2 Vaccine immunology

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To generate vaccine-mediated protection is a complex challenge. Currently available vaccines have largely been developed empirically, with little or no understanding on how they activate the immune system. Their early protective efficacy is primarily conferred by the induction of antigen-specific antibodies (Box 2-1). However, there is more to antibody-mediated protection than the peak of vaccine-induced antibody titers. The quality of such antibody responses, eg, their avidity or specificity, has been identified as a determining factor of efficacy. In addition, long-term protection requires the persistence of vaccine antibodies and/or the generation of immune memory cells capable of rapid and effective reactivation with subsequent microbial exposure. The determinants of immune memory induction, as well as the relative contribution of persisting antibodies and of immune memory to protection against specific diseases, are essential parameters of long-term vaccine efficacy. The predominant role of B cells in the efficacy of current vaccines should not overshadow the importance of T-cell responses: T cells are essential to the induction of high-affinity antibodies and immune memory and will be the prime effectors against novel vaccine targets such as tuberculosis.

New methods have emerged allowing the assessment of a growing number of vaccine-associated immune parameters, including in humans. This development raises new questions about the optimal markers to assess and their correlation with vaccine-induced protection. The identification of immune correlates—or at least surrogates—of vaccine efficacy is a major asset for the development of new vaccines or the optimization of immunization strategies using available vaccines. Thus, their determination generates a considerable amount of interest at all levels, from an immunologist working at the bench to a physician who wants to optimize a vaccine schedule for a specific patient. During the last decade, the increased awareness of the complexity of the immune system and its determinants, including at the host genetic level, suggested that using system biology approaches to assess how various processes and networks interact in response to immunization could prove more successful than trying to isolate and characterize a few components of vaccine responses. Delineating the specific molecular signatures of vaccine immunogenicity may highlight novel correlates of protective immunity and better explain the heterogeneity of vaccine responses in a population. The tailoring of vaccine strategies for specific vulnerable populations, including very young, elderly and immunosuppressed populations, also largely relies on a better understanding of what supports or limits vaccine efficacy under special circumstances at the population and individual levels. Last, the exponential development of new vaccines raises many questions that are not limited to the targeted diseases and the potential impacts of their prevention, but address the specific and nonspecific impacts of such vaccines on the immune system and, thus, on health in general. These immune-related concerns have largely spread into the population, and questions related to the immunological safety of vaccines, ie, their capacity of triggering non–antigen-specific responses possibly leading to allergy, autoimmunity, or even premature death, are being raised. The objective of this chapter is to extract from the complex and rapidly evolving field of immunology the main concepts that are useful to better address these important questions.

How do vaccines mediate protection?

Disease control or elimination requires the induction of protective immunity in a sufficient proportion of the population. This requirement is best achieved by immunization programs capable of inducing long-term protection, a hallmark of adaptive immunity that contrasts with the brisk but short-lasting innate immune responses. Long-term immunity is conferred by the maintenance of antigen-specific immune effectors and/or by the induction of immune memory cells that may be sufficiently efficient and rapidly reactivated into immune effectors in case of pathogen exposure.

Vaccine-induced immune effectors (Table 2-1) are essentially antibodies—produced by B lymphocytes—capable of binding specifically to a toxin or a pathogen.[1] Other potential effectors are cytotoxic $CD8^+$ T lymphocytes* that may limit the spread of infectious agents by recognizing and killing infected cells or secreting specific antiviral cytokines and $CD4^+$ T helper (Th) lymphocytes. These Th cells may contribute to protection through cytokine production and provide support to the generation and maintenance of B and $CD8^+$ T-cell responses. Effector Th cells are commonly subdivided into T helper 1 (Th1), T helper 2 (Th2), and Th helper 17 (Th17) subtypes* (Table 2-1).[2] These Th17 cells essentially defend against extracellular bacteria that colonize the skin and mucosa, recruiting neutrophils and promoting local inflammation.[2] These effectors are controlled by regulatory T cells* (Tregs) involved in maintaining immune tolerance.[3] Most antigens and vaccines trigger B- and T-cell responses, such that there is no rationale in opposing antibody production ("humoral immunity") and T-cell responses ("cellular immunity"). In addition, $CD4^+$ T cells are required for most antibody responses, whereas antibodies exert significant influences on T-cell responses to intracellular pathogens.[4]

Box 2-1: Main immunological definitions

Adjuvant
Agents that increase the stimulation of the immune system by enhancing antigen presentation (depot formulation, delivery systems) and/or by providing costimulation signals (immunomodulators). Aluminum salts are most often used in today's vaccines.

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

Affinity maturation
Processes through which antigen-specific B cells undergo somatic hypermutation and affinity-based selection, resulting in B cells that produce antibodies with increased affinity over germ-line antibodies.

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.

Antigen-presenting cells
Cells that capture antigens by endocytosis or phagocytosis, process them into small peptides, display them at their surface through MHC molecules, and provide costimulation signals that act synergistically to activate antigen-specific T cells. Antigen-presenting cells include B cells, macrophages, and dendritic cells, although only dendritic cells are capable of activating naïve T cells.

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

Carrier protein
A protein that is used as a template to which polysaccharide moieties are chemically conjugated to generate glycoconjugate vaccines. It is believed that carrier proteins provide antigenic epitopes for recognition by $CD4^+$ helper T cells, in particular follicular helper T cells.

$CD4^+$ T helper 1 lymphocytes
$CD4^+$ T cells that on 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 lymphocytes
$CD4^+$ T cells that on activation differentiate into cells that mainly secrete IL-4, IL-5, IL-6, IL-10, and IL-13, exerting direct antimicrobial functions (parasites) and essentially providing support to B lymphocytes.

$CD4^+$ T helper 17 lymphocytes
CD4+ T cells that mainly secrete IL-17, IL-21, and IL-22 are implicated in host defense against extracellular bacteria colonizing exposed surfaces (airways, skin, gut).

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

Chemokines
Small secreted proteins that function as chemoattractants, recruiting cells that express the corresponding chemokine receptors at their surface and thus migrate toward higher concentrations of chemokines.

Costimulatory molecules
Molecules that become expressed at the surface antigen-presenting cells on activation and deliver stimulatory signals to other cells, namely T and B cells.

Dendritic cells
Cells that constantly sample the surroundings for pathogens such as viruses and bacteria, detect dangers, and initiate immune responses. Immature patrolling DCs have high endocytic activity and a low T-cell activation potential. Contact with a pathogen induces maturation and the expression of certain cell-surface molecules, greatly enhancing their ability to activate T cells.

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

Extrafollicular reaction
B-cell differentiation pathways that occur outside of germinal centers in response to protein or polysaccharide antigens. Rapid, it generates B cells that are short-lived (days) and produces low-affinity antibodies, without inducing immune memory.

Follicular dendritic cells
Stromal cells in the spleen and nodes that on activation express chemokines (notably CXCL13) attracting activated antigen-specific B and T cells and thus nucleate the germinal center reaction. Follicular dendritic cells provide antiapoptotic signals to GC B cells and support their differentiation into plasma cells or memory B cells.

Follicular helper T lymphocytes
$CD4^+$ T cells that on activation migrate toward follicular dendritic cells and provide critical help to germinal center B cells, influencing isotype switching, affinity maturation, and differentiation.

Germinal centers
Dynamic structures that develop in the spleen/nodes in response to an antigenic stimulation and dissolve after a few weeks. GCs contain a monoclonal population of antigen-specific B cells that proliferate and differentiate through the support provided by follicular dendritic cells and helper T cells. Immunoglobulin class-switch recombination, affinity maturation, B-cell selection, and differentiation into plasma cells or memory B cells essentially occur in GCs.

Isotype switching
Switch of immunoglobulin expression and production from IgM to IgG, IgA, or IgE, occurring during B-cell differentiation through DNA recombination.

Marginal zone
The area between the red pulp and the white pulp of the spleen. Its major role is to trap particulate antigens from the circulation and present them to lymphocytes.

Pattern recognition receptors
Germline-encoded receptors sensing the presence of infection via the recognition of conserved microbial pathogen-associated molecular patterns and triggering innate immune responses.

Regulatory T cells
T cells that on activation differentiate into cells that express specific cytokines (IL-10, TGF-β/surface markers) and act to suppress activation of the immune system through various mechanisms, maintaining immune homeostasis and tolerance to self-antigens.

Somatic hypermutation
A process that introduces random mutation in the variable region of the B-cell receptor (ie, immunoglobulin) locus at an extremely high rate, during B-cell proliferation. This mechanism occurs through the influence of the activation-induced cytidine deaminase enzyme and generates antibody diversification.

Box 2-1: Main immunological definitions—cont'd

T lymphocytes
Cells that originate in the thymus, mature in the periphery, become activated in the spleen/nodes if their T-cell receptors bind to an antigen presented by an MHC molecule and they receive additional costimulation signals driving them to acquire killing (mainly $CD8^+$ T cells) or supporting (mainly $CD4^+$ T cells) functions.

T-independent B-cell responses
Differentiation pathway of B cells, mainly elicited by polysaccharides, that takes place in the marginal zone and extrafollicular areas of the spleen/nodes. Its hallmarks are to be rapid (days) but to elicit the transient (months) production of antibodies of low affinity, without inducing immune memory.

T-dependent B-cell responses
Differentiation pathway of B cells elicited by protein antigens that recruits T and B cells into germinal centers of the spleen/nodes. Its hallmarks are to be slow (weeks) but to elicit long-lasting (years) production of antibodies of high affinity and immune memory.

Toll-like receptors
A family of 10 receptors (TLR1 to TLR10), present at the surface of many immune cells, that recognize pathogens through conserved microbial patterns and activate innate immunity when detecting danger.

Table 2-1 Effector Mechanisms Triggered by Vaccines

- Antibodies prevent or reduce infections by clear extracellular pathogens through:
  - binding to the enzymatic active sites of toxins or preventing their diffusion
  - neutralizing viral replication, eg, preventing viral binding and entry into cells
  - promoting opsonophagocytosis of extracellular bacteria, ie, enhancing clearance by macrophages and neutrophils
  - activating the complement cascade
- $CD8^+$ T cells do not prevent but reduce, control, and clear intracellular pathogens by:
  - directly killing infected cells (release of perforin, granzyme, etc)
  - indirectly killing infected cells through antimicrobial cytokine release
- $CD4^+$ T cells do not prevent but participate in the reduction, control, and clearance of extracellular and intracellular pathogens by:
  - producing IFN-γ, TNF-α/TNF-β, IL-2, and IL-3 and supporting activation and differentiation of B cells, $CD8^+$ T cells, and macrophages (Th1 cells)
  - producing IL-4, IL-5, IL-13, IL-6, and IL-10 and supporting B-cell activation and differentiation (Th2 cells)
  - producing IL-17, IL-21, and IL-22 and contributing to defense against bacteria on mucosal surfaces (such as *Streptococcus pneumoniae, Bordetella pertussis*, *Mycobacterium tuberculosis*)

What are the main effectors of vaccine responses?

The nature of the vaccine exerts a direct influence on the type of immune effectors that are predominantly elicited and that mediate protective efficacy (Table 2-2).

Capsular polysaccharides (PS) elicit B-cell responses in what is classically reported as a T-independent manner*,[5] although increasing evidence supports a role for $CD4^+$ T cells in such responses. The conjugation of bacterial PS to a protein carrier (eg, glycoconjugate vaccines) provides foreign peptide antigens that are presented to the immune system and, thus, recruits antigen-specific $CD4^+$ Th cells in what is referred to as a T-dependent antibody response*.[67] A hallmark of T-dependent responses,which are also elicited by toxoid, protein, inactivated, or live attenuated viral vaccines (Table 2-2), is to induce higher-affinity antibodies and immune memory. In addition, live attenuated vaccines usually generate $CD8^+$ cytotoxic T cells. The use of live vaccines/vectors or of specific novel delivery systems seems necessary for the induction of strong $CD8^+$ T-cell responses. Most current vaccines mediate their protective efficacy through the induction of vaccine antibodies, whereas BCG-induced T cells contribute to macrophage activation and control of *Mycobacterium tuberculosis*.

The induction of antigen-specific immune effectors (and/or of immune memory cells) by an immunization process does not imply that these antibodies, cells, or cytokines represent surrogates—or even correlates—of vaccine efficacy. This requires the formal demonstration that vaccine-mediated protection is dependent—in a vaccinated person—on the presence of a given marker such as an antibody titer or a number of antigen-specific cells above a given threshold. Antigen-specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many diseases[8] (Table 2-2). Passive protection may result from the physiological transfer of maternal antibodies (eg, tetanus) or the passive administration of immunoglobulins or vaccine-induced hyperimmune serum (eg, measles, hepatitis, varicella). Such antibodies may neutralize toxins in the periphery, at their site of production in an infected wound (tetanus), or the throat (diphtheria). They may reduce binding or adhesion to susceptible cells or receptors and prevent viral replication (eg, polio) or bacterial colonization (glycoconjugate vaccines against encapsulated bacteria) if present at sufficiently high titers on mucosal surfaces.[9] The neutralization of pathogens at mucosal surfaces is mainly achieved by the transudation of vaccine-induced serum IgG antibodies. It requires serum IgG antibody concentrations to be of sufficient affinity and abundance to result in "protective" antibody titers in saliva or mucosal secretions. As a rule, such responses are not elicited by PS bacterial vaccines but achieved by glycoconjugate vaccines, which therefore prevent nasopharyngeal colonization in addition to invasive diseases.

Under most circumstances, immunization does not elicit sufficiently high and sustained antibody titers on mucosal surfaces to prevent local infection. It is only after having infected mucosal surfaces that pathogens encounter vaccine-induced IgG serum antibodies that neutralize viruses, opsonize bacteria, activate the complement cascade (Table 2-1), and limit their multiplication and spread, preventing tissue damage and, thus, clinical disease. That vaccines fail to induce sterilizing immunity is not an obstacle to successful disease control, although it represents a significant challenge for the development of specific vaccines against chronic viral infection.

Table 2-2 Correlates of Vaccine-Induced Immunity

Vaccines	Vaccine type	Serum IgG	Mucosal IgG	Mucosal IgA	T cells
Diphtheria toxoid	*Toxoid*	++	(+)		
Hepatitis A	*Killed*	++			
Hepatitis B (HBsAg)	Protein	++			
Hib PS	PS	++	(+)		
Hib glycoconjugates	PS-protein	++	++		
Influenza	Killed, subunit	++	(+)		
Influenza intranasal	Live attenuated	++	+	+	+ ($CD8^+$)
Japanese encephalitis	Killed	++			
Measles	Live attenuated	++			+ ($CD8^+$)
Meningococcal PS	PS	++	(+)		
Meningococcal conjugates	PS-protein	++	++		
Mumps	Live attenuated	++			
Papillomavirus (human)	VLPs	++	++		
Pertussis, whole cell	Killed	++			
Pertussis, acellular	Protein	++			+?($CD4^+$)
Pneumococcal PS	PS	++	(+)		
Pneumococcal conjugates	PS-protein	++	++		
Polio Sabin	Live attenuated	++	++	++	
Polio Salk	Killed	++	+		
Rabies	Killed	++			
Rotavirus	VLPs	(+)	(+)	++	
Rubella	Live attenuated	++			
Tetanus toxoid	Toxoid	++			
Tuberculosis (BCG)	Live mycobacteria				++($CD4^+$)
Typhoid PS	PS	+	(+)		
Varicella (chickenpox)	Live attenuated	++			+?($CD4^+$)
Varicella (zoster)	Live attenuated				++($CD4^+$)
Yellow fever	Live attenuated	++			

PS, polysaccharide; VLP, virus-like particle.
Note: This table may not be exhaustive and includes only currently licensed vaccines.

Current vaccines mostly mediate protection through the induction of highly specific IgG serum antibodies (Table 2-2). Live viral vaccines such as rotavirus, oral polio, and nasal influenza induce serum IgA and secretory IgA, which help limit viral shedding on mucosal surfaces. Under certain circumstances, however, passive antibody-mediated immunity is inefficient (tuberculosis). There is conclusive evidence that T cells are the main effectors of BCG, despite the fact that specific T-cell frequency and cytokine expression profiles do not correlate with protection in BCG-immunized infants,[10] and of the zoster vaccine. However, there is indirect evidence that vaccine-induced T cells contribute to the protection conferred by other vaccines. $CD4^+$ T cells seem to support the persistence of protection against clinical pertussis in children primed in infancy, after vaccine-induced antibodies have waned.[11-14] Another example is that of measles immunization in 6-month-old infants in whom antibody responses largely are not initiated because of immune immaturity and/or the residual presence of inhibitory maternal antibodies, but significant interferon (IFN)-γ–producing $CD4^+$ T cells are generated.[15,16] The infants remain susceptible to measles infection but are protected against severe disease and death, presumably because of the viral clearance capacity of their vaccine-induced T-cell effectors. Thus, prevention of infection may be achieved only by vaccine-induced antibodies, whereas disease attenuation and protection against complications may be supported by T cells, even in the absence of specific antibodies. The understanding of vaccine immunology requires appraising how B- and T-cell responses are elicited, supported, maintained, and/or reactivated by vaccine antigens.

From innate to adaptive immunity activation: the first steps after immunization

Novel adjuvants essentially enhance vaccine responses by modulating innate immunity, which shapes adaptive responses.[17,18] Indeed, the induction of antigen-specific B- and T-cell responses requires their activation by specific antigen-presenting cells* (APCs), essentially dendritic cells* (DCs) that must be recruited into the reaction. Immature DCs patrol throughout the body. When exposed to pathogens, they undergo brisk maturation,

modulate specific surface receptors, and migrate toward secondary lymph nodes, where the induction of T- and B-cell responses occurs. The central role for mature DCs in the induction of vaccine responses reflects their unique capacity to provide antigen-specific and costimulation signals to T cells; these "danger signals" are required to activate naïve T cells.[19] The very first requirement to elicit vaccine responses is to provide sufficient danger signals through vaccine antigens and/or adjuvants* (Figure 2-1) to trigger an inflammatory reaction that is mediated by cells of the innate immune system.[17]

Dendritic cells, monocytes, and neutrophils express a set of receptors directed against evolutionarily conserved pathogen patterns that are not contained in self-antigens and are readily identified as "danger".[20] Through these pattern recognition receptors*, among which Toll-like receptors* fulfill an essential role (Table 2-3),[20] these host cells sense the potential danger when they encounter a pathogen and become activated (Figure 2-2). They modulate the expression of their surface molecules and produce proinflammatory cytokines and chemokines,[17] which results in the extravasation and attraction of monocytes, granulocytes, and natural killer cells and generates an inflammatory microenvironment (Figure 2-1) in which monocytes differentiate into macrophages and immature DCs become activated.[18] This activation modifies the expression of homing receptors at their surface and triggers DC migration toward the draining lymph nodes (Figure 2-2). In the absence of danger signals, DCs remain immature: on contact with naïve T cells, T cells do not differentiate into immune effectors but into regulatory $CD4^+$ T cells that maintain immune tolerance.[3]

Live viral vaccines most efficiently trigger the activation of the innate immune system through multiple pathogen-associated signals (such as viral RNA), allowing their recognition by pattern recognition receptors (Table 2-3).[21] Following injection, viral particles rapidly disseminate throughout the vascular network and reach their target tissues. This pattern is very similar to that occurring after a natural infection, including the initial mucosal replication stage for vaccines administered through the nasal and oral routes. Following the administration of a live viral vaccine and its dissemination, DCs are activated at multiple sites, migrate toward the corresponding draining lymph nodes, and launch multiple foci of T- and B-cell activation. This sequence provides a second explanation of the generally higher immunogenicity of live vs nonlive vaccines (Table 2-4). Another consequence of this early diffusion pattern is that the site and route of injection of live viral vaccines are of minor importance: for example, the immunogenicity and reactogenicity of measles vaccine is similar following intramuscular or subcutaneous injection,[22] and measles vaccine may be administered by aerosol. Live bacterial vaccines, such as BCG, multiply at the site of injection, where they generate the induction of a prolonged inflammatory reaction, and at a distance, with a preponderance for local draining lymph nodes.

Nonlive vaccines, whether containing proteins, PS, glycoconjugates, or inactivated microorganisms (Table 2-2), may still contain pathogen recognition patterns capable of initiating innate responses. In the absence of microbial replication, however, vaccine-induced activation remains more limited, in both time and space. Nonlive vaccines essentially activate innate responses at their site of injection (Figure 2-1). Their site and route of administration are, thus, more important. The high number of DCs in the dermis allows a marked reduction (eg, 10-fold) of the antigen dose in intradermal immunization. This advantage is applied to the prevention of rabies in many countries and could prove useful against additional targets as novel microneedle and needle-free devices become available.[23] Patrolling DCs are also numerous in well-vascularized muscles, which is the preferred route of injection for nonlive vaccines. They are fewer in adipose tissues, such that subcutaneous injections may be less effective than intramuscular injections under conditions of limited immunogenicity, as demonstrated for adult immunization against hepatitis B.[24]

Despite many efforts, immunization through the mucosal route remains limited to a few live vaccines. The extreme difficulty in producing nonlive mucosal vaccines reflects the need to overcome a large number of physical, immunological, and chemical barriers, which requires the use of live vaccines or strong adjuvants. This fact is not trivial, as unfortunately illustrated by the association of a novel adjuvanted inactivated intranasal influenza vaccine with Bell's palsy.[25]

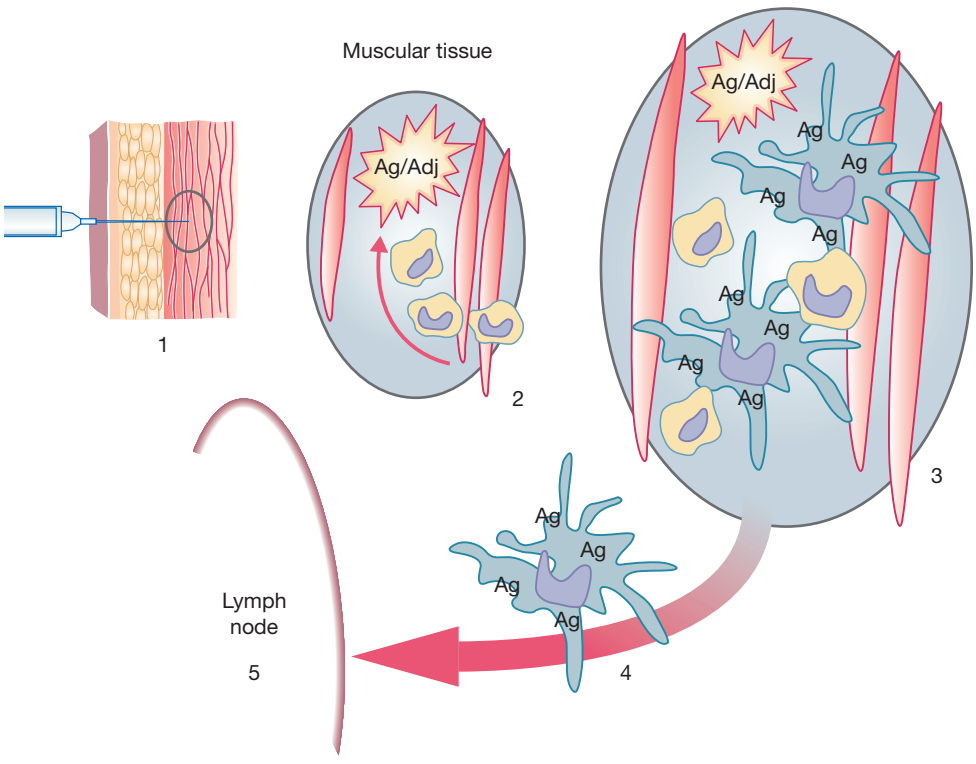

Figure 2-1 Initiation of a vaccine response. Following injection (1), the pathogen-associated patterns contained in vaccine antigens attract dendritic cells, monocytes, and neutrophils that patrol throughout the body (2). Elicitation of sufficient "danger signals" by the vaccine antigens/adjuvants activates monocytes and dendritic cells (3); the activation changes their surface receptors and induces their migration along lymphatic vessels (4), to the draining lymph nodes (5) where the activation of T and B lymphocytes will take place.

Table 2-3 Recognition of Vaccine Determinants by Human Pattern Recognition Receptors

Receptors	Ligands	Demonstrated ligands in vaccines
TLR1	Certain bacterial lipoproteins	
TLR2	Peptidoglycan, lipoproteins, glycolipids, lipopolysaccharide	BCG, Hib-OMP, pneumococcal PS
TLR3	Viral double-stranded RNA	
TLR4	Bacterial lipopolysaccharides	BCG, pneumococcal PS, HPV-VLPs, AS02, and AS04 adjuvants
TLR5	Bacterial flagellins	
TLR6	Lipoteichoic acid, lipopeptides	
TLR7	Single-stranded RNA	Yellow fever, live attenuated influenza, whole cell influenza
TLR8	Single-stranded RNA	Yellow-fever
TLR9	Unmethylated CpG oligonucleotides	Yellow fever
TLR10	Unknown	
NALP3	Multiple	Alum
NOD1, NOD2	Peptidoglycans	Pneumococcal PS

Hib, *Haemophilus influenzae* type b; HPV, human papillomavirus; PS, polysaccharide; VLP, virus-like particle.

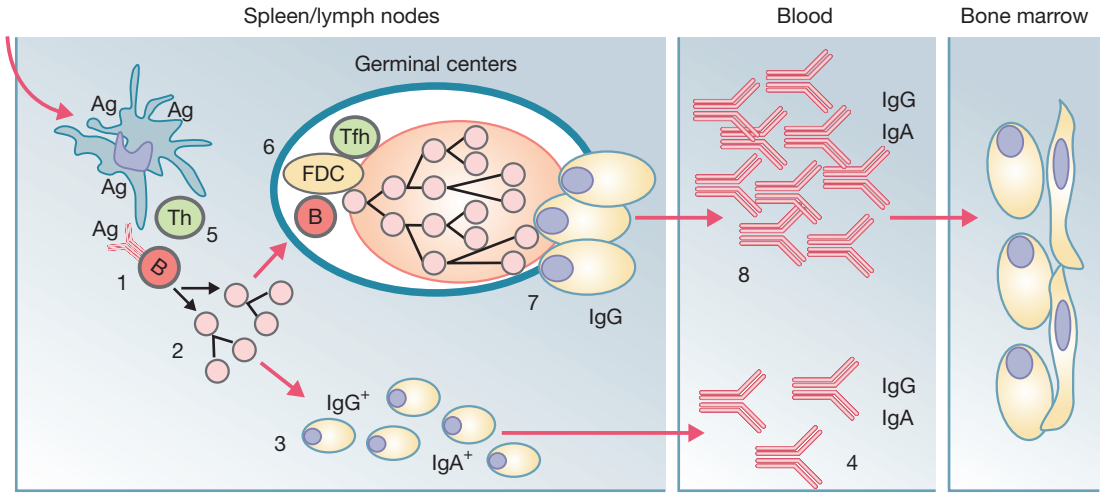

Figure 2-2 Extrafollicular and germinal center responses to protein antigens. In response to a protein antigen reaching lymph nodes or spleen, B cells capable of binding to this antigen with their surface immunoglobulins (1) undergo brisk activation. In an extrafollicular reaction (2), B cells rapidly differentiate in plasma cells (3) that produce low-affinity antibodies (of the IgM +/– IgG/IgA isotypes) that appear at low levels in the serum within a few days after immunization (4). Antigen-specific helper T cells (5) that have been activated by antigen-bearing dendritic cells trigger some antigen-specific B cells to migrate toward follicular dendritic cells (FDCs) (6), initiating the germinal center (GC) reaction. In GCs, B cells receive additional signals from follicular T cells (Tfh) and undergo massive clonal proliferation, switch from IgM toward IgG, IgA or IgE, undergo affinity maturation (7), and differentiate into plasma cells secreting large amounts of antigen-specific antibodies (8). At the end of the GC reaction, a few plasma cells exit nodes/spleen and migrate to survival niches mostly located in the bone marrow, where they survive through signals provided by supporting stromal cells (9).

Following their activation, DCs migrate toward the local draining lymph nodes, eg, the axillary and inguinal area following deltoid and quadriceps injection, respectively. That primary immune responses to nonlive vaccines are essentially focal and unilateral is likely to contribute to the fact that the simultaneous administration of several distinct vaccines may take place without immune interference if vaccines are administered at sites draining into distinct lymph node areas. Most nonlive vaccines require their formulation with specific adjuvants to include danger signals and trigger a sufficient activation of the innate system. The understanding of the mode of action of current and novel adjuvants markedly increased during the last few years, with the long-used aluminum salts revealing some of their secrets.[26] Although the adjuvants currently in use do not trigger the degree of innate immune activation that is elicited by live vaccines, whose immune potency far exceeds that of nonlive vaccines, progress is being made: a single dose of the AS03-adjuvanted influenza H1N1/09 vaccine in healthy children elicited antibody responses similar to those observed in convalescent children.[27]

Vaccine antibody responses

How are primary antibody responses elicited?

B cells are essentially activated in the lymph nodes draining the injection site. Vaccine antigens reaching the subcapsular sinus by free-fluid diffusion are taken up by specific subcapsular sinus

Table 2-4 Determinants of Primary Vaccine Antibody Responses in Healthy People

Determinants	Mechanisms (presumed)
Vaccine type	
Live vs inactivated	Higher intensity of innate responses through the synergistic activation of several PRRs, higher antigen content following replication, and more prolonged antigen persistence generally result in higher Ab responses to live than inactivated vaccines.
Protein vs polysaccharide	Recruitment of T-cell help and induction of GCs, ie, memory induction, results in higher and more prolonged Ab responses to protein or glycoconjugate than to PS vaccines.
Adjuvants	Modulation of antigen delivery and persistence (depot or slow-release formulations) and/or enhancement of Th responses (immunomodulator) may support or limit Ab responses.
Antigen nature	
Polysaccharide antigens	Failure to induce GCs limits immunogenicity.
Protein antigens	Inclusion of epitopes readily recognized by B cells (B-cell repertoire), inclusion of epitopes readily recognized by follicular helper T cells, elicitation of efficient follicular T-cell help, and the capacity of antigen to associate/persist in association with FDCs result in higher Ab responses.
Antigen dose	As a rule, higher Ag doses increase the availability of Ag for B-/T-cell binding and activation and for association with FDCs.
Vaccine schedule	
Interval between doses	A 3-week minimal interval between primary doses avoids competition between successive waves of primary responses.
Genetic determinants	The capacity of Ag epitopes to associate with a large panel of MHC molecules increases the likelihood of responses in the population. MHC restriction may limit T-cell responses. Gene polymorphisms in molecules critical for B- and T-cell activation/differentiation are likely to affect Ab responses.
Environmental factors	Mostly unidentified
Age at immunization	Early life immune immaturity or age-associated immune senescence

Ab, antibody; Ag, antigen; FDC, follicular dendritic cell; GC, germinal center; PRR, pattern recognition response; PS, polysaccharide.

macrophages and translocated into the B-cell zone. The B cells equipped with surface B-cell receptors[28] capable of binding to the vaccine antigens are activated and migrate to the interface between the B-cell (follicle) and the T-cell zones. There, B cells engage T cells and initiate their proliferation. The cumulative amount of costimulation signals received by B cells determines their fate.[29] Protein antigens (which are taken up and displayed on the surface of APCs*) also activate T cells. This induces a highly efficient B-cell differentiation pathway, through specific structures (germinal centers [GCs]) in which antigen-specific B cells proliferate and differentiate into antibody-secreting plasma cells or memory B cells. Polysaccharide antigens that fail to recruit T cells into the response do not trigger GCs, such that they elicit only short-lived plasma cells resulting in weaker and shorter antibody responses and no immune memory.

T-dependent responses to protein antigens

The extrafollicular reaction

Naïve B cells generated in the bone marrow (BM) circulate until they encounter a protein antigen to which their specific surface IgM receptor may bind. Antigen binding initiates B-cell activation and triggers the upregulation of CCR7, a chemokine receptor that drives antigen-specific B cells toward the outer T-cell zone of secondary lymphoid tissues.[30] At this location, vaccine antigen-specific B cells are exposed to recently (<24 hours) activated DCs and T cells that have upregulated specific surface molecules and, thus, provide B-cell activating signals. This T-cell help rapidly drives B-cell differentiation into immunoglobulin-secreting plasma cells that produce low-affinity germline antibodies, in what is called the extrafollicular reaction (Figures 2-2 and 2-3).[31]

Immunoglobulin class-switch recombination from IgM toward IgG, IgA, or IgE occurs during this differentiation of B cells, through the upregulation of the activation-induced deaminase enzyme. Both $CD4^+$ Th1 and Th2 cells exert essential helper functions during the extrafollicular differentiation pathway, and the engagement of their CD40L molecules with CD40 on B cells may skew class-switch recombination into particular immunoglobulin classes and subclasses. In rodents, IFN-γ–producing Th1 T cells promote a switch toward IgG2a, whereas Th2 cells essentially support the generation of IgG1 and IgE (via interleukin [IL]-4) and IgG2b and IgG3 (via TGF-β).[32] The situation is less clear-cut in humans, where IgG1 antibodies frequently predominate regardless of the polarization of T-cell help. The extrafollicular reaction is rapid, and IgM and low-level IgG antibodies appear in the blood a few days after primary immunization (Figures 2-2 and 2-3). These antibodies are of germline affinity, as there is no hypermutation or selection process during the extrafollicular reaction. This extrafollicular reaction is short-lived, as most cells die by apoptosis within a few days. Consequently, it probably has a minor role in vaccine efficacy.

The germinal center reaction

Antigen-specific B cells that receive sufficient help from antigen-specific activated T cells proliferate in specialized structures, the GCs, in which they differentiate into plasma cells or memory B cells.[29,33] The induction of GCs is initiated as a few antigen-specific activated B cells upregulate their expression of CXCR5 and migrate toward B-cell follicles, where they are attracted by CXCL13-expressing follicular DCs (FDCs). The FDCs fulfill an essential role in B-cell responses: they attract antigen-specific B and T cells and capture/retain antigen for extended periods. B cells attracted by antigen-bearing

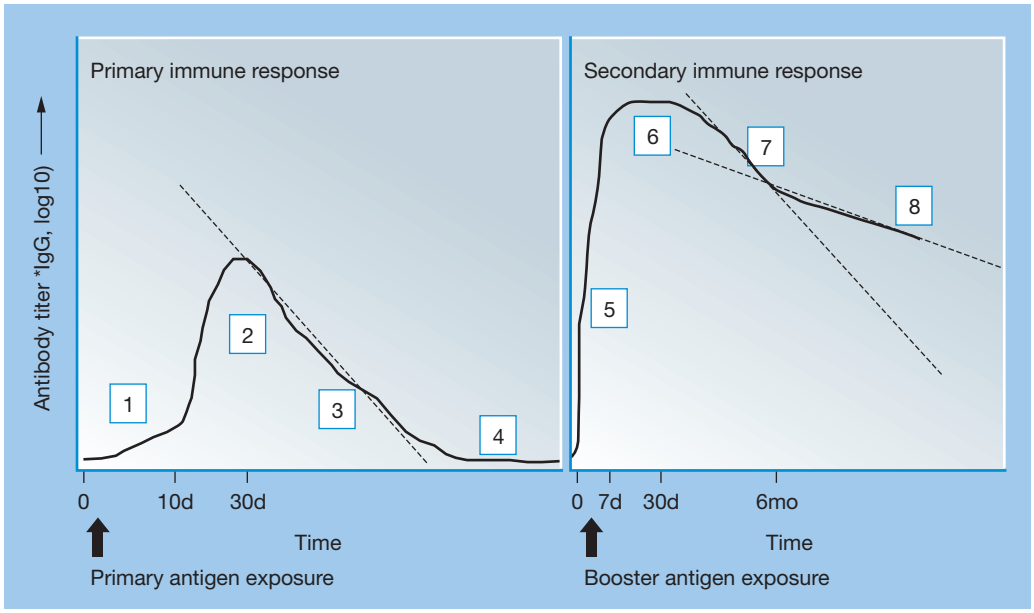

Figure 2-3 Correlation of antibody titers to the various phases of the vaccine response. The initial antigen exposure elicits an extrafollicular response (1) that results in the rapid appearance of low IgG antibody titers. As B cells proliferate in germinal centers and differentiate into plasma cells, IgG antibody titers increase up to a peak value (2), usually reached 4 weeks after immunization. The short lifespan of these plasma cells results in a rapid decline of antibody titers (3), which eventually return to baseline levels (4). In secondary immune responses, booster exposure to antigen reactivates immune memory and results in a rapid (<7 days) increase (5) of IgG antibody titer. Short-lived plasma cells maintain peak antibody levels (6) during a few weeks—after which serum antibody titers decline initially with the same rapid kinetics as following primary immunization. Long-lived plasma cells that have reached survival niches in the bone marrow continue to produce antigen-specific antibodies, which then decline with slower kinetics. Note: This generic pattern may not apply to live vaccines triggering long-term IgG antibodies for extended periods.

FDCs become the founders of GCs (Figure 2-2). Receiving additional activation and survival signals from the FDCs and follicular helper T (Tfh) cells*,[34,35] they undergo massive clonal proliferation—such that each GC is constituted by the progeny of a single antigen-specific B cell. This intense proliferation is associated with two major events: immunoglobulin class-switch recombination from IgM toward IgG, IgA, or IgE and maturation of the affinity of B cells for their specific antigen. This process results in the higher production of antibodies with a higher antigen-binding capacity (Figure 2-3).

The maturation of B-cell affinity results from an extensive somatic hypermutation process within the variable-region segments of immunoglobulin genes.[29] In most B cells, this stochastic process results inadvertently in a decline of the affinity of B-cell immunoglobulin for antigen. In a small minority of B cells, however, the introduction of mutations in their immunoglobulin genes increases the affinity* of their surface IgG for antigen. This enables these B cells to efficiently compete for binding to the small amounts of vaccine antigens that are associated with the surface of FDCs (Figure 2-2). B cells process these vaccine antigens into small peptides that they display at their surface through MHC class II molecules. MHC-peptides complexes thus become available for binding by a specific subset of $CD4^+$ T cells, Tfh cells.[34,35] These Tfh cells, which express CXCR5, migrate toward CXCL13-expressing FDCs. Differing from Th1 and Th2 cells by their chemokine receptors, transcription factors, surface markers, and interleukins,[34,35] they are uniquely equipped to provide efficient B-cell help through a series of costimulation molecules, including CD40L, ICOS (inducible T-cell costimulator), the IL-10 B-cell growth factor, and IL-21.[34,35] The cellular interactions between antigen-specific GC B cells, antigen-bearing FDCs, and antigen-specific Tfh cells (Figure 2-2) result in the proliferation, survival, and selection of B cells that have the highest possible antigen-specific affinity. They also provide the signals required for the subsequent differentiation of GC B cells toward plasma cells secreting large amounts of specific antibodies or toward memory B cells.

The development of this GC reaction requires a couple of weeks, such that hypermutated IgG antibodies to protein vaccine antigens first appear in the blood 10 to 14 days after priming (Figure 2-3).[36] Feedback mechanisms terminate GC reactions within 3 to 6 weeks, a period during which a large number of antigen-specific plasma cells will have been generated. It is the magnitude of GC responses, ie, the quality of DC, B-cell, Tfh-cell, and FDC interactions, that controls the intensity of B-cell differentiation into plasma cells and thus the peak of IgG vaccine antibody reached within 4 to 6 weeks after primary immunization (Figure 2-3).

T-independent responses to polysaccharide antigens

Bacterial (*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Salmonella typhi*) PS antigens released from the injection site essentially reach the marginal zone of the spleen/nodes through the blood, an area that is equipped by macrophages exhibiting a unique set of scavenger receptors. There, PS bind to marginal zone B cells, and their repetitive structure cross-links the immunoglobulin receptors at the B-cell surface.[31] This activates marginal zone B cells in extrafollicular foci (Figure 2-4).[31] During the week following immunization, B cells differentiate into plasma cells, undergo some degree of isotype switching from IgM to IgG/IgA, and—in rodents—rapidly produce essentially nonmutated, low-affinity, germline antibodies. Thus, PS vaccines are generally known as triggering T-independent responses characterized by the induction of moderate titers of low-affinity antibodies and the absence of immune memory.

In humans, PS immunization does generate the production of intermediate-affinity IgG antibodies bearing some somatic mutations in their variable regions.[37,38] The production of

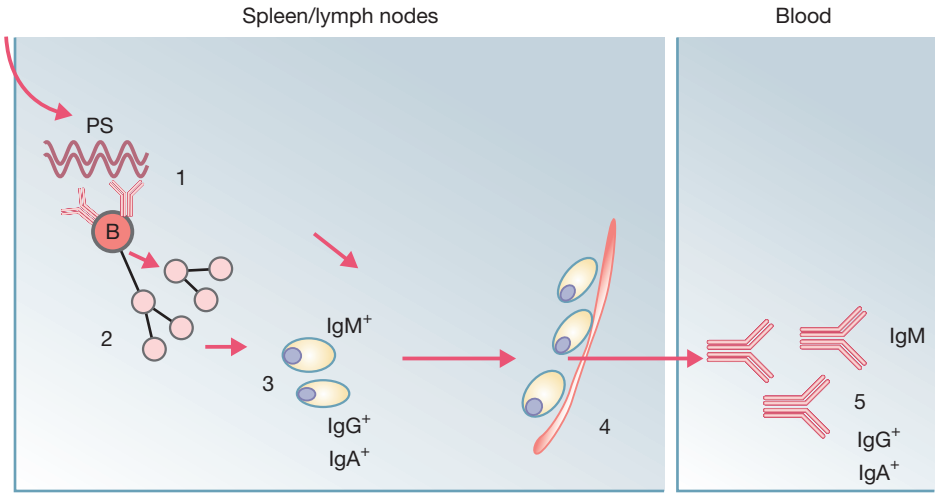

Figure 2-4 Extrafollicular B-cell responses to polysaccharide antigens. B cells use their specific immunoglobulin surface receptors (1) to bind to the repetitive structures of polysaccharides reaching the marginal zone of spleen/nodes. In the absence of antigen-specific T-cell help, B cells are activated, proliferate (2), and differentiate in plasma cells (3) without undergoing affinity maturation in germinal centers. These plasma cells migrate toward the red pulp of the spleen (4), where they survive for a few weeks/months, secreting low levels of low-affinity IgM, IgG, or IgA antibodies (5).

mutated antibodies is not expected during a T-independent immune response, as somatic mutations essentially take place in GCs. One hypothesis is that PS immunization activates "memory" B cells that have been previously primed by cross-reacting PS bacterial antigens somehow linked to protein moieties—and thus eliciting GC responses.[39] An alternative possibility is that the IgM^+, IgD^+, $CD27^+$ memory B cells that appear in the blood in response to PS immunization may be recirculating splenic marginal zone B cells.[40] These cells would diversify their immunoglobulin receptor to a certain extent in the absence of cognate T-B interaction. This hypothesis is concordant with the fact that bacterial PS vaccines are poorly immunogenic in young children, ie, before the maturation of the splenic marginal zone.[41,42]

After their differentiation in the extrafollicular pathway, PS-specific plasma cells move toward the red pulp of the spleen (Figure 2-4) where they persist for some time, before their death by apoptosis and the waning of corresponding antibody responses after a few months. As PS antigens do not induce GCs, bona fide memory B cells are not elicited. Consequently, subsequent reexposure to the same PS results in a repeated primary response that follows the same kinetics in previously vaccinated as in naïve people.[43] Revaccination with certain bacterial PS may even induce lower antibody responses than the first immunization, a phenomenon referred to as hyporesponsiveness,[44] with molecular and cellular bases that are not fully understood and have uncertain clinical significance.[45,46]

What are the determinants of primary vaccine antibody responses?

Numerous determinants modulate the intensity of vaccine-induced GCs and, thus, of peak antibody responses (Table 2-5). The main determinants are the nature of the vaccine antigen and its intrinsic immunogenicity. For example, tetanus toxoid is intrinsically a stronger immunogen than diphtheria toxoid, which becomes apparent when immunocompetence is more limited, such as in preterm infants.[47] Whether this difference reflects a higher capacity of tetanus toxoid to provide antigenic epitopes that may be bound by naïve B cells, to generate cognate promiscuous T-cell help for B cells, and/or to associate with FDCs is unknown.

The drastically distinct outcomes of immunization with plain bacterial PS or with protein-conjugated glycoconjugates[44] highlight the differences between the extrafollicular and the GC reactions. It is only when capsular PS are conjugated to a protein carrier driving effective Th differentiation that PS-specific B cells are driven toward GC responses, receive optimal cognate help from carrier-specific Tfh cells, and differentiate into higher-affinity antibody-producing cells, longer-lived plasma cells, and/or memory B cells. Protein antigens exhibit markedly distinct carrier properties—regardless of their capacity to induce B- and Th-cell responses.[48] That these differences may reflect differences in Tfh induction is an interesting hypothesis that is supported by the enhanced immunogenicity of a synthetic polyepitope carrier containing optimal Th epitopes.[49] The limited number of potent carrier proteins implies that an increasing number of conjugate vaccines rely on the same molecules (eg, CRM_{197}, tetanus or diphtheria toxoids), with the risk of limiting anti-PS responses to individual conjugate vaccines (carrier-mediated epitope suppression) and resulting in vaccine interference.[50,51] This phenomenon may be abrogated by replacing full-length proteins with peptides lacking B-cell epitopes,[52] suggesting that carrier-mediated epitope suppression essentially reflects the competition of carrier- and PS-specific B cells for activation/differentiation signals and factors.

Another determinant of the magnitude of primary vaccine antibody responses (Table 2-5) is the use of an optimal dose of vaccine antigen, which may be determined only experimentally. As a rule, higher doses of nonlive antigens—up to a certain threshold—elicit higher primary antibody responses. This may be particularly useful when immunocompetence is limited, eg, for hepatitis B immunization of patients undergoing dialysis.[53,54] Remarkably, a limiting dose of vaccine antigen may restrict primary antibody responses but increase B-cell competition for FDC-associated antigens and, thus, result in a more stringent selection of higher-affinity GC B cells and stronger secondary responses (see subsequent text). Alternatively, adjuvants increasing inflammation at the injection site and, thus, cell recruitment and cell-mediated antigen transport toward lymph nodes, improve antibody responses despite a reduced antigen dose.[55] Little is known about factors that support or limit the affinity maturation process.[56] Interestingly, carrier proteins[57] and adjuvants may modulate the affinity maturation process, as observed following the addition of CpG oligonucleotides to an alum-adsorbed hepatitis B vaccine.[58]

The nature of the vaccine directly influences the activation of innate immunity and, thus, vaccine responses. The strongest antibody responses are generally elicited by live vaccines that are "naturally adjuvanted", better activate innate reactions, and, thus, better support the induction of adaptive immune effectors in addition to providing a replicative expansion of antigen. Nonlive vaccines frequently require formulation in adjuvants that enhance and shape vaccine immune responses through a variety of mechanisms.[17] The potency of the immune system indeed resides in its highly polymorphic nature, enabling sufficient immunological diversity to overcome a high number of diverse pathogens.

Table 2-5 Determinants of the Duration of Vaccine Antibody Responses in Healthy People

Determinants	Mechanisms (presumed)
Vaccine type	
Live vs inactivated	Live vaccines generally induce more sustained Ab responses, presumably through Ag persistence within the host.
Polysaccharide antigens	Failure to generate GCs limits the induction of memory responses and of high-affinity long-lived plasma cells.
Vaccine schedule	
Interval between primary doses	A minimal interval of 3 wk between primary doses allows development of successive waves of Ag-specific primary responses without interference
Interval before boosting	A minimal interval of 4 mo between priming and boosting allows affinity maturation of memory B cells and thus higher secondary responses
Age at immunization	Early life immune immaturity and age-associated immunosenescence limit the induction/persistence of long-lived plasma cells
Environmental factors	?

Ab, antibody; Ag, antigen; GC, germinal center.

This diversity impacts vaccine responses.[59] Probing how host genetic markers may result in variations of vaccine-induced responses is expected to identify gene polymorphisms that predict the likelihood of successful or adverse vaccine outcome, whereas epigenetic studies may help reveal how environmental influences affect innate and adaptive immune responses.[59] This work is still in infancy but holds great promise, especially when combined with novel system vaccinological approaches.[60,61] Immune competence obviously affects vaccine antibody responses, which are limited at the two extremes of life (see subsequent text), by acute or chronic diseases, by acute or chronic stress, and by a variety of factors affecting innate and/or B- and T-cell immunity.

Few nonlive vaccines induce high and sustained antibody responses after a single vaccine dose, even in healthy young adults. Primary immunization schedules therefore usually include at least two doses, optimally repeated at a minimal interval of 3 to 4 weeks to generate successive waves of B-cell and GC responses. These priming doses may occasionally be combined into a single "double" dose, such as for hepatitis A or B and for human papillomavirus (HPV) immunization.[62,63] In any case, however, vaccine antibodies elicited by primary immunization with nonlive vaccines eventually wane (Figure 2-3).

What controls the persistence of vaccine antibody responses?

Antigen-specific plasma cells elicited in spleen/nodes after immunization have only a short lifespan, such that vaccine antibodies rapidly decline during the first few weeks and months after immunization. A fraction of plasma cells that differentiated into GCs, however, acquire the capacity to migrate toward long-term survival niches mostly located within the BM, from where they may produce vaccine antibodies during extended periods.[64,65]

Some GC-induced plasma cells are attracted toward the BM compartment by cells that provide the signals required for their long-term survival.[29,66,67] In such BM niches, plasma cell survival and antibody production may persist for years. Whether the persistence of vaccine-induced plasma cells reflects the long-term persistence of the plasma cells that were initially generated, or the maintenance of a BM reservoir of plasma cells through homeostatic mechanisms that maintain cell numbers through low level replication, remains undefined. Regardless of the exact mechanisms supporting BM plasma cell survival, the duration of antibody responses reflects the number and/or quality of long-lived plasma cells generated by immunization:[65] in the absence of subsequent antigen exposure, antibody persistence may be reliably predicted by the antibody titers that are reached 6 to 12 months after immunization, ie, after the end of the short-term plasma cell response (Figure 2-3). This is illustrated by the accuracy of mathematical models predicting the kinetics of anti-HBsAg,[68] anti–hepatitis A,[69] or anti-HPV[70,71] antibodies.

A few determinants of the persistence of vaccine antibody responses (Table 2-5) have been identified. The nature of the vaccine has a crucial role: only live attenuated viral vaccines or virus-like particles induce antibody responses that persist for several decades, if not lifelong, in absence of subsequent antigen exposure and reactivation of immune memory. This could reflect the in vivo persistence of viral antigens that continuously trigger B-cell responses, although other mechanisms may be at play. In contrast, the shortest antibody responses are elicited by PS antigens, which fail to trigger GC responses and do not elicit high-affinity plasma cells capable of reaching the BM survival niches. Vaccine schedules also control antibody magnitude and persistence. Closely spaced (1-2 weeks) primary vaccine doses may be administered when a rapid induction of protection is desirable, eg, before travel. However, this raises less persisting responses than when the same number of vaccine doses are given at longer intervals (1-2 months),[72,73] reflecting the generation of fewer post-GC B cells capable of long-term survival and thus requiring later boosting.

Age at immunization also modulates vaccine antibody persistence, which is shorter at the two extremes of life (see subsequent text). Certain disease conditions may also limit the persistence of vaccine antibody responses because of enhanced catabolism (as in malaria) or the loss of antibodies in the urinary or digestive tract. The identification of the mechanisms that support or limit the persistence of vaccine antibody responses represents a major challenge for vaccinologists, as resources are lacking for most immunization programs throughout the world to include the booster doses otherwise required to maintain vaccine efficacy.

What are the hallmarks of B-cell memory responses?

Memory B cells are generated during primary responses to T-dependent vaccines.[29,74] They persist in the absence of antigens but do not produce antibodies, ie, do not protect, unless reexposure to antigen drives their differentiation into antibody-producing plasma cells. This reactivation is a rapid process, such that booster responses are characterized by the rapid increase to higher

Table 2-6 Hallmarks of Memory B-Cell Responses

Are generated only during T-dependent responses inducing germinal center responses
Memory B cells are resting cells that do not produce antibodies.
Memory B cells undergo affinity maturation during several (4-6) months.
Memory B cells rapidly (days) differentiate into antibody-secreting plasma cells on reexposure to antigen.
Memory B cells differentiate into plasma cells that produce high(er)-affinity antibodies than do primary plasma cells.

titers of antibodies that have a higher affinity for antigens than do antibodies generated during primary responses (Table 2-6).

With the possible exception of responses to live viral vaccines, vaccine antibody responses are deemed to wane and eventually decline below protective thresholds, unless antigen retention (for example on FDCs) or repeated antigen exposure reactivates immune memory. Memory B cells are generated in response to T-dependent antigens, during the GC reaction, in parallel to plasma cells (Figure 2-5).[29,75] At their exit of GCs, memory B cells acquire migration properties toward extrafollicular areas of the spleen and nodes. This migration occurs through the blood, in which postimmunization memory B cells are transiently present on their way toward lymphoid organs.

It is essential to understand that memory B cells do not produce antibodies—ie, they do not protect. Their participation in vaccine efficacy requires an antigen-driven proliferation and differentiation process.[74] This reactivation may occur in response to endemic or frequent pathogens, to colonizing or cross-reacting microorganisms ("natural boosters"), or to booster immunization. The antigen-driven activation of memory B cells results in their rapid proliferation and differentiation into plasma cells that produce very large amounts of higher-affinity antibodies.[75] As the affinity of surface immunoglobulin from memory B cells is increased, their requirements for reactivation are lower than for naïve B cells: memory B cells may thus be recalled by lower amounts of antigen and without $CD4^+$ T-cell help. Antigen-specific memory cells generated by primary immunization are much more numerous (and better fit) than naïve B cells initially capable of antigen recognition.[29] Thus, a first hallmark of memory responses (Table 2-6) is to generate significantly higher antibody levels than primary immunization. Should this not be the case, the effective generation of memory B cells should be questioned.

The reactivation, proliferation, and differentiation of memory B cells occur without requiring the induction and development of GC responses. This process is, thus, much more rapidly completed than that of primary responses. A window of 4 to 7 days after *Haemophilus influenzae b* (Hib) PS immunization was reported as sufficient for high levels of PS-specific vaccine antibodies to appear in the blood of previously primed infants.[76] The rapidity with which antigen-specific antibodies appear in the serum is, thus, another hallmark of secondary responses (Table 2-6). Slower kinetics suggests that memory B-cell induction, persistence, and/or reactivation may have been suboptimal.

Another hallmark of memory B cells is to display and secrete antibodies with a markedly higher affinity than those produced by primary plasma cells, as a result of somatic hypermutation and selection. The affinity maturation process that is initiated within the GCs extends during several months after the end of the GC reaction. Consequently, vaccine antibodies with higher than baseline avidity (defined as the sum of epitope-specific affinities) for antigen are induced only when sufficient time has elapsed after priming.[77-79] A "classical" prime-boost immunization schedule is, thus, to allow 4 to 6 months to elapse between priming and booster doses, hence the generic "0-1-6 month" schedule. Secondary antigen exposure (Table 2-6) thus results in the production of higher-affinity antibodies than primary responses.[80] Notably, this may not be the case when "natural" priming, eg, through cross-reactive bacteria, has taken place before immunization.

What are the determinants of B-cell memory responses?

The factors that drive the differentiation of antigen-specific GC B cells toward plasma cells or memory B cells are poorly understood.[29] In response to protein antigens, both cell populations are generated in the same GCs, and their differentiation pathway differs only late in the GC reaction. As a rule, factors enhancing plasma cell differentiation and primary antibody responses (such as increasing the antigen dose or using adjuvants) therefore also support the induction of memory B cells (Table 2-7). Postbooster antibody titers are, therefore, higher in people with stronger primary responses. For example, higher postbooster anti-HBsAg responses are observed in people with high (eg, ≥100 UI/L) rather than intermediate (10-99 UI/L) anti-HBsAg postprimary responses.[81,82] This is likely to reflect the induction of a larger pool of antigen-specific memory B cells. An interesting question is whether this may confer specific

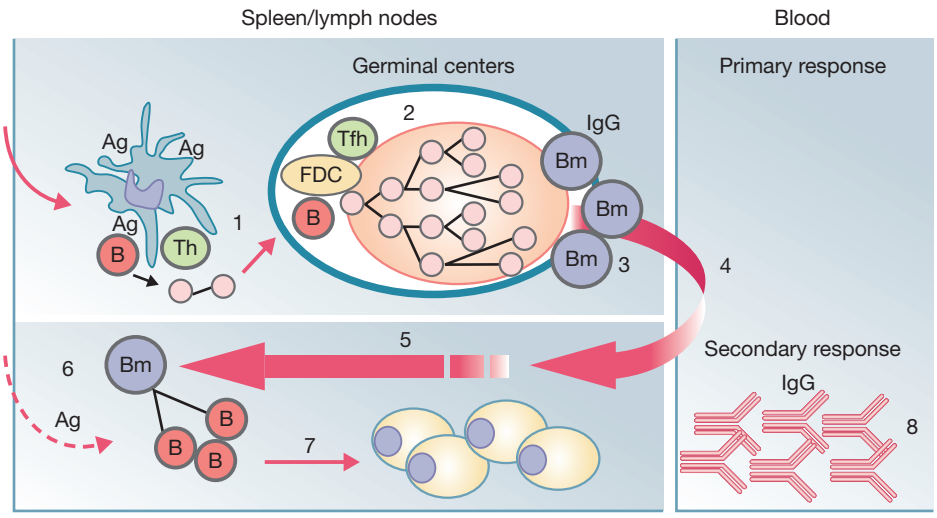

Figure 2-5 Generation of B-cell memory responses. Memory B cells are generated in response to T-dependent antigens (1), during the germinal center (GC) reaction (2), in parallel to plasma cells. At their exit of GCs, these B cells do not differentiate into antibody-secreting plasma cells but into memory B cells (3) that transiently migrate through the blood (4) toward the extrafollicular areas of spleen and nodes (5). They persist there as resting cells until reexposed to their specific antigens (6). On secondary antigen exposure, memory B cells readily proliferate and differentiate into plasma cells (7) secreting large amounts of high-affinity antibodies that may be detected in the serum (8) within a few days after boosting.

Table 2-7 Determinants of Secondary B-Cell Responses

Determinants	Mechanisms (presumed)
Postprimary antibody titers	As plasma cells and memory responses are generated in parallel in GCs, higher postprimary Ab titers reflect stronger GC reactions and generally predict higher secondary responses.
Residual antibodies at boosting	Neutralization of live viral vaccines; negative feedback mechanisms on nonlive vaccines
Lower antigen dose at priming	A limited quantity of antigen may induce B cell differentiation away from PCs, toward memory B cells(?).
Longer intervals before boosting	A minimal interval of 4-6 mo is required for optimal affinity maturation of memory B cells.
Higher antigen dose at boosting	A higher availability of antigen may drive higher numbers of memory B cells into differentiation.
Antigen availability	
Exogenous exposure	Exposure to exogenous antigens may reactivate or favor the persistence of memory B cells.
In vivo persistence	Antigen persistence may reactivate or favor the persistence of memory B cells.

Ab, antibody; GC, germinal center; PC, plasma cell.

advantages in terms of protection: the protective threshold of serum antibodies could be reached more rapidly with the reactivation of a larger number of memory B cells.

The dose of antigen is an important determinant of memory B-cell responses (Table 2-7). At priming, higher antigen doses generally favor the induction of plasma cells, whereas lower doses may preferentially drive the induction of immune memory.[83] Thus, a lower antigen content may be preferred if the rapid induction of protection is not required. Closely spaced primary vaccine doses may also be beneficial for early postprimary antibody responses but not for postbooster antibody responses, as illustrated with meningococcal group C glycoconjugates.[84] As a rule, accelerated schedules in which a 4 to 6 month window is not included between priming and boosting result in significantly lower booster responses[79] (Table 2-7). At the time of boosting, a higher antigen content raises stronger booster responses, presumably by recruiting more memory B cells into the response. This is illustrated by higher antibody responses of children immunized with a high-antigen-dose pertussis vaccine[85] or primed with a glycoconjugate vaccine and boosted with PS (20-50 μg of PS) than glycoconjugate (1-3 μg of PS) vaccines.[86,87]

Residual titers of vaccine antibodies present at time of boosting directly influence vaccine antibody responses. As a rule, secondary responses to live attenuated viral vaccines are minimal, as preexisting antibodies mostly neutralize the vaccine load before its in vivo replication. Consequently, even multiple doses of live attenuated vaccines remain without undesirable effects. Responses to nonlive vaccines are also negatively influenced by residual vaccine antibody titers. This may reflect the formation of antigen-antibody complexes that reduce the load of antigen available for B-cell binding and/or antibody-mediated negative feedback mechanisms acting directly on B cells. Consequently, people with residual antibodies to a given antigen may show only a limited increase of their antibody responses—such that vaccine responses are better described by the proportion of people with titers above a given threshold than by people with a 2- or 4-fold increase of their antibody titers.

The persistence of memory B cells is of utmost importance for long-term vaccine efficacy. Antigen persistence may extend for prolonged periods on the surface of FDCs (Table 2-7) and contribute to the duration of immune memory,[88] probably by extending the period during which antigen remains available for memory B-cell induction and reactivation. This is likely to contribute to the extended (indefinite?) memory to live attenuated vaccines, recently exemplified by repeated administration of smallpox vaccines decades after priming.[89] Fortunately, memory B cells survive for prolonged periods (eg, several decades), even in the absence of reexposure to antigen.[90] It has been suggested that memory B cells undergo a certain degree of homeostatic polyclonal activation.[91] Although this does not seem sufficient to maintain antibody responses, ie, to drive their differentiation to immunoglobulin-secreting cells persisting despite the depletion of memory B cells,[92] it likely contributes to their persistence and the replenishment of BM plasma cells.

The demonstration of the persistence of memory B cells long after vaccine antibodies have eventually disappeared, and of their brisk reactivation on antigen exposure, has direct consequences for immunization programs. First, it implies that an immunization schedule should never be started all over again—but continued where interrupted, regardless of the duration of the interruption. Consequently, regular booster doses are not required to maintain immune memory during low-risk periods, which has direct implications for travelers who may simply need a single booster dose before departure. Second, it implies that certain immunization schedules may not need to include booster doses, should exposure provide regular natural boosters. Importantly, however, successful immunization programs may eventually reduce opportunities for natural boosters and, consequently, modify booster requirements. This issue will doubtlessly be an area of intense investigation in the next decades. Furthermore, intriguing observations indicate that in the absence of childhood boosters, up to 50% of adolescents or young adults primed against tetanus or hepatitis B in infancy might not raise anamnestic responses, suggesting that infant-induced vaccine memory, albeit prolonged, may not last forever.[93,94]

Immune memory and vaccine-induced protection: a race between reactivation and microbial invasion?

All existing vaccines, with the exception of T-independent PS, induce immune memory. Nevertheless, vaccine efficacy may be short-term, as illustrated following infant immunization against group C meningococcus.[95] Demonstration of priming—or "boostability"—is therefore not a surrogate marker for long-term vaccine efficacy. This requires identifying the determinants that contribute to—or limit—the persistence of vaccine efficacy. One hypothesis is that this essentially results from the race between the reactivation of immune memory and disease pathogenesis, as recently summarized.[96]

It is generally considered that protection by toxoid-based vaccines requires the presence of antitoxin antibodies at time of toxin exposure. This is supported by the observation that despite the occurrence of many adult cases of diphtheria during a large outbreak in the former Soviet Union, a single vaccine dose raised strong antibody responses to this relatively poor immunogen. This confirmed that most patients had been immunized in childhood and had lost vaccine antibodies over time but had persistent immune memory.[97] This immune memory was, however, not sufficient to protect against diphtheria, a disease characterized by a short incubation period (1-5 days). The same requirement for protective antibodies at the time of exposure is frequently applied to protection against tetanus. However, tetanus does not seem to occur in previously immunized people (ie, three doses as adults). Whether this reflects a longer incubation time or the frequent administration of a booster dose at the time of a wound is unknown.

Persisting immune memory is not sufficient to protect against acute hepatitis B after the waning of vaccine-induced antibodies. When anti-HBsAg antibodies reach titers less than 10 IU/L, acute viral infection occurs, reflected by the appearance of anti-HBc antibodies.[98-100] However, progression to chronic liver disease has not been reported in fully immunized vaccine responders. This suggests that viral replication and reexposure to HBsAg efficiently drive vaccine-induced memory cells into effector cells before the end of the viral incubation period (4-12 weeks). This process requires a sufficient number of HBsAg-specific memory B cells to be elicited, to persist, and to be capable of reactivation even several decades after infant priming. Analyses of secondary responses elicited late after priming demonstrate that earlier and stronger booster responses are achieved when postprimary anti-HBsAg antibodies had reached higher titers.[81,82] This suggests the induction and long-term persistence of a higher number of memory B cells, such that protective neutralizing antibody thresholds are reached faster. Whether this confers an advantage in the race against chronic hepatitis requires investigation. Another essential unresolved issue is whether the size of the pool of memory B cells elicited by primary immunization influences their long-term persistence, particularly in absence of antigen exposure, eg, in low-endemicity countries. It also remains to be defined whether T-cell memory responses contribute to the maintenance of vaccine-induced protection after waning of anti-HBsAg antibodies.

Glycoconjugate vaccines against encapsulated bacteria illustrate the importance of immune memory for vaccine efficacy and some of its limitations. Glycoconjugate priming elicits a bona fide GC reaction, with the induction of high-affinity memory B cells that can be rapidly (4-7 days) recalled on PS immunization.[76] Efficient priming, ie, induction of immune memory, is readily demonstrated in children primed in infancy.[101,102] However, immune memory is also evidenced in children with Hib vaccine failure,[103] indicating that their reservoir of memory B cells did not protect them against invasive disease, perhaps through a failure of avidity maturation.[104] The discrepancy between the existence of memory B cells and the lack of protection may again reflect the race against microbial invasion: the time required for production of sufficient levels of circulating antibodies could be too long to interrupt bacterial invasion. Notably, secondary vaccine failures have been relatively rare and primarily observed in countries using an early accelerated infant schedule without a booster dose,[105] the use of DTaP/Hib vaccines with lower Hib immunogenicity resulting in additional risks.[106] Thus, these priming conditions are not optimal for sustained individual protection: it is tempting to conclude that they may not elicit a sufficiently large pool of memory B cells for a sufficiently rapid interruption of bacterial invasion. Similarly, glycoconjugate vaccines against group C meningococcal disease proved much more efficacious during the first year after infant priming than during the following 3 years.[95] Thus, infant immunization fails to induce sustained protection against group C meningococcus, despite the demonstration of the induction and persistence of immune memory.[107] The requirement for boosters to confer long-term vaccine protection is also well illustrated for pertussis, for which boosters are required to extend protection beyond childhood.[108] The prompt reactivation of immune memory is also not sufficient to control viral replication in the digestive tract: fecal excretion patterns were similar in subjects who were seronegative at time of oral challenge with poliovirus vaccine, whether or not they raised prompt anamnestic serum antibody responses attesting to the persistence of immune memory.[109]

Live attenuated viral vaccines (measles, rubella) are considered the prototype inducers of lifelong immunity, although prolonged immunity is also induced by certain nonlive vaccines (hepatitis A, HPV, inactivated poliovirus vaccine, rabies). This derives in part from the induction of sustained antibody responses, which, however, tend to slowly decline in the absence of recurrent exposure[110] and might eventually result in a growing proportion of seronegative vaccinated young adults, including women of childbearing age. Whether the reactivation of immune memory will be sufficient to curtail the replication process and confer protection against measles, rubella, or varicella and whether adult booster doses may become needed after microbial control are essential questions. The resurgence of mumps outbreaks in fully vaccinated young adults, however, demonstrates that secondary vaccine failure may occur even with live attenuated vaccines.[111] These questions, which are central to sustained vaccine efficacy, are usually unresolved at the time of registration of a new vaccine. For example, to vaccinate young girls against HPV requires reassurance that vaccine protection will extend during several decades. The claim that "HPV equals HBV" and, thus, that booster doses will not be required is unlikely to prove correct: HPV infection of the basal epithelial cells can occur within minutes and is not followed by any antigen exposure to the immune system. Thus, antibody persistence will be required for sustained protection. Remarkably, however, the concentration of vaccine antibodies required to neutralize HPV at the site of entry is so minute[112] that boosters may indeed not be needed for several decades.

Altogether, one may thus expect questions related to the nature (size, type, responsiveness) of the pool of memory cells elicited by various immunization schedules and the relative contribution of long-term antibodies and immune memory to protection to be at the core of many vaccine studies in the next decade.

T-cell vaccine responses

How do vaccines induce CD4$^+$ and CD8$^+$ T-cell responses?

The generation of CD4$^+$ Th-cell response begins when DCs capture antigen in peripheral tissue and migrate to draining lymph nodes, where T-cell vaccine responses are elicited in parallel to B-cell responses (Table 2-1). Thus, DCs fulfill a pivotal role in initiating and shaping the immune response to vaccine antigens.

Protein vaccine antigens are taken up by immature DCs activated by local inflammation, which provides the signals required for their migration to draining lymph nodes (Figure 2-1). During this migration, DCs mature and their surface expression of molecules changes.[113] Simultaneously, antigens are processed into small fragments and displayed at the cell surface in the grooves of MHC (HLA in humans) molecules. As a rule, MHC class I molecules present peptides from antigens that are produced in infected cells, whereas phagocytosed antigens are displayed on MHC class II molecules.[114-116] Thus, mature DCs reaching the T-cell zone of lymph nodes display MHC-peptide complexes and high levels of costimulation molecules at their surface.[117]

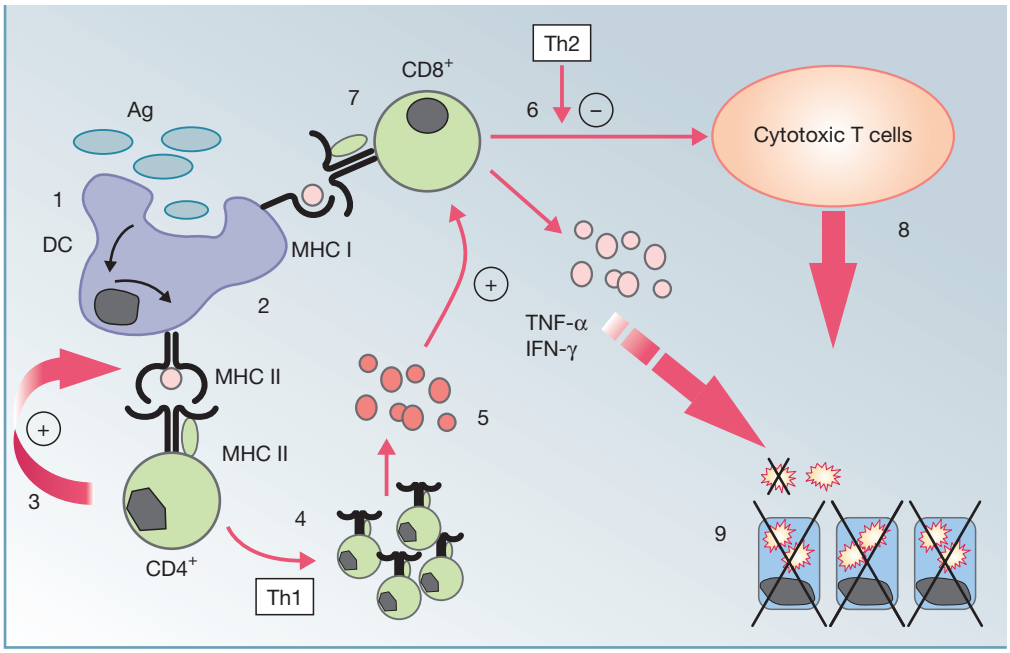

Figure 2-6 Generation of T-cell effector responses. Antigens are phagocytosed by dendritic cells (DCs) (1), processed into small peptides, and displayed at the cell surface in the groove of MHC class I and/or class II molecules (2). $CD4^+$ T cells with the appropriate MHC-peptide specificity are activated, provide activation signals to DCs (3), and differentiate in effector cells (4) that produce preferentially T helper (Th)1 or Th2 cytokines. Th1 $CD4^+$ T cells support (5) $CD8^+$ T-cell differentiation, which is in contrast inhibited (6) by Th2-like cytokines. $CD8^+$ T cells recognize MHC class I-peptide complexes (7) and differentiate into cytotoxic effector cells (8) capable of killing infected cells (9) or pathogens.

$CD4^+$ T cells recognize antigenic peptides displayed by class II MHC molecules, whereas $CD8^+$ T cells bind to class I MHC-peptide complexes (Figure 2-6).[118] Their recognition is restricted to short peptides (8-11 [$CD8^+$] or 10-18 [$CD4^+$] amino acids) displayed on specific MHC class I or II molecules, respectively. Antigen-specific T-cell receptors may bind only to specific MHC molecules (eg, HLA A2), which differ among individual people and populations. Consequently, T-cell responses are highly variable within a population. These T-cell epitopes may be generated from any region of the vaccine antigens, whether the peptide sequence is located within or at the surface of the protein. This is in contrast with B-cell recognition, which is essentially limited to conformational determinants constituted by amino acids at the antigen surface. This MHC-peptide signal (signal 1) is not sufficient for activation of T cells, which remain anergic or become tolerized in absence of costimulation (signal 2). This ensures that only naïve T cells binding to the surface of activated DCs, ie, DCs that have sensed danger signals through their Toll-like receptors and responded by a modulation of their surface or secreted molecules, receive the costimulation signals required for their activation.[117]

Activated $CD4^+$ T cells essentially exert supportive functions for DCs, to which they provide signals (CD40L, etc) resulting in further activation, for B cells (Figure 2-2) and for $CD8^+$ cytotoxic T cells (Figure 2-6 and Table 2-8). They are elicited by each vaccine type, with the exception of plain PS, which are not properly displayed by MHC molecules. Thus, the demonstration of postimmunization $CD4^+$ T-cell responses does not imply a direct role in vaccine efficacy. $CD4^+$ T-cell activation by DCs triggers their differentiation along distinct differentiation pathways.[117,119] By default, DCs essentially trigger the induction of Th2-type $CD4^+$ T cells producing IL-4, IL-5, and IL-13,

Table 2-8 T-Cell Responses to Vaccines

Type	Mechanisms (presumed)	Function
$CD4^+$ helper T cells		
Th1	IFN-γ production	Extrafollicular B-cell help
Th1	Cell contact, IFN-γ	Activation of $CD8^+$ T cells
Th1/Th2	Cell contact, CD40L	Dendritic cell activation
Th2	IL-4, IL-5, IL-13	Extrafollicular B-cell help
Th2	Cell contact, IL-4	Suppression of $CD8^+$ T cells
Th17	IL-17, IL-21, IL-22	Mucosal inflammation
$CD4^+$ follicular helper T cells		
Th1	IFN-γ	Germinal center B-cell help
Th2	IL-4, IL-5, IL-13	Germinal center B-cell help
$CD4^+$ regulatory T cells	Multiple mechanisms	Suppression of $CD4^+$/$CD8^+$ responses
$CD8^+$ T cells	IFN-γ, TNF-α	Killing of infected cells
Effector memory T cells	Th1/Th2 cytokines, perforin	Rapid secondary effector responses in periphery
Central memory T cells	IL-2, IL-10, CD40L	Delayed activation/proliferation in lymph nodes

which are implicated in the defense against extracellular pathogens such as helminths.[120] More potently activated DCs release IL-12p70, which induces the differentiation into Th1 cells that essentially produce IFN-γ and TNF-α and, thus, contribute to the elimination of intracellular pathogens directly (cytokine responses) and indirectly through macrophage activation and support to $CD8^+$ T-cell differentiation (Figure 2-6).[121] Th1 and Th2 cells support B-cell activation and differentiation during extrafollicular responses, whereas Tfh $CD4^+$ cells provide critical help to GC B cells (Figure 2-3).[122] Activated DCs may also release IL-23, supporting the induction of inflammatory Th17 cells by TGF-β and IL-6.

Numerous factors influence the preferential differentiation of $CD4^+$ T cells toward the Th1, Th2, or Th17 pathway.[123] Lower vaccine doses are classically associated with preferential Th1 responses, and the route of administration may target distinct DC subsets. However, the main determinant of $CD4^+$ T-cell differentiation is the extent and type of DC activation by the innate system.[117] Consequently, DCs are the primary target for specific adjuvants, which may preferentially skew $CD4^+$ responses toward Th1, Th2, or TH17 responses and impact the differentiation of Tfh cells, requiring their careful design and selection.[17,19,124]

$CD8^+$ T-cell responses are essentially (although not exclusively) induced as a result of cross-presentation elicited by vaccines that introduce antigens within the cell cytosol, ensuring their access to MHC class I molecules.[125] The induction of strong $CD8^+$ T-cell responses is, thus, currently limited to infectious, live attenuated viral or bacterial vaccines. However, novel delivery systems such as live-vectored vaccines or DNA vaccines are now in human trials.[126] As $CD8^+$ T cells are unique in their ability to kill cells that are chronically infected, novel vaccine targets such as HIV, hepatitis C virus, or malaria require their induction.

The activation of naïve T cells by vaccine-bearing DCs may also induce their differentiation into Tregs (Table 2-8), a heterogeneous population with many levels of complexity.[3,127] Vaccine-induced Tregs may use multiple mechanisms to suppress T-cell induction or proliferation: in draining lymph nodes, they may prevent DC maturation, block the priming of effector T cells, or destroy antigen-bearing DCs. These Tregs may be induced by "tolerogenic" DCs, which capture antigen in the absence of danger signals and, thus, remain immature or are elicited as feedback mechanisms to avoid excessive and, thus, potentially harmful inflammatory responses. By suppressing immune responses, Tregs may limit the efficacy of vaccines, for example, when danger signals are insufficient to elicit immunity, eg, in chronic infections and cancer. This was formally demonstrated in humans by the enhancement of anti–cancer vaccine responses following Treg depletion.[128] Defining the determinants of Treg differentiation may be needed for novel immunization strategies such as therapeutic vaccines.

What are the determinants of vaccine-induced T-cell memory?

Effector T-cell responses are short-lived, and most (>90%) effector T cells die by apoptosis within a few days. Thus, immune memory is essential to T-cell vaccine efficacy. It is dependent on three main parameters: the frequency of antigen-specific memory T cells, their phenotype, and their persistence (Table 2-9).[126,129] Memory T cells may persist lifelong, even in the absence of antigen exposure and despite their quality and amount being set during the primary immune response.

The frequency of memory T cells directly reflects the magnitude of the initial T-cell expansion and that of its subsequent contraction during which few surviving cells differentiate toward memory T cells. The main determinant of the expansion phase is the level of or duration of antigen stimulation present during priming.[130] This is a major limitation for nonreplicating vaccines, which fail to reach sufficient antigen content and typically require adjuvantation and/or booster doses. The contraction phase and the transition toward memory cells take place soon after antigen is cleared, which occurs faster for nonreplicating vaccines. Current efforts are, thus, oriented toward the optimization of the primary expansion phase through adjuvantation and/or booster administration. As vaccine-induced immunity limits the subsequent "take" of a live vaccine by inducing its rapid neutralization, one attractive approach is the use of distinct vaccines for priming and boosting.[131-133]

Table 2-9 Determinants of Memory T-Cell Responses

Main factors	Determinants
Frequency of memory T cells	Magnitude of T-cell expansion (initial antigen load, antigen persistence)
Phenotype of memory T cells	
Effector memory	Induction favored by prolonged antigen persistence
Central memory	Induction favored by rapid antigen clearance
Persistence of memory T cells	Supported by IL-15, IL-7

The phenotype of memory T cells is also important. Two main types of memory T cells have been identified (Table 2-8) based on their phenotype and function.[134] Effector memory cells (Tem) traffic through nonlymphoid organs, where they monitor tissues for the presence of specific microbial peptides.[135] They have a high cytotoxic potential that enables them with immediate action on pathogen recognition. In contrast, central memory T cells (Tcm) preferentially traffic through lymph nodes and BM and do not exhibit much cytotoxic capacity but have a high proliferative potential. Their role is to recognize antigens transported by activated DCs into lymph nodes and to rapidly undergo massive proliferation, generating a delayed but very large wave of effector cells.[135] Antigen persistence essentially controls the proportion of Tcm and Tem memory cells (Table 2-9): Tcm cells predominate when antigen is rapidly cleared, whereas Tem cells become preponderant when antigen persists, such as in chronic infections.[126] This is also a challenge for novel nonreplicating vaccines that should induce and maintain sufficient Tem cells for immediate clearance in infected tissues. The long-term persistence of memory T cells is well established. Through homeostatic proliferation supported by specific cytokines such as IL-15 and IL-7, memory T cells may persist lifelong, even without antigen exposure.[136] Recent studies of the persistence of vaccinia-induced immune memory have confirmed that this applies to humans.[137-139]

How specific are vaccine immune responses?

The specificity of vaccine responses is at the center of many debates. Ideally, one would want vaccine-induced responses to be sufficiently broad to extend protection to nonvaccine strains (eg, for influenza, rotavirus, *S. pneumoniae*, or HPV vaccines) and sufficiently restricted to not elicit cross-reactions to allergens or self-antigens or other undesirable nonspecific effects. The specificity of vaccine responses has received added interest as a number of studies reported positive or negative nonspecific effects of vaccinations in low income countries.[140,141]

As B cells recognize conformational epitopes constituted by distant amino acids, they may bind to antigenic peptides with distinct sequences: it has been estimated that roughly 5% of

monoclonal antibodies made against 15 kinds of viruses cross-reacted with human proteins.[142] That any viral infection is not followed by the induction or flare of an autoimmune disease highlights the importance of regulatory mechanisms suppressing responses directed against self-antigens. Indeed, the specificity of antibody responses is well controlled. Although polyclonal stimulation was suggested as capable of activating memory B cells of distinct specificities,[91] which could contribute to their homeostasis, this nonspecific activation was not associated with antibody responses. Similarly, the administration of hepatitis B vaccine with CpG oligonucleotides, ie, a potent DC activating adjuvant, did not drive preexisting tetanus-specific B cells into antibody-producing plasma cells.[58] Vaccination with tetanus toxoid was found to expand specific and bystander memory T cells but did not modulate antibody responses to unrelated antigens such that antibody production remained vaccine-specific.[143] Altogether, this indicates that the induction of cross-reactive antibody responses is extremely limited, which may be important in preventing undesirable reactions, but it limits the efficacy of vaccine-induced antibody responses to very few nonvaccine serotypes.[144]

T cells need to recognize only a few amino acids of antigenic peptides displayed by MHC molecules, which offers a much greater potential for cross-reactivity. It has been estimated that each T lymphocyte could potentially bind to a million different peptides.[142] In addition, memory T cells readily respond to homeostatic cytokines, such that bystander memory T cells of distinct antigen specificity may be transiently activated and expand during a flu-like illness or an immunization process.[143,145] Despite the likelihood of cross-reactive responses to infectious agents or vaccines and the relative ease with which autoreactive lymphocytes may be elicited, vaccine-induced exacerbations of autoimmune diseases remain extremely rare, which probably reflects the efficacy of regulatory mechanisms limiting their intensity, scope, and duration.[146,147]

The induction of cross-protective T cell–mediated responses has been repeatedly observed in murine experimental models, which suggested that wide spectrum viral vaccines could be based on T-cell responses.[148] Convincing examples of heterologous protective immunity in humans were more limited: neonatal BCG protects against leprosy,[149] and people vaccinated against smallpox seem to be protected against monkeypox.[150] In contrast, the sharing of several T-cell determinants is not sufficient for a single oral polio vaccine or influenza strain to confer cross-protection. It is, thus, tempting to conclude that heterologous protective immunity essentially comes into play for T-cell rather than for antibody-mediated protective responses. Accordingly, the heterosubtypic immunity conferred by live attenuated influenza vaccines[151,152] could be mediated by T cells and/or by mucosal IgA antibodies.

Nonspecific effects of vaccines are occasionally associated with the fear of immune overload and subsequent enhanced vulnerability to infections, a theory not supported by evidence.[153,154] A series of studies in several low-income populations, particularly in West Africa, reported nonspecific beneficial effects of BCG and live measles vaccines and nonbeneficial effects of DTP on childhood mortality.[155] Randomized controlled trials would be needed to define the impact that nonspecific vaccine effects may exert on all-cause mortality in low-income countries and how they may vary by sex.

Vaccine responses at the extremes of age

The challenges of neonatal and early life immunization

According to UNICEF estimates, 4 million infants younger than 6 months die yearly of acute infections.[156] In more privileged countries, mortality has been reduced, but infections represent a significant proportion of infant hospitalizations. This disease burden is caused by a limited number of pathogens, such that the availability of a few additional vaccines that would be immunogenic soon after birth would make a huge difference. Although antigen-specific B- and T-cell responses may already be elicited in utero, early life responses markedly differ from those elicited in mature hosts. The blunting of neonatal immune responses was long considered as resulting from "neonatal tolerance", then as reflecting the antigen naïveté of the immune system and, subsequently, its immaturity. Recent work has prompted a change of perspective, leading to the recognition that the neonatal and early life immune system is, in contrast, specifically adapted to the unique challenges of early postnatal life and developing over time through poorly defined but tightly regulated processes.

These specific neonatal features first affect innate responses as pattern-recognition receptors elicit responses biased against the induction of proinflammatory cytokines, which could cause harmful alloimmune reactions against maternal antigens or excess inflammatory reactions.[157,158] In addition, many factors determine the quality and quantity of infant antibody responses: this includes the state of prenatal and postnatal development of the immune system, the type of vaccine and its immunogenicity, the number of doses and their spacing, and the influence of maternal antibodies (reviewed by Siegrist and Aspinall[159]).

Early life immune responses are characterized by age-dependent limitations of the magnitude of responses to all vaccines (Table 2-10). Antibody responses to most PS antigens are not elicited during the first 2 years of life, which is likely to reflect numerous factors, including the slow maturation of the splenic marginal zone,[42,160] limited expression of CD21 on B cells, and limited availability of the complement factors.[161] Although this may be circumvented in part by the use of glycoconjugate vaccines, even the most potent glycoconjugate vaccines elicit markedly lower primary IgG responses in young infants.[162]

Early life antibody responses are directly determined by the prenatal (eg, gestational age[163]) and the postnatal age at immunization.[161] Accelerated infant vaccine schedules in which three vaccine doses are given at 1-month intervals (2, 3, 4 or 3, 4, 5 months) thus result in lower responses than schedules in which more time elapses between doses (2, 4, 6 months) or between the priming and boosting dose (3, 5, 12 months). However, the magnitude of infant antibody responses to multiple dose schedules reflects the interval between doses, with longer intervals eliciting stronger responses, and the age at which the last vaccine dose is administered. That postnatal immune maturation is required for stronger antibody responses is best demonstrated by comparing antibody responses to single-dose vaccines given to antigen-naïve infants of various ages.[164,165] These studies may be confounded by the persistence of maternal antibodies, which negatively influence infant antibody responses in epitope-specific– and titer-specific–dependent manners.[166] Thus, multivariate analyses of the data for a large number of infants are required to identify the main determinants of vaccine antibody responses. The induction of strong antibody responses to a single vaccine dose that would be given soon after birth, unfortunately, remains an elusive goal, and adult-like responses may eventually be elicited only in older infants.

The induction of B-cell responses is critically dependent on components of the local microenvironment, whereas blood is the only accessible compartment in infants. Thus, factors that specifically limit the magnitude of early life antibody responses are difficult to study in human infants. Studies in which human infant vaccines were administered at various stages of the postnatal maturation of infant mice indicated that the same limitations of antibody responses affect early life human and murine responses, probably reflecting similar postnatal constraints.[161] These models showed that limitations of antibody responses in early life result from the limited and delayed induction of GCs in which antigen-specific B cells proliferate and differentiate. This was shown to essentially reflect the delayed development

Table 2-10 Limitations of Vaccine Responses at the Extremes of Life (Mechanisms Presumed)

In early life	
Limited magnitude of Ab responses to PS	Immaturity of marginal zone; low CD21 expression on B cells; limited availability of complement
Limited magnitude of Ab responses to proteins	Limited GC responses (? delayed FDC development); inhibitory influence of maternal antibodies
Short persistence of Ab responses to proteins	Limited establishment of bone marrow plasma cell pool (? survival niches)
Shorter duration of immune memory (?)	Limited GC responses (? magnitude of initial memory B-cell pool)
Limited IFN-γ responses	Suboptimal antigen-presenting cell/T-cell interaction (IL-12, IFN-α)
Limited $CD8^+$ T-cell responses (?)	Insufficient evidence
Influence of maternal antibodies	Inhibition of B-cell but not T-cell responses
In elderly people	
Limited magnitude of Ab responses to PS	Low reservoir of IgM^+ memory B cells; weaker differentiation into plasma cells
Limited magnitude of Ab responses to proteins	Limited GC responses: suboptimal $CD4^+$ helper responses, suboptimal B-cell activation, ? limited FDC network development; changes in B-/T-cell repertoire
Limited quality (affinity, isotype) of antibodies	Limited GC responses; changes in B-/T-cell repertoire
Short persistence of Ab responses to proteins	Limited plasma cell survival?
Limited induction of $CD4^+$/$CD8^+$ responses	Decline in naïve T-cell reservoir (accumulation of effector memory and $CD8^+$ T cell clones)
Limited persistence of $CD4^+$ responses	Limited induction of new effector memory T cells (IL-2, IL-7)

Ab, antibody; FDC, follicular dendritic cell; GC, germinal center; PS, polysaccharide.

of FDCs required to nucleate and support GC reactions.[167] This would explain the stepwise increase of antibody responses elicited in older infants, although direct evidence is difficult to obtain and is limited[160] in human infants.

In contrast with this blunting of early life antibody responses, the neonatal immune system readily allows the induction of immune memory, thus reflecting preferential differentiation of early life GC B cells toward memory rather than immunoglobulin-producing plasma cells. Neonatal priming may, thus, be used to initiate vaccine responses against hepatitis B or poliomyelitis. Recent work demonstrated that acellular pertussis vaccines may similarly effectively prime neonatal responses, resulting in faster acquisition of infant immunity.[168-170] However, neonatal priming with a combined DTaP vaccine blunted rather than primed subsequent infant pertussis responses[171] and somewhat reduced Hib and HBsAg responses, being also observed following neonatal acellular pertussis priming.[169,172] Thus, vaccine interference issues may be exacerbated in early postnatal life, requiring further studies.[173]

Although immune priming may be readily elicited at birth, memory responses elicited in early life could quantitatively differ from those elicited later. The persistence of immune memory has important implications, especially for infant immunization programs such as for hepatitis B that are intended to protect throughout adult life. The duration of such responses (eg, the boostability of hepatitis B vaccine antibody responses primed in infancy) extends for at least one decade. However, recent observations suggest that in the absence of childhood boosters, the boostability of infant-induced immunity may not persist lifelong.[93,94]

Antibody responses elicited before 12 months of age rapidly wane, and antibody titers soon return to near baseline levels,[107,174] which may be associated with a resurgence of vulnerability to infection.[95] This short duration of infant responses reflects the limited survival of antigen-specific plasma cells. This hypothesis was recently confirmed in infant mice,[167] in which early life BM stromal cells provided insufficient survival signals to plasma cells reaching BM niches.[175] Whether this similarly limits the induction of long-lived plasma cells in human infants is unknown, but short-lived antibody responses are a hallmark of early life immunization with most, although not all (eg, hepatitis B), infant vaccines. Isotype switching and somatic hypermutation, ie, the affinity maturation of vaccine induced B cells, are already functional in the first year of life,[78,176-178] including in preterm infants.[163] Few studies have compared the affinity-maturation process of vaccine responses in infants and adults, which seems to be similar (C.-A.S., unpublished observations). However, several months are required for affinity maturation of vaccine antibody responses, even in adults,[58] such that high-affinity responses are not observed in very young infants.

Neonatal and infant T-cell responses also differ from those elicited later in life, in particular in the induction of lower IFN-γ responses.[161] As examples, IFN-γ responses to oral polio vaccine are significantly lower in infants than in adults,[179] hepatitis B vaccine induces lower primary IFN-γ responses and higher secondary Th2 responses in early life than in adults,[180] and tetanus-specific IFN-γ $CD4^+$ T-cell responses progressively increase with age.[181] Comparing neonatal and infant priming with acellular pertussis vaccines indicated the preferential induction of Th2 responses on neonatal priming.[182] Whether this results essentially from the fact that neonatal APC responses to Toll-like and other pathogen-associated molecular pattern receptors produce less IFN-α, IFN-γ, and IL-12p70 and more IL-10 than adult cells[183-185] as a result of complex epigenetic controls or from the predominance of recent thymic emigrants in neonatal human and murine blood[186] is unknown. Other factors, such as the predominance of Tregs that are abundant during fetal life,[187] remain to be studied. Remarkably, certain vaccines may elicit potent IFN-γ T-cell responses in infants, and adult-like neonatal responses are notoriously elicited by BCG.[188] This suggests that adult-like $CD4^+$ Th1 responses are elicited only by vaccine formulations (ie, adjuvants or delivery systems) capable of circumventing the specificities that characterize neonatal

patterns. Whether neonatal CD4+ T cells have higher intrinsic requirements for antigen-specific activation and how neonatal features affect human neonatal CD8+ T-cell vaccine responses, which are readily elicited following viral infections, require further investigations. Such studies will be especially important for the development of novel T cell–based vaccines.

Importantly, the induction of early life B- and T-cell vaccine responses takes place in an environment that may be influenced by the presence of antibodies of maternal origin. IgG antibodies are actively transferred through the placenta, via the FcRn receptor, from the maternal to the fetal circulation.[189] On immunization, maternal antibodies bind to their specific epitopes at the antigen surface, competing with infant B cells and, thus, limiting B-cell activation, proliferation, and differentiation. The inhibitory influence of maternal antibodies on infant B-cell responses affects all vaccine types, although its influence is more marked for live attenuated viral vaccines that may be neutralized by even minute amounts of passive antibodies.[190] This inhibition is epitope-specific, such that infant responses to nonimmunodominant maternal epitopes may still be elicited.[191] Consequently, maternal antibodies to carrier proteins (ie, to tetanus toxoid) mediate a specific inhibitory influence on infant responses to tetanus toxoid, but not to the Hib PS moiety.[192,193] Maternal antibodies were recently reported as inhibiting cotton-rat B-cell responses by interaction with the inhibitory/regulatory FcγRIIB receptor on antigen-specific B cells.[194] Whether this regulatory mechanism similarly accounts for the inhibition of human infant responses is yet undefined.

The inhibitory influence of maternal antibodies is antibody titer–dependent, or rather reflects the ratio of maternal antibodies to vaccine antigen.[58] This was elegantly demonstrated in a study in which Israeli infants were immunized with hepatitis A vaccine at 2, 4, and 6 months and blood was sampled immediately before each vaccine dose and at 7 months of age.[195] The first vaccine dose induced detectable responses only in infants immunized in the absence of detectable maternal antibodies. The second vaccine dose induced detectable responses in infants primed in the presence of maternal antibodies less than 1,999 mIU/mL and the third dose in infants primed in the presence of maternal antibodies less than 3,999 mIU/mL. Overall, infant responses were elicited only when maternal antibodies declined to a threshold of 300 to 400 mIU/mL.[195] The maternal antibody titer at which infant responses may be elicited can be defined only experimentally by comparing antibody responses in infants stratified according to maternal antibody titers at the time of priming.

The extent and duration of the inhibitory influence of maternal antibodies, therefore, increase with gestational age,[163] eg, with the amount of transferred immunoglobulins, and decline with postnatal age, as maternal antibodies wane.[58] Increasing the dose of vaccine antigen may also be sufficient to circumvent the inhibitory influence of maternal antibodies, as illustrated for hepatitis A[196] and measles[197] vaccines.

Although maternal antibodies interfere with the induction of infant antibody responses, they may allow a certain degree of priming, ie, of induction of memory B cells. This likely reflects the fact that limited amounts of unmasked vaccine antigens may be sufficient for priming of memory B cells but not for full-blown GC activation, although direct evidence is lacking. Importantly, however, antibodies of maternal origin do not exert their inhibitory influence on infant T-cell responses, which remain largely unaffected or even enhanced.[198-200] This is best explained by the fate of maternal antibodies–vaccine antigen complexes: immune complexes are taken up by macrophages and DCs, dissociate into their acidic phagolysosome compartment, and are processed into small peptides. These peptides are displayed at the surface of APCs and are available for binding by CD4+ and CD8+ T cells.

Thus, the challenges for further improvement of early life immunization strategies are to identify vaccine formulations and strategies capable of inducing, after one or two early doses, the strong primary antibody responses required against certain early life pathogens. To elicit prolonged vaccine efficacy, such formulations/strategies would have to overcome the inhibitory influence of maternal antibodies for sufficient priming to occur and to elicit more long-lived plasma cells despite the limitations of the early life BM compartment. T cell–based infant vaccines will have to meet the challenge of bypassing the factors that limit the induction of Th1 early life responses. Importantly, these immunological objectives should be reached by formulations/strategies demonstrated as safe in immunologically immature hosts, adding to the challenges.[201]

Age-associated changes in vaccine responses

Innate and adaptive antibody and T cell–mediated cellular immune responses decline with age, which increases the frequency and severity of infections and reduces the protective effects of vaccinations. Aging affects the magnitude and the persistence of antibody responses to protein vaccines,[202,203] as reflected by lower serum antibodies to influenza,[204,205] tetanus, and tick-borne encephalitis (TBE) vaccines.[206] It also affects responses to pneumococcal PS vaccines, although differences in methodological issues have yielded contradictory results.[207] Remarkably, the aging process that limits antibody responses is initiated early: after the age of 20 years, each 10-year period reduced by 31% antibody titers elicited by a potent adjuvanted pandemic influenza vaccine in healthy control subjects and immunosuppressed patients.[208] In contrast with infants whose antibody responses are quantitatively limited but seem qualitatively similar to those of mature people, limitations of antibody responses in elderly people are also associated with qualitative changes that affect antibody specificity, isotype, and affinity, ie, functional efficacy (Table 2-10).[209,210]

The age-associated limitations of antibody responses result from the influence of a large number of underlying events that have been recently reviewed.[159,211] Responses to T-independent PS vaccines are directly conditioned by a decline in the reservoir of IgM+ memory B cells that are present in reduced numbers, differentiate less efficiently into antibody producing cells, and, thus, limit the IgM responses to PS of aged people.[212] Antibody responses relying on the induction of GCs are also limited in elderly subjects.[213] This limitation of GCs limits B-cell proliferation and differentiation, limiting the magnitude and function of antibody responses. It also restricts hypersomatic mutations in immunoglobulin genes, such that antibodies are of weaker affinities/functional capacities than those generated in younger people.[210] Last, limitations of GCs prevent efficient immunoglobulin class switching, resulting in age-associated differences in IgG1 and IgG2 subclass antibodies, eg, to pneumococcal PS.[214] Numerous factors contribute to limiting the induction of GCs in elderly persons, including factors that are intrinsic to B cells[215] and that affect other cell types. For example, studies in aged mice have convincingly demonstrated the existence of age-related changes in FDCs, whose molecular interactions with B cells are critical for the induction and maintenance of GCs.[216,217] The limited ability of aged subjects to generate high-affinity antibody responses also reflects changes in their antibody repertoire as a result of differences in B- and CD4+ T-cell response capacity.[217,218]

Age-associated changes in T-cell responses are reflected by a progressive decline in naïve T cells, reflecting declining thymic output. This is associated with a marked accumulation of large CD8+ clones presumably resulting from prior infections. These large T-cell clones, eg, elicited in response to cytomegalovirus, have reached a state of replicative senescence, and homeostatic mechanisms negatively influence the size of the naïve and effector memory T-cell subsets.[203] In response to influenza

immunization, healthy elderly people mount CD4+ responses initially similar to those of young adults but that fail to maintain or expand such that T-cell responses assessed 3 months after immunization are markedly lower than in younger adults.[219] This does not reflect a functional impairment of CD4+ T memory cells[220] but a shift of the T-cell pool from naïve to memory effector CD4+ T cells. The failure to maintain CD4+ responses reflects a lower induction of new effector memory T cells in relation to lower IL-7 levels.[219,220] Other studies indicated that frail elderly subjects mount blunted and delayed Th1 responses to influenza vaccination, which correlated positively with their reduced total and IgG1 antibody response.[221] Limitations also affect the expansion of infection-driven influenza-specific CD8+ T cells.[221] Strategies to enhance vaccine-induced protection in aging people include the development of vaccine formulations capable of a stronger induction of specific B- and T-cell responses, for example through the selection of specific adjuvants. Nevertheless, changes in the repertoire may prove difficult to circumvent, and limitations of effector memory responses and of GC responses may continue to require the more frequent administration of certain vaccine boosters (eg, against tetanus or TBE[221]) to compensate for the brevity of B- and T-cell vaccine-induced responses in elderly people.

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