Cell mediated immunity in laboratory vaccine surveillance

A tool to better predict immunity to vaccine preventable diseases?
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A tool to better predict immunity to vaccine preventable diseases?
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Preface

This report aims at investigating the potential benefit of cell-mediated immunity (CMI) in monitoring the Swedish national immunization programs (NIP) and at the time of new introductions of vaccines into the program. We reviewed current knowledge about the vaccine-induced cell-mediated immune responses by a systematic search of the scientific literature, complemented with ad hoc literature, and consulted Nordic and European Public Health Agencies to map what is currently being done in the field.

At present, vaccine program-related surveillance includes monitoring of vaccine coverage, incidence and morbidity of vaccine preventable diseases (VPD), vaccine effectiveness and vaccine-related adverse events. Laboratory surveillance of the NIP includes seroepidemiological studies, serotyping and molecular typing of viral and bacterial isolates and monitoring of antimicrobial resistance.

This review focuses on the potential added value of including CMI as a surveillance tool of the VPDs included in the NIP, currently available methods to measure cellular immunity and laboratory resources required.

The report provides primarily an information basis for future decision-making regarding the role of CMI in the surveillance of VPDs, at the Public Health Agency of Sweden. It may also be of value to professionals and specialists such as managers of NIPs and managers of laboratory surveillance of NIPs, as well as national and international communicable disease control and prevention authorities and public health agencies.

The report was written by Karina Godoy-Ramirez, Ingrid Uhnoo and Karl Ljungberg and, contributions and comments were given by experts in different areas (Appendix 1). Ann Lindstrand, Head of the Unit for Vaccination Programs, and Mia Brytting, Head of the Unit for Laboratory Surveillance of Viral Pathogens and Vaccine Preventable Diseases, participated in the final revision of the report.
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<tr>
<td>Ab</td>
<td>Antibody</td>
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<tr>
<td>aP</td>
<td>Acellular pertussis vaccine</td>
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<tr>
<td>BCG</td>
<td>Bacille Calmette Guérin</td>
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<tr>
<td>CBA</td>
<td>Cytometric Bead Array</td>
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<tr>
<td>CD1</td>
<td>Cluster of Differentiation 1</td>
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<tr>
<td>CMI</td>
<td>Cell-mediated immunity</td>
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<tr>
<td>CTL</td>
<td>Cytotoxic T Lymphocyte</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>ELISpot</td>
<td>Enzyme-linked immunospot</td>
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<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
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<tr>
<td>FAMA</td>
<td>Fluorescent antibody to membrane antigen</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration (USA)</td>
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<tr>
<td>FC-LPA</td>
<td>Flow cytometric Lymphoproliferation assay</td>
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<tr>
<td>FHA</td>
<td>Filamentous hemagglutinin</td>
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<tr>
<td>HAI or HI</td>
<td>Hemagglutinin inhibition</td>
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<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
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<td>HLA</td>
<td>Human Leukocyte Antigen</td>
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<td>HPV</td>
<td>Human Papillomaviruses</td>
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<td>HZ</td>
<td>Herpes Zoster</td>
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<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>ICP</td>
<td>Immunological correlate of protection</td>
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<td>ICS</td>
<td>Intracellular cytokine staining</td>
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<td>LPA</td>
<td>Lymphoproliferation assay</td>
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<tr>
<td>LAIV</td>
<td>Live attenuated influenza vaccine</td>
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<tr>
<td>MBC</td>
<td>Memory B cell</td>
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<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<tr>
<td>MHRA</td>
<td>Medicines &amp; Healthcare products Regulatory Agency (UK)</td>
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<tr>
<td>MMR</td>
<td>Measles, Mumps and Rubella</td>
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<tr>
<td>NIP</td>
<td>National immunization program</td>
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<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
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<td>PHAS</td>
<td>Public Health Agency of Sweden</td>
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<td>PHN</td>
<td>Post herpetic neuralgia</td>
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<td>PRN</td>
<td>Pertactin antigen</td>
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<td>PT</td>
<td>Pertussis toxin/toxoid</td>
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<td>SFC</td>
<td>Spot-forming cells</td>
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<tr>
<td>SOP</td>
<td>Standard operating procedure</td>
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<tr>
<td>TB</td>
<td>Tuberculosis</td>
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<tr>
<td>Tc</td>
<td>Cytotoxic T cell</td>
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<tr>
<td>Th</td>
<td>Helper T cell</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>TCR</td>
<td>T-Cell Receptor</td>
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<tr>
<td>TIV</td>
<td>Trivalent influenza vaccine</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>VLP</td>
<td>Virus-like particles</td>
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<tr>
<td>VPD</td>
<td>Vaccine-preventable diseases</td>
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<tr>
<td>VZV</td>
<td>Varicella-zoster virus</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>wP</td>
<td>Whole-cell pertussis vaccine</td>
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Glossary

**Antibodies**: Proteins of the immunoglobulin family, present on the surface of B lymphocytes, secreted in response to stimulation, that neutralize antigens by binding specifically to their surface. Antibodies prevent or reduce infections by extra- and intracellular agents and clear extracellular pathogens through several mechanisms.

- Antibody affinity refers to the tendency of an antibody to bind to a specific epitope at the surface of an antigen, i.e., the strength of the interaction.
- Antibody avidity is the sum of the epitope-specific affinities for a given antigen. It directly relates to its function.

Non-neutralizing antibodies are also produced after viral infection. They bind specifically to virus particles, but do not neutralize infectivity. Non-neutralizing antibodies may enhance infectivity following interaction with receptors on macrophages or may inhibit virus replication in macrophages and immature dendritic cells.

**B lymphocytes**: Blood cells that originate in the bone marrow, mature in secondary lymphoid tissues, and become activated when their surface immunoglobulins bind to an antigen, and differentiate either into antibody secreting cells (plasma cells) or memory B cells.

**Booster (or revaccination)**: Additional dose of vaccine given at a certain time interval post-primary vaccination, in order to induce immune memory and improve long-term protection against infection.

**Cell-mediated Immunity (CMI)**: An immune response that involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes and the release of various cytokines in response to an antigen. In this report, we define CMI as an immune reaction driven by either memory CD4- and/or CD8-expressing T cells or memory B cells.

**Central memory T cells (Tcm)**: Memory T cells trafficking through the lymph nodes, ready to proliferate and generate a high number of effector cells in response specific microbial peptides.

**Correlate of protection (CoP)**: An immune response that is statistically correlated with protection.

**Effector memory T cells (Tem)**: Memory T cells patrolling through the body to detect specific microbial peptides and capable of an immediate cytotoxic function in case of recognition.

**Herd immunity**: Reduction of disease in an unimmunized segment of a population in which a large proportion has been immunized.
Immunological Correlate of Protection (ICP): An immune response that is responsible for and statistically interrelated with protection.

Immunosenescence: An age-related decline in immune functions. This increases the frequency and severity of infections and reduces the protective effects of vaccinations.

Innate and adaptive immune system: The innate immune system consists of cells and proteins that are always present and ready to mobilize and fight microbes at the site of infection. The adaptive immune response is the response of antigen-specific lymphocytes to antigen, including the development of immunological memory.

Memory B cells: Blood cells that are generated during primary responses to T-dependent vaccines. They persist in the absence of antigens, are resting cells that do not produce antibodies but can rapidly differentiate into antibody-secreting plasma cells on reexposure to antigen.

Surrogate of protection: An immune response that substitutes for the true immunologic correlate of protection, which may be unknown or not easily measurable.

Surveillance: Continuous collection, compilation and analysis of data, with or without subsequent action.

T lymphocytes: T cells that originate in the thymus, mature in the periphery and become activated in the spleen/nodes when their T-cell receptors bind to an antigen presented by a Major Histocompatibility Complex (MHC) molecule and receive additional costimulation signals. The T cells can be driven to acquire either supporting (mainly CD4+ T cells) or killing (mainly CD8+ T cells) functions. Main subpopulations of T cells are:

CD4+ T helper cells (Th) do not prevent but participate to the reduction, control and clearance of extra- and intracellular pathogens:

- CD4+ T helper 1 (Th1) lymphocytes: T cells that upon activation differentiate into cells that mainly secrete IL-2, IFN-γ and TNF-β exerting direct antimicrobial functions (viruses) and essentially providing support to cytotoxic T cells and macrophages.
- CD4+ T helper 2 (Th2) lymphocytes: T cells that upon activation differentiate into cells that mainly secrete IL-4, IL-5, IL-6, IL-10, IL-13, exerting direct antimicrobial functions (parasites) and essentially providing support to B lymphocytes.
- CD4+ T helper 17 (Th17) lymphocytes: T cells that upon activation differentiate into cells that mainly secrete IL-17, IL-21, IL-22, and are implicated in host defense against extracellular bacteria colonizing exposed surfaces (airways, skin gut).
CD4+ regulatory T cells (Tregs): T cells that upon activation differentiate into cells that express specific cytokines such as IL-10 IL-25, TGF-b/surface markers, and act to suppress the activation of the immune system through various mechanisms, maintaining immune homeostasis and tolerance to self-antigens.

CD8+ cytotoxic T cells (CTLs) do not prevent but reduce, control and clear intracellular pathogens.

- CD8+ T cells that upon activation differentiate into cells that exert directly killing of infected cells by release of perforin, granzyme, etc.
- CD8+ T cells that upon activation differentiate into cells that exert indirectly killing of infected cells through antimicrobial cytokine release.

**Vaccine efficacy and vaccine effectiveness**: Vaccine efficacy is the percentage reduction in disease incidence attributable to vaccination, measured in an individually randomized, placebo-controlled clinical trial.

When the percentage reduction is measured through observational studies under program conditions, the term vaccine effectiveness is used.

**Vaccine failure**: Primary vaccine failure (non-responsiveness) is the inability of an individual to mount an initial immunological response after primary or booster vaccination. Secondary vaccine failure is the ability of an individual to mount an initial response after vaccination but the protective response wanes over time.
Summary

Purpose of this review

The purpose of this review is to summarize current knowledge on cell-mediated immunity (CMI) against vaccine-preventable diseases (VPD) included—and considered to be included - in the National Immunization Program (NIP) in Sweden and, to investigate the possible added value of CMI in vaccine surveillance and in research to address specific issues that might be useful in the future for design of optimized immunization schedules, timing of booster immunizations and evaluation of novel vaccine concepts and adjuvants targeted at eliciting CMI responses. CMI analysis are also discussed in a broader perspective, and as part of a strategic and long-term work with the development of the NIP. Also the applicability of CMI methods for large-scale surveillance of vaccine-induced immunity is addressed.

Data collection

The report is based on a comprehensive scoping literature review on cell-mediated immunity including scientific peer-reviewed literature from the past 12 years, available grey literature, and ad-hoc search. A survey on CMI methods established at the Public Health Agency of Sweden and the Nordic countries was also performed.

Added value of analysis of CMI for vaccine surveillance?

Humoral immune responses have been established as the primary immunological correlate of protection against most VPD in the Swedish NIP, and protective antibody levels are defined for several of these. Vaccine-induced cell-mediated immunity (CMI) may also correlate to protection against certain diseases, where antibodies either do not provide protection such as tuberculosis or have waned to non-protective levels over time after vaccination. However, CMI analysis suffers from a lack of defined correlates of protection. Only a few studies have been able to propose protective levels of some CMI parameters (tuberculosis, influenza).

Large-scale analysis of CMI is costly and labor-intensive, and can thus not replace serological immune surveillance in the general population, which is currently performed by high throughput serological assays.

Added value of analysis of CMI for research and knowledge gaps?

Studies aiming at better understanding protective immunity in individuals or specific target populations are important.

Conventional CMI methods will remain essential in evaluation of vaccine-induced responses in the future. However, these approaches are generally limited to the analysis of a small number of components of the immune system and are not
sufficient to assess the full complexity of structures and dynamics of the immune system as a whole. Recent advances in high throughput technologies and deeper understanding of the immune system have given rise to a new approach, the so-called systems immunology/vaccinology, with a holistic view of the immune system, where measurement of cell mediated immunity is only one of several arms to assess.

Conclusions

In conclusion, CMI has currently no role in routine surveillance of the VPDs in the NIP in Sweden. For knowledge gaps and research purposes it may be useful in different areas, as summarized in the sections CMI in protection for vaccine preventable diseases under Results. Selected examples of suggested use of CMI:

- Estimation of duration of protection of MMR vaccine induced immunity in the absence of epidemics
- In the development of new vaccines against tuberculosis
- To investigate the optimal timing of pertussis booster vaccination
- To measure long-term immunity against Hepatitis B and HPV after vaccination

In planning and carrying out research studies using cmi, several issues need to be considered, such as:

- Data from national serosurveillance studies on population-level immunity should be taken into account for decision on any future study based on CMI analysis
- Explore the possibility of collaboration with the Nordic and other European countries to create a collaborative CMI platform
- Jointly planned CMI studies to be carried out through collaboration with the Nordic and other European countries or other stakeholders
- Continuously surveil the scientific literature on new vaccines and CMI with specific focus on the development of national immunization programs.
Introduction

Vaccination is one of the most cost-efficient intervention against infectious diseases and has greatly improved global health by decreasing disease burden, disability, death and inequity. Vaccination campaigns have eradicated smallpox and are on the brink of ridding the world of polio. Immunization is used to elicit an immune response with the aim of providing an individual as well as an indirect (herd immunity) protective immunity from the pathogen at a subsequent exposure.

Vaccines currently in use consist of live attenuated microorganisms, inactivated (killed) microorganisms or derivatives of those such as subunit vaccines, toxoid vaccines, conjugate vaccines or vaccine-like particles (VLP) vaccines. These derivatives could be purified proteins or polysaccharides as well as recombinant proteins or peptides. Vaccines are frequently formulated with an immunostimulatory agent, i.e. an adjuvant, to enhance the immune response.

The type of immune response induced by a vaccine largely depends on the design of the vaccine and the type of adjuvant used. Most current vaccines mediate protection through highly specific serum antibodies and their functional characteristics as well as quantity are important for protection. However, vaccines may protect through multiple mechanisms and vaccine-induced cell mediated immune memory may be crucial for long-term protection. Cell mediated immune memory may also be of relevance for protection against long-incubation diseases such as hepatitis B (1).

A specific immune response or immune function known to mediate protection against infection or disease is referred to as an immunological correlate of protection, while an immune response that substitutes for the true immunologic correlate of protection and is easy to measure, is referred as a surrogate marker (2). There is a traditional distinction between functional antibody (measured by a neutralization assay) and non-functional, binding antibody (measured by ELISA). A defined antibody level measured by ELISA could only be acceptable if the relationship with functional antibody is defined. However, recent data from animal and human studies on various viral infections and vaccine studies suggest that non-neutralizing antibodies (nNAb) without neutralizing activity in vitro may play an important role in protection against viral infection in vivo (3).

Correlates of protection may differ quantitatively and qualitatively, depending on whether the aim is to prevent systematic infection, mucosal infection, disease, or severe disease. Correlates may also vary depending on individual characteristics, such as age, gender, and major histocompatibility complex (MHC) group. For instance, demographic factors including gender, race, and ethnicity influence variations in both innate and adaptive cellular immune responses to measles (4). Further, complex factors including host genetics, infectious dose and route of transmission can influence vaccine-induced protection. Widely accepted immunological correlates of protection only exist in the absence of host factors that might increase susceptibility to an infectious agent. However, in certain
subpopulations with impaired immune responses such as the elderly, transplanted patients or those under chemo- or radiotherapy, it may be of importance to distinguish between correlates and surrogates since surrogates identified in a healthy population may not be relevant for the immunocompromised. Hence, it is important to know whether one is studying a correlate or a surrogate, and to define what the correlates of protection are for a specific pathogen and a specific population. In addition, the mechanism of protection, at least in experimental models, may differ from the mechanism of recovery from infection, where cellular immunity often plays a key role (reviewed in (2)).

The role of cell-mediated immunity

The role and protective efficacy of antibodies is currently unclear for some VPDs such as herpes zoster, mumps, and pertussis. Cell-mediated immunity (CMI) is considered to be of particular importance for VPD where protection persists also after the seroprotective antibody titers are no longer detectable, such as hepatitis B.

In this report, we define CMI as an immune reaction driven by either memory B cells, or CD4- and/or CD8-expressing T cells. Information about memory B and T cells after vaccination may also help designing immunization schedules and determine the optimal timing for booster immunizations. This report will explore the potential added value of CMI in laboratory vaccine surveillance and research.

Other aspects that may require studies of CMI include introduction of new vaccines into national immunization programs. An increasing body of evidence is indicating that T cells are crucial in the control of, protection against and clearance of intracellular pathogens that establish a persistent infection. If effective vaccines against hepatitis C, tuberculosis, herpes viruses or HIV are developed, it is likely that CMI based monitoring will be of relevance. In addition, there may be future demands from regulatory agencies such as EMA or FDA to characterize the CMI responses to new vaccines (5). To date, no specific requirements regarding CMI have been stated.

The Swedish National Immunization programs

The current childhood immunization program in 2017 offers all children in Sweden vaccination against nine diseases: polio, diphtheria, tetanus, whooping cough, infections caused by Haemophilus influenzae type b, measles, mumps and rubella and severe diseases caused by pneumococcus. Girls are also offered vaccine against human papillomavirus (HPV). Immunizations are given according to schedule in Table 1. In addition, selective programmes are recommended for at-risk populations against tuberculosis, hepatitis B, influenza, and invasive pneumococcal disease. Of these selective programmes tuberculosis, influenza, and invasive pneumococcal disease are currently assessed for inclusion in the national immunization program.
Table 1. Vaccination schedule recommended in the national immunization program for children in Sweden

<table>
<thead>
<tr>
<th>Age</th>
<th>Child Health Services</th>
<th>School Health Services</th>
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<tbody>
<tr>
<td></td>
<td>3 mths</td>
<td>5 mths</td>
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<tr>
<td>Grade</td>
<td></td>
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<tr>
<td>VPD</td>
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<tr>
<td>Diphtheria,</td>
<td>Dose 1</td>
<td>Dose 2</td>
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<td>tetanus,</td>
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<td>pertussis</td>
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<tr>
<td>Polio</td>
<td>Dose 1</td>
<td>Dose 2</td>
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<tr>
<td>Hib (Haemo-</td>
<td>Dose 1</td>
<td>Dose 2</td>
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<td>philus</td>
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<tr>
<td>influenzae</td>
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<td>type b</td>
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<tr>
<td>Pneumococcal</td>
<td>Dose 1</td>
<td>Dose 2</td>
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<tr>
<td>disease</td>
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<tr>
<td>Measles,</td>
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<td>mumps,</td>
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<tr>
<td>rubella</td>
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<tr>
<td>HPV (human</td>
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<tr>
<td>papillomavirus)*</td>
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</table>

* Girls only.
Aim

The aim of this report is to investigate the utility of CMI in the surveillance of vaccine preventable diseases included in the national immunization program in Sweden, and for vaccine preventable diseases that are expected to be assessed for inclusion in the national immunization program in the near future.

Our aim is to answer the following questions:

- Would analysis of CMI provide an added value for vaccine surveillance?
- Is there a need for the Public Health Agency of Sweden to implement CMI-based methods for laboratory surveillance of vaccine preventable diseases within the universal and targeted immunization programs?

Specific objectives

- To identify which, if any, cell mediated immune parameters that would be of value to assess in order to determine immunity to vaccine preventable diseases in the current and future national immunization program
- To provide an inventory of CMI methods currently in use, including pros and cons, to assess CMI responses after vaccination or natural infection.
- To identify the capacity and competence of the CMI laboratory methods that is currently available at the Public Health Agency of Sweden
- To identify CMI methods that could be considered to be established at the Public Health Agency of Sweden to meet future demands
- To investigate if any current or planned CMI-related work within the areas of VPD immune surveillance in the Nordic countries is performed
Background

T and B cell-mediated immune responses

The first steps and requirement to elicit vaccine responses reviewed by Claire-Anne Siegrist (6) involve the innate immune system, including natural killer (NK) cells, antigen-presenting cells (APC) such as dendritic cells and macrophages expressing toll-like receptors (TLR), that modulates the quantity and quality of long-term T and B cell memory and protective immune responses to pathogens.

Current vaccines mostly mediate protection through the induction of highly specific IgG serum antibodies produced by plasma cells (PC). The antibodies have the ability to block virus at the site of infection or prevent systemic spread. At the mucosal surface IgA plays a significant role (2, 7).

Numerous determinants modulate the magnitude and quality of immune response of vaccine antibody response, such as the antibody functionality, avidity, the nature of the vaccine antigen and its intrinsic immunogenicity, dose of vaccine antigen, the nature of the vaccine (live vs non-live), genetic determinants such as MHC restriction, and immune competence, which are limited at the two extremes of life. A few determinants of the persistence of vaccine antibody response have been identified: the nature of the vaccine, the age at immunization (shorter at the two extremes of life) and certain disease conditions such as immunosuppressive conditions. In addition, vaccine schedules also control antibody magnitude as well as antibody persistence.

The role of CD4+ and CD8+ T cells in vaccine-induced immunity

Virus- and bacteria specific CD4+ T cells and CD8+ T cells represent, together with memory B cells, the cellular arm of adaptive immunity triggered during infection or vaccination (7).

T-cell priming is a crucial event in the initiation of the immune responses to vaccination influencing both the magnitude and the quality of the immune responses induced (8). CD4+ T-cell priming is influenced by several factors such as the vaccine formulation, nature and dose of antigen and route of immunization, and, is required for the induction of high-affinity antibodies and immune memory. CD8+ T-cell priming needs various stimuli to become fully activated and induce differentiation and proliferation. These stimuli consist of T cell receptor (TCR) signals and environmental cues, including but not limited to dendritic cell (DC) activation and costimulation, CD4 ‘help’, and soluble inflammatory and growth factors (9).

CD4+ T cells play a central role in coordinating the adaptive immune response by providing support to the generation of and maintenance of B and CD8+T-cell responses. The activated CD4+ T cells differentiate into functional subtypes called T helper 1 (Th1), T helper 2 (Th2), T helper 17 (Th17), or regulatory T (Treg) cells (10-12). The development of CD4+ Th subsets is characterized by their cytokine
secretion. Th1 responses are sustained by cytokines such as IFN-γ, IL-2 and IL-12. Th1 responses mainly drive the development of a cytotoxic T cell-response and thus play a role in the defense against intracellular pathogens. Th2 responses are supported by IL-4 and the effector cytokines produced include IL-4, IL-5, IL-9, IL-10 and IL-13. Th2 cells play a key role in immunity to extracellular pathogens. Th17 cells, which are characterized by the production of IL-17 (11), play a key role in protective immunity toward extracellular bacteria and fungi (10). Tregs are involved in maintaining immune tolerance by suppressing activation of the immune system. Moreover, CD4+ Cytotoxic T-cells (ThCTL) with cytotoxic phenotype and function have repeatedly been described in humans and other species as cells that can potentially contribute to immune surveillance, recently reviewed by Marshall (13).

There is also ample evidence that CD8+ cytotoxic T lymphocytes (CTL) are key players in the adaptive immune response (14). One main property of CTL is in killing cells infected with intracellular pathogens, and thus contributing to clearance of infected cells. Similar to CD4+ T cells, CD8+ T-cells can also differentiate into unique and identifiable subsets, Tc1, Tc2 and Tc17 (12). As with Th1 CD4+ T-cells, Tc1 cells also produce high levels of IFN-γ and are considered to constitute the classic CTL. Tc1 cells kill target cells through either perforin- or Fas-mediated mechanisms. The cytokine profile associated with Tc2 cells include IL-4, IL-5, and IL-13, kill their targets predominantly through the perforin pathway and have been found in situations of chronic pathology such as persistent viral infections and allergic diseases. As their name suggests, Tc17 cells are characterized by expression of the IL-17 and have fewer cytotoxic effector functions. Thus, similar to CD4+ T cells, also CD8+ T cells also play a role in orchestrating the immune response.

Both CD4+ and CD8+ T-cells develop into memory cell subsets following resolution of viral infection/clearance, and the overall number of memory T cells may correlate with a protective immune response (15). Memory T cells have several key functional characteristics: they can produce a wide array of inflammatory and antiviral cytokines, elicit rapid cytolytic activity, and home efficiently to either lymphoid or non-lymphoid compartments.

Virus-specific T cell subsets identified at or near the peak of an antiviral T cell response have been defined as activated “effector” T cells while T cell subsets following resolution of viral infection/viral clearance are defined as resting “memory” T cells. These memory T cells respond vigorously and rapidly to recall stimulation with an antigen, and the memory T cell response thus typically peaks faster than the initial response to the same antigen and reaches a higher magnitude. Still, this response has to undergo several rounds of clonal expansion and requires several days to mature. Memory T cell populations have been further divided into “central memory” (TCM; CCR7+/CD62L+CD28+CD95+) or “effector memory” (TEM; CCR7−/CD62L−CD28−CD95+) subsets that differ by phenotype and function (12, 16). Recently, yet another memory phenotype has been described; the tissue resident memory T cell (Trm) (17). The Trm are thought to act as a first line
of T cell defense in epithelial surfaces such as the lung or respiratory tract and the gastrointestinal mucosa, which frequently are the first tissues infected with an invading pathogen.

Analysis of antigen-specific CD4+ and CD8+ T cell responses and their contribution to vaccine-mediated immunity is complex. Multiple parameters must be considered such as the expression of different HLA haplotypes, T cell epitope processing and presentation, the magnitude and the breadth of the total vaccine-mediated T cell responses. Another complication is the use of different approaches when quantitating T cell memory. However, the development of standardized and validated assays will likely expedite the analysis of vaccine-mediated immunity and future vaccine evaluation and facilitate unbiased comparisons between different studies.

The role of memory B cells (MBC) in vaccine-induced immunity

Memory B cells play an important role in the maintenance of antibody levels and the ability to respond rapidly after antigen exposure by either booster vaccination or re-infection with the corresponding pathogen. The long-term presence of vaccine-induced antigen-specific MBC and its relation with the presence of specific antibodies in serum has been studied by several groups (18-20). It appears as if the antibody-producing plasma cells and MBC are two different and independent pools, since the correlation between serum antibody concentrations and vaccine-elicited MBC is low. Further, vaccine-induced MBC do not provide direct protection against infection as antibody-secreting plasma cells do (18). Instead, MBC act as an important second line of immune defense that is initiated only if the pre-existing antibody levels are too low to prevent infection or if a pathogen breaches the critical first line of defense (21). It is sometimes speculated that MBC may have an important role in protection against pathogenesis caused by infectious diseases with long incubation time, such as HBV where memory B-cells have time to differentiate into antibody secreting plasma cells before onset of symptoms. The long incubation period of the disease thus allows the anamnestic response to be highly protective (2).

Although detection of antigen-specific memory B-cells is technically challenging, the analysis of antigen-specific memory B-cell responses in addition to the analysis of antibody levels/titers may be useful to understand the longevity and stability of the vaccine-induced immune responses. A better understanding of the mechanisms and maintenance of MBC in humans is important for the choices of optimal vaccination schedule and choices of methods for surveillance of immunization programs.
Method

Literature search and selection
We have systematically screened the original research articles focused on T-cells published since 2003 as well as the CMI-related review articles since 2008, until April 2015, in the PubMed and Cochrane databases. We have specifically searched for literature relating to correlates of protection and cell-mediated immunity induced by vaccination. For these searches, we have had assistance from an information specialist at the Agency. To guide the literature searches, we provided the information specialist with reference articles selected by the project group. In order to reduce the number of hits in the databases, search strings presented in Appendix 2 were created for each of the infectious diseases that are included in the national immunization program (diphtheria, tetanus, pertussis, polio, Haemophilus influenzae type b, measles, mumps, rubella, pneumococcal disease and HPV for girls (Table 1)), in the selective programmes recommended for at-risk populations (tuberculosis hepatitis B, influenza and pneumococci) and for vaccines under consideration to be included in the national vaccine program (varicella and rotavirus).

Inclusion criteria were time period, T-cells, memory B-cells, review and original articles. Articles in other languages than English, Swedish, Danish or Norwegian were excluded as well as preclinical experimental vaccine. Thus, articles addressing (i) antibody-mediated protection, (ii) diseases not included or considered for the immunization programmes, (iii) experimental vaccines or (iv) preclinical vaccine studies were used as exclusion criteria (Figure 1).

All articles were collected in an endnote library containing 1314 articles. Of these, 179 were review articles and 1135 were original research articles. These were subsequently divided into two separate libraries, one containing the original articles and one for the review articles. Among the 1135 original research articles, 840 were duplicates. After sorting out duplicate articles, all remaining 295 abstracts were read. MeSH terms and key words were used to focus the selection of articles. After the selection process outlined above, 27 review articles and 82 original research articles remained.

A similar systematic search was performed October 2014 for memory B cells using the search terms in Appendix 2. This search produced an additional 259 articles of which 120 were selected based on the same exclusion criteria and incorporated into a separate third EndNote library. Thus, our starting point for the literature survey was three libraries of totally 246 original research and review articles.

Ad hoc literature searches until 2018
Since the literature searches performed did not comprise all relevant literature, assessed by random sampling, more specific ad hoc literature searches were also performed using PubMed or Google scholar. Ad hoc searches were performed
using search terms including cell mediated immunity, correlate of protection, vaccine etc. In addition, ad hoc searches were also based on articles that were referenced in both review and original research articles that appeared to be of interest for this report. Many relevant articles were found this way.

In addition, ad hoc searches were necessary to find older literature (before 2008) than specified in our original searches, as well as articles describing experimental and animal vaccine studies in preclinical research needed as a proof of concept. Moreover, recent literature (2015-2017) - including both articles and books - in specific areas have been used in this review. In total, these ad hoc literature searches added an additional of 114 articles to our original search.

**Figure 1.** Flow chart of literature search and review process

<table>
<thead>
<tr>
<th>T-cells</th>
<th>B-cells</th>
<th>Ad hoc literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1314 articles</td>
<td>257 articles</td>
<td>116 articles</td>
</tr>
<tr>
<td>(179 reviews &amp; 1135 originals)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Duplicate s 840

Abstracts 474

*Excluded 120

109 articles (27 reviews and 82)

137 articles

In total 362 articles and reports

Time period (2003-2015), T- and B-cells, review and original articles. *Exclusion criteria: Languages other than English, Swedish, Danish or Norwegian; antibody-mediated protection; diseases not included or considered for the immunization programmes; experimental vaccines; preclinical vaccine studies. Ad hoc literature, time period (1960-2018).

**Contacts with pharmaceutical industry**

To add to our ad hoc searches and to understand what is being done at some of the main vaccine manufacturers, key persons within Sanofi-Pasteur MSD and GSK in Sweden were interviewed about their thoughts on and activities in CMI in vaccine evaluation. Both organizations provided us with a total of 24 original scientific publications of which 12 did not meet our inclusion criteria. Six of these articles did not appear in our original literature searches.

**Contacts with other Public Health agencies**

To gain knowledge on what is being done at other public health agencies, key persons within sister organizations such as Public Health England, The Dutch National Institute for Public Health and the Environment in the Netherlands, Norwegian Institute of Public Health in Norway, National Institute for Health and Welfare in Finland, Island, and Scientific Institute of Public Health in Belgium as
well as the Public Health Agency of Canada and the Center for Disease Control and Prevention in the US were contacted and interviewed regarding their activities in CMI-related methods used or investigated for laboratory surveillance of VPDs. These contacts resulted in indicating literature of a total of 26 additional articles, which did not appear in our original literature searches.

Delimitations of the review
A main delimitation of this report is the choice to focus on protection and surveillance of VPDs included or being considered to be included in the Swedish NIP. Other issues and areas of research not addressed in this review are adjuvants, innate immunity, autoimmunity as well as maturation of the immune system in neonates and immunosenescence regarding elderly.

Inventory of in-house capacity
Key staff at the Department of Microbiology, PHAS, was interviewed regarding equipment, GCLP validated methods and laboratory capacity available for CMI-related analyses as well as current personnel and competency. Results are summarized in Appendix 3.
Methods to study cellular immune responses

Most of the currently licensed vaccines use antibody levels of neutralizing, opsonophagocytic or bactericidal antibodies as the primary endpoint for determining vaccine immunogenicity. Serological analysis for surveillance are in general easy to perform and amenable to high throughput analysis. Moreover, serum and plasma samples are routinely collected at hospital laboratories, can be frozen and stored long-term at -20°C and are thus readily available for analysis.

Regarding CMI responses, the development and studies of vaccines faces several difficulties. One of them is the lack of standardized T-cell assay formats to be used and the issue of which parameters to be measured. Vaccine efficacy studies will be needed to establish an ICP. However, there are numerous techniques developed for quantification of T- and B-cell-responses (22-25), and each method has its relative advantages and disadvantages. Traditional and commonly used CMI assays measure T/B-cell function by (1) detection of cytokine responses; (2) phenotyping and characterization of T/B-cells; (3) assessing T-cell proliferation (4) assessing antigen-specific cytotoxicity as well as (5) novel systems biology approaches which include differential gene or microRNA expression. Appendix 4 includes a short description of the most commonly used assays described in the literature – and many of them also established at PHAS – for the analysis of immunological parameters characteristic for CMI responses as well as and pros and cons of each method. These techniques are described in details in Appendix 4 and summarized in Table 3.
Results

Correlates of protection

It is important to bear in mind that protection against infection may relate to different immune markers than protection against disease (26). In addition many different functions and mechanisms by which antibody may act, may serve as a correlation of protection (CoP). For the majority of the vaccines included in the Swedish national immunization program, except for HPV, mumps and pertussis, immunological correlates have been established and consist of a defined antibody level above which there is a high likelihood of protection (Table 2).

Table 2. Quantitative levels of correlates and surrogates of protection after vaccination (adapted from Plotkin 2010 (2))

<table>
<thead>
<tr>
<th>VPD</th>
<th>Vaccine</th>
<th>Test</th>
<th>Antigen</th>
<th>Protective level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphteria</td>
<td>Purified toxin</td>
<td>ELISA</td>
<td>Diptheria toxoid</td>
<td>0.1 IU/ml</td>
</tr>
<tr>
<td>Hepat B</td>
<td>Recombinant protein</td>
<td>ELISA</td>
<td>rHBsAg</td>
<td>10 IU/ml</td>
</tr>
<tr>
<td>HiB</td>
<td>Conjugated polysac</td>
<td>ELISA</td>
<td>Polysaccharide</td>
<td>0.15 ug/ml</td>
</tr>
<tr>
<td>HPV</td>
<td>Virus like particles</td>
<td>ELISA</td>
<td>Virus like particles</td>
<td>Not defined</td>
</tr>
<tr>
<td>Measles</td>
<td>Live attenuated virus</td>
<td>NT</td>
<td>Whole virus</td>
<td>120 IU/ml</td>
</tr>
<tr>
<td>Mumps</td>
<td>Live attenuated virus</td>
<td>ELISA</td>
<td>Whole virus</td>
<td>Not defined</td>
</tr>
<tr>
<td>Pertussis</td>
<td>Purified PT, PRN, FHA, Fim</td>
<td>ELISA</td>
<td>Pertussis toxoid</td>
<td>5 IU/ml*</td>
</tr>
<tr>
<td>Pneumo- cocci</td>
<td>Conjugated polysac</td>
<td>ELISA</td>
<td>Polysaccharide</td>
<td>0.35 ug/ml</td>
</tr>
<tr>
<td>Polio</td>
<td>Inactivated virus</td>
<td>Neutralization</td>
<td>1/4 dilution</td>
<td></td>
</tr>
<tr>
<td>Rubella</td>
<td>Live attenuated virus</td>
<td>ELISA</td>
<td>Whole virus</td>
<td>10-15 IU/ml</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Live attenuated virus</td>
<td>No antibody test</td>
<td>Rotavirus antigen</td>
<td>Not defined</td>
</tr>
<tr>
<td>Tetanus</td>
<td>Purified toxoid</td>
<td>ELISA</td>
<td>Tetanus toxoid</td>
<td>0.1 IU/ml</td>
</tr>
<tr>
<td>Tuber- culosis</td>
<td>BCG</td>
<td>Gp-ELISA</td>
<td>Mycobacterium bovi/ s BCG</td>
<td>Not defined</td>
</tr>
<tr>
<td>Varicella</td>
<td></td>
<td>&gt;1/64 dil; &gt;5 IU/ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not clearly defined, suggested level
Bacterial vaccines

For vaccines targeting encapsulated bacteria such as *Haemophilus influenzae* (Hib) and pneumococci, protection correlates to the presence of opsonophagocytic or bactericidal antibodies at a certain threshold level, although binding antibodies as measured by ELISA are used as surrogates (2). Protection against toxin-producing bacteria such as diphtheria and tetanus correlates to defined levels of post vaccination anti-toxin antibodies circulating in the blood (2). In the case of protection against pertussis, the situation is more complex. There is no established correlate of protection but antibodies against pertussis toxin (anti-PT) and attachment factors have been proposed as surrogate markers of immunity (2). However, an exact level of antibodies required for protection has not been possible to determine.

It is generally hold that T-cell responses against Bacille Calmette-Guérin (BCG) vaccine and tuberculosis in general have a protective function and that the presence of IFN-γ-producing CD4+ cells correlates with immunity (2). The BCG vaccine - recommended to defined risk groups in Sweden - provides protective immunity against meningitis and disseminated TB in children, although, it does not prevent primary infection or reactivation of latent pulmonary infection (27).

Viral vaccines

The first viral vaccine that is delivered in the national immunization program is the inactivated polio vaccine (Table 1). Neutralizing antibodies mainly directed to the polio VP1 protein block interaction with the virus’ receptor and seroprotective antibody levels have been defined.

At the age of 18 months, the measles, mumps and rubella (MMR) live attenuated vaccine is administered. The role of antibodies in the protection against measles is indisputable as evidenced by the fact that gamma globulin has been effective in preventing infection (2). However, T cells are required for recovery through blunting replication of the measles virus. In addition, T cell deficient children may suffer complications from vaccination, indicating a role for T cells in protection. For protection against mumps, neutralizing antibodies may not be sufficient. Passive immunization has not been demonstrated to provide protection (2), and there is no accepted immunological correlate of protection. The protective efficacy of T cells has yet to be demonstrated. Protection against rubella is primarily dependent of neutralizing antibodies, but T cell as well as memory B cell immunity may have a role in long-term protection.

The human papillomavirus (HPV) vaccine, a recombinant vaccine containing virus-like particles (VLP) of the LI capsid protein, was introduced in 2012 into the national immunization program in Sweden. Although antibodies are considered to be protective, there is no defined level that correlates with protection. Protection appears to persist after antibodies have waned, which suggests a role for local immunity in the genital area (28).
The hepatitis B virus (HBV) vaccine, is recommended to defined risk groups in Sweden. Antibodies against HBsAg (anti-HBs) mediate protection to HBV infection. Although antibodies wane with time after vaccination, protection appears to be solid and long-lasting (2). Postvaccination antibody levels correlate with induction of T helper responses that mediate the memory B cells. Thus, upon exposure to HBV, there is an anamnestic response that prevents disease, and often infection as well (2).

The rotavirus (RV) vaccine, an orally administered live attenuated vaccine, has been introduced in regional vaccination programs in some counties in Sweden and is to be enrolled in the national immunization program in 2018. Studies in humans and animals have reported correlations between rotavirus antibody levels and protection, the most consistent of which has been with serum rotavirus IgA (29). In addition RV vaccination studies in animals and humans show that there is a good correlation of protection with the levels of RV-specific IgA antibodies in the gut lumen (30). In general, assessment of mucosal responses is difficult, while measurement of serum IgA antibodies is easy. Thus, in the absence of a generally agreed mCoP, serum IgA provides a useful nCoP (26).

The seasonal influenza vaccine formulations contain either inactivated influenza antigens or live attenuated influenza virus (LAIV) from two influenza A strains and one influenza B strain. Recently quadrivalent vaccines incorporating an additional B strain have been developed. The vaccines induce strain-specific protection and have to be updated annually. Inactivated influenza vaccines (IIV) either contain subvirion (or split virus) or purified surface antigen (subunit). The IIV primarily stimulate humoral responses against the major surface protein, hemagglutinin (HA), and fail to induce strong anti-influenza CTL responses. Antibody response to HA have been shown to correlate with protection, but an exact antibody threshold value has not been established and may vary according to individual characteristics, populations, age groups and vaccine types. LAIV is for intranasal administration and is indicated for children from 2 to 18 years of age. LAIV replicates in host cells and induces strong CD4+ and CD8- T-cell immunity. No immune correlate of protection for LAIV has been identified.

Varicella vaccination will be considered in the future for being included in the national immunization program in Sweden. There is an overall consensus among clinicians that intact cellular immune responses have a crucial role in recovery from primary infection by varicella-zoster virus (VZV). However, antibodies play also an important role in protection against varicella, and there is a good correlation between the strength of vaccine-induced antibody response and the protection following exposure (2). In addition, children often withstand infection when vaccine-induced antibodies have waned to undetectable levels, suggesting a protective function for CMI (2).

In contrast, herpes zoster – the result of reactivation of varicella virus from dorsal root ganglia – occurs later in life when the cellular immunity wanes, especially CD4+ T cells, despite the persistence of antibodies (1). The two current zoster
vaccines contain either live varicella virus in a large quantity or the most immunogenic antigen glycoprotein E of the virus. Both induce antibodies and cellular response. The best CoP is a CD4$^+$ T-cell lymphocyte proliferation index, although there is also a statistical correlation with antibodies to viral glycoprotein E (31).

The role of CMI in some of the above mentioned vaccine-preventable diseases and vaccines will be addressed in separate sections below.
CMI in protection from vaccine-preventable diseases

The terminology regarding correlates of protection (CoP) is somehow confusing, since variety of definitions are being used. Plotkin and Gilbert (31) have reviewed the field and presented detailed definitions: CoP is described a variable immune response that is statistically associated with protection, and may be either absolute - a threshold value above which protection is certain - or relative, where higher values are quantitatively more protective than lower. However, the important distinction is between a mechanistic correlate of protection (mCoP) - directly responsible for protection – and non-mechanistic correlate of protection which is easy to measure but may only be a substitute for a mCoP that is unknown or difficult to measure. Moreover, the mechanism of vaccine induced protection is not necessarily the same mechanism as that for recovery from infection.

Vaccines included in the NIP

Correlates of protection are particularly clear for some of the vaccine-preventable diseases and the role of CMI will thus not be further addressed in separate sections below. For instance, the toxin-producing bacteria tetanus and diphtheria provides virtually complete protection at an antibody level of 0.1 µg/ml (2). After three doses of diphtheria toxoid, most children achieve antibody titers greater than the minimally protective level of 0.01 µg/ml. As regards Haemophilus influenzae type b, the main component of the capsule is polyribosyl ribitol phosphate (PRP). Anti-PRP antibodies have a protective effect against Hib infections, but the response is T cell-independent and of short duration. PRP covalently linked to a protein carrier elicit a greater immune response, including T-cell recruitment and development of a memory B cell response. Hib conjugate vaccines thus, stimulate both protective antibody responses and anamnestic responses on challenge. Serum anti-Hib capsular antibody levels greater than 1.0 µg/ml are associated with long-term protection and 0.15 µg/ml with short-term protection (32).

The pneumococcal conjugate vaccines containing 7 to 13 serotypes were developed according to the same principle as the Hib conjugate vaccines. Determination of serological correlates or surrogates of protection has been complicated considering that they vary depending on serotype and disease manifestation. Efficacy against invasive pneumococcal disease (IPD) was considered the gold standard when calculating serological correlates. A pooled model based on three efficacy studies was used and an ELISA antibody concentration of 0.35 µg/ml was found to correlate with protection against IPD (ref). Alternative cut-off values by another ELISA have subsequently been proposed (33). More recently the indirect cohort model has been used to develop serotype-specific correlates from protection (34). These correlates predict protection on a population level but cannot be used to predict protection in the individual case since this depends on multiple host and pathogen factors.
There are two vaccines against poliomyelitis, inactivated (IPV) and oral (OPV). The vaccines work in different ways but both elicits serum antibodies that prevent viremia, and neutralization at titers of 1/8 is protective (2). In Sweden, IPV - highly effective in eliciting humoral antibody responses - was used to eliminate poliovirus (35).

Measles

Vaccines against measles

There are several live attenuated measles vaccine available either as monovalent or measles-containing vaccine (MCV) in different combinations. The measles vaccine is commonly delivered as a component of the measles-mumps-rubella (MMR) vaccine. In Sweden, there are two licensed MMR vaccines, both containing live attenuated strains but different strains of measles virus (Schwarz and Enders Edmonston B). The vaccines are reported to protect equally well (1). In 1982 Sweden adopted a two-dose schedule in the NIP with vaccinations at the age of 18 months and at 12 years. In 2007 the vaccine schedule was modified and the second dose is given at age 6-8 years.

The aim of the measles vaccine is to induce protective antibody responses. Internationally, microneutralization titers of 120 mIU/ml is used to define the protective level. In order to survey the immune status of measles in the population and to monitor the effectiveness of the measles immunization programs, measles IgG seroepidemiological studies have been widely used. The effectiveness of the MMR vaccine is high (36): one dose of MMR is at least 95% effective in preventing clinical measles among preschool children and at least 92% (one dose) and 95% (two doses) effective in preventing secondary measles cases.

Although effective vaccination strategies are practiced, sporadic outbreaks of measles have continued to occur in even highly vaccinated populations due to importation of disease from other countries, failure to vaccinate, and in some cases vaccine failure (37, 38).

A number of factors influence the responses to natural and vaccine-induced infection, respectively. Demographic factors including gender, race, and ethnicity influence variations in both innate and adaptive cellular immune responses to measles (4). In addition, factors related both to the measles vaccine and the host may affect the quality and durability of immunity. In considering these factors, it is important to distinguish between primary and secondary vaccine failure, respectively. Primary vaccine failure due to lack of development of detectable antibodies occurs in approximately 5% of measles vaccinated individuals when the MMR vaccine is administered during the second year of life (4{Organization, 2017 #254, 39, 40}). Secondary failure occurs when an individual mounts an initial response to the vaccine but the protective response wanes over time (41). However, studies in revaccination shows that approximately 95% of children develop protective immunity after a second vaccine dose (42). An important cause of vaccine failure is the presence of maternal antibodies at the time of vaccination.
which leads to neutralization of the vaccine strain (43, 44). Thus, the recommended age at vaccination must consider and balance optimizing vaccine-induced protection while minimizing the risk of disease by delaying vaccination. However, Bertley et al. (43) have shown that early measles vaccination in the presence of maternal antibodies can sometimes prime for a balanced humoral and cellular immune response to subsequent revaccination.

Immune responses following natural infection - measles

The long-lasting immune responses and immunological memory following measles infection includes both continuous production of measles-specific neutralizing antibodies as well as measles-specific CD4+ and CD8+ T lymphocytes. The role of antibodies in protection against measles is well illustrated by studying vaccine failure.

The complexity of the T cell response has been illustrated in studies showing that while CD8+ T cells are mainly responsible for viral clearance in the lungs, CD4+ T cells are responsible for viral control in the central nervous system (45, 46). Previous studies demonstrate that natural measles infection can result in an acute and profound state of immune suppression and skew the immune response towards the Th2 phenotype, characterized by high production of IL-4 and increased immunoglobulin levels (47). In addition, in a recent study, Mina and colleagues (48) showed that measles infection induces a long-term immunosuppression (immune memory loss) consistent with recent experimental work that attributes the immunosuppressive effects of measles to depletion of B and T lymphocytes. In children, humoral immunity has been evaluated extensively, however, little is known about the ontogeny of cell-mediated responses to viruses during the first year of life (49).

Natural measles infection generally elicits much higher antibody titers than vaccination. Thus, for the increasing proportion of infants born to vaccinated mothers, the loss of transplacentally acquired measles antibodies occur at an earlier time point and render infants susceptible at a younger age. Several countries have therefore adjusted the timing of the first MMR to the age of 9 months or shortly thereafter (42, 49, 50). However, the vaccine effectiveness and immune responses are lower when vaccinating children below 12 months of age. WHO recommends that the first dose should be given at 12 months or later in countries with low circulation of measles. Recent studies have shown that the most optimal antibody response is achieved if the first dose is administered to children above 15 months of age (51).

Other studies also question the long-term effects of maternal antibodies on the efficacy of measles vaccination, indicating that maternal antibodies inhibit infant antibody responses without inhibiting the development of T cell responses (49, 52, 53).
Immune responses following vaccination - measles

The measles vaccine induces both humoral (long-term persistent neutralizing antibodies) and cellular immune responses similar in most respects to that observed following natural infection. Although antibody levels are usually lower and CMI responses less pronounced than those after natural infection.

The importance of cellular immunity as a complement to humoral immunity with regard to durability of vaccine immunity, providing long-lasting protection against measles, is well recognized (37, 54-56) (55, 56) (37, 54). Vaccination induces measles virus-specific CD4+ and CD8+ cells responses. As following natural infection, first, CD8+ T cells are activated for viral clearance followed by CD4+ T cell activation for support of antibody production. Vaccine-induced responses begins with a transient production of IL-2 and IFN-\(\gamma\), followed by a sustained production of IL-4, corresponding to an initial Th1-type response with a shift to a Th2-type response. Unlike the wild-type measles following natural infection, the MMR vaccine does not exerts any suppressing effect on cell-mediated immune responses and does not increase the risk of invasive bacterial or viral infection after the vaccination (57).

Studies in both animal models and humans have demonstrated that some individuals with poor or undetectable antibody titers following vaccination have readily detectable cellular responses (29, 37, 43, 44, 49, 53, 58). For instance, Bautista et al., studied the development of measles-specific antibody and lymphoproliferative (LP) responses in children for 6 months after MMR vaccination (37). They demonstrated that measles-specific LP responses can be detected in about two thirds of children at six months after MMR vaccination, although there was no correlation between measles antibody titers and lymphoproliferative responses. The value of early lymphoproliferative response for predicting the durability of immunity remains, however, to be determined. Dhiman et al., examined measles-specific antibodies, lymphoproliferation and the Th1 (IFN-g)/Th2 (IL-4) cytokines in a population-based cohort after two doses of MMR, demonstrating the presence of both measles-specific cellular and humoral immunity in the majority of the study population (47). In addition, a significant positive correlation between lymphoproliferation and secretion of IFN-\(\gamma\) and IL-4 were observed suggesting that immunity to measles may be maintained by both Th1 and Th2 cells. Moreover, measles antibody levels were correlated with lymphoproliferation but lacked correlation to either cytokine type. Ward et al. (56) have also demonstrated measles antigen-specific lymphoproliferative responses despite limited serum antibody production after a second dose suggesting that cellular responses to measles virus may be better maintained than humoral immunity after immunization and revaccination in some subjects (56, 59).

In general, memory B cells provide protection when the pre-existing antibody levels are low or if the pathogen is able to overcome the existing antibody titers (60). However, low but significant correlation have been found between numbers of antigen-specific memory B-cells and the circulating antibody titers for measles
virus (18, 21), while others reported no correlation at all (61). A Swedish study reported that antibody titers and memory B-cells (MBCs) against measles and rubella have different kinetics, indicating that the MBC pool and the corresponding antibody titers are regulated independently (62).

The quality and durability of vaccine-induced immunity depends on several factors related to both the vaccine and the host. The antibody titers induced by vaccination decline over time and may become undetectable (2, 63). However, persons who do not have detectable measles antibody may have protection via cellular immunity. The duration of vaccine-induced immunity has been studied by many research groups (2), but there are, however, discrepancies in reported findings, partly due to the use of different type of assays.

**Conclusions – measles**

Antibodies are most important for protection against infection and 2 doses of MMR vaccine are at least 95% effective against measles. However, if infection takes place cellular immunity must be functional to protect against severe disease. The importance of cellular immunity as a complement to humoral immunity regarding durability of vaccine immunity is well recognized. It has also been demonstrated that individuals with poor or undetectable measles antibody titers following vaccination have readily detectable cellular responses and may have protection via cellular immunity. Thus, data points to the important contribution of CMI for an efficient protection, especially in populations with waning or undetectable antibody levels. However, currently CMI does not seem to have a role in the regular and routine surveillance of the NIP.

**Knowledge gaps - areas for further research**

- CMI could be used in defining duration of immune protection, as a complement or instead of expensive clinical studies evaluating a booster dose.

- Investigation of the role of cellular immunity in measles and following measles vaccination in long-term studies to assess duration of the immune response and protection from measles.

**Mumps**

**Vaccines against mumps**

There are safe and efficacious live, attenuated vaccines against mumps since the 1960s (64). Mumps vaccines are available as monovalent, bivalent measles–mumps vaccine, and trivalent measles-mumps-rubella MMR vaccine which is used in most countries. In Sweden, there are two licensed MMR vaccines, both containing live attenuated strains but different strains of parotitisvirus virus (RIT 4385 and Jeryl/Lynn). The vaccines are reported to protect equally well (2). Sweden have adopted a two-dose schedule in the NIP with vaccinations at the age of 18 months and the second dose by the time of school entry (about 6-8 years of age).
It is a live attenuated vaccine, and the primary aim is to elicit neutralizing antibodies to the mumps virus. However, a precise seroprotective level has not been determined. The vaccine efficacy has been estimated to induce protection of over 90% of vaccinees that have received two doses (65). Nevertheless, outbreaks occur in vaccinated populations due to waning immunity. In addition, the level of neutralization varies against different mumps virus genotypes (66).

**Immune responses following natural infection - mumps**

The humoral response to mumps infection follows a pattern typical of that to respiratory viruses, including initial salivary IgA production, followed by virus-specific serum IgA/IgM and finally IgG production 2-3 weeks after symptoms onset, detectable for several years to lifetime (67). Neutralizing antibodies which appear during convalescence were previously believed to provide lifelong protection but this view is changing now.

The significance of cell-mediated immune responses in mumps infection has not been established. However, mumps virus-specific CMI responses such as cytotoxic T-cell activity, lymphoproliferation, and IFN production has been observed in persons following natural infection (67). It is reported that IFN-γ levels detected in serum, saliva and CSF do not correlate with illness severity (67). In general the presence/absence of mumps specific CMI responses correlate with the presence/absence of mumps virus-specific antibody detection. There are, however, some cases of seronegative persons with detectable cell-mediated immune responses (67).

Mumps-specific memory B cells can be detected decades after natural infection, although data suggest low robustness of this response as compared to that following measles or rubella infection.

**Immune responses following vaccination - mumps**

Neutralizing antibodies following mumps vaccination is considered a correlate of protection, however, the precise level of antibody required has not been established since titers of vaccine-induced neutralizing antibody associated with protection have varied widely (2). Also, the level of neutralization varies against different mumps virus genotypes (66).

Cell-mediated immune responses following mumps vaccination are similar in scope and magnitude to that detected after natural infection. During the 1950s and onwards, a delayed type hypersensitivity test known as the mumps skin test was used to determine immunity to mumps (68). This test was later shown to correlate with the development of antibody responses to mumps (69). As more modern methods to study T cell immune responses were developed, it has been shown that mumps-specific lymphocyte proliferation and IFN-γ production were induced following vaccination of infants (49, 70). It has been demonstrated that the lymphoproliferative responses persist for more than 20 years and do not differ significantly from the immune response induced by infection with the wild type
virus (54, 71). The longevity of the CMI response has been corroborated by Hanna-Wakim et al. (72), although natural infection induced stronger interferon-γ release, predominantly by memory CD4+ T cells. Whether the cells were effector or central memory or other type of memory phenotype was not determined. Thus, the immune responses induced by vaccination and natural infection was comparable with the exception of IFN-γ production. The relevance of IFN-γ release to protection is unknown and should be a topic for further investigations. In addition, there is at least one study that has demonstrated mumps-specific MBC (18). Their role in protection against mumps virus infection, remains, however, unclear.

Large outbreaks of mumps in MMR vaccinated populations raise the question on whether memory T cell responses are able or sufficient to prevent outbreaks in the absence of antibodies (73). A Finnish study of 20-year follow-up clearly showed a decrease in the proportion of individuals with seroprotective levels of mumps antibodies, despite a 2-dose vaccine schedule (63). Thus, the pool of susceptible individuals will increase annually.

Conclusions – mumps

No defined serologic correlate of protection has been defined, despite the presence of vaccine-induced neutralizing antibodies. The vaccine efficacy has been estimated to induce protection of over 90% of vaccinees that have received two doses. T-cell responses following mumps vaccination have been demonstrated, but their protective effect is unclear. Thus, waning mumps immunity and the occurrence of outbreaks in highly vaccinated populations (66) suggest a need to define a correlate of immunity but also to further investigate other approaches such as the benefit of a third vaccine dose (74-76).

CMI does not seem not to have a role in the regular and routine surveillance of the NIP. There are, however, important gaps in knowledge regarding understanding of mumps virus pathogenesis and the role of immunological factors involved in protection.

Knowledge gaps - areas for further research

- Waning of anti-mumps antibodies in vaccinated populations raises the question whether the cellular immunity against mumps wanes as well. Further investigations on the role of cellular immunity for protection would be of relevance.
- Further studies are needed to confirm if CMI alone can provide protection against severe infection.
- Knowledge on the persistence of vaccine-induced immunity to mumps virus is of great importance, especially when circulating virus disappears and natural booster infections no longer occur.
- Further studies to investigate immunological correlate of protection since studies of antibody duration yield wide ranging results.
• Passive immunization has not been demonstrated to be effective suggesting that other factors are important for protection against mumps disease.

• Further studies on the influence of different circulating genotypes (G and F) regarding level of protection by current mump vaccine based on genotype A (77).

Rubella

Vaccines against rubella
Rubella vaccines are available either as monovalent or in combination with other vaccine viruses. Most commonly, the rubella vaccine is delivered as a component of the MMR vaccine. It is a live attenuated vaccine, and the primary aim is to elicit protective antibody responses. The antibody response is measured by a variety of methods, although the immune response that correlates best with protection is neutralizing antibodies (2). However, neutralization test are not always routinely available so an ELISA test has become the predominant test in recent years, and an IgG response of 10 mIU/ml is considered to be protective (78), although there is some variation among countries in regarding the concentration of IgG antibodies considered to be protective. WHO estimates the vaccine efficacy to 90-100%, after one dose and protective antibody levels persist for years (78, 79). However, some investigators claim that the continued incidence of congenital rubella syndrome globally, despite the presence of anti-rubella antibodies, highlights the need to define better correlates of protection (80).

Immune responses following natural infection - rubella
Rubella infection occurring just before conception and during early pregnancy may result in miscarriage, fetal death, or congenital rubella syndrome (CRS) (78). Otherwise, rubella is a mild self-limited illness that usually occurs during childhood. Antibody responses are detectable about 14-18 days after acquired rubella infection, including initial IgM and IgG production. A rubella-specific T-cell response begins a week after the humoral response, and the cell mediated immunity appears to persist throughout life.

Immune responses following vaccination - rubella
Overall, rubella vaccine induces immune responses similar in quality but lesser in quantity to those elicited following natural infection. Rubella vaccination induces humoral responses of both IgM and IgG type as well as secretory IgA antibodies. However, the induction of neutralizing antibody is particularly significant and detected 6-8 weeks after administration. Up to 5% of all vaccinees fail to seroconvert; this may be due to either concurrent infection or, in young infants, to preexisting maternal rubella antibodies (78).
Vaccine-induced immunity is generally assumed to be lifelong, although rubella antibodies may fall below detectable levels (78). Several studies on the duration of protection over 10–21 years after vaccination, documented persistent seropositivity ≥95%.

Cellular immune responses to the rubella vaccine have been reported (81), although the significance to protection has not been established (80). Dhiman et al have shown that rubella vaccination elicits cellular immune responses, although the frequency of long-term lymphoproliferative responders was about half compared to the measles and mumps components of the MMR vaccine (54). HLA-restricted T-cell cytotoxicity has also been detected after immunization (309). Restimulated PBMC secrete Th1 cytokines such as IL-6 and TNF-a, but responses by memory T cells were fairly weak (80). Increase of TNF-a, IL-4 and IL-10 after vaccination have been reported (82). The data indicate that cytokine production after rubella vaccination correlates poorly to antibody responses. This finding was corroborated by Almenndinger et al. who failed to correlate IFN-γ ELISpot to rubella-specific IgG in serum (81). However, they demonstrated that almost all seronegative individuals had IFN-γ-producing T cells, resulting in virtually 99% vaccine responders at a mean of 10.5 years after vaccination. Interestingly, in a multi-cohort study investigating relationships between rubella vaccine-specific humoral and cellular immunity, a significant correlation between neutralizing antibodies and IFN-γ production has been observed. Curiously, the development of CMI responses after rubella vaccination has been reported to be faster in men, as measured by lymphoproliferation (83). The kinetics of the rubella CMI may thus be regulated by hormonal status, and may provide an explanation why adverse outcomes of rubella vaccination such as joint manifestations are rarely seen in males or prepubertal children (84).

Conclusions – rubella
Neutralizing antibodies is the immune response that correlates best with protection (85). There is, however, a broad variation in terms of protection since vaccinees with low levels of serum antibodies are often protected, while reinfection may occur in some individuals with detectable antibodies. Cellular immune responses have been studied, although they seem to have limited significance to protection (85). Neither antibody nor CMI status alone is sufficient to predict vaccine induced immune response, thus, evaluation of both arms of immunity is important for predicting the duration of immunity and the risk of reinfection in vaccinated populations. At the current stage, CMI seem not to have a role in the regular surveillance of the NIP.
Knowledge gaps - areas for further research

- Measurement of cellular responses after vaccination would be informative to better understand the potential role of T and memory B cells in vaccine-induced protection against rubella infection.
- Study the persistence of vaccine induced immunity against rubella.

Human Papilloma Virus

Vaccines against HPV

Human papillomaviruses (HPV) infections of the reproductive tract is the cause of a variety of conditions in both men and women including epithelial lesions that may progress to cancer of the cervix, vulva and vagina, anus, penis and the oropharynx (86). The HPV types 16 and 18 are the main causative agents of most cervical (70%) and anal cancers (85%), and HPV6 and 11 account for 90% of genital warts. Currently, there are three licensed HPV vaccines: Cervarix, a bivalent vaccine (bHPV) against HPV16 and 18, Gardasil, a quadrivalent vaccine (qHPV) against HPV 6, 11, 16, and 18, and recently a nine-valent vaccine (9HPV) providing protection against further HPV types (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58). These vaccines all contain virus-like particles based on the HPV L1 major capsid protein from the respective virus types. The qHPV and 9HPV vaccines are adjuvanted with alum (aluminum hydroxide, aluminum phosphate) whereas the bHPV vaccines contain an adjuvant system AS04 (3-O-desacyl-4'-monophosphoryl lipid A (MPL) adsorbed on aluminium hydroxide).

Immune responses following natural infection - HPV

The available data on immune responses to natural HPV infection is not completely understood (87). The humoral immune response is generally slow to develop (8-12 months), of low titre and avidity and, detectable serum anti-HPV levels are only induced in 70 to 80% of women (86). The appearance of anti-HPV responses is usually associated with clearance of infection but is not thought to contribute to viral clearance (87). There is an uncertainty regarding the effectiveness of neutralizing antibodies in preventing infection or reinfection with the same HPV-type (86, 88). In men there is little response to HPV infection and the antibodies produced are not protective.

Cell-mediated immune responses to HPV infection are not well defined. The severe outcome of HPV infection or reinfection in immunosuppressed individuals suggests that cell-mediated immunity is important (Einstein 2009), and that host factors as well as and viral mechanisms, play crucial parts in these inter-related processes. On the other hand it has been observed that defective cell-mediated immunity, for instance in patients with HIV, is associated with increased risk of persistent HPV infection and also with progression to invasive cancer. Thus, this implies that the host immune response mediates clearance of HPV infection (85),
although the main effector mechanisms involved in regression of benign HPV lesions are not well understood.

**Immune responses following vaccination - HPV**

HPV vaccines induce robust antibody responses in young women with practically 100% seroconversion against HPV types included in each vaccine. These vaccines are very effective in protecting immunocompetent subjects against infections with the vaccine HPV types and to some extent related HPV types (cross-protection). However, the exact role of various immune mechanisms in the protective efficacy of the HPV L1 VLP-vaccine remains to be determined. It is believed that the vaccine provides protection by inducing type-specific antibodies that interfere with transmission by binding to and neutralizing contaminating HPV prior to entry into basal cells. Currently, the assumption is that HPV VLP vaccines protect via antibody, although, evidence that antibody is the mechanism of protection is based, on preclinical studies in animals (89). In addition successful passive transfer experiments have been performed suggesting that the efficacy of L1 VLP-vaccines is mediated by humoral immune response.

Immune correlates of protection have not been identified in efficacy trials, partly due to the small numbers of vaccine failure. Nevertheless, the HPV vaccines stimulate type-specific neutralizing antibodies, which are considered the primary immune effector for the VPL vaccines and consequently important for protection. Vaccine-elicited serum antibodies are thought to reach the site of infection in the genital tract by active transudation (86). A randomized blinded study, in healthy woman aged 18-45 years, compared the immunogenicity and safety one month after completion of the three-dose vaccination course of Gardasil and Cervarix, (88). Both vaccines elicited serum neutralizing antibody responses, although Cervarix induced significantly higher antibody titers. The importance of differences in magnitude of immune response between the vaccines is unknown. Data on long-term studies evaluating duration of immunogenicity and efficacy after vaccination are needed. Both HPV vaccines have been shown to induce immune memory (90, 91).

The vaccine-induced antibody titers decrease rapidly during the first two years reaching a plateau level above those seen in natural infection (92). However, there are reports that protection remains also when serum antibodies have waned to undetectable levels (28). This suggests induction of a memory response, i.e. priming has been achieved. Several studies have shown that HPV vaccination induces systemic and genital T cell as well as memory B cell responses (88, 93-95). Cervarix vaccine has been reported to induce significantly more memory B cells in B cell ELISpot analysis than Gardasil, which may reflect a difference in adjuvant properties between the two vaccine formulations (88, 93, 96, 97). Moreover, Gardasil induces predominantly Th2 responses while Cervarix induces predominantly Th1 responses due to the differences in adjuvants. In addition, stronger antibody responses have been reported to be elicited by the AS04-adjuvanted vaccine (Einstein 2009), as well as increased CD4+ T cell responses
(93). However, although Cervarix is more immunogenic, it is not known whether it entitles greater clinical benefit since ICP is unknown and there is uncertainty what the immune memory stands for. Since HPV-induced cancers are slowly developing diseases, it seems plausible that there would be a theoretical window for both memory B cells and T cells to significantly contribute to vaccine-induced protection against disease, for instance by maintaining long-term vaccine-induced antibody protection. Both vaccines have been shown capable of eliciting CD8+ T cell responses (98, 99). This may be an important parameter in the protection against HPV-related tumors although this has not yet been supported in therapeutic immunization in humans (100), while other studies shows data in support of this finding (101, 102).

Also in immunocompromised individuals, HPV vaccines induce CMI. For instance, there were no significant differences in antibody or CD4+ T cell responses between healthy and asymptomatic HIV positive adult women (HIV viral load ≤400 copies/mm³) in South Africa (103). In a study in HIV positive children, however, antibody responses appeared to wane faster over time, although CD8+ T cell responses persisted (99).

The immune response to HPV infection is not completely understood; neutralizing antibodies probably help preventing reinfection but may not contribute to clearance of HPV virus (87), and defective cell-mediated immunity is associated with increased risk of persistent HPV infection. On the other hand, HPV VPL vaccines induces robust T-cell responses to L1.

Conclusions – HPV

A variety of efforts are ongoing to monitor biologic outcomes of HPV, including prevalence to cancer, genital warts and cervical precancers – given that the major outcome to be prevented (cancer) occur many years after infection. The mechanism of protection following HPV VPL vaccines is assumed to be mediated by type-specific neutralizing antibodies. However, the level of antibody needed for protection or immune correlate of protection is not established. HPV immunization also elicits memory B cells, but their contribution to long-term protection is unclear. Cell-mediated immunity seem not to be directly involved in protection following vaccination, despite the fact that HPV VLP vaccines induce robust T-cell responses (87). To summarize, currently CMI does not seem to have a role in the regular surveillance of the NIP.

Knowledge gaps - areas for further research

- Further research is needed on the long-term clinical effectiveness and the duration of protection.
- Potential added value of in situ cellular immune responses.
Pertussis

Vaccines against pertussis

Two types of vaccines against pertussis are available: whole-cell (wP) vaccines and acellular (aP) vaccines. Whole cell vaccines consist of killed \textit{B. pertussis} bacteria and are most commonly used in low- and middle income countries. Acellular vaccines have gradually become the predominant type used in Europe and industrialized countries (104). aP vaccines contain up to five different purified components of the bacterium: pertussis toxoid (inactivated pertussis toxin; PT), filamentous hemagglutinin (FHA), pertactin (PRN) and fimbriae serotype 2 and 3 (Fim2/3).

Globally pertussis vaccination coverage is high with 86% of the children receiving the vaccine (105). In Sweden, vaccination with diphtheria-tetanus-acellular pertussis (DTaP) vaccines started in 1996 after a 17-year period of no pertussis vaccine being included in the NIP following the withdrawal of the wP vaccine due to concerns about its safety and efficacy (106). Children are vaccinated with a primary series at ages 3, 5 and 12 months, and a booster dose (DTaP-polio) is administrated at the age of 5. In 2016 an additional dose (dTap) at the age of 14-16 years was introduced in the NIP.

During the last few years, several countries have however reported a resurgence of pertussis despite high vaccination coverage (107). This indicates that the currently available vaccines are not optimal in providing protection against pertussis. Therefore studies on optimization of acellular vaccines (eg. including additional antigen or altering adjuvants as reviewed by Locht et al. (108) as well as development of live attenuated vaccines by an ongoing phase I clinical study of a live attenuated 	extit{Bordetella pertussis} vaccine BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers, aiming to increase the vaccine induced protection (109).

Immune responses following natural infection - pertussis

Following natural pertussis infection, antibody against pertussis toxin (PT) is detected in 80%-85% of patients (104). The actual duration of protection conferred by natural infection in humans is not fully established, but is thought to range between 10 and 20 years (110). The induction of a Th1 type response may be an essential element in generating a long-lasting immunity. In children, pertussis infection is associated with induction of predominantly Th1 cells and release of IFN-\(\gamma\) secretion (111, 112). More extensive studies of the relationship between CMI and protection obtained by natural infection have been performed in a baboon model, where previously infected animals display a sterilizing immunity without the increase of pertussis-specific antibodies during the first days after reinfection (113). The same model has also been used to further demonstrate that the response evoked by natural infection is of the CD4+ Th1/Th17-type (113). Similar findings have also been seen in the mouse model, as reviewed by Fedele et al.(114).
Like many pathogens causing persistent infections, B. pertussis has developed several mechanisms to subvert host immunity, such as defects in memory T–cell homing (115), and induction of regulatory T cells (116-118) that may negatively affect immune priming, including humoral responses.

**Immune responses following vaccination - pertussis**

Even if no serological correlates of protection have been widely accepted for pertussis vaccines, it is well documented that antibody responses are critical effectors to mediate protection and antibodies against PT are particularly correlated with protection (119, 120). However, no seroprotective threshold value for anti-PT have been identified.

Generally, both aP- and wP-vaccines elicit pertussis-specific antibody responses and protect against disease. There are, however, many different types of aP- and wP-vaccines on the market with varying degree of efficacy. In general, the aP vaccines differ from wP vaccines regarding antigen content and quantity, production methods and type of adjuvant used, which contributes to the differences in the elicited immune responses (104, 121). In addition, the number and concentration of components included in the aP-vaccines differ between vaccines and have in some studies been shown to affect the efficacy (122). An extensive Cochrane report summarizes that multi-component acellular vaccines are more effective than low-efficacy whole-cell vaccines, but may be less effective than the highest-efficacy whole-cell vaccines (123). A direct comparison between different pertussis vaccines must therefore be done with caution.

The vaccine-induced antibody responses wane quickly after aP vaccination to low, but detectable, levels (124-126). In Sweden, aP immunization consisting of a primary 2-dose series and a booster at age 12 months, provided protection against pertussis for approximately 5 years (127). Therefore, booster immunizations at defined intervals are necessary to maintain the antibody responses. Thus, like natural infection, pertussis vaccination does not induce lifelong protection and there is no known single correlate of protection (119, 120).

T-cell and memory B-cell responses are detectable in individuals following infection or vaccination. Natural infection as well as wP-vaccines have been shown to induce a cell-mediated Th1 response whereas aP-vaccines induce an antibody-mediated Th1/Th2 response in humans (111, 112, 120, 128). This type of response profile has also been shown in a non-human primate model (113, 129). In addition, this model has been used to evaluate the contribution of the Th17-response in pertussis immunity, showing that Th17-responses are important for the clearance of pertussis (113, 130). This type of response has however not been thoroughly studied in humans.

The different T helper cell profiles evoked by the Pa-vaccine and the Pw-vaccine (Th1/Th2 and Th1, respectively) have been suggested to be a plausible reason behind the recent resurgence of pertussis. Using the baboon model, it has been shown that Pa-vaccinated animals were only protected against disease but not
against colonization of the bacteria whereas the Pw-vaccinated animals obtained sterilizing immunity (129). This indicates that in a Pa-vaccinated population it will be a large number of subclinical infections and consequently exposure to unprotected individuals, like the infants. As Pw-vaccination evokes a more cell-mediated Th1-type of response this suggests that CMI is an important contributor to the protection against pertussis infection.

Several studies have tried to propose recommendations regarding the pertussis vaccination schedule based on CMI data (131-134). However, as no epidemiology data was collected in these studies it is difficult to draw any conclusion on what type of CMI is crucial for protection in humans. Typically immune correlates are determined within the scope of clinical vaccine efficacy trials, where subjects can be followed for long-term protection against disease. Unfortunately, trials of this systematic approach on CMI are scarce for pertussis. There are however several studies on CMI induced after infection (111, 135, 136) and vaccination (112, 131-133, 135, 137-143) that shows an active CMI response. T-cell activity has been reported for up to 5 years after infection (112) or vaccination (132, 142). Moreover, vaccinated subjects without any PT-specific antibody response have been shown to have an active T-cell response, indicating that immunity against pertussis is not dependent on humoral immunity only (132). Similar findings have also been found with regards to the memory B-cell response (21, 144).

**Long-term pertussis immunity**

Most of the studies on the longevity of pertussis-specific immune responses following vaccination are based on cross-sectional sampling and only a few are available on paired sampling (132, 141, 145). The lack of stringent data is a limitation to the conclusions that can be drawn from these studies.

The long-term follow-up of pertussis-specific immunity following vaccination is also compromised by the ongoing circulation of the pathogen. Following vaccination both the memory B- and T-cell response is present, but the increase of pertussis-specific immune cells is transient as detectable in blood (146-149). However, several studies investigating the long-term effect have reported higher levels of pertussis-specific immune cells several months or years after the primary vaccination peak levels, indicating that the subjects have been boostered by natural infections (132-134, 147). Interestingly, natural boosting has also been reported as early as 15-19 months after primary vaccination (145), illustrating the ongoing circulation of pertussis in the population.

**Conclusions – pertussis**

There are many limitations in addressing the duration of pertussis-specific immunity following both natural infection and vaccination. For instance, no clear serologic marker has been defined for protective immunity against pertussis. In addition, by 2-3 years following natural infection or vaccination with either wP or aP antibodies have waned to barely detectable levels although immunity against
pertussis remain. The interplay between waning immunity and boosting of pertussis immunity by natural infection and vaccination is another limiting factor to evaluate duration of immunity. The data available today is insufficient to draw any final conclusions on what type of CMI parameters are important to assess when evaluating pertussis vaccine efficacy. Further clinical studies are needed to identify those parameters. More knowledge on pertussis-specific CMI, especially Th1 and Th17, will most likely contribute to development of better vaccine formulations and optimized vaccination programs.

In conclusion, even though more and more data points to the important contribution of CMI for an efficient protection against pertussis, its role in the regular surveillance of the NIP is unclear. Existing methodology for surveillance of CMI is extremely costly and time consuming and therefore unsuitable for the type of large scale investigations required in the NIP. With more research and improved methodology, more accurate parameters of protection can be identified and possible to be considered for NIP surveillance in the future.

Knowledge gaps - areas for further research

- No clear serologic marker has been defined for protective immunity against pertussis. Studies are thus needed to define a CoP.
- Since pertussis vaccination does not induce lifelong protection, further studies are needed for long-term evaluation of duration of immunity
- In animal models Th17-reponses have been shown to be important for the clearance of pertussis. This type of response need to be thoroughly studied in humans. CMI data may help to guide decision on optimal time for booster vaccines
- Additional investigations on possible differences in time and degree of waning of protection from pertussis in children who receive different aP vaccines.
- In addition, deeper understanding of pertussis maternal immunization in human is needed, e.g. the duration of the transferred protective immunity in the infant and on potential interference with routine immunization programme,

Vaccines - at risk populations

Tuberculosis (TB)

Vaccines against tuberculosis

Bacille Calmette Guérin (BCG) – a live attenuated strain of *Mycobacterium bovis* vaccine was developed in 1921 and remains the only vaccine against *Mycobacterium tuberculosis* (Mtb) available for clinical use. BCG is routinely administered to infants in most countries and provides significant protection against severe forms of TB and a high degree of efficacy against disseminated TB during childhood when vaccinating within the first few months of life (150, 151).
However, this vaccine has shown variable and inconsistent levels of efficacy against pulmonary TB in adolescents and adults (152). Research on the duration of the vaccine effect is limited and the results vary, but overall there are good evidence for a protective effect of up to 10 years (153). Individual studies have been able to detect protection over 15 years following vaccination (154). Disease in latently infected adults and adolescents accounts for most of the disease burden, and transmission, worldwide. Sweden practices a risk group vaccination policy of children of family origin in a country with an increased incidence of tuberculosis (154), from 6 months of age to 18 years, given as a single dose of BCG vaccine according to the WHO’s recommendations.

**Immune responses following natural infection - tuberculosis**

Due to the intracellular location of *Mycobacterium tuberculosis* infection, antibodies against TB have only been attributed a minor role in control of the disease. Monocytes and T cells have been shown to be the important in the response against infection (2). In general, it is relatively well established that CD4+ T cells play a critical role in protective immunity by production of Th1 cytokines including IFN-\(\gamma\) and IL-2 (155). However, the exact immunologic mechanisms and nature of T cell responses required for an effective immune response and protection remains unclear (156-158). It seems that progressive Tb is more commonly associated with a Th2 or a mixed Th1/Th2 T-cell response, while Th1 response mediates protection (2). However, CD8+ cells are also believed to contribute to protection (159), although no firm conclusion can yet be drawn.

The tuberculin skin test, which involves assessment of the immune reaction to an intradermal injection of purified protein derivative (PPD), remains the most widely used method for detecting immune responses to or infection with *Mycobacterium tuberculosis* and for tuberculosis surveillance since its development in 1930s. However, a variety of host-related factors such as age, immunosuppression, poor nutrition and viral infections can decrease tuberculin reactivity resulting in false-negative reactions. False-positive reactions also occur due to cross-reactivity to environmental mycobacteria. In addition, the tuberculin test cannot distinguish responses caused by BCG vaccination from those caused by infection.

The detection of antigen-induced IFN-\(\gamma\) in cultures of whole blood or PBMC has become a standardized method to assess immune responses against TB following exposition (60). These Interferon-Gamma Release Assays (IGRAs) can distinguish between latent infection and BCG vaccine response since they react to the antigens ESAT-6 and CFP-10 which are not present in the BCG vaccine. This makes the IGRAs more specific for M. tuberculosis than the PPD which also may react to previous BCG vaccination or infection with atypical mycobacteria. Note that the test cannot be used to assess BCG vaccination status. The IGRA assays are not recommended for children below two years old due to specificity problems (154). The assays may however be suitable for evaluation of new Tb vaccines.
**Immune responses following vaccination – tuberculosis BCG**

BCG induces a Th1-like CD4+ T cell response consisting of multiple patterns of cytokine production although single expression of IFN-γ is most frequently described (157, 160, 161). CD8+ T-cells are also activated following BCG vaccination with predominant IFN-γ production, but at lower levels than CD4+ T cells. BCG-induced CD4+ and CD8+ T cells may also have a cytotoxic function as well as proliferative potential (162, 163) and there is evidence suggesting that induction of polyfunctional CD4+ and/or CD8+ T cells is important for vaccine-induced memory responses. In addition, vaccine-induced IL-17 expression by CD4+ cells is also detectable (160). A study in BCG-immunized infants revealed no association between the frequency of polyfunctionality expressed as IFN-γ, TNF, IL-2, and/or IL-17 and risk of TB disease (160).

IFN-γ is essential for protective immunity, and thus immunological assays currently used in clinical trials focus on evaluation of the IFN-γ production by Th1 cells (164). However, animal and human studies have found that IFN-γ alone is not sufficient to prevent TB-related disease (165). Jasenosky et al (157) reviewed findings from vaccine trials showing that most novel vaccines induce Th1 responses, mainly characterized by IFN-γ, TNF, as well as IL-2 production. In some cases, CD8+ T cell immunity is also elicited. The authors question the common practice of measuring conventional CD4+ and CD8+ T-cell responses as outcomes of vaccination, because there is no identified correlate of protection against TB. Efficacy studies (RCT) are needed to improve our understanding on immunological markers and to investigate/define an immune correlate of protection.

Functional assays measuring in vitro mycobacterial growth is another area of interest for evaluation of BCG vaccination (166). In addition, alternative strategies to gain insight into effective adaptive immune parameters are needed. These could involve assessment of non-conventional T-cell responses such as induction of CD1 and HLA-E mediated T-cell responses, γδ T-cell responses, or populations of T cells that primarily reside in the lung, for instance mucosal-associated T cells or Trm cells (167).

A number of reports on TB biomarkers or surrogate end-points of protection have recently been presented (152, 168-172). Ottenhoff et.al (152) has summarized, and briefly discussed the major challenges for biomarker identification and validation. Fuhrmann and colleagues (173) have reviewed the influence of flow cytometry in the study of TB immunology and clinical diagnosis.

The BCG vaccine has been shown to provide relatively good protection in children but not in adults. Its efficacy in certain regions and against disseminated TB and TB meningitis supports the ongoing use and further development. The fact that protection in preclinical challenge models does not reflect field efficacy data urges the need for a human challenge model to facilitate selection of candidates to proceed to efficacy studies (152). In addition, improved vaccination strategies under development will also require broader characterization of the antigens.
involved in the early phase of TB infection, increased and precise understanding of protective immunity and protective host defense against TB, as well as deeper understanding of the features of T cell memory necessary for induction of effective and long-term immune responses against TB.

Awaiting a more effective vaccine, Sweden has adopted public health practices of TB control including timely contact investigation, early detection of infected people and treatment of active pulmonary TB as effective measures to reduce the risk of spreading infection.

Current TB vaccine research and development has been directed towards two main areas: 1) improvement of the level of efficacy of the current BCG by novel vaccine formulations and 2) development of new vaccines to improve or replace the current BCG vaccine by co-administration or boosting. This includes vaccine designs such as: live attenuated mycobacteria (rMTB, rBCG); inactivated whole-cell vaccine; viral vector vaccines with TB antigen (MVA, Adeno); and subunit vaccine with adjuvant and DNA based vaccines. Improving the immunogenicity of the vaccine has led to several versions of BCG vaccines for boosting that increase the T cell response after initial BCG vaccination early in life (174, 175). Boosting BCG with MVA-85A or Ad5-85A resulted in improved efficacy over the use of BCG alone (176). However, a recently performed phase 2b study in infants previously vaccinated with BCG showed that MVA85A did not provide any significant effectiveness against either disease or M. tuberculosis infection (177). Due to the complex nature of Mtb several novel live attenuated mycobacterial vaccine candidates including *M. tuberculosis* gene deletion mutants and recombinant strains are currently being developed as alternatives to the BCG vaccine (178). There are currently more than 15 new TB vaccine candidates in various phases of clinical trials in humans: six vaccines in phase I, six in phase II, two in phase IIb and one in phase III clinical trials (166, 179).

**Conclusions – tuberculosis**

Currently, a number of Tb vaccine candidates are in various phases of clinical trials. This provide an opportunity for assay development of new exploratory assays as well as the standardization, validation and harmonization of assays to allow comparison between vaccine candidates. Thus, the development of assays parallel the development of new vaccines and technical advances achieved simultaneously with broadening understanding of vaccine immunity. Efficacy studies are however needed to improve our understanding on immunological markers and to define an immune correlate of protection. A phase IIb/III clinical trial require a robust clinical and laboratory environment.

At present, CMI seem not to have a role in the regular and routine surveillance of the NIP.
Knowledge gaps - areas for further research

- Today, a main difficulty in studying and monitoring BCG immunization is the lack of a correlate of protection but also lack of a reliable and standardized immunoassay.

- CMI could in the future be a useful tool to assess and determine vaccine efficacy of new vaccines, based on other antigens than BCG.

- Immunology assays currently used for the evaluation of Th1 responses in vaccine trials for new tuberculosis vaccines includes whole-blood cultured IFN-γ-ELISA, IFN-γ-ELISpot and IFN-γ-ICS. These assays can be combined with polyfunctional flow cytometry to evaluate IFN-γ, TNF-α and IL-2 producing cells in conjunction with their effector functions and surface markers profile.

- Functional assays assessing in vitro mycobacterial growth inhibition (MGIAs), are also of interest for evaluation of BCG (180).

- Incomplete understanding of protective responses despite the notion that cellular immunity induced by BCG vaccine is the protective arm against tuberculosis.

Hepatitis B Virus

Vaccines against Hepatitis B

The currently available safe and effective vaccines can prevent disease caused by hepatitis B virus (HBV). The HBV vaccine consists of recombinant hepatitis B surface antigen (HBsAg) proteins that are typically produced in mammalian cell lines or in yeast cells, and form virus-like particles (VLP). These VLPs have a high content of host cell lipids, which promotes conformational integrity as well as antigenicity. HBV vaccination typically results in long-lasting protective antibody responses in 90-95% of healthy individuals (181-183) after completion of the standard vaccination regimen (0, 1, 6 months schedule).

From a public health point of view, the main objective of vaccination against hepatitis B is to prevent the development of persistent carriage of HBV – thereby preventing chronic liver disease and its long-term complications cirrhosis and hepatocellular carcinoma – and to eliminate the pool of chronic carriers in order to eliminate the transmission of hepatitis B infection to susceptible contacts.

Since Sweden is a low incidence-country, a risk group vaccination strategy is practiced. However, regional HBV vaccination programs of children given as hexavalent vaccines (DTaP-HepB-IPV-Hib) have been implemented by all counties in Sweden.

The HBV vaccination regimen of infants consists of three immunizations administered at 3, 5, and 12 months. The need for further booster immunizations is currently unclear. Measurement of vaccine-induced anti-HBsAg antibody levels is used to assess the effectiveness of anti-HBV-vaccination. Anti-HBs concentrations of 10 mIU/ml or more, preferably measured 1-3 months after completion of a
primary vaccination course, is considered a reliable immunological correlate of protection against HBV infection. How long time antibody levels persists related to the initial peak level of anti-HBs after primary immunization (184).

Immune responses following natural infection – Hepatitis B
Most HBV infections are self-limiting and followed by development of hepatitis B-specific antibodies (anti-HBs anti-HBc) (185). T cell mediated immunity is considered essential for adequate control and clearance of HBV infection (186). Chronic HBV infection progress through different disease phases which are strongly associated with patient age, including a so-called immune tolerance phase with active viral replication without active liver disease in children who acquire the infection perinatally (187). High frequencies of HBV-specific CD4+ helper and CD8+ effector T cells can be detected in the acute phase, but not in the chronic phase of HBV infection. Virus-specific CD8+ T cells are recognized as the main effector cells and experimental depletion delays the clearance of acute HBV (188, 189). Furthermore, epidemiological studies support the notion that HBV-specific T cells might compensate for a lack of humoral protection (190).

Immune responses following vaccination – Hepatitis B
A variety of hepatitis B vaccine schedules have been shown to induce a protective long-lasting antibody response in more than 95% of infants and adolescents, and in >90% of healthy adults aged less than 40 years, after the third dose (187). Anti-HBs is an easily measurable correlate of protection using serologic assays. Immunogenicity is generally known to persist approximately 10-31 years depending on age, body mass index, gender and smoking status at the time of initial vaccination (191). Typically, anti-HBs responses decline rapidly during the first year and more slowly thereafter (192, 193). Although, disappearance of anti-HBs does not necessarily indicate loss of protection, given the evidence that immune memory in immunocompetent adults is maintained for at least 30 years despite decrease or loss of vaccine-induced anti-HBs antibodies over time (183, 185, 194).

There is also a small proportion of healthy individuals, both in adults and children, whose response to the HBV vaccine is poor or undetectable (non-responders) (195). However, attempts to elicit responses in non-responders have been made by alternative strategies including double dosing, more frequent dosing, intradermal vaccine, adjuvant vaccines and recombinant vaccine with variable efficacies (196, 197) and some of the strategies show improved response and lasting immunity.

Despite these additional regimens some individuals remain non-responders. Accumulated data from a range of follow-up studies including altogether more than 7000 infants, children and adults showed that despite that certain vaccinees had lost their vaccine-induced HBV antibodies none developed clinical manifestations of HBV disease (183). Moreover, natural boosting has also been proposed to contribute to prolonged persistence of anti-HBs and protection in high incidence
countries (198). There is however no data that highlights the importance of a possible natural hepatitis B booster for the long-term immunity or protection.

On rare occasions, HBV breakthrough infections, illustrated by the seroconversion to anti-HBc, have been reported in young adults, who initially had low responses to the HBV vaccine (199, 200). No clinically symptomatic infection, acute or chronic, were however reported. The studies showed that after 15–18 years following the primary vaccination course at infancy, only 50 percent of the individuals had detectable levels of anti-HBs which again raises the question on booster vaccination later in life. However, breakthrough infections are rare, and more studies of the duration of the immunological B cell memory are warranted to understand if and when booster doses are needed for those vaccinated during infancy. Samandari et al. have shown that there are challenges to successfully boost the anti-HBV antibody responses with booster vaccinations in older adolescents (non-responders) (201). Many factors are related to HBV vaccination non response, among others: age, gender, infectious, obesity, smoking etc, (202, 203).

A number of in vitro experiments performed by Van Hattum and Boland have shed light on the mechanisms of immune memory (204). Anti-HBs titers were investigated 10 years following vaccination and peripheral mononuclear cells were also collected to test lymphocyte proliferation to HBsAg. The study showed that T lymphocyte proliferative responses to HBsAg were positive in the majority of vaccinated persons whose anti–HBs titers were below 10 mIU/mL. Additional studies have provided evidence of both B and T cell lymphoproliferative responses elicited by vaccination (181) as well as long-term persistence of IFN-γ and IL-5 secreting Th1 and Th2 lymphocytes (200), and the presence of memory B cells in persons with undetectable specific antibodies (191). Using ELISpot assays, the presence of circulating memory B cells and anti–HBs-producing cells have been shown in vaccinees who have lost their detectable antibody levels (183, 191, 205).

Anamnestic responses correlates with lymphoproliferative T cell responses (183) and the strength of the response is correlated to the primary antibody response and antigen dose (206). The HBV vaccine induces an immune memory that can rapidly be activated following natural infection as well as revaccination. Thus, generation of immunological memory following vaccination is relevant in addition to the generation a particular level of anti-HBs and defining good markers of a relevant memory response would greatly facilitate studies on hepatitis B long-term protection. However, the long-term persistence of immune memory is not known, since recent studies have indicated that the memory can eventually be lost (207, 208). Studies in the fourth decade following primary hepatitis B vaccination are awaited to clarify the need of booster doses.

Today little is known about the quality and quantity of HBsAg-specific T cell responses and their relation to antibody protection in immunosuppressed individuals, such as kidney transplant patients (209). Immunosuppressed patients with dialysis or advanced HIV infection have a suboptimal response to standard doses of hepatitis B vaccine. These persons are recommended modified dosing
regimens, including double doses of HBV vaccine or administration of additional doses, and regular serological controls (210).

Conclusions – Hepatitis B
In general, studies demonstrate high effectiveness of vaccination against hepatitis B. Also a number of studies indicate that despite antibody decline or loss, immune memory exhibits long-term persistence for up to at least 30 years after vaccination, among healthy adults (193, 199-201, 211-214). Nevertheless, more studies on the duration of the immunological B cell memory are warranted to understand if and when booster doses are needed for those vaccinated during infancy.

Currently, CMI does not seem not to have a role in the regular surveillance of the NIP.

Knowledge gaps - areas for further research
• Studies for deeper understanding on the absence of immune responses among non-responders
• Monitoring of the immune memory in selected vaccinated populations for information and help predict the need for booster doses and booster vaccination, as a complement or instead of costly booster studies.
• Evaluation of long-term protection and the role of CMI in sustaining immunity against hepatitis B.

Influenza virus vaccines
Vaccines against influenza
Current seasonal trivalent vaccine formulations contain either inactivated influenza antigens or live attenuated influenza viruses from two influenza A strains (A/H1N1, A/H2N3) and one influenza B strain. During the 1990s, influenza B viruses diverged into two antigenically distinct lineages based on the hemagglutinin (B/Victoria, and B/Yamagata lineages) (215). This led to the development of quadrivalent vaccines that incorporate two B strains, which have occupied an increasing proportion of the global market since their introduction (216).

A key feature of the influenza virus is its error-prone polymerase that results in accumulation of genetic mutations that are selected for in hemagglutinin (HA) and to a lesser extent neuraminidase (NA), the major surface glycoproteins of the virus. Influenza A viruses exhibit both antigenic drift and antigenic shift, whereas influenza B viruses show only antigenic drift. The continuous antigenic drift of the HA protein is the basis for the annual updating of the composition of seasonal influenza vaccines. Recommendations for the antigenic composition are made by the WHO semiannually to ensure that current influenza vaccines are effective against recently circulating strains in both the northern and southern hemispheres (217, 218).
Vaccination is the primary strategy for the prevention and control of influenza. National influenza programs, including the Swedish, generally target specific persons who are at elevated risk of severe or complicated disease based on age, underlying medical conditions, or pregnancy status (219). In addition, some EU countries have introduced childhood influenza vaccination programs (220). A few countries, such as the United States and Japan, have implemented a universal program recommending routine annual influenza vaccination for all persons aged ≥6 months (221).

In Sweden the available seasonal vaccines include trivalent inactivated influenza vaccines (TIVs), two recently licensed quadrivalent inactivated influenza vaccines (QIV) (222) and one quadrivalent live attenuated influenza vaccine (QLAIV).

Inactivated influenza vaccines

Inactivated influenza vaccines (IIVs) can be categorized based on their production methods into three types: whole cell (virions), subvirion (or “split virus”) and purified subunit (purified surface antigen vaccines) (223). Whole-cell vaccines have become less common because of relatively high reactogenicity (224). Split virus is most widely used among TIVs and has an advantage containing all virus proteins, whereas the subunit vaccines only contain the HA and NA proteins. The production of IIVs involves growth of influenza in embryonated hens’ eggs, followed by concentration of the virions, chemical inactivation and disruption of the envelope and partial purification of the HA and NA proteins. In recent years there are also IIVs produced in cell culture (225-229). The surface glycoprotein hemagglutinin is the main immunogen in IIVs and its level is standardized at 15 µg of each HA antigen per dose (45 µg of total antigen per dose for TIV and 60 µg of total antigen per dose for QIV). IIVs also contain surface glycoprotein neuraminidase (NA), matrix protein (M), and nucleoprotein (NP) in varying amounts, depending on the process methods, but their levels are not specifically quantified. Most IIVs are administered by intramuscular route in a one-dose schedule for adults and in a 2-dose schedule for previously unexposed children less than 9 years of age. For young children 6 to 36 months of age an HA dose of 7.5 µg is recommended for some trivalent vaccines (230, 231).

Some licensed IIVs contain adjuvants such as oil-in-water emulsions (MF59), alum or virosomes (232). An MF-59-containing TIV was first approved for adults ≥ 65 years of age in Italy in 1997 and thereafter in several EU countries (233), Canada and the USA (232)(WHO ref 122). Vaccines containing MF-59 are also approved for use in children aged 6–23 months in Canada (234). Another oil-in-water adjuvant, AS03 (235), has been previously used with vaccines against 2009 pandemic H1N1 influenza, but as of 2017, it is not present in seasonal influenza vaccines.
Live attenuated influenza vaccine

The rationale for developing the live attenuated influenza vaccine (LAIV) was to create a vaccine that mimics natural infection, and in doing so theoretically induce both cellular and humoral immunity. The LAIV is based on the A/AA ca (ca A/Ann Arbor/6/60) and B/AA ca (ca B/Ann Arbor/1/66) master donor viruses, which are temperature sensitive (ts) (replication reduced at the higher temperature (39°C)), cold-adapted (ca) (grow well at a reduced temperature (25°C)) and attenuated (att) strains (restricted replication in the upper and lower respiratory tract of ferrets) (236, 237). The trivalent seasonal intranasal LAIV was approved by the EMA for use in children from 2 to less than 18 years of age in 2011 (238). It was replaced by the quadrivalent vaccine (QLAIV) in 2013. The vaccine is for intranasal administration using a syringe-like device that delivers a 0.1-mL volume of a large-particle aerosol into each nostril for a total volume of 0.2 mL. For children 2 to 8 years of age it is recommended to give two doses at least 4 weeks apart in their first year of influenza vaccination. Thereafter and in older children only one dose is required.

Vaccine efficacy and effectiveness

Evaluation of the protection against influenza-related illness conferred by the vaccine is complicated by multiple variables including the population being assessed, the study design and the endpoints used, as well as the particular season and the predominant viruses involved. Therefore, published vaccine efficacy/effectiveness point estimates have varied between studies and comparison of results requires consideration of multiple factors. Earlier assessments using suboptimal designs and endpoints have overestimated vaccine efficacy. Laboratory-confirmed infection is the most specific outcome for both influenza vaccine efficacy and effectiveness studies, and RT-PCR is now widely used for this purpose. Currently, the test-negative case-control approach in observational effectiveness studies is widely used, which is intended to minimize biases related to access of health care and to minimize misclassification of influenza cases (239). WHO has published a guide on the evaluation of influenza vaccine effectiveness to encourage and facilitate the use of standard methods (240). Currently, influenza vaccine effectiveness studies are performed annually in a number of countries, including countries in the northern hemisphere (e.g. Canada, USA and several countries in Europe) and in the southern hemisphere (e.g. Australia and New Zealand).

The measurement of influenza vaccine efficacy and effectiveness (VE) can be affected by virus, such as drift or egg–adaptive mutations in vaccine strain, and host factors, such as age, medical conditions and history of prior infections/vaccinations.

Overall the current seasonal influenza vaccines have moderate efficacy and effectiveness. Even in years when influenza vaccines are well matched to circulating viruses, estimates of vaccine effectiveness range from 40 to 60%, which
is lower than that for most licensed non-influenza vaccines. Some years effectiveness plunges to as low as 10%. A recent meta-analysis identified eight studies of IIV in adults aged 18–64 years conducted over nine influenza seasons, found a pooled vaccine efficacy of 59% (CI: 51–67%) in prevention of laboratory confirmed influenza (241).

For older adults over 65 years, only results from observational studies are available. One recent meta-analysis of vaccine effectiveness studies in the elderly demonstrated an average VE of 50% against symptomatic influenza (WHO ref 55. In studies of community-dwelling elderly persons, it has been found that IIV may reduce secondary complications, hospitalizations, cardiovascular events and death (242-246)(WHO ref 241-245). VE of IIV in elderly nursing home residents is generally lower than in healthy younger adults and has been estimated to range from 20% to 40% (247).

In children higher levels of VE for LAIV was found in a comparative study and improved performance of 35% to 55% above efficacy seen with IIV (241, 248-250). Meta-analyses of vaccine effectiveness and efficacy studies in children aged 2–17 years have reported VE estimates of between 40% and 90% depending on antigenic vaccine match (248, 249, 251, 252). Relatively few efficacy studies have been performed in younger children 6-35 months of age, but in recent RCTs, evaluating two QIIVs, VE of 56.6% (CI: 37–70.5%) and 49.8% (CI:41.8; 56.8 ), respectively, was found. A Finnish study conducted with 2-year old children for the 2015-2016 season showed vaccine effectiveness to be 50 % for LAIV and 61 % with TIV (253).

An observational study in the USA in children aged 2–17 years indicated that the effectiveness of the H1N1pdm09 component of seasonal LAIV was lower from season 2010-2011 than that for the corresponding component of IIV (254). Based on these results the ACIP recommended that the LAIV should not be used in the USA during the 2016-17 and 2027-18 seasons. The reasons for the poor effectiveness of LAIV in the USA are not yet clear, but the H1N1pdm09 component has been changed and therefore ACIP decided to recommend the LAIV from season 2018-2019 (221).

The strategy of maternal influenza immunization recommended by the WHO and widely implemented can protect pregnant women and newborn children with a single vaccination (255). RCTs of maternal influenza immunization have demonstrated that pregnant women have a good immunological response to IIV, and that anti-HA antibody titres in newborn infants can exceed maternal titres due to active antibody transport across placenta. Clinical trials have shown VE against laboratory-confirmed influenza in pregnant women ranging from 31 to 70% (256, 257), with vaccine efficacy against the same outcome in infants during the first 6 months ranging from 30 to 49% (256, 257).
Immune responses following natural infection – influenza

Multiple mechanisms provide resistance to influenza in humans (258). Acquired immunity to influenza is mediated by serum antibodies, antibodies at mucosal surfaces and influenza-specific T cells. Antibody alone is sufficient for protection, and T cells play a role in recovery. During a primary exposure to an influenza virus, both B and T cells are able to generate memory toward the specific virus strain.

Humoral (B cell) and mucosal immune responses to infection

Upon infection, mucosal immunity acts as the first line of host defense by blocking influenza virus from infecting the upper respiratory tract and spreading to the lower respiratory tract (259). The neutralizing antibody response in nasal secretions is primarily locally produced IgA, but also IgM and IgG HA antibodies. Both mucosal IgA and IgG antibodies and serum IgG independently contribute to resistance to influenza (260-262). The level of serum HA-and NA-antibody correlates with resistance to illness and with the restriction or virus replication. HA antibody prevents infection by neutralization, whereas NA antibodies mediate their antiviral effect (262, 263). Antibodies are also induced against highly conserved structural proteins including matrix protein 2 (M2), nucleoprotein (NP) and regions of the HA stalk of the virus. NP is an important target for T cell immunity.

For decades the level of anti-HA antibodies in serum has been measured by the hemagglutination inhibition test (HAI) but in recent years neutralization (NT) assays have been used in parallel. IgG serum antibodies against the HA is widely viewed as the best indicator of protection against influenza disease and a HAI antibody titre of 1:40 is considered to provide protection from infection in about 50% of the individuals (264), but higher titres may be required in young children and in other populations (265). In contrast, antibodies against the NA act to reduce virus spread from cell to cell (266) and has also been shown to correlate with protection from infection (266, 267).

During the last few years it has been found that antibodies against the HA, NA and M2 proteins, following infection or vaccination, can to a certain extent recognize heterologous influenza viruses (268, 269). Recently, antibodies that recognize multiple influenza virus subtypes were discovered (270), some of these bound the HA stem region of group 1 influenza A viruses while others bound to the stem region of most of group 2 influenza A viruses.

The initial exposure to an influenza virus affects the antibody response to subsequent exposures to new strains. This concept termed ‘antigenic sin’ was proposed in 1960 (271) to describe the impact of immunological memory related to an initial encounter on exposure to a slightly different version of the infectious agent. Recent data provide strong epidemiologic evidence that infection with the influenza strain circulating during one’s childhood elicits lifelong immunological imprint that impacts responses to novel strains and can help protect against unfamiliar HA subtypes from the same phylogenetic group as the original infecting
virus (272). The phenomenon called `immunological imprinting’ has important implications for public health because it affects responses to subsequent influenza infections and probably to influenza vaccinations.

While antibody-mediated protection against the infecting virus is strong, protection against viruses that have undergone significant antigenic drift in the HA is reduced.

Long-term immunity

An immune response to influenza virus does not induce life-long broadly protective immunity to future infection and individuals can be successively reinfeected by distinct influenza viruses. This is attributed primarily to the extensive antigenic diversity that exists among the influenza viruses. However, antibodies can induce strain-specific protection that can be very long-lasting immunity. Thus, epidemiologic surveys revealed that persons with prior exposure to certain strains (H1N1) conferred immunity to the nearly identical strains that reemerged after a 20-50 year absence (269).

Cell-mediated (T cell) immune responses to infection

CMI play an important role in immunity against influenza, and in contrast to the strain-specific response of antibodies, tend to be more cross-reactive among subtypes, recognizing more conserved epitopes on the surface proteins and internal viral proteins (NP, M and polymerase) (273). Thus, CMI may contribute to so-called heterosubtypic immunity and induce protection against antigenically distinct influenza viruses by CD4+ and CD8+ T cells.

Upon infection with influenza virus CD4+ T cells, CD8+ T cells and regulatory T cells (Tregs) are induced. Different subsets of Th cells (Th1, Th2, Tregs and Th17) are distinguished based on their cytokine expression profiles and their different functions following infection. Also after influenza vaccination Tregs are induced that do not alter the B cell response, but suppresses the T helper response induced by vaccination (274). The main function of virus-specific CD8+ T cells is that of cytotoxic T lymphocytes (CTL) mediated by the release of perforin and granzymes (e.g. GrA and GrB). Recently it was demonstrated that even in the absence of GrA and GrB influenza virus-specific CTL were able to lyse target cells (275-277).

Heterosubtypic immunity to influenza A virus infection has been a research topic for more than four decades (273). The contribution of T cells to protection from infection has been evaluated in some recent studies which indicate that preexisting T-cell immunity independent of baseline antibodies protects against symptoms and viral shedding associated with influenza (278-280).

Once the infecting influenza virus has been cleared, the majority of virus-specific CD8+ T cells die while the remaining 5–10% form a stable, long-lived pool of memory T cells that can be activated rapidly in the face of another influenza virus infection. A recent study has shown that the presence of memory CD4+ T cells specific for influenza in humans correlated with decreased virus shedding (278).
Immune responses following vaccination – influenza

Inactivated influenza vaccines

Unlike natural infection which stimulates multiple components of the immune system resulting in both humoral and cell-mediated responses, currently licensed IIVs primarily stimulate humoral responses. The inactivated vaccines do not infect cells and therefore only partial activation of cellular immunity occurs and they fail to induce strong anti-influenza CTL responses. However, the IIVs are manufactured either as split-virion or subunit vaccines and, the latter contain more internal proteins and has been shown to stimulate a greater cellular immune response. IVIs can boost HLA-restricted CTL in adults, but their ability to stimulate CTLs in unprimed subjects has not been evaluated.

Humoral response to Inactivated influenza vaccines

Current IIVs primarily induce antibodies to the globular head of HA and depending on formulation, to NA, NP and M1 proteins. The antibodies against HA and NA are strain-specific and not effective in combating the yearly variants of the virus. The HAI test is most commonly used for measuring the antibody response to IIVs, mainly because serum antibody has been shown to correlate with protection in infection, vaccine efficacy and challenge studies (241, 264). The neutralization test (NT) is an alternative method and has the added advantage of being more sensitive than HAI assays and measuring the overall neutralizing antibody response (281). However, a general problematic issue is the lack of standardized and validated HAI and NT assays. Although antibodies to NA do not efficiently neutralize influenza infection, they restrict virus release from infected cells, reduce the intensity of infection, and enhance recovery (282). Improved assays to measure NA inhibiting (NAI) antibodies in sera are being employed (283, 284) but as yet there are no standardized assays. Furthermore, anti-NA and anti-HA antibodies have been found to be independent predictors of immunity to influenza infection (262).

For IIV vaccines, an HAI titre of 1:40 was previously suggested to represent a reasonable correlate for an efficacy of 50-70% against clinical symptoms of infection based on challenge studies in healthy adults (264). There is however a need to better define correlates of protection, which may vary according to individual characteristics, populations, specific age groups (e.g. no correlate has ever been identified in the pediatric population and for the elderly HAI titers correlate poorly to protection) and vaccine types(285). In addition, it is essential that NT antibody titres are determined in parallel with HAI. No established seroprotective levels have been defined based on NT antibody levels. No immune correlates of protection have either been defined for NA.

The duration of protection after vaccination with IVI has not been evaluated systematically, but the relatively rapid decline in antibody levels suggest that the protective immunity is of short duration (6 -12 month) (286). The magnitude of the
immune response to IVI is dependent on several factors such as age, health status, level of immunocompetence and level of pre-existing HA antibody. The serum antibody response is low in unprimed children and requires two vaccine doses (287, 288). In primed (previously infected or vaccinated) individuals, the response is predominantly anti-HA IgG, but in young, unprimed children, systemic IgM antibody may be more evident (289, 290). In primed healthy adults, one dose of IIV stimulates good HAI antibody responses in individuals with low pre-existing HA antibody levels, whereas increase in antibody titres is lower in individuals with high pre-existing HA antibody (286).

Adults aged 65 and older have less vigorous serum antibody responses than younger recipients, reflecting the lower vaccine effectiveness observed in elderly. Older adults’ ability to respond to immunogens is compromised by natural immunosenescence (291). Potent antigens, use of adjuvants, higher doses, and multiple vaccine doses may be needed to increase the quality and quantity of their immune responses (232). Adjuvanted vaccines are of particular interest for use in populations that may respond less well to unadjuvanted IIVs. Adjuvanted vaccines were used in many countries during the 2009 H1N1 pandemic, (292) and immunogenicity data indicated that adjuvanted 2009 H1N1 vaccines produced high antibody titers after a single dose (293).

Cell-mediated response to Inactivated influenza vaccines

Cell-mediated immune responses to IVIs have been studied much less extensively in humans than the corresponding antibody responses, partly due to more cumbersome measurement as well as lack of standardized CMI methods. Consequently, many of the assumptions regarding the T cell response to influenza vaccination are extrapolations from natural infection or immunization studies in animal models. Because of the importance of the T cell response for recovery from infection and for protection from serious disease it is thought that priming these responses may affect outcomes such as disease severity, hospitalization and death. It is therefore important to elucidate the T cell response. The role of cell responses after vaccination with IIV, remains unclear.

Immunization of healthy adults with inactivated whole-virus vaccine resulted in enhanced CTL responses in peripheral blood, whereas immunization with subunit vaccine resulted in a poor CTL response (294). It has been found that the amount of antigen per dose and the number of doses of whole-virus vaccine administered prior to influenza activity have an effect on the magnitude of the T cell response, and that addition of certain adjuvants can increase the T cell responses in adults. The 2009 pandemic H1N1 vaccine with AS03 adjuvant enhanced both antibody and CD4+ T cell responses in adults compared with non-adjuvanted vaccine (295) and a similar effect has also been shown for adjuvanted influenza vaccine in elderly adults (296). Cross-reactive and polyfunctional H5N1-specific CD4+ T cells to conserved sequences in HA protein of clade 2 H5N1 HA were identified after a single dose of adjuvanted vaccine and amplified by a second vaccination (297).
It has been demonstrated that pre-existing influenza-specific CD4+ T cells determine the magnitude of the CD4+ T cell response following TIV vaccination (298). The absence of pre-existing CD4+ T cells could negatively affect post-vaccination CTL and antibody responses. This is relevant when studying TIV vaccine responses in young children and infants, because these individuals may be naïve to influenza antigens and there might not be any primary response to boost. Accordingly, vaccination of children with TIV promotes virus-specific CD4+ T cell and antibody responses, while CD8+ T cell responses are not affected (299). However, in a study comparing CD8+ T cell responses in children and adults after vaccination with IIV and LAIV, it was found that responses were variable and dependent on both vaccine type and age (300). In this study, TIV induced a significant increase in influenza-specific CD8+ T cells in children aged 6 months to 4 years, but not in those aged 5 to 9 years, reflecting the diversity of the immune response to IIV in different age groups and the need for more research.

The decrease in response to the influenza vaccine in the elderly may be related to several factors such as smaller B cell populations, T cell populations with impaired antigen-specific activity and a demonstrated shift from production of Th1 cytokines such as IFN-gamma to Th2 cytokines such as IL-10 resulting in decreased CTL activity with increasing age (301). Although the number of virus-specific CD8+ T cells decreases in people aged 65 years and older, there is growing evidence that T cell responses may provide a degree of protection after IIV immunization (302). Influenza vaccination may transiently enhance CTL responses in this population. In a study including community dwelling older adults it was shown that IFN-γ:IL-10 ratio was ten-fold higher in those who did not develop influenza illness. (301). In addition, decreased granzyme B production, was associated to increased risk of influenza illness in the elderly (303). In another study of adults ≥60 years with congestive heart failure, post-vaccination production of granzyme B, was significantly lower in subjects with influenza compared with those without laboratory-confirmed influenza (304). Granzyme B level was a better indicator of protection than HAI antibody titre (303). These results suggest that the evaluation of cell-mediated immune responses to vaccination, particularly in older adults, warrants further investigation.

Live attenuated influenza vaccines

*Humoral response to live attenuated influenza vaccines*

IIVs induce higher titers of serum HAI and NA antibodies than LAIV in primed individuals, but LAIV is more efficient in inducing mucosal IgA antibody responses (261). In influenza-naïve children, LAIV elicits a robust serum antibody response in both HAI and NT assays (305). Lee et al (306) and Belshe et al (305) compared serum antibody responses after LAIV in 1- to 5-year-old seronegative children by NT or HAI, and they reported seroconversion rates of 58% and 16% against H1N1, 100% and 92% against H3N2, and 100% and 88% against influenza B, respectively, after a single dose of the trivalent LAIV vaccine. Administration of
a second dose of LAIV 28 to 60 days later improved the seroconversion rate against the H1N1 virus in these studies to 77% and 61%, respectively.

Several studies have found that seroconversion following vaccination with LAIV occurs more frequently in immunologically naïve individuals than in primed adult subjects (307). It is of note that in Europe the LAIV vaccine is only indicated for children 2-18 years of age. In human challenge studies and in field studies, LAIV was highly efficacious even in persons with modest serum HAI responses (308) and either serum antibody or nasal wash IgA was a predictor of protection (261).

Cell-mediated response to live attenuated influenza vaccines

Vaccination with LAIV induces superior T cell responses compared to IIVs (309) including both CD4+ and CD8+ T-cell immunity. However, assays for the assessment of cellular immune responses to LAIV in clinical trials have lagged behind antibody assays and have primarily focused on the induction of T-helper cell type 1 (Th1)-mediated immunity by enumeration of interferon gamma (IFNγ)-producing T cells and NK cells by flow cytometry, ELISA and ELISPOT assay. Significant increases in IFNγ+, CD4+, and CD8+ T cells after LAIV have been observed in both children and adults.

The induction of influenza-specific T cells by LAIVs has been demonstrated in children (261). The LAIV vaccine have been shown to be a strong inducer of long-lasting CD4+ and CD8+ T cell responses (310). In a study, children 6–35 months old were administered, 1 month apart, 2 doses of either TIV or LAIV, or combinations of LAIV and TIV in both prime/boost sequences, and all LAIV and/or TIV combinations induced similar HAI responses. In contrast, only regimens containing LAIV induced influenza-specific CD41, CD81, and cd (γδ) T cells, including T cells specific for highly conserved influenza peptides (310). In a large field study of seasonal LAIV administered to young children, more than 100 spot-forming cells per 10⁶ peripheral blood mononuclear cells in an IFNγ ELISPOT assay was associated with protection against culture-confirmed influenza illness (311). In addition, it has been shown that LAIVs alter the expression of IFN-related genes, suggesting that the innate immune response plays an important role in protection mediated by LAIVs (312). A robust Type I (IFNγ) interferon response was observed when peripheral blood mononuclear cells from LAIV recipients were cultured with LAIV (313). A comparison of genome-wide transcript profiles in whole blood from young children 7 days following vaccination with LAIV or TIV revealed that LAIV induced higher expression of Type I IFN and interferon-stimulated genes than TIV (314). Our understanding of LAIV-induced immunity will be enhanced as assays that permit evaluation of cellular and innate immune responses are incorporated into clinical studies designed to explore immune correlates of protection for LAIV and biomarkers of early responses to LAIV.

Considering that vaccination with LAIV is known to elicit a broad range of immune responses against conserved influenza virus proteins, it is would seem plausible that LAIV may confer heterosubtypic immunity. A study designed to
address the impact of heterosubtypic immunity induced by LAIV in children suggests that LAIV do not induce heterosubtypic immunity (315). Larger population-based studies are underway to determine whether robust heterosubtypic immunity exists in humans.

Conclusions – influenza
Current seasonal influenza vaccines aim primarily at the induction of strain specific neutralizing serum antibodies to HA. Inactivated influenza vaccines are very safe, but only moderately efficacious and have to be updated regularly to match circulating influenza strains. A serum HAI antibody titre of 1:40 have since decades been considered a correlate of protection against influenza, but this correlate has to be re-defined adjusted to individual characteristics, different populations, specific age groups and vaccine types. The IIVs only partly activate cellular immunity and fail to induce strong anti-influenza CTL responses. Pre-existing influenza CD4+ T cells determine the magnitude of the CD4+T cell responses following IIV vaccination and its absence can negatively affect post-vaccination CTL and antibody response. Data in the elderly have shown that granzyme B level might be a better indicator of protection than HAI antibody titre, which suggest that evaluation of CMI responses to vaccination in the elderly warrant further investigation. The LAIV is more efficient than IIVs in inducing mucosal IgA responses and T cell responses including CD4+ and CD8+ T cell immunity. LAIV is also more efficacious than IIV in young children. Our understanding of LAIV-induced immunity will be enhanced as assays that permit evaluation of cellular and innate immune responses are incorporated into clinical studies designed to explore immune correlates of protection for LAIV and biomarkers of early responses to LAIV.

Cell-mediated immunity play an important role in immunity to influenza, and in contrast to the strain-specific response of antibodies, tend to be more cross-reactive among subtypes, recognizing more conserved epitopes on the surface proteins and internal viral proteins (NP, M and polymerase). T cell mediated immunity therefore may contribute to heterosubtypic immunity and may afford protection against antigenically distinct influenza viruses. Developing influenza vaccines that provide broader protection against heterotypic strains and more durable protection would eliminate the need to update and administer the seasonal flu vaccine each year and substantially improve influenza prevention programs.

The CMI responses to influenza infection and vaccination need to be further investigated. However, there are technical difficulties in using the complex assays of CMI and there are no validated and standardized assays to assess CMI responses to influenza. Various methods have been employed in the study of T cells responses including IFN-γ ELISpot, FACS staining, cytokine release and CTL assays. There is however no agreement on which assays to run as best correlates and how to run them. In an attempt to address this critical issue of identifying cellular immune correlates of protection, an international group with participants from public health agencies in the EU and researchers from Canada has made an
attempt to standardize assays determining cellular immune responses against influenza (316).

At the current state CMI seem not to have a role in the regular surveillance of the influenza vaccination program. However, surveillance of population immunity to influenza might benefit from CMI data rather than HAI titers, at least in selected populations such as infants, young children and the elderly.

Knowledge about CMI immune status and vaccine CMI induction may have an impact on how we immunize potentially influenza-naïve children, immunocompromised populations as well as the elderly.

Knowledge gaps – areas for further research

- There are major gaps that remain in our understanding of the various components of the human immune system and its response to influenza viral antigens. Expanded knowledge of the humoral and cell-mediated immune response to influenza infection and vaccination must be the foundation for strategies to develop game-changing 21st century vaccines.

- New analytical tools are being developed that could give insights in the protection induced by influenza vaccines. Research devoted to new tools, which include systems biology, definition of biomarkers, etc. may uncover still unknown parameters that should be tested as potential correlates of protection.

- Next-generation vaccines will require new biomarkers and in particular new correlates of protection and need to be assessed with new assays. The data available today is insufficient to draw any final conclusions on what type of CMI parameters are important to assess when evaluating influenza vaccine efficacy. Identification of alternative correlates of protection is an important step toward the development of cross-reactive influenza vaccines. Further clinical studies are needed to identify those parameters.

- A correlation between antibody responses and CMI, both in terms of T cell and memory B cell response need to be established

- There are increasing focus on producing universal influenza vaccines that will provide substantial cross protection, either by inducing antibodies to preserved structures such as the matrix protein 2, NP and the HA stem region, or by inducing cross-protective cellular immune responses. In preparation for these novel vaccines, methods to evaluate CMI responses need to be set up and validated for applied for laboratory surveillance.
Vaccines – considered to be included in the NIP

Varicella zoster virus vaccine

Vaccines against varicella zoster virus

In Sweden, two monovalent vaccines are licensed for the prevention of chicken pox, Varivax and Varilrix. There is also, Priorix Tetra, a combined tetravalent vaccine against measles, rubella, mumps and varicella (MMRV). In addition, Zostavax is licensed to prevent herpes zoster (HZ). All vaccines consist of live attenuated virus derived from the Oka/Merck strain. As with natural infection, the vaccine strain is able to establish latent infection in the vaccinees. The HZ vaccine differs from the chicken pox vaccines in that the dose (number of virus particles) is about ten-fold higher.

Varicella vaccine is given in Sweden primarily to specific high-risk groups or provided in private vaccination clinics. However, introduction of the chickenpox vaccine in the national childhood immunization program is under consideration. In Sweden, the current varicella vaccination regimen of infants consists of two doses and can be given to children from the age of nine months (Varilrix) or 12 months (Varivax). The second dose would be administered at least 1-3 months after the 1st dose to ensure long-term protection against chickenpox. Several studies on the effectiveness of varicella vaccines have presented data showing that although 1 dose of varicella vaccine provides good protection, a second 2 doses helps to reestablish very high levels of effectiveness and to reduce the risk of breakthrough varicella (317, 318).

Zostavax vaccine is approved for people aged 50 years or older. In addition, Shingrix, a new vaccine candidate – an adjuvanted non-live recombinant subunit vaccine – for prevention of shingles has recently been evaluated in a comprehensive phase III clinical trial program regarding its efficacy, safety and immunogenicity (319).

Immune responses following natural infection

Varicella (chickenpox)

The primary infection with varicella-zoster virus (VZV) causes varicella (chickenpox), which in temperate climates mainly occurs during childhood. VZV infection induces VZV-specific antibody and T-cell-mediated immunity, important for recovery. However, the severity and progression of the disease is in general different in different populations or risk groups. Varicella in healthy children is usually not severe, but the disease has a variety of manifestations or complications (320). Varicella may also be more severe in adults, particularly in pregnant women. Maternal varicella developed just before or after delivery is potential more serious for the newborn infant as the baby will not be protected by maternal antibodies. In immunodeficient patients varicella can become very serious (320) like in those with congenital deficits of cell-mediated immunity, and leukemic children are at greatest risk of developing severe varicella disease.
The exact roles of humoral and cell-mediated immunity in protection against VZV infection are not completely understood. In general, antibodies are considered to be protective against varicella, and passively administered varicella-zoster immunoglobulin can prevent infection (reviewed in (2). Serum antibodies - IgM, IgG and IgA - are detectable in most patients after varicella infection. Although, a common protective level has not been defined it has been suggested that an ELISA titer of 5 U/ml could be used as a threshold for protection and vaccine efficacy (Watson, 2008 #90).

Cellular immune responses plays a crucial role in recovery from primary infection by defending the infected person from spread of the virus (2). Cellular responses also contribute to protection following re-exposure to VZV (321).

Following natural varicella infection, immunity mediated by both CD4+ and CD8+ T cells, is commonly maintained for decades demonstrated by proliferation and cytokine production. T lymphocytes from varicella immune individuals usually produce Th1 cytokines, such as IL-2 and IFN-γ CD4+ T cells provide help to develop and maintain the VZV-specific antibody response. Memory T-cells with the phenotype CD45R0+CCR7- may be maintained partly due to periodic exogenous re-exposure with varicella or HZ and partly due to endogeneous re-exposure to the virus during subclinical reactivation of VZV (320).

Cell-mediated immunity appears to be of major importance in control of VZV disease by maintaining the balance between the host and latent VZV. Cellular immunity to VZV can be assessed by lymphoproliferation, CTL assay and ELISpot (320). In addition, using tetramer analysis, the major role of CD4 response and cellular immunity to glykoprotein E (gE) in recovery from varicella have been demonstrated. The strongest CMI responses to VZV appear to be in early adulthood.

Herpes zoster (shingles)

The secondary infection Herpes zoster usually occurs later in adult life due to waning VZV-specific CMI which enables symptomatic reactivation of the virus. (322). This occurs as with aging or certain medical conditions or treatments. HZ presents when reactivation occurs and VZV-CMI is below an undefined but critical level. A possible potential role of VZV-CMI is to directly prevent, rather than limit, VZV reactivation.

Immune responses following vaccination

Varicella (chickenpox)

Studies suggest that both the cell-mediated and humoral immunity are responsible for the vaccine-induced protection (322). Following vaccination serum IgG antibody responses are detected in the majority of healthy children (322). Protective antibody levels (>5 units/mL by gp-ELISA) are induced in more than 99% of children after 2 doses and, lasts for months to years. The IgG antibody
titers are, however, 10-30 times lower than those after natural infection, depending on the dosage, number of doses and the age of immunization (320). In addition, it has been reported that antibody levels may decrease below the threshold for herd immunity in vaccinated young adults unexposed to wildtype VZV as compared to unvaccinated individuals (323). Serum IgA antibody is detectable only occasionally and at low levels after immunization. The development of IgG antibodies to VZV as measured by the ELISA is considered a surrogate marker of protection. There is good correlation between the antibody response after varicella vaccine and the protection after later exposure. Antibody titers measured by the fluorescent antibody to membrane antigen (FAMA) assay give best indication of protection against disease. Although specific antibodies against VZV confer protection against the primary VZV infection, they appear to play little to no role in the host resistance against reactivation of the latent infection i.e., the development of herpes zoster (324).

Passive immunization leads to partial efficacy showing that antibody is at least partly responsible for protection, although cell-mediated killing is also important. B and T cells after vaccination probably collaborate in protection. For instance, vaccinated children often resist infection after vaccine-induced antibodies are undetectable. In a study on HIV positive but immunocompetent children on antiretroviral treatment, it was observed that anti-VZV antibody titers waned relatively quickly, and that CMI responses measured as lymphocyte proliferation was a better measure of vaccine-induced protection (325). Likewise, in another study in leukemic children protection remained after antibody titers had disappeared (326). In a cohort of HIV positive children, the development of HZ was associated with the loss of VZV-specific CD8+ T cells (327).

It has been demonstrated that vaccine strain, has the capacity to establish latent infection, and can later, reactivate and cause herpes zoster (2). However, studies have demonstrated considerable lower risk of HZ among vaccinees (328).

Herpes zoster (shingles)

In several studies of elderly populations, it has been shown that HZ-CMI responses drop while antibody levels remain, and that robust CMI responses can blunt or prevent HZ (329-331). Levin and coworkers also provided evidence that a booster vaccination with live attenuated Oka/Merck varicella-zoster virus vaccine strengthened the VZV-specific CMI response and had a suppressive effect on HZ development that lasted for four years (329). In addition, all responses, assessed by γ-interferon ELISPOT, responder cell frequency assays and glycoprotein ELISA, were significantly boosted after vaccination and a roughly twofold increase in VZV-CMI was reported in the early pilot studies (329). The vaccine-induced VZV-CMI persisted during the 3 years of follow-up, although their magnitude decreased over time. In a study a second dose was administered 10 years after first dose to individuals above 70 years of age. IFN-γ and IL-2 results were significantly higher at baseline and after vaccination in the booster-dose group, compared with the first-dose group, indicating that a residual effect of zoster vaccine on VZV-specific CMI
persisted for ≥ 10 years and was enhanced by the booster dose (332-334). Further, it was not possible to determine a protective level. These observations suggest that HZ develops when CMI to VZV falls below a critical threshold. Supportive of this data are also the findings by Weinberg and colleagues that strong CMI to VZV at onset of symptoms was associated with reduced HZ severity and decreased risk of post-herpetic neuralgia (PHN) (331). Moreover, diseases and treatments resulting in compromised cellular immunity increases the risk of HZ as well as severity (reviewed in (335)). In contrast, acquired reductions in antibody responses are neither associated with an increased incidence of HZ nor with increased severity.

Conclusions – varicella and herpes zoster
Traditionally, immunity to VZV following disease or immunization is assessed by measuring antibody titres. Although antibodies can neutralize extracellular virus, the role of CMI is critical in defense against the intracellular forms of and in long-term protection against this VZV. It is, however, more difficult, complicated, and expensive to measure CMI to VZV than to assess antibody titres. It has been proposed that decisions on new zoster vaccine formulations and the use should be based on evidence of the induction of VZV-CMI, because attempts to assess HZ vaccines using VZV antibody assays may provide erroneous information (335). Infection with varicella zoster virus (VZV) produces lifelong immunity, but duration of post-vaccination immunity has not been established. In addition, cell-mediated immunity appears to be of main importance in maintaining the balance between the host and latent VZV to directly prevent reactivation later in life. However, currently, CMI seem not to have a role in the regular surveillance of the NIP.

Knowledge gaps - areas for further research
- For certain cohorts, CMI may provide a better measure of immunity to VZV, especially regarding the role of CMI in limiting reactivation of latent VZV and thereby preventing HZ and its complications. A better understanding of these relationships could influence design of vaccination regimen and timing of booster doses.
- Studies are needed to assess duration of the immune response and protection from HZ.
- CMI studies provides opportunities to understand the importance of both exogenous and endogenous boosting (subclinical reactivations).
- Optimal time for a second vaccine dose.

In addition, the following areas will benefit from further research as suggested by ECDC (336).
- Duration of vaccine-induced immunity beyond 14 years
- Potential need for further doses later in life
• Impact of vaccine coverage on the long-term epidemiology (i.e. shift to older ages) of the disease

• Also risk of an increased number of complications due to varicella following shifts to older ages of infection after vaccine introduction (including congenital and maternal varicella)

• Severity of breakthrough varicella with an increase in time since varicella vaccination (including BV outcomes in previously vaccinated pregnant women)

• Immunological mechanisms for HZ development including studies on the vaccine strain.
CMI activities at PHA in Sweden and other Nordic countries

Sweden

At the Public Health Agency of Sweden, there are currently no CMI assays routinely performed for the monitoring and laboratory surveillance of the vaccines included in the national immunization programs. However, there is a history of vaccine trials and clinical research studies with a strong focus on cellular immune responses at the Swedish Institute for Infectious Disease Control (SMI), before it merged to become the Public Health Agency in 2014. A detailed description of these studies and on the current laboratory capacity and facilities at PHA Sweden is presented in the Appendix 3. A short summary is presented below.

A variety of methods for the analysis of cell-mediated immune responses have been used in different research and/or vaccine studies to measles, rubella, herpes zoster and pertussis (109, 148) at SMI and PHAS, respectively. Also, clinical studies on influenza, CMV, herpes simplex, M. tuberculosis and VZV have been performed using CMI assays (337-341). Currently, a novel nasal live attenuated pertussis vaccine is being tested in two phase I/II clinical trials, and the laboratory analyses have included T and B cell analysis. Outside the national immunization program, measurement of CMI responses in clinical phase I/II trials of a candidate HIV vaccine, originally developed at SMI, has been performed (342).

In short, the CMI methods established at PHAS are: i) validated methods for IFN-γ and IL-2 ELISpot; ii) 4- and 8-color intracellular cytokine staining (ICS) including detection of cytokines, chemokines and cytolytic markers (IFN-γ, IL-2, TNF, MIP-1β and CD107a, perforin and granzyme B); iii) flow cytometry-based lymphoproliferation (LPA) assays including staining for surface markers (CD3, CD4, CD8) and memory markers (CD45RA, CCR7, CD27 and CD28).

B-cell responses were assessed using the B cell ELISpot by IFN-γ and IL-2 Fluorospot and/or IFN-γ-ELISpot and phenotypic analysis of memory B cells by flow cytometry (148, 149, 343). For VZV analysis, a novel method was developed based on whole blood antigen stimulation and subsequent immune phenotyping and flow cytometry analysis. In addition, ELISA or Multiplex could be used to measure cytokine release in culture supernatants (340).

Nordic countries

The national immunization programs in the Nordic countries are relatively similar especially regarding the vaccines that have been included in the NIPs for a long time. New vaccines have, however, been introduced in the Nordic countries at different time-points depending on the regional burden of disease and other local considerations. Surveillance of the VPDs and the immunization programs is also carried out in a in very much the same way in the Nordic countries and there are many similarities on background parameters such as disease epidemiology, vaccine coverage and socioeconomic status.
In order to collect information from the other Nordic countries on ongoing and planned CMI activities for laboratory surveillance of VPDs included in their NIPs, managers and/or key persons in charge of the NIPs in Denmark, Finland, Island and Norway were contacted and interviewed using the following questionnaire:

- Q1. Are there any CMI-related methods in use at your institution/agency?
- Q2. If so, which methods are used?
- Q3. Why are they used instead of serological methods/assays i.e. what is the added value of using CMI?
- Q4. Are there any (new) CMI methods under development for surveillance of the immunity to VPDs?
- Q5. What is the scalability of the method used i.e. can it replace serological methods in population based seroimmunity surveys, or is it a complement to such surveys aiming at specific subpopulations?

The detailed answers from each country are summarized and presented below. In short, similar to Sweden, there are currently no CMI assays in use for routine laboratory surveillance of VPDs included in the NIPs in any of the other Nordic countries. There are, however, CMI assays established and ongoing and planned research studies including CMI analysis.

**Finland**

At the National Institute for Health and Welfare (THL), a variety of CMI methods have been used in studies of naturally induced immunity to respiratory pathogens. In addition, there are plans for CMI analysis in studies of duration of vaccine-induced immunity to MMR following a 3rd vaccine dose, in specific cohorts.

In short, the methods used are: i) stimulation of PBMC cultures with bacterial or viral antigens or vaccine components, ii) flow cytometric analysis of cytokine expression in specific cell populations, especially CD4+ T and CD8+ T cells, iii) analysis of RNA expression in PBMC cultures or in specific cell populations (isolated with FACSAria cell sorter) – the RNA expression is measured by using qRT-PCR and RNA-seq methodologies, iv) measurement of cytokine concentrations in PBMC cell culture supernatants by Luminex technology. In addition, IFN-gamma ELISPOT assay is planned to be set up.

**Island**

The National University Hospital of Iceland and University of Iceland, Dept. of Immunology, is not doing any CMI-based vaccine surveillance of the NIP. CMI-based assays are up and running for scientific research projects in relation to vaccine research in man and mouse.

The established CMI assays include: i) T-cell proliferation assays; ii) T cell responses by cytokine secretion by ELISPOT; iii) T cell responses by Flow cytometry as intracellular staining of cytokines and transcription factors combined
with surface staining for determining responding T cell subsets (Th1, Th2, Th17, Thh); iv) Cytokines in supernatants from lymphocyte/T-cell cultures measured by ELISA or Luminex; v) B cell responses as antibody secreting cells by ELISPOT; and vi) B cell responses by Flow cytometry as intracellular staining of cytokines combined with surface staining for naive, memory, transitional and plasma cell markers.

In addition, at *deCODE genetics, vaccine related research is being performed mainly on effects of genetics, age and gender on vaccine responses and the role of genetics to adverse reactions to vaccines and infections. T cell assays are up and running as well as flow cytometry and antibody measurements by ELISA. *deCODE genetics is a private research company, and not involved in surveillance of the NIP in Iceland.

**Norway**

The Norwegian Institute of Public Health (NIPH), have established a variety of CMI methods for studying cytokine profiles and responses, T cell markers, TB Spot assay, validated Granzyme B assay, T cell stimulation (Thymidin incorporation), Flow cytometry and Multiplex assays (Cytokine profiles), for research studies. Discussions regarding a study on the role of CMI in influenza are ongoing.

**Denmark**

The Statens Serum Institut (SSI) in Denmark uses no CMI-related methods in surveillance of the NIP.
Discussion and conclusions

The purpose of this study was to investigate whether analysis of cell mediated immunity in vaccine surveillance might be a tool to better predict immunity to vaccine preventable diseases included in the NIP in Sweden and diseases considered for the NIP in the future. The specific aims were to 1) review the current knowledge and status of the field; 2) to investigate potential added value of CMI analysis, and 3) to examine whether there is needed to establish new assays or methods for this purpose.

After reviewing the literature, there is little doubt that after administration of nearly all vaccines, prevention of infection of clinical disease correlates with the induction of functional antibodies. However, the picture is far more complex, as the antibodies must be at relevant sites and also have sufficient breadth and, the functional characteristics of antibodies as well as quantity, are important. In addition, CD4+ T cell responses are sometimes better correlates of protection than antibody titers for instance cellular immunity against tuberculosis (2).

Nevertheless, surveillance of vaccine-induced immunity to VPDs can be performed using antibody tests. There is an overall assumption that antibodies prevent infection/clinical disease whereas cellular responses control infection and disease (2). There is, however, evidence that both humoral and cell-mediated immune responses contribute to protection from infection, for diseases such as rubella. In addition, there are several VPDs for which there are no defined immunological correlates of protection, such as pertussis, mumps and tuberculosis. In the case of tuberculosis and herpes zoster, T cells responses correlate to protection, although protective levels or specific cellular markers have not been defined yet. It has also been shown that T cells responses correlate with protection against culture-confirmed influenza virus in infants and young children, wherein efforts were made to establish a possible protective level of CMI (311).

Finally, there is a growing body of evidence that memory B and T cells may correlate to long-term protection. Although, it is important to keep in mind that correlates may differ both quantitatively and qualitatively, depending on whether the goal is to prevent systemic infection, mucosal infection, disease, or severe disease (2).

For many VPDs, protection also persists after antibody responses have waned. Analysis of memory cells using CMI assays would be informative for understanding the duration of protection for different VPD after antibody response have waned below protective levels. For instance, in HBV infection, information about memory B and T cells would be useful to determine the need and the timing of boosting vaccine responses. When it comes to slowly developing VPD caused by viruses such as HBV and HPV, booster doses may not be needed if MBC are present and ready to mount an anamnestic response upon recall stimulation. In addition, CMI might provide an added value when analyzing the immunity to certain VPD in subpopulations such as the elderly or immunocompromised, in
which antibody response may correlate poorly to protection. Hence, CMI analysis may be suitable as a complement to serosurveillance studies; to understand if and when to administer booster doses; to avoid costly booster studies: to design optimal formulations for new vaccines such as DNA and viral vector vaccines; and to increase our overall knowledge of the human immune response and the immunology of protection from infectious diseases. There is an interest in developing vaccines and adjuvants that better stimulate cellular immunity, as well as in documenting the cellular immune responses, predominantly the CD8+ T cell responses and cytotoxic T lymphocytes.

A key public health priority is to develop a ‘universal influenza vaccine’ that would protect against most or all seasonal strains of influenza and potential pandemic strains and provide long-lasting protection (344-346). Researchers are exploring antigens such as the HA stalk, NP and the matrix 2 protein all of which contain segments that are conserved across influenza strains (347). Investigators have proposed that a universal vaccine should exploit cellular immune responses and that induction of cross-reactive T cell responses maybe a promising approach for development of more broadly protective influenza vaccines (273). An important consideration in developing T-cell based vaccines is that T cell immunity does not by itself generate sterilizing immunity similar to that provided by NT antibodies (348). T cells recognize only antigens generated after viral entry and infection of cells. Thus, T cell immunity will be delayed until influenza infection is established and will require different correlates of protection.

To summarize, seroimmunity surveillance is better suited to study the immunity in the general population since large-scale CMI analysis is indisputably both more costly and labor-intensive than high throughput serological assays. However, in smaller studies directed towards specific populations or specific questions, analysis of cellular immune responses might be cost-effective and of added value. From a health economics perspective, there is a lot to gain from optimized immunization schedules (such as decreased disease burden, fewer sick days, less hospitalization etc), and understanding the long-term CMI may guide decisions about the number and timing of booster vaccine doses. In general, however, the lack of identified protective levels for cellular immune responses makes CMI analysis more difficult since one has to deal with quantitative differences without knowing if they correlate to protection. Thus, the field would benefit from more data generated with validated and standardized methods.

Several validated methods to analyze the CMI are already in place at the Public Health Agency of Sweden (Table 3). Notably, the vaccine-induced CMI responses to VPD caused by pertussis, varicella, measles and rubella as well as responses induced by an experimental HIV vaccine can be analyzed. The methods could relatively easily be adapted for other VPDs, although this would require optimization and validation steps. However, the current capacity does not allow for large-scale analysis, but smaller directed studies could be and are already conducted. The sample processing, laboratory capacity and overall competency is being maintained as long as vaccine trials and research studies are conducted.
Currently, there are several scientists and medical laboratory scientists with solid experience in CMI assays working with the ongoing pertussis phase I clinical trial. However, several limiting factors need to be addressed in case of expansion of CMI activities in the future. See Appendix 3 for more details on equipment and facilities.

The Public Health Agency of Sweden has the overall responsibility for the national immunization program, which includes not only monitoring and surveillance of VPD but also development of the program. Thus, studies aiming at better understanding protective immunity in individuals or specific target populations should be seen in a broader perspective and carefully considered and formulated into research questions.

Conventional immunological methods such as ELISA, ELISpot, flow cytometry-based, Luminex-based etc have been invaluable tools and will remain essential in evaluation of vaccine-induced responses in the future. However, these approaches are generally limited to the analysis of a small number of components of the immune system and are not adequate to analyze the full complexity of structures and dynamics of the immune system as a whole.

In recent years, advances in high throughput technologies and deeper understanding of the many components of the immune system have opened up for a broader approach, the so-called systems immunology, with a holistic view of the immune system (349, 350). Systems immunology or systems vaccinology enables evaluation of the immune system in a more in-depth and detailed way with the potential of improving our understanding of the mechanisms of protection following vaccination or natural infection and thus, decrease the length and costs of current clinical. These holistic approaches include analysis of antibody profiling (protein and peptide microarrays); immune cell phenotyping (by flow and mass cytometry); Ig (immunoglobulin) and TCR repertoire analysis (by next-generation sequencing); immune cell gene expression (by microarray and RNA sequencing); and immune cell metabolic status (by metabolomics).

Suggestions for future work

This review provides a foundation for guidance on the future work with the development of the NIP regarding characterization and surveillance of immune responses. Several issues need to be considered and a number of steps are suggested below:

- Await the results and analysis of the ongoing national serosurveillance for population-level immunity as a basis for decision on any future study based on CMI analysis.
- Consider CMI in research studies of new vaccines.
- Decide what to study and design well thought-out proposals. Consider the current knowledge on changing epidemiology in universally vaccinated
populations were waning antibody levels may not always be indicative of susceptibility to infection.

- Explore the possibility of collaboration with the Nordic countries and possibility to create a collaborative CMI platform.
- Jointly planned CMI studies to be carried out through collaboration with the Nordic countries or other stakeholder
- Include and maintain different competencies such as vaccinologist, immunologist and bioinformaticians.
- Create a function or designate a competent person to continuously surveil the literature on CMI and technology advancement and keep updated relevant information on the vaccine-field with specific focus on NIP development and current data.

To conclude, understanding the long-term CMI will be needed in the future at the introduction of new vaccines into the NIP, especially those vaccines based on new concepts and adjuvants, and also to guide decisions about the timing of booster vaccine doses. Future development of the NIP could partly focus on new vaccine constructs against diseases such as TB, HIV and malaria where vaccines must “do better than nature”. In view of the development of the vaccinology field, CMI analyses will be needed and development of CMI capability and competency should be considered within the framework of preparedness; including new vaccine concepts; future perspectives and updated knowledge of systems vaccinology.
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Appendices

Appendix 1 – Contributors

The report was commissioned by Ann Lindstrand and Mia Brytting, and was written by Karina Godoy-Ramirez, Ingrid Uhnoo and Karl Ljungberg, at The Public Health Agency of Sweden.

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Katarina Widgren, Physician Infectious Diseases, Unit for Immunization Programs (Varicella and herpes zoster)

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Tecleab Teghesti, Biomedical Scientist, Unit for Laboratory Surveillance of Vaccine Preventable Diseases

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Hanna M. Nohynek, MD PhD, Team leader Vaccine Programme Development, National Institute for Health and Welfare THL, Finland
Appendix 2 – Search strings

Original research articles October 2003 - April 2015

((((((vaccine>Title OR vaccines>Title OR vaccination>Title)) AND (Diphtheria>Title OR Tetanus>Title OR Pertussis>Title OR Haemophilus>Title OR Tuberculosis>Title OR Staphylococcal>Title OR Poliovirus>Title OR Measles>Title OR Mumps>Title OR Rubella>Title OR Rotavirus>Title OR Hepatitis B>Title OR Influenza>Title OR Chickenpox>Title OR Herpes Zoster>Title OR Papilloma>Title OR pneumoniae>Title))) OR "Diphtheria-Tetanus-Pertussis Vaccine"[Majr:noexp] OR "Haemophilus Vaccines"[Majr:noexp] OR "Tuberculosis Vaccines"[Majr:noexp] OR "Inactivated"[Majr:noexp] OR "Measles-Mumps-Rubella Vaccine"[Majr:noexp] OR "Rotavirus Vaccines"[Majr:noexp] OR "Hepatitis B Vaccines"[Majr:noexp] OR "Influenza Vaccines"[Majr:noexp] OR "Chickenpox Vaccine"[Majr:noexp] OR "Herpes Zoster Vaccine"[Majr:noexp] OR "Papillomavirus Vaccines"[Majr:noexp] AND ((("Immunity, Cellular"[Majr:noexp] AND immunology[sh])) OR (safety & immunogenicity>Title OR immune responses>Title OR Safety & immunogenicity>Title OR cellular immune>Title OR immune response>Title OR (Protection>Title) AND (vaccine>Title OR vaccines>Title OR vaccination>Title))) AND "last 10 years"[PDat]) AND ("clinical trial"[Publication Type] OR "Clinical Trials as Topic"[mesh]) AND "Double-Blind Method"[Mesh] OR (randomized[TAI] AND (trial[TAI] OR trials[tiab])) OR ((single[TAI] OR double[TAI] OR doubled[TAI] OR triple[TAI] OR tripled[TAI] OR treble[TAI] OR treble[TAI]) AND (blind*[TAI] OR mask*[TAI])) AND "last 12 years"[PDat]).

Review articles 2008-2015

OR Meta-Analysis[ptyp] OR ((review[tiab] AND (rationale[tiab] OR evidence[tiab])) OR review[pt])

Original and review articles on memory B cells 2003-2014


An overview of the literature search and review process is presented in Figure 1.
Appendix 3 – Capacity and laboratory competence at PHA Sweden

At the Public Health Agency of Sweden, there are currently no CMI-related assays that are routinely performed in the surveillance activities of the national immunization programs. However, as a legacy from the Swedish Institute for Infectious Disease Control (SMI), there is history of vaccine trials and clinical research studies with specific focus on cellular immune responses. Some of the laboratory capacity that was built up before SMI was merged into the Public Health Agency of Sweden remains intact.

CMI methods

Methods for the analysis of cell-mediated immune responses to measles, rubella, varicella and pertussis have been used in research studies (109, 148, 149, 343). Also, clinical studies on influenza, CMV and VZV have been performed using CMI assays (337-341). For pertussis as well as measles and rubella, the MBC responses were assessed by B cell ELISpot. For measles and rubella, it was demonstrated that the kinetics of the MBC responses induced by MMR vaccination did not follow the same kinetics as antibody induction (62). A novel nasal live attenuated pertussis vaccine is currently being tested in phase I clinical trials, and the laboratory analyses are performed at the Public Health Agency of Sweden on cryopreserved PBMC samples. These included T cell analysis using IFN-γ and IL-2 Fluorospot and/or ELISpot, lymphoproliferative assays, and B cell analysis by B cell ELISpot and phenotypic analysis of memory B cells by flow cytometry (148, 343).

For VZV, a novel method was developed and implemented in 2003 based on whole blood antigen stimulation and subsequent immune phenotyping and flow cytometry analysis (340). This assay had advantages in low inter and intra assay variations and the possibility to scale up to large scale analysis.

Outside the national vaccination program, there is also a big effort to evaluate CMI responses in clinical phase I/II trials with an HIV vaccine candidate originally developed at the SMI (342). Both humoral and cellular parameters of the immune response are addressed. CMI is investigated using standardized and validated methods for IFN-γ and IL-2 ELISpot, 4- and 8-color intracellular cytokine staining (ICS) with staining for CD3, CD4, CD8, IFN-γ, IL-2, TNF, MIP-1β, and CD107a, in different combinations, followed by flow cytometry analysis (342, 351-355). Of note is that the 8-color staining is validated on fresh cells and cannot be performed on cryopreserved samples. In addition, several new methods are implemented, but not yet published, including ICS using a panel of antibodies designed to identify CTL; CD3, CD8, IFN-γ, IL-2, MIP-1β, perforin and granzyme B. A new flow cytometric PBMC lymphoproliferation (FC-LPA) assay is also in use, in which T cells are stained for surface markers: CD3, CD4, CD8, in combination with memory markers CD45RA, CCR7, CD27 and CD28. This proliferation assay has also been used in pertussis vaccine studies and has replaced the radiolabeled
thymidine incorporation test. Moreover, a panel of antibodies is used for phenotyping of PBMC (can also be performed on whole blood) to analyze the T, B and NK cells in the sample and their expression of memory markers CD45RA, CCR7, CD27 and CD28.

To summarize, the CMI methods established at PHAS are: Validated assays for IFN-γ and IL-2 ELISpot; 4-8-color intracellular cytokine staining (ICS) with staining for surface markers (CD3, CD4, CD8), and/or cytokines/chemokines (IFN-γ, IL-2, TNF, MIP-1β), and/or cytotoxicity markers (MIP-1β, CD107a perforin and granzyme B) - in different combinations. Flow cytometric based lymphoproliferation assays (using whole-blood or PBMC) in which T cells are stained for surface markers CD3, CD4, CD8, and if desirable also for memory markers CD45RA, CCR7, CD27 and CD28. In addition, assay for MBC responses using the B cell ELISpot by IFN-γ and IL-2 Fluorospot and/or IFN-γ-ELISpot have been established.

Currently processing capacity and laboratory competency at PHAS

Currently, there are at least seven senior scientists (PhD) and four laboratory scientists with experience in one or more CMI-related method.

In the ongoing pertussis vaccine trial, there are 2-3 staff dedicated to purify PBMC from blood samples and to cryopreserve cells for later analysis. The influx of samples is relatively low and equals about 15 patients per week during a limited time period. Despite the limited sample flow, this occupies a large proportion of the dedicated laboratory space and facilities. A conservative estimate is that the laboratory would be able to handle 2-3-fold the amount of samples.

For the analyses, ELISpot assays (pertussis and HIV studies) could be set up three times per week. The bottleneck is not the number of samples that can be included in the analysis per se, but rather how many samples that can be prepared in one day, especially if freshly isolated (non-frozen) cells are used. A limiting factor is that most analyses can only be performed by one or two persons. Thus, the capacity to perform and analyse CMI methods is vulnerable to staff changes. This negatively influences the capacity of the Public Health Agency to perform large-scale CMI analysis today, and competence transfer will be needed in case of expansion of CMI activities.

The sample processing and laboratory capacity is being maintained as long as vaccine trials and research studies are conducted. However, several limiting factors need to be addressed in case of expansion of CMI activities and large-scale CMI in the future.

Equipment and facilities

Currently there is a limited laboratory space dedicated for work related to CMI. The laboratory holds two laminar airflow benches suitable for sterile work for up to three staff simultaneously, centrifuges, cell counters, incubators, microscopes etc.
The laboratory includes one FACS Canto 8-color flow cytometer as well as a CTL ELISpot reader. The FACS Canto has an HTS module and can read one 96-well plate at a time, which facilitates high throughput screening. Although the equipment is closer to 10 years old, it is kept in good condition through the use of SOPs and service maintenance once or twice per year. In addition, there are ELISA readers and two MagPix instruments available to study cytokine release in cell cultures.

A limiting factor for large-scale CMI analysis is the lack of a backup flow cytometer, especially if assays are run using fresh cells. Previously, there was an identical back-up instrument, which was valuable especially at occasions when there were problems with one of the instruments. Another limiting factor for large-scale analysis is that the cell laboratory is too small to hold more than six-eight staff working simultaneously, and that a maximum of three persons can work in the laminar airflow hoods.
Appendix 4 - Methods to study cellular immune responses

Cytokine-based assays

Cytokine assays are the most frequent type of T/B-cell assays based on a variety of principles and platforms.

ELISpot (enzyme-linked immunosorbent spot)

ELISpot is one of the most commonly used immunoassays for the analysis of antigen-specific T and B cells (24), and is currently used in many clinical vaccine trials. The ELISpot enumerates antigen-specific lymphocytes by measuring secretion of specific immune proteins involved. The assay is functional and quantitative and the platform is adaptable to the evaluation of different cell types (T cells and B cells), secreted analytes (cytokines, chemokines, granzyme B and antibodies such as IgG or IgA), detection systems (fluorophore-based, immunoenzymatic) and mode of analysis (single or multicolor detection). In addition, the assay can be standardized across multiple laboratories and can also be used in low-income countries or settings with poor resources (354, 356). In addition the use of the ELISpot reader instead of manual counting of spots by light microscopy has contributed to the standardization of the assay (24).

In short, B or T cells isolated from a blood sample (or from some other available tissue) are stimulated with an antigen. The antigen can be either a whole protein, a peptide or a pool of peptides. Upon stimulation, activated cells will produce cytokines or antibodies that are then released. The well where the stimulation takes place has been coated with capture antibodies that will bind the secreted analytes. The cells are subsequently removed, and a secondary detection antibody is added. The detection antibody is coupled to a detection system, usually through biotinylation, and the biotinylated antibody can then be detected through an enzyme-linked reaction. Technological advances in recent years have resulted in the FluoroSpot assay, in which multiple cytokines/analytes secreted by the same cell can be detected in the same well (357).

Pros: The major advantages of the ELISpot lie in the possibility to standardize the assay, the robustness of the assay and its relative high throughput capacity. In addition, the platform can be adapted to several cell types and multiple secreted products. The assay measures a cell function in a quantitative manner and is inexpensive.

Cons: The major limitation of the ELISpot is that it provides no information on the phenotype of the responding cells and still a limited number (currently no more than three) of parameters can be detected simultaneously. However, since the method does not involve a fixation step, the stimulated cells can be recovered to be further studied in subsequent assays.

Equipment: A critical step in ELISpot analysis is the washing procedure which can be performed either manually or (semi)automatically using a multichannel washer or an ELISA microplate autowasher. Also, the use of an ELISpot reader – a
computer-based system for semi-automatic counting and interpretation of spots – helps in obtaining more reproducible results as compared to manual reading.

ICS (Intracellular Cytokine Staining)
ICS is a flow-cytometry based method that provides identification of antigen-specific cytokine secreting cells on a single-cell basis. It is possible to combine individual cell phenotyping for identification of specific cell subsets such as Th1, Th2, Th17, CD4+ or CD8+ T-cells with staining for other populations and functions such as memory cells; cytolytic markers (CD107), for natural killer cell activity and, for antigen-specific stimulation of CD4+ T cells (CD154). Central memory cells are identified by surface markers such as CD45RO, CD62L, CCR7 while effector memory cells are identified by the absence or dim (week) expression of these markers.

Advances in flow cytometry instrumentation and analysis software allows the routine combination of 8-12 (or up to 18) markers, which also implies technical and analytical challenges.

Beside fresh or cryopreserved peripheral blood mononuclear cells (PBMC), ICS can be run using whole blood samples, which offers a broad range of samples that can be used. The general procedure is relatively similar for assessment of responses to different pathogens, but the specific details of individual protocols may vary and thus require optimization. In brief, cells are antigen-stimulated to produce cytokines. The cells are treated with a substance that prohibits golgi transport to block secretion of the cytokines. Then, antibodies specific for surface molecules coupled to various fluorophores are used to phenotype the cell before the cells are fixed and permeabilized. In the last step, antibodies for detection of cytokines are used before flow cytometry analysis.

Pros: This ICS adds a qualitative aspect to the quantitative data provided by the ELISpot assay with its information not only on number of responding cells (“how many?”) but also on the extent of the responses (“how much?”) and the type of cells involved (“by whom?”).

Cons: Although the ICS is rather straightforward once all assay parameters have been optimized, it is considered less sensitive than ELISpot and has a lower throughput capacity. Also, the assay requires more expensive and sensitive equipment than the ELISpot.

Equipment: For sample collection a polychromatic flow cytometer with digital signal processing is required. A plate loader facilitates semi-automatic sample acquisition at a large-scale. Software for data analysis is also required.

In general, there is a lack of standardization of the ICS including sample staining, data collection and automated gating for analysis, which could increase power, reduce variability, and streamline the analysis for the ICS assay.
CBA or CTA (cytometric bead array)

CBA or CTA is another flow-cytometry based method for simultaneous quantification of cytokines and chemokines in serum or cell supernatant from antigen-stimulated cells. The assay is based on the use of antibody-coated beads, in which each bead has a fluorescent code to indicate the analyte to be assessed. Different beads can be combined to detect different cytokines in a small sample volume.

Analysis can also be performed using specialized platforms such as the Luminex xMAP system, which can analyse up to 500 analytes per sample. The basic multiplex assay method uses protein, peptide, or antibodies to tag individual analytes in the sample and is subsequently detected by using different labeled reporter ligands. Luminex xMAP is a bead array method that uses a fixed size microsphere, which are fluorescently dyed and dually act as analyte identifier, as well as to provide a solid surface to build the assay.

Pros: Multiplex analysis offers the benefits of ELISA, but also enables higher throughput, increased flexibility, reduced sample volume, and lower cost, with a similar workflow. The format also has advantages in low inter and intra assay variations and the possibility to scale up to large scale analysis.

Cons: Large amount of data generated by multiplex bead-based assays provides many challenges regarding the analysis procedure.

Equipment: Bead array assays must be performed using either flow cytometers or specialized platforms for bead-based assays such as the Luminex systems. In addition, a semi-automatic washer is needed depending on the type of assay format.

Flow Cytometry/Cell Phenotyping Assays

Flow cytometry is a powerful technology for analyzing antigen-specific cellular responses in a quantitative manner, capable of defining lineage and activation state. The biggest advantage of flow cytometry is the combination of multiparameter assessment and high-speed analysis, allowing detection of up to 18 colors at an analysis rate of more than 20,000 events/second (25). The more complex instruments (cell sorters) can also recover specific cells after measurement for further cell culture and biochemical analysis. The enormous advances and use of multiparameter flow cytometry in detailed and simultaneous characterization (phenotype, function, pathways and role) of immune responses have been reviewed by several groups (25, 358, 359).

Immunophenotyping

Immunophenotyping is useful to characterize and assess the frequency of T and B cell memory cell subsets using flow cytometry (23). Memory T cells are generally classified into subcategories including central memory (Tcm) and effector memory (Tem).
Tetramer staining

Tetramer staining is a method for identification of antigen-specific CD8+ T cells (CD8 tetramer staining). Similar CD4 tetramer approaches have been developed for identification of antigen-specific CD4+ T cells. Tetramers are synthetic MHC antigen-specific complexes, which can bind and identify antigen-specific T cell receptors. The technique can provide valuable information on the frequency and phenotype of the antigen-specific T-cell population, and in combination with ICS additional information regarding the functionality of the T cells can be obtained (25).

Pros: Tetramer assays have been shown to have minimal intra-assay variation, better precision and linearity than ICS or ELISpot performed using frozen PBMCs from the same donors.

Cons: The disadvantages of using tetramers are that they are epitope- and T cell receptor specific. Depending on the disease of interest, this assay thus requires epitope mapping and knowledge of the HLA status of the subject analyzed. Consequently, analysis is restricted to one epitope per tetramer. Moreover, tetramer assays do not test for functionality of the T cells such as cytokine release or ability to kill target cells.

In general, a limitation of flow cytometry is the reliance on fluorescent labels. Despite careful panel design, loss of resolution may occur due to issues regarding autofluorescence and spectral spillover. The greater the number of fluorochromes used, the larger these problems become magnified.

Time-of-flight mass spectrophotometry (CyTOF)

CyTOF is a cytometry-based technology, which has the capacity to measure up to 60 parameters simultaneously at a single-cell level, including surface and activation markers and intracellular molecules (23). Instead of traditional fluorescently labeled antibodies, CyTOF uses antibodies labeled with heavy metal isotopes that can be detected with mass spectrometry, eliminating the problems with spectral overlap in flow cytometry. Although the CyTOF technology is at an early stage, its ability to combine many different assessments is very promising. At this point, CyTOF suffers from a high price and few available instruments.

Pros: The advantages of the CyTOF technology include: i) traditional labeling techniques can be used – minimal change to current protocols; ii) no ‘autofluorescent’ equivalent in mass spectrophotometry – overcome problems regarding autofluorescence and spectral spillover; iii) minimal or no spectral compensation is needed; and iv) panel design is much easier than for traditional flow cytometry.

Cons: Limitations of the CyTOF include: i) slower sample acquisition compared to traditional flow cytometry – about 1,000 events per second, ii) sample must be “cleaner” than traditional flow cytometry, iii) so far limited (but growing) catalog of commercially labeled antibodies, iv) complex data structure requires new
methods of data analytical tools, and v) no equivalent scatter (FSC and SSC) measurements since cells are vaporized.

Lymphocyte proliferation assays (LPA)

Proliferation of whole blood or PBMC following cell culture stimulation with antigen (protein or peptides) has traditionally been used as an important measure of an essential feature of the adaptive immune response (23). In a cell proliferation assay, the number of cells or the change in the proportion of cells that are dividing due to cell activation is measured. Proliferation can be assessed in different ways, for instance, by measuring the incorporation of nucleosides into DNA and then detected using 1) radioactivity, 2) antibodies, 3) click-chemistry (a method for attaching a probe or substrate to a specific biomolecule) or 4) fluorescent dyes. 3H-thymidine incorporation has been the gold standard for assessment of proliferative responses. However, the 3H-thymidine assay is only semiquantitative, laborious, requires the use of radioisotopes, and does not provide information on the type or function of the proliferating cells. A number of alternative proliferation assays have been developed to avoid the use of radioactivity and obtain greater flexibility, using antibody-mediated detection or click-chemistry. Proliferation can also be assessed by Ki67-staining. Ki67 is a nuclear antigen, which is expressed throughout the proliferation phase but is absent in resting cells, and can consequently be used as a marker of recently dividing cells. Another alternative flow-cytometry based method is the CFSE-assay that utilizes carboxyfluorescein succinimidy1 ester (CFSE) to the fluorescently stained cells prior to culture. Following cell proliferation, the intensity of CFSE is halved with each cell division. This approach can also be combined with cell phenotyping.

Recently, the LPA have been further developed by the PHAS including two flow cytometry-based assays; a whole blood assay (340, 360) and a PBMC assay (351) (Teclab et al. in preparation)) which measures the proportion of lymphoblasts after stimulation of purified PBMCs. Both assays allow the determination of the immunophenotype of the proliferating lymphoblasts and the flow cytometry-LPA (FC-LPA) can be used in combination with memory profiling (e.g. CD45RA/CCR7/CD28/CD27) or other parameters.

Pros: In general, proliferation assays are sensitive, informative, and if flow-cytometry based, provide the ability to further characterize the responding cells by phenotyping. Small amount of blood volume are required if run using whole blood instead of PBMCs.

Cons: The assays suffer often from high inter- and intra-assay variability due to the differences in initial cell count and culture conditions. In addition, LPAs are long-term assays, requiring culture of 5-7 days.

Equipment: The instruments used for read-out varies depending on the type of assay format. For instance, sample collection using fluorescence labeling can be performed using different types of flow cytometers while a MicroBeta counter for
liquid scintillation counting is used if radioactive 3H-thymidine incorporation is performed.

**Cell-mediated cytotoxicity assays**

Cytotoxic T lymphocyte (CTL) responses are important, as the ability of CTL to destroy infected or tumor cells may correlate with vaccine efficacy (23). Thus, cell-mediated cytotoxicity is the main immune mechanism of protection from various pathogens and cancer. CTL kill target cells by two main mechanisms: 1) release of lytic granules containing perforin and granzymes and 2) triggering target cell death receptors such as TNF-α or FasL. The chromium release assay ($^{51}$Cr) has traditionally been used to measure antigen-specific CTL activity. However, the $^{51}$Cr release assay has a low level of sensitivity, does not provide information about individual cell phenotypes and there are also issues with the use of radioactive materials.

Numerous nonradioactive alternative assays have been developed, and the field has been reviewed by Zaritskaya et al. (25). These assays are in general based on the detection of markers of degranulation of effector CTL or markers of apoptosis or necrosis of target cells. These include for example the measurement of enzymes that are released into the cell supernatant following cytolysis. Alternatively, fluorometric methods can be used and similar results can be achieved by assessment of colorimetric assays or luminescence following transfection of cells with antigen conjugated to fluorescent proteins. In addition, ELISpot can be used to detect either granzyme B or perforin release. However, the drawback of the above CTL assays is their inability to assess other phenotypic features of the responding cells.

A number of alternative CLT-assays have been developed. Some of the approaches include the detection of apoptosis in target cells by staining for surface molecules (Annexiv V), caspase activation or by measuring the uptake of membrane impermeant fluorescent dyes that intercalate DNA when cell membrane integrity has been compromised. Target cells can also be labeled with fluorescent dyes that bind to the cell membranes. Indirectly, flow cytometry can be used to measure CTL-function by immunostaining T cells for a marker of T cell degranulation (CD107) together with a DNA probe. In addition, immunostaining for both CD107 and subsequent intracellular staining for perforin and/or granzyme B is considered a stable system for demonstrating CTL function (23).

**Pros:** Similar to proliferation assays, flow cytometry based cytotoxicity assays provide a number of advantages, including: the avoidance of radioactive compounds; the capacity of simultaneous assessment of CTL function and characterization of the phenotype of involved CTL cells; and evaluation of all stages of the cytotoxic process.

**Cons:** Direct measurement of cytotoxic activity requires highly skilled staff and advanced laboratory capacity. CLT assays are cumbersome and time-consuming. On the other hand, many assays have been developed for the evaluation of cell-
mediated target cell death to overcome some of the limitations of CTL assays. However, most of these assays do not directly measure cytotoxic capacity but rather a surrogate marker of cytotoxic activity. An assay that allows simultaneous evaluation of several parameters of the cytotoxic process may be more advantageous for clinical and vaccine monitoring.

**Equipment:** The instruments used for read-out of cytotoxic activity varies depending on the type of assay format. For instance, sample collection using fluorochrome labeling can be performed using different types of flow cytometers while a β-plate scintillation counter is used for the $^{51}$Cr release assay.

**Systems biology**

Until recently, the immune response have been studied in fragments, with focus on major components such as antibody responses to pathogens. In recent years, advances in high throughput technologies and deeper understanding of the many components of the immune system have opened up for a broader approach, the so-called **systems immunology**, with a holistic view of the immune system, reviewed by Furman et.al (Furman 2015) and Hagan el.al (Hagan 2015). This holistic approach enables evaluation of the immune system in a more in-depth way including: i) analysis of antibody profiling (protein and peptide microarrays); ii) immune cell phenotyping (by flow and mass cytometry); iii) Ig (immunoglobulin) and TCR repertoire analysis (by next-generation sequencing); vi) immune cell gene expression (by microarray and RNA sequencing); and v) immune cell metabolic status (by metabolomics).

Although this holistic systems biology applied to human disease offers a unique approach and the range of possible applications seem to be vast, yet, it is in its very early stages, has yet to be realized and be evaluated in the future.
Table 3. Most commonly used immunoassays for CMI analysis and availability at PHAS

**Characteristics of the immunoassays**

<table>
<thead>
<tr>
<th>Assays</th>
<th>Sample</th>
<th>Read-out</th>
<th>Sensitivity</th>
<th>High throughput</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Resources</th>
<th>Competency</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokine-based assays</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Plasma or serum</td>
<td>Total T cell response</td>
<td>++</td>
<td>+</td>
<td>Simple Well validated</td>
<td>One cytokine per assay No cell phenotyping (subsets or activation markers) Low sensitivity for low frequency cells</td>
<td>Up and running. Currently used for several agents.</td>
<td>Several staff</td>
<td></td>
</tr>
<tr>
<td>CBA</td>
<td>Plasma or serum</td>
<td>Total T cell response</td>
<td>++</td>
<td>+++</td>
<td>Multiplexing capability Require small samples sizes</td>
<td>Low sensitivity for low-frequency cells No cell phenotyping</td>
<td>Not in use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminex/ MagPix</td>
<td>Plasma or serum</td>
<td>Cytokines Multiparameter readout</td>
<td>++</td>
<td>+++</td>
<td>Multiplexing capability DNA probing Dedicated platform for analyzing data</td>
<td>Dedicated instrumentation and trained personnel Low sensitivity for low-frequency cells No phenotyping Requires cell culture</td>
<td>Up and running. To be used for ongoing national serosurvey.</td>
<td>Several staff (4 persons)</td>
<td></td>
</tr>
<tr>
<td>ELISpot</td>
<td>PBMC</td>
<td>Total T-cell response</td>
<td>+++</td>
<td>+++</td>
<td>Qualitative and quantitative Most sensitive technique for low-frequency cells No cell fixation: cells can be restudied</td>
<td>No phenotyping Single- or dual detection (no multiplexing)</td>
<td>Validated for vaccine trials, HIV and pertussis.</td>
<td>Several staff (4 persons)</td>
<td>(148, 149, 354)</td>
</tr>
<tr>
<td>FluoroSpot (T and B cells)</td>
<td></td>
<td>Number of secreting cells. Single or dual readout</td>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracellular cytokine staining (ICS)</td>
<td>Whole blood or PBMC</td>
<td>Surface and intra-cellular markers. Multiparameter</td>
<td>+++</td>
<td>(++)</td>
<td>Multiple cytokines for single cell level. Cell phenotyping (subsets, activation markers)</td>
<td>Cells cannot be sorted for further analysis due to fixation and permeabilization. Validated for HIV clinical trials (SOPs)</td>
<td>Used in HIV trials. To be used in Pertussis clinical trial</td>
<td>Several staff (4 persons)</td>
<td>(353-356)</td>
</tr>
<tr>
<td>Tetramer staining</td>
<td>Whole blood or PBMC</td>
<td>Epitope specific CD8 and CD4</td>
<td>+++</td>
<td>+</td>
<td>Identify antigen-specific T-cell population Can use to enrich for rare cell populations</td>
<td>Limited by peptide choice Requires individual MHC allele typing Not well validated in human studies</td>
<td>Not in use</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Resources and competency at PHAS**
### Characteristics of the immunoassays

<table>
<thead>
<tr>
<th>Pheno-</th>
<th>Whole</th>
<th>Multiparameter</th>
<th>Phenotypic characterization of immune cells; T, B, NK, others and effector, central memory cells, etc</th>
<th>SOPs</th>
<th>Resources and competency at PHAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>typing</td>
<td>blood or PBMC</td>
<td>Differentiation</td>
<td></td>
<td></td>
<td>Used in many studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Several staff (4 persons)</td>
</tr>
</tbody>
</table>

#### Lymphoproliferation assays (LPA)

<table>
<thead>
<tr>
<th>3H thymidine Incorporation</th>
<th>PBMC</th>
<th>Total T cell response. Single parameter</th>
<th>++++ ++</th>
<th>Conventional Well validated</th>
<th>Radioisotope Semi-quantitative No phenotyping</th>
<th>Not in use. Validated for HIV clinical trial</th>
<th>Several staff (2 persons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFSE (FC-based)</td>
<td>PBMC</td>
<td>Multiparameter parameter Functional and differentiation</td>
<td>++ ++</td>
<td>Non-radioactive Cell phenotyping possible. Long term (4–6 days) in vitro stimulation</td>
<td>CFSE is toxic Relatively insensitive. Lack of standardization. Long term (4–6 days) in vitro stimulation</td>
<td>Not in use. Validated for HIV clinical trial</td>
<td>Several staff (2 persons)</td>
</tr>
<tr>
<td>K67 (FC-based)</td>
<td>PBMC</td>
<td></td>
<td>++ ++</td>
<td>Non-radioactive, non-toxic. Does not require cell culture. Cell phenotyping possible Cells must have recently proliferated Cells must be fixed Relatively insensitive</td>
<td></td>
<td>Not in use</td>
<td></td>
</tr>
<tr>
<td>FASCIA, FC-LPA (FC-based)</td>
<td>Whole blood or PBMC</td>
<td>Blast-formation</td>
<td>+++ ++</td>
<td>Non-radioactive, non-toxic. Cell phenotyping possible Long term (4–6 days) in vitro stimulation Sensitive</td>
<td>Validated HIV clinical trials.</td>
<td>Several staff (4 persons)</td>
<td></td>
</tr>
</tbody>
</table>

#### Cytotoxicity assays (CTL)

<table>
<thead>
<tr>
<th>Chromium release (Cr51)</th>
<th>PBMC</th>
<th>Lytic activity. T- or NK cell-mediated activity</th>
<th>+ +</th>
<th>Conventional Directly measures cytotoxic activity</th>
<th>radioactive No cell phenotyping</th>
<th>Not in use. Validated for HIV clinical trial</th>
<th>Several staff (2 persons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL/flow (FC-based)</td>
<td></td>
<td></td>
<td>++ ++</td>
<td>Allows comprehensive phenotyping of CTLs</td>
<td></td>
<td>Not in use. Validated for HIV clinical trial</td>
<td>Several staff (2 persons)</td>
</tr>
</tbody>
</table>
The purpose of this review is to summarize current knowledge on cell-mediated immunity (CMI) against vaccine-preventable diseases (VPD) included – and considered to be included – in the National Immunization Program (NIP) in Sweden and, to investigate the possible added value of CMI in vaccine surveillance and in research to address knowledge gaps.

The review is based on a comprehensive scoping literature review including scientific peer-reviewed literature from the past 12 years, available grey literature, and ad-hoc searches from 1960 to 2018.

The report provides primarily an information basis for future decision-making regarding the role of CMI in the surveillance of VPD, at the Agency. It may also be of value to professionals and specialists such as managers of NIPs and managers of laboratory surveillance of NIPs, as well as national and international communicable disease control and prevention authorities and public health agencies.