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Novel HLA-A2-restricted human metapneumovirus epitopes reduce viral titers in mice and are recognized by human T cells

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Abstract

Human metapneumovirus (HMPV) is a major cause of morbidity and mortality from acute lower respiratory tract illness, with most individuals seropositive by age five. Despite the presence of neutralizing antibodies, secondary infections are common and can be severe in young, elderly, and immunocompromised persons. Preclinical vaccine studies for HMPV have suggested a need for a balanced antibody and T cell immune response to enhance protection and avoid lung immunopathology. We infected transgenic mice expressing human HLA-A*0201 with HMPV and used ELISPOT to screen overlapping and predicted epitope peptides. We identified six novel HLA-A2 restricted CD8⁺ T cell (T_{CD8}) epitopes, with M₃₉₋₄₇ (M39) immunodominant. Tetramer staining detected M39-specific T_{CD8} in lungs and spleen of HMPV-immune mice. Immunization with adjuvant-formulated M39 peptide reduced lung virus titers upon challenge. Finally, we show that T_{CD8} from HLA-A*0201 positive humans recognize M39 by IFN γ ELISPOT and tetramer staining. These results will facilitate HMPV vaccine development and human studies.

Keywords

human metapneumovirus; paramyxovirus; vaccine; T cell response; respiratory infections

Introduction

Acute respiratory infections are a leading cause of childhood death worldwide, especially in low and middle-income countries [1]. Human metapneumovirus (HMPV) is a paramyxovirus discovered in 2001 and a major cause of acute respiratory infection [2]. The

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very young, elderly, immunocompromised persons, and those with underlying cardiopulmonary conditions are at the highest risk for severe HMPV disease [3-11]. Currently, no licensed vaccines or therapeutics exist for the virus, despite serological evidence that all individuals have been exposed to HMPV by the age of five years [2, 12]. Humoral immunity alone appears to be insufficient for protection from infection with HMPV, since the level of neutralizing antibody response in previously infected individuals do not protect from reinfection with this virus [13-15]. The CD8⁺ T cell (T_{CD8}) response contributes to control of HMPV, as is the case for the related respiratory syncytial virus (RSV); in the absence of T_{CD8}, mice exhibit higher viral titers and delayed clearance [16, 17] and adoptively transferred CTL clones can reduce viral titer [18]. Moreover, humans with impaired T cell immunity experience more severe and fatal HMPV infection [6, 7, 9, 19]. Recently, it has been shown that mechanisms exist which drive impairment of the lung T_{CD8} following HMPV infection in mice and humans [20, 21] during primary and secondary infection, further bolstering evidence of the importance of this response in viral control.

Substantial progress has been made toward an effective vaccine, but HMPV vaccines have not yet entered clinical trials. Similar to the formalin-inactivated RSV vaccine tested in the 1960s [22], formalin-inactivated HMPV leads to enhanced lung pathology upon challenge in animal models [23, 24]. Subunit vaccines based on HMPV F protein produce neutralizing antibodies [25, 26], but T cell responses are thought to be important for protection. Live attenuated HMPV has a risk of reversion, which is particularly dangerous in immunocompromised populations, and often viral attenuation leads to significantly reduced immunogenicity [27-30]. CTL epitope vaccines can significantly reduce viral titers in mice [31], and recently a method utilizing virus-like particles (VLP), which incorporates benefits from both subunit and epitope-based vaccines, has shown promise for protection from HMPV [32, 33]. In order to study T_{CD8} responses to vaccines and to natural HMPV infection in humans, it is necessary to map MHC I-restricted viral epitopes.

In this study, we used transgenic mice expressing human HLA-A*0201, and lacking major mouse MHC molecules (Kb^{-/-}/Db^{-/-}), to identify novel immune T_{CD8} epitopes recognized during HMPV infection. Immunization of mice with the immunodominant epitope reduced lung viral titers following challenge. We found that human T_{CD8} of HMPV-exposed HLA-A2 positive subjects were capable of recognizing the epitope by IFN γ ELISPOT and tetramer staining. Our results show that the transgenic mouse is a useful model for identification of HLA-A2-restricted HMPV epitopes, and suggest novel target/s for vaccination against HMPV.

Materials and Methods

Mice, infections, and cells

HLA-A*0201 transgenic mice on a C57BL/6 (B6) background were obtained from Jackson Laboratories and MHC I K^b/D^b double knockout mice were obtained from Taconic. All animals were bred and maintained in specific pathogen-free conditions under guidelines approved by the AAALAC and the Vanderbilt Institutional Animal Care and Use Committee. Six to twelve-week-old age- and gender-matched animals were used in all experiments. HMPV (pathogenic clinical strain TN/94-49, genotype A2) was grown and

titered in LLC-MK2 cells as previously described [34]. For all experiments, mice were anesthetized with intraperitoneal ketamine-xylazine and infected intranasally (i.n.) with 1×10^6 PFU of HMPV. For peptide vaccination (TriVax), mice were injected i.v. with a mixture of 200 μ g M39 peptide, 50 μ g anti-CD40 Ab (clone FGK4.5; BioXCell), and 50 μ g polyinosinic:polycytidylic acid (InvivoGen) and allowed to rest for 4 weeks before infection. The dose of 200 μ g peptide was used based on prior reports of TriVax in mice [35, 36]. Viral titers in infected mouse lungs were measured by plaque titration as described previously [34]. HLA-characterized PBMCs from 5 unique human donors were obtained from Cellular Technology Limited (C.T.L.).

Epitope prediction and peptides

We used both predictopes and overlapping peptide approaches to identify MHC I-restricted HMPV epitopes. A previous report generated predictopes for the nucleoprotein (N), phosphoprotein (P), matrix (M), M2-1, M2-2, fusion (F), short hydrophobic (SH), and glycoprotein (G) into three separate online epitope prediction databases: SYFPEITHI (<http://syfpeithi.de>) [37]; BIMAS (<http://www.bimas.cit.nih.gov>) [38]; and PREDEP (<http://margalit.huji.ac.il/Teppred/mhcbind/index.html>). To perform initial screening, 15-mer peptides overlapping by 9aa covering the entire HMPV M and N open reading frames were synthesized (GenScript). For predictopes, 9-mers were synthesized (GenScript).

IFN γ ELISPOT

ELISPOT assays were performed as previously described [20]. The mitogen concanavalin A (ConA, Sigma) was used as a positive control, while stimulation with an irrelevant peptide served as a negative control. The average number of spots counted from the negative control wells was subtracted from each of the HMPV epitope wells, and the data were expressed as spot-forming cells (SFC) per 10^6 lymphocytes. The antibodies used for the murine ELISPOT were anti-interferon- γ (IFN- γ) monoclonal antibody (mAb) clone AN-18 (5 μ g/ml; eBioscience) and biotinylated anti-IFN- γ mAb R4-6A2 (2 μ g/ml; eBioscience). For the human ELISPOT, the antibodies were anti-IFN- γ mAb 1-D1K (5 μ g/ml; Mabtech) and biotinylated anti-IFN- γ mAb 7-B6-1 (2 μ g/ml; Mabtech).

Flow Cytometry

Cells were isolated from lungs and spleens of infected animals as previously described [20]. Briefly, lungs were rinsed in R10 medium (RPMI-1640 [Mediatech] plus 10% FBS, 2 mM glutamine, 50 μ g/ml gentamicin, 2.5 μ g/ml amphotericin B, and 50 μ M β -mercaptoethanol [Life Technologies]), minced with a scalpel, and incubated with 2 mg/ml collagenase A (Roche) and 20 μ g/ml DNase (Roche) for 1 hour at 37°C. Single-cell suspensions of spleens and digested lungs were obtained by pressing through a steel screen (80 mesh) and then passing over a nylon cell strainer (70- μ m pore size). Erythrocytes were lysed using Red Blood Cell Lysis Buffer (Sigma-Aldrich). For labeling of HMPV-specific T_{CD8} single cell suspensions of mouse lung or spleen were stained with violet LIVE/DEAD dye (Life Technologies), Fc blocked with 1 μ g per 10^6 cells anti-CD16/32 (BD Biosciences), and incubated with PE-labeled HLA-A*0201 tetramers (0.1-1 μ g/mL), anti-CD8 α (clone 53-6.7, BD Biosciences), and anti-CD19 (clone 1D3, eBioscience) [20]. Surface/tetramer staining was performed for 1.5 hours at room temperature in PBS containing 2% FBS [39]. Staining

for HMPV-specific T_{CD8} was normalized to the binding of an irrelevant PE-labeled tetramer loaded with a vaccinia virus (VACV) peptide to T_{CD8} (typically 0.05-0.1% of T_{CD8}). For all cell populations, FSC and SSC gating were used to define cells of appropriate size and shape. All flow cytometric data were collected using an LSRII or Fortessa (BD Biosciences) and analyzed with FlowJo software (Tree Star).

Tetramer Enrichment

Biotinylated HLA-A*0201 human monomers loaded with HMPV epitope were prepared by the exchange of conditional peptide ligand and then tetramerized with streptavidin-PE [40]. Briefly, recombinant HLA-A*0201 heavy and β 2m light chain production [40], class I refolding with conditional peptide ligand KILGFVFJV [41], biotinylation and purification [42], UV-mediated exchange of conditional peptide with HMPV-derived peptides and quantification of peptide exchange were performed as described previously. Tetramerization with phycoerythrin (PE)-, or allophycocyanin (APC)-streptavidin conjugated fluorochromes (Life Technologies) were performed as described [43].

PBMC were briefly thawed, washed once with RPMI, re-suspended in 200 μ l FACS (PBS containing 2% FBS and 50 nM dasatinib), and stained 30 min at RT with 10 μ l of tetramer (25 μ g/ml stock concentration) followed by addition of 50 μ l FACS containing anti-CD8-Alexa Fluor700 (Life Technologies, 1:200 final dilution), anti-CD4-FITC (BD, 1:80 final dilution), anti-CD14-FITC (BD, 1:32 final dilution), and 1 hour incubation at 4°C. Cells were washed, re-suspended in 100 μ l MACS buffer (PBS containing 0.5% BSA and 2 mM EDTA, pH 8.0), and incubated with 10 μ l of anti-PE beads (Milenyi). Enrichment for tetramer+ (PE) T_{CD8} was performed with gravity MS magnetic column (Milenyi). Both enriched and depleted fractions were collected for flow cytometric analysis; the entire enriched fraction and 10% of depleted fraction were analyzed. Accucount counting beads (Life Technologies) were used to account for sample losses during flow cytometry and for more precise population counts. Dead cells were discriminated using propidium iodide. Up to 250,000 events were acquired using a 3-laser LSR-II (BD Biosciences).

Statistical Analyses

Data analysis was performed using Prism v4.0 (GraphPad Software). Groups were compared using unpaired t-test or one-way ANOVA with post-hoc Tukey test for multiple comparisons. $P < 0.05$ was considered significant by convention.

Results

HMPV peptides are recognized by IFN γ -producing cells in HLA-A2 transgenic mice

To generate mice expressing only the HLA-A*0201 transgene, we first crossed two commercially available mouse strains, one expressing the human HLA-A*0201 molecule as well as the WT murine H2-Kb/Db, and another lacking expression of the mouse MHC I molecules (H2-Kb^{-/-}/Db^{-/-}). The F1 heterozygotes were crossbred and homozygote offspring selectively bred for successive generations to generate HLA-A*0201^{+/+}/Kb^{-/-}/Db^{-/-} (A2tg). Genotypes were confirmed by PCR and flow cytometry at each generation (not shown).

A2tg mice were infected with HMPV i.n. and their spleens and lungs were harvested on day 10 post-infection, the peak of the T_{CD8} response [20].

Splenocytes and lung lymphocytes from HMPV infected animals were plated in triplicate in an IFN γ ELISPOT with both predictopes and overlapping peptides. There was a response significantly above background to six peptides (Table 1), with peptide M85 showing the highest response in both spleen (Fig. 1A) and lung (Fig. 1B). However, the length of the overlapping peptides, at 15aa, would allow intracellular processing machinery to load the peptides in either class I or II HLA molecules present on antigen-presenting cells (APC) to stimulate T_{CD4} or T_{CD8}. Thus, the cellular specificity of the epitope response was unclear.

Predicted MHC I binding HMPV peptides are recognized by IFN γ producing cells in transgenic HLA-A2 mice

To identify the MHC I-restricted T_{CD8} epitopes, the amino acid sequences of the antigenic peptides discovered above were analyzed using epitope prediction algorithm software to generate 9-mer peptides that might be presented by HLA-A2 (2-3 9-mers from each original 15-mer peptide). A2tg mice were infected with HMPV i.n. and spleens and lungs harvested at the peak of T_{CD8} response (day 10). Shorter peptides derived from the top two hits in the initial ELISPOT, M₈₅₋₉₉ (M85) and N₂₁₇₋₂₃₁ (N217), did not induce a potent IFN γ response (Fig. 2A, B), suggesting that the IFN γ response to the 15-mer may have been T_{CD4} responding to peptides presented by class II H2-IA^b molecules. However, IFN γ -producing cells were elicited by several peptides that were predicted by the algorithms to be MHC I restricted (Table 2). Two 9-mer peptides, M₃₉₋₄₇ (M39) derived from M37 and N₃₉₋₄₇ (N39) induce the most substantial IFN γ response in both splenocytes (Fig. 2A) and lung lymphocytes (Fig. 2B). The number of IFN γ -secreting cells was greater in the lung than the spleen, reflecting the localized nature of HMPV infection. These data suggested that M39 and N39 might represent true HLA-A2 restricted epitopes recognized by T_{CD8}.

HMPV-specific T_{CD8} are identified with fluorescently labeled HLA tetramer molecules in A2tg mice

To assess whether T_{CD8} that are capable of recognizing the M39 and N39 HMPV-derived peptides in a direct binding assay, we generated fluorescently labeled pM39/A2 and pN39/A2 tetramers loaded with the antigenic epitopes of interest. A2tg mice were infected with HMPV and their lungs and spleens were harvested on day 10 post-infection. Cells within the lymphocyte gate by forward/side scatter were gated on CD8 and CD19⁺ cells while excluding dead cells, and tetramers loaded with an irrelevant VACV peptide [44] were used to set the gate for tetramer⁺ cells (Fig. 3). Both M39 and N39-specific T_{CD8} were detected in HMPV-infected A2tg animals (Fig. 3). Similar to the ELISPOT analysis, we found that M39-specific T_{CD8} were more abundant than N39-specific T_{CD8}. Both populations of epitope-specific T_{CD8} were more frequent in the lung compared to the spleen (Fig. 3). These data indicate that M39 and N39 epitopes were recognized by T_{CD8} in infected animals, but do not address whether T_{CD8} responding to these epitopes contribute to viral clearance.

TriVax vaccination with HMPV peptide is protective in transgenic HLA A*0201 mice

To test the function of virus-specific T_{CD8}, we used an epitope vaccine strategy. Administration of a short peptide with poly(I:C) and α -CD40 mAb (TriVax) generates a robust epitope-specific T_{CD8} response against tumor antigens and viruses [35, 36]. We vaccinated A2tg mice intravenously with M39 peptide using this strategy, and then waited 4 weeks to allow time for the development of memory T_{CD8} response and for the innate immune response to the adjuvant to wane. Control mice were vaccinated in the same manner with an RSV peptide restricted to the mouse H2-K^b/D^b. All mice were then infected with HMPV, and their lungs were harvested at the peak of viral replication (day 5). We previously found that epitope vaccination in the HLA-B7tg and WT C57BL/6 mice did not reduce nasal virus titers ([20] and unpublished data), so only lung titers were measured in these experiments. The lungs were processed and viral titer quantified by plaque assay. Mice vaccinated with the HMPV peptide M39 had significantly lower viral titers compared to control mice vaccinated with the RSV-derived peptide (Fig. 4). These data indicate that that vaccination with M39 elicited protective immunity against HMPV challenge that was able to reduce viral titers *in vivo*.

Human HLA-A*0201 PBMCs recognize HMPV M39 and N39 peptides

To test whether epitopes identified using the A2tg mice would be recognized in humans, we used HLA characterized PBMCs from 5 individual donors. Each of the donors contained at least one allele encoding HLA A*0201 and varied in age (27-59 years), gender, and ethnicity (not shown). The PBMCs were plated in triplicate for a human IFN γ ELISPOT, with an RSV peptide restricted to murine H2-K^b/D^b as a control. IFN γ -producing cells were detected when donor PBMCs were stimulated with the N39 and M39 epitopes, with a frequency ranging from 24 to 72 M39-specific SFC per million PBMCs (Fig. 5A) and 12 to 52 N39-specific spot-forming cells (SFC) (Fig. 5B). Since all humans have been infected with HMPV by 5 years of age [2, 12, 13], and reinfections are common [4, 5, 10, 15], these data indicate that human T_{CD8} recognizing M39 and N39 peptides were elicited by prior HMPV infection.

Frequency of immune CD8 T cells in human HLA-A*0201 PBMCs recognizing the HMPV M39 epitope

Since we observed that the frequency of HMPV-specific CTLs is low in the peripheral blood of the uninfected human donors, it was necessary to enrich these cells using a magnetic affinity column before analysis by flow cytometry. A magnetic column with α -PE beads was prepared and samples containing >10 million PBMCs per donor were incubated with a PE-labeled human HLA tetramer molecule loaded with the M39 HMPV-derived peptide. PBMCs were passed through the column and both the enriched and depleted fractions were collected (Supp. Fig. 1). We analyzed 10% of the depleted fraction (due to the excessive total cell number) and 100% of the fraction enriched for tetramer-specific cells to determine the percentage of T_{CD8} recognizing the M39 peptide in both total PBMCs and T_{CD8} for each donor. We normalized the HMPV-specific T_{CD8} to total input PBMC for each donor. HMPV M39-specific T_{CD8} were detected in all donor samples, ranging from 1 to 32 M39-specific T_{CD8} per million PBMCs (Fig. 6A) and from 7 to 166 M39-specific T_{CD8} per million T_{CD8}

(Fig. 6B). These data suggest that the M39 peptide is recognized and elicited immune CD8 T cell response during normal human HMPV infection, and that the frequency of this virus specific T_{CD8} response is highly variable within the HLA-A2 positive population. Interestingly, despite varying numbers of immune T_{CD8} between individuals, this peptide was recognized by each of five tested subjects.

Discussion

HMPV is an important human pathogen, and thus a safe and effective vaccine is desirable. The optimal immune response to an ideal HMPV vaccine in humans is not known. However, previous human data with the formalin-inactivated RSV vaccine, and preclinical data for both HMPV and RSV, suggest that a balanced antibody and T_{CD8} response are desired. Neutralizing antibodies alone can protect in animal models, and palivizumab is effective at reducing RSV lower respiratory infection in premature infants. However, T_{CD8} are required to clear an established infection, emphasizing the need for an effective vaccine to elicit both antibody and T_{CD8} responses.

A critical first step in designing T_{CD8}-specific vaccines is identification of immune epitopes. Epitope discovery can be approached using several methods, including screening of peptide sets (overlapping or predictopes) by ELISPOT or characterizing peptides eluted from MHC class I molecules expressed by infected cells [44, 45]. We show here that a combination of peptide screening approaches combined with *in vivo* experiments can identify epitopes that are recognized during HMPV infection. We discovered six new epitopes, including the dominant M39 and N39.

We only identified a few epitopes presented by the HLA-A*0201 molecule. Other studies have similarly found a limited number of epitopes presented by a given MHC class I molecule from small RNA viruses such as influenza, RSV, HMPV, or West Nile virus [18, 46-50]. Notably, studies using overlapping peptides spanning the entire RSV genome identified a number of other potential T_{CD4} and T_{CD8} epitopes [51], suggesting that other HMPV epitopes may exist. One study tested PBMCs from 5 A2 adult donors against HMPV predictopes and found that 1 of 5 responded to F₁₅₇ and G₃₂, while 2/5 responded to M2-1₁₅₇. We detected no response against F₁₅₇ or M2-1₁₅₇ in the A2tg mice, and did not screen these peptides against human PBMCs. We did not test any G peptides, as we have failed to identify MHC class I-restricted G-derived epitopes in our prior studies ([20, 33] and not shown). A study of *in vitro* binding affinity of HMPV predictopes against recombinant MHC class I molecules predicted binding of N39 to HLA-A*02:01, but did not test the M39 peptide [52]. Moreover, due to the small number of cells, we did not perform *in vitro* functional assays (e.g., intracellular cytokine staining) to confirm that these were functional T_{CD8}. Due to the variability in peptide recognition between individuals, more extensive testing of human anti-HMPV T_{CD8} responses would be required to define the full HLA-A2 restricted repertoire in a human population. Notably, the M39 epitope was conserved among 197 HMPV M genes and N39 epitope was conserved among 173 HMPV N genes, including viruses of all four subgroups, but neither was present in any avian metapneumovirus strains in GenBank (not shown).

The M39 epitope exhibited antiviral function in a vaccine-challenge model, with approximately 1-log₁₀ reduction in lung viral titer. These experiments do not determine the optimum dose, preparation, or route of epitope vaccination; however, not all T_{CD8} epitopes can be functionally protective, even if immunodominant [44]. Thus, the confirmation that M39 represents an epitope that elicits T_{CD8} capable of reducing viral titer will guide future studies. The identification of an HMPV epitope recognized by human T_{CD8} will facilitate further studies on the human response to HMPV during primary and secondary infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. HMPV peptides are recognized by IFN γ -producing cells in HLA-A2 transgenic mice
 A2tg mice were infected i.n. with 1×10^6 PFU HMPV and lungs and spleens collected on day 10 post-infection. (A) Splenocytes and (B) lung lymphocytes were stimulated with $10 \mu\text{M}$ overlapping M and N peptides (15-mers overlapping by 9) or algorithmically predicted (9-mer) HMPV peptides for 18 hours, IFN γ spots counted, and SFC/10 6 immune cells calculated and corrected by subtracting SFC from RSV negative control peptide wells. Data are representative of two independent experiments performed in triplicate with $N = 3-5$ mice per experiment.

Figure 2. HMPV immune A2tg mice recognize predicted HLA-A*0201 binding HMPV peptides A2tg mice were infected i.n. with 1×10^6 PFU HMPV and lungs and spleens collected on day 10 post-infection. (A) Splenocytes and (B) lung lymphocytes were incubated with 10 μ M HMPV 9-mer peptides for 18 hours, IFN γ spots were counted, and SFC/10⁶ total immune cells were calculated for each peptide corrected by subtracting SFC from RSV negative control peptide wells. Data are representative of two independent experiments performed in triplicate with N = 3-5 mice per experiment.

Figure 3. HMPV-specific T_{CD8} identified with fluorescently labeled HLA tetramer molecules in A2tg mice

A2tg mice were infected i.n. with 1×10^6 PFU HMPV and lungs and spleens collected on day 10 post-infection. Live/dead dye and fluorescently labeled CD8 antibodies were used to identify live CD8⁺ T lymphocytes and fluorescently labeled HMPV-specific M39 and N39 tetramers, as well as an irrelevant vaccinia virus (VACV) epitope-loaded tetramer, were used to identify epitope-specific T_{CD8}. Data are representative of two independent experiments with N = 3-5 mice per experiment.

Figure 4. TriVax vaccination with HMPV peptide is protective in transgenic HLA A*0201 mice A2tg mice were vaccinated with 200 μ g of the M39 peptide with 50 μ g α -CD40 antibody and 50 μ g of poly(I:C), allowed to rest for 4 weeks, and then infected i.n. with 1×10^6 PFU HMPV. Lungs were collected on day 5 post-infection and HMPV titer determined using plaque assay. Data are combined from two independent experiments with N = 4 per group. Error bars represent SEM. *** P<0.001, unpaired T-test.

Figure 5. Human HLA-A*0201 PBMCs recognize HMPV N39 and M39 peptides

Human HLA-A*0201 PBMCs were incubated with 10 μ M HMPV (A) N-39 or (B) M-39 peptide for 24 hours, IFN γ spots counted, and SFC/10⁶ total PBMC expressed as average SFC/10⁶ human PBMC in triplicate wells corrected by subtracting SFC from irrelevant peptide control wells.

Figure 6. Frequency of immune T_{CD8} in human HLA-A*0201 PBMCs recognizing the HMPV M39 epitope

Human HLA-A*0201 PBMCs were incubated with PE-labeled HMPV M-39 tetramer and fluorescently labeled CD8 antibodies to enumerate HMPV-specific T_{CD8}. Cells were enriched through a gravity MS magnetic column with anti-PE beads and both depleted and enriched fractions were collected. The entire enriched fraction and 10% of the depleted fraction were analyzed via flow cytometry. (A) Frequency of tetramer+ cells per 10⁶ total PBMC, as well as the (B) frequency of tetramer+ cells per 10⁶ total T_{CD8} for each sample.

Table 1

Overlapping and predictope HMPV peptides recognized by IFN γ producing cells in A2tg mice. M and N overlapping peptides are 15-mers overlapping by 9 aa. Algorithmically predicted peptides are 9-mers with high scores in SYFPEITHI, BIMAS, and PREDEP prediction software.

Name	Protein	Position	Sequence
M-37	M	37-51	PLFQANTPPAVLLDQ
M-85		85-99	PKKFEVNATVALDEY
N-13	N	13-27	YKHAILKESQYTIKR
N-39		39-47	SLQQEITLL
N-61		61-75	YAAEIGIQYISTALG
N-217		217-231	KVYYRSLFIEYGKAL

Table 2

Algorithmically predicted HMPV 9-mer peptides recognized by IFN γ producing cells in A2tg mice.

Name	Protein	Position	Sequence	SYFPEITHI	BIMAS	PreDep
M-39	M	39-47	FQANTPPAV	15	32.438	2.61
M-86		86-94	KKFEVNATV	18	5.129	-3.04
N-13	N	13-21	GIQYISTAL	19	2.937	-3.82
N-39		N-39	SLQQEITLL	26	49.134	-3.66
N-217		N-217	KVYYRSLFI	14	56.233	-5.73
N-223		N-223	LFIEY GKAL	17	-	-5.83