THIRD WORKSHOP

"Immunology and Structural Vaccinology"

24th-25th June 2020

PROCEEDINGS

DISSECTION has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675106.

VADEMA has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 675879

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INTRODUCTION

VADEMA (Doctoral Industrial School for Vaccine Design through Structural Mass Spectrometry) aims at delivering industry-oriented PhD training in the field of structural mass spectrometry applied to vaccinology. The four recruited Early-Stage Researchers (ESRs) experience an intersectoral training programme encompassing a 18-months internship in Glaxo Smith Kline Vaccines (GSKVACSRL), based in Siena, Italy, and 18-months internship at Department of Pharmacy, in University of Copenhagen (UCPH), DK. The students are enrolled in the Graduate School of Health and Medical Sciences and affiliated to the graduate programme in pharmaceutical sciences, the Drug Research Academy PhD school at UCPH. The 4 ESRs are trained in the growing field of Structural Mass Spectrometry and Vaccinology.

DISSection (Doctoral Industrial School on Human Skin models for Staphylococcal infections) aims at delivering industry-oriented PhD training in the area of Infection and Immunity. The four ESRs experience an intersectoral training programme encompassing a 18 months of internship in GSKVACSRL, based in Siena, Italy, and 18 months internship at Department of "Universitair Medisch Centrum Utrecht" (UMCU), NL. The students are enrolled in the PhD school Program at Universitair Medisch Centrum Utrecht for a period of 4 years and will receive a PhD degree in the area of Infection and Immunity.

For more details and news, visit the websites www.vadema.eu and www.dissection.eu.

WORKSHOPS

Three workshops have been organized during VADEMA and DISSection projects. In these workshops, the ESRs have attended seminars from internationally recognized experts in scientific areas related to the network objectives and participated to practical courses. The topics selected for the workshops aim at covering the state-of-the-art disciplines strongly connected with the VADEMA and DISSection programs.

In particular, the third Workshop course was organised jointly by VADEMA and DISSection Grants. This joint event represented an occasion to connect EU funded projects in order to strengthen the interactions among the fellows of the two networks and create an environment of active communication outside the network.

The Third Workshop focused on "Immunology & Structural Vaccinology" and has been organized by GSKVACSRL and was open also to other students from GSK Academy and other GSK sites.

Due to the epidemiological situation following the Covid-19 outbreak, the Workshop was organised online through video conferencing. The projects consider that the Covid-19 emergence is a serious threat to public health. Restrictive measures are necessary to save lives. In order to respect project deadlines and ensure progression of the VADEMA and DISSection projects while respecting the containment measures and the social distancing, the joint Third Workshop was planned as a virtual event.

The two-days training event entailed extensive and comprehensive lectures about theory and applications related to Immunology and Structural Vaccinology given by eleven experts in the field. Together with the basic concepts about Immunology and Structural Vaccinology, the course covered various techniques and their application, also with actual examples from ongoing projects.

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Day 1 - Wednesday 24th June

14.00-14.30 (08.00-08.30, EDT time) **Introduction - DISSection and VADEMA Projects**
Fabio Bagnoli and Nathalie Norais - GlaxoSmithKline Vaccines S.r.l., Italy

Immunology: How vaccines work

14.30-15.15 (08.30-09.15, EDT time) **What every vaccinologist needs to know about B cell responses**
Monia Bardelli - GlaxoSmithKline Vaccines S.r.l., Italy

15.15-16.00 (09.15-10.00, EDT time) **What every vaccinologist needs to know about T cell responses**
Elisabetta Soldaini - GlaxoSmithKline Vaccines S.r.l., Italy

16.00-16.15 (10.00-10.15, EDT time) *Break*

16.15-17.00 (10.15-11.00, EDT time) **What every vaccinologist needs to know about innate immune responses**
Francesca Schiavetti - GlaxoSmithKline Vaccines S.r.l., Italy

17.00-17.45 (11.00-11.45, EDT time) **Advances in delivery systems and adjuvants**
Diego Piccioli - GlaxoSmithKline Vaccines S.r.l., Italy

17.45-18.00 (11.45-12.00, EDT time) *Break*

18.00-19.00 (12.00-13.00, EDT time) **Fellows Networking activity: round table on Immunology of Covid-19 based on selected papers by DISSection fellows**
Moderator: Fabio Bagnoli - GlaxoSmithKline Vaccines S.r.l., Italy

Day 2 - Thursday 25th June

Structural Vaccinology

14.00-14.45 (08.00-08.45, EDT time) **EM and cryo EM and vaccines**
Fabiola Giusti and Ilaria Ferlenghi - GlaxoSmithKline Vaccines S.r.l., Italy

14.45-15.30 (08.45-09.30, EDT time) **Mass spectrometry and vaccines**
Lucia Eleonora Fontana and Nathalie Norais - GlaxoSmithKline Vaccines S.r.l., Italy

15.30-15.45 (09.30-09.45, EDT time) *Break*

15.45-16.30 (09.45-10.30, EDT time) **X-ray crystallography and vaccines**
Lucia Dello Iacono and Daniele Veggi - GlaxoSmithKline Vaccines S.r.l., Italy

16.30-17.15 (10.30-11.15, EDT time) **Structural vaccinology and vaccines**
Maria Scarselli - GlaxoSmithKline Vaccines S.r.l., Italy

17.15-17.30 (11.15-11.30, EDT time) *Break*

17.30-18.30 (11.30-12.30, EDT time) **Fellows Networking activity: round table on Structural studies on Sars-Cov2 based on selected papers by VADEMA fellows**
Moderator: Nathalie Norais - GlaxoSmithKline Vaccines S.r.l., Italy

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What every vaccinologist needs to know about B cell responses

Monia Bardelli - GlaxoSmithKline Vaccines S.r.l., Italy

Memory B cells (MBCs) are a B cell sub-type that are formed within germinal centers following primary infection. Memory B cells can survive for decades and repeatedly generate an accelerated and robust antibody-mediated immune response in the case of re-infection (also known as a secondary immune response). In a T-cell dependent development pathway, naïve follicular B cells are activated by antigen presenting TFH cells during the initial infection, or primary immune response. After activation, the B cells move into the secondary lymphoid organs (i.e. spleen and lymph nodes). Within the secondary lymphoid organs, most of the B cells will enter B-cell follicles where a germinal center will form. Most B cells will eventually differentiate into plasma cells or memory B cells within the germinal center. Plasma cells produce a first wave of protective antibodies and help clear infection. A fraction of the B cells differentiate into memory B cells that survive long-term in the body. Memory B cells relocate to the periphery of the body where they will be more likely to encounter antigen in the event of a future exposure. Many of the circulating B cells become concentrated in areas of the body that have a high likelihood of coming into contact with antigen, such as the Peyer's patch. The memory B cells produced during the primary immune response are specific to the antigen involved during the first exposure. In a secondary response, the memory B cells specific to the antigen or similar antigens will respond. When memory B cells reencounter their specific antigen, they proliferate and differentiate into plasma cells, which then respond to and clear the antigen. The memory B cells that do not differentiate into plasma cells at this point can reenter the germinal centers to undergo further class switching or somatic hypermutation for further affinity maturation. Differentiation of memory B cells into plasma cells is far faster than differentiation by naïve B cells, which allows memory B cells to produce a more efficient secondary immune response. The efficiency and accumulation of the memory B cell response is the foundation for vaccines and booster shots.

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What every vaccinologist needs to know about T cell responses

Elisabetta Soldaini - GlaxoSmithKline Vaccines S.r.l., Italy

T cells belong to the adaptive immune system, which is characterized by the high degree of specificity in antigen (Ag) recognition and by the ability to generate a memory upon the first encounter with the Ag. This last characteristic makes the response to the subsequent encounters with the Ag quicker and more efficient and is the basis of vaccination. T cells derive from the bone marrow and develop early in life in the Thymus where they undergo genetic rearrangements of the T cell receptor (TCR) as well as positive and negative selection. T cells then enter the circulation where they greatly outnumber B cells. T cells can express either CD4 or CD8 as TCR co-receptor that allows the recognition of the Ag, in the form of a short peptide derived from the enzymatic digestion of the Ag inside the Ag presenting cell (APC), in the context of major histocompatibility complex class II (MHC-II) or class I (MHC-I) molecules, respectively. While all nucleated cells express MHC-I, only professional APCs express MHC class II. Therefore, while endogenous Ags (including viral and tumor Ags) can be presented to CD8 T cells by all nucleated cells, only professional APCs can present exogenous Ags to CD4 T cells. Therefore, while endogenous Ag-based vaccines, like live attenuated, viral vectors- and SAM-based vaccines, induce CD8 T cells, exogenous Ag-based vaccines, like whole inactivated or subunit vaccines, do not. CD4 T cells play a critical role in helping B cells to produce high affinity antibodies (Abs) and are therefore called T helper (Th) cells. Non proteinaceous Ag, like polysaccharydes (PS), are not recognized by CD4 T cells and thus are T-cell independent Ags that generate a faint and short-lived Ab response and no response under 2 years of age. To overcome these problems PS can be conjugated to carrier proteins. The T cell response consists of 3 phases: 1) Activation, 2) Clonal Expansion/Differentiation, and 3) Contraction/Memory. 1) Upon Ag encounter, naïve T cells express activation markers and produce IL-2. 2) As a result, activated T cells proliferate giving rise to Ag-specific T cell clones and, under the influence of cytokines, differentiate into different subsets of effector T cells (Teff) specialized for a particular effector function (e.g. Th1, Th2, Th17). 3) Once Teff have eliminated the pathogen, most Teff die. Surviving Teff differentiate into memory T cells that persist for long time. Central memory T cells (TCM) recirculate among secondary lymphoid organs only while effector memory T cells (TEM) recirculate also in non-lymphoid tissues. More recently tissue-resident memory T cells (TRM) have been identified. TRM reside for long time in the tissue where they have encountered the pathogen conferring to it a local adaptive immunity that can promptly defend against a subsequent infection with the same pathogen.

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What every vaccinologist needs to know about innate immune responses

Francesca Schiavetti - GlaxoSmithKline Vaccines S.r.l., Italy

Assessing innate cell response to vaccination in humans implies, beforehand, understanding why measuring innate immunity in the context of vaccination might be extremely helpful, figure out what are the main cellular subsets to investigate and determine how to measure innate immune response in human clinical exploratory trials with the more appropriate assays.
Taking into account that the purpose of a successful vaccine is to induce long-term protective immunity against a given pathogen, we can say that an ideal vaccine should induce both antigen-specific humoral and cellular memory response, long-lived plasma cells that produce neutralizing antibodies and persisting CD4+ and CD8+ memory T cells. The achievement of these goals is promoted by an effective interaction of innate and adaptive immune response and mediated by the crosstalk between these two arms of the response.
Innate immune response is marked by its ability to recognize several components from pathogens, which are present exclusively in these microorganisms and lacking in the host cells. Detection of pathogens by the innate immune system is made by a class of immune-sensor molecules referred to as pattern recognition receptors (PRRs).
The Toll-like receptors (TLRs) are a class of PRRs that detect a broad range of pathogens. TLR ligands have been widely studied and tested as components of immune adjuvants in many human vaccines, because they can polarize and amplify the resulting immune response, thanks to their stimulatory activity on innate immune cells.
The innate system is composed by many cell subsets and, among them, dendritic cells (DCs), monocytes (MCs) and macrophages (their differentiated counterparty) are key players in inducing an effective adaptive immunity by producing cytokines and chemokines and expressing activation markers that can, in turn, activate adaptive immune cells. In humans, circulating monocytes are classified into three different subsets based on the expression level of CD16 and CD14 surface markers, namely classical, intermediate and non-classical monocytes. Classical MCs have a high antimicrobial capability due to their potent capacity of phagocytosis, and secrete IL-1, IL-6, TNFα and IL-10 upon LPS stimulus. Intermediate and non-classical MCs secrete inflammatory cytokines, IL-6, TNFα and IL-1β upon inflammatory stimulation. During inflammation, classical and intermediate MCs invade tissues. MCs then mature to macrophages and present antigens via MHC-I/II to TCR leading to T cells activation.
On the other hands, DCs play also a pivotal role in initiating the immune response to foreign antigen. They are divided into two major subsets: myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC). Myeloid dendritic cells are professional antigen presenting cells (APC) and produce also large amount of pro-inflammatory cytokines and, similarly to activated monocytes, express for the majority TLR4 and TLR2 on their surface. pDCs are long-lived cells found in blood, bone marrow, and lymphoid organs. Constitute < 0.4% of peripheral blood mononuclear cells (PBMC). pDCs selectively express endosomal Toll-like receptors TLR7 and TLR9, which sense viral RNA and DNA respectively. Their numbers increase during infection (and active immunization) and they are rapidly activated by viral nucleic acids. They are specialized to respond to viruses by producing massive amounts of the type I interferons (IFN-α and IFN-β). After producing interferons, they are still able to differentiate into mature DCs that can stimulate naïve T cells. Therefore, including in vaccines formulations the toll like receptors ligands expressed by the various cellular subsets, might help polarize the adaptive response that follows.
Monitoring the quality and the amplitude of immune response generated by a vaccine can give interesting insights. Advances in the field of immune read-outs and new technologies have dramatically changed our understanding of the role of the multiple innate immune cell subsets on the vaccination outcome. In this context multiparametric flowcytometry combined with high-sensitivity cytokine and chemokine multiplex tests are suitable assays for to the detection and the monitoring innate and adaptive immunity after vaccination in exploratory clinical trials.
In conclusion, different monocytes and dendritic cell subsets are extremely interesting cells to be analyzed in the context of immunization and particularly the process of the antigen presentation, the overview of the various subsets with distinct functions, in addition to their plasticity in responding to signals given by adjuvant components.
Moreover, this exploratory research approach in vaccine development may represent an opportunity to formulate novel vaccines that can generate a more effective and long-lasting immunity.

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Advances in delivery systems and adjuvants

Diego Piccioli - GlaxoSmithKline Vaccines S.r.l., Italy

The adjuvant technology is considered critical to design effective vaccines. An adjuvant is anything which may improve the clinical effectiveness of a vaccine. Modern subunit vaccines are often designed with the addition of an adjuvant because these vaccines contain in general only one or some pathogen's antigens that on one hand are considered critical to elicit a protective immune response, but on the other hand might be non-sufficiently immunogenic in the absence of an adjuvant. In other words, an effective vaccine is often a combination of a good antigen and a good adjuvant. Three examples recently highlighted the importance of this combination: the development of the Shingrix vaccine against Zoster virus of adult life, the first Malaria vaccine implemented in routine immunizations within some countries of Africa and the novel Tubercolosis vaccine that successfully passed the Phase II trial. All of these very important vaccines were possible thanks to the identification of good antigens and to the adjuvant AS01, which is able to promote good cellular and humoral immune responses. An adjuvant works as immunopotentiator and/or as delivery system of immunostimulatory molecules and/or antigens. The Immunopotentiator effect (obtained also by the delivery of immunostimulatory molecules) is based on eliciting stimulation of immune cells, whereas the antigen delivery system is based on the improvement of antigen uptake by antigen presenting cells. Both of these mechanisms are fundamental to generate an optimal and effective immune response. The delivery of vaccines to individuals is prevalently executed via intramuscular injection. However, research to find out alternative strategies of vaccine administration remain an important aspect. Intramuscular administration, in fact, poses some problems such as the need of skilled personnel for vaccine administration (which increases costs of vaccination campaigns), the risk of transmission of infections in poor hygiene conditions (i.e. in low-income countries), some adverse events, as fainting or needle injuries and the absence of mucosal immunity. The transdermal administration with microneedle patches is one of the most interesting alternative strategy to take into account, but several problems should be solved, such as skin irritation, confirmation of dose delivered, scale-up, huge inter- and intraperson hetreogeneity in stratum corneum thickness/hydration levels, hair-follicle density and compatibility of vaccines with the microneedle manufacture process/cost. The mucosal administration is certainly the most important alternative strategy for vaccine delivery because some mucosal vaccines already exist and because the mucosal administration is able to elicit both systemic and mucosal immunity. However, also the mucosal administration has several hurdles to overcome, such as the high dilution of the vaccine and its degradation in the acidic environment of the stomach for oral administration and the fast clearance rate of the vaccine for the intranasal administration. Certainly the development of effective mucosal adjuvants may help to obtain effective mucosal vaccines.

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EM and cryo EM and vaccines

Fabiola Giusti - GlaxoSmithKline Vaccines S.r.l., Italy

Electron microscopy (EM) is a technique for achieving high resolution images and it represents the only technique that allows for direct visualization of samples. In transmission EM the beam, consisting of high energy electrons, passes through a thin specimen showing the inner structures while in scanning EM the beam scans the surface of the sample. In transmission EM we can analyze the inner ultrastructures performing ultrathin sections or we can observe the morphology using Negative staining EM. NS EM allows for rapid morphological identification of the sample and it is able to accommodate the highest magnification images. In negative staining EM the contrast is not applied to the object but to its environment, using heavy metal salts. The electron beam can cross biological material easier than the surrounding space. Here, in GSK, most of the samples are analyzed using NS EM in order to establish integrity, state of aggregation, morphology, dimension and to define the structure. NS EM is a key technique to screen the samples before moving to Cryo-EM. Our goal is to drive highly specific antigen identification for structural characterization of a huge amount of new proteins thus obtaining the structure of virulence factors at high resolution in the native form.

Ilaria Ferlenghi - GlaxoSmithKline Vaccines S.r.l., Italy

The selection in silico of vaccine candidates by Reverse Vaccinology can be challenged by sequence variability of the pathogens, the immune evasion strategies adopted by the pathogens, the low abundance of surface antigens and eventually their low stability once expressed as recombinant antigens. These challenges can be faced with a deep knowledge of the three-dimensional structure of the candidates. The advances in cryo-EM together with well-set X-ray crystallography and HDx-MS allowed recently the structural characterization of a huge amount of new proteins. The use of 3D structure is a key tool to drive highly specific antigen identification.

Thanks to the new approaches in the field of structure determination of molecular systems we can now obtain the structure of virulence factors at high resolution and in the native form presented to the immune system

Moreover, a precise epitope mapping can be obtained at atomic level resolution using a combined approach based on the HDx-MS, cryo-EM and X-ray Crystallography.

At the same time, we can profile the answer of the immune system to the infective agent.

Combining these two knowledges with AI/ML/semantic discovery tool, will enable the prediction of the protective immune response and therefore design improved vaccine candidates and/or therapeutics mAbs – by boosting the discovery phase.

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Mass spectrometry and vaccines

Lucia Eleonora Fontana and Nathalie Norais - GlaxoSmithKline Vaccines S.r.l., Italy

Vaccination is the most effective strategy to prevent infectious diseases. From Pasteur's intuition of using inactivated microorganisms to modern recombinant subunit antigens, vaccine efficacy and safety have continuously improved, mainly thanks to technical advances sustaining antigen discovery and characterization. In this context, mass spectrometry (MS) has been a fundamental tool.

This presentation will illustrate how mass spectrometry is involved in the first part of a project through the identification of potential vaccine candidates among a set of pathogen-secreted or surface-exposed proteins. Once a vaccine candidate is selected, targeted proteomics, native mass spectrometry and hydrogen deuterium exchange coupled to mass spectrometry are involved to assess expression level of selected antigens in clinical circulating strains, to elucidate antigen function, identify potential binding sites to human interactors and epitopes recognized by protective or non-protective sera. All these achievements are pivotal to ensure a broad coverage of the vaccine, to avoid any potential undesired effects, to wuth engineer molecules with higher surface of interaction with the host immune systems and to improve the design of a more efficient antigen with multiple protective epitopes. Specific examples of how these approaches are applied is reported. A view for potential use of mass spectrometry in the next few years will be evocated and discussed with the participants.

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X-ray crystallography and vaccines

Lucia Dello Iacono and Daniele Veggi - GlaxoSmithKline Vaccines S.r.l., Italy

X-ray crystallography is currently one of the two most widely applied techniques for the determination of macromolecular structures at high resolution, playing a crucial role in the structural biology field. The knowledge gained through X-ray crystallography has considerably advanced our understanding of biological processes and greatly facilitated the development of drugs and vaccines.
Herein, an overview of the basic concepts of X-ray crystallography is provided. Protein crystals are able to diffract X-rays into many specific directions. By measuring the angles and intensities of these diffracted beams, the density of electrons within the crystal can be calculated and the 3D structure of the protein can be determined. The structure of a protein is very informative, and can unveil details on the nature of the chemical bonds established by atoms, their degree of disorder, as well as providing info on the interactions established by a protein in complex with a small ligand or a protein interactor.
Finally, some applications of the X-ray crystallography technique to the Structural Vaccinology field are described, by showing case studies where the structural determination of antigen proteins or antigen-antibody complexes was useful to (1) understand the immuno-response at atomic details, (2) remodel the antigen or the epitope by reverse molecular engineering.

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Structural vaccinology and vaccines

Maria Scarselli - GlaxoSmithKline Vaccines S.r.l., Italy

In the last decades vaccinology received new impulse from the emergence of new technologies. One of such innovative tools is represented by reverse vaccinology, which thanks to the advance of bacterial genome sequences allows now the rapid identification of protein candidates. Specific algorithms dedicated to the prediction of sub-cellular localization, putative structure and function of each member of the entire predicted bacterial proteasome allow the rapid identification of potential virulence factors and surface exposed proteins, which can be rapidly expressed and tested in suitable animal models or by in vitro assays.
Knowledge and analysis of 3D structure can then provide the opportunity to modify promising candidates in order to further improve their performance as immunogens. A second generation of optimized molecules can be now routinely designed and tested, leading to compounds able to better present epitopes targets of neutralizing immune response.

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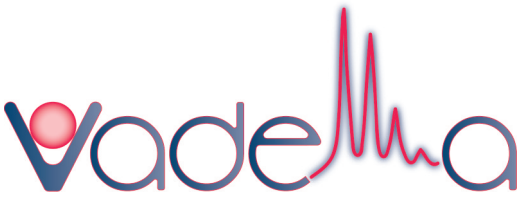

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