Algemene gegevens / General Information

Programma / Programme	:	**COVID-19 Programma**
Subsidieronde / Subsidy round	:	**Bottom-up ronde COVID-19 aandachtsgebied 1**
Projecttitel / Project title	:	**Boosting mucosal defense against SARS-CoV-2 using HDAC inhibitors**
Projecttaal / Project language	:	**Engels / English**
Geplande startdatum / Planned start date	:	**01-07-2020**
Geplande duur / Planned duration	:	**24 maanden / months**
Datum indienen / Date of application	:	**13-05-2020**
Projecttype / Project type	:	**Fundamenteel onderzoek**
Vervolg eerder ZonMw-project / Continuation previously funded project ZonMw	:	**Nee / No**

Projectleden / Project members

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Projectgegevens / Project information

Aandachtsgebieden / Focus

1.1 Thema's aandachtsgebied 1
- Virus, immuniteit, immuunrespons en pathogenese

1.3 Setting
- Anders

Samenvatting / Summary

The nasal mucosa is the site of first contact between SARS-CoV-2 coronavirus and the host and a key reservoir for viral replication and transmission. For successful infection, SARS-CoV-2 uses its spike (S)-protein to engage the epithelial ACE2 receptor induce uptake. However, the respiratory epithelium has an elaborate mucosal defense system that consists of secreted gel-forming mucins produced by mucosal glands and epithelial transmembrane mucins such as MUC1. Together, mucins can form a barrier that prevents pathogen invasion. Our preliminary data show that in the human respiratory mucosa, many ACE2-positive cells also express the large glycosylated transmembrane mucin MUC1. We previously published that SARS-CoV-1 specifically targets serous and mucosal glands in the respiratory epithelium). Cellular targeting of SARS-CoV-2 remains to be established. Boosting expression of defensive mucins or altering mucin glycosylation could prevent SARS-CoV-2 infection and improve infection outcome.

Histone deacetylases (HDACs) Trichostatin A (TSA), Suberoylanilide Hydroxamic Acid (SAHA) and Valproic Acid (VPA) have been shown to upregulate mucin gene expression and have been applied to increase mucosal defenses against viral infections. We hypothesize that HDAC inhibitors could be used as new therapeutic drugs to boost mucosal responses and prevent SARS-CoV-2 infection or improve disease outcome. The HDAC inhibitors TSA, SAHA and VPA are already approved anti-inflammatory, anti-cancer, and anti-epileptic drugs. If our preclinical studies support their role in enhancing mucosal defense during COVID-19, these compounds could be rapidly applied in the clinic.

The goals of this project are to:
-Identify the protective components in respiratory mucus during SARS-CoV-2 infection.
-Preclinical testing of HDAC inhibitors to enhance mucosal defense during SARS-CoV-2 infection.

Project deliverables:
-A putative novel class of therapeutics to boost respiratory defenses against SARS-CoV-2.

Trefwoorden / Keywords

SARS-CoV-2, mucosal defense, mucins, MUC1, HDAC, HDAC inhibitors, novel therapeutics

Samenwerking / Collaboration

Samenwerking tussen onderzoek en praktijk / Cooperation between research and practice:

Nee / No

Inhoud / Content

Disciplines / Disciplines

- Infecties, parasitologie, virologie / Infections, parasitology, virology
- Immunologie, serologie / Immunology, serology

Financiële gegevens / Financial data

ZonMw budget

	Jaar / Year								
Kostenpost	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	**Totaal / Total**
Personeel	(10)(1c)								
Materieel									
Implementatie									
Apparatuur									
Overig									
Totaal / Total									

Co-financiering / Cofinancing

Naam co-financier / Name of cofinancier	Bedrag / Amount	Status

Bijzondere gegevens / Additional information

Vergunningen / Permits

	Verklaring nodig / Statement required?		Status verklaring / Statement status		
	Ja / Yes	Nee / No	Verkregen / Acquired	Aangevraagd / Applied	Nog niet aangevraagd / Not applied yet
METC	X		X		
DEC	X		X		
WBO		X			

Onderschrijvingen / Assents

	Ja / Yes	Nee / No	N.v.t. / N.A.
Code biosecurity / Code Biosecurity	X		
Code openheid dierproeven / Code Transparency of Animal Testing	X		

Andere vergunningen / Other permits

AANVRAAGFORMULIER PROJECTIDEE – BOTTOM-UP RONDE

COVID 19 programma

Deadline voor indiening: 14 mei 2020 (14:00 u)

LEES ALSTUBLIEFT ALLE INSTRUCTIES IN BIJLAGE "TOELICHTING INDIENING PROJECTIDEE" VAN DE OPROEPTEKST ZORGVULDIG!

Wanneer u het formulier heeft ingevuld:

1. **Zet het formulier om naar een PDF file en controleer de details**
2. **Upload het complete formulier als een bijlage bij uw indiening in Projectnet (Let op: dit zijn twee verschillende links, gebruik maar 1 van de 2!)**

ProjectNet: Aandachtsgebied 1 (voorspellende diagnostiek en behandeling)
ProjectNet: Aandachtsgebied 2 (zorg en preventie)

BASISGEGEVENS (voorpagina)

NAAM VAN DE HOOFDAANVRAGER:

Dr. (10)(2e)

ORGANISATIE:

Department of Biomolecular Health Sciences
Division Infectious Diseases and Immunology
Faculty of Veterinary Medicine
Utrecht University

PROJECTTITEL:

Boosting mucosal defense against SARS-CoV-2 using HDAC inhibitors

DATASTEWARD:

Wie is de datasteward die de open science en FAIR data planning in uw project ondersteunt? Zie de webinars op de ZonMw website om de datastewards te informeren en ondersteunen.

☐ Ik betrek een datasteward bij mijn project:
Naam: Klik of tik om tekst in te voeren.
Instituut: Klik of tik om tekst in te voeren.
E-mail: Klik of tik om tekst in te voeren.
Was aanwezig bij de webinar: ☐ Ja ☐ Nee

☒ Ik heb nog geen datasteward.

ONDERZOEKSVOORSTEL max 3 pagina's A4 (inclusief literatuurreferenties)	(voorpagina met basisgegevens niet meegerekend - font type Arial 10 pts)

1. PROBLEEMSTELLING EN DOELSTELLING(EN):

The nasal mucosa is the site of first contact between SARS-CoV-2 coronavirus and the host and a key reservoir for viral replication and transmission. For successful infection, SARS-CoV-2 uses its spike (S)-protein to engage the epithelial ACE2 receptor and associated TMPRSS2 protease to induce uptake (Hoffmann et al., 2020). However, the respiratory epithelium has an elaborate mucosal defense system that consists of secreted gel-forming mucins (MUC5AC, MUC5B) produced by mucosal glands and epithelial transmembrane mucins (MUC1, MUC4, and MUC16). The glycans of gel-forming mucins bind microbes whereas transmembrane mucins sense the external environment, act as a releasable decoy, block the binding of pathogens to the underlying receptors and activate intracellular signal transduction pathways (Van Putten and Strijbis, 2017, Thornton et al., 2008). Together, mucins can form a barrier that prevents pathogen invasion (Rogers, 2009, Denneny et al., 2020). In the human respiratory mucosa, many ACE2-positive cells also express the large glycosylated transmembrane mucin MUC1 (Fig. 1A, B). We previously published that SARS-CoV-1 specifically targets serous and mucosal glands in the respiratory epithelium (Fig. 1C). Cellular targeting of SARS-CoV-2 and the role of MUC1 remain to be established. Boosting expression of defensive mucins or altering mucin glycosylation could prevent SARS-CoV-2 infection and improve infection outcome.

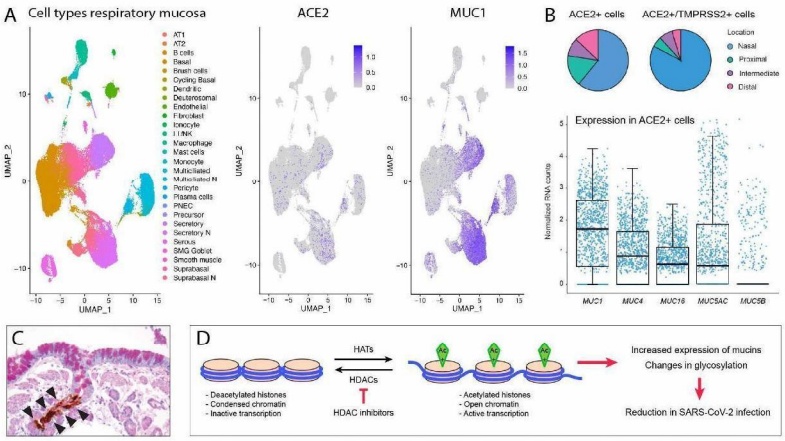

Figure 1. A: Single cell sequencing analysis of respiratory mucosa samples from nasal cavity, proximal and distal sites in the lungs. Reanalyzed dataset from Deprez et al., bioRxiv, 2019. Left: clustering of cell types. Middle and right: cells expressing ACE2 or MUC1. B: The majority of ACE2+ and ACE2+/TMPRSS2+ is located in the nasal cavity and express the transmembrane mucin MUC1. C: PAS and IHC staining of bronchi of SARS-CoV-infected cat. SARS-CoV (black arrows) targets serous and mucosal glands in the respiratory epithelium (Van den Brand et al., 2008). D. Hypothesis of effects of HDAC inhibitors on histone acetylation, induction of mucin expression and reduction of SARS-CoV-2 infection.

Histone deacetylases (HDACs) are a family of enzymes that induce a "closed" chromatin state and downregulate gene expression. HDAC inhibitors can therefore be used to stimulate gene expression (Lin et al., 2015). Inhibitors such as Trichostatin A (TSA), Suberoylanilide Hydroxamic Acid (SAHA) and Valproic Acid (VPA) have been shown to upregulate mucin gene expression (Saco et al., 2018, Kageyama-Yahara et al., 2014, Méndez et al., 2019) and have been applied to increase mucosal defenses against viral infections. TSA protects mice against post-influenza pneumonia (Yagi et al., 2016) and was able to attenuate airway infection in the Chronic Allergic Airways Disease model (Royce et al., 2012). Treatment with TSA/SAHA can significantly reduce respiratory syncytial virus replication (Feng et al., 2016). We hypothesize that HDAC inhibitors could be used as new therapeutic drugs to boost mucosal responses and prevent SARS-CoV-2 infection or improve disease outcome (Fig. 3D). The HDAC inhibitors TSA, SAHA and VPA are already approved anti-inflammatory, anti-cancer, and anti-epileptic drugs. If our preclinical studies support their role in enhancing mucosal defense during COVID-19, these compounds could be rapidly applied in the clinic.

The goals of this project are to:

- Identify the protective components in respiratory mucus during SARS-CoV-2 infection.
- Preclinical testing of HDAC inhibitors to enhance mucosal defense during SARS-CoV-2 infection.

2. PLAN VAN AANPAK:

The project consists of 4 work packages (WP1-4).

Identify cell types in the human nasal and lung mucosa susceptible to SARS-CoV-2 (WP1).
Using an interdisciplinary approach, we will determine which cell types in the nasal and lung mucosa are susceptible to SARS-CoV-2 infection and correlate this to the mucin repertoire and mucin glycosylation.

A. Determine ACE2 expression and the mucin repertoire of epithelial cells, serous glands and mucosal glands. We will combine bioinformatics analysis of a single cell database of respiratory tissue, histology of healthy and virus-infected patient samples and specific antibodies for soluble mucins MUC5AC and MUC5B and transmembrane mucins MUC1, MUC4 and MUC16.
B. Characterize respiratory mucin glycosylation using bioinformatics, lectin arrays, histology and mucin-specific antibodies.
C. Quantify infection of epithelial cells, serous glands and mucosal glands in human nasal and lung biopsies during SARS-CoV-2 infection. Histology with PAS and immunohistochemistry will be performed on patient nasal and lung samples.

Uncover protective components in soluble mucus during SARS-CoV-2 infection (WP2).
Our unique expertise with mucus and mucin proteins allows us to isolate mucus and identify which specific mucins or sugar groups in the mucus are responsible for SARS-CoV-2 binding and prevention of virus entry into respiratory epithelial cells. Many viruses interact with mucins via sialic acid that are the terminal sugars on the mucin O-linked glycans. We will make use of a VSV-based pseudovirus with SARS-CoV-2 spike protein on the surface that expresses luciferase for quantification.

A. Isolate mucus from human lung tissue (available through our collaborators at UMC Utrecht).
B. Treat mucus with specific glycosidases and N-acetyl cysteine (NAC). We will use sialidases and fucosidase to remove the terminal sialic acids and fucose moieties of mucins and NAC, a mucolytic compound that reacts with disulfide groups and decreases mucus viscosity.
C. Virus-mucus interaction experiments with treated and non-treated mucus.
D. Perform infection assays with pseudotype virus in human Calu-3 lung cells with and without mucus.
E. Determine viral entry in nasal or lung organoids and explants after treatment with glycosidases or NAC.

Elucidate the role of epithelial transmembrane mucins during viral entry (WP3).
Large glycosylated transmembrane (TM) mucins are expressed on the apical surface of the respiratory epithelium. We need to determine if specific TM mucins and their glycans form a barrier that prevents viral entry or if they serve as a docking sites that facilitate viral entry.

A. Knockout TM mucins MUC1, MUC4 and MUC16 in human Calu-3 cells using CRISPR/Cas9. We are routinely doing CRISPR/Cas9 on mucin genes in different epithelial cell types.
B. Determine infection of SARS-CoV-2 pseudotype virus in wild type and MUC1, MUC4 and MUC16 knockout cells. Does the removal of TM mucins increase or decrease infection?
C. Overexpress different TM mucins constructs that lack the extracellular domain or cytoplasmic tail (MUC1-FL, MUC1-dTR or MUC1-dCT). Determine which domain is responsible for barrier and/or receptor functions during SARS-CoV-2 infection.

Apply HDAC inhibitors to boost mucus production and protect against SARS-CoV-2 infection (WP4).
HDAC enzymes regulate the chromatin structure and generally suppress gene expression. HDAC inhibitors have been shown to increase mucus production and can be protective during infection with respiratory viruses. In this work package, we will perform preclinical testing to determine the efficacy of HDAC inhibitors in boosting mucus production and improving outcome of SARS-CoV-2 infection.

A. Determine the effects of HDAC inhibitors TSA, VPA and SAHA on expression of soluble and TM mucins and mucin glycosylation in Calu-3 cells by qRT-PCR for MUC genes and specific MUC antibodies.
B. Investigate the effects of HDAC inhibitors on infection with SARS-CoV-2 pseudotype virus in Calu-3 cells and explants.
C. Perform animal experiments to investigate effects of HDAC inhibitors on SARS-CoV-2 infection. Hamsters are a suitable model to study SARS-CoV-2 infection (Roberts et al., 2005 and personal communication with Viroclinics). Experiments will be performed at Viroclinics Biosciences. TSA, VPA and SAHA will be administered through IP injection and hamsters will be infected with SARS-CoV-2 according established protocols. Nasal and lung tissues will be collected at day 2 and 4 post-infection for viral quantification, histology analysis with PAS staining for serous and mucosal glands, IHC for MUC1, MUC4 and MUC16 and MUC5AC expression and lectin arrays to determine mucin glycosylation.

Together, these 4 workpackages will establish which components in the respiratory mucosa are protective during SARS-CoV-2 infection and will investigate if HDAC inhibitors are promising drugs to boost mucosal defense against the virus. We envision that HDAC inhibitors could be further developed as prophylactic drugs or applied in early stages of infection to reduce symptoms and prevent spread of the virus from the nasal cavity to the lungs.

Budget
The total budget requested for this project is 335.000. This includes the salary of one postdoc for 2 years, explant, organoid and animal experiments, histology, bioinformatics analysis and bench fee.

3. HAALBAARHEID VAN HET PROJECT:

Start of the project: July 1st, 2020. Total running time: 24 months.

Project GANTT chart	July 2020		January 2021		July 2021		January 2022	
Project months:	0-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24
Work package 1. Infected cell types	1A, 1B	1C						
Work package 2. Soluble mucus		2A	2B, 2C	2D, 2E				
Work package 3. TM mucins	3A		3B		3C			
Work package 4. HDAC inhibitors		4A, 4B			4C			

Feasibility - This project will be conducted by a highly qualified research group consisting of skilled infection biologists, virologists and pathologists of the Department of Biomolecular Health Sciences of the Faculty of Veterinary Medicine at Utrecht University in close collaboration with the clinical groups at UMC Utrecht and the company Viroclinics Biosciences in Rotterdam. A postdoc who is highly skilled in mucus techniques and infection assays is currently working in the Infection Biology group. She will be available for this project starting July 1st, 2020. The experiments proposed in this project are already ongoing for other pathogens as part of an ERC starting grant and a One Health project, therefore all the tools and techniques are up and running. The application of HDAC inhibitors to battle COVID-19 is innovative and high-risk. However, these approved drugs could be swiftly included in clinical studies if our preclinical studies demonstrate potential benefits of HDAC inhibitors during SARS-CoV-2 infection. HDAC inhibition might have additional effects in the respiratory mucosa. Further analysis of single cell transcriptomic data will provide new insights into transcriptional regulation of mucins. This will enable us to identify alternative (more specific) druggable pathways to manipulate mucin expression and reduce viral infections.

Interdisciplinary project group with unique human and veterinary expertise:

- Mucologists Dr. Maitrayee Chatterjee and Dr. Karin Strijbis (Infection Biology, UU)
- Pathologist and coronavirus expert Dr. Judith van den Brand (Pathology, UU)
- Single cell sequencing experts Dr Bart Westendorp and Prof. Alain de Bruin (Pathobiology, UU)
- Coronavirus expertise: Dr. Berend (10)(2e) (10)(2e) and Prof. (10)(2e) (Virology, UU)
- Human samples available through collaboration with clinical groups (UMC Utrecht)
- Viroclinics Biosciences (Rotterdam)

4. RELEVANTIE VOOR DE PRAKTIJK:

The first preclinical results will emerge in the first year of the project (see GANTT chart). Because HDAC inhibitors are approved drugs, we can fast track these compounds into clinical studies. We will team up with Sigma/Merck and initiate the development of HDAC inhibitors as a spray for human application. We envision that such a spray can be used as a prophylactic treatment, not only to decrease severe illness and improve disease outcome but also to decrease spread of virus. The spray is an easy and rather cheap method and may be used in both high risk and low risk groups. This may have large societal and economic impacts since the spread of the virus may be hampered, less people may become seriously ill with subsequent less hospitalization. If successful against SARS-CoV-2, enhancing mucosal defense of the respiratory system can also prove instrumental during future pandemics with different types of (corona) viruses.

While interactions with the mucus layer are essential during viral pathogenesis, very few groups in the world work on mucosal defense and even fewer have access to coronavirus expertise. Therefore, we have a unique research team, the idea is novel and innovative and to our knowledge this type of project is not being conducted elsewhere.

5. DEELNAME VAN DE STAKEHOLDER(S) (e.g. patiënten, zorgprofessionals, etc.):

SARS-CoV-2 is a zoonotic virus. In this unique project, disciplines from both human and veterinary research are combined, and our expertise at the level of coronaviruses, mucology, pathology and molecular research is highly complementary. Additionally, the collaboration with industry such as pharmacy (Sigma/Merck), preclinical research facilities (Viroclinics Biosciences) and UMC Utrecht for clinical studies provides a broad network will ensure the implementation of this application in a safe and fast way.

6. LITERATUURREFERENTIES (optioneel):

See text.