VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D14.1

Quantity of access provided over the duration of the project to TPI

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Revision: V1

Dissemination level	
Public	**X**
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

TNA Provided

Name of the TNA project	Name of TNA user	Organisation of TNA user	Country of TNA user	Installation from the RI	Start date	End date	Number of units of access provided
Harnessing local immunity for protection against influenza	Matthias Tenbusch	University Hospital Erlangen, Institute of Virology, Germany	DE	ISO10&11	Jan 2022	Apr 2022	28 (+10.900,00 € for histopathology and student accommodation)

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TNA 1: Harnessing local immunity for protection against influenza

1. User-Project Title: Harnessing local immunity for protection against influenza
2. User Name: Matthias Tenbusch
3. User Institute: Universitätsklinikum Erlangen
4. Service Provider Institute: Pirbright/APHA
5. Starting date of the Access: 2.1.22
6. Finishing date of the Access: 3.4.22
7. Biosafety level (BSL) / Containment of the Access: BSL 3
8. Details of users involved in the research project, including users who did not stay at the Service provider:

	User1	User2
Family_Name	Tenbusch	Schmidt
First_Name	Matthias	Anna
Gender (M/F)	M	F
Birth_year	1978	1995
Nationality	German	german
Researcher_status (1)	EXP	PGR
Scientific field 1 (main)	Virology	Virology
Scientific field 2 (optional)	Immunology	Immunology
Scientific field 3 (optional)		
Home_Institution_Type (2)	UNI	UNI
Home_Institution_Name	UK Erlangen	UK Erlangen
Home_Institution_Town	Erlangen	Erlangen
Home_Institution_Country	Germany	Germany
Email (3)	Matthias.tenbusch@fau.de	tri.a.schmidt@fau.de
New_User (Y/N)	Y	Y
Group_leader (Y/N)	Y	N

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Remote_user (Y/N) (4)	Y	N
Number_of_visits (5)	0	1
Duration_of_stay (6)	0	91
Travel_and_Subsistence reimbursed (Y/N) (7)	N	Y
Additional_Information		

(1) UND: Undergraduate; PGR: Postgraduate; PDOC: Post-doctoral researcher; TEC: Technician; EXP: Experienced researcher.
(2) UNI: University; RES: Public research organisations: SME: Small or Medium Enterprise; PRV: Other Industrial and/or profit Private organisation; OTH: Other organisation.
(3) E-mail address of the user
(4) Indicate N (no) for users who stayed at the service provider, and Y (yes) for persons involved in the research project but who did not stay at the service provider.
(5) Number of visits to the service provider in the frame of this user-project. Remote users must write "0".
(6) In days, including week-ends and public holidays.
(7) Remote users must write "N" (no).

9. Reporting of scientific aspects

a. Summary:

The development of a universal influenza vaccine to combat the rapid evolution of influenza viruses is a highly desirable goal. In this study, we addressed whether mucosal vaccination with adenoviral vectors encoding for influenza A Virus (IAV) antigen and the immunomodulatory molecule Interleukin-1beta (IL-1b) can provide protection against non-vaccine related IAV. This immunisation approach induced strong local T-cell responses and demonstrated high vaccine efficacy in mice. In the highly relevant large natural host animal pig model, we could confirm the beneficial effects of IL-1b on the generation of mucosal T-cells, but this did not translate into protection against heterologous IAV infection. Furthermore, a previous infection with H1N1 virus did not provide protection against infection with heterologous H3N2 virus. This sharp contrast between the two animal models is of high scientific importance and supports the notion that vaccine candidates must be validated in more than one animal model and certainly in at least one large animal model. The study also generated crucial information on the prerequisites for mucosal vaccines to induce local T cell resident memory responses. The different inflammatory conditions of a prior H1N1 infection and the IL-1b adjuvanted adenoviral vector immunization resulted in different mucosal T- and B-cell responses which have not been described before in this highly relevant large animal model.

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b. Introduction & Aim of project:

Tissue-resident memory T-cells (TRM) have been shown to be essential for control of influenza viral replication and disease progression in the absence of strain-specific neutralizing antibodies in mice and ferrets. TRM directed against conserved epitopes (e.g. nucleoprotein, NP) can be induced by natural influenza infection or mucosally delivered vaccines. However, local inflammation and the kinetics of antigen-presentation, both essential for TRM priming, will differ greatly between these. In this study, we compared qualitatively and quantitatively TRM induced either by prior pH1N1 infection or pulmonary immunisation with of Ad-HA/NP and Ad-IL-1ß.

Our hypothesis was that pre-exposure with pH1N1 virus or pulmonary immunisation with Ad-HA/NP and Ad-IL-1ß generate lung TRM which are capable of mediating heterosubtypic protection against H3N2 virus and that IL-1β potentiates the induction of TRM.

We used immunisation with Ad-HA/NP and influenza infection, to address the following questions:

Objective 1. Are the lung TRM responsible for heterosubtypic protection?

Objective 2: Are the TRM generated by Ad-HA/NP immunisation and pH1N1 infection different?

Objective 3: Does IL-1β enhances the generation of TRM and what factors potentiate their induction?

c. Materials & Methods of experiments performed:

Due to difficulties in breeding of the Babraham pigs insufficient numbers of pigs were available and we had to adapt our initial plan and separated the immunogenicity and the efficacy parts of the study. The immunogenicity study with the detailed T-and B-cell analyses was performed as initially intended in inbred Babraham pigs allowing the measurement of antigen-specific TRM-cells by tetramers which has rarely been reported in pigs. The efficacy study was performed in outbred pigs which for translational reasons is even better suited than the inbred model mimicking the higher genetic diversity in humans. As described in the accompanying manuscript, the immunological data are of high quality and confirmed the adjuvant properties of IL-1b in the large natural host animal model. All methods were in line with the original application. In addition, in-depth analyses on antigen-specific memory B-cells were performed, exceeding the original plan. This further underscores the high value of our study to provide more detailed knowledge on the mucosal immunity induced by mucosal vaccination or infection. The challenge infection was performed as described in the original proposal and viral load in tissues was analysed by plaque assay and qRT-PCR. Lung gross- and histo- pathology was evaluated including immunohistochemistry for NP.

d. Ethical Clearance & Justification of experiments performed:

Given the nature and localisation of the TRM it is not possible to use an *in vitro* system to study how these cells are induced following immunisation, nor is it possible to measure immune responses to or protective efficacy of a vaccine without the use of animals.

The results of this study clearly underscore the importance of confirmatory studies in a large natural host animal species. Our adenoviral vector immunization or prior H1N1 infections protects well against

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heterologous IAV challenges in mice, but both failed to provide heterotypic immunity in pigs. Since the pigs are susceptible to a variety of IAV strains and a possible “mixing vessel” for reassorting of zoonotic IAV with pandemic potential, it is crucial to understand the mechanisms and correlates of protection in this relevant large animal model and whether they are the same as those in humans.

The experiments were performed in line with all regulations to minimize the burden for the animals as much as possible and the gain of knowledge in regard to develop a universal flu vaccine outweighs this.

e. Results:

The results of this study are presented in detail in the attached manuscript which is currently under review at Mucosal Immunology. In brief, we confirmed the adjuvant effect of adenovirus encoded Il-1B on the induction of antigen-specific TRM in the lungs, which was one of the primary objectives of our proposal. The TRM responses against the H1N1-derived epitopes were significantly higher in the Ad-IL-1b treated animals than in the non-adjuvanted group and in the H1N1 pre-exposed animals. Furthermore, we demonstrated higher frequencies of HA-specific antibodies and memory B-cell responses in Ad-IL-1b immunised animals. Surprisingly, the strong T-cell responses against the conserved NP-epitopes and enhanced Ab responses were insufficient to control viral replication after heterologous H3N2 infection. The lung gross and histopathology showed enhanced pathology in Ad-IL-1b treated animals following H3N2 challenge. However, prior infection with H1N1 did not induce sufficient heterotypic immunity either, which supports the notion that the correlates of protection differ between the small rodent and the large pig animal model.

f. Conclusions:

The study was well designed and the established models allowed the specific answering of our three objectives formulated in the original application. Although the results, especially the efficacy data, were unexpected, this new information is of high relevance for the further development of universal flu vaccines. Our study also raises new questions about the suitability of different animal model for predicting translational outcomes in human clinical trials. To decipher modalities which are able to induce long-lasting and broadly protective mucosal immunity is still urgently needed and will be part of future studies of the applicant and the service provider.

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