SARS-CoV-2 and human retroelements: a case for molecular mimicry?

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Research Article

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Abstract

Background

The factors driving the late phase of COVID-19 are still poorly understood. However, autoimmunity is an evolving theme in COVID-19's pathogenesis. Additionally, deregulation of human retroelements (RE) is found in many viral infections, and has also been reported in COVID-19.

Methods

The relationship of coronaviruses (CoV) to RE was explored by comparing CoV genomes to a comprehensive RE database. Next, the aligning results were matched to epitope signatures from severely vs. mildly affected COVID-19 patients. In addition, transcriptomes from healthy controls, COVID-19 patients as well as lung- and immune cells were investigated for changes in RE expression after SARS-CoV-2 infection.

Results

Unexpectedly, CoV – including SARS-CoV-2 – harbour many RE-identical sequences (up to 35 base pairs), and some of these sequences are part of SARS-CoV-2 epitopes associated to COVID-19 severity. Furthermore, RE are expressed in healthy controls and human cells and become deregulated after SARS-CoV-2 infection, showing mainly changes in long interspersed nuclear element (LINE1) expression, but also in endogenous retroviruses.

Conclusion

CoV and human RE share coding sequences, who are targeted by antibodies in COVID-19 and thus could induce an autoimmune loop by molecular mimicry. To the best of my knowledge these findings have never been reported before and should be explored further in CoV-related diseases.

Background

At the end of 2019, a severe acute respiratory syndrome (SARS)-like disease was noted in eastern China and a novel coronavirus (later designated SARS-CoV-2) recognized as the factor for the disease, COVID-19[1]. By the autumn of 2021, 245 million people have been infected globally, with 5 million casualties[2]. COVID-19 can be divided into an early viral replication phase and a late stage of organ failure[3, 4]. While the inhibition of SARS-CoV-2 replication has already been achieved[5–10], the factors driving the late phase of the disease are poorly understood[11, 12]. However, it has been reported that autoimmunity[13–27] and deregulation of human retroelements (RE) might contribute to the outcome of COVID-19 patients[28–31].

The RE share a reverse transcriptase as a common denominator. Together with an endonuclease, they can move by “copy and paste.” Based on the presence of an envelope gene, they can be divided into long terminal repeat (LTR) positive and LTR negative retrotransposons. The latter and endogenous retroviruses (ERV) belong to LTR positive elements, long interspersed nuclear elements (LINE), short interspersed nuclear elements (SINE) and SVA elements (SINE-R, VNTR and Alu) belong to LTR negative elements[32–35]. The LINE contain at least two open reading frames (ORFs), ORF1, coding for a nucleic acid binding protein with chaperone activity (ORF1p) and ORF2, which codes for a reverse transcriptase/endonuclease (ORF2p)[35, 36]. Importantly, RE make up 50 – 70 % of the human genome[37, 38]. About 20 % of the genome is made up from LINE sequences (c. 500,000 copies), of which more than 100 LINE1 family members are still intact and about 68 active in humans. The LINE1 show strong interpersonal differences[39, 40] and an age-dependent expression pattern[41–43]. By comparison, ERV make up about 8 % of the human genome. Despite – similar to LINE – predominant inactivation, there are still hundreds of intact viral promoters and open reading frames from which the expression of ERV transcripts and proteins is possible[44–46]. The RE activation is known from many viral infections, such as HIV[47], dengue[48], influenza A[48], Zika virus[48], West Nile virus[48], measles[48], Epstein-Barr[49] virus and cytomegalovirus[50]. Therefore, I looked for the relationship of coronaviruses (CoV) to human RE based on genome, transcriptome, epitope and peptide array data. Here, transcriptome analysis coincidentally revealed many RE-identical sequences and shared epitopes in the CoV family members investigated, such as SARS-CoV-2, MERS-CoV and HKU1. To the best of my knowledge, these findings have never been reported. Importantly, epitopes are shared between human LINE1- and SARS-CoV-2 proteins and antibodies against some of these epitopes have been found to be correlated to COVID-19’s severity. In addition, RE are expressed in healthy controls and deregulated in COVID-19 patients, as well as in SARS-CoV-2-infected human cells.

Methods

Genome analysis

Genome sequences from SARS-CoV-2 (isolate NC045512.2 = Wuhan-Hu-1), SARS-CoV-1 (AY291315.1 = FFM1), MERS-CoV (NC_019843.3 = EMC2012), human pathogenic CoVs (NC-006577.2 = HKU1; AY391777.1 = OC43, NC-002645.1 = 229E; NC-005831.2 = NL63) and bat CoVs (MN996532.2 = RaTG13, KC881005.1 = RsSHC014; MG916904.1 = Ra1359) were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). Retro.hg38.v1 (https://github.com/mlbendall/telescope_annotation_db/tree/master/builds) was employed as an RE database. The database contains 28,513 RE and is made of “RepeatMasker” hits for 60 HERV families (RepeatMasker Open-4.0, <http://www.repeatmasker.org/>) and all LINE elements from “L1base v2” (<https://l1base.charite.de/>)[51]. Alignment of the retro.hg38.v1 database to CoV genomes was done by the genome sequence aligner “nucmer”[52] (4.0.0beta2) on galaxy.org[53] and a local installation of “LAST” (v1250), a programme for genome scale sequence comparison[54]. The minimum sequence length cut-off (with 100 % sequence identity) was stepwise chosen at 12, 15, 18, 21, 24, and ³ 27, based on an immuno-relevant epitope size of about 4 – 12 amino acids (aa) (many epitopes are less than 8 aa,

about 25 % £ 6 aa, but only a few at 4 aa[55]). The nucmer “-b” and “-L” variables were used accordingly, and “Show-Coords” as well as “Mummerplot” from the “MUMmer 4” package[52] employed to extract and plot data. Regarding “LAST,” firstly, an RE database was built (“lastdb -uNEAR -c RE_db retro.hg38.v1.fa”) and then CoV genomes were compared to the RE database (“lastal -D100 RE_db CoV_genome.fa > RE_db_CoV.maf”).

Epitope-specific antibody data in COVID-19 patients

The SARS-CoV-2 epitope-specific antibody data (IgG) in severely vs. mildly affected COVID-19 patients are from Schwarz et al.[56] “Peptide microarray data – severe vs. mild – IgG,” with the peptides: 1060 (NSP12, QTVKPGNFKDFYDF, LogFC 5,3, p-value 2,4E-04, FDR-adj. p-value 2,8E-02), 1243 (NSP16, ENDSKEGFFTYICGF, LogFC 2,2, p-value 4,0E-02, FDR-adj. p-value 5,2E-01), 1227 (NSP13, IPARARVECFDKFKV, LogFC -0,9, p-value 3,2E-01, FDR-adj. p-value 5,3E-01) and 1690 (Spike, AQVKQIYKTPPIKDF, LogFC 0,2, p-value 8,3E-01, FDR-adj. p-value 8,5E-01). “L1base v2” was used for comparison with coding LINE1 sequences (<https://l1base.charite.de/>)[51]. Known SARS-CoV-2 B- and T-cell epitopes are from Phan et al.[57] and Griffoni et al.[58]. The PDB data for the SARS-CoV-2 RdRp (PDB ID: 7BW4), helicase (PDB ID: 7NNG), 2'-O-ribose methyltransferase (PDB ID: 7JYY) and -spike protein (PDB ID: 7LSS) were downloaded from <https://www.rcsb.org> and epitopes displayed by “UCSF Chimera v1.15” (for Mac OS).[59]

Transcriptome analysis

Total RNA sequencing data from SARS-CoV-2-infected macrophages (BioProject ID PRJNA637580, Sequence Read Archive (SRA) ID mock: SRR11934391, SRR11934392, SRR11934393, infected: SRR11934394, SRR11934395, SRR11934396)[60], Calu-3 adenocarcinomic lung epithelial cells (PRJNA615032, mock: SRR11517744, SRR11517745, SRR11517746, infected: SRR11517747, SRR11517748, SRR11517749)[61] and bronchoalveolar lavage (BALF) samples from intensive care COVID-19 patients (PRJNA605983SRA, SRA: SRR11092056, SRR11092057, SRR11092058, SRR11092059, SRR11092060, SRR11092061, SRR11092062, SRR11092063, SRR11092064)[62] compared to healthy controls (PRJNA316136, SRA: SRR3286988, SRR3286989, SRR3286990, SRR3286991, SRR5515942, SRR5515943, SRR5515944)[63] were downloaded from SRA (<https://www.ncbi.nlm.nih.gov/sra>), quality controlled by FastQC (Babraham Institute, Cambridge, UK, <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and Illumina adapters trimmed by Trimmomatic[64]. Salmon[65] and DESeq2[66] were employed for differential RE analysis, with standard parameters after indexing the retro.hg38.v1 database (“salmon index -t retro.hg38.v1.fa -i retro.hg38.v1_index -k 31”). Heatmaps were done by iDEP v0.92[67] and graphs by GraphPad Prism software version 8.0 for OS X (GraphPad Software Inc., USA).

Results

The CoV genomes harbour a large number of RE-identical sequences. Several of these sequences represent shared RE-SARS-CoV-2 epitopes. Importantly, antibodies against some of these epitopes are

correlated to the severity of COVID-19. In addition, RE are widely expressed in healthy controls and deregulated in COVID-19 patients, as well as in SARS-CoV-2-infected human cells.

Sequence identity between retroelements and coronaviruses

A sequence identity (≥ 12 bp, range 12 – 35 bp, **Fig. 1A**) of human RE sequences to CoV genomes from SARS-CoV-2, SARS-CoV-1, MERS-CoV, NL63, 229E, OC43, HKU1, bat CoV RA13591, bat CoV RATG13 and bat CoV RSSHC014 was found by sequence alignment of human RE sequences and different CoV genomes (**Fig. 1 and 2, Table 1**). Very high counts of RE-identical sequences in CoV were seen at ≥ 12 , ≥ 15 and ≥ 18 bp (**Table 1**).

Table 1. Number of retroelement-identical sequences in CoV genomes dependent on sequence length (12 – 27 bp, based on 100 % sequence identity (alignment by nucmer). Underlines indicate the highest score at the respective cut-off.

CoV	Genbank ID	RE-identical sequences (nucmer)					
		³ 12 bp	³ 15 bp	³ 18 bp	³ 21 bp	³ 24 bp	³ 27 bp
229E	NC_002645.1	16992	4663	155	8	5	<u>4</u>
NL63	NC_005831.2	17751	5758	206	4	0	0
OC43	AY391777.1	<u>19428</u>	5709	162	3	0	0
HKU1	NC_006577.2	19112	<u>6843</u>	<u>332</u>	<u>13</u>	0	0
MERS-CoV	NC_019843.3	18435	4252	106	3	1	0
SARS-CoV-1	AY291315.1	18446	4731	122	2	1	0
SARS-CoV-2	NC_045512.2	18917	5358	191	11	<u>3</u>	2
RsSHC014	KC881005.1	18425	4651	114	5	1	0
Ra13591	MG916904.1	17971	4644	142	2	0	0
RaTG13	MN996532.2	18950	5327	167	4	0	0

A cut-off ³ 18 bp (correlating to potential epitopes of at least 6 aa) was chosen for downstream analysis for sensitivity and epitope size reasons. A 6 aa cut-off corresponds well to a known immuno-relevant linear epitope length of 4 – 12 aa, as about 50 % of them have a length ≥ 8 aa (about 25 % ≥ 6 aa, and only a few of 4 aa)[55]. At this cut-off point, the majority of RE-identical sequences are seen in HKU1 (332), followed by NL63 (206) and SARS-CoV-2 (191) (**Fig. 2A and 2B, Table 1**). SARS-CoV-2 and RE sequence data were further explored by “LAST” in order to allow single nucleotide polymorphisms to be included, thereby alignments to RE sequences up to 35 bp were seen (**Supplementary Table 2**). In the RE-CoV data, LINE1 represent the majority of all shared sequences, while alignment to ERV sequences is a

relevant minority and includes the 35 bp hits (**Fig. 1B, Supplementary Tables 1 and 2**). In conclusion, genome analysis revealed the presence of many short RE-identical sequences in CoV genomes, including SARS-CoV-2.

Shared epitopes between SARS-CoV-2- and retroelement proteins

Subsequently, all RE-identical sequences ³ 18 bp were compared to the coding regions of the genome of SARS-CoV-2. Accordingly, 70 sequences showing identical aa sequences in CoV and RE were identified (**Supplementary Table 1**). These sequences were then compared to results from a peptide array, which investigated epitope signatures in COVID-19 patients (severe vs. mild)[56]. An overlap of human LINE1 proteins to SARS-CoV-2 epitopes from the RNA-dependent RNA polymerase (RdRp), helicase and 2'-O-ribose methyltransferase was detected for epitopes targeted with > 2-fold elevated antibody levels in severe cases (**Fig. 3**). Importantly, antibodies targeting an epitope of the SARS-CoV-2 RdRp polymerase, which is identical to an epitope of the LINE1 ORF2p endonuclease domain, were 39-fold elevated in severely compared to only mildly affected COVID-19 patients (**Fig. 3A**). The same is seen with antibodies targeting the shared CoV-RE epitopes from the 2'-O-ribose methyltransferase (**Fig. 3C**) and helicase (**Fig. 3D**). The latter is also a known B cell epitope, aa "PARARVECFDKEKV" (B cell epitope underlined)[57]. Many other shared RE-CoV peptides (similar to those depicted in **Fig. 3B**) were not targeted by antibodies in severe vs. mild COVID-19 (**Supplementary Table 2**), but some are known as T cell epitopes, such as the one present in all three chains of the spike protein shown in **Fig. 3B** (aa VKQIYKTPPIKDF, known T cell epitope sequence underlined)[58].

Taken together, SARS-CoV-2 and RE share peptide sequences, of which some are epitopes correlated to COVID19 severity.

Transcriptome analysis of retroelements in SARS-CoV-2-infected cells

An RE analysis of COVID-19 patient data (bronchoalveolar lavage fluid, BALF), SARS-CoV-2 infected lung epithelial cells and SARS-CoV-2 infected macrophages was performed to explore the presence of and changes in RE expression after SARS-CoV-2 infection. Infection resulted in a highly significant (adjusted p-value ≤ 0.05) and relevant (fold change ≥ 2) deregulation of human RE in all samples. Transcriptome data from COVID-19 patients' BALF compared to healthy controls shows an upregulation of 2035 and downregulation of 3144 RE (**Fig. 4A**). Among the top deregulated RE are mainly LINE1 (**Fig. 4D**). SARS-CoV-2-infected epithelial lung cells (Calu-3) show 34 up- and 29 downregulated RE (**Fig. 4E**), while infected human macrophages have 8 up- and 24 downregulated RE. Among the top de-regulated RE for both are also mainly LINE1 (**Fig. 4E, 4F**).

In conclusion, RE are expressed in COVID-19 patients and human cells and become deregulated after SARS-CoV-2 infection, showing mainly changes in LINE1 expression.

Discussion

The factors driving the late phase of COVID-19 are still not fully understood[11, 12]. However, there is evidence that autoantibodies and autoreactive lymphocytes could contribute to the disease's final outcome[13–27]. Therefore, the question of autoantibody formation in COVID19 has to be asked. The employment of a comprehensive RE database revealed many RE-identical sequences in ten CoV family members investigated, such as in SARS-CoV-2, MERS-CoV and HKU1 (Fig. 1 and 2). Crucially, it was found that the LINE1 proteins ORF1p and ORF2p have peptides identical to SARS-CoV-2 epitopes (Fig. 3), and that some of these epitopes are associated with COVID-19's severity, as shown by correlation to COVID-19 patients' antibody titres (Fig. 3). In addition, RE are deregulated in COVID-19 patients (Fig. 4A), as well as SARS-CoV-2-infected human epithelial lung cells and macrophages (Fig. 4B and C), which has occasionally been reported in the last few months for cell lines and patients[28–31]. Among the analysed REs, LINE1 are strongly represented in all results (Fig. 2 – 4, **Supplementary Table 1 and 2**). The LINE1 code for at least a nucleic acid binding protein with chaperone activity (ORF1p) and a reverse transcriptase/endonuclease (ORF2p). Importantly, autoantibodies targeting the LINE1 ORF2p endonuclease domain have been reported in 41 % of SARS-CoV-1 ptients[68]. The RE are also targeted by autoantibodies in several connective tissue diseases, for example, antibodies against LINE1's ORF1p or ERV HERV-K's envelope protein have been described in patients with systemic lupus erythematosus, lupus nephritis, rheumatoid arthritis, Sjogren's syndrome and mixed connective tissue disease[69–78]. Relating to SARS, the autoantibodies' target, LINE1 ORF2p, was prominently stained post-mortem in lung macrophages (residing in blood vessels), leading the authors to suspect a build-up of autoreactive CD4+ Th cells and, thus, an autoimmune loop in SARS[68]. Importantly, there is also increasing evidence for an autoimmune pathogenesis in severe COVID-19[13–27, 79, 80]. One explanation for autoantibody formation is by molecular mimicry, i.e. shared epitopes between pathogens and hosts[81–85]. The evolution of mimicry epitopes in pathogens could be based on chance. However, although the RE-identical sequences in CoV observed are short (12 – 35 bp), the sequence lengths observed make formation by chance highly unlikely. Exemplarily, taking the genetic code (A, T, C, G) raised to a sequence of 18 bp (4^{18}) results in 68,719,476,736 possible bp combinations, thus, the chance of getting one identical sequence is 1:69 billion. Additionally, a myriad of 12 bp events (Table 1) occurring by chance is stochastically very unlikely ($4^{12} = 16,777,216$) at more than 18,000 events. Moreover, an observed 35 bp hit such as ERVL_Xq21.31b (4^{35}) corresponds to 1.18 E21 possible bp combinations, thus, the chance of getting an identical sequence is 1:1.1 trilliard – without accounting for all the other matching sequences. Therefore, recombination activities more probably account for the phenomena observed. The exchange of genetic material by recombination in RNA viruses is generally associated with virulence, host range and host response[86]. It is known that recombination in CoV can take place during co-infections at a high frequency by homologous and non-homologous recombination[87–89]. Mechanistically, an explanation could be the switching of the RdRp between multiple available RNA strands during replication[90]. This could have happened in a CoV host/ancestor with relevant LINE1 expression, as this is possible in some bat species. The black-bearded tomb bat (*Taphozous melanopogon*), for example, harbours two active LINE families[91] and shows relevant SARS-CoV-2 infection efficiency[92]. Moreover, lots of ERV families also reside in bats[93]. Therefore, serial acquisition of RE sequences, possibly taken from CoV in host animals (starting many million years ago) is a feasible scenario. Relating to the rather

short sequence lengths observed, there might be an evolutionary functional constraint working against the uptake of longer RE sequences, but a benefit for the virus by coating itself with host self-antigens (“self-peptide coat”). This would dampen the innate and adaptive immune response by the presentation of “viral but self-like” peptides. The consequence of this hypothesis is in line with the view of autoimmune disease as a breakdown of self-tolerance[94, 95]. Based on the findings, autoantibodies targeting human RE could be a factor in CoV-induced disease, like COVID-19. However, this report has limitations, as the data basis for a more extensive analysis of anti-RE autoantibodies in COVID19 still does not exist.

Conclusion

In conclusion, it was found that CoV – including SARS-CoV-2 – harbour many RE-identical sequences, and that some of these sequences are part of SARS-CoV-2 epitopes associated with COVID-19 severity. To the best of my knowledge, these findings have never been reported, they deserve future research.

Abbreviations

229E

Human coronavirus 229E

aa

Amino acids

BALF

Bronchoalveolar lavage

bp

Base pairs

CoV

Coronaviruses

COVID-19

Coronavirus disease 2019

ERV

Endogenous retroviruses

HKU1

Human coronavirus HKU1

LINE1

Long interspersed nuclear elements

LTR

Long terminal repeat

MERS-CoV

Middle East respiratory syndrome-related coronavirus

NL63

Human coronavirus NL63

OC43

Human coronavirus OC43

ORF

Open reading frame

Ra1359

Bat coronavirus Ra1359

RaTG13

Bat coronavirus RaTG13

RdRp

RNA-dependent RNA polymerase

RE

Human retroelements

RsSHC014

Bat coronavirus RsSHC014

SARS-CoV-1

Severe acute respiratory syndrome coronavirus type 1

SARS-CoV-2

Severe acute respiratory syndrome coronavirus type 2

SINE

Short interspersed nuclear elements

SRA

Sequence read archive

SVA

SINE-R, VNTR and Alu

Declarations

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Authors' contributions

Conceptualization, investigation, formal analysis, writing: BK.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author declares no competing interests.

Availability of data and materials

All data generated in this study is included in this published article and its supplementary files.

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Figures

Figure 1

Sequence alignments of retroelements to CoV genomes by LAST. **A.** Length distribution of alignment results by LAST. **B.** Longest aligning RE-CoV sequences (LAST).

Figure 2

Sequence alignments of CoV genomes to retroelements by nucmer (cut-off ≥ 18 bp). **A.** Proportion of LINE1 (L1) and endogenous retrovirus sequences, showing a dominance of L1 sequences in all virus genomes (nucmer) analysed. **B.** Dot plot of shared RE sequences in CoV genomes, showing the highest RE-identical sequences in HKU1, followed by NL63 and SARS-CoV-2 (nucmer). Each dot represents an ≥ 18 bp retroelement sequence also found in the respective CoV genome.

Figure 3

A. Mapping of the shared RE-CoV epitope “FNKDFY” to the SARS-CoV-2 RdRp (epitope in red), orange box depicting IgG antibody levels of severe vs. mild COVID-19 disease, with anti-FNKDFY antibodies showing 39-fold elevation in severe COVID-19. **B.** Mapping of the shared RE- CoV epitope “VKQIYK” to the SARS-CoV-2 spike protein (epitope in red), no reported significantly elevated anti-epitope antibodies in severe COVID-19. **C.** Mapping of the shared RE-CoV epitope “TYICGF” to the SARS-CoV-2 2'-O-ribose methyltransferase (epitope in red), orange box depicting reported antibody levels of severe vs. mild COVID-19 disease, with anti-TYICGF antibodies showing 4.6-fold elevation in severe COVID-19. **D.** Mapping of the shared RE-CoV epitope “ECFDKFKV” to the SARS-CoV-2 helicase (epitope in red), orange box depicting reported antibody levels of severe vs. mild COVID-19 disease, with anti-ECFDKFKV antibodies showing 2-fold elevation in severe COVID-19 **E.** Structure of a human LINE1 element with proteins ORF1p (orange) and ORF2p (green).

Figure 4

A. Heatmap of the most highly deregulated retroelements in bronchoalveolar lavage fluid (BALF) from COVID19 patients (red = upregulated, blue = downregulated). **B.** Heatmap of the most highly deregulated retroelements in SARS-CoV-2-infected epithelial lung cells (Calu-3). **C.** Heatmap of the most highly deregulated retroelements in SARS-CoV-2-infected macrophages.

D. Top 10 up- and downregulated retroelements in COVID19 BALF. **E.** Top 10 up- and downregulated retroelements in SARS-CoV-2-infected epithelial lung cells. **F.** Top 10 up- and downregulated retroelements in SARS-CoV-2-infected macrophages.

Supplementary Files

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- [Supplementarytable1nucmer.xlsx](#)
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