonaute protein); RdRP (RNA-dependent RNA Polymerase); TEP (Thioester-containing protein); STAT (Signal Transducer and Activator of Transcription protein); Imd (Immune deficiency); IKKb (Inhibitor of nuclear factor Kappa-B Kinase subunit Beta); TF (transcription factor).
[b]Number of genes in the family or group identified by homology search against canonic and noncanonic immunity genes in *Drosophila melanogaster*, *Tetranychus urticae* and *Metaseiulus occidentalis* (www.flybase.org; Palmer & Jiggins, 2015) in our in-house *D. pteronyssinus* (*Dp*) genome assembly (accession numbers are provided in the following columns).
[c]Significantly different expression in the virus-infected *Dp* colony compared to the control colony as assessed by RNAseq (false discovery rate adjusted *p*-value ≤0.05). Fold-change differences are shown in brackets, negative values indicate lower expression in the viral-infected colony.

TABLE 3 Differential expression of stress-related genes upon viral infection

Functional group[a]	Number of genes[b]	Differentially expressed genes (fold-change)[c]
Protection of intracellular proteins		
Heat shock proteins	24	derpt01g07660 (4.47); derpt06g03860 (2.29); derpt11g02040 (1.47); derpt06g03870 (1.31); derpt22g01030 (1.31)
Autophagy		
Atg-like	18	derpt08g01180 (1.19); derpt01g08270 (1.15); derpt07g04610 (1.18); derpt16g01860 (1.19)
Tsc1-like hamartin-like protein	1	derpt01g09160 (1.17)
Uvrag-like UV-resistance associated gene protein	1	derpt04g00600 (1.19)
Ectopic P granules protein 5-like	1	derpt04g05390 (1.26)
Cell death/apoptosis		
Caspases	3	derpt10g0600395 (1.36); derpt16g00740 (1.17)
Apoptotic protease-activating factor	1	derpt05g02940 (1.28)
DIAP1-like	1	derpt05g02120 (1.09)
Ninjurin-like	4	derpt22g00700 (1.48)
LITAF-like	5	derpt02g01450 (1.46); derpt02g01470 (1.26)
Peritrophic membrane integrity/function		
Chitin binding peritrophin A-domain proteins (excluding chitinases)	48	derpt99g00030 (1.54); derpt17g0241295 (1.45); derpt25g00880 (1.32); derpt10g00650 (1.32); derpt08g00520 (−1.19); derpt04g03170 (−1.24)
Mucin-like proteins	n.a.	derpt24g00980 (1.76)
Regulation epithelial barrier homeostasis		
JNK-like MAPK	2	derpt24g01470 (1.40); derpt10g00570 (1.26)
Zyx-like LIM domain protein	1	derpt04g01810 (1.15)
Dachs-like myosin family protein	1	derpt50g00090 (1.16)
Dco-like Ser/Thr kinase	1	derpt19g00210 (−1.14)
MOB kinase activator	4	derpt31g00180 (−1.12)
Epithelial barrier remodelling/repair		
Innexin-like gap junction proteins	11	derpt08g02340 (1.97)
Tetraspanin-like smooth SJ proteins	20	derpt04g02630 (1.24); derpt05g421380 (1.21); derpt11g01340 (1.10)
Mesh-like smooth SJ protein	2	derpt05g02220 (1.23)
Dlg-like SJ proteins	3	derpt05g02230 (1.48)

[a](Atg) Autophagy related protein; LITAF (LPS-induced TNF-activating factor); DIAP1 (Death-Associated Inhibitor of Apoptosis 1); JNK (c-Jun N-terminal Kinase pathway); MAPK (Mitogen-Activated Protein Kinases); SJ (Septate Junction); Dlg (discs large).
[b]Number of genes in the family or group identified by homology search against selected *Drosophila melanogaster* genes (www.flybase.org) and/or by screening of protein domain features in our in-house *D. pteronyssinus* (*Dp*) genome assembly (accession numbers for differentially expressed genes are provided in the following columns). 'n.a.' denotes 'not analysed'.
[c]Significantly different expression in the virus-infected *Dp* colony compared to the control colony as assessed by RNAseq (false discovery rate adjusted *p*-value ≤0.05). Fold-change differences are shown in brackets, negative values indicate lower expression in the viral-infected colony.

functional patterns, yet the species transcriptome was only partially represented (35.1% of the genes with GO terms in the category 'Biological Process'). Viral infection-induced upregulation of genes involved in: signalling and regulation (GO:0007165, GO:0050794); transmembrane transport and localization (GO:0055085, GO:0051179); phosphorous metabolic process (GO:0006793) and protein phosphorylation (GO:0006468); lipid metabolism (GO:0006629); cell adhesion (GO:0007155); and autophagy (GO:0006914) (Figure S2). On the other hand, viral infection repressed genes related to metabolic processes in general (GO:0008152), including primary metabolism (GO:0044238), and, specifically, proteolysis (GO:0006508), carbohydrate metabolism (GO:0005975), alpha-amino acid biosynthesis (GO:1901607), and DNA-related processes (GO:0006260, GO:0015074), among others (Figure S3). KEGG annotation of the *Dp* proteome assigned KEGG Ontology (KO) terms to 56.1% of the genes, related to 399 KEGG reference pathways. Of note, most of the KO terms that were related to differentially expressed genes in the 'Carbon metabolism' KEGG pathway (map01200) corresponded to genes downregulated upon infection, 7 out of 72 identified KO terms (Figure S4). Similarly, 6 out of

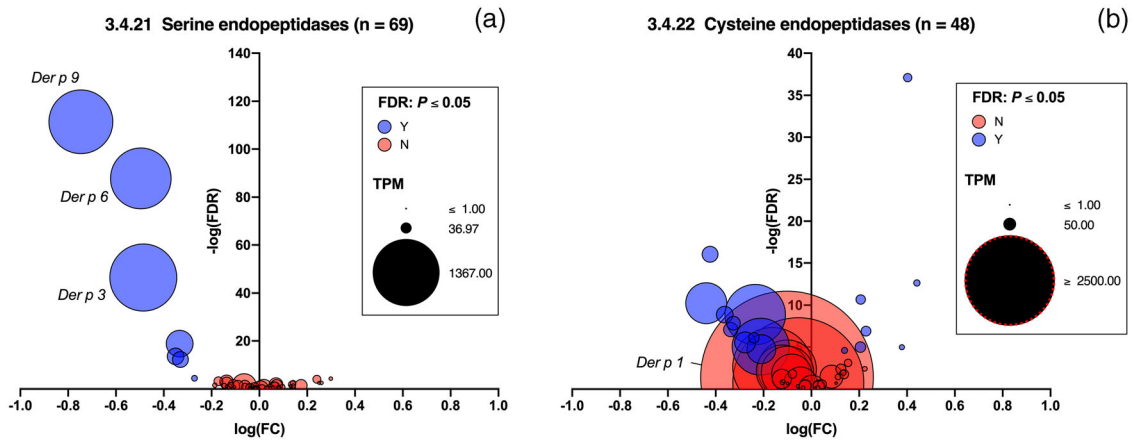

FIGURE 2 Regulation of *D. pteronyssinus* serine and cysteine endopeptidase genes as a result of viral infection. Transcription data are represented using modified volcano plots where the *X*-axis refers to the $\log_2$ of fold-change (FC; negative values indicate downregulation in the viral-infected colony; positive values indicate upregulation); the *Y*-axis refers to -$\log_2$ of the adjusted *p*-value (corrected by the Benjamini-Hochberg procedure for false discovery rate, FDR). Bubble colours indicate statistical significance (blue if adjusted *p*-value ≤0.05; red if no significant differences); bubble sizes denote the total expression level of each gene as estimated by the transcripts per million parameter (TPM; average from the four control replicate libraries).

39 KO terms identified in the 'Biosynthesis of amino acids' pathway (map01230) were related to downregulated genes (Figure S5).

Impact of virus infection on allergen expression

Seven recognized *Dp* allergen genes were differentially expressed as a result of viral infection (Table 1). Upregulated genes in the infected colony, as assessed by both RNAseq and RT-qPCR, included those encoding Der p 28 (the top upregulated gene in the study) and major allergen Der p 23. On the other hand, genes coding for the major allergen Der p 2, the three serine protease allergens Der p 3, 6 and 9 (top downregulated gene in the study), the glutathione S-transferase (GST) Der p 8, the apolipophorin-like Der p 14, and the chitinase Der p 15 (two copies in tandem) were downregulated in the infected colony based on RNAseq, though RT-qPCR analysis did not confirm results for Der p 2 and 8. In addition, two genes coding for putative isoallergens, Der p 8 and 30-like, and two genes coding for allergen orthologs, Der f 34 and 35-like, were differentially expressed based on RNAseq (Table 1).

The impact of viral infection on allergen production was further explored by ELISA and allergen-related enzymatic activity assays. The accumulation of major allergen Der p 23 in virus-infected cultures was significantly higher than that of control cultures ($F_{1,10} = 104.0$, $p < 0.0001$), ranging the increase from 19% to 29% along the culture, as estimated by ELISA on WMC extracts (Figure 1). Estimates for major allergens Der p 1 and 2 did not vary significantly as per the culture's virus content ($F_{1,10} = 0.4951$, $p = 0.498$; and $F_{1,10} = 4.825$, $p = 0.053$; respectively). Similarly, the cysteine protease activity corresponding to Der p 1 did not change significantly at any time point (Sidak's adjusted $p > 0.164$), nor Cathepsin D activity (Sidak's adjusted $p > 0.207$) (Figure 1). On the other hand, trypsin and chymotrypsin serine protease activities (linked to Der p 3 and 6, respectively) were significantly reduced in infected cultures ($F_{1,10} = 123.4$, $p < 0.0001$; and $F_{1,10} = 54.96$, $p < 0.0001$; respectively) ranging from 14% to 21%, and 14% to 20% reduction, respectively. In the case of extracts from purified mite bodies (cleaned from media and faecal fractions), none of the major allergen titres estimated by ELISA did significantly change with the infection status (for Der p 1, $t(5) = 1.164$, $p = 0.297$; for Der p 2, $t(5) = 1.189$, $p = 0.288$; for Der p 23, $t(5) = 2.194$, $p = 0.080$). Enzymatic activity significantly decreased for trypsin (43%; $t(5) = 4.891$, $p = 0.004$) and GST (6%; $t(5) = 2.785$, $p = 0.039$; related to Der p 8) in infected mite bodies, but not for Der p 1-like ($t(5) = 1.362$, $p = 0.231$), chymotrypsin ($t(5) = 0.869$, $p = 0.424$) or α-amylase ($t(5) = 0.668$, $p = 0.533$; Der p 4) (Figure S6).

Defence-related transcriptional responses

Regulation of immunity-related genes

RNAseq analysis detected up to 34 immune-response genes significantly regulated under viral infection. The identified genes were related to: RNAi pathway; recognition receptors; components of the Toll, JAK/STAT and Imd signalling pathways; Nup98 antiviral response; and, effector proteins (putative antimicrobial peptides, including defensin and ixodidin homologues) (Table 2).

Regulation of other stress-related genes

A number genes involved in protection of intracellular proteins, autophagy, cell death and apoptosis, peritrophic membrane function and integrity, and gut epithelial barrier homeostasis were differentially expressed after viral infection. Results are summarized in Table 3; extended results for each group are provided in Text S2 (Section A). In addition, our transcriptomic survey detected other significantly regulated genes for which a mechanistic link to infection could be

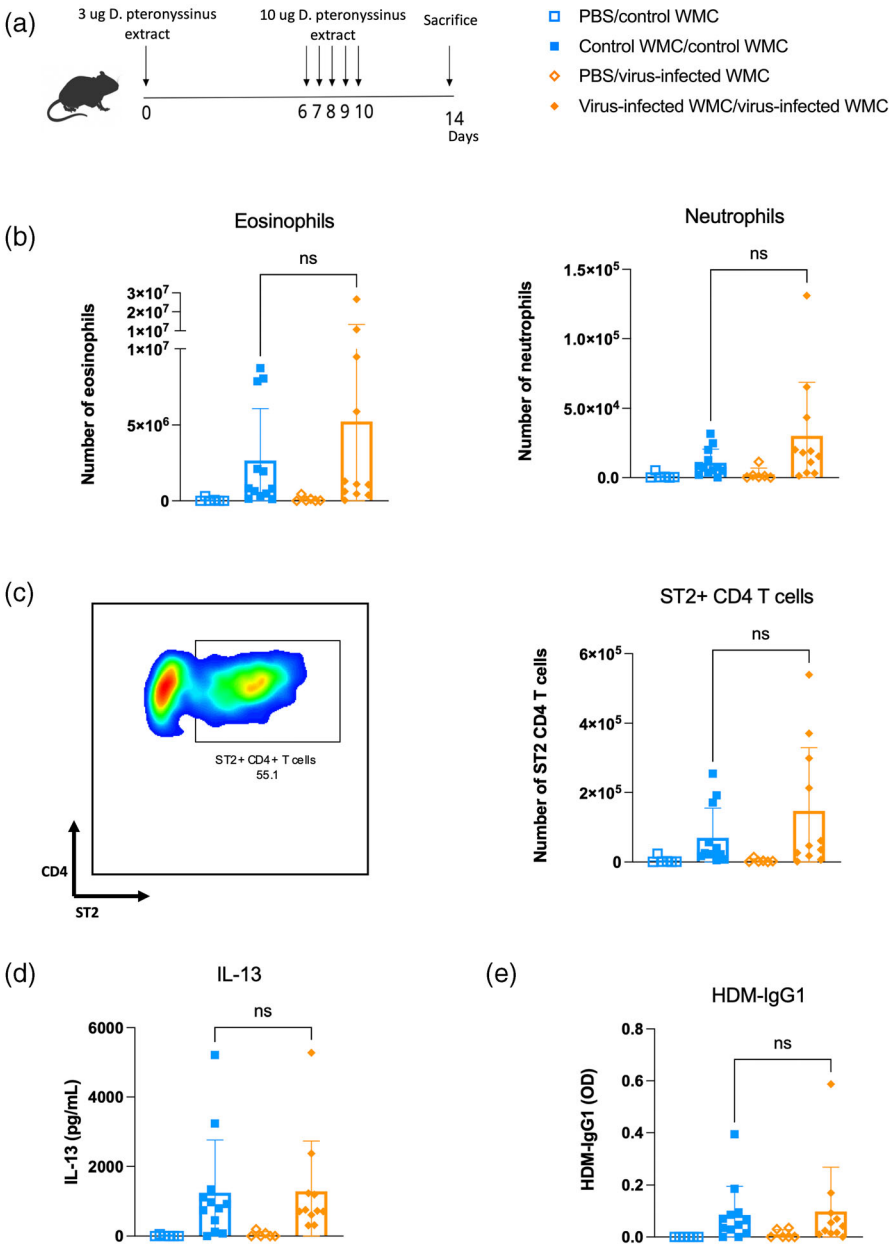

FIGURE 3 Presence of RNA viruses in *D. pteronyssinus* whole mite culture extracts does not affect asthmatic airway inflammation in a mouse model of house dust mite driven asthma. (a), experimental set-up: at day 0, mice were intratracheally sensitized with PBS or 3 μg of protein from whole mite culture extracts (WMC; derived from either control or virus-infected colonies). From day 6 to 10, all mice were daily challenged with 10 μg of WMC, and consequently sacrificed for analysis at day 14. (b), eosinophil and neutrophil numbers in BAL fluid. (c), ST2+ CD4+ T cells in BAL fluid; ST2+ CD4+ T cells were gated on live CD3+ T cells. (d), protein levels of IL-13 secreted by mLN cells during restimulation with HDM extract. (e), serum HDM-specific IgG1 levels (OD value). Data were pooled from two independent experiments ($n = 6$ for PBS/control WMC, 12 for control WMC/control WMC, 6 for PBS/virus-infected WMC, and 11 for virus-infected WMC/ virus-infected WMC). Mann–Whitney statistical test was performed between groups sensitized with WMC. Data are shown as means ± SD; 'ns' denotes 'not significant'.

tentatively proposed; including RCC1/BTB domain proteins, ion transmembrane transporters and regulators, or Rho and Rab GTPases. Results are summarized in Table S7 and extended in Text S2 (Section B). Of note, half of the genes among the top 30 downregulated genes in the virus-infected colony encoded uncharacterized proteins of unknown function (no significant homology to nonmite sequences; no InterProScan protein domain motif; no structural homologue using the Phyre[2] server; more details in Text S2, Section C).

Transcriptional changes in proteolysis-related genes

As indicated earlier, the GO biological process 'proteolysis' was significantly enriched among the group of downregulated genes in the infected colony (Figure S3). Further screening of the genome assembly detected 317 peptidase-encoding genes (EC 3.4 hydrolases; Table S6), 34 of which were significantly regulated under viral infection (Table S8). Notably, none of the peptidase genes showing high overall

expression (54 genes above the 90th percentile of most expressed genes, as estimated by the Transcripts Per Million RNAseq metric, TPM) was upregulated in infected mites, whereas 11 were significantly downregulated: 2 metallocarboxypeptidases, 4 serine endopeptidases, and 5 cysteine endopeptidases. In the case of serine endopeptidases, the top 3 more expressed genes encode Der p 3, 9 and 6 allergens, which were all significantly downregulated in the infected colony (Figure 2a). This result is consistent with the decrease of Der p 3 and Der p 6-like enzymatic activities indicated earlier (Figure 1). By contrast, for cysteine endopeptidase-encoding genes, the top 3 more expressed genes, including Der p 1, were not differentially expressed (Figure 2b), which is also consistent with the lack of significant differences for Der p 1 content and its enzymatic activity (Figure 1).

Impact of virus infection on the allergenicity of mite extracts: Murine asthma model

To assess the effect of virus infection on the allergenicity, we tested two types of mite-derived extracts (WMC or purified bodies) from both the control and infected colonies in a model of HDM-driven allergic asthma (Figure 3a, Figure S7A). Upon treatment with WMC or purified bodies, mice from the sensitized group exhibited clear Th2-driven airway inflammation compared to mock-sensitized mites, as defined by airway eosinophilia, presence of Th2-skewed CD4 T cells (ST2 + CD4+) and production of Th2 cytokines in mLN restimulation culture (Figure 3b–d, Figure S7B-D). In addition, increased levels of allergen-specific IgG1 immunoglobulins could be detected in the serum of allergen-sensitized compared to mock-sensitized mites (Figure 3e, Figure S7E). However, we could not detect any significant differences in these inflammatory parameters comparing the groups that were exposed to extracts from control versus virus-infected colonies ($p > 0.05$). Additional readouts are available in Figure S8.

Impact of virus infection on the allergenicity of mite extracts: Binding of human IgE

Binding of IgE from sera of *Dp*-sensitized human subjects (Table S9) was assessed using the Phadia ImmunoCAP system with custom-made ImmunoCAPs loaded with WMC or purified body extracts from both the control and infected colonies as allergen source. Estimates of IgE concentration in sera were not significantly different when comparing the four types of extract, or when considering the origin of the extract or its viral content, as assessed by 2-way analysis of variance (ANOVA) with matched values per patient coupled with Tukey post-test (Figure 4).

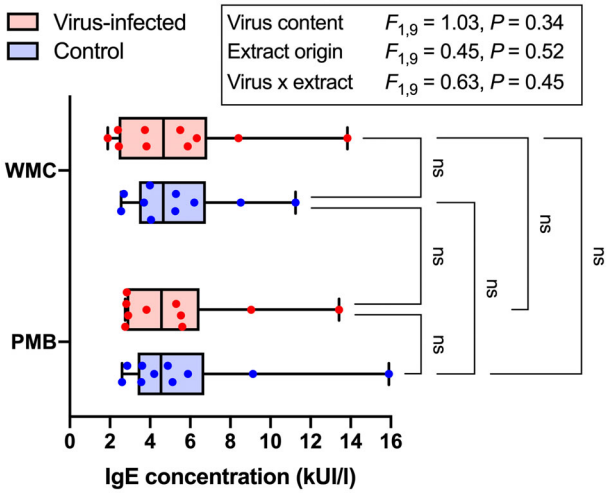

FIGURE 4 Binding of IgE from *D. pteronyssinus* sensitized patient's sera. IgE-binding was assessed using custom-made ImmunoCAPs from whole mite culture (WMC) or purified mite body (PMB) extracts from both the control (blue) and virus-infected (red) colonies. Estimates of IgE concentration in sera are shown for each extract source as box-and-whiskers plots ($n = 10$ patients). Concentrations (dependent variable) were compared by two-way ANOVA (virus content and extract origin as independent variables) considering matched values per patient, followed by Sidak's post-test for comparisons between each extract type; 'ns' denotes 'not significant'. p values and statistics for each independent factor, and their interaction are shown in the upper box.

DISCUSSION

Virus infection, general considerations

In a previous study, we showed that the association of RNA viruses with HDM is remarkably widespread, being found in laboratory or commercial cultures and in natural environments (Vidal-Quist, Vidal, et al., 2021). In the present report, we study the consequences of viral infection after artificially-infecting a control colony for which no RNA virus could be detected by RT-PCR. The analysis by RNAseq confirmed the presence of five viral genotypes in the infected colony, with the dicistro-like picornaviruses DerpV3 and 1 being the most abundant, whilst the viral load in the control colony was almost negligible when compared to the infected colony. Mite counts conducted in the previously cited work suggested no significant impact of viruses over mite population growth. Likewise, no differences were found on the Cathepsin D activity profile of both colonies during culture in this report. Notably, this activity can be used as a physiological marker for mite population growth level due to its decay at the early saturation phase of the culture (Vidal-Quist et al., 2017).

By contrast, the extensive annotation of our high-quality assembly of the *Dp* genome followed by RNAseq have provided evidence that mite-associated RNA viruses have significant impact on the mite transcriptome and affect allergen expression. Nearly 10% of the genes annotated in our genome assembly were significantly regulated as a result of viral infection. It is worth to note that whilst RNA was purified from complete mite bodies, virus particles have only been visualized in some epithelial cells of the midgut (Vidal-Quist, Vidal, et al., 2021). Thus, given that infected cells were homogenized together with a majority of noninfected, it is possible that our transcriptomic analysis may have underestimated gene expression differences. As a matter of fact, fold-change estimates were generally moderate. The low virulence of the studied viruses towards *Dp* could have also contributed to this result.

Mite-virus interaction

The overall transcriptional pattern reported here was in agreement with other observations on pathogenic viral infections in the digestive tract of arthropods, in which defence-related processes are commonly upregulated at the expense of reducing general functions related to primary metabolism, such as digestion (Chtarbanova et al., 2014; Li-Byarlay et al., 2020). However, in a previous study using the same colonies, we demonstrated that the fitness of the population was not compromised by viral infection (Vidal-Quist, Vidal, et al., 2021), despite the repression of primary metabolism. This result could be related to the generally mild intensity of the observed transcriptional change, but also to the type of genes being regulated. As a matter of fact, regarding digestion, previous studies using protease inhibitors have pointed out that proteolytic gut digestion in *Dp* is probably ruled by Der p 1 and other cysteine proteases (which were not affected by infection in this study), whilst serine proteases (which were repressed in this study) appear to play a minor role on gut digestion (Erban et al., 2017; Vidal-Quist, Ortego, & Hernández-Crespo, 2021).

Despite the apparently low virulence of these RNA viruses on *Dp*, extensive screening of immunity and stress-related genes in our study revealed a number of defence/adaptation mechanisms that were regulated after viral challenge, and that could have contributed to the observed tolerance to infection. Immunity to viruses on the model species *D. melanogaster* appears to rely on two main arms: the degradation of viral RNA by the short interfering (siRNA) RNAi pathway (Palmer & Jiggins, 2015; Wynant et al., 2017), and an inducible immune response mediated by the Toll and JAK/STAT signalling pathways (Lemaitre & Hoffmann, 2007; Sparks et al., 2008), and possibly the Imd pathway (Rückert et al., 2014). In *D. pteronyssinus*, many of the genes regulated upon viral infection were involved in these pathways, as well as pathogen recognition receptors that activate the immune system, and antimicrobial peptides (AMPs) that act as effector proteins (Palmer & Jiggins, 2015). Beyond the canonical immunity set, transcriptomic surveys on *D. melanogaster* have also found several noncanonical genes also contributing to defence against pathogens (Keehnen et al., 2017). Our work suggests a similar response in *D. pteronyssinus*, in which some differentially expressed genes in the infected colony indicated stimulation of autophagy, a mechanism that allows the elimination of cytoplasmic components via autophagosomes in infected cells (Shelly et al., 2009), or apoptosis, an antiviral mechanism that can suppress virus-infected cells and for which some viral genomes have evolved proteins interfering its regulation (Liu et al., 2013). Other regulated genes included those related to the integrity of the peritrophic membrane (PM), an important host defence factor associated to the susceptibility to oral pathogenic viruses and for which some viruses are able to secrete PM-degrading chitinases (Liu et al., 2017), as well as genes promoting homeostasis (Royet, 2011) and cell-to-cell adhesion in the gut epithelial barrier (Güiza et al., 2018; Izumi et al., 2019), the site of viral infection detected in *D. pteronyssinus* (Vidal-Quist, Vidal, et al., 2021). In addition, five different heat shock proteins (Hsp) were induced by viral infection, including the two top upregulated genes in our study. Interestingly, these proteins are regarded as a 'double-edged sword': on the one hand, they protect intracellular proteins from degradation under stress conditions maintaining homeostasis in the infected cell, but, on the other, they can be highjacked by the virus for its own benefit to complete different stages of its cycle (Shang et al., 2020; Wan et al., 2020). For the sake of conciseness, further discussion on additional defence-related genes has been addressed as supplementary material (Text S3).

Impact of virus infection on allergenicity

Virus infection affected the expression of several allergens and allergen-related genes. Seven recognized *Dp* allergens were differentially regulated as a result of infection, as supported by RNAseq, RT-qPCR, ELISA, and enzymatic assays (Vidal-Quist, Ortego, Castañera, & Hernández-Crespo, 2017). The expression of Der p 1 and 2, two of the three major allergens described in *Dp* (Thomas, 2018), was unaffected by infection. However, the expression of major allergen Der p 23 was substantially induced by viral infection as confirmed by ELISA on WMC extracts. Der p 23 is the most recently identified major allergen and several studies have confirmed a high prevalence of Der p 23-specific IgE in HDM-sensitized human cohorts from different geographical locations (Celi et al., 2019; Muddaluru et al., 2021; Mueller et al., 2016; Romero-Sánchez et al., 2022; Weghofer et al., 2013), which could even include a small percentage of monosensitized patients (Matos-Semedo et al., 2019; Romero-Sánchez et al., 2022). However, prevalence and serodominance (contribution to the total IgE binding) for Der p 23 have been very variable across studies, being substantially lower in some cases (Batard et al., 2016; Eder et al., 2020; Thomas, 2016). Der p 23 gets accumulated in the spent culture after being excreted by the mite (Weghofer et al., 2013), most probably for this reason its increase after infection was detected only on WMC samples and not on purified bodies.

Genes encoding minor allergens, based on reported allergenicity hierarchy (Thomas, 2018), were also regulated in the virus-infected colony. The highest upregulation in our study corresponded to the Hsp allergen Der p 28. In addition, the three serine protease allergens, Der p 3, 6 and 9 were among the top downregulated genes in the infected colony. Consistently, trypsin (Der p 3) and chymotrypsin (Der p 6) enzymatic activities were reduced in virus-infected cultures. As for Der p 23, these two allergens are concentrated in faecal pellets (Herman et al., 2014; Zhan et al., 2010), thus their activity is much higher in WMC than in purified bodies (Vidal-Quist et al., 2017). Despite that the prevalence and serodominance (i.e., potency) of these minor allergens is expected to be low in human cohorts (Thomas, 2018), a clinically relevant contribution of Der p 3, 6 and 9 to allergy cannot be discarded since their serine protease activity exhibits additional IgE-independent immunoactivity (Jacquet, 2021; Reithofer & Jahn-Schmid, 2017).

Other allergen-related proteins of unknown impact over allergenicity were also differentially expressed, including putative Der p 8-like isoallergen GST (>67% sequence identity (Radauer et al., 2014); with total GST activity being reduced in infected body extracts), as well as orthologs to *Dermatophagoides farinae* (Hughes) Der f 34 and 35 allergens. As

mentioned earlier, in addition to Der p 28, four Hsp genes were significantly upregulated in response to infection. Besides their suspected role in mite-virus interactions, *D. pteronyssinus* Hsp other than Der p 28 might also play a role in mite-induced allergy. Although yet to be explored, some evidences point in this direction: first, allergens have been identified from different Hsp families in a heterogeneous range of sources (www.allergen.org). Second, binding to allergic patients' IgE has been reported for three distinct *D. farinae* group 28-like proteins that show relatively low sequence identity: Der f 28.0101 (An et al., 2013), Der f 28.0201 (Lin et al., 2015) and Der f Mag29 (partial) (Aki et al., 1994). And, third, electrophoresis of *Dermatophagoides* spp. proteins and immunoblotting with allergic patient's sera have revealed IgE reactivity to more than one Hsp70 (An et al., 2013), but also to Hsp20 (Liu et al., 2018) and Hsp60 (Liao et al., 2018).

Predicting the overall effect of virus infection on the allergenicity of mite extracts based on the impact that they have on the allergen content in cultures is challenging. Moderate variations in the exposure to Der p 23 as a result of viral infection (in environmental mites or in commercial extracts) could potentially be of significant clinical relevance considering the high immunogenic potential reported for this allergen (Batard et al., 2016; Martín-López et al., 2021; Mueller et al., 2016) and the low cutoff levels for anti-HDM extract IgE used to infer HDM allergy (Thomas, 2016). However, at the same time these effects might be compensated by the repression of other allergens, some of them also eliciting IgE-independent immune responses, as discussed above. On top of that, although yet unknown, direct effects on the human immune system of components from the viruses themselves are also possible (Iwasaki, 2012). Hence, in vivo and in vitro experiments were needed to confirm potential effects on allergenicity. The allergenicity of control and infected-mite extracts did not differ based on the results from our murine model of HDM-driven allergic asthma. In addition, IgE-binding tests to sensitized human's sera showed no differences between control and infected-mite extracts. These results suggest that HDM-infecting viruses are of minor clinical importance, and that current clinical practice is not to be affected by RNA viruses likely present in the manufacture HDM-based diagnostic products. However, HDM-derived extracts are also clinically used to induce allergen-specific desensitization, and we cannot fully rule out the possibility that mite-infecting viruses could influence outcomes of AIT. In addition, since our IgE-binding assays do not contemplate human innate immune responses and in vivo mouse models may not be fully extrapolated to humans, the possibility of having missed other immune effects attributable to viruses cannot be discarded.

Concluding remarks

Based on the presented results, despite significant transcriptomic effects, the presence in our *Dp* colony of two dominant picornaviruses infecting digestive cells (DerpV3 and 1) and three minority RNA viruses from other families (DerpV4, 5 and 6) is not expected to substantially compromise the allergenicity of mite-derived extracts. However, we demonstrate that viruses are able to alter allergen expression, and particularly major allergen Der p 23. From a pharmaceutical perspective, it remains to be studied if viral infections in industrial mite cultures might have consequences on product consistency within and between companies. Some manufacturers have assessed Der p 23 in their AIT products but, to our knowledge, not for standardization (Batard et al., 2016; Stranzl et al., 2021). We cannot predict how other virus species potentially infecting mite cultures may affect their performance or allergenicity, as this should be studied case-by-case. In this regard, refreshing mite stock cultures by introducing naturally-derived mites should be taken with caution to avoid the introduction of viruses or other pathogens. It is worth noting that the effects of one virus on the host will depend on the rest of the virome; for example, persistent mild infections could prevent proliferation of more pathogenic ones (Escobedo-Bonilla, 2021).

Finally, the high-quality assembly and thorough annotation of the *Dp* genome described here will be publicly available through the Online Resource for Community Annotation of Eukaryotes (ORCAE), where each gene has a specific entry including protein domains, GO terms, or Blast homology information; and where continuous community curation of the annotation will be available (Sterck et al., 2012). This release, together with that of other recently annotated mite genomes (https://bioinformatics.psb.ugent.be/orcae/), represents an outstanding tool to assist future molecular biology research on this and other allergy-causing mites.

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CONFLICT OF INTEREST

BNL has received research grants or consulting fees from GSK, OncoArendi, ArgenX and ALK. PHC leads a R&D support contract with ALK. In all cases, these relationships were conducted outside the submitted work, thus they are not directly relevant or directly related to the work described in this manuscript. The rest of authors have no potential conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The sequencing, genome assembly and annotation data that support the findings of this study will be openly available in NCBI and ORCAE repositories, accession numbers and URLs are indicated in the text and supplementary information. Any other data that is not available as supplementary information will be available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All animal procedures were approved by the Ethical Committee of Ghent University (Belgium). The estimation of HDM-specific IgE in allergic patient's sera at Complejo Hospitalario Universitario de Santiago (Spain) was approved by the Institutional Ethics Committee (Code 2018/434) and complied with the recommendations of the Declaration of Helsinki; informed consent was obtained from all patients to conduct the analysis. All the activities conducted with dust mites at CIB (Spain) were compliant with the rules of the Bioethics and Biosecurity Committee of the host institution, Consejo Superior de Investigaciones Científicas (CSIC, Spain). When appropriate, this information has been added into Experimental Procedures.

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REFERENCES

Aki, T., Fujikawa, A., Wada, T., Jyo, T., Shigeta, S., Murooka, Y. et al. (1994) Cloning and expression of cDNA coding for a new allergen from the house dust mite, *Dermatophagoides farinae*: homology with human heat shock cognate proteins in the heat shock protein 70 family. *Journal of Biochemistry*, 115, 435–440.

An, S., Chen, L., Long, C., Liu, X., Xu, X., Lu, X. et al. (2013) *Dermatophagoides farinae* allergens diversity identification by proteomics. *Molecular and Cellular Proteomics*, 12, 1818–1828.

Barber, D., Arias, J., Boquete, M., Cardona, V., Carrillo, T., Gala, G. et al. (2012) Analysis of mite allergic patients in a diverse territory by improved diagnostic tools. *Clinical and Experimental Allergy*, 42, 1129–1138.

Batard, T., Baron-Bodo, V., Martelet, A., le Mignon, M., Lemoine, P., Jain, K. et al. (2016) Patterns of IgE sensitization in house dust mite-allergic patients: implications for allergen immunotherapy. *Allergy*, 71, 220–229.

Casset, A., Mari, A., Purohit, A., Resch, Y., Weghofer, M., Ferrara, R. et al. (2012) Varying allergen composition and content affects the *in vivo* allergenic activity of commercial *Dermatophagoides pteronyssinus* extracts. *International Archives of Allergy and Immunology*, 159, 253–262.

Celi, G., Brusca, I., Scala, E., Villalta, D., Pastorello, E., Farioli, L. et al. (2019) House dust mite allergy in Italy - diagnostic and clinical relevance of Der p 23 (and of minor allergens): a real-life, multicenter study. *Allergy*, 74, 1787–1789.

Chtarbanova, S., Lamiable, O., Lee, K.Z., Galiana, D., Troxler, L., Meignin, C. et al. (2014) *Drosophila* C virus systemic infection leads to intestinal obstruction. *Journal of Virology*, 88, 14057–14069.

Debeuf, N., Haspeslagh, E., van Helden, M., Hammad, H. & Lambrecht, B. N. (2016) Mouse models of asthma. *Current Protocols in Mouse Biology*, 6, 169–184.

Eder, K., Becker, S., Gellrich, D., Zieglmayer, P. & Gröger, M. (2020) The role of Der p 23 sensitization: an analysis of 474 patients sensitized to mite. *International Archives of Allergy and Immunology*, 181, 689–698.

Erban, T., Harant, K. & Hubert, J. (2017) Detailed two-dimensional gel proteomic mapping of the feces of the house dust mite *Dermatophagoides pteronyssinus* and comparison with *D. farinae*: reduced trypsin protease content in *D. pteronyssinus* and different isoforms. *Journal of Proteomics*, 162, 11–19.

Escobedo-Bonilla, C.M. (2021) Mini review: virus interference: history, types and occurrence in crustaceans. *Frontiers in Immunology*, 12, 674216.

Evans, J.D., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J.L., Jiang, H. et al. (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology*, 15, 645–656.

Güiza, J., Barría, I., Sáez, J.C. & Vega, J.L. (2018) Innexins: expression, regulation, and functions. *Frontiers in Physiology*, 9, 1414.

Guo, L., Lu, X., Liu, X., Li, P., Wu, J., Xing, F. et al. (2021) Metatranscriptomic analysis reveals the virome and viral genomic evolution of medically important mites. *Journal of Virology*, 95, e01686–e01620.

Harris, H.M.B. & Hill, C. (2021) A place for viruses on the tree of life. *Frontiers in Microbiology*, 11, 604048.

Herman, J., Thelen, N., Smargiasso, N., Mailleux, A.C., Luxen, A., Cloes, M. et al. (2014) Der p 1 is the primary activator of Der p 3, Der p 6 and Der p 9 the proteolytic allergens produced by the house dust mite *Dermatophagoides pteronyssinus*. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1840, 1117–1124.

Hubert, J., Nesvorna, M., Klimov, P., Dowd, S.E., Sopko, B. & Erban, T. (2019) Differential allergen expression in three *Tyrophagus putrescentiae* strains inhabited by distinct microbiome. *Allergy*, 74, 2502–2507.

Iwasaki, A. (2012) A virological view of innate immune recognition. *Annual Review of Microbiology*, 66, 177–196.

Izumi, Y., Furuse, K. & Furuse, M. (2019) Septate junctions regulate gut homeostasis through regulation of stem cell proliferation and enterocyte behavior in *drosophila*. *Journal of Cell Science*, 132, jcs232108.

Jacquet, A. (2021) Characterization of innate immune responses to house dust mite allergens: pitfalls and limitations. *Frontiers in Allergy*, 2, 662378.

Keehnen, N.L.P., Rolff, J., Theopold, U. & Wheat, C.W. (2017) Insect antimicrobial defences: a brief history, recent findings, biases, and a way forward in evolutionary studies. In: Ligoxygakis, P. (Ed.) *Advances in insect physiology, vol. 52 insect immunity*. London: Academic Press, pp. 1–33.

Lemaitre, B. & Hoffmann, J. (2007) The host defense of *Drosophila melanogaster*. *Annual Review of Immunology*, 25, 697–743.

Liao, Z., Liu, H., Liu, J., Cai, M., Chen, T., Hong, Q. et al. (2018) Heat shock protein 60: identification of an undetected allergen from *Dermatophagoides farinae*. *Iranian Journal of Biotechnology*, 16, e1697, 235–240.

Li-Byarlay, H., Boncristiani, H., Howell, G., Herman, J., Clark, L., Strand, M.K. et al. (2020) Transcriptomic and epigenomic dynamics of honey bees in response to lethal viral infection. *Frontiers in Genetics*, 11, 566320.

Lin, J.L., Wang, Y.Y., Xiao, X.J., Wu, Y.L., Sun, B.Q., Gao, A.J. et al. (2015) Characterization of a new subtype of allergen in *Dermatophagoides farinae*-Der f 28. *Journal of Thoracic Disease*, 7, 1842–1849.

Liu, B., Behura, S.K., Clem, R.J., Schneemann, A., Becnel, J., Severson, D.W. et al. (2013) P53-mediated rapid induction of apoptosis conveys resistance to viral infection in *Drosophila melanogaster*. *PLoS Pathogens*, 9, e1003137.

Liu, F., Huang, W., Wu, K., Qiu, Z., Huang, Y. & Ling, E. (2017) Exploiting innate immunity for biological pest control. In: Ligoxygakis, P. (Ed.) *Advances in insect physiology, vol. 52 insect immunity*. London: Academic Press, pp. 199–230.

Liu, X.Y., Yang, K.Y., Wang, M.Q., Kwok, J.S., Zeng, X., Yang, Z. et al. (2018) High-quality assembly of *Dermatophagoides pteronyssinus* genome and transcriptome reveals a wide range of novel allergens. *Journal of Allergy and Clinical Immunology*, 141, 2268–2271.

Martín-López, L., López-Matas, M.A., Calzada, D., Benjumeda-Maira, A., Martín, E., García, E. et al. (2021) Environmental exposure of Der p 23 in household dust samples. *Clinical and Experimental Allergy*, 51, 1–3.

Matos-Semedo, F., Dorofeeva, Y., Pires, A.P., Tomaz, E., Taborda-Barata, L., Inácio, F. et al. (2019) Der p 23: clinical relevance of molecular monosensitization in house dust mite allergy. *Journal of Investigational Allergology and Clinical Immunology*, 29, 314–316.

Möllmann, J.S. & Colgan, T.J. (2022) Genomic architecture and sexually dimorphic expression underlying immunity in the red mason bee, Osmia bicornis. *Insect Molecular Biology*, 31, 686–700. Available from:https://doi.org/10.1111/imb.12796

Muddaluru, V., Valenta, R., Vrtala, S., Schlederer, T., Hindley, J., Hickey, P. et al. (2021) Comparison of house dust mite sensitization profiles in allergic adults from Canada, Europe, South Africa and USA. *Allergy*, 76, 2177–2188.

Mueller, G.A., Randall, T.A., Glesner, J., Pedersen, L.C., Perera, L., Edwards, L.L. et al. (2016) Serological, genomic and structural analyses of the major mite allergen Der p 23. *Clinical and Experimental Allergy*, 46, 365–376.

Palmer, W.J. & Jiggins, F.M. (2015) Comparative genomics reveals the origins and diversity of arthropod immune systems. *Molecular Biology and Evolution*, 32, 2111–2129.

Park, G.M., Lee, S.M., Lee, I.Y., Ree, H.I., Kim, K.S., Hong, C.S. et al. (2000) Localization of a major allergen, Der p 2, in the gut and faecal pellets of *Dermatophagoides pteronyssinus*. *Clinical and Experimental Allergy*, 30, 1293–1297.

Radauer, C., Nandy, A., Ferreira, F., Goodman, R.E., Larsen, J.N., Lidholm, J. et al. (2014) Update of the WHO/IUIS allergen nomenclature database based on analysis of allergen sequences. *Allergy*, 69, 413–419.

Reithofer, M. & Jahn-Schmid, B. (2017) Allergens with protease activity from house dust mites. *International Journal of Molecular Sciences*, 18, 1368.

Romero-Sánchez, L., Otero, A., González-Rivas, M., Lojo, S., González-Quintela, A. & Vidal, C. (2022) Der p 23 sensitisation in patients with house dust mite respiratory allergy. *European Annals of Allergy and Clinical Immunology*. Available from: https://doi.org/10.23822/EurAnnACI.1764-1489.264

Royet, J. (2011) Epithelial homeostasis and the underlying molecular mechanisms in the gut of the insect model *Drosophila melanogaster*. *Cellular and Molecular Life Sciences*, 68, 3651–3660.

Rückert, C., Bell-Sakyi, L., Fazakerley, J.K. & Fragkoudis, R. (2014) Antiviral responses of arthropod vectors: an update on recent advances. *Virus-disease*, 25, 249–260.

Sánchez-Borges, M., Fernández-Caldas, E., Thomas, W.R., Chapman, M.D., Lee, B.W., Caraballo, L. et al. (2017) International consensus (ICON) on: clinical consequences of mite hypersensitivity, a global problem. *World Allergy Organization Journal*, 10, 14.

Shang, Q., Wu, P., Huang, H.L., Zhang, S.L., Tang, X.D. & Guo, X.J. (2020) Inhibition of heat shock protein 90 suppresses *Bombyx mori* nucleopolyhedrovirus replication in *B. mori*. *Insect Molecular Biology*, 29, 205–213.

Shelly, S., Lukinova, N., Bambina, S., Berman, A. & Cherry, S. (2009) Autophagy as an essential component of *drosophila* immunity against vesicular stomatitis virus. *Immunity*, 30, 588–598.

Sparks, W.O., Bartholomay, L.C. & Bonning, B.C. (2008) Insect immunity to viruses. In: Beckage, E. (Ed.) *Insect immunology*. London: Academic Press, pp. 209–242.

Sterck, L., Billiau, K., Abeel, T., Rouze, P. & van de Peer, Y. (2012) ORCAE: online resource for community annotation of eukaryotes. *Nature Methods*, 9, 1041.

Stranzl, T., Ipsen, H., Christensen, L.H., Eiwegger, T., Johansen, N., Lund, K. et al. (2021) Limited impact of Der p 23 IgE on treatment outcomes in tablet allergy immunotherapy phase III study. *Allergy*, 76, 1235–1238.

Thomas, W.R. (2016) House dust mite allergens: new discoveries and relevance to the allergic patient. *Current Allergy and Asthma Reports*, 16, 69.

Thomas, W.R. (2018) IgE and T-cell responses to house dust mite allergen components. *Molecular Immunology*, 100, 120–125.

Vidal-Quist, J.C., García, M., Ortego, F., Castañera, P. & Hernández-Crespo, P. (2019) Inbreeding of house dust mites, a tool for genomic studies and allergy-related applications. *Allergy*, 74, 198–201.

Vidal-Quist, J.C., Ortego, F., Castañera, P. & Hernández-Crespo, P. (2017) Quality control of house dust mite extracts by broad-spectrum profiling of allergen-related enzymatic activities. *Allergy*, 72, 425–434.

Vidal-Quist, J.C., Ortego, F. & Hernández-Crespo, P. (2021) Contribution of cysteine and serine proteases to proteolytic digestion in an allergy-eliciting house dust mite. *Journal of Insect Physiology*, 133, 104285.

Vidal-Quist, J.C., Ortego, F., Lombardero, M., Castañera, P. & Hernández-Crespo, P. (2015) Allergen expression in the European house dust mite *Dermatophagoides pteronyssinus* throughout development and response to environmental conditions. *Medical and Veterinary Entomology*, 29, 137–146.

Vidal-Quist, J.C., Ortego, F., Rombauts, S., Castañera, P. & Hernández-Crespo, P. (2017) Dietary shifts have consequences for the repertoire of allergens produced by the European house dust mite. *Medical and Veterinary Entomology*, 31, 272–280.

Vidal-Quist, J.C., Vidal, C., Escolar, F., Lambrecht, B.N., Rombauts, S. & Hernández-Crespo, P. (2021) RNA viruses in the house dust mite *Dermatophagoides pteronyssinus*, detection in environmental samples and in commercial allergen extracts used for *in vivo* diagnosis. *Allergy*, 76, 3743–3754.

Wan, Q., Song, D., Li, H. & He, M.L. (2020) Stress proteins: the biological functions in virus infection, present and challenges for target-based antiviral drug development. *Signal Transduction and Targeted Therapy*, 5, 125.

Weghofer, M., Grote, M., Resch, Y., Casset, A., Kneidinger, M., Kopec, J. et al. (2013) Identification of Der p 23, a peritrophin-like protein, as a new major *Dermatophagoides pteronyssinus* allergen associated with the peritrophic matrix of mite fecal pellets. *Journal of Immunology*, 190, 3059–3067.

Wynant, N., Santos, D. & vanden Broeck, J. (2017) The evolution of animal Argonautes: evidence for the absence of antiviral AGO Argonautes in vertebrates. *Scientific Reports*, 7, 9230.

Zhan, Z., Ji, K., Liu, X., Liu, Z., Li, M., Chen, J. et al. (2010) Monoclonal antibodies against recombinant Der f 3 reveal localization of Der f 3 in the gut and faecal pellets of *Dermatophagoides farinae*. *Experimental and Applied Acarology*, 52, 63–71.

Zimmer, J., Bridgewater, J., Ferreira, F., van Ree, R., Rabin, R.L. & Vieths, S. (2021) The history, present and future of allergen standardization in the United States and Europe. *Frontiers in Immunology*, 12, 725831.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting information.

Table S6. Supporting information.

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